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Response of freshwater communities to hydrology and temperature regime changes accelerated by climate change and human activities

Habilitation thesis

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Content

Introduction

Over recent decades, impacts of climate change on all ecosystems around the globe have become indisputable. Amongst these, freshwater habitats are being threatened by increasingly unstable hydrological and thermal regimes, with increased flow regime variability and stochasticity, especially when accompanied by warm episodes, causing previously permanent freshwater habitats to dry out and shift episodically to terrestrial environments. Such significant changes are reflected in both taxonomic and functional composition changes in recent communities. In addition, these changes may also be derived from contemporary spatial arrangements of genetically divergent lineages of cryptic species persisting in refuges over long geological periods (**Copilas–Ciocianu et al. 2017**). Further, past changes may also be inferred based on former sub–recent communities preserved in the paleoarchives of long–persisting lakes (e.g. from the last glaciation). These aquatic systems may have passed through multiple climate–driven environmental shifts during their quaternary development (e.g. temperature oscillations accompanied by dry and wet phases). Complex and mechanistic studies analysing these previous climate and environment events can help predict current and future changes in ecosystem functioning, allowing for evidence–based informed decisions for effective conservation measures aimed at minimising potential negative impacts and trends induced by climate change.

Freshwater benthic communities represent a suitable model group for tracking such hydrological and thermal regime changes (and their mutual interaction) as they are disposed by adaptation to withstand complete water loss or temperature fluctuations. Such adaptations to changing environments are reflected in a range of species ecological and biological traits, readily described in available databases, enabling the construction of robust models that enable future predictions incorporating climate change scenarios.

Analysis of environmental variability, as reflected in changes to communities, should always be assessed on temporal scales that reflect different levels of biological processes. Biological cycles occurring within the lifespan (months/years) of typical benthic macroinvertebrates (MIV) can be captured over daily, seasonal or annual scales, while long–term processes modifying communities, or even taxa and their phylogeographic distribution, occur over long–term centennial, millennial or geological scales. Only by incorporating and complementing recent and historical processes shaping present communities (and their fundamental units, i.e. species) at different time–scales can we gain a complex understanding of the impacts governed by different processes at regional– and global–scales during the present, highly dynamic Anthropocene period.

In recent decades, and especially in temperate regions of Central Europe, dynamic lotic ecosystem communities have had to cope with the novel phenomena of extensive stream drying (**Crabot et al. 2021**) during pronounced supra–seasonal droughts (*sensu* Lake 2003) between 2014 and 2019 (Moravec et al. 2021). As such, the communities of previously perennial streams are currently reflecting recent short–term climate shifts. Moreover, the gradual cessation of flows (i.e. a shift from lotic to lentic habitats, accompanied by siltation) and the onset of dry phases in what were traditionally permanently aquatic habitats, boosts the impact of additional natural or human–induced stressors, such as pollution and eutrophication (amplified by reduced dilution). Additionally, the temperature moderation (increase in mean, maxima and oscillation amplitude; see **Pařil et al. 2010a**) leading in the final stage to complete desiccation.

On the other hand, inter–annually more stable standing water communities in lake paleoarchives complement current rapid climate change shifts by offering a long–term perspective, because these lentic habitats having passed through repeated warm/cold and wet/dry oscillations over millennial or longer time–scales. Lake habitats that have persisted for thousands of years also preserve archives of complementary biotic proxies, mostly lacking in river sediments due to their highly dynamic flow regimes, that enable more complex views of past environments.

Aquatic MIV communities are useful not only as bioindicators of such changes but also as mediators of biochemical processes closely associated with ecosystem services, such as organic matter processing, CO_2 emissions, etc.). Linkages between in–stream processes and functional or taxonomic composition of assemblages facilitate informed insights into present community changes and enable well–founded predictions of their future development. As Central Europe is presently experiencing turbulent climate shifts manifested in increased air temperatures and the occurrence of more frequent extreme weather events (flash floods, hurricanes, heat and cold waves, long–term droughts), accurate predictions of their future impacts and trajectories are becoming crucial. In the Czech Republic (CR), the onset of dry episodes is likely to have been caused not only by rising air temperatures and unstable/decreasing precipitation (Trnka et al. 2016), but also by complex shifts in land use and river network management, resulting in lowered water retention in landscape structures (Petřík et al. 2015).

The search for answers in complex aquatic ecosystems calls for a comprehensive approach based on community analysis approaches that complement each other. With regard to the above–mentioned climatically and anthropogenically induced trends, I have focused my work on the responses of aquatic communities to these drivers, with the aim of i) understanding of their response mechanisms, and ii) developing tangible tools for monitoring and efficiently managing such communities.

This habilitation thesis is based on a collection of 23 papers published over the past sixteen years. Conducted primarily in Czech running waters, with additional studies in Slovak paleolakes, with a special emphasis on the effect of current and past climate change on aquatic communities, incorporating the impact of human alterations to aquatic habitats. The main model group used, i.e. benthic MIVs, are sensitive indicators of shifts in environmental conditions. In my published papers, these are primarily represented by permanent fauna (mostly aquatic oligochaetes and gammarids) and temporal fauna as Ephemeroptera, Plecoptera and Trichoptera (EPT taxa) or Ditera–Chironomidae. Each group react differently to the examined drivers, thus providing a comprehensive variety of biotic responses. In several studies, community or species responses to environmental change were complemented by parallel analyses of autotrophic community components (e.g. in paleoreconstructions papers mostly by diatoms, green algae or macrophytes analysed by my co–authors). Incorporation of multilevel responses by different trophic groups provided a more complex overview of the numerous interconnected environmental factors and their impacts, allowing more robust reconstructions of climate and environment development.

Part I: Macroinvertebrate communities of Central European intermittent streams

Part I.I: Response of macroinvertebrate communities and sensitive groups to the novel phenomena of stream drying

Naturally intermittent rivers and ephemeral streams (IRES) are surprisingly prevalent in river networks all over the globe (Messager et al. 2021). While they are a dominant landscape feature in Mediterranean and arid regions they are also abundant in more humid climates (Stubbington et al. 2017). The present “Anthropocene period” is characterised by increasing human alterations to natural flow regimes and increasing water consumption related to industrial processes, food production and urban settlement, which in turn have led to increased flow fluctuations. These changes, alongside the growing impacts of a changing climate, have caused the proportion of drying streams in river networks to grow over time (Boergens et al. 2020, Možný et al. 2020).

In recent decades, many previously perennial rivers in Central Europe have become intermittent (Döll & Schmied 2012, Laaha et al. 2017), and this has become especially relevant in countries with continental humid climates, such as the CR (Peel et al. 2007), where IRES were uncommon in the past. The increasing occurrence of summer heatwaves, resulting in raised evapotranspiration (Brázdil et al. 2009), as well as increased pressure on water resources and land–use changes over recent decades, have increased the extent of stream drying in Central Europe both spatially and temporally (Fiala et al. 2010, Laaha et al. 2017). As a result, many streams have started to dry out for the first time, particularly during the extremely harsh dry episodes that occurred in 2003, 2015 and 2018 (Van Lanen et al. 2016, Moravec et al. 2021). Consequently, around 50% of the total stream length in the CR is presently at risk of drying **(Zahrádková et al. 2015)**.

Prior to 2007, the effects of drying on aquatic invertebrate communities in Central Europe had been understudied, with most authors focussing on simple descriptions of community composition in IRES (e.g. Pastuchová 2006 in Slovakia; Bohle 2000 & Meyer et al. 2003 in Germany). Most previous research on flow cessation in the CR has focused on low flow impacts (e.g. Kubíček et al. 1999), from which threshold values for minimal ecological flows have been inferred (e.g. Mrkvičková & Balvín 2011), while streams that dried out completely were typically excluded from studies and monitoring programmes (**Stubbington et al. 2018**). However, in 2003, the first extreme Czech drought of the 21st century (Řičicová et al. 2004) raised serious questions about the possible impacts of drying on stream communities, and generate the urgent need for extensive research into these jeopardised ecosystems, previously ignored by most limnologists in temperate climates.

Based on this limited knowledge of drying stream communities, the initial phase of my research was focused on quantification of the temporal effects of drying on MIV communities. In the first study, we compared two reaches (downstream near–perennial and upstream intermittent) that dried out over different spatial and temporal extents over two successive years (**Paper 1: Řezníčková et al. 2007**). In this stream, dry episodes resulted in a decrease in MIV density over the short intermittent reach (hundreds of metres), but had no marked effect on taxa number, which remained comparable to a nearby (2 km downstream) near–perennial site, a pattern also observed by previous studies (e.g. Miller & Golladay 1996).

Regarding functional composition, we detected a rapid decline in permanent fauna and an increase in r–strategists before the onset of the dry phase at the intermittent site, while the near–perennial site hosted mostly K–strategists. This pattern lasted up to the first post–drying samples. Permanent and temporary fauna groups also differed in their ability to recolonise the subsequently rewetted reach. While the dominant crustaceans (*Gammarus* spp.) rapidly returned to the site after the short dry episode, aquatic insects with a long life–cycle, i.e. EPT taxa, only gradually recolonised the stretch, continuing up to the end of sampling in late autumn. This initial case study showed clear differences in response between MIV fauna in near–perennial and intermittent river sections, despite their proximity, suggesting the importance of perennial refuges and recolonisation pathways for enabling the persistence of MIV communities in intermittent reaches.

Closer insights and better understanding of potentially hidden mechanisms for long–term MIV survival at intermittent sites was provided through the analysis of selected traits favouring the Ephemeroptera (mayflies), an extensively studied model group due to their i) well–known traits, ii) well–defined taxonomy, and iii) sensitivity to drying (**Paper 2: Řezníčková et al. 2010**). This study, undertaken in the Podyjí (Thayatal) National Park, one of driest region in the CR, examined mayfly assemblages in two independent perennial and intermittent streams with comparable physico–chemical characteristics, hydromorphology and low anthropogenic alterations, situated ca. 10 km apart. Mayfly taxa from both sites were compared using five relevant biological and ecological traits favouring their ability to cope with drying, that were scored according to their expected benefit for species survival.

The mayfly taxocene of the intermittent stream was short of nine species comparing to perennial one, when mostly taxa with rheobiontic and oxyphilic ecological traits absent in drying stretch. While intermittent stream taxa exhibited the highest sum of favourable traits enabling dry–phase survival, we also found several low–scoring taxa persisting in the drying reach. These exceptions probably being related to different dry impact selection mechanisms within a group of closely related congeners. We conclude that the improved desiccation adaptations of these exceptional taxa probably originate from some of their “more powerful” traits eliminating the disadvantages of other unfavourable traits. This finding led us to the conclusion that complex taxonomic and functional analyses of whole MIV communities would be necessary to obtain more consistent results.

Just such a comparison, based on the whole MIV community, was performed on the same pair of streams in our next paper (**Paper 3: Řezníčková et al. 2013),** using taxonomic and functional community characteristics. In some aspects, this comparison showed a similar pattern to that revealed in the first study (**Řezníčková et al. 2007)**, i.e. reduced MIV densities in the intermittent stream. However, unlike the initial single stream study, taxonomic diversity was significantly higher in the perennial stream, as was the proportion of temporary fauna. Furthermore, the representation of drying–sensitive community traits exhibited similar expected differences between both stream types to those of the previous single–group mayfly study (**Řezníčková et al. 2010**), i.e. an absence of rheobionts and taxa with a high oxygen demand taxa on intermittent stream. We also recorded a significantly lower representation of grazers/scrapers in the regularly drying stream, possibly linked to the slower current conditions during the onset of the pool phase, when increased siltation may limit algal growth (**Pařil et al. 2010b**, Piano et al. 2019).

Part I.II: Stream drying bioindication and monitoring tools developed and tested in Central Europe

The three initial studies outlined above implied some general patterns differentiating MIV communities of perennial and IRES in our target temperate region, and from this arose the idea to use these differences to construct a bioindication approach discriminating between communities impacted and unimpacted by river drying. In 2011, during preparation of the first project (biodrought.eu, sucho.eu) in which I acted as principal investigator, we found in the literature only few references to applicable methods for retrospective bioindication of antecedent drying. Most available results at that time came from initial studies or technical reports comprising only lists of potential bioindicator taxa; these works lacking any detailed community analysis with defined accuracy of stream classification to flow state enabling practical use for stream monitoring (e.g. USA – Mazzacano & Black 2008; Germany – Bohle 2000; Australia – Rose et al. 2008).

Consequently, we based our approach on climatically conditioned differences between perennial streams and IRES originating from communities displaying low adaptation to drying in temperate climates. Unlike arid and Mediterranean regions, where intermittent rivers dominate and communities are well adapted due to frequent drying over evolutionary long periods, we expect MIV communities in humid temperate regions to be less well adapted with longer post–drying recovery periods (Bonada et al. 2007). Owing to the irregular occurrence of river drying in temperate regions, which can vary markedly in spatiotemporal extent from year to year, we expected fewer drying–adapted communities with less well developed resistance/resilience (RR) traits (**Crabot et al. 2021, Stubbington et al. 2022**). Moreover, unlike Mediterranean rivers, which are rapidly recolonised (Vander Vorste et al. 2016), we expected temperate regions to display long–lasting dry–episode fingerprints, that would be detectable by shifts in taxonomic and functional community structure for several months to a year after flow resumption.

To test these hypotheses, we sampled in BIODROUGHT project different regions across the CR using a paired design, i.e. a perennial and intermittent site in each region no more than 20 km apart to compare regionally specific species pools. Only near–pristine streams were included in the study to eliminate interference with other stressors (e.g. pollution, hydromorphology and hydrology alterations). By comparing state monitoring data within the SALAMANDER database (Kokeš et al. 2006) and samples collected during our own project, we were able to show a general pattern differentiating MIV communities of perennial streams and IRES (**Pařil 2015**). In IRES communities, we observed i) a marked decrease in MIV diversity, ii) a slight but still significant decrease in MIV abundance (for both see Fig. 1), iii) increased representation of bioindicator taxa able to cope with drying, and iv) a prevalence of species traits enabling tolerance to drying. These differences were less pronounced in spring samples (community had a longer recolonisation time) than autumn early post–dry recovery samples, which led us to separate analyses for each season.

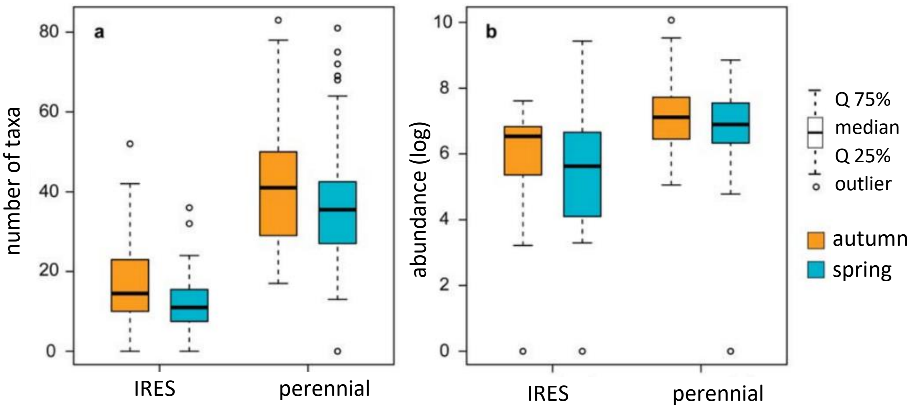

Figure 1. Differences in number of taxa *(a)* ***and abundance*** *(b)* ***between perennial and IRES sites in spring*** *(blue)* ***and autumn*** *(orange)* ***seasons, based on the SALAMANDER database analysed in the BIODROUGHT project.*** *Adapted from Pařil 2015.*

The spatial and temporal extent of drying, i.e. the length of dry stretch and the duration of the dry episode, differed between sites along an intermittence gradient, which also had to be considered in the development of the multi–metric index discriminating between drying impact. We arbitrarily defined flow regime categories according to annual regularity and duration of the dry phase in the 365 days before sampling, with drying defined by an absence of surface flow on the riverbed. Wee distinguished three stream categories along the intermittence gradient: i) intermittent (duration of dry phase typically less than week), ii) near–perennial (duration of dry phase more than week), and iii) perennial stream (continual flow without a dry phase).

The multi–metric index was based on the most efficient combination of taxonomic composition descriptors and functional trait representation, and completed by an index expressing the proportion of indicator taxa characteristic for intermittent and perennial flow regimes. Sensitive indicator groups of EPT taxa played a crucial role in this approach as they significantly discriminated between the three stream types based on a) total abundance, b) taxa number, and c) proportional representation in the whole community (Fig. 2).

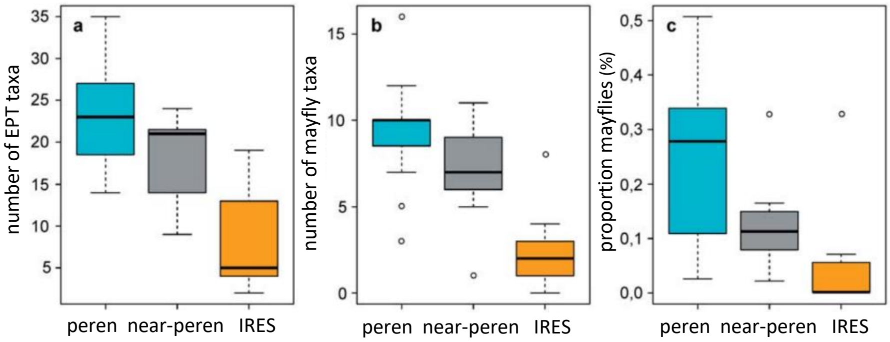

Figure 2. Gradual decrease in the number of EPT taxa *(a)*, ***number of mayfly taxa*** *(b)* ***and proportional representation of Ephemeroptera*** *(c)* ***along an intermittence gradient between three defined stream types.*** *peren = perennial, near–perennial and IRES = intermittent streams. Adapted from Pařil 2015.*

EPT taxa are frequently employed as indicators for assessment of river ecological quality and are used as such within the EUs Water framework directive (WFD; European commission 2000). Indeed, EPT taxa have been used within the CR to discriminate between ecological states in several Czech river types as part of WFD implementation (**Brabec et al. 2004**). In addition to their high sensitivity to drying, they respond strongly to multiple anthropogenic stressors, including organic pollution, eutrophication and morphological degradation, which could complicate their targeted use for indication of drying. For their effective use in this role, therefore, it is essential that we disentangle the impacts of dry episodes from those of other anthropogenic stressors (**Stubbington et al. 2022**). Robust and sensitive tools for identification of antecedent dry episodes can only be obtained through the inclusion of complex metrics including the whole MIV community, identified to the highest possible level (predominantly species or genus) with linked species traits. During development of the first version of the multi–metric index (BIODROUGHT), we used a combined approach for assembling permanence or intermittence indicators employing indicator species analysis of available datasets. In addition, we included taxa from a literature search, with only those indicators repeatedly reported from perennial or IRES streams being included in the indicator list.

We also employed functional community characteristics alongside this traditional taxonomic approach, using traits favouring those MIVs surviving in IRES. Based on a linear discriminant analysis combining several community descriptors in one metric, we developed a robust method characterising antecedent flow regimes on site and discriminating MIV samples from three predefined stream categories with a high probability of correct classification (between 80 and 90% in the CR; see **Straka et al. 2019, 2021**; **Zahrádková et al. 2015**). This high classification accuracy was enabled by two versions of the multi–metric, each adjusted separately for the traditional spring and autumn sampling seasons, each of which reflect different levels of post–drought recovery.

To exclude inappropriate use of the method in practical monitoring, its applicability was restricted to geographical, hydromorphological and natural characteristics of sites used for method development (i.e. it was not suitable for heavily impacted streams displaying strong pollution, acidification, or morphological or hydrological alterations). In addition to the BIODROUGHT method, which has been certified by the Ministry of the Environment of the CR (**Pařil et al. 2015**), we also developed a freeware BIODROUGHT calculator for analysing imported samples (see Fig. 3; http://biodrought.eu/BScalc.php). This has reached a broad international audience, as shown by the analysis of several hundred imported samples to date.

BIODROUGHT index calculator

This BIODROUGHT calculator allows calculation of the BIODROUGHT multimeric index, which determines the probability that a drying event occurred in the year preceding sampling). The calculation is based on analysis of the taxonomic and functional composition of macroinvertebrate samples taken < 1 year after the suspected flow resumption.

The calculator analyses single samples comprising a list of taxa and their abundances. Either import your data as a CSV (comma delimited) file or manually enter each taxon and its abundance.

If importing a .csv file, enter "taxa names" in cell 1A and "abundances" in cell 1B, with data in cells 2A and 2B onwards. During the calculation, any additional information (e.g. species traits) will be added to your taxa list and any necessary taxonomic adjustments will be made automatically. The taxa in your data file must be included in the calculator database, which you can download HERE. If an entered taxon is not in the database, a warning indicating mismatched names and suggested alternative taxa names is printed.

Ensure the correct season is selected, because the calculator uses different formulas to calculate the index for spring and autumn seasons; the calculator is not suitable for samples collected in other seasons.

File entry

Vybrat soubor Soubor nevybrán

Please, select season:
○ Spring ○ Autumn
Spring samples: March 1- May 15; Autumn samples: September 15- November 15

SUBMIT FILE

Taxon entry

Enter taxon name Abundance

ADD ROW

Please, select season:
○ Spring ○ Autumn
Spring samples: March 1- May 15; Autumn samples: September 15- November 15

SUBMIT TAXALIST

The calculator will produce the following output. Further information on each metric can be found HERE:

Sum of indicator taxa weights ☐
Czech saprobic index ☐
Number of individuals ☐
BIODROUGHT score ☐
Probability of classification as intermittent stream ☐
Probability of classification as stream vulnerable to drying ☐
Probability of classification as permanent stream ☐

Figure 3. Front page of the BIODROUGHT calculator enabling calculation all components of multi–metric from imported samples and showing the probability of classification into one of three flow categories along an intermittence gradient *(www.biodrought.eu).*

This new method could help fill gaps in water agencies monitoring of small streams drying as traditional hydrological monitoring using gauging stations suffers from low spatial coverage of river networks, especially as regards low–order streams. In such small streams, water levels can decrease rapidly (e.g. daily; Dvořáková et al. 2012), especially during the increasingly frequent summer heat waves affecting Central Europe, where intensive vegetative evapotranspiration can lead to rapid flow cessation. Our method is sensitive enough to retrospectively capture even short dry episodes lasting several days, and thus reveal stream intermittence in catchments lacking any other relevant tool (e.g. gauging stations) for capturing antecedent drying.

The second important contribution of the BIODROUGHT project to the monitoring of Czech IRES has been the "Map of stream drying risk" (**Zahrádková et al. 2015**), developed as a 1 : 200,000 resolution GIS layer classifying all 4th order catchments in the CR (https://heis.vuv.cz/data/webmap/datovesady/projekty/Biosucho/default.asp). Classification of the ca. 8,700 small Czech catchments into one of three drying risk categories (low, middle, high risk) was accomplished using two complementary analyses. The first involved classification of 1 362 MIV samples from 332 sites across the CR, originating from the SALAMANDER database, into the three flow categories, according to the BIODROUGHT index. Owing to the high classification accuracy of our BIODROUGHT index (< 10% misclassifications for this database), we were able to substitute any missing direct observations of drying on small streams from gauging stations by deriving BIODROUGHT index flow categories. The second input for drying risk classification of IV. order catchments (differs from Strahler stream order) involved GIS analysis of important factors driving the occurrence of drying streams (e.g. geology, land–use, climate, presence of standing water bodies). Classification tree analysis confirmed a combination of precipitation deficit, land–cover type, proportion of clay sediments in catchment geology, geomorphological characteristics and (partially) a higher representation of sandstone or karstic geology as the most important factors contributing to an increased frequency of stream drying. Classifications resulting from this combination of factors were then used to delimit each small catchment into one of the three drying–risk categories, the final risk categories and combination of factors leading to the classification being displayed on a map (Fig. 4).

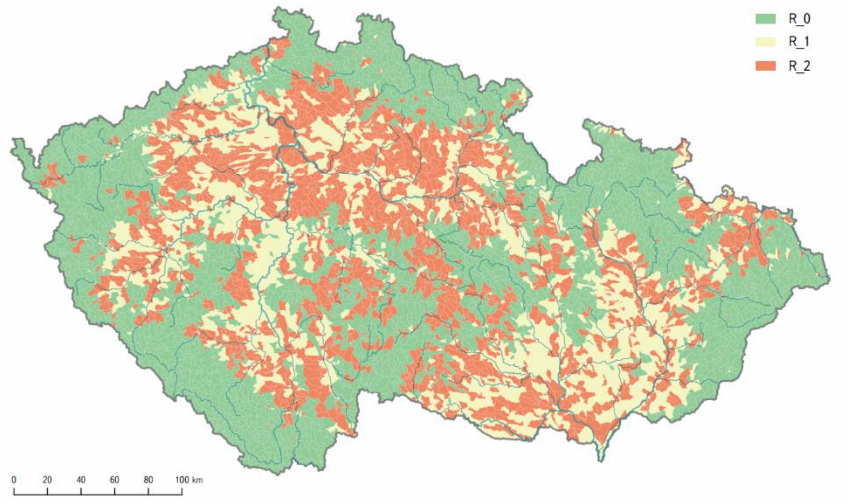

Figure 4. Stream drying–risk classification for all 4th order Czech catchments, developed as part of the BIODROUGHT project. *Stream drying–risk categories: R0 (green) = low, R1 (yellow) = intermediate, R2 (orange) = high. Adapted from Zahrádková et al. 2015.*

This initial approach for retrospective identification of antecedent drying episodes based on MIV community (see schematic representation in Fig. 5; **Pařil et al. 2015**) was later substantially improved through international collaboration with leading European specialists in intermittent river ecology, i.e. Rachel Stubbington from the UK and Thibault Datry from France. The main aim of the method upgrade (**Paper 4: Straka et al. 2019**) was to increase the "succession rate of correct flow–regime classifications", achieved through modification of indicator taxa list used (i.e. indicators acquired solely from a literature search were excluded, and those selected by Indicator species analysis kept). In addition, we tested for the effect of season and potential differences between communities from two different ecoregions, i.e. the Carpathians and the Central highlands (*sensu* Illies 1967). Despite we found significant differences for both, the season and ecoregion as well, they did not reduce index classification accuracy into three flow categories. We then cross–validated the upgraded version using an independent dataset derived from the SALAMANDER state monitoring program. Owing to lack of precise drying information in that database, we were only able to achieve a rough sample classification based on two categories, i.e. intermittent and perennial.

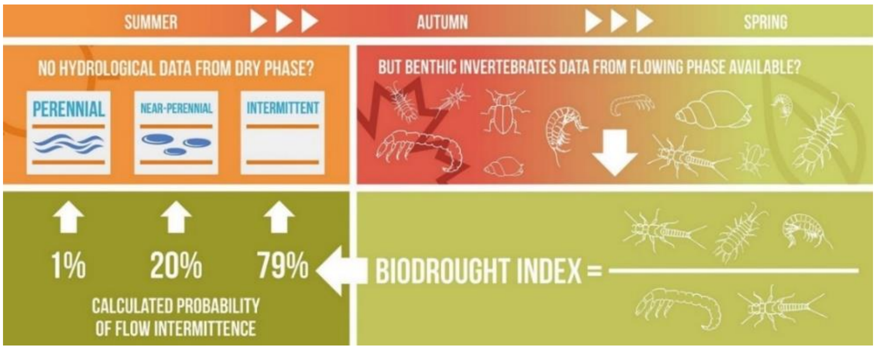

Figure 5. Schematic illustration showing practical use of the BIODROUGHT index for bioindication of antecedent stream drying in river networks lacking gauging stations *(calculated probability of sample classification to flow categories as an example only). Adapted from Straka et al. 2019.*

While some components of the BIODROUGHT multi–metric index remained unchanged, the advanced approach used a slightly modified list of permanent/intermittent indicator taxa and differing in some selected trait characteristics (e.g. body flexibility or organic substrate preference). As with the previous version, we identified several important community characteristics gradually changing along intermittence gradient, including lowered abundance and taxa richness (Fig. 6) or changes in the representation of the drying–sensitive mayfly group Heptageniidae (see **Řezníčková et al. 2010**). In addition components of this new method worked consistently over both seasons spring and autumn. After the index modifications, the accuracy of sample classification to flow category increased to 92% and 96% for samples from perennial and non–perennial sites, respectively (the accuracy of the previous multi–metric version usually not exceeded 90%).

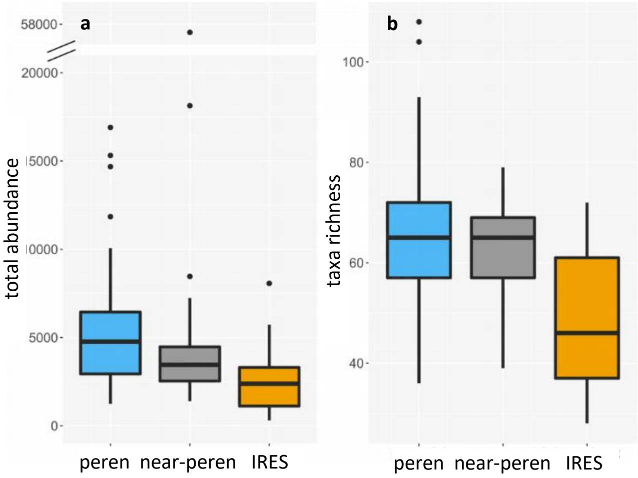

Figure 6. Differences in macroinvertebrate abundance *(a)* ***and taxa richness*** *(b)* ***over three flow categories*** *(peren = perennial and IRES = intermittent). Adapted from Straka et al. 2019.*

As the new metric displayed higher classification accuracy, and as the traits included in the improved multi–metric formula were better covered in the most of European MIV trait databases, we decided to test the validity of the method over a wider geographical scale within Central Europe (**Paper 5: Straka et al. 2021**). Prior to 2021, no biota–based tool discriminating between intermittent and perennial stream communities had been applied in this region (**Straka et al. 2019**); consequently, we decided to test our innovative approach over five biogeographical regions (Dinaric Western Balkans, Central Highlands, Hungarian lowlands, Central Plains and the Carpathians) covering the three major climatic zones, i.e. Continental, Pannonian and Alpine. We obtained suitable datasets with i) known flow regimes from the antecedent year, ii) taxonomic resolution (predominantly species/genus) and iii) appropriate timing of sampling within season from four neighbouring countries, i.e. Austria, Germany, Slovakia and Hungary. In addition, we decided to retest the Czech dataset, which only had the rough discrimination of the antecedent flow regime, i.e. intermittent vs. perennial.

Though the samples all originated from different climatic and biogeographical regions, in most cases the BIODROUGHT index clearly reflected the previous flow state and identified stream drying. The misidentification rate between intermittent and perennial samples was very low, ranging from 0% to 6%, i.e. just six of 330 samples classified in the wrong opposite category. Although the foreign datasets utilised different taxa identification levels (ranging from 100% species level identification to > 50% of MIVs identified to higher than family level), there was no marked effect of taxonomic resolution on the probability of correct classification. Thus, our study confirmed the robustness and reliability of the BIODROUGHT index (Fig. 6), showing it to be an effective tool for assessing the recent hydrological history of small– and medium–sized (up to 4th Strahler order) unpolluted streams across the whole Central European region.

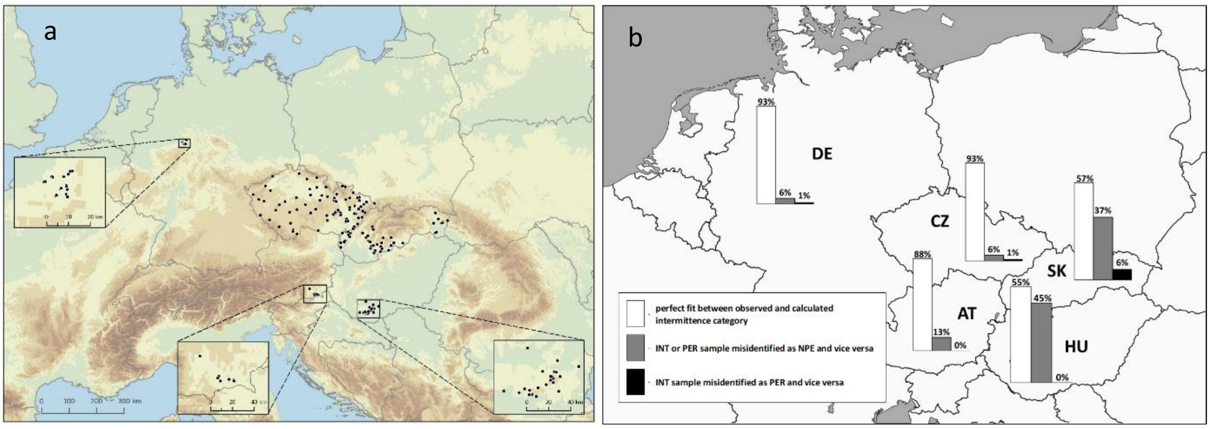

***Figure 6. Maps showing the location of datasets obtained from five Central European countries** (a) **and the percentage of correct sample classifications in each country according to the BIODROUGHT index** (b). Columns indicate success rate (%) of sample classification: white columns = correct classification; grey = misclassification to the neighbouring category; black = misclassification to opposite category (i.e. perennial sample in intermittent category or vice versa). Adapted from Straka et al. 2021.*

A similar approach, inspired by our method, was later used for intermittence recognition in low order streams in both temperate (England et al. 2019 – UK) and more arid zones (Chessman et al. 2022 – Australia, Theodoropoulos et al. 2020 – Greece, Milіša et al. 2022 – temperate and Mediterranean Europe, Fritz et al. 2020 – USA). Our approach, which offers relevant information on the antecedent flow regime of monitored streams, differs from traditional biomonitoring methods used for classification of river “ecological status” according to WFD as these were designed primarily for perennial streams. Consequently, currently used WFD assessment methods are not adapted for IRES and frequently produce incorrect assessments that typically underestimate intermittent stream ecological state, placing it in a worse category (**Stubbington et al. 2018, Stubbington et al. 2022**).

During the BIODROUGHT index development process, we also observed increasing variability in community composition with increasing flow intermittence gradient (Fig. 7). This raises the question of which mechanisms shape Central European stream communities in previously perennial catchments, as opposed to river networks historically exposed to regular drying in arid regions since the Holocene.

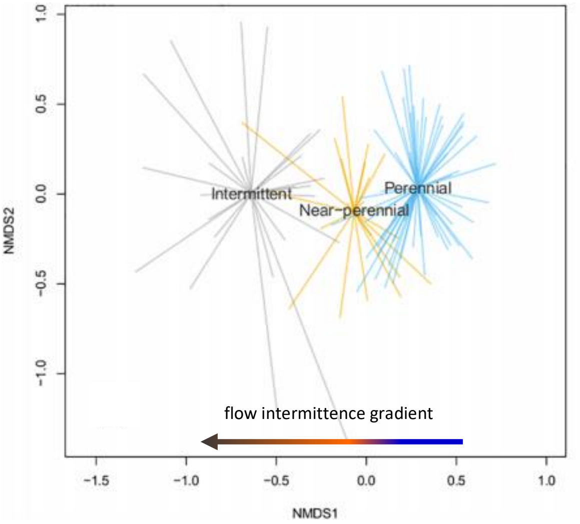

***Figure 7. Increasing MIV community taxonomic variability in three flow categories** (blue = perennial, orange = near–perennial and grey = intermittent) **along a gradient of increasing flow intermittence**. (NMDS ordination plot–based Bray–Curtis distance matrix; adapted from Straka et al. 2021).*

Part I.III: Specific responses of temperate IRES fauna to drying in the continental climate zone: from populations to communities, from aquatic to terrestrial groups.

Previous analyses of Czech IRES have shown that drying decreases the taxonomic richness of aquatic MIVs at the local scale (alpha diversity; Fig. 6b); however, this metric presents a static view that fails to provide insights into the underlying mechanisms driving community structure (Aspin et al. 2018). To fill this gap, we initiated a Czech–French collaborative research (**Paper 6: Crabot et al. 2021**) focused on the IRES beta diversity, aimed at clarifying the relationship between local and regional patterns by comparing taxonomic and functional composition among habitats and over time. To obtain a more detailed view of the processes shaping communities, we also partitioned spatiotemporal variability into turnover (taxa replacement between localities or dates) and nestedness (indicating if sites harbour subsets of taxa from richer sites), according to Baselga (2010).

To the best of our knowledge, this is the first study to examine the differences in responses of aquatic communities to drying in recently–drying (RD) river networks (CR) and historically–drying (HD; hundreds to thousands of years) river networks (France). We expected aquatic communities from RD rivers to be less adapted to desiccation and, consequently, more affected by drying than those from HD rivers (Fig. 8). Specifically, we hypothesised reduced functional redundancy in RD networks compared with HD due to a lack of functional traits enabling species to cope with drying. Thus, functional differences between intermittent and perennial sites would be stronger in space and time in RD networks.

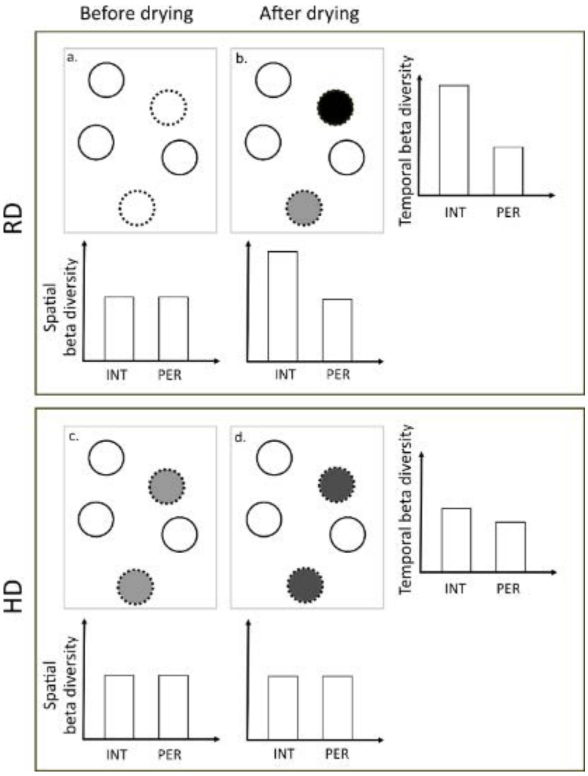

Figure 8. Expected differences in community structure response to drying in intermittent *(INT)* ***and perennial*** *(PER)* ***rivers over space*** *(regional beta–diversity)* ***and time*** *(before and after dry episodes)* ***in recently–drying*** *(RD)* ***and historically–drying*** *(HD)* ***river networks****. Adapted from Crabot et al. 2021.*

Dotted circles = intermittent sites, solid circles = perennial sites; darker circles represent community composition more affected by drying and differs from perennial sites. In RD before drying (a), intermittent and perennial sites have similar community composition, but after drying (b), intermittent sites show high stochastic impacts. Temporal variability between (a) and (b) would be higher on intermittent sites due to a greater difference in alpha diversity, and thus higher temporal nestedness. In HD before drying (c), community composition already differs between intermittent and perennial sites due to pre–adapted taxa, though they could still be similar after drying (d). Temporal taxonomic and functional variability between (c) and (d) is slightly higher for intermittent sites. Adapted from Crabot et al. 2021.

Most of these assumptions were confirmed, with the results highlighting a stronger local effect of drying on RD communities (i.e. a decrease in taxonomic alpha diversity), along with higher temporal and spatial beta–diversity. Perennial sites in Czech RD networks showed higher taxonomic richness than intermittent sites, while this pattern was not significant in French HD rivers. Furthermore, the effect of drying was stronger in the CR than France, despite milder conditions during the drying process in Central Europe, where dry events are typically shorter and bed sediments tend to retain a higher percentage of moisture (**Pařil et al. 2019b**).

As expected, we also recorded a lower proportion of resistant taxa in Czech RD networks than French HD rivers. Thus, MIV communities in RD rivers appear to be mainly driven by resilience processes (note the decrease in resilient taxa with increasing intermittence in Fig. 9), with taxa partially tolerating dry conditions whilst lacking specific dry–resistance traits (**Pařil et al. 2019b**).

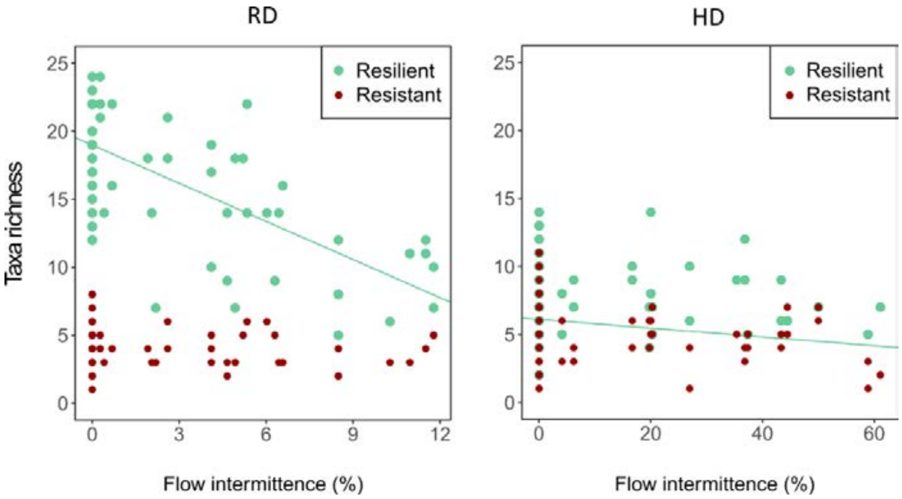

Figure 9. Effect of flow intermittence** (percentage of dry days during a year)* ***on taxa richness of resilient *(larger green points)* ***and resistant taxa*** *(small red points)* ***on recently–drying (RD) and historically–drying (HD) networks, showing the linear regression*** *(green line)* ***of resilient taxa richness against flow intermittence****. Adapted from Crabot et al. 2021.*

As with most previous MIV research in IRES, most of my previous work concentrated on responses of the whole MIV community to drying (**Pařil et al. 2019b, Straka et al. 2019, 2021, Loskotová et al. 2021)**. However, a few papers analysed adaptations and responses of model taxa (**Loskotová et al. 2019**) or individual taxonomic groups (e.g. mayflies; **Řezníčková et al. 2010**) more deeply. As detailed analyses of the responses of single taxa populations to drying are scarce, especially in the temperate zone, we tried to fill this gap in the last study presented here (**Paper 7: Pařil et al. 2019a)**.

In this study, we analysed spatiotemporal changes in population structure for one of the keystone taxa inhabiting nearly all low–order undisturbed streams in the CR, the freshwater shrimp *Gammarus fossarum* (Crustacea: Amphipoda). Owing to its naturally high densities in perennial streams, and its importance in trophic cascades, the taxa is considered a crucial ecosystem engineer in low–order streams (Syrovátka et al. 2020). Gammarids mediate energy flows from allochthonous food sources by shredding leaf litter, and act as opportunistic predators controlling community composition through selective predatory pressure, especially in fishless streams (Syrovátka et al. 2020). Due to its high sensitivity to drying (Poznaňska et al. 2013), and its key ecosystem role, any drying–induced alteration in its population structure could lead to substantial changes in MIV community composition and functioning.

We examined *G. fossarum* population structure in Czech low–order intermittent streams before, during (residual pools and dry riverbed sediments) and after a dry phase. Surface sampling was complemented by freeze cores to compare the vertical distribution of gammarids during wet and dry phases. We found that population densities increased during flow recession, potentially reflecting both a decline in submerged habitat availability and seasonal increases in juvenile abundance (Fig. 10b). As expected, persistence within dry surface sediments was minimal; however, high population densities were recorded in pools and saturated subsurface sediments, when the gammarids becoming increasingly concentrated in the shrinking aquatic habitat. During the dry phase, juveniles prevailed (80%) in the subsurface sediments, probably due to their ability to inhabit small interstitial spaces, and dominated (> 90%) in the residual pools (Fig. 10b). However, very low post–drying densities in the freeze cores suggested that the subsurface sediments may act as a graveyard for all gammarid classes.

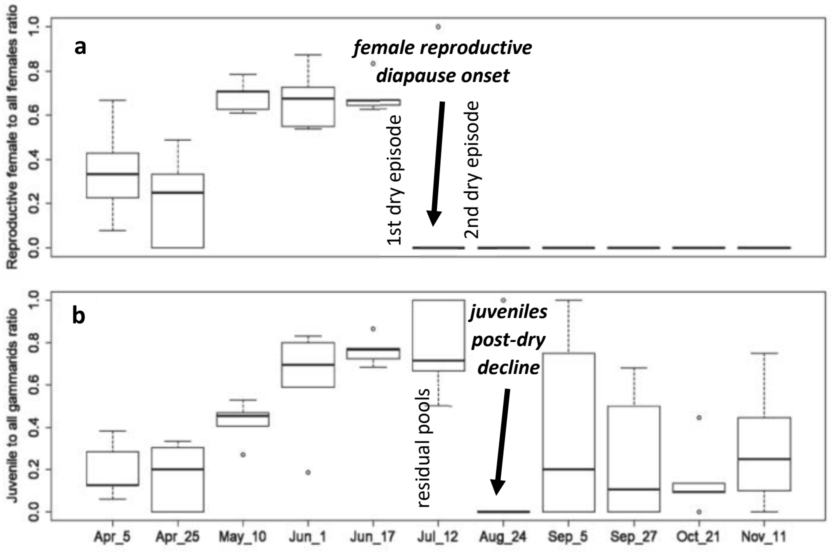

***Figure 10. Proportion of reproductive females to all females** (a) **and juveniles as a proportion of the whole population** (b) **in benthic samples.** A marked decrease in reproductive females is visible immediately after the first dry episode (July 12) and in juveniles after the second dry episode (August 24). Adapted from Pařil et al. 2019a.*

Compared with pre–drying samples, post–drying population structure was dominated by adults, presumably because of its better upstream swimming capability and high downstream drift. On the other hand, we recorded low numbers of spring–recruited juveniles and reproductive females, the latter probably suffering from early onset of reproductive diapause induced by drying stress (Fig. 10a). Both effects could lead to a long–term reduction in population recruitment in this “ecosystem engineer”, possibly leading to marked alterations in taxonomic and functional community structure. We observed not only reduction of gammarid population densities by streambed drying, but in addition, we also proved for the first time the long persistence of the drying effect on their population structure in intermittent temperate streams.

Despite the fact, that higher latitude rivers also cease flowing during freezing episodes, studies of flow intermittence have traditionally focused on drying river networks in arid regions. In Central Europe, climate change has mainly manifested itself through increasing winter temperatures (Zahradníček et al. 2021); however, there is also evidence that dry episodes are lasting longer up to the first freezing days. Furthermore, artificial snowmaking on ski slopes at the late autumn is increasingly leading to water over–abstraction, which in turn induces stream drying and subsequent freezing of the riverbed surface (De Jong 2015).

Both ecosystem types characterised by temporal absence of flowing surface water provide dynamic habitats for biota. As both freezing and drying result in the loss of water flow, however, research into the ecological effects of intermittent freezing on biota has remained relatively scarce compared to the recent increase in studies exploring the ecology of intermittent streams. Based on my experience in both, research in IRES ecology and investigation of artificial snow–making impacts on aquatic biota, I was invited to join a group of European limnologists developing a review comparing the effects of both freezing and drying events (**Paper 8: Tolonen et al. 2019**).

The lack of research into freezing intermittent rivers and ephemeral streams (FIRES) is especially concerning as the percentage of rivers experiencing annual ice–processes in the northern hemisphere may be as high as 60% (Allard et al. 2011). In this case, the absence of free–flowing water affects ecosystem structure and functioning in a similar manner as with IRES (von Schiller et al. 2017). In the review, we summarise the responses of stream biota at different levels, ranging from individual adaptations to population effects, community biodiversity variation and food webs. The paper highlights the main convergences and divergences in aquatic community adaptation to flow intermittence caused by both freezing– and drying–induced absence of water flow. At the individual organism level, aquatic taxa use a range of physiological, morphological, life–history and behavioural responses to persist in the face of changing habitat conditions. In addition to major contrasts in environmental conditions due to different temperatures (which directly regulate physiological processes), dry channels are exposed to direct interactions with the riparian zone during the dry phase, while freezing streams become enclosed during the freezing process (Fig. 11).

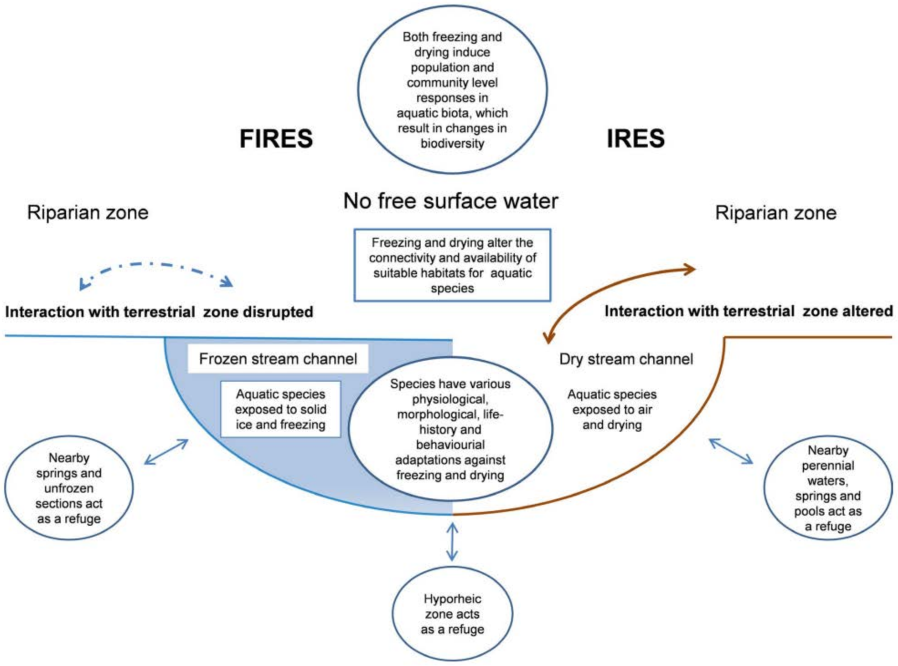

Figure 11. Comparison of habitat connectivity in intermittent rivers and ephemeral streams (IRES) vs. freezing IRES (FIRES)*. Adapted from Tolonen et al. 2019.*

In this review, special emphasis was placed on MIV response to the absence of free water, particularly as regards alterations in population structure caused by flow intermittence (**Pařil et al. 2019a**). Secondly, we examined anthropogenic alterations to flow regimes (joint impact of climate change and water abstraction), particularly those related to the novel phenomena of artificial snow making in winter and recent summer stream drying. Intensive water abstraction for artificial snow making at ski resorts during natural low–flow periods in late autumn (de Jong et al. 2009) can lead to more frequent freezing–drying episodes which, in turn, result in deteriorating aquatic biota, particularly in temperate climates where the biota is not adapted to harsh freezing. These changes could result in unpredictable cascading effects throughout freezing aquatic ecosystems.

Future climate scenarios predict that, globally, FIRES ecosystems will become less fragmented and warmer; if biota cannot adapt to such changes, the structure of ecological communities in freezing streams could experience substantial alterations. Furthermore, due to the ecological parallels between intermittently drying and freezing streams, approaches developed currently in research into drying streams may also be applied in freezing stream ecosystems.

Despite the increasing number of IRES across Europe, they are poorly represented in biomonitoring programmes assessing the ecological status of rivers (e.g. the EU WFD). These streams support a surprisingly high biodiversity and provide substantial ecosystem services; nevertheless, they have been underrepresented in research and omitted from monitoring programmes, especially in humid regions such as the CR.

During the international initiative “COST Action SMIRES” (www.smires.eu), I participated on a survey to identify current challenges to IRES status assessment, examples of best management and priorities for future research (**Paper 9: Stubbington et al. 2018**). We identified several major challenges and barriers limiting modern scientific–based management of these river types, many of which (listed below) are relevant to Central European temperate streams. First is the exclusion of IRES from WFD biomonitoring due to their small catchment size (< 10 km^2); however, in the CR, there are cases of drying rivers with catchments > 100 km^2, e.g. Velička near Strážnice or Loučka near Tišnov. Secondly, there is a lack of river typologies that distinguish between IRES types, leading to difficulties in defining "reference conditions" representative of this unimpacted ecosystem. Thirdly, there is the false perception of intermittent rivers as new phenomena of anthropogenic origin, especially in more humid climates, despite regular stream drying also occurring naturally in temperate climates, especially in sandstone or karstic regions, both relatively common in the CR.

As an example, most non–Mediterranean countries (n=13) included in our study using WFD status assessment, failed to recognise flow intermittence in river typologies (including the CR). As a response to this, we developed the first index in temperate Europe, distinguishing IRES from perennial systems, i.e. the BIODROUGHT index. Use of such indices is needed across Europe as hydrological metrics are being hampered by poor IRES representation in gauging station networks (Leigh & Datry 2017). Our study also showed that, in addition to distortions to ecological state assessments caused by drying, deviations from natural states originated by parallel impact of drying and anthropogenic alterations (e.g. pollution) also need to be identified (**Stubbington et al. 2022, Loskotová et al. 2021**). In the case of anthropogenic stressors, we were one the first European countries detecting organic pollution impacts in intermittent streams through the analysis of MIV communities using Pol–Int software (Polášek 2018), which successfully distinguished unimpacted and polluted IRES.

In addition to all identified gaps and challenges, we also recognise in the paper examples of innovative practice and define priorities for future research, including the inclusion of spatiotemporal community fluctuations in the reference condition approach, and modification of indices assessing ecosystem state (**Stubbington et al. 2022**). In this respect, I contributed substantially to the recognition of both taxon–specific sensitivities to intermittence (**Straka et al. 2019**) and taxa dispersal abilities within a landscape context (**Sarremejane et al. 2021**).

For most of the presented studies, we used aquatic MIV as the main model group; however, I also co–led a bilateral Czech–Spanish study focused on the utilisation of dry riverbeds by vertebrates (mostly large mammals) in two contrasting climatic zones, temperate Central Europe and the Mediterranean Iberian Peninsula (<u>**Paper 10:**</u> **Sánchez–Montoya et al. 2022**)

The main aim of this study was to test whether intermittent rivers provide crucial ecological functions for terrestrial vertebrates (e.g. corridors, refuges, food and water supplies) that have been mostly attributed to perennial rivers (Fig. 12). This traditional perception is highly biased as more than half the length of the global river network presently dries up naturally or because of climate change and human activities (Datry et al. 2014). A newly emerging river paradigm highlights that both wet and dry phases contribute substantially to overall biodiversity (Corti & Datry 2016) and ecological functions (Sánchez–Montoya et al. 2017). However, to the best of our knowledge, this assumption had not been tested before as regards terrestrial vertebrates (Steward et al. 2012).

***Figure 12: Examples of dry riverbed ecological functions for terrestrial vertebrates in Spain and the CR, e.g. food and water sources, migration, resting sites**. Adapted from Sánchez– Montoya et al. 2022.*

We compared ecological functioning and habitat utilisation of intermittent and perennial rivers by terrestrial vertebrates by analysing both their presence and behaviour recorded by camera traps during different flow phases (flow, dry, pools). Animal occurrence and behaviour in the river channel during wet and dry phases of different duration were compared with data recorded in riparian zones of the same reach.

We found that intermittent reaches, especially those with a short dry phase, harboured a higher species richness and abundance of terrestrial vertebrates than their perennial counterparts in both study areas (see Fig. 13). These findings suggest that, in addition to aquatic and semi–aquatic MIV communities (Corti & Datry 2016, Leigh & Datry 2017), terrestrial vertebrate biota should also be considered when characterising the biodiversity of intermittent rivers. As expected, even those reaches subjected to low anthropogenic disturbance (e.g. recreational use or alteration of riparian zone) showed a reduced species richness than undisturbed reaches (Marzano & Dandy 2012; Fig. 13).

The ecological functioning of rivers monitored through terrestrial vertebrate behaviour was mainly explained by the duration of the dry period rather than its presence. While riparian zones were key habitats in perennial rivers, acting as migratory corridors and food sources, dry channels played a more important role in intermittent rivers. The downside of the attractiveness of dry channels to vertebrates was the high abundance of omnivorous wild boar using the sites, these consuming large quantities of aquatic MIVs concentrated in the shrinking pools and dry riverbeds. This predatory pressure could have a strong effect on MIV communities (Motta et al. 2020), especially in temperate climates where intermittent streams were relatively uncommon before climate change (**Crabot et al. 2021**). As this study was undertaken in two contrasting climatic zones exhibiting substantial differences in frequency, extent and predictability of dry phases, it is likely that the results could be extrapolated over wider geographical scales.

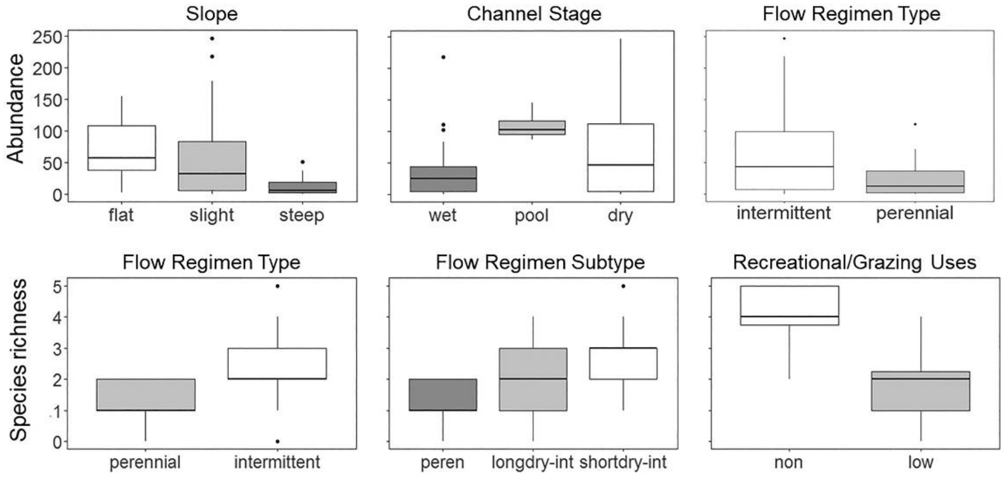

***Figure 13. Main factors** (GLMM model) **affecting vertebrate species richness and density in Czech rivers**. Adapted from Sanchez Montoya et al. 2022.*

Slope = bank slope; Flow regime type – perennial, intermittent; Flow regime subtype = perennial, long dry, short dry; Recreational use = anthropogenic disturbance – extensive tourism or cattle grazing; Channel stage = wet (i.e. flowing), pools (isolated pools), dry (bare riverbed); boxes are interquartile ranges (25th percentile to 75th percentile), range bars show maximum and minimum values, horizontal lines show the median value.

Part I.IV: Hyporheic habitats as crucial refuges for macroinvertebrate communities in drying rivers

The streambed plays a crucial role for aquatic fauna, not just during the flow phase but especially during drying, providing important refuges that support an important part of aquatic biodiversity. While the role of IRES bed sediments has been intensively studied across different climate zones around the globe over the last two decades (Stubbington & Datry 2013), very little was known about its functioning and importance in temperate continental Europe until our first studies from the CR (**Pařil et al. 2019b, Loskotová et al. 2019. 2021**). Unlike arid climates, the humid Central European continental climatic zone has only experienced frequent river drying in recent decades. Thus, there is a lack of relevant information on the contribution of dry sediments to the survival and recovery of MIV communities after flow resumption. Previous studies, mostly in arid climates, have identified the most important factors affecting the so–called “seedbank” (different MIV life stages surviving in dry sediment) and controlling its survival in sediment. These factors characterise drying reach by habitat characteristics (e.g. substrate humidity, air temperature, organic matter content and channel shading) or over a wider area climatic characteristics such as precipitation frequency and drying intensity also come into play. Together, these drivers could shape MIV communities in different climatic zones via different mechanisms.

We conducted several *in situ* and laboratory studies investigating the taxonomic and functional composition of the dry streambed seedbank and its contribution to community recovery in near–pristine streams. Our results from unimpacted habitats were complemented by studies from polluted streams (mostly nutrient enriched). Furthermore, we incorporated our findings into a software discriminating near–pristine and polluted intermittent streams, however the effects of both stressors on MIV communities was not considered as easily distinguishable.

As proportionally comparable dry riverbed samples are needed for dry sediment studies, we developed a new sampler that was capable of taking quantitative samples from a defined area, volume and substrate depth. The sampler was successfully tested during the BIODROUGHT project (“Sampling set for dry riverbed sediment” **Pařil et al. 2014**), where we gathered representative stratified samples enabling quantitative analysis of surviving MIV propagules. The equipment has been patented through the Industrial Property Office in Prague (Utility model no. 27576). We have subsequently used the sampler in several studies (e.g. **Pařil et al. 2019b**), and the equipment has been successfully adapted for intermittent river research abroad (Pecs University in Hungary).

As a first step to gaining a deeper insight into the mechanisms driving MIV survival in dry riverbeds was the experimental approach developed with my MSc and PhD student, Barbora Loskotová (**Paper 11: Loskotová et al. 2019**). We examined utilisation of dry sediment refuges using four model MIV taxa (the amphipod *Gammarus fossarum,* the mayfly *Ephemera danica,* the bivalve *Pisidium* spp. and oligochaetes Tubificinae spp.) differing in drying survival traits (i.e. different pore size penetration and survival with decreasing water content) exposed to experimental mesocosms with gradual water loss in subsurface layers. Specifically, we examined the vertical movement of the model taxa, each of which differed in body diameter, mobility and drying resistance, after being placed on the sediment surface during initial dewatering of the experimental mesocosms and subsequently exposed to a 32 days dry episode. We used three substrate sizes (sand, fine and rough gravel) and left the lower sediment layers saturated with water to attract the model taxa and encourage vertical migration.

We observed significant differences in the vertical distribution of all model taxa among three substrate treatments (Fig. 14). Despite the expected overall pattern of higher survival rate in deeper, more saturated moist layers, we only observed significant differences related to pore size for larger taxa such as *G. fossarum* and *E. Danica*. Substrate effect was less clear for bivalves *Pisidium* spp. and especially oligochaetes Tubificinae spp., as these taxa naturally utilise moist hyporheic refuges that are easily accessible due to their small body diameter. When body diameter was approximately the same as pore size, individuals were located throughout the upper and lower substrate layers, indicating that some individuals could migrate vertically whereas others became stranded in sediment.

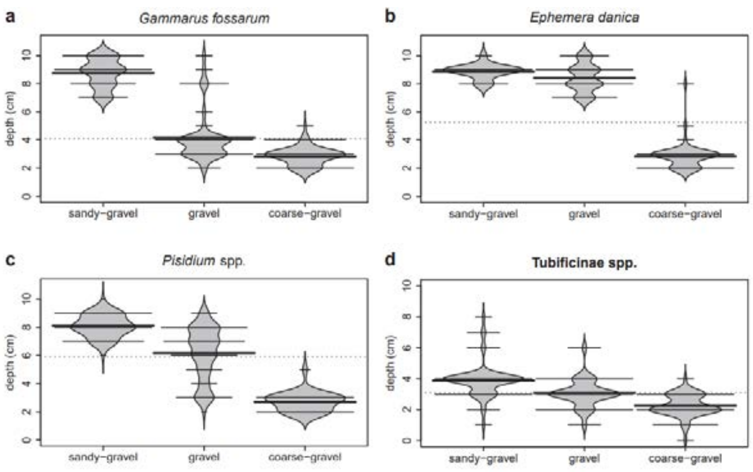

Figure 14. Proportional representation of four model taxa recorded at different sediment depths in experimental mesocosms with three sediment types *(increasing in size from left to right). Adapted from Loskotová et al. 2019.*

Our study demonstrated the importance of sediment characteristics, which can affect the availability of streambed refuges for MIVs, and taxon–specific penetration and utilisation of subsurface sediments. The CR has the highest proportion of uniform farming blocks within the EU, and the large proportion of drying rivers are in this unified farmland (**Zahrádková et al. 2015**). Streams in these catchments suffer increasingly from clogging of interstitial spaces due to riparian erosion. Our study highlights the crucial role of dry riverbed habitats, and the importance of protecting such sites against erosion that causes extensive siltation of interstitial spaces.

Following an initial laboratory study, we undertook a series of field studies focused on quantifying the contribution of the surface sediment MIV "seedbank" to MIV dry phase survival (**Paper 12: Pařil et al. 2019b**). Unlike previous studies, which have included exclusively "seedbank elements cultivated from re–watered dry sediments" (mostly resistant stages as eggs or cysts), we concentrated on that part of MIV community that is able to survive in dry riverbeds as an active aquatic form (adult or larvae) not specifically adapted to dry phase persistence. To the best of our knowledge, this is the first time this has been done in the temperate zone.

I led the study addressing the ability of such MIV active forms to cope with climate change–induced river flow intermittence in continental humid climates, such as that found in the CR. The high relative air humidity, dense riparian cover and relatively short duration of drying events (compared to arid climates) in the CR contributed to the formation of taxonomically and functionally rich communities of such living forms, with > 80% of all taxa (and 70% of all specimens) collected in the dry riverbed surviving dry phases without specific desiccation–resistance forms. After persisting in dry streambeds during dry phases, these are then able to contribute substantially to community recovery upon flow resumption.

On the other hand, we recorded an exponential decrease in riverbed community taxonomic and functional richness during such dry phases (Fig. 15a, b), with an initial rapid loss of sensitive taxa in dry riverbeds and a pool of less sensitive taxa persisting longer. It is these resistant taxa that contribute to rapid community recovery upon re–wetting (Fig. 15c,d), especially during the initial two week phase after flow resumption when they contribute ca. 50% of taxonomic and 70% of functional community diversity. While such species contribute substantially to MIV community persistence in temperate IRES, the significantly lower taxonomic richness than perennial streams, especially during the post–dry flow phase, indicates that such streams are strongly affected by drying (**Crabot et al. 2021**).

To conclude, the high survival rates of active aquatic MIVs lacking desiccation–resistance adaptations has rarely been quantified (but see Boulton 1989 for Australia). The surprisingly high rates observed are most likely due to the relatively benign environmental conditions prevailing in the drying stream riverbeds in our study, i.e. high moisture and air humidity in dry riverbed interstitials and shading from dense riparian vegetation. As such, our results highlight the need to protect the adjacent riparian environments in addition to the dry riverbed communities. Our study suggests that the role of dry sediments as refuges has been under–valued, while refuges in perennial stream sections during periods of low–flow have been overrated, despite the poor water quality in those sections typically associated with low dilution of pollution during extremely low–flows.

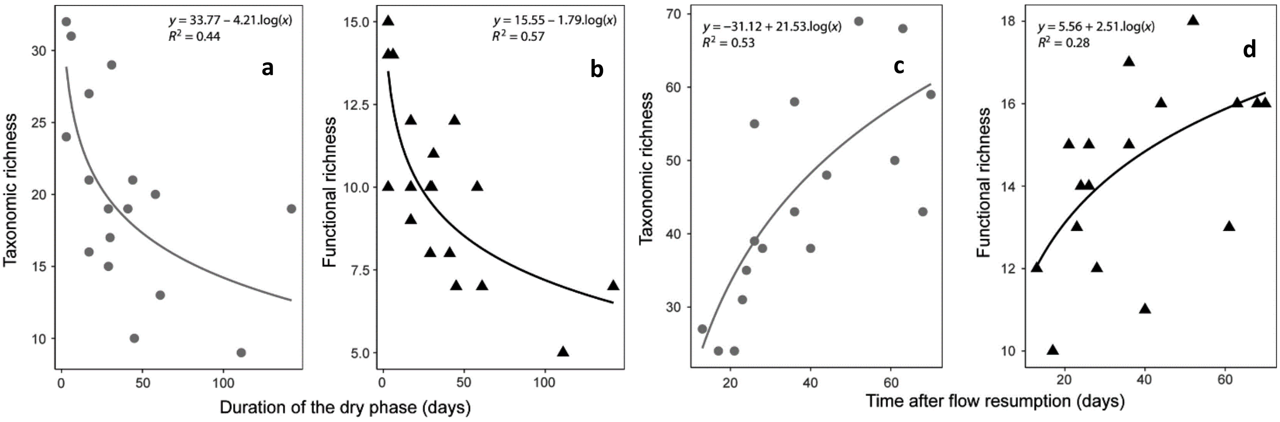

***Figure 15. Changes in MIV community taxonomic and functional richness related to duration of riverbed dry phase** (a, b) **and flow resumption re–watering** (c, d). Adapted from Pařil et al. 2019b.*

Part I.V: Interaction of stream drying and anthropogenic pressures

To avoid interference from other artificial impacts, our previous studies were focused on riverbed MIVs from near–pristine streams; however, most small watercourses in the CR are in urban or agricultural catchments with higher human impact. Consequently, we undertook a series of studies to assess the most common anthropogenic impacts on dry riverbed refuges due to poor water quality, i.e. organic pollution and eutrophication.

In a study conducted by my PhD student (**Paper 13: Loskotová et al. 2021**), we compared the survival of the two main MIV seedbank groups persisting in dry riverbeds, i.e. i) active forms (*sensu* **Pařil et al. 2019b**) and ii) dormant life–stages that only become activate after rewetting (Stubbington & Datry 2013). While dormant MIV stages must undergo some form of transformation before becoming active (e.g. hatching; Strachan et al. 2015, Williams 2006), active MIVs revive quickly after inundation.

Up until now, little has been published on the influence of “intermittency” on MIV communities in nutrient–enriched streams (Datry et al. 2014, Steward et al. 2012), partly as IRES nutrient cycling has mainly been studied in anthropogenically unimpacted streams (**Datry et al. 2018, von Schiller et al. 2019, Shumilova et al. 2019**). This is particularly true in temperate zones with a humid continental climate (Sabater et al. 2011), such as the CR. Consequently, we examined the impact of four selected environmental factors (substrate moisture, dry period duration, nutrient enrichment and proportion of fine sediment <2 mm) on MIV species richness in two groups of sites, near pristine and nutrient enriched.

Based on our previous results (**Pařil et al. 2019b**), we evaluated seedbank contribution to community recovery, with active living MIV forms treated separately from those with dormant stages. The roles of three characteristic stream mesohabitats (riffle, pool and marginal) in defining community composition and its contribution to recovery were also analysed separately to assess the contribution of each to taxa persistence. The seedbank consisted of around one–third the taxa recorded during flow phases, with slightly higher taxa numbers in nutrient–enriched streams. Moisture content positively affected seedbank taxa richness, while dry period duration had a negative impact. Finally, taxa richness displayed a unimodal response to nutrient enrichment, with the proportion of fine sediment having no significant effect. Our results indicated a richer MIV seedbank in drying sediments under moist conditions with mild nutrient enrichment, with raised nutrient levels limiting MIV survival.

The higher seedbank taxa diversity observed in nutrient–enriched streams (Fig.16) was somewhat surprising; however, as polluted stream MIV communities are partially adapted to harsher environments, its seedbank is predisposed to withstand drying better than those in pristine streams. On the other hand, the reduction in seedbank diversity observed in autumn samples in pristine streams (compared with spring) was more pronounced than in enriched streams (Fig. 16). As in previous studies (**Pařil et al. 2019b**, Verdonschot et al. 2015), we recorded a significant decline in seedbank richness with duration of dry period, with this decline occurring faster than that in harsher Mediterranean or arid regions (Bonada et al. 2007). Unlike nutrient–enriched streams, mesohabitat diversity played a crucial role at near–pristine sites, where variable habitat conditions enabled survival of different taxonomic groups adapted to persist in specific dry refuges declining physical and chemical conditions.

All the above–mentioned factors had a significant influence on seedbank taxa richness and, consequently, each was important for community composition, with the leading effect being moisture followed by duration of the dry period and nutrient enrichment. This study not only highlights the crucial role of refuges in dry streambeds for MIV survival but also showed the importance of both living forms and resistant stages in the MIV seedbank. Additionally, we were able to demonstrate different MIV community responses to drying in undisturbed and eutrophized streams, where survival is driven by different factors; habitat heterogeneity important in pristine streams and nutrient levels in enriched streams.

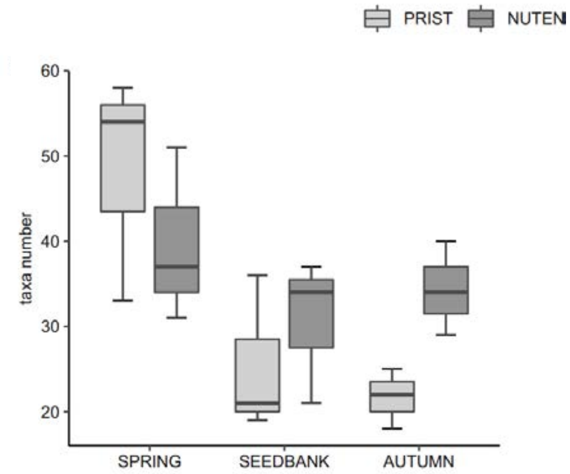

Figure 16. Comparison of seasonal changes in taxa number between nutrient enriched *(NUTEN – dark grey)* ***and pristine*** *(PRIST –light grey****) sites during pre–dry flow*** *(SPRING),* ***summer dry riverbed*** *(SEEDBANK)* ***and post–dry flow phases*** *(AUTUMN). Adapted from Loskotová et al. 2021.*

Parallel impacts and mutual interactions of both pollution (nutrient enrichment) and drying can substantially alter MIV communities and thus distort ecological assessments by MIV–based indices used for WFD biomonitoring programmes. Prior to this study, almost nothing was known about the interaction of these stressors in newly drying temperate streams. We expected that the impacts from these drivers would be almost impossible to disentangle, whether using traditional bioassessment MIV metrics or our own BIODROUGHT index, which was originally developed for near–pristine streams. Specifically, we expected lowered BIODROUGHT performance (i.e. increased misclassification) due to the mutual interaction and impact similarity of both stressors resulting in conditions such as increased hypoxia or eutrophication.

To test the performance of the BIODROUGHT index on gradients of intermittence and pollution (nutrient enrichment), we undertook a new project entitled InterStream, where we assessed four stream types categorised by contrasting flow regime and level of organic pollution: 1) perennial unpolluted, 2) perennial polluted, 3) intermittent unpolluted and 4) intermittent polluted.

Despite the additional impact of pollution, the BIODROUGHT index performed well in recognising antecedent dry phases. Furthermore, our findings strongly suggest that the pollution (characterised by Czech saprobic index; Zelinka & Marvan 1961) and flow intermittence (BIODROUGHT index) gradients act independently. Based on these results, we developed the Pollution–Intermittence (Pol–Int) calculator software package (available on http://sucho.eu/polint.php; Polášek 2018). This package not only enables classification of flow regime based on MIV samples in both polluted and unpolluted streams, it is also able to derive the BOD_5 values (with 95% confidence intervals) from the MIV-based saprobic index, that express levels of organic pollution.

Many of the metric–based methods presently available for WFD reporting were originally developed for perennial rivers, and thus are not adapted for assessing ecological status in rivers previously affected by dry episodes or cessation of flow (Chadd et al. 2017). In addition, stream drying can be caused by anthropogenic activities or be climatically mediated, whether through natural climate variation or as a novel phenomenon associated with climate change (**Crabot et al. 2021**). What is more, multiple stressors may interact in IRES to influence the river ecosystem and, as such, disentangling the ecological responses to such stressors will be challenging but necessary to effectively manage ecosystem adaptation to global change.

As part of a collaborative European initiative (**<u>Paper 14:</u> Stubbington et al. 2022**), we analysed both independent and interactive effects of human impacts and natural drying on aquatic MIV communities using six frequently used biological metrics (e.g. family richness, BMWP, ASPT and its modifications) typically used as descriptors of taxonomic and functional richness or functional redundancy. The responses of these indices were tested on more than 15 datasets comprising 400 communities across eight European countries. Alongside metrics calculated for the whole community (adjusted to family level), we also selected for the new metric a group of taxa with resilience/resistance traits to drying (RR taxa) and examined their responses to human stressors in relation to climate type.

While our results indicated that most of these community metrics decreased in response to human impacts and drying, taxa richness–independent indices (e.g. ASPT) showed improved potential for biomonitoring purposes. These traditional indices should be considered alongside the new metrics representing drying RR taxa diversity in intermittent streams as the combination of both metric types provide the best assessment of ecological status in streams affected by both drying and anthropogenic stressors. It should be noted, however, that drying RR taxa may sometimes overestimate ecological status by responding to human impacts only rather than drying; thus, the results of these indices have to be interpreted with caution.

Unfortunately, this newly developed drying RR taxa metric was able to explain limited variance in community response to human alterations, though its sensitivity could be enhanced by using regionally–adapted metrics based on a higher taxonomic resolution than family level (Soria et al. 2020). As IRES exhibit high between–type variability, we recommend using both richness–independent biomonitoring indices and the new drying RR taxa richness metrics for characterising region–specific river types (**Stubbington et al. 2018**). The interactions observed between climate type (expressed by aridity), additional human impacts and drying confirm the flexibility of these new metrics, enabling their ongoing adaptation as climate change progresses.

Part II: Spatiotemporal variation in aquatic macroinvertebrate distribution driven by a changing climate, anthropogenic activities and taxa dispersal

As I documented in the previous chapters, MIV community trajectories in freshwater ecosystems in current Anthropocene period are strongly driven by the concurrent interaction of human activities and ongoing climate change. These drivers, which may act alone or, more frequently, in combination, can trigger shifts in species distribution, manifested as the expansion or reduction of their original distribution area. Alternatively, taxa may colonise new types of habitat not typically utilised within their original distribution area (Karatayev et al. 2009, **Pařil et al. 2008**). Many MIV taxa are currently invading both terrestrial and aquatic habitats from remote geographical regions, and even different continents. On the other hand, many taxa only have spread into Central Europe by northward or eastward expansion of their original distribution areas in the neighbouring Mediterranean or more distant Ponto–Caspian regions. These colonisation processes have been triggered by both natural and anthropogenic mechanisms, including climate change, shipping, habitat changes and intentional or accidental introduction by humans, with many having been well documented for permanent MIV fauna such as crustaceans, molluscs and annelids.

On the other hand, many insect invasions in running waters have gone unrecorded (Pyšek et al. 2010) due to logistic reasons (e.g. complicated taxonomy and identification, insufficient geographical and temporal sampling) or biological limitations of expansions as regards temporary fauna (Karatayev et al. 2009). In many cases, it is not possible to distinguish recent area expansion/contraction from "noise" signals in data originating from different sources with varying quality and geographic coverage (e.g. WFD monitoring, scientific projects, accidental records).

During the early years of the 21st century, I documented such rare expansions of one riverine aquatic insect, the Atlanto–Mediterranean stonefly *Leuctra geniculata*, and summarised its ecological preferences in its newly colonised territory. While this exceptional example enabled us to demonstrate some general expansion patterns, not all the causes and consequences associated with this case could be fully known. As stonefly larvae and adults of this taxon are easily distinguished from other congeners by unique characteristics, we were able to avoid previous oversights in the historical records. Furthermore, the larger rivers preferred by this taxon have been regularly and continuously monitored by water agencies over recent decades and species populations tend to be relatively dense, excluding possible oversights due to low sampling intensity.

Since its first recording in western Bohemia in 2003 (**Paper 15: Pařil et al. 2008**), the species has rapidly colonised other larger rivers across the western part of the CR (Bohemian massive). By 2010, the species had extended its range eastward from Bohemia to the border of the Western Carpathians in Moravia, maintaining a similar colonisation pattern as that observed in the western part of the CR. Initially, the species colonised river sections immediately below large dams, before spreading upstream and downstream from this reach (**Pařil et al. 2011**; Fig. 17). This use of human–modified habitats during the initial phase of colonisation highlights the substantial contribution reservoir–related alterations to riverine habitats have played in the settlement of such "incomers", e.g. through modification of hydrological and thermal regimes. Though we still do not fully understand the exact mechanisms enabling taxa adaptation, factors such as higher winter water temperatures below the dam may eliminate stream freezing, ensuring egg survival. Anthropogenic alterations to the natural flow and temperature regime of rivers, together with the simultaneous impact of climate change through increased water temperatures (Novický et al. 2009), could "open the first gate" for incomers from warmer southern (Mediterranean) or milder western (temperate oceanic) climates, allowing them to colonise previously unsuitable regions (Ott 2001).

Another non–typical pattern observed in the *L. geniculata* case was the use of an expansion pathway independent of the river network structure. Unlike many other examples of aquatic MIV invasion, this stonefly did not follow the river network through upstream migration, typical for permanent fauna such as oligochaetes, which are restricted to aquatic dispersal (**Schenková & Pařil 2010**). Temporal succession of records from taxa expansion within the Elbe basin suggest the species first entered the country by crossing the catchment divide from Austria, i.e. the Šumava (Bohemian Forest) mountain ridge (Fig. 17). While most of stoneflies in the *Lectra* genus, including *L. geniculata*, are classified as poor fliers (**Sarremejane et al. 2021, Pařil 2011**), passive aerial transport may also have played a part as the dominant part of winds blow from west in the CR.

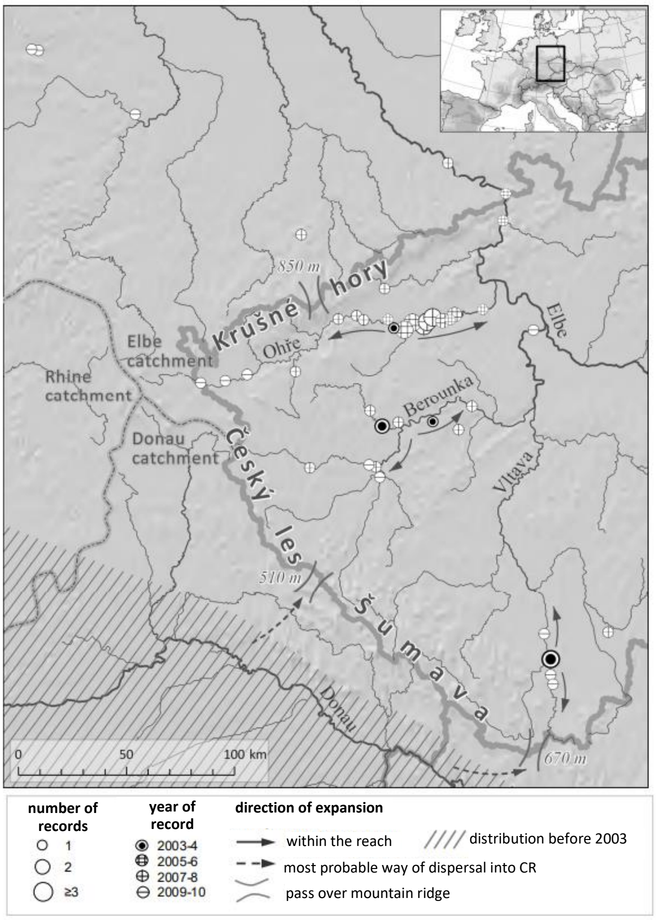

Figure 17. Chronology of Leuctra geniculata expansion into the CR between 2003 and 2010. *The size of circles shows the abundance of recorded larvae, lines across the circles indicate the year of record, dashed arrows indicate possible pathways of dispersal into the CR, and full arrows indicate expected direction of expansion from reaches below dams. Adapted from Pařil 2011.*

Unlike the presumably dominant aerial dispersion of temporary fauna, as documented in the case of *L. geniculata*, the natural dispersion pathways of permanent fauna such as the oligochaetes is mostly restricted to aquatic environments (and more rarely zoochory). Partly due to this dispersal limitation, many taxa have been accidentally transported in ship ballast water (Norf et al. 2010), enabling them to passively spread along navigation channels across Europe by routes used typically by invasive or alien species.

In our study of the lower Elbe river (**<u>Paper 16:</u> Straka et al. 2015**, Straka & Špaček 2009), close to the German border, we documented the invasion process of the Ponto–Caspian polychaete, *Hypania invalida*. This stretch of the Elbe is the most frequently used Czech river corridor and connects with major European navigation routes. *H. invalida* has only recently colonised many large European rivers (from the 1950s on) and we were the first to report it in the CR in 2014 (the river having been monitored regularly from 2010). The four records were all located close to an inland ship port, with no records in nearby Elbe sections with similar hydromorphology. Large navigable reaches tend to be highly susceptible to invasion by non–indigenous aquatic taxa due to easy accessibility mediated by high transportation frequency and the presence of disturbed habitats suffering from anthropogenic impacts such as pollution, siltation, channel modification and damming.

Our assumption that shipping plays a crucial role in taxa dispersion was supported not only by the location of records close to the port (up to 4 km) but also by species high dispersion rate compared to the mostly terrestrial spreading stonefly taxa. Despite using aquatic pathways only, this polychaete managed to migrate 440 km upstream from its first recording in the German Elbe in 2007 (Eggers & Anlauf 2008) to the Czech border seven years later. This aquatic dispersion rate is comparable to that of the flying stonefly, which covered ca. 70 km each year, demonstrating the high effectivity of passive shipping transport for this alien taxa. In subsequent years, *H. invalida* gradually disappeared from the lower stretch of the Elbe, possibly due to the decrease in shipping intensity resulting from extremely low flows during a supra–seasonal hydrological drought between 2015 and 2019 (Moravec et al. 2021).

While the case of *H. invalida* represents the most recent example of an invasion, similar dispersal pathways have been used by other alien clitellates, as documented in our analysis of the historical and current distribution of Czech aquatic oligochaetes (**<u>Paper 17:</u> Schenková et al. 2010, Schenková & Pařil 2017**). Many non–indigenous taxa from this group have used the Elbe navigation corridor as the first step for invasion into Czech inland waters. As these are generally transported in ballast water, they have tended to be recorded for the first time (and in some cases exclusively) in the lower Elbe and its tributaries (e.g. species such as *Pristina osborni*, *Paranais frici, Quistadrillus multisetosus)*.

In addition to these human–mediated invasions, we also found some questionable examples of taxa considered non–indigenous to Europe (Drake 2009), despite historical records in Bohemia stretching back to the start of 20th century, e.g. *Potamothrix bavaricus* and *Potamothrix moldaviensis* (Hrabě 1981). Indeed, the second of these, *P. moldaviensis*, that is considered as alien taxa, has previously been described from Central Bohemia and is in fact named after the region's second largest Bohemian river, the Moldau (the German name for the River Vltava). Similarly, another Ponto–Caspian species, *Psammoryctides moravicus*, was first described by Hrabě in 1934 and named according to the region where it was first recorded, i.e. Moravia (eastern part of the CR). Nevertheless, we cannot exclude its introduction by shipping prior to taxa description; thus, its non–indigenous status in Central Europe remains unclear. Consequently, these potentially alien taxa cannot automatically be considered as alien/invasive in the CR as there is a lack of evidence for typical invasive behaviour, with low population densities, low frequency of occurrence and unknown impact on native communities.

In comparison, there is a general consensus on the non–indigenous status of some other aquatic oligochaetes, such as the thermophilic species *Branchiura sowerbyi*. Despite this species being known to have spread via shipping, the evidence suggests its distribution area has only expanded slowly since the first Czech record in the 1960s (Gruszka 1999). Increasing temperatures due to climate change are also likely to result in an increase in the number of habitats suitable to this non–native species; however, there is lack of empirical evidence for large–scale expansion to date. As the dispersal strategies of taxa typically inhabiting warmer pools in tropical and Mediterranean climates do not differ from local oligochaete taxa (**Sarremejane et al. 2021**), this suggests that its expansion may still be limited by low temperatures.

On the other hand, rare taxa with a restricted distribution, such as *Stylodrilus absoloni* or *Trichodrilus pragensis* in the CR (Hrabě 1981), typically exhibit reduced dispersion abilities, and thus tend more toward endemism. Aquatic systems typically hosting such taxa are frequently sub–terrestrial habitats such as caves or interstitials, where dispersion is limited by physical barriers. Taxa with a shrinking distribution include also specialists inhabiting vanishing habitats, such as periodic pools in large river floodplains that are gradually being eliminated by river regulation (e.g. *Lamprodilus mrazeki*) or populations once having a continuous distribution during historically colder periods (e.g. *Peipsidrilus pusillus*) that are now restricted to glacial refuges (Hrabě 1981, Timm 1999).

Though we documented a range of factors besides environmental drivers and biotic interactions promoting oligochaete area expansion or reduction, dispersion ability was one of the most important factors controlling distribution and enabling further expansion. Dispersion ability is difficult to measure in the field, and relevant information on dispersal remains scattered or unpublished. However, specific biological traits related to the various species' morphology, life history and behaviour offer useful dispersal proxies.

While participating in a range of projects, together with my team we assembled a database of aquatic MIV traits specifically connected with river drying (BIODROUGHT project; www.biodrought.eu) or general ecological information related to anthropogenic stressors and climate change (RIVERCHANGE project; https://hydro.chmi.cz/riverchange/). Based on this work, I was invited, as an "Oligochaeta" specialist, to join an international effort aimed at compiling information on selected dispersal–related biological traits of European aquatic MIVs into a unique source entitled the "DISPERSE database" (**Paper 18: Sarremejane et al. 2021**).

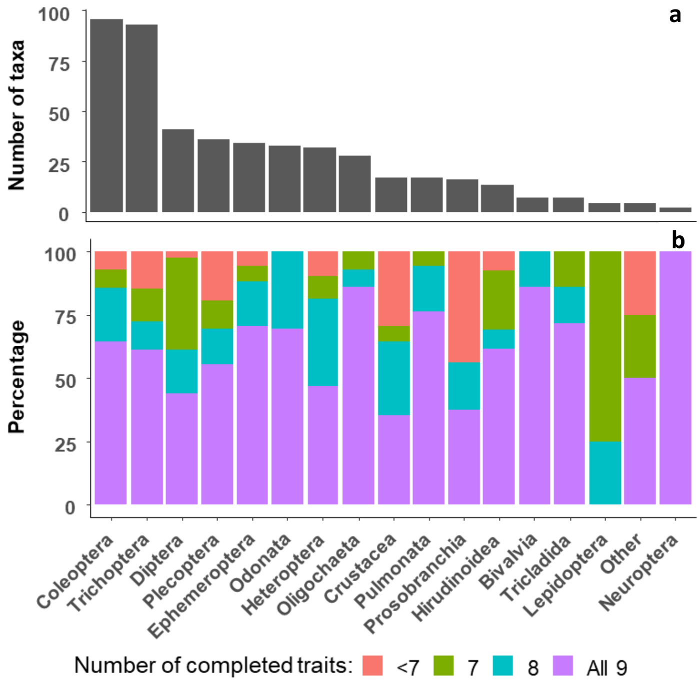

***Figure 18. Total number of taxa** (a) **and completeness (%) of nine traits** (b) **for each MIV group.** The group "Other" includes Hydrozoa, Hymenoptera, Megaloptera and Porifera, each having just one taxon in the database. Adapted from Sarremejane et al. 2021.*

Initially, we selected 39 dispersal–related MIV biological traits grouped into nine categories (e.g. maximum body size, life span, number of reproductive cycles, fecundity, dispersal mode, drift propensity etc.). Based on structure of comparable databases, we collected and fuzzy–coded robust genus–level information for all aquatic MIV groups across Europe (except some taxonomically complicated groups). The "Oligochaeta" are traditionally considered one of the groups that are hardest to distinguish due to their limited number of identification characters. Likewise, they are only considered a moderately diverse group compared to other aquatic MIVs (Fig. 18a). Despite these limitations, I was able to achieve one of the most complete trait information within examined MIV taxonomical groups, covering > 75% of all traits (Fig. 18b).

Further examination of dispersal traits, in combination with spatial distance measurements, could improve our understanding of dispersal limitations on biodiversity patterns, while information on taxa dispersal capacities could improve conservation strategies by enabling them to establish priorities for habitat spatial connectivity. From an applied perspective, the incorporation of dispersal proxies could improve ecological response predictions to global change and contribute to more effective biomonitoring and conservation management.

Part III: Reconstruction of past climate development in the Western Carpathians using recent and paleo–communities

It is now generally accepted that the world's climate is warming more rapidly than it ever has in the past (Portner et al. 2022). Consequently, the study of temperature shifts captured in paleoarchives may help identify potential consequences, leading to a better understanding of the current period of climate change. Climate is never stable over time and all continents have experienced multiple changes thorough geological history, though such events tend not to impact different regions simultaneously or with similar extent or timing. The Pleistocene, and the succeeding Holocene period, the closest geological period to the present, are the most suitable model epochs for reconstruction of past climatic events, enabling potential climate trajectory predictions for the future. Reliably dated and archived paleorecords can help determine whether current warming has exceeded the temperature change range of the last glaciation and Holocene warming periods. Further, the comprehensive combination of biotic and abiotic proxies (indirectly recording climate conditions when formed) from aquatic environments might allow one to assemble a complex outline providing the most reliable information on rate and amplitude of climatic oscillations.

One of the most prominent climate drivers directly and indirectly affecting most biological proxies is air temperature, which also controls water temperatures in surface water bodies. Lakes accumulate sequential sedimentary layers containing the remains of such proxies. Changes in summer air temperatures (i.e. three warmest months in year) are well reflected by midge larvae (Diptera: Chiromomidae) as their larval development, and later adult emergence, are closely linked with water temperature because in lakes of appropriate depths are both temperatures closely interconnected. However, chironomid–based temperature reconstruction can be biased by interference with other environmental factors influencing lake development, such as switching between lotic and lentic environments, trophic conditions and depth oscillations (including drying). Consequently, simultaneous changes in these drivers can sometimes lead to misinterpretation of target parameters, such as summer air temperature. The undesirable interference of these factors could potentially be eliminated using a multi–proxy approach, leading to an improved interpretation of seemingly contradictory changes when each individual proxy is affected by multiple environmental factors. To do so, all the paleoreconstructions in which I participated employed a multi–proxy approach to avoid such inconsistencies. Such studies require the participation of many specialists, both for the various taxonomic groups and for issues related e.g. to hydrochemistry, hydrogeology or archaeology, and thus we assembled larger expert team for each respective project.

The main proxy group used in my studies were the well–preserved chitinous head capsules of chironomid larvae, which are frequently used in paleoreconstructions from lake sediments. This group has several advantages over other proxies, including their relatively short life cycle and high dispersal ability, enabling rapid responses to changing environments (Brooks et al. 2007). The numerous stenotopic species of this family can provide reliable reconstructions of past environments and, in addition, identification of genera or species groups (morphotypes) with known ecological preferences is achievable using broadly–accepted European taxa lists and identification keys (Brooks et al. 2007). Several calibration datasets (i.e. recent taxa lists of regional species pools with known ecological preference) have been used for July air temperature (T_{July}) reconstruction in Eurasia (e.g. Heiri et al. 2011, 2014). Consequently, chironomid–based paleo–temperature reconstructions in the northern hemisphere are considered one of the most suitable tools for deriving past climatic changes, not just in Europe but across the world (Heiri et al. 2014).

Our research group were forced to focus on filled paleolakes due to the lack of suitable recent Central European lakes persisting from the last glaciation. In addition, the best suitable lakes must be free of human impact in early Holocene and ideally with suitable depth without extensive riparian vegetation during reconstructed period. If such lakes fulfil mentioned criteria, despite have been filled with sedimentary deposits (e.g. have become peat bogs), are located in small catchments, then they become highly suitable for reflecting local climatic changes. My first chironomid–based paleoreconstruction (**Paper 19: Hájková et al. 2016**) filled a gap in knowledge on climatic development from the end of the Pleistocene to the first half of the Holocene in East–Central Europe.

Very few quantitative T_{July} temperature reconstructions exist in Central Europe till our study, and those that do tend to cover different climate types or biogeography, e.g. the Retezat Mts. in the Eastern Carpathians, (latitude 45°N; Toth et al. 2012, 2015) or the Polish lowlands (latitude 52°N; Płóciennik et al. 2011), neither of which are climatically relevant to our study region (latitudes 47–52°N). As there is no chironomid calibration dataset based on recent local assemblages for our region (**Hájková et al. 2016**), we employed a chironomid–inferred model from the nearest geographically suitable region (Swiss–Norwegian model; Heiri et al. 2011) for our T_{July} reconstructions, this having been previously used in other Carpathian studies (Toth et al. 2012, 2015).

In this study, we analysed a seven–metre core sample from a filled paleolake (now a peatbog at altitude 820 m) in the Vihorlat Mts, the site having remained without human impact until 6,000 cal. yr. (calibrated years) BP (before present). Thanks to a high core resolution (after 5 cm) and exact dating by using the carbon–14 (^{14}C) isotope, we were able to precisely time–place 11 layers with an error rate of only 50–100 years. The most pronounced reconstructed temperature shifts (see Fig. 19) fit well with the expected timing of changes documented in the North Greenland Ice Core Project based on the delta oxygen–isotope record (d^{18}O; Rasmussen et al. 2014), as well as other Central and East Central European chironomid paleoreconstructions (Toth et al. 2012, 2015; Płóciennik et al. 2011). During the Younger Dryas (YD), temperatures appeared to fluctuate between 7 and 11 °C, which agrees with other European paleoclimatic records. The site was slightly colder than expected from the general south–to–north YD European temperature gradient, possibly due to the north–facing exposure of the paleolake on hillslope. During the Late–Glacial/Holocene transition, T_{July} increased steeply between 11,700 and 11,400 cal. yr. BP from 11 to 15.5 °C, this rapid climate change being reflected by all proxies that exhibiting a compositional change and increased diversity. The following Early–Holocene climate remained relatively stable, and the lake appears to have been productive with a well–developed littoral zone, as indicated by both diatoms and chironomids.

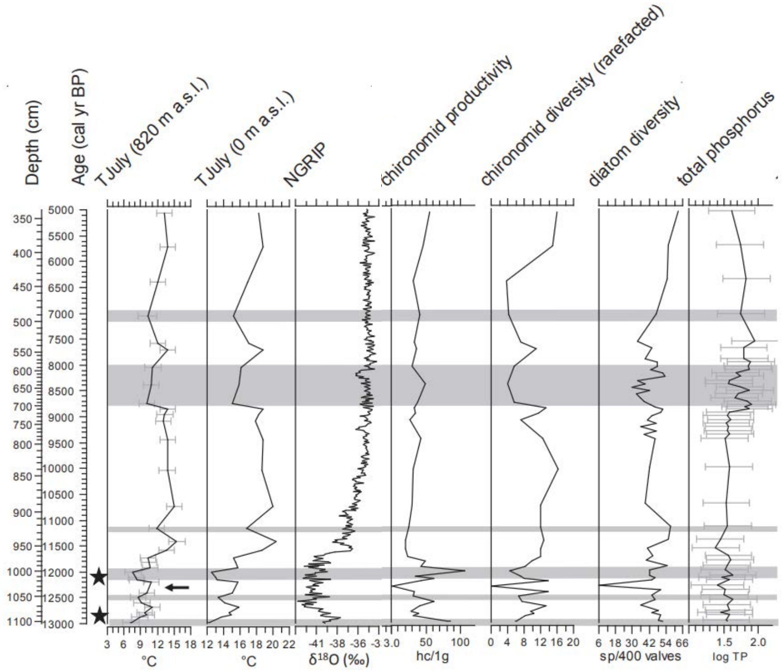

Figure 19. Reconstructed chironomid–based T_{July} *(original values and adjusted to 0 m a.s.l.)* ***from the Hypkaňa profile between 13,000 and 5,000 cal. yr. BP*** *(first two curves). Subsequent curves show changes in NGRIP (North Greenland Ice Core Project), chironomid diversity (number of taxa per 1g of dry sediment), chironomid productivity (number of heads per 1g of dry sediment), diatom diversity (number of taxa per 1g of dry sediment) and reconstructed total phosphorus. Arrow showing possible dry episode. Adapted from Hájková et al. 2016.*

Unlike reconstructions from other parts of Western Europe, our results suggest that the Holocene thermal maximum occurred unusually early in the Holocene (compare to **Kaufmann et al. 2020**), though its timing may have been affected by local topography and mesoclimate. In Central Europe, a distinct and rapid cooling ca. −3 °C took place between 8,700 and 8,000 cal. yr. BP, which we assigned to the rarely captured “8.2 ka event” (Płóciennik et al. 2011) associated with decreasing chironomid diversity, though some other biotic proxies did not reflect this oscillation.

We also eliminated possible T_{July} reconstruction interferences from other factors, such as nutrients (Heiri et al. 2011). To assess this, my co–authors reconstructed the trophic status of the lake using diatom–inferred total phosphorus (P). The results showed that P remained relatively constant throughout the core; hence, we could exclude T_{July} bias from any positive interaction with P oscillations. A further factor potentially influencing T_{July} reconstruction are oscillations in lake depth, as the shallow areas warming significantly during the summer, while in deeper stratified lakes cold–demanding chironomids remain isolated in profundal zone (Velle et al. 2010). In our reconstruction, lake shallowing could potentially have led to a distortion in TJ_{uly}, particularly during the warmer phases of the Younger Dryas (12,200–12,400 cal. yr. BP), when the lake was characterised by low water levels or even complete desiccation, as documented by the lowest chironomid diversity recorded and the dominance of terrestrial taxa (Fig. 19).

In conclusion, by using a combination of biotic and abiotic proxies assembled by multiple specialists, we were able to obtain a complex view of historical lake environmental trajectories, enabling us to build the first widely accepted T_{July} paleoreconstruction of the Late Glacial and early Holocene for the Western Carpathians.

The second paleo–chironomid study extended the temporal coverage of the first Central European Late Glacial–Holocene T_{July} reconstruction back to the Allerød and Older Dryas, i.e. before 13,000 cal. yr. BP (**Paper 20: Šolcová et al. 2020**). We again used a filled Slovakian paleolake (Santovka) on the Western Carpathian/Pannonian ecoregion border. Prior to this study, no detailed summer temperature reconstruction for this region was available for lowland sites below 400 m a.s.l. Complementary mountain and lowland reconstruction studies in the same region can be very useful as the region's characteristics could differ in many ways, not exclusively related to local mesoclimate. For example, they can reflect different components of climate oscillations (e.g. temperature and humidity), which may differ in proxies sensitivity due to topography, geochemistry or overall ecological setting within the lake or whole catchment.

In this case, the chironomid T_{July} reconstruction was combined with records from unique travertine deposits (to our knowledge, the first study analysing paleochironomids from these sediments), also using a multi–proxy approach. The filled paleolake was formed when increased accumulation of travertine deposits dammed the stream, and this has led to a specific type of paleoarchive rarely used for paleoclimate reconstructions. Due to the complex hydrothermal water circulation systems in travertine springs, the influence of climate on carbonate precipitation (travertine) is considered less obvious than with karstic carbonate (Capezzuoli et al. 2014). We eliminated any potential issues related to distortion of paleoreconstruction by collaborating with hydrogeologists, who used stable $d^{18}O$ and $d^{13}C$ isotopes from the travertine to reconstruct whether the basin had been influenced by deep or shallow water circulation, which then enabled us to derive additional information on climate humidity.

Overall, Late Glacial Interstadial temperatures reconstructed for Santovka were slightly colder than expected (ca. 2° C) when compared with other European chironomid reconstructions at 48° N (Heiri et al. 2014). This could be related to the specific type of locality, which may have been influenced by significant input from a cold oxygen–saturated stream feeding a very shallow riverine lake. Such running–water conditions are likely to have enabled the persistence of more cold–adapted taxa that do not typically occur in fully lentic lake environments traditionally used for temperature reconstructions, thereby implying a lower than expected temperature. Occurrence of at least slow–flowing water conditions was supported by frequent records of blackflies larvae (*Simulidae*).

The most important finding resulting from the reconstruction was the 2.2 °C temperature increase at the MIS 2/MIS 1 (Marine Isotope Stage) transition at 14,560 cal. yr. BP (Fig. 20), which coincided with that found by NGRIP (Rasmussen et al. 2014). This prominent and abrupt change in the local environment occurred due to increased precipitation together with a rise in reconstructed T_{July}, and coincided with a rapid increase in the $d^{18}O$ isotope and vegetation turnover, with macrophyte fossils also indicating rising temperatures. The overall robustness of the reconstruction was supported by the complex multi–proxy approach used and the involvement of numerous specialists in geochemistry, pollen, plant macrofossils and molluscs, which also enabled us to minimise any potential bias in chironomid inferred T_{July}. This high–resolution paleoecological data, alongside $d^{18}O$ and $d^{13}C$ stable isotope dating, provided a precise reflection of abrupt climatic and environmental changes in the travertine deposits, while at the same time demonstrating the value of these rarely–used travertine paleoarchives for reconstructions.

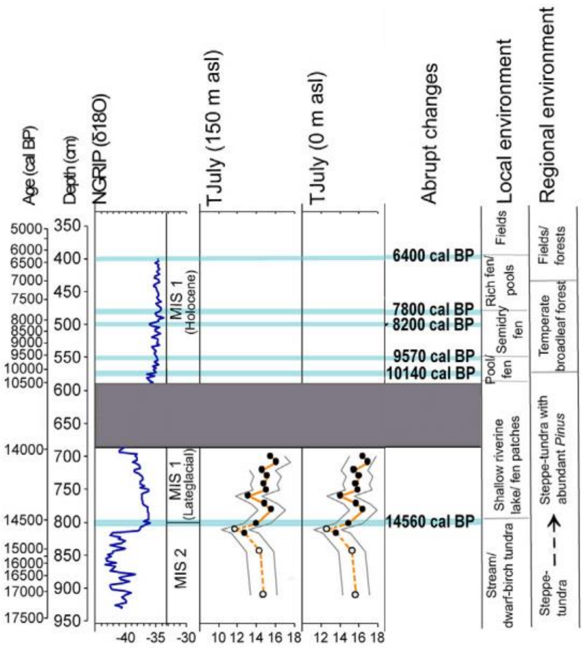

Figure 20. Summary diagram showing results of the Santovka climate chironomid–based paleoreconstruction compared with NGRIP ($d^{18}O$) reconstruction and changes in local and regional environment. Reconstructed chironomid–based T_{July} between ca. 17,000 and 14,000 cal. yr. BP is adjusted to 0 m a.s.l. *(second and third curve). The grey block indicates a hiatus in the data caused by massive tufa precipitation. Upper part of core above hiatus was not suitable for chironomid reconstruction due to lake shallowing. Adapted from Šolcová et al. 2020.*

To obtain a complex understanding of the multiple drivers shaping communities under environmental change, transfer functions used for temperature paleoreconstructions must employ not only known temperature preferences of recent taxa but also knowledge on their complex ecological traits. As we demonstrated in the two previous works, information on taxa–specific preferences helped us interpret changes in the paleoarchive over a broader context, and eliminating reconstruction distortions resulting from the multiple interactions of abiotic factors (e.g. temperature, flow velocity, substrate). Recent community responses to factors along sharp spatial environmental change in spring–brooks and temporal turnover (i.e. seasonality) in these habitats with contrasting physico–chemical parameters could help us to build relevant reconstructions of past environments and climate change.

In 2010, I collaborated as a chironomid specialist in a case study examining two types of fens (basic spring with tufa precipitation *vs.* acid spring with *Sphagnum* mosses) in the West Carpathians (**Paper 21: Křoupalová et al. 2011**). We examined the impact of dramatic physico–chemical changes along a gradient from spring–source to spring–brook transects (hundreds of metres), and compared the temporal stability of both systems within one season using different aquatic MIV groups. We expected a different importance of seasonal changes and environmental heterogeneity primarily linked to strong tufa precipitation in the calcareous fen, which causes stronger environmental filtering.

The tufa fen chironomid community was in some aspects similar to the paleocommunity recorded at the Santovka tufa paleolake analysed by **Šolcová et al. (2020)**. In **Křoupalová et al. (2011),** we, amongst other taxa, recorded high representation of the Stempellini tribe (e.g. *Stempellinella*), frequently recorded in tufa, and terrestrial/semiterrestrial Orthocladinae taxa (*Limnophyes, Metriocnemus, Smittia*), identified during dry phases in the Hypkaňa paleolake (**Hájková et al. 2016**). In some aspects, this spring brook resembled the Santovka paleolake, which was also fed by springs and supported tufa precipitation, and could be considered as its modern analogue.

Our results from the tufa fen revealed relatively stable chironomid assemblages within the season, again supporting the suitability of the chironomid group for temperature paleoreconstructions. The tufa chironomid community appeared to be also seasonally less variable compared with the other MIV groups analysed and also to the second studied habitat (*Sphagnum* fen), which exhibited an increased temporal turnover over the season. The chironomid assemblages also responded significantly to the change in discharge along the spring–source/spring–brook gradient, which corresponds with the Santovka paleorecords, where chironomids also clearly reflected changes in the flow regime. Close relationships between abiotic environmental factors forming chironomid assemblages in tufa habitats, together with their low seasonal variability, confirmed the reliability of the chironomid–based paleoreconstructions in non–typical paleoarchives, such as the naturally dammed Santovka riverine lake (**Šolcová et al. 2020**).

As climate change gradually progresses, there is a growing need for assessments of recent warming and long–term records of natural climate variability. To address this, there is a clear call for comprehensive databases of paleoclimatic records based on quality–controlled, peer–reviewed and temperature–sensitive proxy records dating back to the last glacial period. Based on my quantitative T_{July} paleoreconstructions from the poorly covered Western Carpathian region, I was invited to provide support to a global initiative assembling Holocene period temperature paleorecords (**Paper 22: Kaufmann et al. 2020**). The resultant world database covers the last 12,000 years of past climate history, and is based on different archive types from around the world, with most inland water contributions originating from lake sediments (51%) and peat bogs (11%), the rest being based on marine sediments, data from glacier ice or other sedimentary archives. While the same two biotic sedimentary environments (filled paleolakes and peatbogs) were also used for my Central European T_{July} reconstructions (**Hájková et al. 2016, Šolcová et al. 2020**), only those from the Vihorlat Mts. (Hypkaňa) sufficiently covered the Holocene period.

The database only included time series' that fulfilled a series of minimum criteria: 1) they covered at least 4,000 years, 2) with a resolution at sub–millennial scale (median interval between samples of 400 years or less), and 3) with at least one layer dated every 3,000 years. The assembled data can be used to reconstruct the spatiotemporal evolution of Holocene temperatures at both global and regional scales as climate development of both, global and local scales, frequently exhibits distinct geographical or climatic gradient patterns. In Europe, for example, latitudinal (North–South), longitudinal (East–West; oceanic *vs.* continental climate) and altitudinal (lowland *vs.* mountainous regions) gradients are all observable, with declinations from the general pattern caused by specific local conditions (e.g. **Hájková et al. 2016**). The mid–altitude, Central European region was represented in the database only by our Hypkaňa record, which also covers the transition from the last glacial to the Holocene (unlike many other chironomid reconstructions). Two other available records, those of Toth et al. (2015) and Płóciennik et al. (2011), were situated in high mountains or lowlands, respectively, and included less suitable lakes for reconstruction (i.e. relatively shallow lakes limiting reconstruction due to interference from littoral development).

While all the biotic proxies in the assembled database showed a warming trend during the Holocene, some indicated peak warmth as early as ca. 10,000 years ago (including chironomid records such as mine – Fig. 21a; **Hájková et al. 2016**) and others are delayed at around 6,000 years ago (mainly pollen records – Fig. 21b).

This inconsistency between proxies could be due to known differences in the rate of community change between faster responding chironomids (short lifespan, good dispersers; **Sarremejane et al. 2021**) and the slower vegetation reaction related to its longer lifespan (e.g. trees). Furthermore, most of the database record only covers the Holocene period (especially pollen), with the Late glacial–Holocene transition being far less represented – Fig. 21c (my reconstruction helps fill this gap). Similarly, seasonal temperature variability is poorly covered by some proxies as most of them can only be used for annual temperature reconstructions. Hence, proxies that cover seasonal variability (e.g. chironomid–based T_{July}) are of great help for building a more complex picture of climate variability.

The Pleistocene climatic oscillations (namely glaciations) have left a strong signature, not only in paleoarchives but also on contemporaneous patterns of European freshwater biodiversity. It is generally assumed that lower latitudes served as refuges during these glaciations, and that species diversity gradually increases the further south one moves from the formerly glaciated regions.

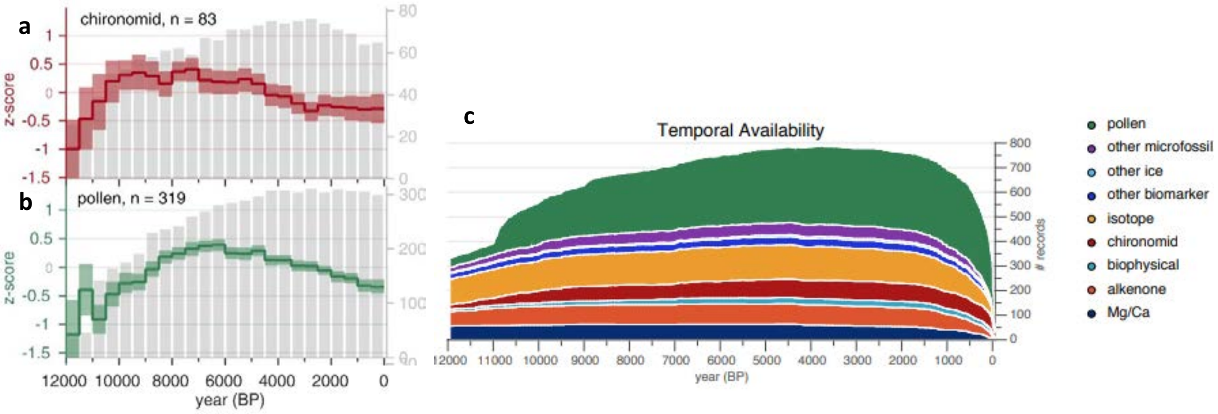

***Figure 21. Comparison of major temperature change trends during the Holocene period (last 10,000 cal. yr. BP), reconstructed from (a) chironomid** (n=83) **and (b) pollen** (n=319) **records** (z–score = mean of zero and variance of 1 SD over the entire record); **Temporal data availability of different proxy records (c) for the temperature database covering 12,000 cal. yr. BP** (proxy type coded by colour). Adapted from Kaufmann et al. 2020.*

While groups of cold–adapted species persisted in these previously glaciated areas in cryptic refuges (Provan & Bennett 2008), such refuges have not been documented for aquatic communities in Central Europe. In the Western Carpathians, however, fossil material for molluscs (Juřičková et al. 2014) and terrestrial insects (Drees et al. 2016) suggests that similar refuges may have existed for terrestrial invertebrate groups. Prior to our study (**Paper 23: Copilas–Ciocianu et al. 2017**), similar evidence for aquatic refuges was inconclusive, though some studies had documented surprisingly divergent gammarid lineages in the Western Carpathians (Copilas–Ciocianu & Petrusek 2017).

Based on my previous population–genetic research (supervising of Bc and MSc thesis), I was invited by a group of molecular ecologists to collaborate as a specialist on gammarid ecology in a study examining the population structure of gammarids on the Czech–Slovak border. Despite our model taxa freshwater amphipod *Gammarus fossarum* species complex is common in the Bohemian massif and Western Carpathians, we expected shifts in its lineage representation based on the borderline between these biogeographic regions (Illies 1967) previously documented for permanent aquatic groups such as oligochaetes (**Schenková et al. 2010**). Furthermore, the intersection of three major European sea basins (the Baltic, Black and North Seas) in this region could also support our expectations of the molecular–genetic structural shifts in the *G. fossarum* complex.

We compared lineage diversity spatial patterns for this widely–distributed taxa between the adjacent biogeographically and geomorphologically distinct regions using molecular data (mitochondrial and nuclear markers). The main aim of the study was to determine whether the observed patterns of spatial diversity were more likely to stem from historical processes enabling long–term survival within glacial refuges (related to climate changes and orogenesis) or present processes related to post–glacial recolonisation and habitat selection (based on ecological preferences).

Based on the results, we distinguished eight phylogenetically diverse *G. fossarum* lineages in the species complex, two of which inhabited both regions and exhibited signatures of recent demographic expansion. The other six lineages were found exclusively in the Western Carpathians and showed a relict distributional pattern (Fig. 22). Molecular dating indicated that these lineages were old, and had probably diverged throughout the Miocene, 7–18 Ma (millions of years). Furthermore, their distribution was not constrained by the present catchment boundaries or topography, thereby excluding the possibility of any recent expansion or response to catchment environmental factors. Such contrasting spatial patterns between two regions thus more likely result from historical rather than contemporaneous or recent factors. Therefore, our results surprisingly indicate that the higher latitude Western Carpathians could have served as long–term glacial refuges for permanent freshwater fauna over millions of years of drastic climatic fluctuations.

This was the first study to document such northern refuges for aquatic fauna based on the high cryptic diversity of lineages (with basal position within the species complex), and to highlight the most important diversity hotspots for this species complex in Europe. The functioning of these long–term refuges could be explained by the heterogeneous topography of the Western Carpathians, which provided shelter during the adverse climatic conditions of the Pleistocene (Juřičková et al. 2014). Further, running waters would have been buffered against freezing during cold periods by the widespread thermal springs (Pauls et al. 2006), which are more frequent across the Western Carpathians than the Bohemian Massif and will have maintained flows and temperature stability during the cold and dry periods associated with the glaciation cycles (Franko et al. 2008).

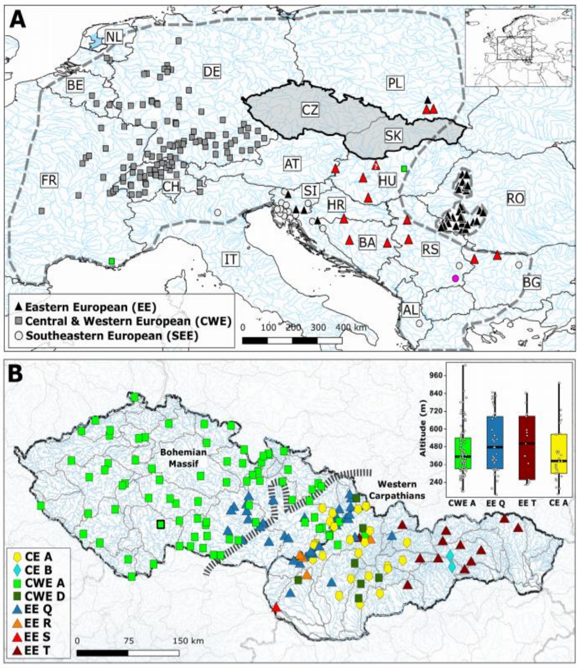

Figure 22. Distribution of eight cryptic Gammarus fossarum lineages across the Czech and Slovak Republics, with the marked biogeographical boundary between the Bohemian Massive and Western Carpathians*. The overlap in lineage altitudinal distribution indicates poor differentiation of ecological preferences in this parameter (shown in top–right corner of B). Adapted from Copilas–Ciocianu et al. 2017.*

A – geographical distribution of the three main lineage groups in Eastern, Central & Western and South–eastern Europe; B – geographical distribution of the eight described cryptic lineages in Bohemian Massive and Wester Carpathian in the area of the CR and Slovakia.

Research outlook and future perspectives

As I have shown in the overview of my current research, myself and my team have contributed substantially to the present understanding of biodiversity responses and patterns shaped by stream intermittence in the humid continental climate of temperate Central Europe; indeed, in many respects, some of these studies were pioneering works. While the general perception of stream drying in this region has traditionally been negative, we demonstrate different aspects of this phenomena and its interaction with common anthropogenic stressors, such as organic pollution/eutrophication, and show that such conditions need not always lead to substantial deterioration on riverine MIV communities. Some mechanisms and interactions described in our publications were far from trivial expectations, demonstrating that the impact of drying cannot always be interpreted without deeper insights into community functioning and responses to, what is in many aspects, a novel stressor.

Progressive climate change has affected recent river communities in many ways, not just through flow cessation but also by rising temperatures, changing water biochemistry and climate triggered biological invasions/expansions, which have had an increasingly important impact in recent decades. In response to these climatically driven changes, both alone and acting together with anthropogenic stressors such as pollution or habitat modification, many taxa have changed their original distribution areas or completely disappeared from some regions. The subsequent novel “mixing” of communities could lead to dynamic changes, not only in their taxonomic composition but also in their functioning, with many unpredictable consequences. In my studies I have tried to contribute an explanation to some current or historical routes of taxa dissemination, along with related mechanisms leading to their recent redistribution.

Only by providing complex descriptions of current climate impacts, and placing them within an historical context, can we better understand the past and make better predictions of future changes. In the third part of my research summary, I outlined how we have reconstructed historical air temperature trajectories in Central Europe, which were accompanied by shifts in taxa distribution and subsequent radiation from refuges. While the end results of the various projections of climate change can never be 100% certain, most models predict substantial warming until at least the end of this century. This will affect not only aquatic communities but certainly all areas of human existence too. In my work, I have tried to contribute to our understanding of past temperature shifts and related taxa distribution oscillations, in the hope that it will help us better predict changes in Central European freshwater ecosystems in the future, by which time they will be experiencing substantial deviations from their current state.

I constantly incorporate all the knowledge gained from my research activities in both the lectures and practical courses I lead at Masaryk University (ca. 10) and in my intermittent teaching at Charles University and the University of South Bohemia. As more than 20 of my students have been directly involved in national and international projects (some being co–authors on my publications), I hope most of these novel results and practical experiences will be disseminated and applied by them in the future.

My future research plans are mostly focused on the broader interaction of the multiple climate and anthropogenically–driven stressors affecting communities in drying river networks using new approaches and tools. As we have shown in some of our papers, dry riverbeds and their adjacent riparian zones can also play a crucial role for intermittent streams. Thus, I will continue to study these terrestrial habitats with my team and examine their contribution to the long–term persistence of aquatic and semiaquatic riverine fauna. As we want to include a wider range of organisms in our studies, including those not traditionally monitored in freshwater ecology (e.g. terrestrial invertebrates, terrestrial vascular plants), we aim to employ in limnology novel methods such as pitfall traps and terrestrial vegetation mapping. Furthermore, we will use new and rapidly developing tools such as eDNA analysis and water biochemistry to complement data from traditional biota observations and sampling using classic limnological techniques. While I presently use mainly aquatic MIVs as model groups in my research, I also plan to fill gaps in our knowledge of traditionally less studied groups in IRES, such as algae (phytoplankton and phytobenthos) and fish (despite their low representation in drying rivers) due to the lack of available datasets for the continental temperate climate of Central Europe. Finally, we have collected long–term datasets for aquatic MIVs in near–pristine IRES, enabling future assessments of their communities and any shifts related to accelerating climate change.

In the future, one area of our research will focus on the presently understudied increase in autumn and winter stream drying resulting from a prolonged dry episodes and/or water over–abstraction (e.g. for artificial snowmaking). I have already participated in applied research examining the measures mediating the impact of stream drying on IRES biota (e.g. building fishponds, riparian vegetation management and land–use modification).

I will continue this research by examining the responses of aquatic MIV, algae, vascular plants and fish to abiotic factors resulting from mediation measures (e.g. flow modification, shading, hydromorphological improvements and nutrient management). With respect to specific factors associated with river drying and low flows, I will study drivers such as water temperature oscillation, temperature refuges and anthropogenic or naturally induced salinisation. While the extent of my future research will always depend on successful funding, thanks to my long–term participation in many international projects, I believe that my team and I will continue to undertake successful research on these topics well into the future.

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Abbreviations:

ASPT – Average Score Per Taxa index
BMWP – Biological Monitoring Working Party index
BOD_5 – 5 days Biological Oxygen Demand
CR – Czech Republic
EPT – Ephemeroptera, Plecoptera, Trichoptera
EU – European Union
HD – historical drying
IRES – Intermittent Rivers and Ephemeral Streams
MIV – Macroinvertebrates
NGRIP – North Greenland Ice Core Project
RD – recent drying
RR – Resistance/Resilience
T_{July} – reconstructed July air temperature
WFD – Water Framework Directive

List of papers used for this habilitation thesis

Part I:

Paper 1: Řezníčková, P., **Pařil,** P. & Zahrádková, S. (2007). The ecological effect of drought on the macroinvertebrate fauna of a small intermittent stream – an example from the Czech Republic. International Review of Hydrobiology 92, 514–526. DOI: 10.1002/iroh.200610997

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Paper 4: Straka, M., Polášek, M., Syrovátka, V., Stubbington, R., Zahrádková, S., Němejcová, D., Šikulová, P. Řezníčková, L. Opatřilová, Datry T. & **Pařil**, P. (2019). Recognition of stream drying based on benthic macroinvertebrates: A new tool in Central Europe. Ecological Indicators, 106, 105486. DOI: 10.1016/j.ecolind.2019.105486

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Appendixes

Part I: Macroinvertebrate communities of Central European intermittent streams

Paper 1: Řezníčková, P., **Pařil,** P. & Zahrádková, S. (2007). The ecological effect of drought on the macroinvertebrate fauna of a small intermittent stream – an example from the Czech Republic. International Review of Hydrobiology 92, 514–526. DOI: 10.1002/iroh.200610997

Paper 2: Řezníčková, P., Soldán, T., **Pařil,** P. & Zahrádková, S. (2010). Comparison of mayfly (Ephemeroptera) taxocenes of permanent and intermittent Central European small streams via species traits. Biologia, 65, 720–729. DOI: 10.2478/s11756–010–0067–x

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Paper 4: Straka, M., Polášek, M., Syrovátka, V., Stubbington, R., Zahrádková, S., Němejcová, D., Šikulová, P. Řezníčková, L. Opatřilová, Datry T. & **Pařil**, P. (2019). Recognition of stream drying based on benthic macroinvertebrates: A new tool in Central Europe. Ecological Indicators, 106, 105486. DOI: 10.1016/j.ecolind.2019.105486

Paper 5: Straka, M., Polášek, M., Csabai, Z., Zweidick, O., Graf, W., Meyer, E.I., Elexová, E.M., Lešťáková, M. & **Pařil**, P. (2021). Stream drying bioindication in Central Europe: A Biodrought Index accuracy assessment. Ecological Indicators, 130, 108045. DOI: 10.1016/j.ecolind.2021.108045

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Paper 9: Stubbington, R., Chadd, R., Cid, N., Csabai, Z., Miliša, M., Morais, M., A. Munné, **Pařil**, P., Pešić V., Tziortzis I., Verdonschot R. C., & Datry, T. (2018). Biomonitoring of intermittent rivers and ephemeral streams in Europe: Current practice and priorities to enhance ecological status assessments. Science of the total environment, 618, 1096–1113. DOI: 10.1016/j.scitotenv.2017.09.137

Paper 10: Sánchez–Montoya, M. M., Gómez, R., Calvo, J. F., Bartonička, T., Datry, T., & **Pařil**, P. (2022). Ecological values of intermittent rivers for terrestrial vertebrate fauna. Science of The Total Environment, 806, 151308. DOI: 10.1016/j.scitotenv.2021.151308

Paper 11: Loskotová, B., Straka, M., & **Pařil**, P. (2019). Sediment characteristics influence benthic macroinvertebrate vertical migrations and survival under experimental water loss conditions. Fundamental and Applied Limnology 193, 39–49. DOI: 10.1127/fal/2019/1138

Paper 12: Pařil, P., Polášek, M., Loskotová, B., Straka, M., Crabot, J., & Datry, T. (2019b). An unexpected source of invertebrate community recovery in intermittent streams from a humid continental climate. Freshwater Biology, 64, 1971–1983. DOI: 10.1111/fwb.13386

Paper 13: Loskotová B., Straka M., Polášek M., Dostálová A. & **Pařil** P. (2021). Macroinvertebrate seedbank survival in pristine and nutrient–enriched intermittent streams and its contribution to flow phase communities Hydrobiologia 848, 1911–1923. DOI: 10.1007/s10750–021–04566–1

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The Ecological Effect of Drought on the Macroinvertebrate Fauna of a Small Intermittent Stream – An Example from the Czech Republic

key words: macrozoobenthos, abundance, taxa richness, hydrology

Abstract

The impact of an unstable hydrological regime on macroinvertebrates in an intermittent stream (with periodic summer droughts in particular stretches) was investigated in two successive years with differing climatic conditions. Ten series of macroinvertebrate samples were taken from two sampling sites between April 2002 and October 2003. Total abundance, taxa richness, main faunal groups, and life strategies were assessed in order to describe the changes in the macroinvertebrate assemblages. Periodic dry periods caused a decrease in macroinvertebrate density in the intermittent stretch, whereas they had no negative influence on number of taxa. Before the drought, the density of permanent fauna decreased and the number of r-strategists rose and stayed at a high level in first samples in the after-drought period.

1. Introduction

Drying up of streams is not a common phenomenon in natural conditions in Central Europe. Natural low flows are more frequent in Australia (Brock *et al.*, 2003, McMahon and Finlayson, 2003), South and North America (Miller and Golladay, 1996; Del Rosario and Resh, 2000; Smith *et al.*, 2001; Covich *et al.*, 2003) and the south of Europe (Pires *et al.*, 2000), generally in karst and chalk streams (Wright *et al.*, 2004). Low flows can also be caused by anthropogenic activities (river regulation, dams, *etc.*) and these changes in ecosystems are more rapid and dramatic (Williams and Feltmate, 1994; Kubíček *et al.*, 1999; McMahon and Finlayson, 2003).

In this study, two types of habitats within stream were distinguished according to the natural periodicity of their discharge conditions. The middle stretch of the Gránický brook has intermittent flow but the lower stretch is perennial. The intermittent stream type dries up periodically and is characterised by greater frequency and variability of disturbances. The development of macroinvertebrate assemblages in such a "stream type" is seasonal and predictable. Perennial streams are more common in Central Europe and drying up of this stream type is unpredictable, and only occurs in extremely dry years. As a result, such drought events are less frequent (Miller and Golladay, 1996; Del Rosario and Resh, 2000; Hansen, 2001).

Drought conditions lead to changes in abiotic parameters such as decreases in dissolved oxygen and phosphorus concentrations and increases in conductivity, water temperature, and nitrogen concentrations (Williams and Feltmate, 1994; Humphries and Baldwin, 2003).

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The drying up of the streambed impacts on all stream organisms. Particularly important are the duration and intensity of the dry periods (depending on the depth of the water table under the streambed surface, the length of the dry stretch, *etc*.).

During the drying up process habitat diversity decreases. This phenomenon is closely associated with the lack of food sources and induces stronger intra- and interspecific competition (Williams and Feltmate, 1994; Humphries and Baldwin, 2003). Organisms can respond to changes in different ways. Some can survive using resistant eggs, seeds or spores (Brock *et al*., 2003) whilst other, *e.g.*, insect species, can reduce their life cycle duration. Another possibility is moving to refugia (*e.g*., downstream drift, active upstream movement or downward movement in the hyporheal). Drift is used, for example, by insect larvae and the crustacean *Gammarus fossarum* Koch, 1836. Fish commonly use active upstream movement (Magoulick and Kobza, 2003; Matthews and Marsh-Matthews, 2003) but upstream macroinvertebrate migration is also known (Söderström, 1987). The hyporheal can also serve as a refugium for invertebrates with small, hard bodies (Thorp and Covich, 1991; Williams and Feltmate, 1994; Bretschko, 1995; Rulík, 1995; Del Rosario and Resh, 2000; Fowler, 2004) but this possibility mainly depends on the porosity of the substratum and the permeability of the stream bed.

The aims of this study were to find out if there were (i) differences in taxon composition of macroinvertebrate community of intermittent and perennial stream type, (ii) changes in life strategies of macroinvertebrates induced by drought, and (iii) structural changes of the assemblage caused by drought.

2. Methods

2.1. Study Area

This study was carried out in the Gránický brook (Fig. 1), which is situated in the south of Moravia (Czech Republic). It is a tributary of the River Dyje (Thaya), in the Danube Catchment, and it is situated on the boundary of Hercynian and Pannonian subprovinces (Culek, 1996).

This region is one of the driest parts of the Czech Republic (average annual rainfall is only 665 mm) and the middle part of the brook dries up every summer due to climatic, geological and geomorpho-

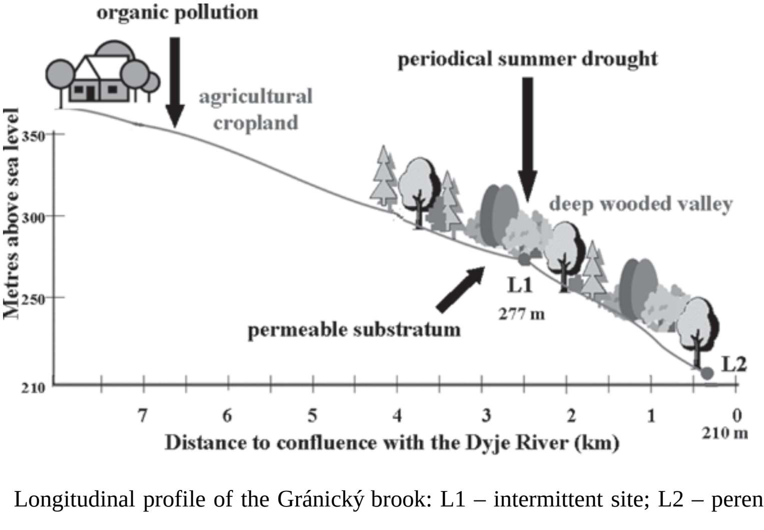

Figure 1. Longitudinal profile of the Gránický brook: L1 – intermittent site; L2 – perennial site.

logical conditions. Annual precipitation in 2002 was higher than the long-term average (691 mm), while in 2003 it was very low (402 mm).

The total length of the brook is 13 km and its catchment area is 20.5 km^2. In the section studied, the brook has a high slope and a cobble-gravel-sand substratum. The watercourse here meanders through a deep wooded valley with no organic loading or human impact. Its high conductivity is natural in origin and results from the geological conditions (sandy clay sediments and schistose biotic granites with high concentrations of sulphates).

Two sites of different character were observed in two successive years: (i) intermittent (middle stretch, altitude 277 m), and (ii) perennial (lower stretch, altitude 210 m). The intermittent site was 1.9 km upstream of the perennial site, which is situated 300 m above the confluence of the brook with the River Dyje (Thaya). The hydrological regime of the middle intermittent stretch is typically unstable, with periodic summer drying up. In contrast, the lower perennial stretch only dries up in extremely dry years, such as in 2003. The dry period lasted for approximately two weeks in 2002, when only the intermittent site dried up. In the following year, the whole brook dried up but the duration of the drought was different at each site. The intermittent site was dry for more than 3 months, from mid July to the end of the investigation in October. However, the perennial site dried for only six weeks, from mid August to the beginning of October, when flow resumed in all reaches except the intermittent site.

2.2. Sampling Methods

Ten series of samples were taken from April 2002 to October 2003. Samples were taken only when the site was flooded with water.

Benthic macroinvertebrates were sampled using the PERLA method (Kokeš *et al*., 2006) that comprised semi-quantitative, multihabitat 3 minute kick samples gathered with a hand net (25 × 25 cm aperture, mesh size 0.5 mm, sack length 75 cm). Samples were collected from an approximately 20 m long, representative stretch of the site and each type of mesohabitat was sampled proportionally to its occurrence.

For percentage substratum cover estimates, the following six size categories were used: boulders (>256 mm), cobbles (64–256 mm), coarse gravel (16–64 mm), fine gravel (2–16 mm), sand (0.1–2 mm) and mud (<0.1 mm) (Furse *et al*., 1986).

Basic abiotic parameters were also measured during the sampling visits, comprising actual air and water temperatures, pH, conductivity, mean stream width and depth. Monthly precipitation data were provided by the Czech Hydrometeorological Institute.

2.3. Data Analysis

Both abiotic characteristics and biotic metric values (*e.g.,* total abundance, taxonomic richness, relative frequency of main faunal groups) of the sites were compared over the whole sampling period using nonparametric Wilcoxon tests for paired samples (Statistica version 7.0. software; StatSoft, Inc. 2004).

The benthic fauna was divided into two groups: permanent (non-insect fauna: Turbellaria, Crustacea, Nematoda, Enchytraeidae, Oligochaeta, Hirudinea, Crustacea and Mollusca) and temporary (insect larvae: EPT taxa, Megaloptera, Diptera, and Coleoptera, including adults). Species were classified into (i) “r-selection”, “K-selection and “unspecified-selection” groups (Šporka *et al*., 2003), and (ii) six feeding groups: grazers/scrapers, shredders, gatherers/collectors, filter feeders, predators and others (Moog, 1995).

A multivariate approach was applied to highlight biological and functional similarities among samples using CANOCO software (Ter Braak and Šmilauer, 2002). Principal Component Analysis (PCA) was used to analyse spatial (*i.e.* “between sites”) and temporal (*i.e.* “between dates”) variation in community trait selection (*e.g.*, r-strategists *vs*. K-strategists) and feeding habits. The arrays gathering abundance of the mentioned groups in the samples were used for the analyses.

Table 1. Minimum, maximum and mean values of the main abiotic parameters at each site, computed for the whole sampling period. Physical and chemical parameters were compared using the nonparametric Wilcoxon paired test.

	Intermittent site			**Perennial site**			**Wilcoxon test**
	MIN	MAX	MEAN	MIN	MAX	MEAN	*P*-values
water temperature (°C)	4.0	14.7	9.8	4.0	15.1	9.6	0.592
pH	5.7	8.5	7.6	6.2	8.6	7.7	0.208
conductivity ($\mu S \cdot cm^{-1}$)	755	930	810	756	1003	861	0.025
dissolved oxygen ($mg \cdot l^{-1}$)	9.7	19.5	13.6	9.9	17.0	13.1	0.401
water depth (cm)	2.1	8.6	6.9	4.2	11.8	8.4	–
stream width (m)	1.4	2.6	1.9	1.5	2.9	2.1	–

3. Results

3.1. Abiotic Factors

Basic abiotic parameters from both sites were compared and no statistically significant differences between the main physico-chemical parameters, excepted conductivity were observed (Table 1). Differences between substratum cover at each site throughout the sampling period and also between sites on each sampling date were not statistically significant (Wilcoxon paired test, $P > 0.05$). Consequently, the samples were suitable for comparison, due to proportional sampling design, identical sampling time and effort and minimum differences, throughout the year, in the substratum cover of each stretch sampled. The sites differed mainly in the duration of the dry period.

3.2. Abundance and Taxa Composition of Macroinvertebrate Community

The total number of processed individuals was 77655 and 83 taxa were found. Total abundances and numbers of taxa per sample were compared.

A lower total abundance (Fig. 2) was found at the intermittent site (between 956 and 2653 ind.). Abundance was several times higher (between 1230 and 18500 ind.) at the perennial site throughout the whole sampling period. The highest observed density at the perennial site, in summer 2002, was due to the high number of *Gammarus fossarum*. The following year, after the long-lasting drought, the summer peak did not occur and abundance values were much lower during both the summer and autumn. In the dry year of 2003, total abundances at the perennial site decreased only before the period of drought. In 2002, the year of permanent flow, the summer abundances at the perennial site were higher than at the intermittent site. The total abundances for samples from both the intermittent and perennial sites and for all sampling dates were compared by the nonparametric Wilcoxon pair test and a statistically significant difference ($P = 0.017$) was found.

In contrast, the numbers of taxa (Fig. 2) were higher during the major part of the year at the intermittent site. These varied between 28 (winter 2002) and 37 taxa (spring 2002). At the perennial site, the number of taxa varied from 16 (spring 2002) to 36 taxa (autumn 2003). Numbers of taxa at the intermittent and perennial sites were significantly different (Wilcoxon paired test, $P = 0.027$).

The composition of the macroinvertebrate community (for main faunal groups see Fig. 3) at the intermittent site was heterogenous, without evident trends, except for the strong

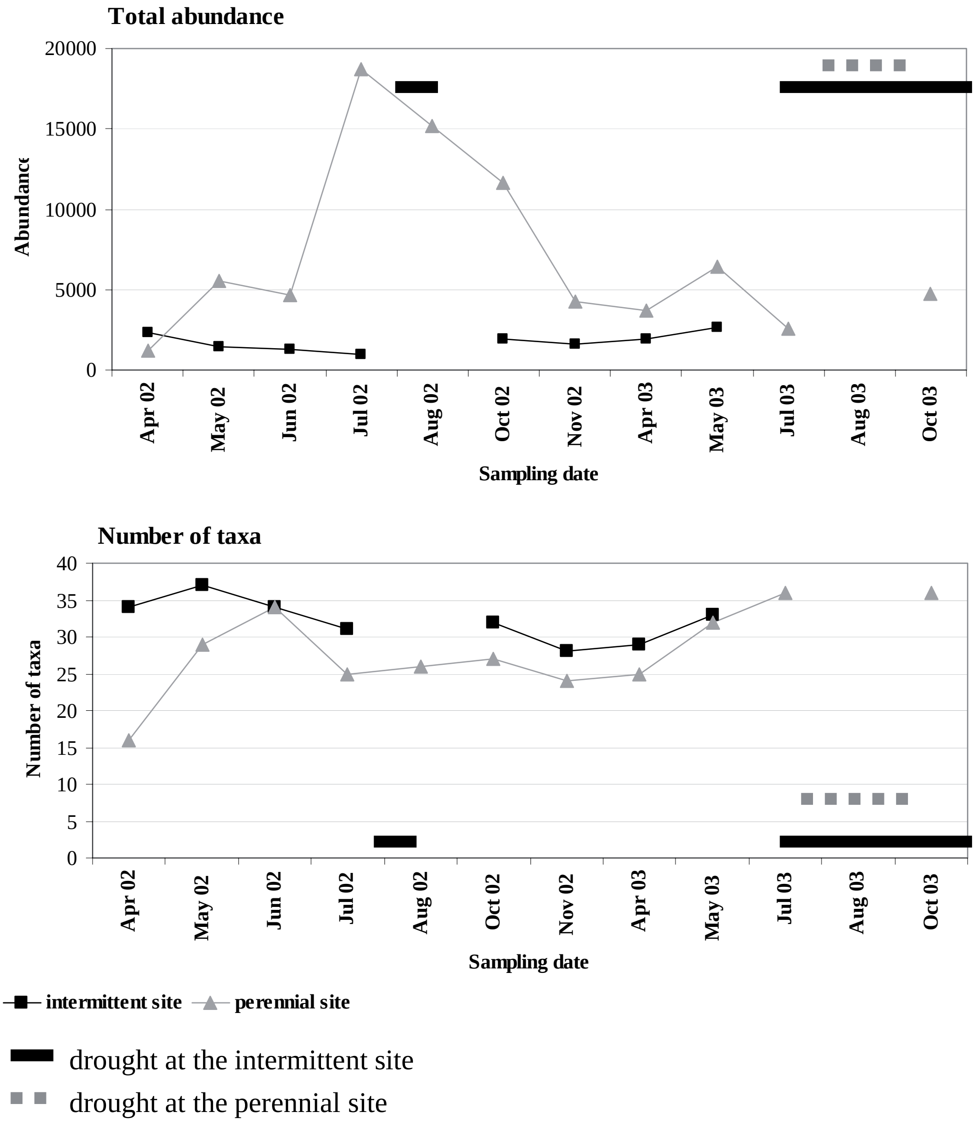

Figure 2. Comparison of the total abundance of individuals and numbers of taxa per sample unit at the intermittent and perennial sites during two sampling years.

increase in dipteran (mainly chironomid) taxa in the pre-drought period. The perennial site was dominated mainly by Crustacea (represented by *Gammarus fossarum*) during the whole first year and the number of dipteran taxa (again represented mainly by chironomids) increased only in the very dry year of 2003, particularly in the spring and summer pre-drought samples. Ephemeroptera, Plecoptera and Trichoptera were represented in all samples; changes in their relative abundance were most probably caused by seasonal effects.

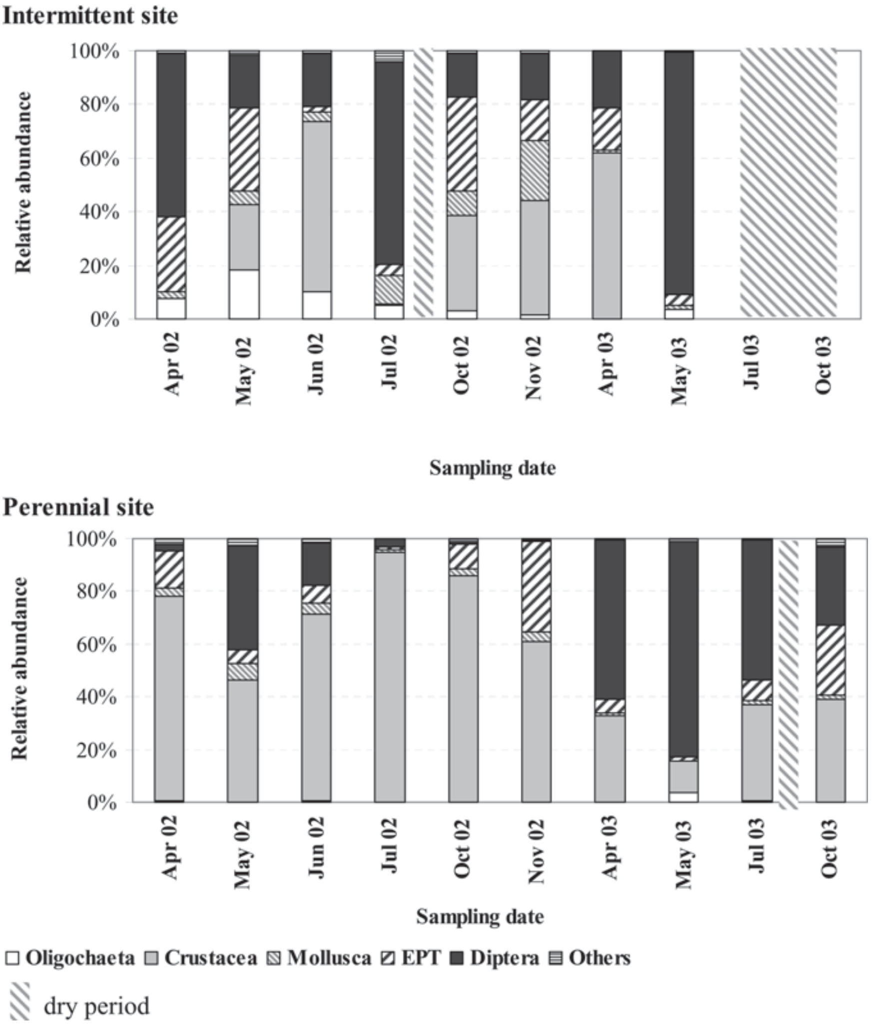

Figure 3. Comparison of the relative abundance values of the main faunal groups (Oligochaeta, Crustacea, Mollusca, Diptera, EPT taxa and others) at the intermittent and perennial site.

3.3. Life Strategies

A comparison of the number of individuals of permanent and temporary fauna formed part of the life strategy analyses. Generally, the permanent fauna (Fig. 4) predominated at the perennial site but their relative abundance was very low in spring 2003, before the 2003 drought, in comparison with spring 2002. The proportion of temporary invertebrate taxa was higher in the intermittent than in the perennial site samples throughout 2002. The proportion of permanent fauna dropped before the dry period at both sites.

Life strategies (number of individuals of r-, K- and unspecified group) for each site were evaluated by PCA (Fig. 5) The 1st axis explained 77.4% and the 2nd axis 22.6% of the known

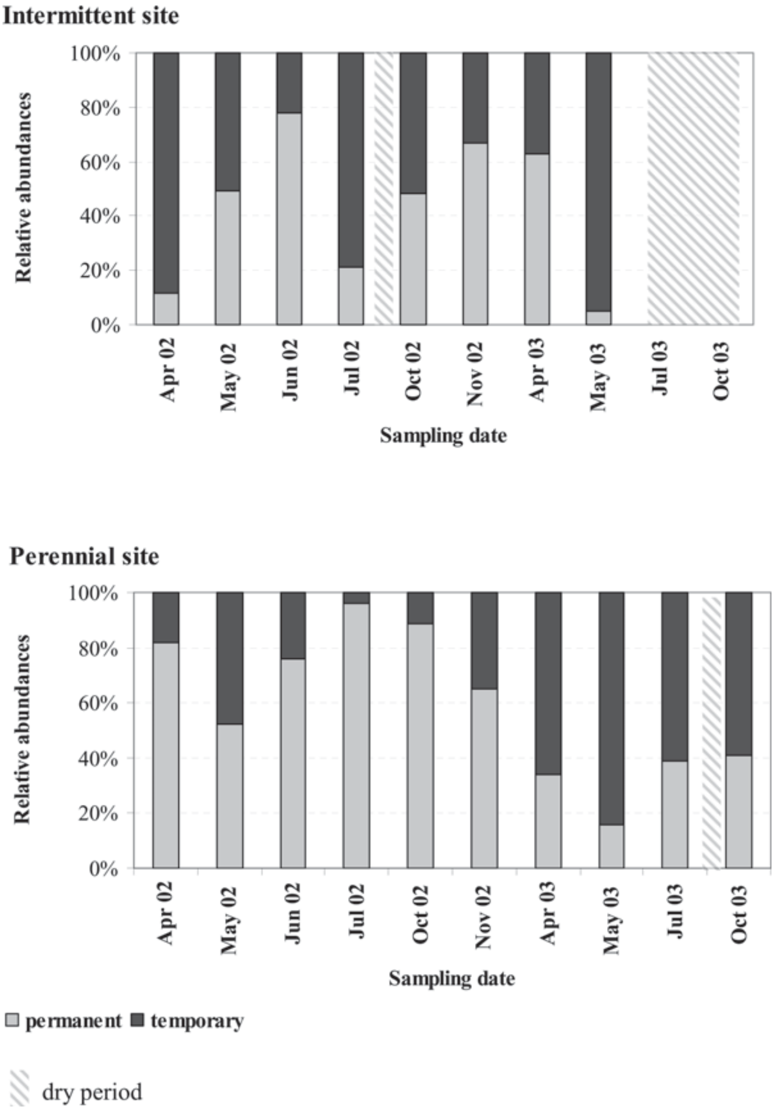

Figure 4. Relative abundance values of temporary and permanent fauna at the intermittent and perennial site.

variability. Most of the samples from the perennial site were situated in the lower part of the ordination graph in the K-strategy factor direction but the samples from the extremely dry year, 2003, were partially shifted in the opposite direction. The samples from the intermittent site were predominantly oriented towards r-strategy factor.

Representation of feeding groups (number of individuals) is evident from Figure 6. Both sites were dominated mainly by shredders and/or gatherers/collectors during most of the sampling period. Nevertheless, before the drought, the relative numbers of gatherers/collectors markedly increased at both sites and in both years. The comparison of feeding groups

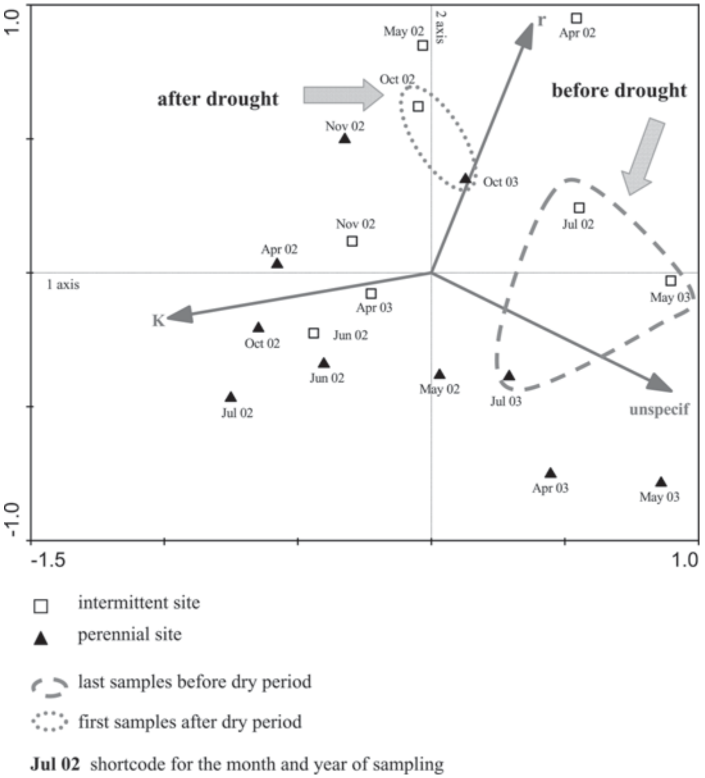

Figure 5. PCA life strategies – comparison of total abundances of K-strategists, r-strategists and an unspecified group at the intermittent and perennial site.

by PCA (the 1st axis and the 2nd axis explained 90.9% of the variation) shows that the sites were very similar and overlapping in this respect (see Fig. 7). Only at the beginning of the dry period, as they are oriented towards gatherer/collector factor, did samples from both sites and years differ from the remaining samples.

4. Discussion

4.1. Abundance and Taxonomic Composition of Macroinvertebrate Community

Throughout the whole sampling period, the lowest abundances of invertebrates were found in samples from the intermittent site (Fig. 2). Miller and Golladay (1996) and Del Rosario and Resh (2000) also found that total abundances of macroinvertebrates were much higher in perennial streams than in intermittent streams. Total abundances fluctuated considerably at the perennial site in both years, whilst abundances at the intermittent site were relatively stable. The highest abundance observed at the perennial site was in the summer of 2002 and was caused mainly by high numbers of *Gammarus fossarum*. From field observations, this could be connected with the in-stream migration of this species and its concentration, during the dry period, in reaches with residual water.

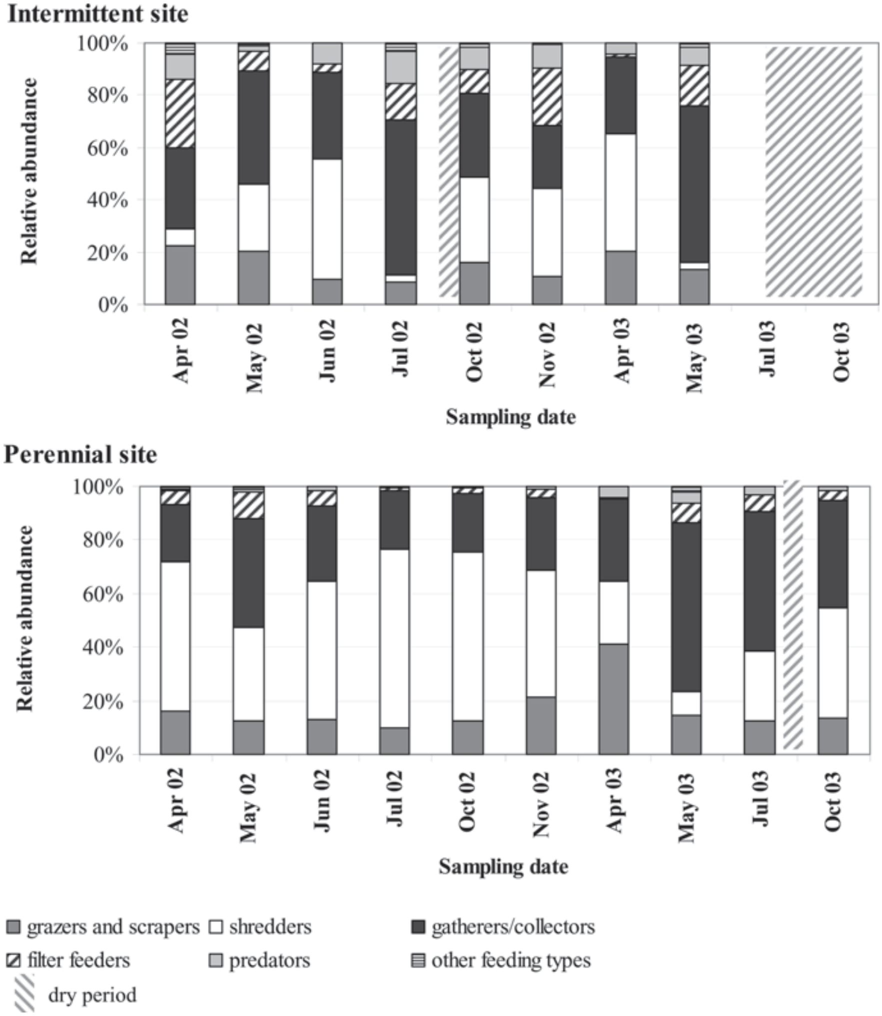

Figure 6. Comparison of relative abundance of six feeding groups (grazers/scrapers, shredders, gatherers/collectors, filter feeders, predators and others) at the intermittent and perennial site.

The number of taxa was relatively stable at both sites (Fig. 2) and rapid decreases did not occur in the samples taken before nor after dry periods. This corresponds with Fowler (2004), who found that the majority of taxa (95%) recovered already after seven days.

Some faunal groups can be more affected than others, for example Chironomidae and Gammaridae can survive short dry periods (Iversen *et al.*, 1978). In the current study, crustaceans (mainly *Gammarus fossarum*) were numerically dominant in most samples, especially at the perennial site (Fig. 3). However, at both sites the number of crustaceans decreased rapidly in samples collected immediately before the drought. As crustaceans were numerous at both sites they were able to compete effectively with other macroinvertebrate groups in recolonization after drought (see the relatively high proportion of this group in

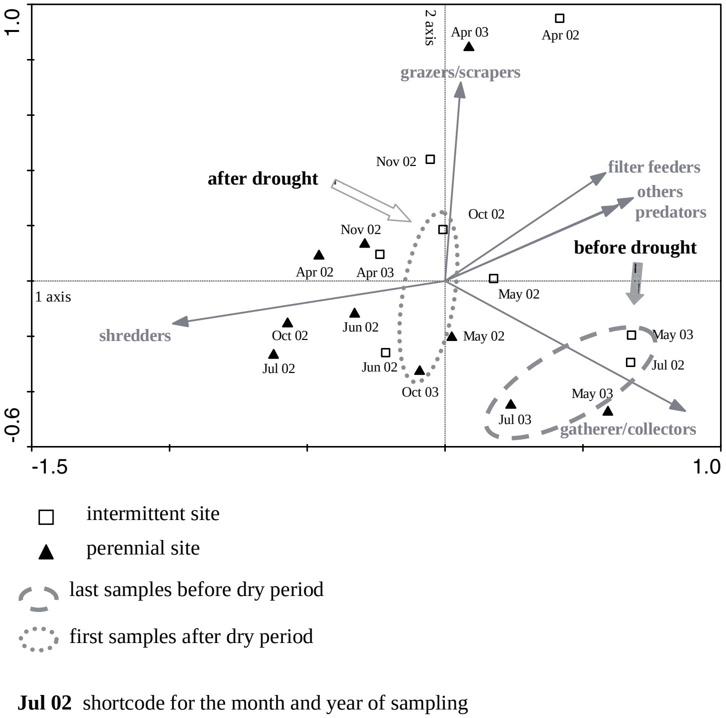

Figure 7. PCA life strategies – comparison of total abundances of six feeding groups (grazers/scrapers, shredders, gatherers/collectors, filter feeders, predators and others) at the intermittent and perennial site.

after-drought samples, Fig. 3). Chironomids are able to adapt their physiology and life cycles to high water temperature and lower oxygen concentration, as documented by HILSENHOFF (1991), therefore they can rapidly respond to very unfavourable environmental conditions (PIRES *et al.*, 2000).

4.2. Life Strategies

Very low relative abundances of the permanent fauna were recorded at the intermittent site in comparison with the perennial site. An apparent decrease in abundance occurred before the intermittent site dried up in summer (Fig. 4). The permanent fauna dominated at the perennial site in 2002 but the situation was different in 2003 and it was probably influenced by the extremely low precipitation that year.

The permanent fauna can survive drought disturbance in the hyporheal or in the upper or lower flooded stretches (KUBÍČEK, 1988a; DEL ROSARIO and RESH, 2000). Representatives of the temporary fauna can respond quickly to the drought by shortening their life cycles or surviving unfavourable conditions in diapause or as eggs (THORP and COVICH, 1991). Macroinvertebrates, which do not have this predisposition for survival, perish.

Droughts represent a substantial environmental disturbance and the survival of freshwater organisms depends on their life strategies. The intermittent site was periodically disturbed by drying up and there were relatively more r-selection species there than at the perennial site (Fig. 5). These species are capable of surviving such disturbances and can rapidly colonize new habitats. K-strategists relatively dominated in samples from the perennial site collected during 2002, the year with permanent flow. The more stable and predictable conditions at the perennial site represent better conditions for K-species (Smith, 1992; Townsend *et al.*, 2003).

Both study sites have similar habitat conditions and probably also food sources. They were dominated by gatherer/collectors and shredders (Fig. 6) but, in the pre-drought period, the relative abundance of shredders always decreased while that of gatherer/collectors increased. There was a reduction of life space with gradual drying up of the streambed and, in relation to such changes in food and habitat resources, feeding groups change too.

4.3. Recolonization and Changes in Community

The rate of recolonization of disturbed habitats, after drying up of the streambed, depends on the length and extent of the drought (Kubíček, 1988b). In the Gránický brook the total abundance values did not decrease markedly after the drought.

Perennial upstream and downstream sections of streams, as well as the hyporheal of the dry stretches, can each serve as refugia and recolonization sources for intermittent reaches (Kubíček, 1988b; Del Rosario and Resh, 2000; Fowler, 2004). In the Gránický brook, in the first year of study, only a short middle stretch dried up. This dry period lasted only two weeks, so the macroinvertebrate fauna had an opportunity to recolonize this reach by drifting down from upstream sections or by active movement from downstream. However, during the following dry year, rapid recolonization was not possible because the whole brook dried up for six weeks, and the intermittent site for 3 months. In this case, the only recolonization options were from the hyporheal or by compensatory flights of insect adults. Colonization from hyporheal could be the slower of the two mechanisms (Kubíček, 1988b). Generally the rate of colonization is mainly dependent on the substratum porosity and bottom permeability (Thorp and Covich, 1991; Rulík, 1995; Del Rosario and Resh, 2000).

The Diptera were the numerically dominant group before the drought but they were not dominant in samples after drought. Chiromomids were the most abundant group of Diptera species in the Gránický brook, the abundances of other dipterans were negligible. Our findings of Diptera (*i.e.* predominantly chironomids) abundance in after-drought samples (4 – 6 weeks after re-flooding) corresponds with findings of Elser (1999) who recorded a very high peak of chironomids on the fifth day after the flow return only, but then their abundance sharply decreased, by more than 50%.

EPT taxa have slower recolonization rates than chironomids (Elser, 1999), which can be caused by their life cycle. Ciborowski and Clifford (1984) found a slight, linear increase in the number of *Hydropsyche* (Trichoptera) after the return of flow, but even after 12 days this had not stabilised. *Hydropsyche* larvae showed the steep growth of number of individuals in the first 5 days and then the growth was only gradual (Ciborowski and Clifford, 1984; Elser, 1999). The lower relative abundance of EPT taxa in Gránický brook was found before drying up than after drought at both sites. The higher proportions of EPT taxa after the return of flow, which were found in our samples taken 4–6 weeks after re-flooding, corresponded with the findings of Ciborowski and Clifford (1984) and Elser (1999) who supposed a gradual growth of abundance after drought. The recolonization rate is specific for each taxon and has to be evaluated in detail.

5. Conclusions

An unstable hydrological regime has significant influence on stream organisms but different natural conditions can generate different community response. Nevertheless, the results of this study are in agreement with observations from other geographic regions. In the case of the Gránický brook, disturbance has no important influence on the number of taxa in both the intermittent and the perennial types of habitat. On the other hand, periodic dry periods caused a significant long-lasting decrease in macroinvertebrate densities at the intermittent site. The composition of macroinvertebrate communities is closely connected to the phenomenon of drying up. Before the drought, the relative abundances of the permanent fauna decreased and relative abundances of r-strategists rose and stayed at a high level in first sample after drought.

Although changes in feeding group composition were found during the pre-drought period (increase of relative abundance of gathering collectors), the exact evaluation of this process needs further and more detailed investigation.

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Comparison of mayfly (Ephemeroptera) taxocenes of permanent and intermittent Central European small streams via species traits

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Abstract: The recurrent drying out of small streams in past decades has shown an urgent need to pay attention to the impact of global climate change. The objectives of this study were to describe the effect of drying out on the composition of the mayfly taxocene and evaluate the relevance of individual species traits for survival of mayflies to drying out. The mayfly taxocenes of two model localities, one at an intermittent and one at a permanent brook, were investigated in 2002, 2003 and 2005. Compared with the permanent stream, the taxocene of the intermittent stream was short of nine species, foremost rheobionts and high oxygen demand species. To explain further differences between both stream types in survival and recolonisation ability, 15 species traits were evaluated. These included so-called "ecological traits" (e.g., habitat and substrate range, density, distribution, current velocity adaptation) and "biological traits" connected with life cycle and larval/adult adaptations. Species showing the highest number of advantageous traits (with only exception of *Electrogena* sp. cf. *ujhelyii* – species of taxonomically unclear status) were able to successfully survive under the unfavourable conditions of the intermittent brook. Biological traits considered more important in many respects seem to be good predictors for assessing sensitivity to extreme temperature changes, hydrological regime fluctuations and the survival/recolonisation ability of species in exposed habitats.

Key words: Ephemeroptera; drought survival; species traits; life cycles; intermittent stream; Czech Republic

Introduction

The climate has globally changed many times over the course of the Earth's history. At present, we are in a warm interglacial period, but over the past two million years there have been numerous glacials. The last one (Würm or Vistula Glaciation) had a greater impact on biota because this period exerted a crucial effect on the selection and composition of contemporary faunas (and biota in general), the phylogeny and biodiversity of most animal groups and numerous processes at population or biosystem levels. Geological records show that past climate changes have not always been gradual; rather there have been numerous rapid changes, sometimes over period as short as centuries or even decades (Bonada et al. 2007b), with principal changes able to be traced even within modern human's history. For instance, starting around 1550 and lasting until around 1850 was a period of cold temperature called the "Little Ice Age" (Lamb 1995).

There are several future climate scenarios for individual regions of the world. Taking into consideration Europe, there are several alternatives, which identically expect local and/or regional temperature increases and more variable precipitation patterns (Beniston et al. 2007; Tapiador & Sánchez 2008). In Central Europe, no substantial changes of annual precipitation are anticipated unlike seasonal precipitation distribution (Pišoft et al. 2004; Kyselý & Beranová 2009). Winter–spring precipitation is expected to increase and summer–autumn precipitation decrease (Bonada et al. 2007b). Temperatures in Europe warmed by 0.5 °C between the mid-1800s and 1940 but cooled again over the next 25 years. Since then temperatures have been rising and current predictions forecast increases of annual mean air temperatures by as much as 8 °C by the end of century in some regions (cf. e.g., IPCC 2001).

Climate change impacts both terrestrial and aquatic parts of the ecosystems. Considerable attention is paid to fluvial systems, which can be affected by changes of hydrologic regimes, primarily in terms of extreme hydrological events such as high discharge and drying out. Over the past decade in Central Europe, besides the phenomenon of summer drought (an unusual event in this region), there have also been rising occurrences of summer floods. For instance, extreme floods occurred in the Czech part of the Danube basin in July 1997 and in the Elbe basin in August 2002. By contrast, the summer in 2003 was extremely hot and dry in Central Europe.

The lack or surplus of water can result in changes in the taxonomical composition and functional structure of assemblages (Bonada et al. 2007a), which could have far-reaching impacts on the self-cleaning processes of water (and consequently on oxygen regime), as well as on the productivity of freshwater biotopes. The impact of extremely low discharges and the drying out of various freshwater habitats was a frequent topic of research in regions with common occurrences of these events, for example, Australia (e.g., Brock et al. 2003; McMahon & Finlayson 2003; see also a review by Boulton 2003) and South and North America (Miller & Golladay 1996; del Rosario & Resh 2000; Smith et al. 2001; Covich et al. 2003). In Europe, the drying out of streams has especially been studied in the south of the continent (e.g., Pires et al. 2000), focusing on the differences in the taxonomical and functional structure of benthic assemblages between streams of temperate region and the Mediterranean, including temporary streams (Bonada et al. 2007a). The Czech Republic, which is situated just in the centre of the European continent and at the boundaries of three sea drainage areas (the North Sea, Baltic Sea and Black Sea), shows a high percentage of small (first to fourth order by Strahler) running waters, which represent approximately 90% of the total length of Czech rivers and streams. These types of streams are vulnerable to anomalous discharges. Small perennial streams, as well as intermittent streams natural in origin can be found in several regions of the Czech Republic. These regions are defined by specific geological conditions (karstic, e.g., the Moravian Karst and Cretaceous regions, e.g., parts of the Czech Plateau). Both perennial and intermittent streams also exist in heavily altered areas (deforested or ameliorated areas, e.g., the Pannonian lowlands in the southeast of the Czech Republic). These regions were most affected by extreme drought and above average summer temperatures in 2003.

In this extremely dry period (2002–2003), studies of macroinvertebrate assemblages of two nearby brooks on the boundary of the Central European highlands and Pannonian lowlands (Illies 1967) were carried out. One of these brooks dried out. The results of the research conducted in the intermittent brook (the Gránický brook) were published (Řezníčková et al. 2007a), whereas the data on the second permanent brook (the Klaperův brook) were left unpublished in a diploma thesis (Nyklová 2006). These unpublished data from the permanent brook, which showed similar abiotic characteristics to the Gránický brook, were used to define the reference state in this study. Comparison of these results stimulated follow-up research, which took place in 2005 in the intermittent brook (the Gránický brook). The results are being processed (Řezníčková et al. 2007b). In comparison with the permanent brook, the intermittent brook showed evident and stabile differences in (among others) the composition and species richness of mayfly taxocenes, which were not interpretable by the impacts of pollution or hydromorphologic dissimilarities of both brooks, which were negligible. Thus, this drying out seemed to be the only disturbance. Surviving a dry period heavily depends on the characteristics of the species and their biological and ecological traits. The species traits important for overcoming this type of disturbance have not yet been summarised, and there is also a lack of detailed information on particular species. Therefore, the objectives of this study were to (i) summarise the information on species traits of species recorded in both study sites, (ii) evaluate the relevance of individual traits for resisting drying out and (iii) verify the results of comparing the taxocenes of permanent and intermittent brooks.

The mayflies (Ephemeroptera) have been chosen as a suitable model group (while other benthic biota will be treated later), among others, the reasons are as follows. (i) First of all, in Central Europe with absolutely prevailing permanent water courses, data describing the effects of drought, on one hand and floods on the other (more precisely, unfavourable prediction scenarios not only in precipitation and runoff but also in temperature fluctuation) are still relatively very scarce or fragmentary; (ii) Mayflies, which first appeared in the fossil records of the Upper Carboniferous represent, with their only about 3,000 described species within 39 families a group in an evident regression with “old” and relatively firmly fixed adaptations, especially in larval stage; (iii) Due to a relatively very low vagility, their distribution is conservative with a very high degree of endemism (although not in the area studied and Europe in general); (iv) The Ephemeroptera order involves in general (as well as in taxocenes studied here) both less sensitive “generalists” with beneficial species traits as well as “specialists” with strict environmental limits and poor powers of dispersal that might be easily become extinct; (v) Mayflies have complex life cycles involving both aquatic and terrestrial phases. This type of life cycle creates evolutionary dichotomy with selection pressure operating in two, more or less, independent environments (cf., e.g., Wilbur 1980; or Brittain 1982, 1990, 1991 and others); (vi) In comparison with other benthic (and aquatic in general) groups of animals their extremely short-lived adult stages represent sole but crucial roles in reproduction, dispersal and recolonisation; (vii) Mayflies have survived, despite problems associated with selection processes operating in both aquatic and terrestrial environments, many climatic shifts and have successfully colonised a very wide range of freshwater habitats from the tropic to the Arctic and from small ponds to large rivers. For instance, in comparison with stoneflies (Plecoptera), they have made a greater intrusion into the tropics, both in terms of diversity and abundance, and are at the same time more abundant and diverse than dragonflies (Odonata) in the Arctic. This fact is extremely important because global climate change indications favour mayflies against Plecoptera and Odonata; (viii) Extremely low vagility of “conservative” mayflies with a very low presentation of purely behavioural adaptations most probably reflects the environmental changes much more sensitively than advanced and “progressive” insects groups such as, e.g.,

Table 1. Environmental parameters in the Gránický and Klaperův brooks.

	Gránický brook (intermittent)			Klaperův brook (permanent)		
	Min.	Max.	Average	Min.	Max.	Average
Water temperature (°C)	6.6	15.2	10.9	3.0	19.0	11.2
pH	6.1	8.6	7.8	6.3	7.9	7.3
Conductivity (µS cm^{-1})	715	1090	884	594	692	643
Dissolved oxygen (mg L^{-1})	7.1	12.1	9.9	8.4	17.4	12.4
Depth (cm)	3	30	12	8	15	11

aquatic dipterans (Diptera), beetles (Coleoptera) and, in a lesser extent, also true aquatic bugs (Heteroptera–Nepomorpha) and caddisflies (Trichoptera) and, last but not least, (ix) mayfly species can be relatively easily identified in contrast with the above aquatic insect orders (perhaps except Heteroptera–Nepomorpha), because the larvae of numerous representatives do not provide us with sufficient critical diagnostic characters or even remain undescribed and (x) principal species traits of numerous mayfly species (or, more precisely, most species living in Central Europe) are relatively well known despite some gaps in knowledge, especially in rare species and often controversial literature data. This enables, in a relative easy way, to trace crucial species traits with inherent plasticity in response to environmental changes.

Study sites

Both the Klaperův and the Gránický brooks are third order tributaries of the Dyje (Thaya) River (the Danube catchment) situated in the warmest (mean annual air temperature is 8–9 °C) and driest area (sum of annual precipitation is 450–500 mm) of the Czech Republic (Gránický brook: 48°51′59″ N, 16°01′32″ E; Klaperův brook: 48°52′24″ N, 15°52″58″ E) (Tolasz et al. 2007). The presence of sandy clay sediments and schistose biotitic granites with high concentrations of sulphates is the main reason for the higher values of conductivity of water typical for the region (Table 1).

The total length and catchment area of both streams is comparable (the Klaperův brook 8.5 km and 17.6 km^2; the Gránický brook 13 km and 20.5 km^2, respectively). The brooks have similar substrate (diverse, but cobbles dominating; boulders are rare). The distance of both study sites, which are situated in the middle stretches of streams, is 10.5 km. The discharge of both brooks is comparable (ca. 0.2–0.3 $m^3 s^{-1}$) during winter and spring. While the Klaperův brook has a permanent water discharge from source to mouth (except for the restricted uppermost segments), the upper part of the Gránický brook has a permanent character, and the middle part, where the study site is located, is intermittent. The lower part of brook is fed by groundwater and has a perennial character with drought events only in extremely dry years. The intermittent stretch became completely dry every summer for the past 12 years (1996–2008).

Material and methods

The data set

The basic data set proceeded from the period April 2002 – May 2003 when the benthic macroinvertebrates were sampled from both brooks at six-week intervals (except in winter). The data from the detailed study of the Gránický brook (2005) were used to confirm the mayfly taxocene composition and study mayfly life cycles. Eleven series of samples were taken between April and November 2005 at three-week intervals. The semiquantitative multihabitat sampling method PERLA was used for both brooks and periods: multihabitat three-minute kick samples gathered with a hand net (25 × 25 cm aperture, mesh size 0.5 mm, sack length 75 cm) (CSN 75 7701; Kokeš et al. 2006). The material collected was fixed in 4% formaldehyde in the field, transferred into 75% alcohol after identification and deposited in collection at the Institute of Botany and Zoology, Faculty of Science, Masaryk University, Czech Republic. Physicochemical parameters (pH, conductivity, dissolved oxygen, water temperature; Table 1) and discharge were measured by portable instruments.

Species traits

Selected species traits of mayfly species found in the Gránický brook and/or in the Klaperův brook were compiled from original data and literature sources. Although species traits themselves have been treated and discussed not so frequently (e.g., Soldán & Zahrádková 2000; Brittain 2008; Zahrádková et al. 2009), appropriate (however, not complete) data concerning ecological requirements, dispersal and distribution of species in question are available in contemporary literature sources (see the list of references in Table 4). The most important seems to be tabular summaries of habitat/substrate preferences, feeding types, emergence, fecundity, oviposition and embryogenesis by Bauernfeind & Humpesch (2001), where numerous additional references can be found along with a biogeographical analysis by Haybach (1998, 2003, 2006) summarising also further references on the spatial and geographical occurrence and distributional types of Central European mayflies (Zahrádková et al. 2009). Although these data are controversial in some respects and some of them are still not known in details, we decided to avoid a detailed discussion in this respect (except the most important species trends concerning life cycles, see below). Moreover, some species traits (egg development and hatching, nymphal development, body size and shape and temperature relationships have been recently discussed by Brittain (2008) in detail with emphasis on their attributes generally advantageous or disadvantageous in disturbed habitats or in rapidly changing environments.

This paper focuses on the relationships between selected species traits on one hand and the effects of drought on the other in order to: (i) select critical species traits, (ii) define their polarity (i.e., which traits are advantageous and which are disadvantageous to survive drought) and (iii) define how much the individual species traits are pronounced within individual species found at the localities investigated.

The following traits were taken into account (see also Table 3 and 4): life cycle flexibility, bi- or polyvoltinism, adaptation in oviposition, egg quiescence, degree of parthenogenesis, asynchrony in emergence, length of winged stage, larval body form, larval body length, feeding type, current velocity adaptation, habitat range, substrate range, density and distribution. The information about these traits was adopted from numerous sources (see Table 4); trait "density" is intended for mayfly taxocenes in the Czech Republic (Zahrádková et al. 2009).

An attribution of individual species traits as advantageous, disadvantageous or indifferent are apparent from Table 4. The attribution follows Brittain (2008) and general principles of r-K continuum concept (MacArthur & Wilson 1967; Pianka 1970).

To define the significance of individual species traits (i.e., how much are they pronounced in individual species), the three-grade scale was used to assess if the trait was advantageous for survival of drought events or not: 2 – advantageous; 1 – indifferent or less pronounced; 0 – disadvantageous or not pronounced. A total score was also calculated for each species in question (see Table 3 for details).

Life cycles represent the most important species traits concerning drought impacts. There are several systems of classification and nomenclature of mayfly life cycles. We used the classification by Clifford (1982), which seemed to be most frequently used in general (cf., e.g., Studemann et al. 1992; Haybach 1998, 2006; Sartori & Landolt 1998; Bauernfeind & Humpesch 2001; Derka 2003c). The Clifford classification evaluating the life cycle type as follows (only categories found in species dealt with in this study are mentioned). Main life cycle categories U – Seasonal univoltine; MB – Seasonal bivoltine, MP – Seasonal polyvoltine; U-MB – Total uni-multivoltine; Y – Total semivoltine; U-Y – Total uni-semivoltine. Life cycle groups (or "subcategories"): Uw – Seasonal univoltine (winter); Us – Seasonal univoltine (summer); Uw-Us – Seasonal univoltine (winter–summer); MBss – Seasonal bivoltine (summer); MBws – Seasonal bivoltine (summer–winter); MB-MP – Seasonal bivoltine or polyvoltine; Us-MBss – Seasonal variable (one or two summer generations); Uw-MBws – Seasonal variable (univoltine winter, possible summer generation); 2Y – Seasonal semivoltine (one generation in two years); 2Y-3Y – Seasonal semivoltine (one generation in one or two years); Uw-2Y – Seasonal variable (univoltine winter or two-year semivoltine)*.

Results

Species composition of the mayfly taxocene

Altogether, 13 mayfly species of nine genera belonging to five families were found at the study sites (Table 2). Four species, namely *Siphlonurus aestivalis* Eaton, 1903, *Baetis rhodani* (Pictet, 1843), *Electrogena* sp. cf. *ujhelyii* (Sowa, 1981) (see bellow) and *Habrophlebia fusca* (Curtis, 1834) formed the Ephemeroptera taxocene of the Gránický brook. Eleven species, namely *Baetis muticus* (L., 1758), *B. rhodani, Ecdyonurus starmachi* Sowa, 1971, *Rhithrogena carpatoalpina* Kłonowska, Olechowska, Sartori et Weichselbaumer, 1987, *Rhithrogena semicolorata* (Curtis, 1834), *Habroleptoides confusa* Sartori et Jacob, 1986, *Habrophlebia lauta* Eaton, 1884, *Paraleptophlebia submarginata* (Stephens, 1836), *Paraleptophlebia werneri* Ulmer, 1919 and *Ephemera danica* Müller, 1764 formed the mayfly taxocene of the Klaperův brook (see also Table 2 and 3).

Except for a single species of the genus *Electrogena*** of the family Heptageniidae (subfamily Heptageniinae), species collected at both sites of the area studied identified without any problems using current identification keys of Central European mayfly fauna (Landa 1969; Bauernfeind 1994, 1995; Bauernfeind & Humpesch 2001; Studemann et al. 1992).

Altogether 15 traits were estimated in all 13 species investigated, altogether 195 values presented (Table 3). The following species show (by the score) the highest ability to drought survival: *S. aestivalis* (24), *B. rhodani* (23), *B. muticus* (23), and *H. fusca* (18). Except for *B. muticus*, all these species were collected in the intermittent stream (Gránický brook). Further species collected in the intermittent stream, namely *E.* sp. cf. *ujhelyii* (12) seems to be much less adapted to surviving drought. The species found in the permanent Klaperův

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* Because alternative classifications are often used (Landa 1968; Sowa 1975a), we consider it necessary to briefly present the equivalency among individual life cycle categories. In other words, to define which Clifford categories agree at least partially with those by Landa (1968) and/or Sowa (1975a). Life cycle subcategories according to the classification by Landa (1968) [main life cycle types A – Seasonal univoltine (winter or summer); B – Seasonal univoltine (winter–summer) and/or seasonal bivoltine and variable; C – Seasonal semivoltine (one generation in two or three years); D – Seasonal semivoltine (one generation in one or two years) and/or seasonal variable (univoltine winter or two-year semivoltine)] shows the following approximate relationships to subcategories by Clifford (1982): A1, A3 = Uw; A2 = Us; B1 = MBws; B2 = MBss and/or Us-MBss; B3 = MB-MP and/or Uw-MBws; B4 = Uw-Us; C1 = 2Y; C2 = 3Y; D1 = Uw-2Y; D2 = 2Y-3Y (here simplified, see Soldán & Zahrádková 2000 for further details). Main life cycle types according to the classification by Sowa (1975a) [main life cycle types: A – Seasonal semivoltine and/or seasonal variable (total uni-multivoltine), B – Seasonal univoltine (winter, summer, winter–summer); C – Seasonal bivoltine and/or polyvoltine and/or seasonal variable (one or two summer generations). Life cycle groups (subcategories) exhibit the following approximative relationships to subcategories by Clifford (1982): A1 = Uw-MBws and/or 2Y; A2 = Uw-2Y and/or 2Y-3Y; B1 = Us; B2 and/or B3 = Uw; B4 and/or B5 = Uw-Us; C1 = MBss; C2 = MBws; C3 = MB-MP (here simplified, see Soldán & Zahrádková 2000 for further details).

** Concerning only the *Electrogena* species, there is a little doubt about its proper taxonomic position. Judging from the arrangement of larval morphological characters (mouthparts, leg and posterior margin of abdominal terga chaetotaxy, gills and cerci) and the exochorionic structure of eggs, this material most probably belongs to *Electrogena samalorum* (Landa, 1982), originally described sub *Ecdyonurus samalorum* Landa (Landa & Soldán 1982), also summarised critical distinguishing characters of this species from the closely related *Electrogena ujhelyii* (Sowa, 1981), originally described sub *Ecdyonurus ujhelyii* Sowa, 1981. Anyway, *E. samalorum* was found conspecific and synonymised with *E. ujhelyii* by Zurwerra & Tomka (1986), however, the type material had never been compared. Moreover, *E. ujhelyii*, originally misidentified as *Ecdyonurus subalpinus* Klapálek, 1907 (by Ujhelyi 1966, see Sowa 1981) had later been confused by the same authors (Tomka & Zurwerra 1985) with the related species *Electrogena gridellii* (Grandi, 1953) and *E. quadrilineata* (Landa, 1969) and described once again from Switzerland sub *Electrogena rivuscellana* Sartori & Landolt, 1991 (Landolt et al. 1991). The opinion of conspecificity of *E. ujhelyii* and *E. samalorum* is followed by some authors (e.g. Belfiore & Desio 1995; Bauernfeind & Humpesch 2001), whereas others (e.g., Derka 2003a, 2003b, 2003c in populations living in Slovakia) recognize the validity of *E. samalorum.* Because *E. samalorum* has still not been formally removed from synonymy with *E. ujhelyii* and its proper taxonomic position thus remains questionable, we present our material as *Electrogena* sp. cf. *ujhelyii.*

Table 2. Selected traits of mayfly species of the area studied.

Species	Site		Feeding group					Current preferences	Life cycle type at studied area	Alternative life cycle types
	Gránický Brook	Klaperův Brook	Grazers – Scrapers	Shredders	Gatherers – Collectors	Passive filter feeders	Active filter feeders			
Siphlonurus aestivalis Eaton, 1903	*		1	+	9	0	0	RL	univoltine summer (Us-MBss)	Uw, Landa (1968): A2, B2
Baetis muticus (L., 1758)		*	5	0	5	0	0	RP	bivoltine winter-summer (MBws)	Uw, MP
Baetis rhodani (Pictet, 1843)	*	*	5	0	5	0	0	RP	bivoltine winter-summer (MBws)	Uw, MP
Ecdyonurus starmachi Sowa, 1971		*	7	0	3	0	0	RP	univoltine winter (Uw)	Sowa (1975b): B3
Electrogena sp. cf. *ujhelyii* (Sowa, 1981)	*	*	7	0	3	0	0	RP	univoltine winter (Uw)	MBws
Rhithrogena carpatoalpina Kłonowska, Olechowska, Sartori et Weichselbaumer, 1987		*	10	0	+	0	0	RB	univoltine winter (Uw)	
Rhithrogena semicolorata (Curtis, 1834)		*	10	0	+	0	0	RB	univoltine winter (Uw)	?MBws, ?Us-Uw, Landa (1968): A1, Sowa (1975a): B2
Habroleptoides confusa Sartori et Jacob, 1986		*	+	0	10	0	0	RP	univoltine winter (Uw)	Landa (1968): A1, Sowa (1975a): B2
Habrophlebia fusca (Curtis, 1834)	*		+	0	10	0	0	RL	univoltine winter (Uw)	Landa (1968): A3, Sowa (1975a): B1
Habrophlebia lauta Eaton, 1884		*	+	0	10	0	0	RL	univoltine winter (Uw)	?Us, Landa (1968): A3, Sowa (1975a): B3
Paraleptophlebia submarginata (Stephens, 1836)		*	+	0	10	0	0	RP	univoltine winter (Uw)	
Paraleptophlebia werneri Ulmer, 1919		*	+	0	10	0	0	RP	univoltine summer (Us)	Landa (1968): A2
Ephemera danica Müller, 1764		*	+	0	0	8	2	RP	semivoltine two years (2Y)	3Y, Uw, Landa (1968): C1, Sowa (1975a): A1

Explanations: Feeding types and current preferences according to Schmedtje & Colling (1996); RB (rheobiont), RP (rheophile), RL (rheo- to limnophile); for abbreviations of life cycle types see Material and methods.

brook only (except for *B. muticus* again) show generally lower scores (16 or less). The lowest adaptation to survive can be found, in this respect, in *R. semicolorata* (11), *E. starmachi* (9), and *R. carpatoalpina* (9).

Discussion

There is no doubt that the taxocene composition as well as survival and density of individual species found in the intermittent Gránický brook are influenced, first of all, by their life strategies and reproductive fitness. These attributes (now currently discussed as "species traits") in fact represent a certain position of the species in r-K continuum. However, despite some exceptions (cf. Derka 2003c), r- and/or K-strategies have still not been discussed within the order Ephemeroptera, and the relative position(s) of species in question remains unknown in detail. Moreover, the situation seems to be rather complicated since, just in the Ephemeroptera, some apparent K-strategists may exhibit a "typical attribute" of r- strategy and *vice versa*. For instance, *E. danica*, a species that might be considered a K-strategist with regards to some (prevailing) very conservative and fixed attributes (e.g., semivoltinism, no egg laying adaptations, no egg quiescence or survival of drought, larval body form and length, habitat, substrate range and others), showed extremely high fecundity (cf. Bauernfeind & Humpesch 2001), a very long winged stage and considerable flight ability – attributes considered "typical" for r-strategists. By contrast, *B. rhodani*, an apparent representative of r-strategists because of body form and length, bi- or polyvoltinism, life cycle flexibility, synchrony in egg hatching, larval development and emergence, pronounced adaptations in oviposition, density and distribution, showed very low fecundity (cf. Bauernfeind & Humpesch 2001), no respiratory and current velocity adaptation and a short winged stage life span – attributes considered "typical" for K-strategists.

Consequently, we avoid discussing the species found from this point of view because only very limited data concerning the other (here "reference" species) are available. Instead, we decided to evaluate species traits

Table 3. Selected species traits of mayfly species found in the (*) Klaperův brook and (**) Gránický brook, compiled from original data and literature sources.

Species found	*Siphlonurus aestivalis*	*Baetis muticus*	*Baetis rhodani*	*Ecdyonurus starmachi*	*Electrogena* sp. cf. *ujhelyii*	*Rhithrogena carpatoalpina*	*Rhithrogena semicolorata*	*Habroleptoides confusa*	*Habrophlebia fusca*	*Habrophlebia lauta*	*Paraleptophlebia submarginata*	*Paraleptophlebia werneri*	*Ephemera danica*
(*) – Klaperův Brook		(*)	(*)	(*)	(*)	(*)	(*)	(*)		(*)	(*)	(*)	(*)
(**) – Gránický Brook	(**)		(**)		(**)				(**)				
Selected traits and scores													
Life cycle flexibility	1	2	2	0	1	0	1	0	1	1	0	0	2
Bi- or polyvoltinism	1	2	2	0	0	0	1	0	0	0	0	0	0
Adaptation in oviposition	1	2	2	0	0	0	0	1	1	1	1	1	0
Egg quiescence	1	0	0	0	0	0	0	0	0	0	0	2	0
Degree of parthenogenesis	2	2	2	0	1	0	0	0	0	0	0	0	1
Asynchrony in emergence	1	2	2	1	2	1	1	1	1	2	1	0	2
Length of winged stage	2	0	0	2	2	2	2	2	1	1	2	1	2
Larval body form	2	2	2	0	0	0	0	1	1	1	1	1	0
Larval body length	1	2	2	1	1	1	1	1	2	2	1	2	0
Feeding type	2	1	1	1	1	0	0	2	2	2	2	2	0
Current velocity adaptation	2	1	1	1	1	0	0	1	2	2	1	1	1
Habitat range	2	1	1	0	0	0	0	1	2	1	1	2	1
Substrate range	2	2	2	1	1	1	1	2	2	1	2	1	1
Density	2	2	2	1	2	2	2	2	1	1	2	0	1
Distribution	2	2	2	1	0	2	2	2	2	1	2	0	2
Total score:	24	23	23	9	12	9	11	16	18	16	16	13	13

Explanations: Score: 0 – disadvantageous manifestation for drought survival; 1 – manifestation for drought survival not clearly pronounced or indifferent; 2 – advantageous manifestation for drought survival.

or, more precisely, selected traits apparently related to survival and (re-)colonisation.

There is a large number of species traits within mayflies, some of them clearly manifested and supported by the numerous data in the majority of Central European species (e.g., flexibility of life cycles, fecundity and length of emergence period), some of them described in detail only in some species (e.g., traits concerning the "ecological range", see bellow) and others insufficiently known supported by concrete data only in a very small number of species (e.g., respiratory adaptation, true nature of quiescence or gene flow).

Furthermore, species traits can be manifested in different ways within different parts of species area. For instance, four types of developmental cycle have been identified in *B. rhodani*: (a) univoltine seasonal winter cycle (Uw) at latitudes above 65° and in mountains above 900–1,200 m a.s.l.; (b) bivoltine seasonal winter life cycle (MBws) or (c) seasonally variable cycle (Uw-MBws) mostly in a central latitudinal belt through Europe; and (d) seasonal polyvoltine cycle (MP) in southern area part, two summer generations have also been observed, for example in the Atlantic Pyrenees. Despite the occurrence of only 1–2 life cycle types within Central Europe in general, (b) and/or (c) (cf. Landa 1968; Sowa 1975a; Studemann et al. 1992; Sartori & Landolt 1998; Bauernfeind & Humpesch 2001), this species shows the highest life cycle flexibility of all remaining species at both localities.

Finally, much literature data seem to be highly controversial. This concerns for example the data on adaptations for oviposition or actual fecundity in *S. aestivalis.* In the latter case, there are differences from several hundreds to 2–3 thousand eggs per female and fecundity largely depends on body size, generation, season and different local conditions (see Soldán & Zahrádková 2000; Bauernfeind & Humpesch 2001 for a complete list of respective references). Nevertheless, *S. aestivalis* shows relatively high fecundity, however, on our scale it was comparable to most species of the Heptageniidae. This was the reason we avoided to consider fecundity in our localities studied, however, it could represent very important species trait in general.

Naturally, the significance of individual species traits is rather different. Besides so-called "ecological species traits", e.g., habitat and substrate range, density, distribution, and current velocity adaptation, we consider so-called "biological species traits" more important to explain survival and recolonisation (see below). Generally, there are three main types of biological traits: (i) those connected with life cycle are the most important and probably determine survival from generation to generation. Besides the type of life cycle (see Material and methods for their delimitation) this

Table 4. Nature (polarity) of species traits with regards to drought survival in species investigated (see Table 3 for the species list), compiled from original data and literature sources.

Selected species trait	Advantageous manifestation for drought survival (score: 2)	Manifestation for drought survival not clearly pronounced or indifferent (score: 1)	Disadvantageous manifestation for drought survival (score: 0)
Life cycle flexibility (2, 3, 5, 12, 18, 19)	flexible (regularly two or several life cycle types)	not strictly fixed (usually a single life cycle type, at most one alternative life cycle type)	strictly fixed (the only life cycle type)
Bi- or polyvoltinism (2, 3, 4, 5, 12, 18, 19)	present (regularly more than 1 gen./year)	possible (usually univoltine with possible complete or incomplete 2nd generation)	absent (regularly a single gen./year or semivoltinism)
Adaptation in oviposition (1)	female underwater, oviposition on substrate, ovoviviparity	female above water, oviposition on substrate	female flying, toughing water surface
Egg quiescence (3, 4, 5, 7, 12, 18)	long (always overwintering eggs, mostly with diapausis)	variable (eggs may overwinter or not, mostly without diapausis)	short (eggs never overwinter, diapausis absent)
Degree of parthenogenesis (6, 7, 13, 15)	high (>30 %)	medium (5–30 %)	negligible (<5 %)
Asynchrony in emergence (1, 8, 9)	high (eggs hatch regularly in several cohorts)	medium (eggs hatch usually in a single cohort)	low (eggs hatch regularly in a single cohort)
Length of winged stage life span (13, 15)	long (more than 2 days)	medium (1–2 days)	short (1 day or shorter)
Larval body form (4, 8, 13)	not specialised, cylindrical (fish-like)	partly specialised (sprawling, walking)	specialised – adapted (flattened, hydrodynamic, burrowing)
Larval body length (4, 9, 13)	small (≤10 mm)	medium (10–20 mm)	large (>20 mm)
Feeding type (8, 16, 19)	gatherers-collectors, omnivores	mixed types (gatherers-collectors and grazers-scrapers)	filter feeders or grazers-scrapers specialists
Current velocity adaptation (8, 16, 19)	rheo- to limnophil	rheophile	rheobiont
Habitat range (1, 8, 14, 16, 19)	broad (colonizing more 5 or more habitat)	medium (colonizing 3 or 4 habitats)	narrow (colonizing usually a 1 or 2 habitat)
Substrate range (16, 19)	broad (living in substrates with different roughness)	medium (living in substrates with similar roughness)	narrow (specialized, living in substrates with defined roughness)
Density (16, 19)	high (eudominant or dominant species)	medium (subdominant or recendent species)	low (subrecendent species)
Distribution (1, 8, 9, 10, 11, 16, 17, 19)	large areas (Palaearctic), very frequent or frequent localities in Central Europe	medium areas (West Palaearctic), medium frequent or scarce localities in Central Europe	small areas (submediterranean), very scarce localities in Central Europe

Explanations: 1 – Bauernfeind & Humpesch (2001), 2 – Brittain (1990), 3 – Brittain (1991), 4 – Brittain (2008), 5 – Clifford (1982), 6 – Degrange (1954), 7 – Degrange (1960), 8 – Derka (2003c), 9 – Haybach (1998), 10 – Haybach (2006), 11 – Landa & Soldán (1985), 12 – Landa (1968), 13 – Landa (1969), 14 – Sartori & Landolt (1999), 15 – Soldán, unpubl., 16 – Soldán & Zahrádková (2000), 17 – Sowa (1975a), 18 – Sowa (1975b), 19 – Zahrádková et al. (2009)

category involves also tendency to polyvoltinism, flexibility of the cycle, length of embryogenesis, egg quiescence, degree of parthenogenesis, asynchrony in egg hatch, development and emergence; (ii) species traits connected with the adult stage, particularly important as far as the vagility of mayflies is concerned (e.g., egg laying adaptation and length of winged stage); and (iii) species traits connected with the larval stage and basic physiological functions (e.g., larval body form and length).

Intermittent and permanent brook: comparison of mayfly taxocene survival and recolonisation

We compared our study site with the adjacent Klaperův brook to evaluate changes in taxocene composition under unstable hydrological regime contra expected (reference) status of permanent brook of the same type. Judging from respective chemical analyses, the conspicuous impoverishment of taxocene components is definitely not caused by the pollution of water and thereby most likely represents a consequence of an anomalous hydrological regime, a key factor governing the mayfly taxocene composition at least in this case. Moreover, the total absence of pronounced rheobiont species indicates that a very important role is played by current velocity.

The differences in survival in the intermittent Gránický brook and adjacent permanent Klaperův brook, which are probably closely related in colonisation and recolonisation cycles, are expressed by the occurrence of only four species in the former while the latter permanent brook is inhabited by additional nine species (Tables 2 and 3), e.g., by rheobionts *R. semicolorata* and *R. carpatoalpina* or high oxygen demand species such as *E. starmachi*, or semivoltine nonseasonal species showing very plastic developmental cycle such as *E. danica* occurring in relatively high densities. The latter species, although exhibiting a relatively high total score (Table 3) enabling larvae to occur and easily survive at high densities in the Klaperův brook, is not able to persist in an intermittent brook. Most probably, larval survival is prevented by their relatively high oxygen demands. Contrary to some other species of *Ephemera*, larvae of *E. danica* never occur in lakes, ponds and other standing waters. In addition, traits such as substrate range, body form, length and especially apparent semivoltinism with no egg dormancy are apparently unfavourable (cf. Brittain 2008).

Total scores (Table 3), clearly show the species with the highest number of advantageous species traits, in other words, the most successful species for survival and recolonisation under the unfavourable conditions of the intermittent brook, namely *S. aestivalis* and *B. rhodani*. However, both species seem to utilise different strategies to survive. Survival of the former is enabled mainly by a large ecological range of larvae with apparently low oxygen demands and respiratory adaptation (movable gills). Larvae are also known to survive in periodic water bodies and usually quickly develop in two months in spring during relatively good water supply. Adults fly in May and July. The eggs (hatching usually early next spring with a high degree of parthenogenesis; cf. Degrange 1954, 1960) are able to tolerate drying up during summer months (cf. Bohle & Potabgy 1992 or Fiedler & Bohle 1994). Sættem & Brittain (1993) even pointed out the summer diapause (aestivation) in populations living in relatively very warm water. *S. aestivalis* was not found in the Klaperův brook at the places sampled during this study but it occurs, however in very small abundance, at several localities situated upstream (in permanent brook segments that might be subjected to severe drought) (Nyklová 2006).

By contrast, *B. rhodani* seems to be short of the pronounced attributes enabling survival of *S. aestivalis* because the larvae are apparently adapted to streamline habitats. Survival is enabled by oviposition adaptation and mainly enormous flexibility of life cycle accompanied with asynchrony in embryogenesis, larval growth and emergence. There is no doubt that at least some larval cohorts are able to survive partial drying up or recolonise brook segments that have been subjected to total drought. The taxocene of the intermittent brook is short of *B. muticus*, although this species exhibits a relatively high number of advantageous species traits. The absence of the species can be explained by (i) a competition with *B. rhodani* – both species have similar ecological requirements, but *B. rhodani* is less specialised (Schmedtje & Colling 1996; Zahrádková et al. 2009) and thus probably more successful in survival; and (ii) *B. muticus*, in spite of relatively high number of "advantageous" traits for drought survival, is considered a species sensitive to anomalous hydrological regimes (Céréghino et al. 2002). This fully agrees with our opinion that *B. muticus* belongs to species first disappearing in intermittent brooks or running waters subjected to non-periodical water level fluctuation, as seen at numerous localities in the same area (Zahrádková unpublished).

As to *E.* sp. cf. *ujhelyii*, its life cycle and details on species traits are far from to be understood, published data are extremely scarce. However, the total score of species traits in *E.* sp. cf. *ujhelyii* is relatively low in comparison with survival of other species, it is the highest within the Heptageniidae studied that represent the most sensitive species in general. Larvae with respiratory adaptation (movable gills of most pairs) living among submerged roots or logs at rather slow current places are probably able to survive partial drought. Moreover, they apparently develop in several cohorts and adults show a pronounced asynchrony in emergence, they fly from April to September (cf. e.g., Bauernfeind & Humpesch 2001)

The occurrence and survival of *H. fusca* (living in the Gránický brook) and *H. lauta* (living in the Klaperův brook) agree with the differences in our total scores (Table 3) as well as with literature data. Larvae of *H. fusca* are evidently much better adapted to survive unfavourable conditions, including at least partial drought as documented, e.g., in Germany (Fiedler & Bohle 1994), Spain (Gallardo-Mayenco & Ferreras Romero 1984) and North Africa (El Agbani et al. 1992). Contrary to *H. lauta*, the species is considered azonal and thermophilous (Haybach 1998; Jacob 1972), generally tolerating large substrate and habitat range (Landa 1957, 1969; Sowa 1975a; Sartori & Landolt 1999; Derka 2003b).

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EFFECTS OF DROUGHT ON THE COMPOSITION AND STRUCTURE OF BENTHIC MACROINVERTEBRATE ASSEMBLAGES – A CASE STUDY

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Abstract

ŘEZNÍČKOVÁ PAVLA, TAJMROVÁ LENKA, PAŘIL PETR, ZAHRÁDKOVÁ SVĚTLANA: *Effects of drought on the composition and structure of benthic macroinvertebrate assemblages – a case study.* Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 2013, LXI, No. 6, pp. 1853–1865

Natural drying up of streams is not common in Central Europe. Nevertheless, the recurrent drying up of small streams in last decades has shown an urgent need to pay attention to the impact of global climate change. This strong disturbance influences conditions in streams markedly and causes changes in the taxonomical and functional structure of biota. The aim of the study was to compare aquatic macroinvertebrate assemblages of one intermittent and one permanent brook in South Moravia. The study was carried out in two stretches with otherwise comparable environmental parameters. Lower densities of macroinvertebrates were found at the intermittent site the difference was statistically significant. The number of taxa and diversity were significantly higher at the permanent site. Functional structure of the assemblages also varied. The shares of rheobionts, grazers and predators differed.

benthic macroinvertebrates, intermittent stream, drought, functional structure, diversity

In this study, we addressed the issue of drying up of small streams. This phenomenon was not typical for the Czech Republic and Central Europe in the past. However, considering the ongoing global climate change, it can be assumed that it will become more frequent in the future. Studies dealing with the effect of natural drought on the aquatic biota are rather scarce in Central Europe (Sommerhäuser & Schuhmacher, 1996; Meyer & Meyer, 2000, Meyer *et al.*, 2003; Pastuchová, 2006; Řezníčková *et al.*, 2007; Řezníčková *et al.*, 2010), but several authors have investigated the influence of fluctuating hydrological regime caused by human activities.

Hydrological regime of watercourses is influenced by various environmental factors (e.g. the character of the catchment, the amount of rainfall and runoff, soil moisture, groundwater level and flow rate etc.) and thus drying up has various reasons (hydrological, climatic or geological). It can be characterized by its frequency, duration of dry and watery period, or by a season in which the watercourses dry up (Lake, 2003).

Based on a combination of physical and biological conditions, Hansen (2001) classified three types of drying streams: perennial, intermittent and ephemeral. In perennial streams water flows during most of the time. These streams dry up only exceptionally in extremely dry years and have a well-defined river bed, in which substrate moves and organic matter does not accumulate (Hansen, 2001). Perennial streams occur on all continents and are common in Europe (Attrill *et al.*, 1996; Wood & Petts 1999; Pinna *et al.*, 2004). Intermittent streams are characterized also by a well-defined river bed, but they dry up in regular cycles, and this disturbance can be predicted (Hansen, 2001; Lake, 2003). Intermittent streams occur on all continents, especially in arid and semi-arid regions of Australia (Closs & Lake, 1994; Hillman & Quinn, 2002; Boulton, 2003), North America (Stehr & Branson, 1938; Delucchi & Peckarsky, 1989; Miller & Golladay, 1996; Rosario & Resh, 2000; Covich *et al.*, 2003; DAHM *et al.*, 2003; Magoulick & Kobza, 2003; Matthews & Marsh-Matthews, 2003; Fritz & Dodds, 2004; Chadwick & Huryn, 2007) or Africa (Arab *et al.*, 2004). Within Europe intermittent stream are often found in the Mediterranean region (Muñoz, 2003; Pinna *et al.*, 2004; Acuña *et al.*, 2005; Bonada *et al.*, 2007; Fenoglio *et al.*, 2007). Ephemeral streams lack stable river-bed, water flow is limited to time periods with extreme rainfall. Organic material does not move or accumulate in ephemeral streams (Hansen, 2001).

Drying up is a disturbance with a crucial impact on both abiotic and biotic factors in aquatic ecosystems. Physico-chemical parameters and biochemical conditions change significantly as the water level decreases; these changes usually include decreasing oxygen saturation, increasing conductivity, deteriorating water quality etc. (Lake, 2003; Larned *et al.*, 2011). Reduced flow velocity and amount of water results in reduced quantity and diversity of habitats and in their changes. The supply of organic matter and basic trophic elements (carbon, nitrogen and phosphorus) from the upper part of the stream is interrupted, the supply of bioavailable carbon, which is important for the metabolism of microorganisms, is reduced (Boulton, 2003; Dahm *et al.*, 2003; Acuña *et al.*, 2005).

Changing conditions significantly affect macroinvertebrate communities. Riffle habitats disappear with decreasing water flow and rheobionts and rheophils loose their living space. Conditions become more lenitic and habitats with slow current and greater depth, which are more suitable for limnobionts tolerating lower oxygen content and water quality, prevail. Drying also causes marked changes in the trophic network of the communities. Formation of isolated pools often leads to a significant increase in the number of predators. Generally, the availability of natural food sources is impaired and competition increases. Interruption of the longitudinal continuum limits migration of organisms from both upstream and downstream parts of the watercourse (Lake, 2003). Benthic invertebrates are adapted to living in water and drying acts as a significant stressor that can eliminate some species from the aquatic ecosystem. Animals that are unable to adapt to adverse conditions or unable to find suitable refugia die. Therefore, abundance and taxonomic composition and functional structure of the community change. The impact on biota and the ecosystem depends on many factors including the presence of refugia (e.g. Rosario & Resh, 2000; Smith & Wood, 2002; Boulton, 2003; Lake, 2003; Magoulick & Kobza, 2003) and also on the extent of the impact of human activities, e.g. river regulation, pollution, water abstraction etc.

Aquatic organisms react to changing conditions in accordance with their characteristics and abilities – they choose different survival strategies (Lake, 2003). Organisms living in habitats with a rather regular hydrological regime usually adapt more easily (Lake, 2003); the same is true for organisms that had the opportunity to adapt over long evolutionary development (Mcmahon & Finlayson, 2003). In this case, the animals can have various physiological and behavioral adaptations or predispositions to survival. Under the conditions of the Czech Republic, macroinvertebrate communities are mostly not adapted to drying from this point of view.

The level of impairment of macroinvertebrate communities depends on local conditions of the disturbed stream and the duration and intensity of drought (Kubíček, 1988). After the dry period, recolonization takes place. Its rate depends on the resistance and resilience of the community. Different recolonization mechanisms exist and usually they are species-specific (Williams & Hynes, 1976). Organisms can colonize re-flooded habitats by drifting from up-stream parts of the watercourse or by active migration from down-stream stretches or hyporheic zone. An important part of the colonization cycle of insect species is the aerial dispersion of egg-laying imagoes. This mechanism is relevant only during the warmer parts of the year and therefore, the recolonization of damaged habitats may be much slower in winter.

Stabilization and full recovery of the communities can take up to three months to several years depending on the extent of the damage, sources in refugia and local conditions (Kubíček, 1978).

Organisms are generally unable to adapt to sudden and unpredictable drying. This type of drying is characterized by low resistance and resilience of organisms (Lake, 2003). Recolonization after such dry episode takes longer. Overall, there are only few papers dealing with non-seasonal and unpredictable drying (Ladle & Bass, 1981; Wood & Petts, 1999; Boulton, 2003; Covich *et al.*, 2003).

The aim of this study was to determine differences between macroinvertebrate communities of one intermittent and one permanent stream. We have assumed that drying is a fundamental disturbance that will be reflected in both taxonomic and functional structure of the communities.

MATERIALS AND METHODS

Study Area

This study was carried out in Granicky and Klaperuv brooks, both stretches are situated in the south of Moravia (Czech Republic) and they are left-hand tributaries of the River Dyje (Thaya), in the Danube Catchment. This region is one of the driest parts of the Czech Republic (average annual rainfall is only 665 mm).

The character of these two brooks is very similar, environmental parameters (e.g. cachment area, discharge, substrate, etc.) are comparable. The distance of both study sites, which are situated in the middle stretches of the streams, is 10.5 km.

Klaperuv brook is permanent. Its total length is 8.5 km and its catchment area is 17.6 km^2. The total length of the Granicky brook is 13 km and its catchment area is 20.5 km^2. The study site is situated within a stretch with a high slope and a cobble-gravel-sand substratum that meanders through a deep wooded valley with minimal organic loading or human impact. This stretch is of an intermittent character. The hydrological regime is typically unstable, with regular summer drying up. The dry period lasted for approximately two weeks in 2002. In the following year 2003, the whole brook dried up, study site was dry for more than 3 months, from mid July to the end of the investigation in October.

Sampling Methods

Samples of macroinvertebrates were taken from April 2002 to May 2003 (8 series). The data set used in this study was obtained as a part of the diploma theses of Department of Botany and Zoology, Masaryk University Brno. Benthic macroinvertebrates were sampled using the PERLA method (CSN 75 7701; Kokeš *et al.*, 2006) that comprised semi-quantitative, multihabitat 3 minute kick samples gathered with a hand net (25 × 25 cm aperture, mesh size 0.5 mm, sack length 75 cm). Samples were collected from an approximately 20 m long, representative stretch of the site and each type of mesohabitat was sampled proportionally to its occurrence. Samples were taken only when both sites were flooded with water.

For the estimation of percentage substratum cover, the following six size categories were used: boulders (> 256 mm), cobbles (64–256 mm), coarse gravel (16–64 mm), fine gravel (2–16 mm), sand (0.1–2 mm) and mud (< 0.1 mm) (Furse *et al.*, 1986).

Basic abiotic parameters were also measured during the sampling occasions, comprising actual air and water temperatures, pH, conductivity, mean stream width and depth. Monthly precipitation data were provided by the Czech Hydrometeorological Institute.

Data Analysis

Both abiotic characteristics and biotic metric values (*e.g.* total abundance, taxonomic richness, Shannon Weaver Diversity Index) of both sites were compared over the whole sampling period using nonparametric Wilcoxon tests for paired samples (Statistica version 7.0. software; StatSoft, Inc. 2009).

The benthic fauna was divided into two groups: permanent (non-insect fauna: Tricladida, Crustacea, Nematoda, Enchytraeidae, Oligochaeta, Hirudinea, Crustacea and Mollusca) and temporary (insect fauna: of Ephmeroptera, Plecoptera, Trichoptera (EPT), Megaloptera, Diptera, and Coleoptera, in the last case including adults). The next step was the analysis of the assemblage functional structure: feeding types, current preferences, stream zonation preferences, saprobity, and microhabitat preferences. Macroinvertebrates were divided into six feeding groups: grazers/scrapers, shredders, gatherers/collectors, filter feeders, predators and others (Moog, 1995).

RESULTS AND DISCUSSION

Abiotic parameters

Drying significantly affects all abiotic factors in streams and subsequently the biota. The main factors significantly affecting local conditions in temporary streams include discharge, water temperature, oxygen content and conductivity (Rocha *et al.*, 2012). Water temperature, pH, and dissolved oxygen were comparable on both studied brooks (Tab. I). Only conductivity and depth showed significant differences.

Generally higher values of water conductivity in both studied brooks are of natural origin and result from the geological conditions (sandy clay sediments and schistose biotic granites with high concentrations of sulphates), nevertheless, higher values in Granicky brook are probably caused by low discharges in summer. Sampling and measurements of physico-chemical parameters were carried out during normal water levels and, therefore, no extreme values were recorded. The character of the channel and the substrate were similar at both studied sites.

Abundance

Drying represents a strong disturbance that may cause significant and long-term changes in the abundance of benthic organisms in streams. Total abundance, taxonomic richness and diversity of both sites were compared using nonparametric Wilcoxon tests for paired samples (Tab. II).

I: *Minimum, maximum and mean values of the main abiotic parameters at each site, computed for the whole sampling period. The values were compared using the nonparametric Wilcoxon paired test.*

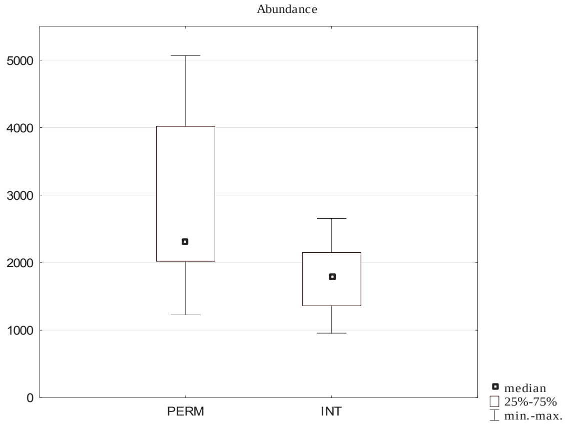
	Permanent brook			**Intermittent brook**			**Wilcoxon test**
	MIN	**MAX**	**Mean value**	**MIN**	**MAX**	**Mean value**	**P-values**
Water temperature (°C)	3,0	19,0	11,2	4,0	14,7	9,8	0,116
pH	6,3	7,9	7,3	5,7	8,5	7,6	0,484
Conductivity (μS.cm^{-1})	594	692	643	755	930	810	**0,012**
Dissolved oxygen (mg.l^{-1})	8,4	17,4	12,4	9,7	19,5	13,6	0,237
Depth (cm)	7,6	15,2	10,5	2,1	8,6	6,9	**0,018**

II: *Total abundance, taxonomic richness and diversity of both sites were compared using nonparametric Wilcoxon tests for paired samples*

	Z	**p-value**
Abundance	1.960	0.049
Number of taxa	2.521	0.012
Diversity	2.240	0.025

The numbers of individuals in samples taken from both studied streams were compared and this comparison shows that the abundance of macrozoobenthos was higher in the permanent Klaperuv brook than in the intermittent Granicky brook (Fig. 1), the difference was statistically significant (p = 0.049).

The highest abundances were recorded in the Klaperuv brook in summer 2002; at the same time abundances were very low in the Granicky brook, probably due to low water level. Regular drying can have lasting effects on benthic communities. Most studies show significantly lower abundances of macroinvertebrates in intermittent streams (Rosario & Resh, 2000; Shivoga, 2001; Smith & Wood, 2002; Smith *et al*., 2003; Řezníčková *et al*., 2007). Arab *et al*. (2004) even found extremely low abundances in intermittent streams in North Africa. On the other hand, some authors did not find significant differences between macrozoobenthos abundance in temporary and permanent streams (Legier & Tallinn, 1973; Miller & Golladay, 1996). This inconsistency may be caused by local conditions and the different character and extent of drying.

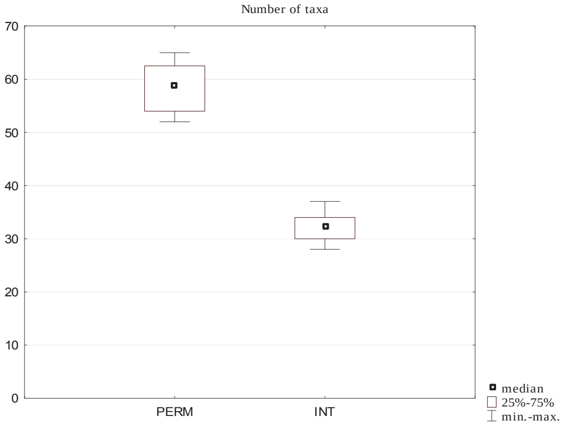


1: *Total abundance of macrozoobenthos in the Klaperuv brook (KB, permanent site) and the Granicky brook (GB, intermittent site)*

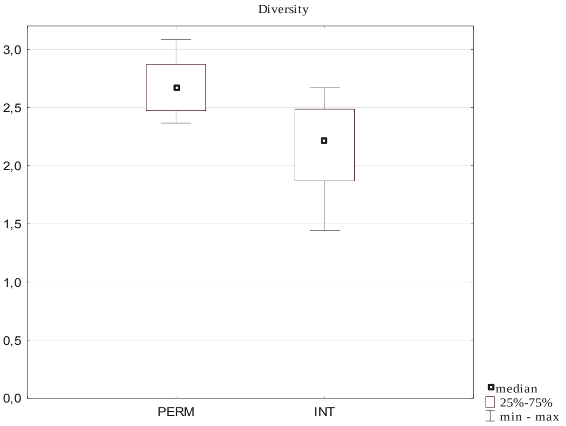
Usually there are greater oscillations in the total abundance during the year at intermittent sites if compared to permanent locations. Abundance often decreases with the decreasing water level before complete drying (Fritz & Dodds, 2004; Muñoz, 2003), and the highest abundances are usually recorded during greater discharge (Pastuchová, 2006; Řezníčková, 2007). In case of slow progressive drying, the stream gradually becomes discontinuous and isolated pools form. Benthic invertebrates can accumulate in these pools and paradoxically the abundance may significantly increase at such habitats. This phenomenon was observed at the study site in Granicky brook during the following research in 2005 and 2007 (undpublished data).

Taxa richness

Several authors compared the number of taxa in intermittent and permanent streams and their results vary. Most authors noted that the number of taxa is negatively affected by drying and, therefore, usually significantly lower in intermittent streams (Wright *et al*., 1984; Miller & Golladay, 1996; Rosario & Resh, 2000; Muñoz, 2003; Smith *et al*., 2003; Wood *et al*., 2005). On the other hand some authors observed lower numbers of taxa only immediately after drying (Boulton, 1989; Griswold *et al*., 2008). Muñoz (2003) recorded low numbers of taxa before drying, but high in the autumn after re-flooding. In contrast to these results, other authors recorded similar numbers of taxa in permanent and



2: *The number of taxa in the Klaperuv brook (PERM - permanent site) and the Granicky brook (INT - intermittent site)*



3: *Diversity in the Klaperuv brook (PERM - permanent site) and the Granicky brook (INT -, intermittent site)* temporary streams (Legier & Tallinn, 1973; Boulton & Suter, 1986; Miller & Golladay, 1996; Bonada *et al.*, 2007).

In our case study, the number of taxa was higher in the permanent stream (Fig. 2) and this difference was statistically significant (p = 0.012).

Diversity (Shannon-Wiever) was also significantly higher (p = 0.025) in the Klaperuv brook at all sampling dates (Fig. 3).

The total number of taxa was almost twice higher in Klaperuv brook (117 taxa) if compared to Granicky brook (only 67 taxa). The lowest number of taxa was recorded at the intermittent site in autumn (i.e. in the first samples collected after drying). Comparing to oscillations on intermittent site no trends or greater fluctuations in the number of taxa were recorded in the permanent Klaperuv brook.

Taxonomic composition

Disturbances usually negatively affect taxonomic composition of macroinvertebrate assemblages. Regardless of the abundance or the number of taxa, taxonomic composition was different at both studied sites. Appendix gives the list of recorded taxa. Only 30 taxa were found at both sites (e.g. *Gammarus fossarum*, *Micropsectra* sp., *Plectrocnemia conspersa, Baetis rhodani*). The freshwater shrimp *G. fossarum* was most abundant in both streams and eudominant in almost all samples. Lower numbers were recorded only in samples collected at the intermittent site during summer just before drying. A total of 55 taxa were found only in Klaperuv brook (e.g. *Brillia flavifrons*, *Baetis muticus*, *Hydropsyche* sp.); and 37 taxa were exclusively found in the intermittent Granicky stream (e.g. *Chaetopteryx* sp., *Isoperla tripartita, Amphinemoura sulcicolis*). In contrast to our findings, Beche *et al.* (2006) reported similar structure and composition of macrozoobenthos communities in intermittent and perennial streams. They found that most taxa were common for both stream types, nevertheless, more unique taxa occurred in intermittent streams.

Larvae of family Chironomidae (especially *Micropsectra* sp., *Tanytarsus* sp.) were abundant in the Granicky brook in summer before drying. This group is often abundant in intermittent streams (Muñoz, 2003; Arab *et al.*, 2004; Rocha *et al.*, 2012). Some of its representatives such as the genus *Polypedilum* are able to produce drought-resistant cocoons (Griswold *et al.*, 2008). *Baetis rhodani* (Ephemeroptera) is also regularly reported from intermittent streams (Bohle, 2000; Meyer & Meyer, 2000; Meyer *et al.*, 2003; Pastuchová, 2006). This species is unable to survive dry periods and has no drought-resistant stages. It occurs in intermittent streams because it is an ubiquist and a good colonizer with a flexible life cycle. *Habrophlebia fusca* is another mayfly species that commonly occurs in intermittent streams in Europe (Bohle, 2000; Meyer & Meyer, 2000; Meyer *et al.*, 2003; Acuña *et al.*, 2005; Buffagni *et al.*, 2007; Sarriquet *et al.*, 2007). This species survives dry periods in the form of drought-resistant eggs (Buffagni *et al.*, 2007). In some intermittent streams other mayfly species were recorded, e.g. *Electrogena* cf. *ujhelyii* (Meyer *et al.*, 2003; Pastuchová, 2006) or *Siphlonurus aestivalis*, which is considered to be an indicator of intermittent streams due to its flexible life cycle, rapid larval development and egg or larval diapause (Bohle, 2000).

Some Trichoptera species are also regularly found in intermittent streams, e.g. carnivorous larvae of *Plectrocnemia conspersa* (Sommerhäuser *et al.*, 1996; Meyer & Meyer, 2000; Meyer *et al.*, 2003; Pastuchová, 2006; Stubbington *et al.*, 2009) that are able to colonize temporary water habitats and are often regarded as indicators of intermittent streams (Sommerhäuser *et al.*, 1996; Bohle, 2000), or *Micropterna nycterobia* (Bohle 2000, Meyer & Meyer, 2000; Meyer *et al.*, 2003; Pastuchová, 2006,). *M. sequax*, which belongs to typical inhabitants of karst intermittent streams in Germany (Meyer & Meyer, 2000; Meyer *et al.*, 2003) and England (Smith & Wood, 2002; Wood *et al.*, 2005; Stubbington *et al.*, 2009).

Remarkably few representatives of Plecoptera were recorded at the intermittent site in the Granicky brook. In the Klaperuv brook, stoneflies were more abundant and a higher number of taxa was recorded (cf. Appendix). According to present knowledge, Ephemeroptera and Trichoptera are much more common in temporary waters compared with Plecoptera (Williams, 1996).

Functional structure

Not only the abundance and taxonomic composition of the macroinvertebrate communities but also their functional structure can be significantly impaired by drying. However, a few studies have dealt with the functional and structural response of aquatic communities to drought (Boulton & Lake, 1992; Wood & Petts, 1999; Churchel & Batzer, 2006; Bonada *et al.*, 2007; Griswold *et al.*, 2008). Therefore, we analyzed the functional structure of the communities from both studied streams and focused on the representation of species living temporarily or permanently in the aquatic environment, current preferences and feeding groups (Tab. III).

Permanent and temporary fauna

Species living permanently in water (permanent fauna) should logically be more affected by drying if compared to species that depend on water for only a certain part of their life cycle (temporary fauna, typically insects) and have the opportunity to complete their development in the aquatic environment before the stream dries up. Generally, these species are also able to colonize reflooded habitats faster.

The proportion of permanent a temporary fauna fluctuated at both studied localities during the sampling season; greater fluctuations were recorded in the intermittent Granicky brook. Permanent fauna, represented mainly by *Gammarus fossarum*, mostly prevailed in the Granicky brook, but its

III: *Comparison of permanent versus temporary fauna, current preferences and feeding types - both sites were compared using nonparametric Wilcoxon tests for paired samples*

		Z	p- value
Permanent versus temporary fauna	permanent	0,56	0,575
	temporal	**1,96**	**0,05**
Current preferences	limnophil	0,14	0,889
	limnorheophil	1,68	0,093
	rheolimnophil	1,26	0,208
	rheophil	1,4	0,161
	rheobiont	**2,366**	**0,018**
Feeding type	grazers/scrapers	**2,38**	**0,017**
	shredders	0,28	0,779
	gatherers/collectors	0,14	0,889
	filter feeders	1,82	0,069
	predators	0	1

proportion decreased markedly in summer before drying. Pastuchová (2006) recorded significant decrease in the number of temporary fauna representatives in Slovak streams during summer when drying typically occurs. However, this decrease may be the result of natural life cycles of insects and may not necessarily be related to drying. Temporary fauna prevailed in the permanent Klaperuv brook. During summer its dominance was even more marked as, similarly to the Granicky brook, the share of permanent fauna (*G. fossarum*) was very low. When comparing the two studied streams, no significant difference in the number of the representatives of permanent fauna was recorded (p = 0.575), but lower share of temporary fauna was recorded in the intermittent Granicky brook and this difference was statistically significant (p = 0.050). Other authors, on the contrary, reported the superiority of the representatives of temporary fauna at intermittent locations (e.g. Smith & Wood, 2002).

Current preferences

At both studied localities rheobionts and rheophils prevailed, which corresponds to the nature of both streams and their hydrological regime. There were no significant differences in the shares of limnorheobionts, rheolimnobionts and rheophils between the two sites. Significant difference were recorded for rheobionts (p = 0.018), which were missing at the intermittent site, probably due to drying. With the decreasing water, level riffle habitats gradually disappear and isolated pools are formed. This process is connected with the loss of living space for rheobionts and rheophils. Under such conditions limnobionts dominate as the remaining aquatic habitats represent lenitic environment in which survivors and new colonizers gather. Stream continuity is interrupted and the migration of organisms (both downstream drift and upstream active movement) is no longer possible (Lake, 2003). Fluctuating hydrological regime is not suitable for rheobiont species that require faster current and high oxygen content. Generally, taxa with pool-like strategies dominate in intermittent streams (Bonada *et al.*, 2007).

Feeding groups

The trophic network in benthic assemblages changes during drying up as the competition for natural food sources increases. The supply of organic matter from the upper parts of drying streams is interrupted; the loss of riffle habitats with coarse and stable substrate leads to the disappearance of filtrators and scrapers. Isolated pools are more suitable for gatherers or (if there is enough of coarse organic matter) for shredders.

When comparing the relative representation of feeding groups at both studied sites, only the share of grazers/scrapers was significantly different (p = 0.017). Shredders represented mainly by *G. fossarum* prevailed in most samples collected at the intermittent site and collectors/gatherers were also abundant there. In summer the share of shredders dramatically decreased for the benefit of collectors/gatherers. The same conclusion was reached by scientists during the research of intermittent streams in Italy (Fenoglio *et al.*, 2007). The share of predators increased just before complete drying, which is a common phenomenon linked to the abundance of prey (Muñoz, 2003). Isolated pools and dry stream bottom are quickly colonized by a large number of predators, both aquatic (Heteroptera, Coleoptera, etc.) and terrestrial (spiders, ants, beetles, amphibians and birds). In the Klaperuv brook the shares of feeding groups were more or less stable throughout the year; gatherers/collectors, grazes/scrapes and shredders prevailed.

Appendix

			PERM	INT
Tricladida	*Dugesia gonocephala*	(Dugès, 1830)	+	-
Mollusca	*Galba truncatula*	(Müller, 1774)	+	+
	Pisidium casertanum	(Poli, 1791)	+	+
	Pisidium personatum	Malm, 1855	+	-
	Pisidium subtruncatum	Malm, 1855	+	-
	Bythinella austriaca	(Frauenfeld, 1857)	-	+
Oligochaeta	*Aulodrilus pluriseta*	(Piguet, 1906)	+	-
	Criodrilus lacuum	Hoffmeister, 1845	+	-
	Eiseniella tetraedra	(Savigny, 1826)	-	+
	Fridericia sp.	Folli, 1658	+	-
	Limnodrilus hoffmeisteri	Claparede, 1862	+	-
	Limnodrilus sp.		-	+
	Lumbriculus variegatus	(Müller, 1774)	+	-
	Nais communis	Piguet, 1906	+	-
	Nais elinguis	Müller, 1773	+	+
	Pristina aequiseta	Bourne, 1891	+	-
	Pristina longiseta	Ehrenberg, 1828	+	-
	Pristina rosea	(Piguet, 1906)	+	-
	Psammoryctides barbatum	(Grube, 1861)	-	+
	Stylodrilus parvus	(Hrabe & Cernosvitov, 1927)	-	+
	Stylodrilus brachystylus	Hrabe, 1928	+	-
	Stylodrilus heringianus	Claparede, 1862	+	+
	Tubificidae g.sp.juv.		+	+
	Rhyacodrilus sp.		-	+
Hirudinea	*Erpobdella octoculata*	(Linnaeus, 1758)	+	-
	Glossiphonia complanata	(Linnaeus, 1758)	+	-
	Haemopis sanguisuga	(Linnaeus, 1758)	+	-
Crustacea	*Gammarus fossarum*	Koch, in Panzer, 1835	+	+
	Gammarus roeselii	(Gervais, 1835)	+	+
Ephemeroptera	*Baetis muticus*	(Linnaeus, 1758)	+	-
	Baetis rhodani	Pictet, 1843–1845	+	+
	Brachyptera risi	(Morton, 1896)	-	+
	Electrogena ujhelyii	(Sowa, 1981)	+	+
	Ephemera danica	Muller, 1764	+	-
	Habroleptoides confusa	Sartori & Jacob, 1986	+	-
	Habrophlebia fusca	(Curtis, 1834)	+	+
	Habrophlebia lauta	Eaton, 1884	+	-
	Paraleptophlebia submarginata	(Stephens, 1835)	+	-
	Paraleptophlebia werneri	Ulmer, 1919	+	-
	Rhithrogena carpatoalpina	(Klonowska *et al.*, 1985)	+	-
	Rhithrogena semicolorata	(Curtis, 1834)	+	-
	Siphlonurus aestivalis	(Eaton, 1903)	-	+
Plecoptera	*Amphinemura sulcicolis*	(Stephens, 1836)	-	+
	Isoperla goertzi	Illies, 1952	+	-
	Isoperla grammatica	(Poda, 1761)	+	-
	Isoperla rivulorum	(Pictet, 1841)	+	-
	Isoperla tripartita	Illies, 1954	-	+
	Leuctra albida	Kempny, 1899	+	-

			PERM	INT
Plecoptera	*Leuctra digitata*	Kempny, 1899	+	-
	Nemoura cambrica	Stephens, 1836	+	-
	Nemoura cinerea	(Retzius, 1783)	+	+
	Nemoura flexuosa	Aubert, 1949	+	-
	Nemurella pictetti	Klapálek 1900	-	+
	Protonemura auberti	Illies, 1954	-	+
Trichoptera	*Protonemura intricata*	(Ris, 1902)	+	-
	Halesus digitatus	(Schrank, 1781)	+	-
	Halesus tesselatus	(Rambur, 1842)	+	-
	Hydropsyche instabilis	(Curtis, 1834)	+	-
	Hydropsyche saxonica	McLachlan, 1884	+	+
	Chaetopteryx fusca/villosa		+	-
	Chaetopteryx maclachlani	Stein, 1874	-	+
	Chaetopteryx major	McLachlan, 1876	+	-
	Ironoquia dubia	(Stephens, 1837)	+	-
	Limnephilus lunatus	Curtis, 1834	-	+
	Lype reducta	(Hagen, 1868)	+	-
	Micropterna nycterobia	(McLachlan, 1875	-	+
	Micropterna sequax	(McLachlan, 1875)	-	+
	Odontocerum albicorne	(Scopoli, 1763)	+	-
	Plectrocnemia conspersa	(Curtis, 1834)	+	+
	Potamophylax cingulatus		+	+
	Potamophylax rotundipennis	(Brauer, 1857)	+	-
	Rhyacophila fasciata	Hagen, 1859	+	-
	Sericostoma sp.		+	+
	Synagapetus moselyi	Ulmer, 1938	-	+
	Stenophylax vibex	(Curtis, 1834)	+	-
	Tinodes rostocki	McLachlan, 1878	+	-
Megaloptera	*Sialis fuliginosa*	Pictet, 1836	+	+
Diptera	*Apsectrotanypus* sp.		-	+
	Apsectrotanypus trifascipennis	(Zetterstedt, 1838)	+	-
	Brillia modesta	(Meigen, 1830)	-	+
	Brillia flavifrons	Johannsen, 1905	+	-
	Ceratopogoninae g. sp.		+	+
	Cladotanytarsus sp.	Kieffer, 1921	+	-
	Corynoneura cf. *celeripes*	Winnertz, 1852	+	-
	Corynoneura lobata	Edwards, 1924	+	-
	Cricotopus tremulus-Gr.		+	-
	Diamesa sp.		+	+
	Dicranota sp.	Zetterstedt, 1838	+	+
	Dixa sp.		+	+
	Empididae g. sp.		+	-
	Epoicocladius flavens	(Malloch, 1915)	+	-
	Eukiefferiella claripennis	(Lundbeck, 1898)	+	-
	Eukiefferiella minor/fittkaui		+	-
	Eukiefferiella sp.		-	+
	Heleniella ornaticollis	(Edwards, 1929)	+	-
	Heleniella sp.		-	+
	Chaetocladius piger-Gr.	(Goetghebuer, 1913)	+	-

			PERM	INT
Diptera	*Chelifera* sp.		+	+
	Limnophila sp.	Macquart, 1834	+	-
	Limnophyes cf. *gurgicola*	(Edwards, 1929)	+	-
	Micropsectra sp.		+	+
	Microtendipes pedellus-Gr.		+	-
	Molophilus sp.	Curtis, 1833	+	-
	Nanocladius rectinervis	(Kieffer, 1911)	+	-
	Natarsia sp.		+	-
	Neolimnomyia batava	(Edwards, 1938)	+	-
	Nilotanypus sp.		+	-
	Odontomyia sp.		-	+
	Orthocladius obumbratus	Johannsen, 1905	+	-
	Orthocladius rubicundus	(Meigen, 1818)	+	-
	Orthocladius sp.	Wulp, 1874	-	+
	Orthocladius thienemanni	Kieffer, in Kieffer, & Thienemann 1906	+	-
	Paracladopelma camptolabis-Gr.		+	-
	Parametriocnemus stylatus	(Kieffer, 1924)	+	-
	Paraphaenocladius sp.		-	+
	Paratrichocladius nivalis	Goetghebuer, 1938	+	-
	Paratrichocladius rufiventris	(Meigen, 1830)	+	-
	Paratrissocladius excerptus	(Walker, 1856)	+	-
	Paratrissocladius sp.		-	+
	Pedicia sp.	Latreille, 1809	+	+
	Pneumia stammeri	Jung, 1956)	+	-
	Polypedilum convictum	(Walker, 1856)	+	-
	Polypedilum laetum-Gr.	(Meigen, 1804)	+	+
	Polypedilum nubeculosum-Gr.		+	-
	Polypedilum pedestre-Agg.		+	-
	Prodiamesa olivacea	(Meigen, 1818)	+	+
	Prosimulium tomosvaryi	(Enderlein, 1921)	+	+
	Psychodidae g. sp.		+	+
	Ptychoptera sp.		+	+
	Rheocricotopus fuscipes	(Kieffer, 1909)	+	-
	Rheocricotopus sp.		-	+
	Rheotanytarsus sp.		+	-
	Scleroprocta sp.	Edwards, 1938	+	-
	Simulium ornatum	Meigen, 1818	+	-
	Simulium vernum	Macquart, 1826	+	-
	Stempellinella sp.		-	+
	Stempellinella brevis-Gr.		+	-
	Stictochironomus sp.		+	-
	Tanytarsus sp.		+	+
	Thienemannia sp.	Kieffer, 1909	-	+
	Thienemanniella cf. *vittata*	(Edwards, 1924)	+	-
	Thienemannimyia Gr., Gen. indet.		+	-
	Tipula maxima	Poda, 1761	-	+
	Tvetenia calvescens	(Edwards, 1929)	+	-
	Tvetenia discoloripes/verralii		+	-
	Zavrelimyia sp.		+	-

			PERM	INT
Coleoptera	*Elmis* sp.		+	+
	Elodes sp. Lv.		+	+
	Hydraena sp. Ad.		+	-
	Limnius sp.		+	-
	Orectochilus villosus Lv.	(Müller, 1776)	+	-
	Platambus maculatus Lv.	(Linnaeus, 1758)	+	-

SUMMARY

The present paper focuses on the impacts of drought on stream macroinvertebrate assemblages. Drying up of watercourses is a strong disturbance that significantly affects both abiotic and biotic conditions in streams. Two brooks in southern Moravia were investigated – the permanent Klaperuv brook, and the intermittent Granicky brook that regularly dries up in summer periods. The study sites were situated only a few kilometres far from each other and except for drying of one of them were very similar. The main objective of the study was to compare the taxonomical and functional structure of the macrozoobenthos communities of both streams. Marked differences between the two assemblages were clearly demonstrated. The total abundance, the number of taxa and diversity were significantly lower in the intermittent Granicky brook. The taxonomic composition of both communities was also different. In total, 151 taxa were recorded, but only 30 taxa were found in both studied streams. 37 taxa were found only in the Granicky brook and 55 taxa were found only in the Klaperuv brook. The two studied communities also differed in their functional structure. The proportion of permanent a temporary fauna fluctuated at both studied localities during the sampling season. When comparing the two streams, no significant difference in the number of the representatives of permanent fauna was recorded, but significantly lower share of temporary fauna was recorded in the intermittent Granicky brook. Rheobionts that require faster current and high oxygen content were completely missing at the intermittent site. Rheophilic and rheolimnephilic species prevailed at both sites, which corresponded with the nature of both streams and their hydrological regime. The trophic network in macroinvertebrate assemblage was also affected by drying. Lower proportions of filtrators and scrapers were recorded in the intermittent stream, but statistically significant difference was observed only in case of grazers/scrapers.

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Original Articles

Recognition of stream drying based on benthic macroinvertebrates: A new tool in Central Europe

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ABSTRACT

Many streams in the extensive Central European region have an intermittent flow regime. Conventional hydrological methods used to identify zero-flow conditions, and in particular drying events, have limited use when assessing large areas dominated by low-order streams. We developed a novel multimetric index to recognise antecedent stream drying based on the analysis of benthic macroinvertebrate communities. The data used to develop the index were collected in pristine streams with different flow permanence regimes between 2012 and 2014, using standard sampling methods for ecological status assessment. The data include 64 perennial, 19 near-perennial and 27 intermittent benthic macroinvertebrate samples. Metrics considered for the index included variables based on (i) the occurrence of indicator taxa, (ii) the proportion of biological and ecological traits, and (iii) structural community metrics. Linear discriminant analysis identified the metric combinations that best discriminated among the three flow permanence categories. Different metrics were used in the final multimetric index calculation for the autumn and spring season that followed stream drying. In both seasons, the index included the proportion of indicator taxa and the proportion of taxa with high body flexibility. In addition, the autumn index included the proportion of taxa with a preference for organic substrates, whereas in spring the index included total abundance. Independent data from regulatory monitoring activity were used to validate the accuracy of the index. The correct classification of independent samples was 92% and 96% for samples from perennial and non-perennial sites, respectively. The index can be calculated using data collected by routine monitoring programmes used to assess ecological status and provides information about stream intermittence where conventional hydrological monitoring is limited. As intermittent streams increase in extent in global regions including Central Europe, the tool may be of particular interest to those who use invertebrates to monitor or manage these ecosystems.

1. Introduction

Intermittent rivers and ephemeral streams (i.e. streams with periodic flow cessation and/or drying; IRES) are mostly associated with arid and semi-arid regions. However, they are also common and widespread in temperate and continental regions with cooler, wetter climates (Datry et al., 2014a; Stubbington et al., 2017). IRES are typically managed using methods developed for perennial waterways, or as if they were part of the terrestrial ecosystems (Acuña et al., 2014; Stubbington et al., 2018), and the need for more effective policies and management strategies has been highlighted around the world (Datry et al., 2017; Marshall et al., 2018). In the European Union, a major objective for water management is the assessment of ecological status in water bodies, to meet the legislative requirements of the EU Water Framework Directive (European Commission, 2000). The characterisation of ‘biological quality elements’ such as benthic macroinvertebrates is one important approach used to evaluate ecological status.

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Aquatic macroinvertebrates are routinely used as indicator organisms for determining ecological status and diagnosing specific environmental pressures (e.g. Rosenberg and Resh, 1993; Birk et al., 2012). However, even short-term (i.e. days to weeks) stream drying can substantially alter benthic macroinvertebrate community composition (Datry et al., 2014b; Hille et al., 2014; Lancaster and Ledger, 2015). The interpretation of ecological status based on metrics developed in perennial streams can thus be misleading (Munné and Prat, 2009; Menció and Mas-Pla, 2010; Wilding et al., 2018). Information about flow permanence is crucial to accurately interpret data used to evaluate status and thus inform effective IRES management. In addition, knowledge about stream intermittence is needed, to anticipate the effects of climate change, to assess human pressures such as water abstraction and to manage protected species and habitats (Wilby et al., 2010).

Using flow gauging stations to monitor the flow permanence of small streams is expensive and technically impractical, because small streams (Strahler orders 1–4) comprise a substantial part of the stream network length (e.g. 92% in Czech Republic; Zahrádková et al., 2015). Moreover, even where hydrological data are collected, they may not distinguish between lentic and dry zero-flow conditions, and alternative methods are therefore needed to recognize IRES and characterize their water regimes (Gallart et al., 2016; Beaufort et al., 2018), for example using stream biota. Loss of surface water acts as an ecological filter (Poff 1997) and benthic macroinvertebrates have taxon-specific quantitative responses to drying (Datry et al., 2014b; Leigh and Datry, 2017). Dry phases can therefore be detected through both structural (i.e. taxonomic) and functional (i.e. trait-based) changes in community composition (Bogan et al., 2013; Schriever et al., 2015; Leigh et al., 2016; Chadd et al., 2017). Presence/absence of indicator taxa of benthic invertebrates has also been used to recognize stream flow duration (NC Division of Water Quality, 2010; Nadeau, 2015), and both taxonomic structure and species trait information have been used to assess flow connectivity in Mediterranean regions (Cid et al., 2016). Such tools may enable the data routinely collected during biomonitoring programmes to provide information about flow intermittence, even if hydrological data are absent. However, no method to detect drying events has been developed for the extensive continental-climate region of Central Europe.

Different metrics can be sensitive to different aspects of stream drying, and a combination of structural, functional and indicator-taxa metrics may therefore provide robust and sensitive insight into the responses of an assemblage to drying (Li et al., 2010). Here, our aim was to develop and test a new multimetric index to identify antecedent stream drying events based on the structural and functional composition of benthic macroinvertebrate communities. We evaluated three groups of metrics as indicators of stream drying: (i) the occurrence of indicator taxa; (ii) the proportion of biological and ecological traits; and (iii) structural community metrics. We identified the metrics and metric combinations that most effectively indicated antecedent stream drying.

2. Materials and methods

2.1. Study area

The study was conducted in 23 small (order 2–4) streams in the Czech Republic (Fig. 1). All were classified as reference streams, i.e. exposed to minimal anthropogenic impacts. Catchment land use was dominated by woodland, with smaller proportions of agricultural land, and with at least 10-m riparian buffer strips of deciduous bushes and trees. The study area has a warm-summer humid continental climate (Köppen-Geiger classification *Dfb*; Peel et al., 2007) and spans the Hercynian (17 streams) and West-Carpathian (6 streams) biogeographical subprovinces (Culek, 2013), which approximately correspond with ecoregions 9 (Central Highlands) and 10 (Carpathians; Illies, 1967). To facilitate comparison of macroinvertebrate communities from different sites, streams were paired, with each pair comprising one

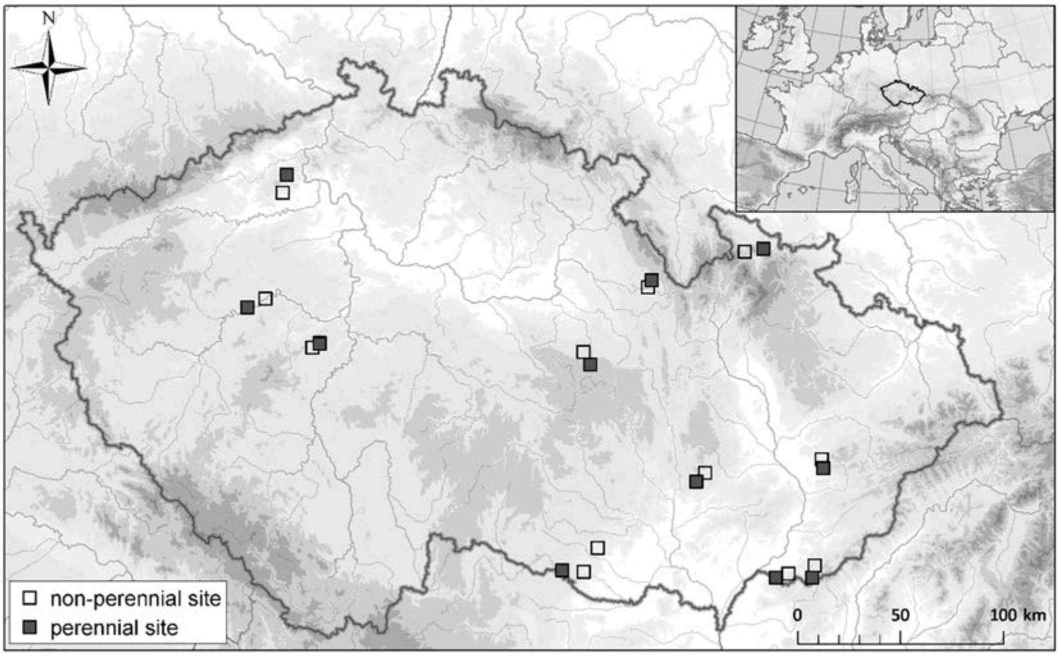

Fig. 1. Location of the study sites in the Czech Republic. Open squares represent non-perennial (i.e. near-perennial and intermittent) sites and filled squares represent perennial sites.

non-perennial and one perennial stream of comparable altitude, size, geology and hydromorphology. In one case, a single perennial stream was paired with two non-perennial streams. A sampling site representative of typical conditions was selected in each stream. The altitude of the sampling sites ranged from 250 m to 560 m a.s.l. and the maximum distance between paired sites was 13 km.

2.2. Sampling strategy

Benthic macroinvertebrate assemblages were sampled in spring (March–April) in 2013 and 2014 and in autumn (September–October) in 2012, 2013 and 2014. Proportional multi-habitat three-minute kick samples were collected with a hand net (25 × 25 cm aperture, 0.5-mm mesh size) according to the standard method used for ecological status assessments in the Czech Republic (Kokeš et al., 2006; Kokeš and Němejcová, 2006). Samples were preserved in 4% formaldehyde and processed in the laboratory. All macroinvertebrates were identified to the lowest possible taxonomic level: 292 out of 421 to species level, 117 to genus level, and the remaining 12 to a higher taxonomic level.

Sites were classified as non-perennial or perennial a priori, based on expert knowledge. In addition, observed instream conditions were recorded from summer 2012 to autumn 2014, including determination of the dry period duration using a water-level logger (Solinst Levelogger Edge) and photo-trap (Acorn 5310MG) installed at each site. Each water-level logger was placed in the lowest part of the streambed, to account for the persistence of surface water in isolated pools. Phototraps were installed in trees in the adjacent riparian zone (at 3 m height) and were facing a water-level gauge board. Logger and phototrap data were collected every 15 min and every 4 h, respectively.

We divided individual samples into three intermittence categories: *perennial*, *intermittent* and an intermediate *near-perennial* category. The allocation of samples into the three categories was based primarily on the dry period duration: i) perennial sites were defined as those which experienced more than one year of continuous flow prior to sample collection; ii) near-perennial sites lost surface water for hundreds of meters for < 7 d in the summer prior to sampling, with disconnected pools occasionally present; and iii) intermittent sites dried for > 1 km for 7–86 d before sampling. The cut-off between near-perennial and intermittent sites was arbitrary, reflecting the gradual nature of stream drying. In total, we collected 27 perennial, 7 near-perennial and 12 intermittent samples in spring and 34 perennial, 12 near-perennial and 15 intermittent samples in autumn. Spring samples from intermittent and near-perennial streams were taken 175–224 d after flow resumption and autumn samples were taken 2–63 d after flow resumption.

2.3. Identification of perennial flow indicators and flow intermittence tolerant taxa

To identify taxa associated with either perennial or intermittent streams, we performed Indicator Species Analysis (IndVal; Dufrêne and Legendre, 1997) using samples from spring 2013 and autumn 2012 and 2013. IndVal was performed using the multipatt function in the R package indicspecies (De Cáceres and Legendre, 2009), which identifies taxa with a strong association with each group based on their abundance and frequency. The calculated indicator value (IV) of a given taxon for its preferred group ranges from zero (no affinity) to one (strongest affinity). Best-matching patterns were tested for statistical significance of the associations. Taxa significantly associated with either perennial or intermittent and near-perennial samples were considered as reliable indicators and were assigned a reliability value of 2 (see “Proportion of indicator taxa” section). Taxa with non-significant IV but found exclusively in either perennial or intermittent and near-perennial streams in at least 10% of samples were considered as indicators with lower reliability and were assigned a reliability value of 1.

To support the IndVal analyses, we conducted a literature review of primary research papers comparing macroinvertebrate assemblages at perennial and non-perennial sites during flowing phases. From these studies, a taxon was identified as indicative of perennial flow if it was absent from intermittent stream assemblages. A taxon was considered as intermittence tolerant if it was found in intermittent stream assemblages. Literature sources describing the autecology of macroinvertebrate taxa were also reviewed to explore taxon-specific responses to intermittence. If a taxon’s relationship with stream intermittence was described once in data-based published sources then the species was assigned a reliability value of 1, and if it was published two or more times, a value of 2 was assigned. The review focused on taxa living in the Czech Republic area; consulted literature is listed in Appendix A. If information in different published sources were contradictory or unclear, then a taxonomic expert decided the reliability of the taxa based on the available evidence.

2.4. Metrics used for index development

Three groups of potential metrics that indicate flow intermittence were calculated, to inform subsequent development of the multimetric index:

i) Proportion of indicator taxa

The proportion of indicator taxa was calculated as the sum of the reliability values of perennial flow indicators present in a sample divided by the sum of the reliability values of all (perennial + intermittence-tolerant) indicators present. The proportion of indicators was rescaled to zero and the resultant values are within the range −1 to +1.

Proportion of indicator taxa

$$= \left(\frac{\sum values\ of\ perennial\ flow\ indicators}{\sum values\ of\ all\ indicators} \times 2\right) - 1$$

ii) Proportion of macroinvertebrate traits in community

The macroinvertebrate community was characterized using traits extracted from available databases (IS ARROW, 2014; Schmidt-Kloiber and Hering, 2015), supplemented by information from the literature review and from experts in different taxonomic groups. Information about fine-sediment-sensitive invertebrates was taken from Extence et al. (2013). To describe the ability to penetrate substrata, taxonomic experts assigned each taxon to one of three body flexibility modalities: (i) none, body flexibility is limited by shell or exoskeleton, e.g. molluscs, adult beetles; (ii) low, body is flexible but cannot turn around, e.g. mayflies, stoneflies; (iii) high, body can turn 360 degrees, e.g. Oligochaetes, Chironomidae (Omesová et al., 2008). Preferences for organic substrate were calculated as preferences for pelal (organic mud) and particulate organic matter. For each trait coded in a single-category assignment system or presence/absence assignment system (Schmidt-Kloiber and Hering, 2015), the community-level trait value was calculated as the number of individuals within the trait modality divided by the total macroinvertebrate abundance of a sample. The community-level trait value of traits coded in ten-point assignment system (Schmidt-Kloiber and Hering, 2015) was calculated as the sum of the trait values multiplied by the abundance of each taxon and divided by the total abundance (Schmera et al., 2014).

iii) Community structure metrics

The number of taxa, ln(x + 1)-transformed total abundance, and abundances and proportion in the community of the most abundant higher taxonomic units was calculated for each sample. This was done also for three drying-sensitive taxonomic groups (Bonada et al., 2007; Datry et al., 2014b): the family Heptageniidae, ET taxa (Ephemeroptera and Trichoptera) and EPT taxa (Ephemeroptera, Plecoptera and Trichoptera).

2.5. Index development

We performed exploratory data analysis to inform later identification of metrics that discriminate among intermittence classes. We used dot charts to assess metric distribution, and pair plots to assess their collinearity. We used nonmetric multidimensional scaling (NMDS) of a Bray-Curtis distance matrix calculated using ln(x + 1)-transformed abundance data to visualize variability in community composition in relation to year, season, subprovince and intermittence. We used the adonis function from the vegan package (Oksanen et al., 2017) to run permutational multivariate ANOVA (PERMANOVA; Anderson, 2001) to identify differences in community composition between seasons (*spring*, *autumn*), subprovinces (*Hercynian*, *West-Carpathian*) and intermittence categories (*intermittent*, *near-perennial*, *perennial*). Because only autumn samples were available for 2012, the difference between years was not tested. One-way ANOVA was used to examine differences in total abundance and taxa richness between perennial, near-perennial, and intermittent samples.

To reduce the number of potential metrics selected for inclusion in the final multimetric index (hereafter, the *Biodrought index*) a pre-selection process was conducted. To identify candidate metrics, values of each potential metric were plotted against intermittence categories. Those that effectively discriminated between intermittent and perennial samples were retained as candidate metrics (Table 1), with Mann-Whitney U tests used to identify differences in metric values between perennial and intermittent samples.

To identify the metric combination that best discriminated among perennial, near-perennial and intermittent sites, we identified linear combinations of candidate metrics using a sequence of linear discriminant analyses (LDA). Because macroinvertebrate community composition differs among seasons (e.g. Straka et al., 2012) we expected different metrics to reflect stream intermittence in autumn and spring. Therefore, all possible combinations of 2–4 metrics were tested for their discriminatory power using the leave-one-out cross-validation procedure (lda function, MASS package; Venables and Ripley, 2002) in each season. The combination of metrics with the best discrimination power was selected and its equation noted. The final index is this equation centred to zero and can be used to calculate a ‘discrimination score’ for an independent sample. To relate the discrimination score to the intermittence categories, we calculated discrimination scores for all samples and fitted probability density functions of a normal distribution to each category, from which the probability of belonging to each category could be derived. Normality of distribution of LDA discriminant scores for each sample category was tested by Shapiro-Wilk tests. The effects of season and sample category on LDA discriminant score were tested using ANOVA. All statistical analyses and graphs were performed using R open-source software version 3.4.4 (R Core Team, 2018).

2.6. Validation by independent samples

The ability of the index to identify antecedent stream drying was tested on an external dataset, provided by the former Czech Agricultural Water Management Authority, and collected using the same method as the original dataset (Kokeš and Němejcová, 2006). The dataset comprised 117 (59 autumn + 58 spring) samples from 59 perennial sites and 26 (16 autumn + 10 spring) samples from 16 non-perennial sites. We could not distinguish between intermittent and near-perennial sites, and all samples for at which stream drying was observed were therefore classified as non-perennial. Samples were taken from 2nd to 4th order streams distributed across the Czech Republic (Fig. 2), at an altitude of 210 to 460 m a.s.l. The streams were not polluted or morphologically modified and monthly observations of hydrological instream conditions in the summer prior to sampling were available. The value of the Biodrought index was calculated for each sample and the probability of its classification as intermittent, near-perennial or perennial was calculated using the probability density function of the normal distribution. The sample was assigned to the category with the highest probability.

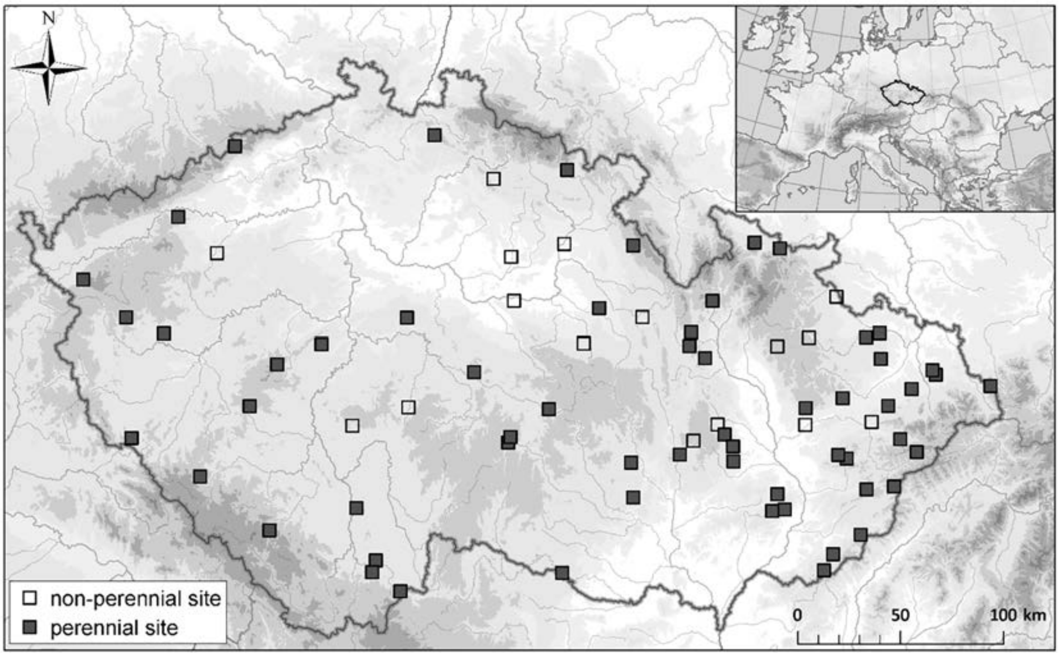

Fig. 2. Location of the sites used for validation within the Czech Republic. Open squares represent non-perennial sites and filled squares represent perennial sites.

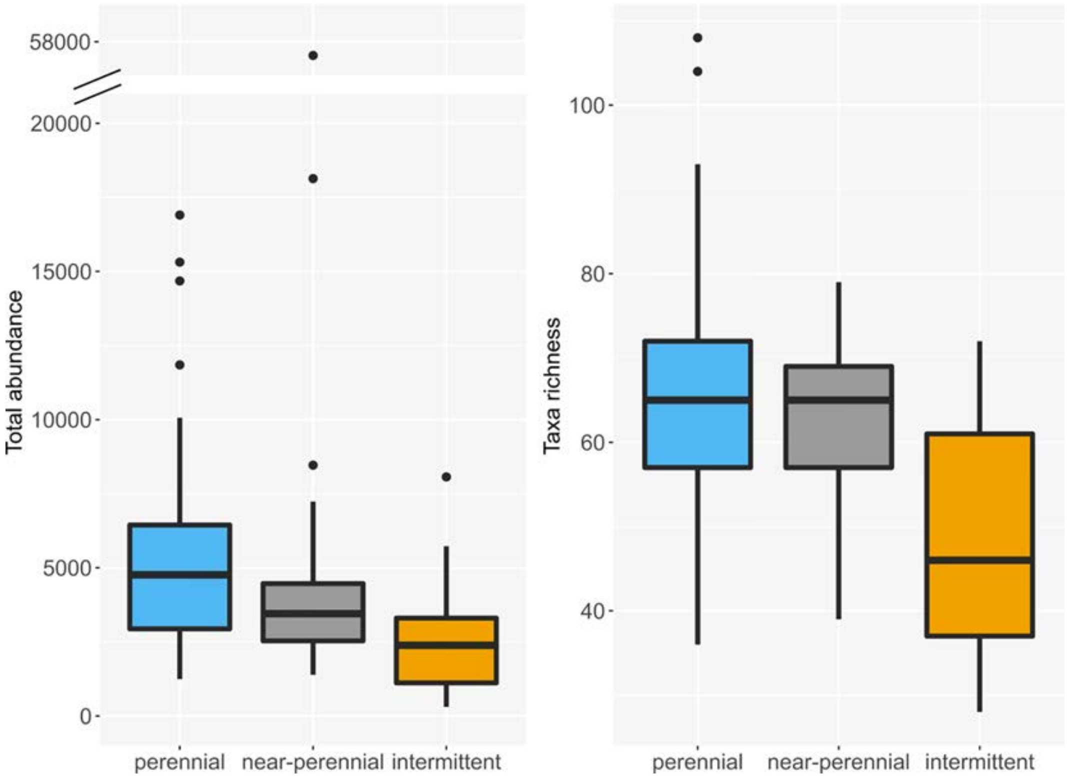

Fig. 3. Total invertebrate abundance (number of individuals) and taxa richness (number of taxa) in samples classified into three flow intermittence categories: i) perennial; ii) near-perennial; iii) intermittent. The box area indicates the first and third quartiles, the central line indicates the median, whiskers represent 95% confidence intervals and circles indicates outliers.

3. Results

3.1. Community structure across years, seasons, and regions

In total, 532 552 benthic macroinvertebrate individuals from 421 taxa were collected (Appendix B). Individual sample abundance values ranged from 307 to 57 581 (mean ± SE, 4997 ± 591) individuals per sample and taxa richness values from 28 to 108 (60.7 ± 1.5) taxa per sample. Macroinvertebrate abundance decreased with increasing flow intermittence (Fig. 3), with significant differences among intermittence categories (one-way ANOVA, $F_{2,104} = 3.71$, $p = 0.028$). Taxa richness was also lowest in intermittent samples ($F_{2,104} = 15.11$, $p < 0.001$). The five most abundant taxa were *Gammarus fossarum* (30% of all individuals), *Micropsectra atrofasciata*-Gr. (9.5%), *Nemoura* sp. (3.5%), *Baetis rhodani* s.l. (3.1%) and *Habroleptoides confusa* (2.9%). Twenty-six taxa were identified as significant indicators of intermittent and near-perennial samples and 33 were identified as significant indicators of perennial samples (Appendix A). The five taxa with the highest IV were *Eiseniella tetraedra*, *Brachyptera risi*, *Parametriocnemus stylatus*, *Paraphaenocladius* sp. and *Marionina* sp. for non-perennial sites and *Dugesia gonocephala*, *Baetis muticus*, *Baetis rhodani* s.l., *Leuctra* sp. and *Hydropsyche* sp. for perennial sites.

Non-metric multidimensional scaling analyses produced a two-dimensional solution (stress = 0.1998; Fig. 4). Community composition differed among perennial, near-perennial and intermittent samples (PERMANOVA; $F_{2,104} = 7.4$, $p < 0.001$). Despite considerable overlap, we detected differences in community composition both between seasons (PERMANOVA; $F_{1,105} = 6.0$, $p < 0.001$) and subprovinces ($F_{1,105} = 5.6$, $p < 0.001$).

3.2. Discrimination of samples from each intermittence category

Because PERMANOVA identified differences in community composition among perennial, near-perennial and intermittent samples, we investigated the metrics that most effectively discriminated between these intermittence categories. Because many potential metrics differed between intermittent and perennial samples, we plotted all calculated potential metrics and selected a subset of candidate metrics based on their ability to discriminate between categories (Table 1).

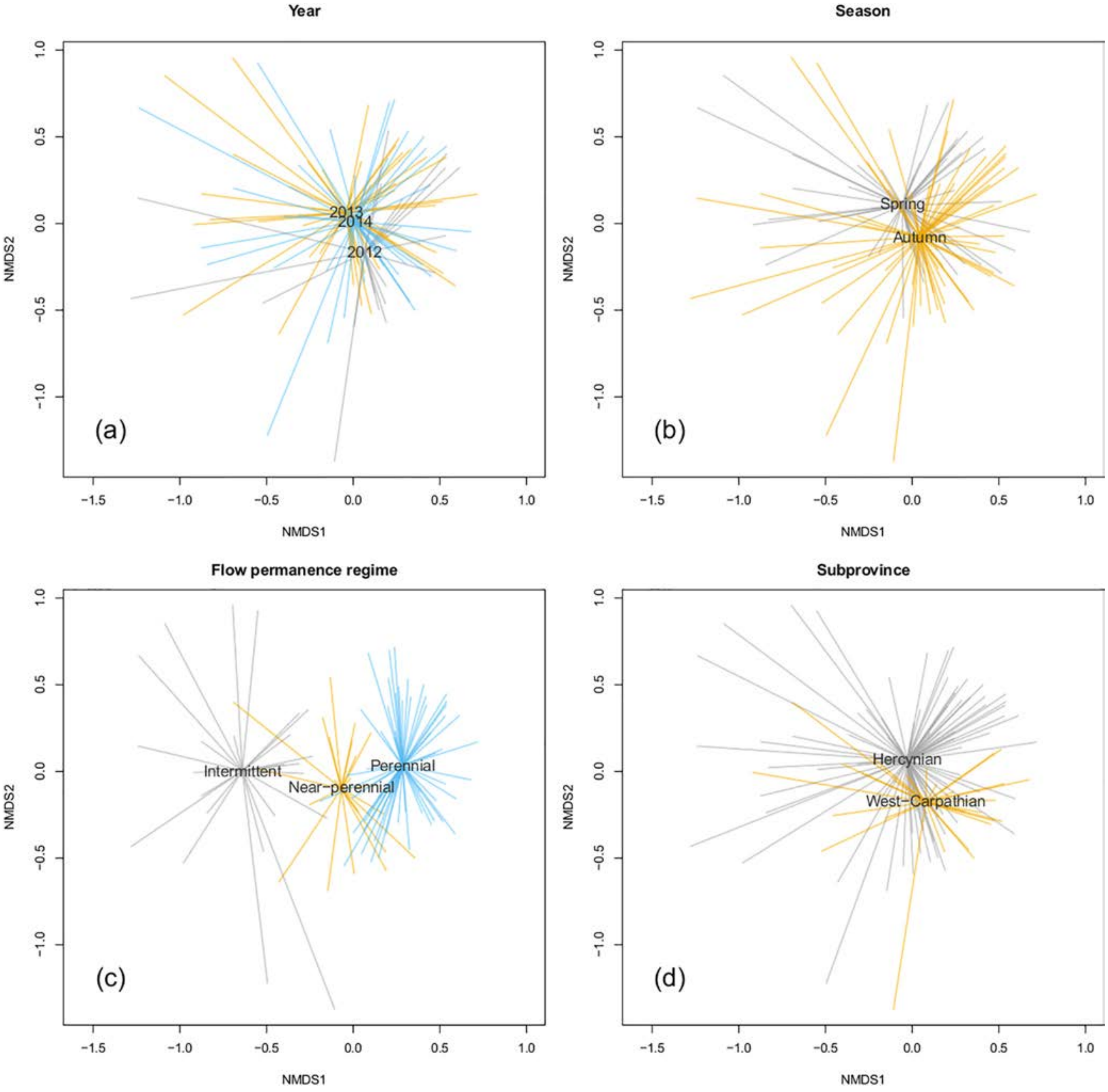

Fig. 4. Non-metric multidimensional scaling ordinations of all samples, showing differences in community composition between: (a) years (2012, 2013, 2014); (b) seasons (autumn, spring); (c) intermittence categories (intermittent, near-perennial, perennial); and (d) biogeographical subprovinces (Hercynian, West-Carpathian). Each point (sample) is connected to the group centroid.

Table 1
Candidate metrics tested as discriminators of macroinvertebrate communities from sites in different intermittence categories. Differences between perennial/intermittent samples were tested with Mann-Whitney U-tests. *** < 0.001, ** < 0.01, * < 0.05.

Metrics selected for index development	Autumn	Spring	Observed state at intermittent sites
% of lithal preference	***	***	lower
% of organic substrate preference	**	**	higher
% of rheophilic preference	***	n.s.	lower
% taxa with respiration with gills	***	***	lower
% taxa with high body flexibility	***	**	higher
% of rhithral preference	***	*	lower
% of epirhithral preference	***	*	lower
Weighted mean of sediment-sensitive invertebrates	***	n.s.	higher
Number of taxa	**	**	lower
Number of ET taxa	***	***	lower
Number of EPT taxa	***	***	lower
Total abundance	***	*	lower
Abundance of Heptageniidae	***	***	lower
Abundance of EPT	***	n.s.	lower
Proportion of Heptageniidae	***	***	lower
Proportion of Oligochaeta	***	n.s.	higher
Proportion of indicator taxa	***	***	lower

From the set of candidate metrics (Table 1), successive LDA revealed important metrics for sample separation. Three metrics were selected for discrimination in autumn: the proportion of indicator taxa (IT), the proportion of individuals with high body flexibility (HBF), and the proportion of taxa with a preference for organic substrates (POS). The formula for calculating the autumn index is:

$\text{Biodrought index} = (4.23*IT) + (-1.69*HBF) + (-2.94*POS) - 0.26$

Three metrics were also selected as discriminators of spring samples: the proportion of indicator taxa, the proportion of individuals with high body flexibility, and the total abundance (TA). The formula for calculating the spring season index is:

$\text{Biodrought index} = (4.09*IT) + (-1.76*HBF) + (0.47*TA) - 4.01$

The Biodrought index was calculated for each sample (Fig. 5b) and the probability function for perennial, near-perennial and intermittent samples was approximated based on the distribution of index values within the intermittence categories. LDA discriminant scores (i.e. the Biodrought index) for each category were normally distributed (Shapiro-Wilk tests, $p > 0.05$). Mean discriminant scores varied between samples in the three intermittence categories ($F_{2,\ 101} = 215.9$; $p < 0.001$) but not between spring and autumn samples ($F_{1,\ 101} = 0.009$; $p = 0.926$), and there was no interaction between intermittence category and season ($F_{2,\ 101} = 0.328$, $p = 0.721$). Because there was no difference in the distribution of sample discriminant scores between spring and autumn, it was possible to calculate probability functions independently of season (Fig. 5a). Values for the probability function were derived from the dataset for intermittent (mean = −3.36, SD = 1.398), near-perennial (mean = -0.231, SD = 0.926) and perennial (mean = 1.559, SD = 0.777) sites.

3.3. Validation by independent samples

To evaluate the performance of the method, we calculated the Biodrought index values for a dataset not used for index development (Fig. 5c). Of 117 perennial samples, 108 (92%) had a probability > 50% of being classified correctly by the index, with eight and one samples classified as near-perennial and intermittent, respectively. The index classified 1 of 26 samples from non-perennial streams incorrectly, with 15 and 10 correctly classified as near-perennial and intermittent, respectively. Because it was not possible to distinguish between intermittent and near-perennial categories in this independent dataset, the correct classification of non-perennial samples was 96%.

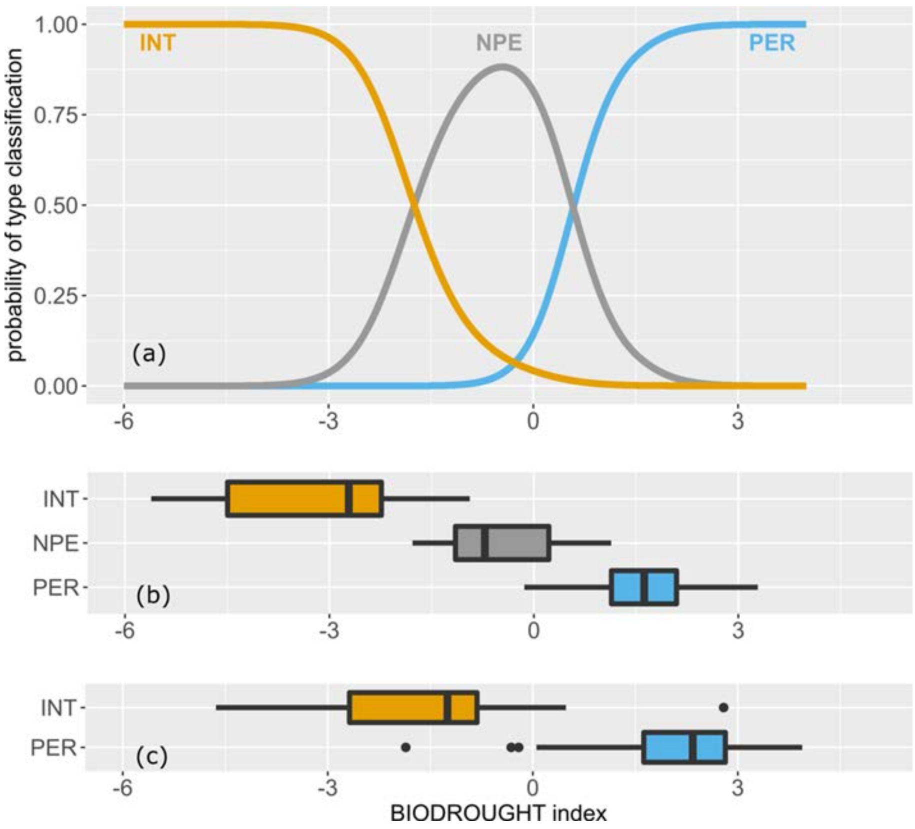

Fig. 5. Biodrought index values for each flow intermittence category: (a) Probability of classification to each category according to the index; index values for each category, for (b) samples used for index development and (c) independent samples used for index validation. INT – intermittent, NPE – near-perennial, PER – perennial.

4. Discussion

Our results agree with previous research demonstrating that benthic macroinvertebrate community composition reflects stream flow intermittence (Arscott et al., 2010; Datry, 2012; Soria et al., 2017) and that the effects of relatively short (7–86 day) drying events can be detected even after seven months after flow resumption (Ledger and Hildrew, 2001; Chester and Robson, 2011). Previous studies from other regions have distinguished perennial and non-perennial streams using the proportional representation of different macroinvertebrate taxonomic groups (Mazzacano and Black, 2009; Nadeau, 2015; Cid et al., 2016; Cañedo-Argüelles et al., 2016) and using traits (Serra et al., 2017; Kelso and Entrekin, 2018). Our results confirm that macroinvertebrate communities in Central Europe can also be explored to identify stream intermittence, and specifically drying events. Our Biodrought index provides a new tool to identify antecedent drying events, which may facilitate interpretation of ecological data including the results of ecological status assessments, especially when hydrological data are missing. The method was developed and validated using Czech Republic data, but it has considerable potential for wider uptake due to the extensive distribution of the continental climate zone across Central Europe

4.1. Metrics used for index calculations

Biotic responses to environmental stress (e.g. water loss) can be species-specific (Lake, 2003) and we examined available information about responses to flow continuity mainly at the species level, and summarized the most sensitive/tolerant indicator taxa (Appendix A). The proportion of these indicator taxa was one of the most effective metrics to distinguish among intermittence categories. Flow intermittence tolerant taxa usually possess behavioural, morphological, physiological and/or life-history adaptations to survive dry phase (Lytle and Poff, 2004). For example, one species strongly associated with intermittent streams in this study was *Brachyptera risi*. This stonefly has the eggs with summer diapause, allowing the species to remain in the sediments in a viable egg stage during dry phases (Khoo, 1964). However, the selective loss of taxa sensitive to flow intermittence (and specifically drying) rather than selection for desiccation-resistant specialists is the primary driver of differences in community composition between perennial and non-perennial sites (Datry, 2012; Vidal-Abarca et al., 2013; Cid et al., 2016).

Although high body flexibility is not typically reported as a crucial trait in relation to stream drying (e.g. Bonada et al., 2007; Díaz et al., 2008; Walters, 2011) our results indicated its relevance. Organisms with flexible body shapes can respond to drying by moving into the subsurface interstices, which may maintain high humidity and can thus act as refuge for aquatic invertebrates during dry phases (Stubbington, 2012; Strachan et al., 2015). Moreover, a highly flexible body enables organisms including oligochaetes and leeches to form globular, desiccation-tolerant cysts (Montalto and Marchese, 2005; Shikov, 2011). Intermittent streams also supported a higher proportion of invertebrates with a preference for organic substrates, which may reflect their dry-phase survival in moisture-retaining patches of organic matter (Stubbington et al., 2009). Invertebrates that use such organic-rich habitats during flowing conditions can therefore persist in this substrate, which acts as a refuge that limits desiccation during dry phases (Boulton, 1989).

The Biodrought index calculated for the spring season also included total abundance. The relationship between total abundance and flow permanence in streams has previously been indicated by several studies (Rüegg and Robinson, 2004; Fenoglio et al., 2007; Datry, 2012). The lower abundance in our study was caused in particular by high mortality of *Gammarus* species, known to be sensitive to stream drying (Meyer and Meyer, 2000; Smith et al., 2003; Pařil et al., 2019).

4.2. Utility of the developed tool

Our index addresses the recognized need (Datry et al., 2011; Cid et al., 2016) for methods to identify antecedent drying events using biological metrics, and thus without using gauging stations or other prohibitively costly infrastructure. Even where gauging station data are available, biotic approaches such as our index complement the continuous, long-term hydrological information by distinguishing dry from lentic zero-flow states (Gallart et al., 2012). Sampling and processing of benthic invertebrate samples is methodologically well-managed and is routinely practised within the scope of monitoring programmes (Smith et al., 1999; Birk et al., 2012; Hill et al., 2017). The method used for taking samples (Kokeš et al., 2006) is comparable to other commonly used European methods such as AQEM/STAR method (Lorenz and Clarke, 2006), and the Biodrought index therefore has high potential for testing and use in other countries within the extensive continental climate zone that spans Central and Eastern Europe. The study dataset spans two biogeographical subprovinces (Hercynian and West-Carpathian; Culek, 2013) and the discriminatory power of the index was sufficient to correctly classify evaluated samples from the independent dataset from whole Czech Republic with high probability. Although working at a finer geographical resolution (e.g. within one subprovince) may achieve higher levels of correct assignment, we also recommend testing of our index in other Central European countries to explore the geographical limits of its reliability. A saprobic system based on common taxa is used in many Central and Eastern European countries (Rolauffs et al., 2004) and the Biodrought index may therefore be useable in Central European region without extensive taxonomic adjustment.

Our index was developed in pristine streams where only a single, natural stressor (drying) was known to influence macroinvertebrate community composition. Elsewhere, factors that may affect index performance include interactions between the hydrological stressor of drying (both natural and anthropogenic) with other human pressures, interactions which can have antagonistic or additive effects on biological communities (Folt et al., 1999; Matthaei et al., 2010; Ormerod et al., 2010). In particular, the typical sequence of hydrological conditions from low flows, to flow cessation and gradual drying can reduce oxygen concentrations, and intermittence and organic pollution can therefore have comparable effects by eliminating sensitive invertebrates (Pardo and García, 2016). Moreover, a reduction in oxygen availability and low flow can have synergistic effects on benthic invertebrates (Calapez et al., 2017). Disentangling the effects of stream drying and anthropogenic stressors (including organic pollution) is therefore challenging, but consideration of the Biodrought index alongside physico-chemical quality elements may improve the accuracy of ecological status assessments that might otherwise incorrectly classify IRES as polluted. Wider testing is needed to explore how the index performs in streams exposed to human stressors.

The spatial distribution of intermittent reaches in relation to perennial reaches and other refuges may have a considerable influence on the rate and extent of community recovery after drying events (Sedell et al., 1990; Bogan et al., 2017; but see Datry et al., 2014b). The spatial proximity of extensive refuges, in particular upstream perennial reaches, may enable rapid recolonization and community reestablishment within weeks to obscure the occurrence of an antecedent dry phase (Fowler, 2004; Fritz and Dodds, 2004; Pařil et al., 2019). In our dataset, the intermittent sites were at least 0.5 km downstream of a perennial reach and the samples were taken at most 7 months after flow resumed. However, recovery at highly isolated sites (e.g. > 10 km from perennial refuges) may take multiple years with repeated drying events often preventing aquatic communities from reaching a stable state (Bogan and Lytle, 2011; Bogan et al., 2017). Our index and comparable tools thus have higher potential for strong performance in IRES with relatively short dry phases, compared to those which may remain dry for years.

4.3. Conclusions and recommendations for index use

We developed the Biodrought index: a novel index to characterize the effect of stream drying on aquatic invertebrate communities. The index enable calculation of the probability of the antecedent stream drying, based on benthic invertebrates sampled using standard methods. We demonstrated the index as robust at national scale. The robustness of our index is evidenced by its registration by the Ministry of Environment of the Czech Republic, and we recommend its testing and adoption (with required adaptation) in other European countries within continental-climate region. In particular, the Biodrought index is intended for use by those with responsibility for monitoring and management of river ecosystems, including water management boards, environmental agencies and private water companies. We recommend adoption of the Biodrought index by such managers, to improve their interpretation of the macroinvertebrate assemblage data collected during ecological status assessments. The index can help avoid misinterpreting a deviation of community composition from reference status caused by a preceding dry phase (Reyjol et al., 2014; Cid et al., 2016; Stubbington et al., 2018). Environmental agencies could employ this tool for characterization of dry phase effects on macroinvertebrate communities in protected areas or for evaluation of the effectiveness of projects intended to restore naturally perennial flow at sites impacted by water resource pressures such as over-abstraction. The index can also be useful when identifying the impacts of water resource use in naturally perennial streams. However, since the method was developed in naturally intermittent streams, its use in non-natural IRES must be carefully assessed and the performance of Biodrought index in such conditions remains to be tested.

Our approach was essentially qualitative, identifying if a drying event had or had not occurred in the the one-year period preceding sample collection. Further research is needed to explore variability within 'intermittent', 'near-perennial' and other flow intermittence categories, in particular to identify species-specific responses to dry phases of differing durations. Identification of such thresholds is crucial to predict biotic responses to increasing intermittence in the context of ongoing climate change, which is interacting with increasing water resource pressures.

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Declaration of Competing Interest

None.

Appendices A and B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2019.105486.

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Short Note

Stream drying bioindication in Central Europe: A Biodrought Index accuracy assessment

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ABSTRACT

With ongoing climate change and increasing water resource pressures, the knowledge and predictability of stream drying is essential for water management. However, the hydrological data for assessing the flow regime of temporary streams are often non-existent or scarce. The flow regime strongly affects stream ecological functioning and ecosystem processes. A few biological indices have been developed in the past decade to assess community responses to flow intermittency. One of them – the Biodrought Index, used to recognise antecedent stream drying based on benthic invertebrate assemblage composition – was initially intended for deployment in the Czech Republic. Here we report on a test of how reliably this index can be used in Central Europe. We used five independent data sets of macroinvertebrate samples collected during the flowing phase in five countries: Austria, Czech Republic, Germany, Hungary and Slovakia. Altogether 89, 26 and 220 samples were assessed from intermittent, near-perennial and perennial sites, respectively. Each sample was assigned to one of three flow intermittence categories according to the Biodrought Index and then compared with the flow regime recognised by hydrological observations and gauging station data. In most cases, the Biodrought Index clearly reflected the previous flow state and identified streamflow cessation. The misidentification rate between intermittent and perennial samples was very low, ranging from 0% to 6%. Classification rate of correctly recognised samples ranged from 54% to 93% and misclassification rate between near-perennial and perennial/intermittent category was 6% to 46%. Overall, this study confirmed the robustness and the reliability of the Biodrought Index, which proved to be an effective tool in assessing the recent hydrological history of small and medium-sized streams. The index can improve the interpretation of the macroinvertebrate assemblage data collected for ecological status assessment, can help to evaluate the effectiveness of river restoration projects or identify water resource pressures. Hence, we consider the Biodrought Index a useful method for indicating antecedent stream drying in the extensive area of Central Europe and thus helpful in monitoring and managing river ecosystems.

1. Introduction

Contrary to general assumptions, intermittent rivers and ephemeral streams (IRES) are not restricted exclusively to arid regions but occur in all biomes and ecoregions. The proportion of IRES in global river networks is likely to be greater than 50% (Datry et al., 2014). In humid climates (oceanic and continental), this proportion has increased continuously in recent decades, parallel with ongoing change in the climate (Stahl et al., 2010; Laaha et al., 2017). The overall high significance of IRES for the global carbon cycle (Marcé et al., 2019), for unique and high biodiversity (Vander Vorste et al., 2019) and essential ecosystem services (Acuña et al., 2014; Datry et al., 2017) has been proved, while the recent onset of supraseasonal drought has raised awareness among public and expert communities (Van Lanen et al., 2016). Nevertheless, IRES are typically poorly represented in biomonitoring programmes in Central European countries (Stubbington

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et al., 2018). Our knowledge of the occurrence of flow intermittence is often limited because hydrological gauging station networks tend to cover only larger streams and rivers that are typically perennial in the temperate zone and have minimal risk of complete surface water loss. Thus, alternative methods to recognise zero-flow periods are greatly needed (Gallart et al., 2016; Beaufort et al., 2018; Fritz et al., 2020). To fill this gap, a few tools using benthic invertebrates have recently been developed (Cid et al., 2016; Chadd et al., 2017; England et al., 2019). Among them, the Biodrought Index (BI) (Straka et al., 2019) has been designed and tested for retrospective recognition of stream drying in the Czech Republic. Index calculation is based on analyses of benthic invertebrate samples collected within the stream's flowing phase (typically spring or autumn). It enables the calculation of the probability that the stream experienced flow cessation in the previous dry season. Its algorithm is season specific and is based on the proportion of indicator taxa, the proportion of taxa with high body flexibility, the proportion of taxa with a preference for organic substrates (autumn season) and total abundance (spring season). The original purpose of the BI was to evaluate benthic invertebrate samples that are routinely collected within the scope of monitoring programmes and to represent a new diagnostic tool that can improve evidence-driven stream management actions (Carvalho et al., 2019). In the Czech Republic, the index has shown a high accuracy of antecedent stream drying bioindication. However, the index's accuracy and reliability can be limited by factors like different sampling methods, taxonomic identification precision or varied species pools in study sites across climatic and geographical gradients. This work's overall aim is to test how well the BI performs outside of its range of development and possibly expand its geographical applicability. More specifically, we test the BI's reliability (i) in samples from five different countries with different ecoregions/biogeographical regions, (ii) sampled with three different sampling techniques, and (iii) effect of taxonomic resolution. We hypothesise that BI efficiency will decrease in samples taken outside Czech Republic with different sampling methods and its reliability will decline with rougher taxonomic resolution.

2. Material and methods

2.1. Data sets

To test the BI's reliability, we compiled several data sets from benthic invertebrate studies across Central Europe. Only data sets of benthic invertebrate assemblages sampled from near-pristine small streams (Strahler orders 1–4) with a known recent hydrological history (i.e. extent of stream intermittence within a year before sampling) were used. Also, species/genus taxonomical resolution was demanded. Data sets from five countries were gathered and analysed: Austria, the Czech Republic, Germany, Hungary and Slovakia (Fig. 1, Table 1). The hydrological status of analysed streams was reconstructed using gauging station data, derived from in-stream temperature and water-level data-loggers as well as personal observations. The dry period duration allowed us to divide sampling sites into three categories: perennial, near-perennial and intermittent. Perennial sites were those with more than one year of continuous flow prior to the sampling campaign. Intermittent sites experienced complete surface water loss for weeks or months (greater than 7 days), and near-perennial sites were stated as an intermediate category when assignation to other two categories was uncertain. Typically the surface flow was lost for < 7 days, and series of disconnected pools were present. The only exception was the Czech data

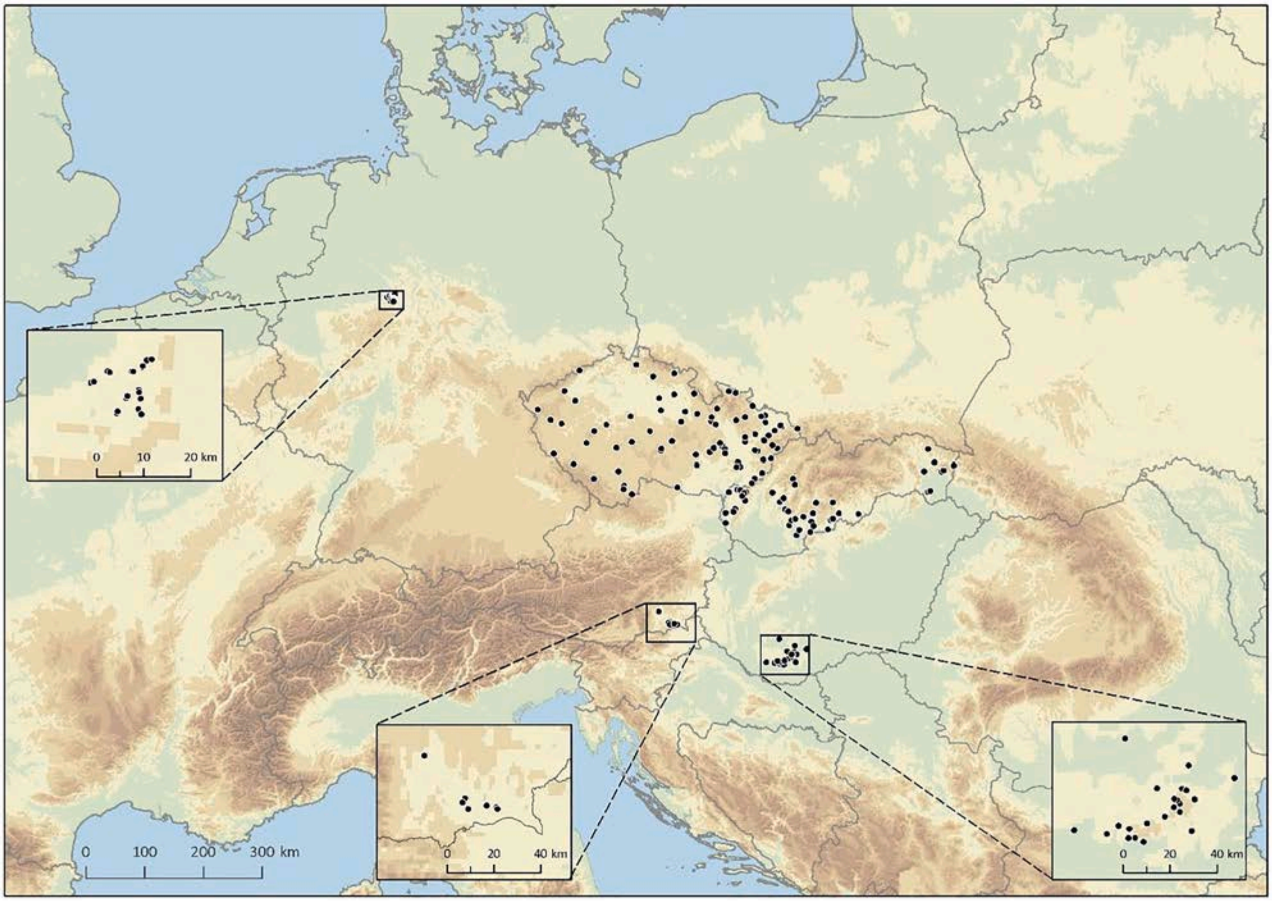

Fig. 1. Location of sites used for BI assessment. Twenty sites are located in East Westphalia (Germany), 76 sites are scattered across the Czech Republic, 8 sites are in Styria (south-eastern Austria), 22 sites are in the Mecsek mountains (southern Hungary) and 46 sites are located in Slovakia.

Table 1
Data sets used to explore the BI's effectiveness.

Country	Source	Number of samples			Hydrological monitoring	Sampling method	Overall taxonomic resolution [%]				Strahler's order	Altitude [m a.s.l.]	Ecoregion (sensu Illies, 1978)	Biogeographical region (EEA, 2016)
		Perennial	Near-perennial	Intermittent			Species	Genus	Family	Higher		Min-Max		
Austria (AT)	Zweidick (2020)	2	3	3	visual observation; temperature dataloggers	modified AQEM; only five subsamples processed	48	12	19	21	1–2	250–340	Dinaric western Balkan	Continental
Czech Republic (CZ)	State monitoring data	117	26		visual observation	PERLA; three-minute multihabitat sampling	86	12	3	0	2–4	210–460	Central highlands, Hungarian lowlands	Continental, Pannonian
Germany (DE)	Meyer & Meyer (2000); Meyer et al. (2003)	29	3	39	visual observation	Surber; three Surber samples pooled together	75	10	15	0	2–4	140–380	Central plains	Continental
Hungary (HU)	Unpublished dataset	41	16	7	visual observation; temperature dataloggers; gauging stations	AQEM	90	4	6	0	1–3	125–380	Hungarian lowlands	Pannonian
Slovakia (SK)	State monitoring data	31	4	14	visual observation; gauging stations	AQEM	83	15	2	0	2–4	100–420	The Carpathians, Hungarian lowlands	Alpine, Pannonian

set, where it was not possible to distinguish between intermittent and near-perennial sites since no precise hydrological data were available. Hence, both categories were assessed together as intermittent.

Because of their different origins, the taxalists were heterogeneous concerning the identification level, sampling season and sampling and sorting methods. Three sampling methods were used during the flowing phase to collect benthic invertebrate samples: PERLA (Kokeš and Němejcová, 2006), AQEM (AQEM Consortium, 2002) and the use of a Surber sampler. While PERLA samples are time-related (three-minute kick sampling), the AQEM and Surber samples are area-related (Table 1). The data sets covered five different ecoregions as introduced by Illies (1978) and adopted in the European Union (EU) Water Framework Directive (European Commission, 2000), namely Dinaric Western Balkan, Central Highlands, Hungarian Lowlands, Central Plains and The Carpathians and three biogeographical regions according to the European Environment Agency (EEA, 2016), namely Continental, Pannonian and Alpine.

2.2. BI calculation and data analyses

The BI was calculated according to Straka et al. (2019). Before the calculation, each data set was adjusted to harmonise the taxalists with the Biodrought source database. This process comprises unification of taxa names, excluding non-relevant taxa (e.g. terrestrial species) and in case the taxon was missing in the database its adjustment to the nearest taxonomical unit.

The BI is season-specific and was originally designed for samples taken in spring or autumn. Given that the samples were taken during all seasons, we arbitrarily decided to assess winter (December, January, February) samples using the spring algorithm and summer (June, July, August) samples using an autumn-specific algorithm. According to the BI, which represents a discrimination score along an intermittence gradient, each sample was classified into one of the three flow intermittence categories (i.e. perennial, near-perennial or intermittent). This calculated classification was compared with the observed flow regime. The threshold values of the BI for classification to perennial/near-perennial and near-perennial/intermittent categories were adopted from Straka et al. (2019) and are 0.675 and −1.645, respectively. Flow permanence expressed as % of days in a year with continuous flow was studied within three data sets (Austria, Germany, Hungary) (Supplementary material 1). The relationship between flow permanence and the BI was examined using linear regression performed on available data from these three countries.

To test whether the BI can distinguish among the three intermittence categories, differences in index values among observed hydrological states were analysed using analysis of variance (ANOVA) for each country separately. The individual differences between categories' pairs were tested using planned contrasts using the R package lsmeans (Russell, 2016). In case of an insufficient number of replicates within a group, the post-hoc test was not performed. To test whether the original boundaries between intermittence categories calculated by Straka et al. (2019) are valid, we counted the percentage of correctly classified samples, the rate of samples misclassified to the closest category (intermittent/near-perennial and near-perennial/perennial) and the percentage of completely misidentified samples to the opposite category (intermittent/perennial and vice versa). In the Czech data set, only the misclassification of perennial/non-perennial was calculated because near-perennial and intermittent samples were not distinguished.

The probability density function of a normal distribution enables calculating the probability of classifying the analysed sample to each of the three intermittence categories (Straka et al. 2019). To test whether the samples' taxonomical identification level influences classification success, we fitted a linear model between the probability of classification to the correctly observed flow category and the identification level. The identification level was expressed as the percentage of macroinvertebrates identified as species. Additionally, we artificially decreased the identification level from the original “best available” to “genus” and “family” levels, respectively. Afterwards, the BI values were calculated and compared among observed flow categories for all three identification levels separately.

3. Results

The BI was calculated for 335 samples from five evaluated data sets. Overall, 220 perennial, 26 near-perennial and 89 intermittent samples were assessed. The index values ranged from −7.4 (sample from a Czech intermittent stream) to 3.9 (sample from a Hungarian perennial stream). We found highly significant differences in BI values among the observed sample categories in each country (Fig. 2). Regarding pairwise differences, we found significant differences between BI values of opposite categories (intermittent and perennial) and also intermittent and near-perennial types. In the case of the Austrian dataset, the difference between perennial and other categories was not tested since the number of replicates in perennial samples was low (N = 2). Differences in the BI between near-perennial and perennial types were significant only in the dataset from Germany (Fig. 2.). However, the index values for the near-perennial category were transitional between the intermittent and the perennial categories in all countries and the BI reflects continuous changes in stream flow duration (Supplementary material 1).

Each sample was classified into one of three categories according to the index. This classification was compared with the known flow regime history, and the success rate of the correct classification was calculated. The correct classification of samples varied from 54% in Hungary to 93% in Germany and the Czech Republic (Fig. 3, Supplementary material 2). The rate of complete misidentification (i.e. intermittent sample misclassified as perennial and vice versa) was very low. Overall, only six of 335 samples were misclassified between intermittent and perennial samples.

The identification level varied considerably among the assessed samples, ranging from samples with 100% species level identification to samples in which more than 50% of invertebrates were identified to family or a higher taxonomic level. However, overall taxonomic resolution was mainly to species or genus level (Table 1) and the probability of classification to the correct category was not affected by the sample identification level (ANOVA $F_{1,328} = 1.54$, $p = 0.22$). Artificially decreased identification level was reflected by decreasing in BI efficiency (Supplementary material 3).

4. Discussion and conclusions

Our study demonstrated the generalizability of a new biomonitoring tool, the BI, to data sets collected in different European ecoregions with various sampling methods and diverse taxonomic resolutions. A wide range of approaches exists for biomonitoring using macroinvertebrates across Europe (Friberg et al., 2006). This lack of methodological uniformity emphasises the importance of comparing the effectiveness of bioassessment indices across different regions (Herbst and Silldorff, 2006). Furthermore, sample processing in a laboratory is known to be a possible reason for bias in environmental assessments (Haase et al., 2010; Ligeiro et al., 2020). Therefore, we gathered independent data sets (collected and analysed by different teams applying differing sampling and processing protocols) to evaluate the potential of the index for future use across a wide geographical range. The BI was originally developed to assess samples taken with the PERLA method; we added another two common sampling methods (AQEM and Surber sampler), processed within different laboratories and according to their own laboratory protocols. The results showed that the BI can effectively distinguish intermittent and perennial samples even though different sampling methods were used (Fig. 3). This finding is in concordance with Lorenz and Clarke (2006), who found time-related PERLA samples to be highly comparable with area-related AQEM samples.

The BI is a multimetric index based on the responses of specific taxa

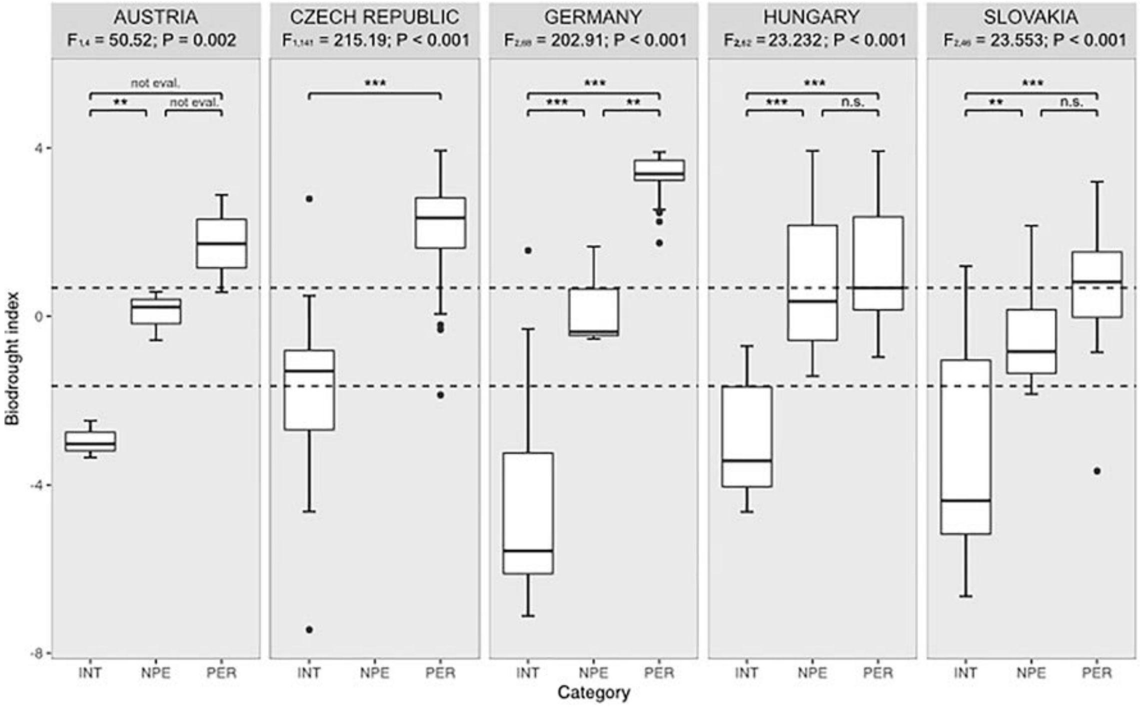

Fig. 2. Distribution of the values of the BI calculated for samples from five countries. Individual boxplots represent the classification of samples observed according to hydrological data (INT = intermittent, NPE = near-perennial, PER = perennial). The higher/lower the index values, the more perennial/intermittent the samples (according to the index). Dashed lines represent the thresholds between the intermittent/near-perennial and near-perennial/perennial categories according to Straka et al. (2019). The F statistic and the P values for ANOVA comparing all three intermittence categories are shown in each country plot’s header; asterisks represent the significance of the pairwise differences tested using planned contrasts (***= $p < 0.001$; **= $p < 0.01$; . = $p < 0.1$; n.s. = $p > 0.1$; not eval. = groups with insufficient number of replicates were not statistically tested).

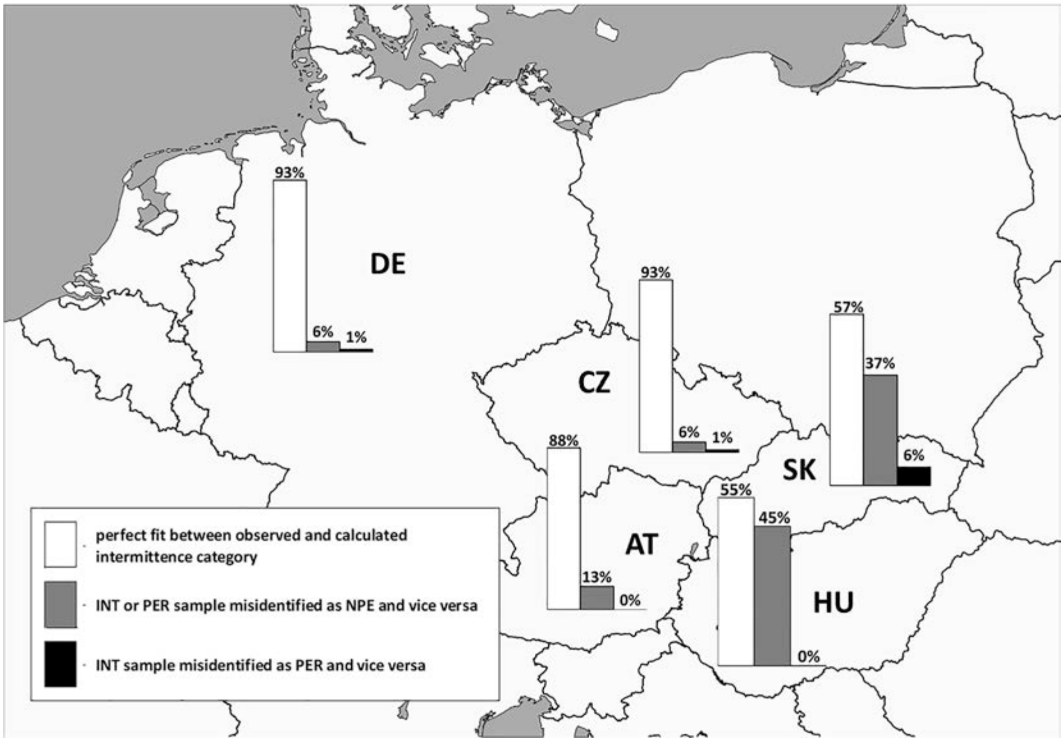

Fig. 3. Percentage of classification of samples according to the BI compared to the observed intermittence categories. White columns represent correctly classified samples. Grey columns represent misidentifications to the neighbouring category (i.e. perennial or intermittent classified as near-perennial and vice versa). Black columns represent complete misidentifications (i.e. perennial sample classified as intermittent and vice versa).

to stream drying (proportion of indicator taxa), assemblage parameters (total abundance) and species traits proportion (body flexibility, preference for organic substrates)(Straka et al., 2019). Metrics based on specific species traits are comparable across geographic regions, as evidenced by applying indices such as LIFE index (Lotic-invertebrate Index for Flow Evaluation) in hydroecological modelling across and beyond Europe (Dunbar et al., 2010; Ncube et al., 2018). The one of the crucial BI metrics is a ratio of flow intermittence sensitive and flow intermittence tolerant taxa. The recognition of these taxa was based on data from the Czech Republic. The similarity of species composition between ecological communities was found to decrease with increasing geographical distance (Astorga et al., 2012). Consequently the use of a taxa-based index can be limited outside the area where it was developed. Thus, it is challenging to determine whether indices developed for small areas can be extended beyond the areas, for which they were initially designed for (Stoddard et al., 2008; Martins et al., 2020). According to the results presented in this study, we can conclude that the geographical limits of the index were not reached. The samples from CZ, DE and AT were classified into intermittence categories with similar success rates, and the BI can be used outside area where it was constructed. However, the high proportion of samples misidentified within the near-perennial category in the Hungarian and Slovakian data sets suggests that wider use of the BI must be carefully assessed. As these data sets origin from the eastern part of the study area it could indicate that invertebrate assemblages during the dry phase form different mechanisms in oceanic-continental climate gradient. This was suggested by Pařil et al. (2019) who recorded high proportion of alive aquatic invertebrates without drought-resistance adaptations in dry stream sediments in continental climate in contrast to previously reported findings from oceanic climates. High similarity of Hungarian perennial and near-perennial samples may be caused by past dry events since severe drought can affect assemblages for years (Bogan and Lytle, 2011) and our study included hydrological status only one year back. These issues remain to be tested at a more extensive time scale and a larger geographic area than the one tested here.

Macroinvertebrate taxonomic resolution can influence the performance of hydrologically sensitive indices (Monk et al., 2012) and considerable bias in index performance has already been recognised for the Saprobic (Sandin and Hering, 2004) or LIFE index (Laini et al., 2018) when a higher taxonomic identification level (family level or above) is used. We did not observe the decreasing reliability of sample classification with reduced taxonomic resolution in the original data sets. However, this might have been due to the fact that even in the data set with the least detailed taxonomic resolution, 60% of individuals were identified to the species or genus level. When the identification level was artificially decreased, the BI reliability deteriorated. Therefore, we consider the fine taxonomic resolution to be a fundamental prerequisite of correct BI performance.

Overall, the results confirmed that biomonitoring tools can indicate flow intermittency (Sarremejane et al., 2019; White et al., 2019). The benthic community metrics reflected the gradient of drying severity (Sarremejane et al., 2020) and the near-perennial category showed intermediate values between intermittent and perennial sites (Fig. 2). Stream drying is a gradual process when available habitats are reduced and community composition and diversity change (Lake, 2003). After the flow resumes, the consecutive rate and the extent of community recovery is influenced by the proximity of perennial refuges (White et al., 2018; Doretto et al., 2019), the length and the severity of drought (Datry, 2012; Bogan et al., 2015), the time since flow resumption (Calapez et al., 2014; Doretto et al., 2019) the sediment character (Vadher et al., 2018; Loskotová et al., 2019) and the site's history (Crabot et al., 2020). These factors can greatly influence community recovery and possibly mask the effect of previous stream drying. The response of the BI can be obscured and its performance under such conditions (e.g. distance to refuges) remains to be tested.

In conclusion, we believe that the BI is an effective index especially to identify streams with continuous flow and streams that have previously experienced long-term (weeks to months) flow cessation. The samples categorised as an intermediate near-perennial category need to be assessed as "suspicious" and the flow regime assessment requires further information (e.g. hydrological data, temperature loggers, visual observation). BI thus represents a diagnostic tool with sufficient capability to identify if a stream drying had occurred in the one-year period preceding sample collection in all studied countries (Austria, Czech Republic, Germany, Hungary and Slovakia). However, the BI's reliability outside of tested conditions range (i.e. in polluted, acidified, morphologically modified streams or streams above 500 m a.sl.) and outside of tested geographical area (e.g. use in Mediterranean, Western Europe) is currently unknown.

CRediT authorship contribution statement

Michal Straka: Conceptualization, Funding acquisition, Visualization, Writing - original draft. **Marek Polášek:** Data curation, Formal analysis, Methodology, Visualization, Writing - review & editing. **Zoltán Csabai:** Data curation, Investigation, Writing - review & editing. **Oliver Zweidick:** Data curation, Investigation, Writing - review & editing. **Wolfram Graf:** Data curation, Investigation, Writing - review & editing. **Elisabeth I. Meyer:** Data curation, Investigation, Writing - review & editing. **Emília Mišíková Elexová:** Data curation, Investigation, Writing - review & editing. **Margita Lešťáková:** Data curation, Investigation, Writing - review & editing. **Petr Pařil:** Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2021.108045.

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ORIGINAL ARTICLE

Drying in newly intermittent rivers leads to higher variability of invertebrate communities

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Abstract

1. Aquatic invertebrate communities inhabiting intermittent rivers that are characterised by recurrent drying events (flow cessation or complete disappearance of surface water) often show rapid recovery upon flow resumption. Such rapid recovery is possible thanks to specific resistance and resilience traits that species adapted to river drying often exhibit. However, differences in community response to drying can be expected between historically drying (HD) networks—those IRs with a long history of flow intermittence—and recently drying (RD) networks, where drying is a novel, often human-induced, phenomenon. The invertebrate species found in RD networks may lack the adaptations that are known to facilitate quick community recovery upon rewetting and could thus be dramatically affected by drying. Unfortunately, the responses of aquatic communities in RD networks are still poorly explored, limiting our capacity to predict and mitigate future biodiversity changes.
2. Here, we compared the responses of aquatic invertebrate communities to drying in nine pairs of intermittent and perennial river reaches from HD networks across France and nine pairs from RD networks across the Czech Republic. Using both taxonomic and functional perspectives, differences in α- and β-diversity patterns between perennial and intermittent sites were evaluated separately for HD and RD groups, and before and after drying over several years.
3. Drying had stronger effects on taxonomic richness in RD compared to HD networks. In addition, drying greatly altered spatial and temporal β-diversity in RD networks, but it marginally affected β-diversity in HD networks. Communities of HD networks showed a higher proportion of resistant taxa than RD networks.
4. These results suggest that recent drying can have, at least on a short time scale (i.e. years), stronger effects on aquatic communities in RD networks compared to those in HD networks.
5. Because drying duration, frequency, and spatial extent are increasing with climate change, RD networks could soon reach tipping points, calling for long-term monitoring of biodiversity in these novel ecosystems.

KEYWORDS

anthropogenic disturbance, aquatic invertebrates, functional diversity, temporary rivers, β-diversity

1 | INTRODUCTION

Climate change scenarios predict increased frequency of droughts in Europe resulting from increased air temperature in northern Europe and decreased precipitation in southern Europe (EEA, 2017; Spinoni et al., 2018). Regions with a continental climate in central Europe may acquire Mediterranean characteristics with milder winters and warmer summers (Alessandri et al., 2014). These climatically induced changes will affect river flow regimes by decreasing run-off in central Europe (Arnell & Gosling, 2013; Giuntoli et al., 2015; Laaha et al., 2017), which could be amplified by higher surface and groundwater abstraction for human needs (Döll et al., 2018; Kundzewicz et al., 2008; Vörösmarty et al., 2010). Seasonal aspects of flow regimes will also be likely to change, with rivers experiencing a decrease in summer flows (EEA, 2017; Giuntoli et al., 2015; Johns et al., 2003), an increase of hydrological drought frequency and magnitude (Spinoni et al., 2018), and, in many cases, shifts from permanent to intermittent flow regimes due to increasing river drying (Larned et al., 2010; Döll & Schmied 2012; Datry et al., 2014).

Intermittent rivers (hereafter IRs) are those rivers experiencing recurrent periods of flow cessation or complete disappearance of surface water (Larned et al., 2010). Aquatic invertebrate communities inhabiting IRs that have historically experienced drying events show a high capacity to recover when flow resumes (e.g. Bogan, Chester, et al., 2017; Datry, Larned, Fritz, et al., 2014; Stanley et al., 1994). This is due to a combination of both resistance and resilience strategies, which evolved over time (Bogan, Chester, et al., 2017; Bonada et al., 2007). Resistance refers to the ability to withstand drying events by tolerating harsh environmental conditions in remaining wet habitats or by entering desiccation-resistant dormant stages (Bogan et al., 2017). Some resistant taxa may survive in remnant pools despite low dissolved oxygen concentrations and high water temperature (Boulton & Lake, 1992), and some may find refuge in the hyporheic zone (Vander et al., 2016). When no wet refuge is left, only desiccation-resistant taxa remain, including resistant eggs, larvae, or adults (Strachan et al., 2015; Stubbington et al., 2017). In IRs with suitable sediment structure and sufficient moisture, an invertebrate seedbank of active and dormant invertebrates surviving in sediments can thus be a crucial source of recolonisation (Pařil, Polášek, et al., 2019; Stanley et al., 1994; Stubbington & Datry, 2013; Tronstad et al., 2005). Other taxa cannot persist during drying, and their recovery after drying instead relies on resilience via spatial dispersal (Bogan, Chester, et al., 2017; Chester & Robson, 2011). Such resilience strategies require traits enabling efficient recolonisation through drift from upstream (Altermatt, 2013; Fagan, 2002; Robson et al., 2008) or overland aerial dispersion (Bilton et al., 2001; Robson et al., 2008).

In recent IRs where drying is a novel, often human-induced phenomenon, a significant part of invertebrate communities may not exhibit such adaptations and could be heavily altered by drying. This is typically the case in continental Europe, such as the Czech Republic, where many once-perennial streams have started to dry in the past decade, mainly because of the joint impact of climate-related change and human pressures (Pařil, Leigh, et al., 2019; Van Lanen et al., 2016; Zahrádková et al., 2015). In rivers exposed to such recent flow intermittence (FI), taxa without adaptation could undergo stochastic extinctions following drying events (Chase, 2007). However, the natural responses of aquatic communities to drying are still poorly understood in these systems (Pařil, Polášek, et al., 2019; Strachan et al., 2015), which hinders our capacity to assess how climate change will affect riverine biodiversity globally.

The effect of drying events on the taxonomic richness of aquatic invertebrate communities at the local scale, also called α-diversity, has been extensively studied (Stubbington et al., 2017). Alpha-diversity typically decreases with increasing FI (proportion of time without surface flow; Datry, Larned, & Tockner, 2014). Although commonly reported, α-diversity does not provide insights into the underlying mechanisms driving community structure, as it focuses on one spatial scale and relies on a static view of biodiversity (Aspin et al., 2018; Ruhí et al., 2017). In contrast, β-diversity highlights the relationship between local and regional patterns of biodiversity and their underlying processes by comparing taxonomic composition among sites and over time (Anderson, 2001; Aspin et al., 2018; Socolar et al., 2016). Additional insights into assembly mechanisms can be obtained by partitioning this measure into two additive components: turnover (taxa replacement between two localities or dates) and richness nestedness (indicating if sites or dates harbouring fewer taxa are subsets of richer ones; Baselga, 2010; Legendre, 2014).

To further understand the effects of FI on aquatic ecosystems, a functional approach using biological traits analysis can complement taxonomic measures by quantifying how ecosystem functions provided by communities respond to drying events (Boersma et al., 2014; Bonada et al., 2017). Biodiversity studies of aquatic ecosystems exposed to drying increasingly include functional analyses (Aspin et al., 2018; Belmar et al., 2019; Rodrigues-Filho et al., 2020; Vander et al., 2016) but most still fall short of assessing the temporal dynamics of functional diversity (Crabot et al., 2020; Leigh et al., 2019). However, this approach allows exploring, together with taxonomic temporal patterns, whether drying eliminates some biological traits over time or induces a turnover of traits (Leigh et al., 2019).

Here, we compared the responses of aquatic communities to drying between river networks recently prone to drying (RD) and those historically prone to drying (HD). We expected aquatic communities from RD networks to be less adapted to desiccation and, therefore, to be more affected by drying than those from HD networks. Specifically, we made the following assumptions concerning the responses of communities to different drying history (Figure 1). In RD networks, drying events should greatly affect community composition and functions. Assuming that taxa that have only recently been exposed to drying do not exhibit the particular set of functional traits required to cope with desiccation, we expected less functional redundancy in RD than in HD networks (Boersma et al., 2014; Vander et al., 2016). Hence, functional differences among sites and among dates would be stronger in RD than in HD networks. In contrast, drying events should have a lesser impact on community composition and functional diversity in HD networks due to adaptation to desiccation. Accordingly,

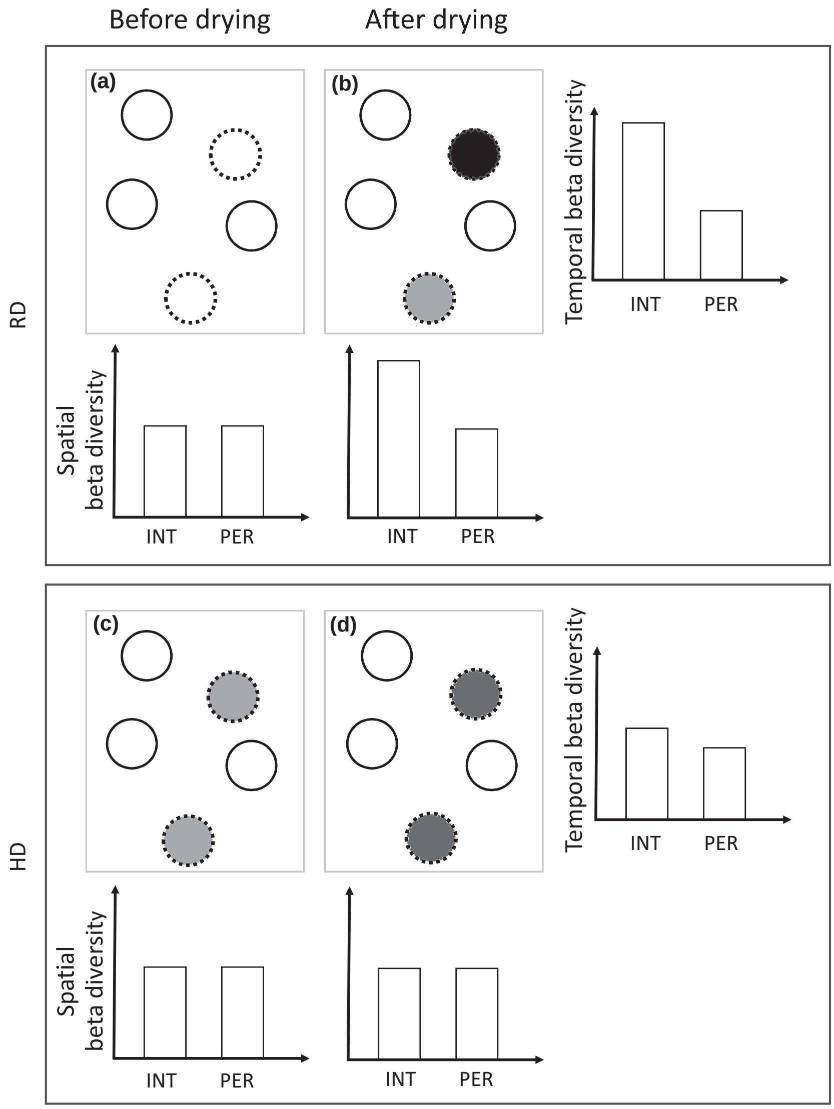


FIGURE 1 Conceptual figure of the expected response of aquatic invertebrate communities to drying in recently drying (RD) networks (a, b) and in historically drying (HD) networks (c, d), and the resulting patterns of taxonomic β-diversity. Dotted circles represent intermittent sites and solid circles represent perennial sites. The darker the circle is, the more the community composition is affected by the drying event and differs from nearby perennial sites. In RD rivers before drying (a), intermittent and perennial sites present similar community composition, and spatial dissimilarity is thus similar among intermittent and among perennial sites from both a taxonomic and functional perspective. In RD networks after drying (b), intermittent sites are greatly impacted in a stochastic way and present a higher spatial dissimilarity than among perennial sites. Temporal variability between (a) and (b) would be higher in intermittent than in perennial sites from a taxonomic and functional perspective, due to a greater difference of α-diversity between (a) and (b) and thus a higher temporal nestedness. In HD networks before drying (c), community composition already differs between intermittent and perennial sites because of the presence of intermittence-adapted taxa in intermittent sites (d), harsh conditions may similarly impact communities across intermittent sites, leading to a similar spatial taxonomic and functional dissimilarity in community composition between intermittent sites and perennial sites (compared to that before drying). Temporal variability between (c) and (d) is slightly higher for intermittent sites from both a taxonomic and functional perspective

we predicted: (1) higher taxonomic and functional differences before and after drying events in RD than in HD networks (Figure 1); (2) contrasted spatial and temporal taxonomic and functional responses to drying between RD and HD networks, with communities being less homogeneous in time and in space in RD than in HD networks (Figure 1); and (3) in RD networks, a lower proportion of taxa resistant to drying compared to the proportion of taxa resilient to drying, and the opposite pattern in HD networks.

2 | MATERIALS AND METHODS

2.1 | Study sites

For RD networks, nine intermittent and nine perennial river reaches (thereafter sites) were selected throughout the Czech Republic. This region is dominated by a continental climate characterised by a temperature of the hottest month above 10°C and cold winters with temperatures below 0°C (Dfb Köppen class; Peel et al., 2007). River basin areas ranged from 3 to 22 km^2 with the mainstem lengths ranging from 3 to 10 km.

For HD networks, nine intermittent and nine perennial sites were selected in south-eastern France. This region is dominated by a temperate climate characterised by a temperature of the hottest month above 10°C and a temperature of the coldest month between 0 and 18°C (Csb and Cfb Köppen class; Peel et al., 2007). River basin areas ranged from 80 to 626 km^2 with the mainstem lengths ranging from 33 to 822 km.

In RD and HD networks, sites were selected to include a diversity of geomorphologic configurations and intensities of drying events, but within a homogeneous biogeographic context and in nearly pristine conditions, using knowledge from local stakeholders and field visits (Table 1, Figure 2). In both RD and HD networks, drying occurs dominantly mid-reach on the catchment.

2.2 | Quantification of FI

Flow intermittence is defined here as the proportion of days without surface flow within a year (Bonada et al., 2007; Datry, 2012; Datry et al., 2007). At each site, we installed a combination of sensors to monitor the occurrence of drying events in RD networks over the whole sampling period: water level loggers (Solinst Levelogger Edge®) in the riverbed in deeper part of the stream (pools), temperature loggers (HOBO® Water Temp Pro V2) in a riffle, and photo-traps (Acorn® 5310MG) placed in an adjacent tree. The level loggers and temperature loggers recorded stream water depth and/or temperature once every 30 min, photo-traps took photos every 4 hr.

In HD networks, FI was quantified with water presence loggers (Onset Hobo®, Intermountain Environmental, Inc., Logan, UT, U.S.A.; Jensen et al., 2019; Vander et al., 2016) installed at each site in riffle heads, as this mesohabitats is the first to be affected by drying (Boulton, 2003). These loggers continuously monitored whether surface water was present from April 2013 to November 2013 and from June 2014 to December 2014. In addition, visual observations of flow state (flowing, non-flowing, or dry) were made on a monthly basis from November 2013 to June 2014 and from December 2014 to July 2015, as described in Datry, Larned, Fritz, et al. (2014). Previous analyses indicated that visual observations and flow state loggers provide similar FI estimates (Datry, Larned, Fritz, et al., 2014).

2.3 | Sampling collection and processing

In RD networks, all sites were sampled once before (April or May) and once after drying events (October or November); there were five sampling instances from autumn 2012 to autumn 2014. For HD networks, all sites were sampled before (April) and after (November) drying events; there were four sampling instances from autumn 2013 to spring 2015.

TABLE 1 Characteristics of recently drying (RD) and historically drying (HD) networks

		RD	HD
Catchment area (km^2)	Mean	9	258
	SD	5	162
	Max	22	626
	Min	3	80
River length (km)	Mean	5	369
	SD	2	286
	Max	10	822
	Min	3	33
% drying length of mainstem	Mean	3	21
	SD	2	14
	Max	8	47
	Min	2	8
Distance between pair of sites (km)	Mean	7.2	4.9
	SD	2.7	0.7
	Max	11.3	3.2
	Min	3.9	1.1
Elevation (m)	Mean	385	386
	SD	71	202
	Max	557	875
	Min	285	187
Prevailing geology		Crystalline complex, Calcareous, alluvial	Calcareous, alluvial
Main land use (Corine landcover)		Broad-leaved and mixed forest	Broad-leaved forest

Electrical conductivity, pH, water temperature, dissolved oxygen concentration, and oxygen saturation were measured with Hach Lange® HQ40d and HQ14d devices at each sampling site and each sampling date in both RD and HD networks. Possible differences in water chemistry between RD and HD networks were assessed with ANOVAs (Table S1).

In RD networks, benthic invertebrates were collected using the Czech national standardised protocol PERLA (Kokeš et al., 2006) based on a multihabitat 3-min kick sampling using a pond net (frame 25 × 25 cm; mesh size 500 μm). Each type of mesohabitat (e.g. riffle, pool) was sampled proportionally to its representation on a 20–50-m stream stretch. Samples were pre-sorted in the field, preserved in 4% formaldehyde and sorted in a laboratory. Taxa were mostly identified at the species level.

In HD networks, benthic invertebrates were sampled at two riffle heads for each site (to reduce the effects of small-scale habitat variability) using a Hess sampler (40 cm diameter; 1,250 cm^2 surface area; 250 μm mesh size). Samples were preserved in 96% ethanol, counted, and identified to the lowest practical taxonomic level. Most insects, most crustaceans and all molluscs were

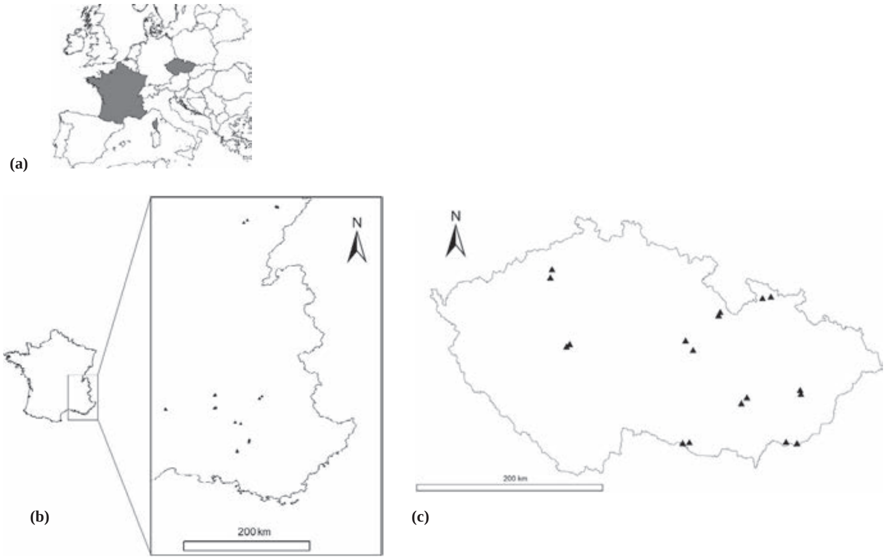


FIGURE 2 Location of both countries in Europe (a), location of sites in France (b) and sites in the Czech Republic (c)

identified to genus except for some insects of the order Diptera, which were often identified at family, subfamily, or tribus level. Annelids were identified to subclass for Oligochaeta and to genus for Achaeta. Mites were identified to the subclass and Tricladida to the genus.

Despite different sampling protocols, the sampling effort was similar in RD and HD networks as shown by accumulation curves in Table S2. An ANOVA test was carried out for low sample numbers (fewer than 10 samples) and showed no difference in taxon richness between RD and HD networks (*F* = 1.306, *p*-value = 0.268). To

TABLE 2 Results of mixed-effects models on taxonomic richness, functional richness, spatial taxonomic and functional β-diversity and their components, with hydrological regime (perennial or intermittent sites) and period (pre-drying or post-drying) as fixed effect, for recently drying (RD) and historically drying (HD) rivers. Estimates, standard deviation and 95% confidence interval are provided. Year and site were random effects when fitting α-diversity, year was a random effect when fitting β-diversity. Degrees of freedom of log-likelihood tests are equal to one

			Fixed effect estimates (full model)											
			Pre-drying								**Post-drying**			
			Perennial				**Intermittent**				**Perennial**			
			Est.	***SD***	**Low**	**Up**	**Est.**	***SD***	**Low**	**Up**	**Est.**	***SD***	**Low**	**Up**
Alpha taxon		RD	44	2	39	49	37	2	33	42	40	2	35	44
		HD	32	3	26	37	26	3	21	31	27	3	22	32
Alpha function		RD	101	17	67	135	65	17	31	99	109	15	78	141
		HD	67	6	55	79	53	6	41	66	62	6	50	74
Beta spatial taxon	RD	Total	0.80	0.01	0.79	0.82	0.84	0.01	0.82	0.86	0.79	0.01	0.77	0.80
		Turnover	0.75	0.01	0.73	0.77	0.77	0.01	0.75	0.80	0.74	0.01	0.72	0.76
		Nestedness	0.05	0.01	0.03	0.07	0.06	0.01	0.05	0.08	0.05	0.01	0.03	0.06
	HD	Total	0.35	0.01	0.33	0.36	0.33	0.01	0.32	0.35	0.43	0.01	0.42	0.45
		Turnover	0.23	0.02	0.18	0.28	0.23	0.02	0.18	0.28	0.29	0.02	0.25	0.34
		Nestedness	0.12	0.02	0.06	0.19	0.10	0.02	0.04	0.17	0.14	0.02	0.07	0.20
Beta spatial function	RD	Total	0.44	0.02	0.39	0.49	0.45	0.02	0.40	0.50	0.39	0.02	0.35	0.43
		Turnover	0.24	0.03	0.16	0.31	0.21	0.03	0.13	0.28	0.14	0.03	0.08	0.20
		Nestedness	0.20	0.02	0.15	0.25	0.23	0.02	0.18	0.29	0.25	0.02	0.21	0.29
	HD	Total	0.47	0.04	0.38	0.56	0.47	0.04	0.38	0.56	0.47	0.04	0.38	0.56
		Turnover	0.22	0.02	0.16	0.28	0.18	0.02	0.12	0.24	0.23	0.02	0.17	0.29
		Nestedness	0.24	0.04	0.14	0.34	0.28	0.04	0.18	0.38	0.26	0.04	0.17	0.36

Significant results are indicated with asterisks as follows: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

further avoid potential bias due to the differences in sampling protocol, spatial and temporal trends were considered separately within RD and HD networks (i.e. no direct comparison of samples collected with different sampling protocols).

2.4 | Taxonomic resolution homogenisation

We described benthic invertebrate communities at each site and for each sampling season in terms of taxonomic richness. Because the taxonomic resolution differed between the RD and HD networks, the taxonomic resolution of RD samples was downgraded to the lowest common taxonomic level, mostly genus or family level in case of several dipterans. To ensure that this did not affect the observed patterns in RD networks, results without taxonomic homogenisation are presented in Table S3.

2.5 | Data analysis

To test our first hypothesis (stronger effect of drying events on richness in RD than in HD networks), we tested for differences in taxon richness and functional richness with mixed-effects models using the *lme4* package (Bates et al., 2019) in R (R Core Team, 2019), with type of hydrological regime (intermittent or perennial) and sampling period (pre- or post-drying) and their interaction as fixed effects, and with year and site as random effects. To describe the trait composition of stream invertebrate communities, 18 categories across seven biological traits were used (Tachet, 2010; Schmidt-Kloiber & Hering, 2015; see traits in Table S4). We only considered biological traits that may be related to drying (i.e. lifespan, resistance forms, Crabot et al., 2020; Datry, Larned, Fritz, et al., 2014; Pařil, Polášek, et al., 2019). Selected traits characterise life-cycle features, resilience and resistance features, physiology, morphology, and reproduction and feeding behaviour. In the database, each taxon was coded according to its affinity to each category of a trait using a fuzzy-coding approach (Chevenet et al., 1994), meaning that the affinity of each genus to each category was coded from 0, for no affinity, to 3 for the strongest affinity, except for feeding and locomotion categories coded from 0 to 5. Taxa with missing trait data were omitted from subsequent functional composition analyses (41 taxa out of 160 in RD networks and 20 taxa out of 111 in HD networks). To calculate functional richness, we first computed taxon-by-taxon Gower distances within each site from the trait matrix and then generated a dendrogram using hierarchical clustering analysis on these distances with the unweighted pair group method with arithmetic mean

				Log-likelihood ratio tests						Random effects significance	
Intermittent				Regime		Period		Interaction			
Est.	*SD*	Low	Up	Chi	*p*-value	Chi	p-value	Chi	p-value	Year	Site
31	2	27	36	5.131	0.024 *	140.656	<0.001 ***	0.727	0.394	>0.999	<0.001 ***
20	3	15	25	3.045	0.081	120.672	<0.001 ***	0.226	0.634	>0.999	<0.001 ***
48	15	17	79	6.661	0.010 **	00.095	0.758	0.786	0.375	>0.999	0.151
42	6	30	54	4.033	0.045 *	70.003	0.008 **	1.463	0.227	>0.999	<0.001 ***
0.85	0.01	0.83	0.86	160.757	<0.001 ***	0.397	0.529	3.771	0.052	0.129	-
0.78	0.01	0.76	0.80	90.381	0.002 **	0.071	0.790	1.272	0.259	0.684	-
0.06	0.01	0.05	0.08	30.723	0.054	0.025	0.876	0.123	0.726	>0.999	-
0.42	0.01	0.40	0.43	50.654	0.017 *	27.379	<0.001 ***	0.003	0.960	0.479	-
0.29	0.02	0.24	0.34	00.005	0.943	10.867	<0.001 ***	0.189	0.664	0.007 **	-
0.12	0.02	0.06	0.19	110.618	<0.001 ***	9.111	0.003 **	1.980	0.159	<0.001 ***	-
0.44	0.02	0.40	0.49	20.430	0.126	1.200	0.273	0.994	0.319	>0.999	-
0.26	0.03	0.19	0.32	20.139	0.144	0.377	0.539	4.332	0.037 *	>0.999	-
0.19	0.02	0.14	0.23	00.780	0.377	0.002	0.966	5.014	0.025 *	0.579	-
0.62	0.04	0.53	0.71	20.119	0.146	2.155	0.142	2.973	0.085	0.013 *	-
0.32	0.02	0.25	0.38	00.588	0.443	1.716	0.190	13.770	<0.001 ***	>0.999	-
0.32	0.04	0.23	0.42	10.312	0.252	0.636	0.425	0.072	0.788	>0.999	-

(Cardoso et al., 2014). Functional richness was calculated as the total branch length of a tree linking all species represented in such community using the function *alpha* of the *BAT* package using the R software (Cardoso et al., 2014; R Core Team, 2019). Being aware that local IR communities could differ between countries due to biogeographical history, we quantified their responses to drying using pairwise comparisons between intermittent and perennial streams, with separate tests for each country, in a before–after–control–impact design with perennial sites as control.

To test our second hypothesis (less functionally homogeneous communities in space and time in RD networks than in HD networks), temporal β-diversity (pairwise differences of composition between sampling periods at the same site) was first computed and partitioned into its two additive components, turnover and nestedness, as suggested in Legendre (2014). The sampling method being different for RD and HD networks, we chose a presence–absence metric for both temporal and spatial β-diversity, the Sørensen-based index, which was computed with the R function *beta.div.comp* of the *adespatial* package (Dray et al., 2018).

To calculate temporal functional β-diversity, we first computed a trait distance matrix based on Gower distance between pairs of species and generated from this matrix principal coordinates analysis trait vectors (Villéger et al., 2013). Temporal functional β-diversity was then calculated on each site and partitioned based on the first two principal coordinates analysis trait vectors and a sample-by-species presence/absence matrix using the R function *functional.beta.multi* of the *betapart* package (Baselga et al., 2018). Separately for RD and HD networks, we tested for differences in temporal β-diversity and its components between intermittent and perennial sites with ANOVA tests, and we tested if this effect of flow regime differed between RD and HD with ANCOVAs.

In addition, to test that communities of intermittent sites should be less homogeneous in space in RD compared to HD networks, spatial β-diversity (pairwise differences of composition between sites at one sampling occasion) was computed among intermittent sites of RD networks, among intermittent sites of HD networks, among perennial sites of RD networks, and among perennial sites of HD networks for each sampling *df*. Differences in spatial β-diversity were tested with mixed-effects models, with type of flow regime (intermittent or perennial), sampling period (pre- or post-drying) and their interaction as fixed effect, and with year as random effect. As these hypotheses rely on a lower functional redundancy in RD than in HD networks, we compared functional redundancy between RD and HD networks as described in Schriever and Lytle (2016). Taxonomic richness was log10-transformed to meet the assumption of linearity and we tested the relationship of functional diversity against log(taxonomic richness) with linear regression. The difference in slopes was tested with ANCOVA.

To test our hypothesis H3 (lower proportion of taxa resistant to drying compared to the proportion of taxa resilient to drying in RD, the opposite in HD), communities were described in terms of resilience–resistance traits. Traits were used to classify taxa into four categories as described in Datry, Larned, Fritz, et al. (2014): resistant; resilient; resistant and resilient; and neither resistant nor resilient. We considered the following traits as resilient traits: long adult lifespan, multivoltine life cycles, and high dispersal abilities. We considered the following traits as resistant traits: egg-laying in terrestrial habitats or in vegetation, desiccation–resistance forms (cyst, cocoons, diapause), plastron/spiracle respiration (see traits and modalities in Table S4). When more resistance trait states were found for a taxon, it was classified as resistant (*n* = 20 taxa in RD and 23 in HD networks). When more resilience trait states were assigned for a taxon, it was classified as resilient (*n* = 54 taxa in RD and 43 in HD networks). If a taxon was assigned an equal number of resistance and resilience trait states, it was classified as resistant and resilient (*n* = 22 taxa in RD and 30 in HD networks). When no trait was assigned to a taxon, it was classified as neither resistant nor resilient (*n* = 23 taxa in RD and 33 in HD networks). We calculated the proportion of resistant and resilient taxa, and they were arcsin-√(*x*)-transformed to correct deficiencies in normality and homogeneity of variance. Proportions were compared with Kruskal–Wallis tests. Linear regressions were then computed separately for RD and HD networks to study the effect of FI on the taxon richness of resilient and resistant taxa and slopes were compared with an ANCOVA test with FI and drying history (RD or HD network) as factors. Statistical analyses were run using the R packages *stats* (R Core Team, 2019) and *adespatial* (Dray et al., 2018).

3 | RESULTS

3.1 | Environmental characteristics of the site

Mean FI ± *SD* in intermittent sites was 5.2 ± 3.7% in RD and 29.7 ± 17.5% in HD networks, spanning a large range of values of FI. The comparison of environmental variables showed that there were not any ecologically meaningful environmental differences between RD and HD networks. Even though differences in conductivity (51.08 ± 27.6 in RD vs. 39.89 ± 20.9 in HD networks), oxygen concentration (9.98 ± 1.38 in RD vs. 10.60 ± 1.45 in HD networks) and saturation (92.2 ± 9.5 in RD vs. 98.6 ± 11.6 in HD networks) were statistically significant, mean values of those variables indicate good environmental conditions (Datry, 2012) and fairly similar conditions in both RD and HD networks (see Table S1).

3.2 | Characteristics of invertebrate communities

In RD networks, 160 taxa were identified (38 taxa on average per sampling) compared to 111 taxa (26 taxa on average per sampling) in HD networks. The most frequently encountered taxa were Orthocladiinae, Oligochaeta, and Tanytarsini in both RD and HD networks; these taxa represented 3, 3, and 2% of total occurrences in RD networks, respectively, while they represented 5, 4, and 4% of total occurrences in HD networks, respectively (See Table S5 for taxa lists of both RD and HD networks). Complementary analyses computed on data from RD networks without the adjustment of taxonomic resolution showed the

TABLE 3 Results of ANOVAs tests (between intermittent and perennial sites within recently drying [RD] and within historically drying [HD] rivers, separately) and ANCOVAs tests (difference of response to flow regime between RD and HD networks) on temporal taxonomic and temporal functional β-diversity and their components. Degrees of freedom of ANOVAs and ANCOVAs test are equal to one

	Taxonomic						Functional					
	RD		HD		ANCOVA		RD		HD		ANCOVA	
	F	*p*-value	F	*p*-value	F	*p*-value	F	*p*-value	F	*p*-value	F	*p*-value
Total	19.210	<0.001***	0.002	0.962	7.864	0.009**	2.648	0.124	0.256	0.620	0.420	0.521
Turnover	17.400	<0.001***	0.001	0.976	9.123	0.005**	2.723	0.120	0.608	0.447	3.061	0.090
Nestedness	0.066	0.800	0.004	0.952	0.030	0.863	0.117	0.737	1.871	0.190	0.726	0.401

Significant results are indicated with asterisks as follows: $^{**}p < 0.01$; $^{***}p < 0.001$.

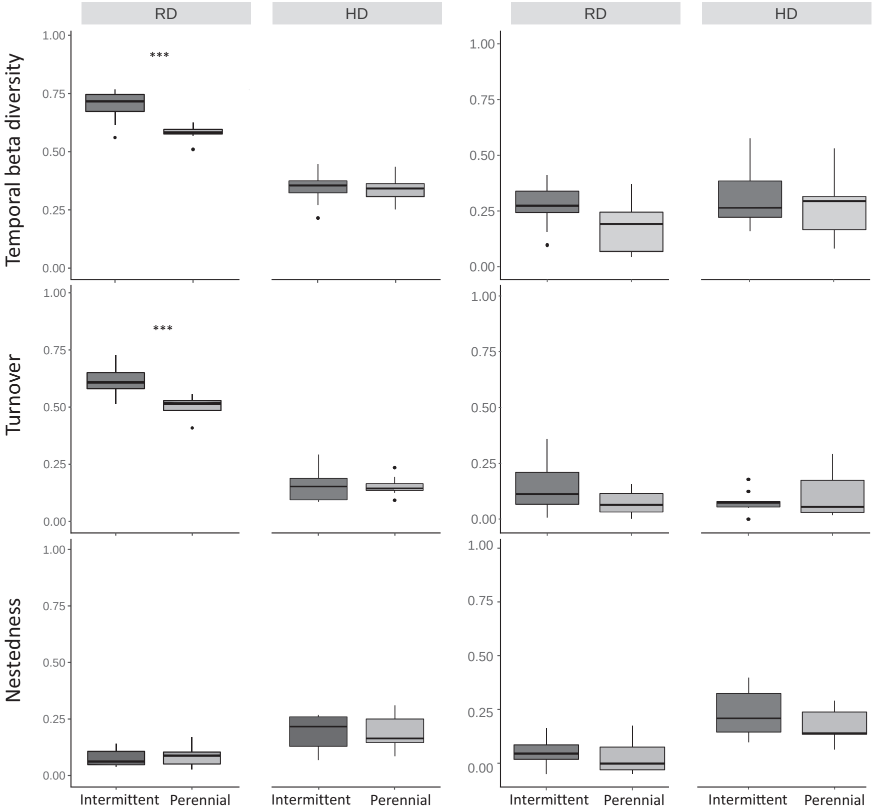


FIGURE 3 Temporal β-diversity in sites from recently drying (RD) and historically drying (HD) rivers and contribution of its two additive components, turnover and nestedness, from a taxonomic (left) and a functional perspective (right). Values are reported with boxes representing the 25, 50, and 75 quartiles, the thick line is the median, whiskers are minimum and maximum values, and black points are outliers

same patterns as those described below, showing the consistency of our analyses based on adjusted data (Table S3).

3.3 | Effects of drying events on α-diversity and functional diversity

Taxon richness and functional diversity had similar responses to drying in RD and HD networks. In RD networks, taxon richness was significantly higher at perennial sites than intermittent sites (42 ± 2 vs. 35 ± 2 taxa). Taxon richness was similar for both groups of sites in HD networks, although the difference was almost significant (29 ± 2 vs. 23 ± 2 taxa; Table 2). In RD and HD networks, taxon richness was significantly higher pre-drying than post-drying (41 ± 2 vs. 35 ± 2 for RD and 29 ± 3 vs. 24 ± 2 for HD networks; Table 2). Functional diversity was significantly higher in perennial sites compared to intermittent sites in both RD and HD networks. In HD networks, functional diversity was significantly higher pre- than post-drying but there was no such remarkable difference in RD networks (Table 2). There was no interaction between fixed effects (hydrological regime and sampling period) in either RD or HD networks (Table 2).

3.4 | Effects of drying on temporal β-diversity

Flow regime had a significantly stronger effect on temporal taxonomic β-diversity and turnover in RD compared to HD networks (Table 3, Figure 3). The temporal taxonomic β-diversity was significantly higher at intermittent than at perennial sites in RD networks (0.61 ± 0.07 vs. 0.49 ± 0.03) while there was no such difference in HD networks (0.34 ± 0.13 vs. 0.34 ± 0.12) (Table 3, Figure 3). In RD networks, temporal taxonomic turnover contributed more than nestedness (87 ± 6% of contribution) to taxonomic β-diversity, and turnover was significantly higher at intermittent sites (Table 3, Figure 3). In HD networks, temporal taxonomic turnover contributed less than nestedness (45 ± 16%) and there was no difference between intermittent and perennial sites (Table 3, Figure 3). In RD and HD networks, temporal functional turnover contributed less than nestedness (44 ± 25% of contribution of turnover to functional β-diversity in RD and 31 ± 18% in HD networks on average). Temporal functional β-diversity and its components were similar in intermittent and perennial sites for both HD and RD networks, indicating similarities in functional responses over time in RD and HD networks (Table 3, Figure 3).

3.5 | Effects of drying on spatial β-diversity

The effect of flow regime on spatial taxonomic β-diversity differed between RD and HD networks. Community composition was more dissimilar (higher spatial taxonomic β-diversity) among intermittent sites compared to perennial sites in RD networks (0.84 ± 0.01 vs. 0.79 ± 0.20), but the opposite was true in HD networks (0.38 ± 0.05 vs. 0.39 ± 0.04; Table 2). In both RD and HD networks, spatial taxonomic β-diversity was mostly due to turnover (93 ± 2% of contribution in RD and 66 ± 9% in HD networks). In RD networks, spatial turnover was 5% higher among intermittent sites compared to perennial sites, but nestedness was similar for both groups of sites (0.05 ± 0.01; Table 2). In HD networks, spatial nestedness was 11% higher among perennial sites compared to intermittent sites, but turnover was similar for both groups (0.25 ± 0.03; Table 2). In RD and HD networks, there was no significant difference in spatial functional β-diversity among intermittent and among perennial sites (0.43 ± 0.04 on average for RD and 0.48 ± 0.09 for HD networks), or for functional turnover and functional nestedness (Table 2). In RD networks, spatial functional turnover contributed 52 ± 14% on average to functional β-diversity; in HD networks, it contributed 50 ± 5%.

Spatial taxonomic β-diversity did not vary between pre- and post-drying in RD networks (0.82 ± 0.03 for both on average) and neither did its two components (Table 2). In HD networks, spatial β-diversity, turnover and nestedness were higher post- than pre-drying (Table 2). In RD networks, functional turnover decreased post-drying (−41%) for perennial sites whereas it increased (+2%) for intermittent sites and the opposite was true for functional nestedness (Table 2). In HD networks, functional turnover was stable pre- and post-drying for perennial sites but it increased (+43%) post-drying for intermittent sites.

3.6 | Functional redundancy

Functional richness was positively correlated to log10-transformed taxonomic richness for RD and HD networks (slope of 144 with $r^2 = 0.90$, $p < 0.001$ for RD; slope of 105 with $r^2 = 0.87$, $p < 0.001$ for HD networks, Table S6). This relationship was stronger for RD compared to HD networks indicating stronger functional redundancy in HD networks (interaction term of ANCOVA test: $F = 8.40$, $p = 0.004$).

3.7 | Difference in proportion of resilient and resistant taxa within RD and HD

Proportion of resilient taxa ($F = 356.9$, $p < 0.001$) was higher, and the proportion of resistant taxa ($F = 25.07$, $p < 0.001$) was lower, in RD compared to HD networks. In RD networks, the proportion of resilient taxa was similar among intermittent and perennial sites (54 ± 6% on average), but the proportion of resistant taxa was higher at intermittent sites (16 ± 5 vs. 13 ± 4%; Table 4). In HD networks, the proportion of resilient and resistant taxa were similar among intermittent and perennial sites (35 ± 7 and 19 ± 8%, respectively, Table 4).

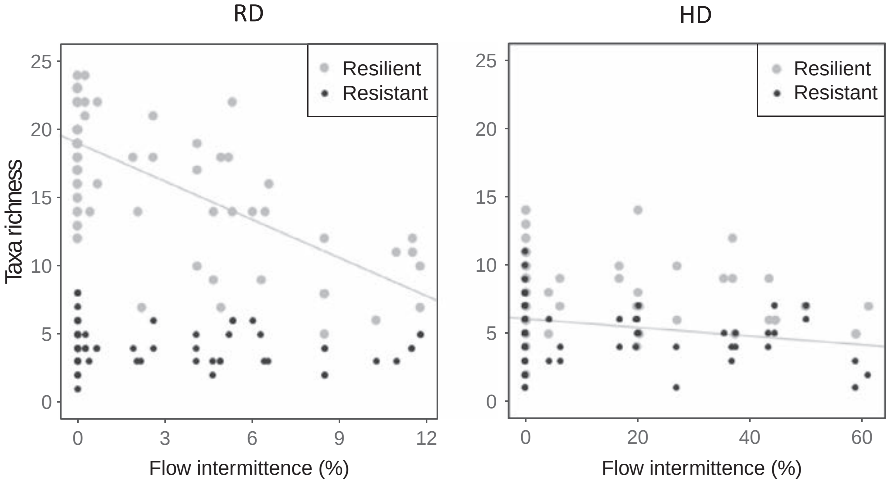
Flow intermittence affected the functional composition of invertebrate communities in RD and HD networks (Figure 4). The richness of resilient taxa decreased with increasing FI in both RD and HD networks (slope of −0.9 with $r^2 = 0.46$, $p < 0.001$ for RD; slope of −0.04 with $r^2 = 0.10$, $p = 0.007$ for HD networks, Figure 4). In RD networks, the trend was strongly linear and the slope was steeper compared to HD (interaction term of the ANCOVA: $F = 79.60$, $p < 0.001$). In both RD and HD networks, FI did not influence the richness of resistant

TABLE 4 Results of Kruskal–Wallis tests on the proportion of resistant and resilient taxa between perennial (per) and intermittent (int) sites for recently drying (RD) and historically drying (HD) rivers

	RD				HD			
	Mean values		Kruskal–Wallis		Mean values		Kruskal–Wallis	
	Int	Per	χ^2	*p*-value	Int	Per	χ^2	*p*-value
Resistant taxa (%)	16	13	8.8	0.004**	20	19	0.8	0.377
Resilient taxa (%)	54	55	0.8	0.370	34	35	0.2	0.666

Significant results are indicated with asterisks as follows: **$p < 0.01$.

FIGURE 4 Effect of flow intermittence on the taxa richness of resilient (large light points) and resistant (small dark points) taxa on sites from recently drying (RD) and historically drying (HD) rivers. Lines represent the linear regression fit of resilient taxa richness against flow intermittence



taxa ($r^2 = 0.03$, $p = 0.136$ in RD and $r^2 = 0.01$, $p = 0.412$ in HD networks, interaction term of the ANCOVA: $F = 0.9$, $p = 0.344$, Figure 4).

4 | DISCUSSION

In this study, we explored the response of aquatic invertebrates to drying in river networks that have historically experienced drying events (HD) and in networks recently affected by FI (RD). As recent drying might not have allowed taxa to acquire resistance and resilience strategies, communities of RD networks were expected to be more altered by drying than those of HD networks which experienced repeated drying over the previous decades or centuries. As local aquatic communities could differ between RD and HD networks due to biogeographical history, we quantified their responses to drying using pairwise comparisons between intermittent and perennial streams, with separate tests for each region, in a before–after–control–impact design with perennial sites as control. We found an effect of local drying on the α-taxonomic diversity of RD network communities but no such response in HD networks. We found a strong effect of flow regime on temporal diversity in RD networks and no such contrast in HD networks. We also found that intermittence induced an increase in community dissimilarities among sites in RD networks while the opposite was true in HD networks. However, how communities will respond in the near future to increasing drying is unknown and thus more research is needed on recent IRs.

4.1 | Lower taxon richness of intermittent sites in recent IRs

In line with our expectation, drying events induced stronger local effect on taxonomic diversity in recent IRs compared to IRs that have historically experienced drying events. Perennial sites in RD networks harboured higher taxonomic richness than intermittent sites, even at short distances (i.e. 7 km in RD), a pattern that is very common in IRs worldwide (e.g. Arscott et al., 2010; Bogan et al., 2013; Datry, 2012; Leigh & Datry, 2017; White et al., 2018), while this pattern was not significant in HD. As shown elsewhere in HD networks (e.g. Datry et al., 2016; García-Roger et al., 2013; Vander et al., 2016), taxonomic richness was higher before than after drying, due to the severe effects of drying on local communities.

The effects of drying were stronger in RD than in HD networks despite potentially milder conditions during drying events in the RD networks considered here and/or differences in drying duration and timing of post-drying sampling. For example, dry riverbeds in RD networks remain with relatively high sediment moisture, up to 12% (Pařil, Polášek, et al., 2019), while sediment moisture in HD dry riverbeds is typically lower than 7% (Datry et al., 2012). Such conditions have recently been shown to favour the persistence of invertebrates in the dry riverbed of these RD streams, even for those lacking the physiological adaptations to cope with drying that are generally found in IRs (Pařil, Polášek, et al., 2019). For example, Pařil, Polášek, et al. (2019) showed that 83% of taxa could tolerate short drying events (<1 month) without possessing desiccation-resistance forms, which then allowed a quick recovery of aquatic communities upon flow resumption. Drying duration can also strongly influence the responses of aquatic communities to drying (e.g. Datry, 2012; Leigh & Datry, 2017; Soria et al., 2017). For instance, intermittent sites in RD networks experienced at most 12% of FI, whereas the majority (87%) of intermittent sites in HD networks experienced FI greater than 12%. As such, the stronger local taxonomic response to drying found in RD networks in spite of milder conditions validates our hypothesis. Moreover, synoptic sampling efforts among countries or climate zones are still, to our knowledge, non-existent in IR research yet most global analyses (e.g. Datry, Larned, & Tockner, 2014; Soria et al., 2017; Stubbington et al., 2019) have previously detected substantial differences in community responses to drying despite differences in drying duration and timing of sampling.

4.2 | Resilience and resistance traits and functional responses to drying events

As expected, the proportion of resistant taxa was lower in RD than in HD networks. However, this did not lead to different functional diversity responses to drying between RD and HD networks, as functional diversity was higher on perennial sites compared to intermittent sites for both types of networks. Communities exposed to long and recurrent drying events (e.g. each year) can be assembled and colonised with taxa exhibiting adaptive traits (Robson et al., 2011). Therefore, we assumed that rivers that have been exposed to FI for a long time would have a pool of taxa more adapted to desiccation than those in recent IRs (Bonada et al., 2007). This prediction was supported; communities in HD networks harboured a higher proportion of resistant taxa traits, whereas communities in RD networks were dominated by resilient taxa. Thus, invertebrate communities of RD networks seem to be driven by resilience processes, which aligns with recent findings indicating that many organisms can tolerate dry conditions in these rivers for weeks or even months (Pařil, Polášek, et al., 2019). However, one should take into consideration that pools were sampled in RD but not in HD networks: riffle communities could harbour a higher proportion of resistant taxa as they are the first habitats to dry (Bonada et al., 2007). Conversely, communities from pools can also experience severe environmental conditions during the dry phases, with increased temperature and intra-/interspecific competition and low dissolved oxygen (Boulton, 2003). In addition, the steeper decline of taxa exhibiting resilience traits with increasing FI in RD compared to HD networks suggests that communities in recent IRs are more sensitive to an increase in drying duration than those from IRs that have historically experienced drying events. Remnant pools in IRs can be refuges as they serve of substitute aquatic habitat for good dispersers to move along the drying river and survive pool conditions, including lower oxygen and higher temperature (Bogan, Chester, et al., 2017). As such, we could have expected a lesser response of resilient taxa in RD networks because pool mesohabitats were sampled in RD but not in HD networks, which implies that the steeper decline we observed is particularly robust. This aligns with findings of Bertoncin et al. (2019), which indicate that unusually prolonged droughts of ponds in Brazil reduced their resilience to other environmental stressors. Exploring whether the recent drying in RD networks alters their resilience to other stressors, such as contaminants, hydromorphological alterations, or biological invasions would deserve further research.

The lower proportion of resistant traits in RD networks was not associated with a more severe response of functional richness to drying in RD compared to HD networks, nor with a higher temporal or spatial functional β-diversity. Communities from both RD and HD networks showed similar functional responses to drying. Ecosystem function could thus be maintained during drying events as it relies on functional diversity (e.g. loss of detritivores invertebrates would translate into lower rates of leaf litter decomposition; Corti et al., 2011; Datry et al., 2018). As environmentally harsh systems such as IRs that have historically experienced drying events harbour taxa with traits that promote resistance and resilience to disturbance and consequently present a high functional redundancy (Boersma et al., 2014; Vander et al., 2016), we expected a lower functional redundancy in RD networks where taxa did not experience such selective pressure. However, RD network communities showed a higher functional redundancy. This further confirms high discrepancy in functional redundancy patterns in intermittent aquatic systems reported by others. For example, Leigh et al. (2016) found no difference in functional redundancy between intermittent and perennial systems within temperate, Mediterranean, and arid regions. This was also previously reported by Schriever et al. (2015) in streams of the U.S.A. Boersma et al. (2014) found higher functional redundancy in drying mesocosms mimicking stream pools from arid south-western U.S.A. compared to control treatment without drying. By contrast, Leigh et al. (2019) reported a lower functional redundancy in invertebrate communities exposed to artificial drying in mesocosms compared to control treatment. Similar results were reported by Belmar et al. (2019) on IRs that have historically experienced drying events in Spain. While such discrepancy is surprising, probably stemming from differences in trait selection, coding, and analytic approaches, it calls for more efforts to describe functional redundancy patterns in long-standing and recent IRs, along with conceptual efforts to explain underlying mechanisms.

4.3 | Higher temporal and spatial variability of community composition in recent IRs

Drying events induced a stronger temporal variability of community composition on intermittent sites in RD than in HD networks. This difference was due to a higher replacement of taxa over time in RD than in HD networks rather than differences in temporal nestedness as initially expected. The theory predicts community composition to be stable over time in frequently disturbed environment as organisms are supposed to possess resistant and resilience traits (Chase, 2007). This is because resistant taxa promote rapid initial community recovery following disturbances while resilient taxa quickly contribute to ecological succession following this initial recovery, which results in high similarity of community composition over time (Bertoncin et al., 2019; Chase, 2007; Vander et al., 2016). Accordingly, we found that intermittent sites in RD networks had a higher turnover of taxa over time compared to perennial sites, while no difference of turnover between intermittent and perennial sites was observed in HD networks. However, our prediction was only partly supported because we predicted a higher temporal nestedness in RD networks. A greater effect of drying would have led to high taxa and traits loss resulting in high temporal nestedness. In contrast, temporal change in community composition in RD networks was found to be driven by temporal turnover, which comprised 80% of temporal β-diversity. By mimicking artificial droughts in mesocosms, Ledger et al. (2012, 2013) reported similar taxonomic turnover rates in response to drought on a pool of taxa from perennial rivers, thus not presenting resistance forms to drying events. They also found a decline of large species sensitive to drought (e.g. amphipods, mayfly larvae) and an increased proportion of opportunistic species (e.g. with shorter life cycles such as chironomids) along the gradient of drying. A possible limitation of our study lies in the differences of invertebrate identification between RD and HD networks, which could have increased our chances to detect turnover in RD networks (given the higher resolution of our identification in these systems). Nevertheless, we homogenised taxonomic resolutions between datasets to reduce such bias. This high temporal variability of community composition might challenge current management approaches that rely on reference conditions assumed to be stable over time and thus average community composition (Datry et al., 2016; Stubbington et al., 2017). Further work with a finer temporal resolution is needed to more deeply explore how the temporal dynamics in flow conditions in recent IRs affect community composition and functions over time (Crabot et al., 2020).

Flow regime also had a stronger effect on the spatial taxonomic dissimilarity of communities in RD compared to HD networks as expected, but functional turnover varied more between periods among intermittent sites in HD networks. Assuming that communities in RD networks would not present adaptions to drying due to their recent exposure to FI (Robson et al., 2011), we hypothesised that extinctions during drying events would be randomly driven, whereas in HD networks it should be rather driven by deterministic processes (Thompson & Townsend, 2006; Bonada et al., 2007). Such determinism and a lack of functional redundancy can explain the stronger functional signal observed in HD intermittent sites. The loss of generalist taxa, sensitive to desiccation, such as mayflies and stoneflies (Arscott et al., 2010; Datry, 2012; del Rosario & Resh, 2000) could lead to a selection of taxa with unique traits combination and more functional turnover between sites upon rewetting. In contrast, stochastic variations in colonisation or extinction can lead to higher spatial taxonomic β-diversity (Chase, 2010), which could explain the high spatial variability in RD networks. However, further work could consider other environmental variables that were not included in this analysis. For instance, the presence and type of riparian vegetation could be considered, as vegetation promotes sediment moisture in the riverbed during the dry phases and thus the survival of organisms without resistant traits (Pařil, Polášek, et al., 2019; Stubbington & Datry, 2013). More frequent drying and stronger severity predicted in the near future (Hänsel et al., 2019; Pařil, Leigh, et al., 2019; Van Lanen et al., 2016) might alter this and could generate more determinism in RD network community dynamics (Chase, 2007).

In this study, we showed that in RD networks, drying had a stronger effect on α-diversity, and community composition was much more variable in time and space, compared to HD networks, suggesting that drying had profound effects on community processes in recent IRs.

While communities in RD networks currently show some resilience to drying events, this might be different in the next decades as climate change intensifies. An increase in the frequency and magnitude of hydrological drought is expected (Spinoni et al., 2018) with many cases of shifts from permanent to intermittent flow regimes as a result (Datry, Larned, & Tockner, 2014; Döll & Schmied 2012; Larned et al., 2010). Increases in drying duration and spatial extent with climate change (Döll & Schmied, 2012; Spinoni et al., 2018) might reduce the number of nearby perennial refuges and the sources of colonists, along with reducing hydrological connectivity (Jaeger et al., 2014). As aquatic invertebrate communities in recent IRs seem highly sensitive to increasing FI, this could jeopardise community recovery of recent IRs in the near future due to climate change. Smol and Douglas (2007) showed that a major ecological threshold has recently been crossed for Arctic ponds associated with climate change, driving them to become ephemeral and dry very early in the year and strongly impacting diverse organisms (diatoms, invertebrates, plants). Recent IRs exposed to changes could be reaching a similar tipping point. Therefore, long-term monitoring is needed in these systems to examine the ongoing shift of FI and the biological implications of this shift for resilience and community recovery after drying events.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHORS CONTRIBUTION

T.D. and P.P. initiated the study, T.D., B.L, P.P., and M.P. established the sampling design, B.L., P.P., and M.P. carried out the sampling and invertebrate processing, and J.C. and M.P. carried out data analyses. J.C., T.D., P.P., and M.P. co-wrote the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Short-term streambed drying events alter amphipod population structure in a central European stream

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With 4 figures and 2 tables

Abstract: Temporary streams are becoming increasingly common, but ecological responses to streambed drying are poorly characterized in the temperate continental region of central Europe. In addition, global research has focused on community responses to drying, whereas effects on individual populations remain unknown. We explored the population structure of *Gammarus fossarum* (Crustacea: Amphipoda) in a central European temporary stream. Benthic gammarids were sampled on five dates during a flow recession, one date between two drying events (of 10 and 20 days, respectively), and five dates after flow resumed. Additional benthic samples were taken from isolated pools and dry sediments during drying events, and freeze cores were collected to compare the vertical distribution of amphipods during wet and dry phases. Gammarids were measured, adults distinguished from juveniles, adults sexed, and female reproductive state determined. Densities increased during flow recession, potentially reflecting both a decline in submerged habitat availability and seasonal increases in juvenile abundance. Persistence within dry benthic sediments was minimal, whereas pools and saturated subsurface sediments supported high population densities. Juveniles comprised 80 % of the subsurface population, suggesting that their ability to inhabit small interstices promotes persistence within the dry reach. Juveniles also comprised 92 % of pool inhabitants, despite their potential exposure to predation. Adults dominated after flow resumed, and population structure was altered post-drying by the loss of spring-recruited juveniles and reproductive females. Our results suggest that streambed drying may have longer-term effects than typically characterized by community-level studies. We recommend management actions that support populations of ecologically important species as they adapt to changing flow regimes.

Keywords: drought; hyporheic zone; intermittent rivers and ephemeral streams; population dynamics; recolonization; refuge; refugium; reproductive diapause; river drying

Introduction

Temporary streams, also known as intermittent rivers and ephemeral streams, are those in which water sometimes stops flowing, and in many cases, surface water is lost to leave sediments partly or completely dry (Datry et al. 2017; Stubbington et al. 2017). Such streams dominate arid zone networks, are common in regions with cooler, wetter temperate climates (Stubbington et al. 2017), and occur in the continental zone of central Europe. Due to increasing drought and heatwave events (Brázdil et al. 2009; Ledger & Milner

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2015), water resource pressures and land use change, the spatial and temporal extent of drying is increasing in such streams in central Europe and other global regions (Fiala et al. 2010; Laaha et al. 2017; Pyne & Poff 2017). However, whereas the effects of drying on aquatic invertebrate communities have been characterized in temporary streams in central Europe (Pastuchová 2006; Řezníčková et al. 2007; Řezníčková et al. 2010; Řezníčková et al. 2013), population-level impacts of drying remain poorly known here and across regions (Lake 2003).

Instream habitat diversity and availability change as discharge declines in a temporary stream (Boulton 2003). Depending on channel shape, flow recession can reduce the submerged habitat area, and as the water table falls an increasing channel area dries, and surface water may become restricted to persistent or temporary isolated pools (Lake 2003). Water quality may be altered, including temperature increases and reductions in dissolved oxygen availability (Bogan et al. 2017). Depending on the extent to which the water table declines, free water may remain within the benthic and hyporheic sediments after surface water is lost.

The upper reaches of central European streams can be densely populated by *Gammarus fossarum* (Crustacea: Amphipoda), an amphipod that supports ecosystem functioning by transferring energy between trophic levels, as a predator, prey, and shredder of particulate organic matter (MacNeil et al. 1997). Gammarids have limited desiccation tolerance, surviving for only a few days in moist sediments after free water is lost (Stubbington et al. 2009; Poznańska et al. 2013). However, as water levels decline, gammarids can remain submerged by migrating over benthic sediments ahead of a receding waterline (Poznańska et al. 2013). This concentrates populations within a diminishing submerged habitat area (Stubbington et al. 2011), exposing vulnerable individuals such as juveniles to greater risk of predation, including cannibalism (McGrath et al. 2007). If submerged habitats become restricted to isolated pools, biotic interactions can be intense, changing the structure of both communities and populations (Lake 2003; Bogan & Lytle 2011). In addition, poor water quality may affect the metabolic health and therefore reproductive activity of organisms persisting in pools, although such effects remain uncharacterized.

Gammarids may also migrate vertically into the saturated interstices of benthic and hyporheic sediments, in particular if surface water is lost (Vander Vorste et al. 2016a; Vadher et al. 2017). Vertical migrations vary in response to environmental drivers (Stubbington 2012), with experimental studies noting that coarse- and fine-grained sediments respectively promote and reduce Gammarid movements (Mathers et al. 2014; Vadher et al. 2015; Vadher et al. 2017; Vadher et al. 2018a). Vertical migrations may also vary within an individual population: during flowing phases, juveniles may dominate subsurface populations due to the risk of cannibalism by larger adults in the surface sediments, a response facilitated by their smaller size and thus their relative ease of movement through interstices (McGrath et al. 2007). During dry phases, an influx of refuge-seeking adults may create a more balanced population structure within subsurface sediments (Stubbington et al. 2011), if interstitial pathway dimensions are sufficient to accommodate these larger individuals (Vander Vorste et al. 2016a). As in pools, the effects of lower oxygen concentrations and different trophic resources on the physiology of individuals within the hyporheic zone are poorly understood (Findlay 1995).

After flow resumption, gammarids can rapidly recolonize a previously dry reach from perennial surface and subsurface refuges (Meyer et al. 2004; Řezníčková et al. 2007). Drift and upstream migration can provide most recolonists (Meijering 1977; Meyer et al. 2004), with the ability of gammarids to move both upstream and downstream increasing with body size (Lehmann 1967) and larger males therefore most capable of longitudinal migration (Elliott 2005). Migration from hyporheic back to benthic sediments has also been shown experimentally (Vander Vorste et al. 2016b), although gammarid metabolism may be affected by dry-phase persistence within suboptimal habitats. However, true recovery of population structure remains largely unknown (Lake 2003; Lancaster & Ledger 2015), and may be influenced by the representation of juveniles, adult males and adult females in different recolonist sources.

We characterized changes in *G. fossarum* population structure in response to flow recession, two streambed drying events, and flow resumption. Our first hypothesis (hereafter, H1) was that gammarid population densities would change over time, in relation to hydrological variables, and between pre-drying and post-drying phases, and would: (i) increase during flow recession in response to submerged habitat contraction, peaking in isolated pools; (ii) be low in benthic sediments during dry phases, but be higher in subsurface sediments during dry phases compared to wet phases; and (iii) increase after flow resumption.

Our second hypothesis (H2) was that differential survival of different population groups (males, reproductive and non-reproductive females, and juveniles) would alter gammarid population structure over time, in relation to hydrological variables, and between phases, including: (i) a decrease in the proportion of juveniles during flow recession, if the submerged habitat area contracted; (ii) a lower proportion of juveniles in the subsurface sediments during dry phases, if substrate characteristics allowed adults to move into interstices; (iii) a lower proportion of females after flow resumption, due to faster recolonization by males.

Methods

Study site

The Gránický stream (length 13 km, catchment area 20 km^2, annual discharge ca 7.6 dm^3 s^{-1}) is a third-order tributary of the Dyje river in the Podyjí National Park in the Czech Republic, 7 km from Czech-Austrian border (Fig. 1; 48° 52.0′ N, 16° 01.5′ E). The stream arises in an elevated plateau (410 m a.s.l.) dominated by arable farmland, and the middle and lower reaches meander through a broad-leaved forested valley with minimal human impacts. Schistose granites dominate the bedrock. The upper and lower reaches of the Gránický stream are near-perennial and dried in two extremely dry years in the period 2002–2017 (Řičicová et al. 2004; Laaha et al. 2017). The studied mid-reach (length 1.5 km, 280 m a.s.l) is temporary due to sediment permeability and a decrease in stream slope from 2.5 % to 1.5 %, and dried in 12 years between 2002 and 2017. The temporary reach is locally groundwater-fed, allowing isolated pools to persist for 1–3 weeks at the start of a dry phase, although pools are lost in years in which dry phases exceed this duration.

Based on 1961–2000 records, the mean annual air temperature in the study area is 9.4 °C and mean annual precipitation is 484 mm (Tolasz et al. 2007). Although annual precipitation was comparable to the long-term average in the study year (i.e. 490 mm in 2005; Czech Hydrometeorological Institute 2006), only 16 % and 43 % of the long-term monthly mean rainfall fell in March (4 mm compared to 27 mm) and June (25 mm compared to 57 mm), respectively. Annual mean air temperature was also comparable to the long-term average in 2005 (9.3 °C; Czech Hydrometeorological Institute 2006), but monthly means were 0.5–1.0 °C above average between April and June.

Field sampling strategy

The study was conducted between April and November 2005, which included two dry phases: 10 days (24 June – 3 July) during which surface water was lost from a 600 m stretch, and 20 days (25 July – 13 August), when > 2 km dried. Isolated pools persisted throughout the 10-day dry phase, and some also retained surface water during the second, longer dry phase. Our study period thus encompassed pre-drying, dry and post-drying phases, and allowed the development of distinct gammarid cohorts to be examined. Based on a visual assessment of the water depth and flow velocity, three dominant instream habitat types were identified within a 20-m stretch of the studied reach: riffle (high velocity, low depth), glide (low velocity, high depth) and marginal (low velocity, low depth). Two riffle, two glide, and (due to its limited spatial extent) one marginal habitat area were each identified by a semi-permanent marker.

Environmental conditions

Water depth and flow velocity (measured with a flow meter at 0.4× depth) were recorded within 50 × 50 cm plots at ≥ 100 points at 50 cm intervals within a 7.5 m long stretch. These data were used to calculate the proportion of the channel that was submerged on each date, compared to the maximum submerged area, which was recorded on 25 April during the period of highest discharge (hereafter, % submerged habitat area [%SHA]).

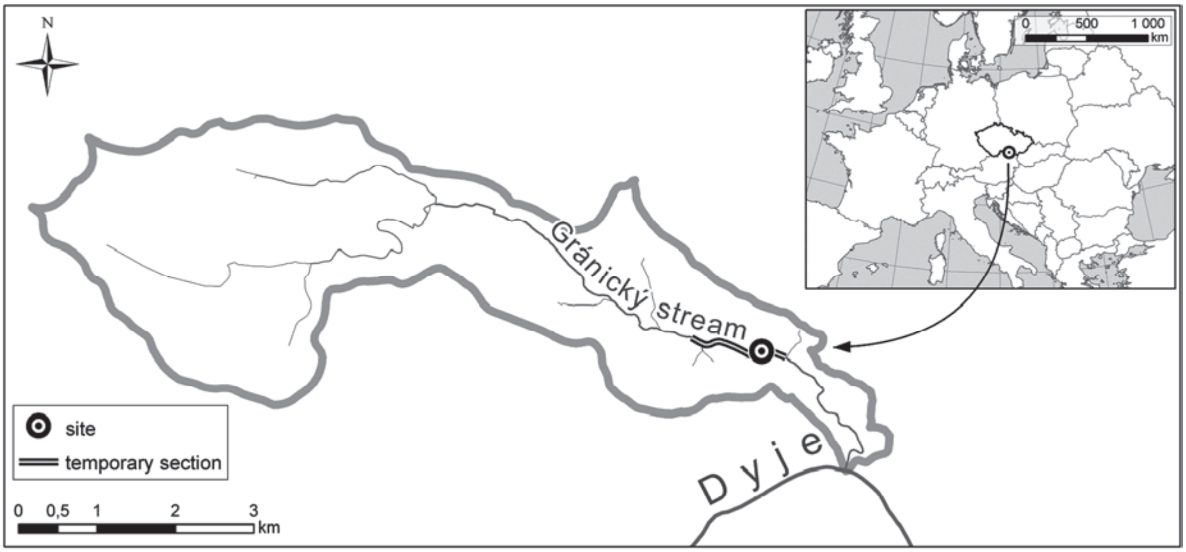

Fig. 1. Map of the study catchment, indicating the position of the study site within the temporary section of Gránický stream, and its location within the Czech Republic and Europe.

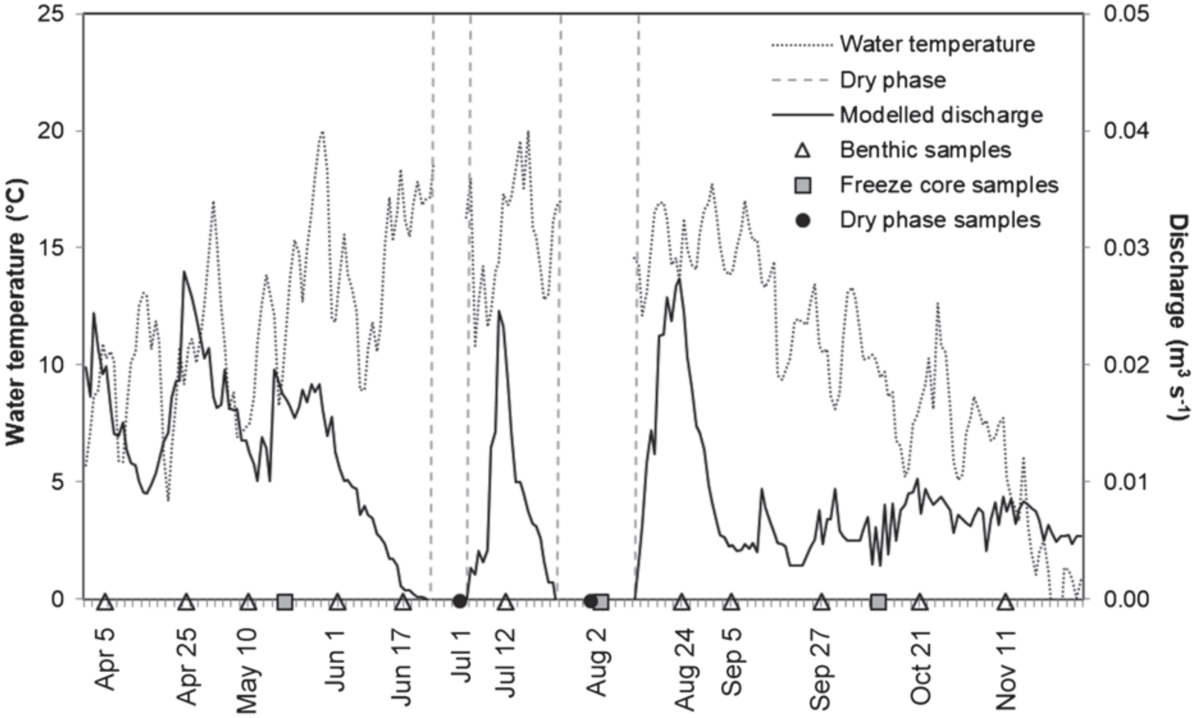

Fig. 2. Water temperature and modelled stream discharge in Gránický stream during the study period (April to November 2005), indicating the timing of flowing and dry phases, and of flowing-phase benthic, freeze-core and dry-phase sample collection.

Discharge was calculated on each sampling date based on flow velocity (at 0.4× and 0.8× depth) and depth measurements from ≥ 8 points at 0.5 m intervals across the channel wetted width. These data and daily precipitation and mean air temperature data from a hydrometeorological station located 4 km from the study area (Kuchařovice 48° 52.8′ N, 16° 5.1′ E) were used to model discharge and water temperature for the study period (Fig. 2).

On each sampling date, pH, conductivity (μS cm^{-1}), dissolved oxygen concentration (mg l^{-1}) and saturation (%) and water temperature (°C) were measured in situ using a multiparameter probe (Hach-Lange HQ40d). In each habitat area, water depth and flow velocity (at 0.4× depth) were measured using a flow meter, and substrate composition characterized by estimating the proportion of each inorganic substrate category in the AQEM manual (Hering et al. 2004).

Gammarid sampling

To characterize the gammarid population, one benthic sample was collected in each of the five habitat areas (i.e. two in riffle, two in glide and one in a marginal habitat type) by manually disturbing the benthic sediments within a 0.1 m^2 frame, 0.5-mm-mesh sampler for 30 s. Samples were collected on each of 11 dates, including five dates in the period preceding drying (hereafter, *pre-drying*), one date during a short flow resumption between two dry phases (hereafter, *between-dry*), and five dates after continuous flow resumed (hereafter, *post-drying*; Fig. 2). In each habitat area, a benthic sample was taken 10–25 cm upstream or downstream of the marker on successive dates, to allow at least 4 weeks for the recolonization of a previously sampled area prior to re-sampling (Johnson & Vaughn 1995).

Additional gammarid samples were collected during dry phases. First, on day 9 of the second dry phase (2 August; hereafter, *dry-2*), one benthic sample was collected from each of four isolated pools located within 150 m of the main sampling stretch. Second, on one date during both the first dry phase (1 July, hereafter, *dry-1*) and dry-2 (2 August), one dry benthic sediment sample was collected in each of the three habitat types, by manually excavating sediments within a 0.1 m^2 area to a depth of 10 cm.

Benthic and hyporheic bed sediments and associated invertebrates were collected in each of the three habitat types, 30 m downstream of the main sampling area, using the freeze-core technique of Bretschko & Klemens (1986). Cores were taken on three dates: five weeks pre-drying (19 May), on day 11 of dry-2 (4 August), and two months after flow resumed (11 October; Fig. 2). Standpipes were driven 70 cm into the bed at least 7 d before each date, to allow gammarids to recolonize prior to sampling. On each sampling date, one core was taken from each habitat type using liquid nitrogen. After 20 min of freezing, cores were extracted using a tripod and winch. Each extracted core was divided in the field into 10-cm horizontal layers. Each layer was submerged in water in a calibrated container to determine its volume, then removed and preserved using 4 % formalin.

In the laboratory, each layer was elutriated following Omesová & Helešic (2004) and a sieve stack (mesh sizes [mm]: 5, 0.5, 0.25, 0.1) used to separate invertebrates and substrate. A ≤ 0.5 l sediment subsample from each layer was oven-dried at 105 °C, separated using consecutive sieves (mesh sizes [mm]: 63, 31.5, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.063) and weighed.

Grain sizes with an intermediate (*B*) axis > 6 cm were removed, to eliminate the stochastic effects caused by the irregular distribution of large grains (Weigelhofer & Waringer 2003). Weights for each sediment fraction were used to calculate the 25th, 50th and 75th percentiles of each grain-size category for each layer. Coarse particulate organic matter (CPOM) in the dry subsample from each layer was separated using a 0.5-cm mesh sieve and weighed.

Characterization of the gammarid population

To distinguish adults from juveniles, the length of each gammarid was measured from the base of antenna 1 to the tip of the telson (resolution 1 µm) using a stereomicroscope (Olympus SZX9, magnification 3–182×) and QuickPHOTO MICRO image analysis software (version 2.3, © PROMICRA Ltd., Czech Republic). All specimens ≥ 6.5 mm were sexed, with adults and juveniles defined as those above and below this length, respectively. Sex was determined based on the presence of oostegites in females (Pöckl 1992) and the sex-specific shape of gnathopod 2 (Goedmakers 1972). Reproductive females were defined as those with embryos in the brood pouch. For each benthic sample (including flowing-phase, pool and dry sediment samples), we explored population structure by calculating the densities of each population group, and the relative densities (i.e. proportion) of: females compared to all adults, reproductive females compared to all females, and juveniles compared to all gammarids.

Statistical analyses

In total, 55 flowing-phase benthic samples were included in statistical analyses used to test H1-2. In addition, specific hypotheses were explored using: the four benthic isolated pool samples (H1[i]), the six dry benthic sediment samples (H1[ii]) and the nine freeze cores (H1[iii]; H2[ii]). Pool and dry-sediment data were not statistically analysed due to low replication. Analyses were done in R (R Development Core Team 2015), using the *nlme* package for linear mixed models (LMM; Pinheiro et al. 2018). Data distributions were transformed as necessary to meet assumptions of the analyses, including log transformation of density data.

We first used LMM to test for differences in hydrological response variables (water depth, flow velocity and discharge) among hydrological phases (specified as a fixed factor with three levels: pre-drying, between-dry, post-drying). We then used repeated-measures (RM) ANOVA to identify differences in flowing-phase benthic gammarid response variables (i.e. H1: total, male, female and juvenile density; H2: female, reproductive female and juvenile proportion) between dates. To test whether flowing-phase benthic gammarid populations responded to hydrological changes and/or differed among phases (H1), we constructed separate LMM for each response variable. We used stepwise selection based on the Akaike Information Criterion (AIC) to select the best set of explanatory variables (depth, velocity, discharge and hydrological phase) for each response variable using the *stepAIC* function in the *MASS* package (Venables & Ripley 2002). Non-significant variables were removed from the best model if the subsequent model had a ΔAIC < 2 from the best model. We also tested if total, male, female and juvenile gammarid densities (H1[i]) and the proportion of juveniles (H2[i]) varied with %SHA during the pre-drying phase (i.e. 5 dates, $n = 25$) by building separate LMM for each response variable, with %SHA as the fixed factor. Similarly, we tested if gammarid densities (H1[iii]) and the proportion of females (H2[iii]) changed during the post-drying phase (5 dates, $n = 25$) using LMM with time since flow resumption (i.e. the number of days since the end of dry-2) as the fixed factor. Date was included as a random factor in each LMM model to account for temporal auto-correlation.

One-way ANOVAs using freeze-core densities standardized to core volume were used to compare total and juvenile densities (H1[ii]) and body length (H2[ii]) between wet and dry phases and among the three cores (pre-drying [wet], dry-2 [dry], post-drying [wet]). Spearman rank correlation coefficients (r_s) were calculated to examine relationships between sediment characteristics (depth of layer; 25th, 50th and 75th percentiles of each grain size category; proportion of each grain size category; proportion of CPOM) and population characteristics (total and juvenile densities, and body length mean, maximum and standard deviation). Males and females were excluded from analyses due to low densities.

Results

Environmental conditions

Modelled discharge varied between 1–28 $dm^3\ s^{-1}$, interrupted by dry-1 and dry-2 (Fig. 2, Table 1). Heavy rain caused the 21-day flow resumption between these dry phases and re-established flow after dry-2. Compared to the maximum recorded on 25 April (100 %), %SHA varied between 54 % and 97 % on other dates, declining gradually from 25 April to 54 % on 17 June, 7 days before dry-1 (Table 1). Although velocity was particularly high on the between-dry date (Table 1), such flow peaks are insufficient to mobilize sediments (P. Pařil, unpublished observations) and all hydrological variables were comparable among phases (LMM, all $p > 0.5$). Chemical characteristics of flowing-phase water quality did not exceed values from unpolluted streams during the study (European Commission 2015; Table 1). Mean water temperature was 10.9 ± 0.30 °C and the maximum daily mean was 20.8 °C (Fig. 2). In isolated pools, minimum dissolved oxygen concentrations were 1.8 $mg\ l^{-1}$, water temperature peaked at 16.2 °C, and conductivity was always > 1000 $\mu S\ cm^{-1}$ (Table 1).

Hypothesis 1: response of gammarid population densities to hydrological changes and drying

Considering the 55 flowing-phase benthic samples, total, juvenile, male and female gammarid population densities all varied among dates (RM ANOVA, all $F < 11.37$, $p < 0.05$; Fig. 3; Table 2; H1). Total, female and juvenile densities were highest on the final pre-drying date, and male densities on the preceding date (Table 2), and densities of all population groups were

Table 1. Hydrological and water chemistry variables in Gránický stream during flowing phases pre-drying, between two dry phases, in isolated pools during a dry phase ($n = 4$), and post-drying. Mean ± 1 SE or mean values.

Timing		Hydrological variables				Water chemistry variables			
Date	**Phase**	**Discharge ($dm^3\ s^{-1}$)**	**Submerged habitat area (%)**	**Velocity at 0.4× depth ($m\ s^{-1}$)**	**Water depth (m)**	**Conductivity ($\mu S\ cm^{-1}$)**	**Dissolved oxygen ($mg\ l^{-1}$)**	**Dissolved oxygen (%)**	**pH**
5 Apr	Pre-drying	19	94	0.10 ± 0.12	0.14 ± 0.10	797	12.1	107	8.0
25 Apr		29	100	0.17 ± 0.13	0.15 ± 0.09	733	9.2	81	8.7
10 May		14	78	0.11 ± 0.16	0.13 ± 0.09	920	11.8	105	7.8
1 Jun		14	75	0.15 ± 0.20	0.12 ± 0.09	835	8.4	80	9.0
17 Jun		1	54	0.06 ± 0.09	0.10 ± 0.09	874	7.1	71	8.4
12 Jul	Between-dry	25	97	0.26 ± 0.25	0.15 ± 0.10	715	7.9	80	8.4
2 Aug	Dry	NA	NA	NA	0.19 ± 0.07	1123 ± 18	5.1 ± 0.9	21–88	8.2 ± 0.15
24 Aug	Post-drying	28	94	0.11 ± 0.12	0.13 ± 0.08	803	7.5	78	8.4
5 Sep		4	61	0.04 ± 0.06	0.11 ± 0.09	1090	9.4	90	8.5
27 Sep		6	65	0.07 ± 0.06	0.10 ± 0.07	998	9.4	86	8.1
21 Oct		10	86	0.06 ± 0.09	0.13 ± 0.08	904	11.8	101	6.0
11 Nov		9	86	0.08 ± 0.08	0.15 ± 0.09	1070	9.8	81	6.5
Mean		**14**	**81**	**0.11 ± 0.14**	**0.13 ± 0.08**	**87**	**9.5**	**885**	**8.0**

Table 2. Mean ± SE *Gammarus fossarum* response variables in flowing-phase benthic sediment samples collected in Gránický stream, pre-drying, between two drying events, and post-drying; values calculated for $n = 5$ samples on each date, except 25 Apr, $n = 3$ for juveniles; 12 Jul, $n = 1$ for reproductive females / females, $n = 3$ for males; 24 Aug, $n = 1$ for all gammarids / juveniles; 5 Sep, $n = 3$ for all gammarids, $n = 1$ for non-reproductive females / females, $n = 2$ for males, $n = 3$ for juveniles; 27 Sep, $n = 3$ for juveniles; 21 Oct, $n = 4$ for juveniles; 11 Nov, $n = 4$ for males / juveniles; NA, $n = 0$.

Date	**Phase**	Density (individuals 0.1 m^{-2})					Length (mm)	Proportion (as a %)*		
		Total	**Juvenile**	**Female**		**Male**	**Total**	**Female**	**Female reproductive**	**Juvenile**
				reproductive	**non-reproductive**					
5 Apr	Pre-drying	85 ± 55	27 ± 22	5.6 ± 0.7	27 ± 18	25 ± 15	9.0 ± 0.7	56 ± 5.7	35 ± 9.9	20 ± 5.9
25 Apr		39 ± 26	8.2 ± 5.3	4.8 ± 3.6	7.4 ± 3.4	19 ± 14	9.4 ± 0.6	47 ± 3.2	21 ± 9.5	17 ± 7.2
10 May		97 ± 32	36 ± 6.8	25 ± 13	11 ± 5.3	25 ± 7.3	7.2 ± 0.3	54 ± 4.4	69 ± 3.2	42 ± 4.4
1 Jun		199 ± 51	139 ± 48	25 ± 3.7	15 ± 5.1	20 ± 4.7	5.9 ± 1.0	65 ± 6.8	67 ± 6.3	62 ± 12
17 Jun		149 ± 120	112 ± 23	14 ± 5.4	5.2 ± 0.9	18 ± 6.4	5.3 ± 0.3	53 ± 7.2	69 ± 3.7	76 ± 3.0
12 Jul	Between-dry	3.4 ± 0.9	2.6 ± 0.7	0.2 ± 0.2	NA	0.6 ± 0.2	4.4 ± 0.8	17 ± 17	1 ± 0	78 ± 9.8
24 Aug	Post-drying	1.4 ± 1.4	1.4 ± 1.4	NA	NA	NA	3.4 ± 0	NA	NA	100 ± 0
5 Sep		3.6 ± 1.7	2.6 ± 1.7	NA	0.4 ± 0.4	0.6 ± 0.4	5.7 ± 1.0	25 ± 25	NA	65 ± 24
27 Sep		15 ± 3.4	5.0 ± 3.2	NA	5.2 ± 1.6	5.2 ± 1.7	9.3 ± 0.9	50 ± 11	NA	26 ± 14
21 Oct		32 ± 11	3.4 ± 1.2	NA	17 ± 6.5	11 ± 4.5	9.6 ± 0.4	60 ± 5.7	NA	15 ± 7.6
11 Nov		9.4 ± 1.9	2.4 ± 1.1	NA	4.4 ± 1.6	2.6 ± 1.0	7.8 ± 1.0	64 ± 10	NA	31 ± 13

* Female = the proportion of females compared to all adults; female reproductive = the proportion of reproductive (egg- or embryo-carrying) females compared to all females; juvenile = the proportion of juveniles compared to all gammarids.

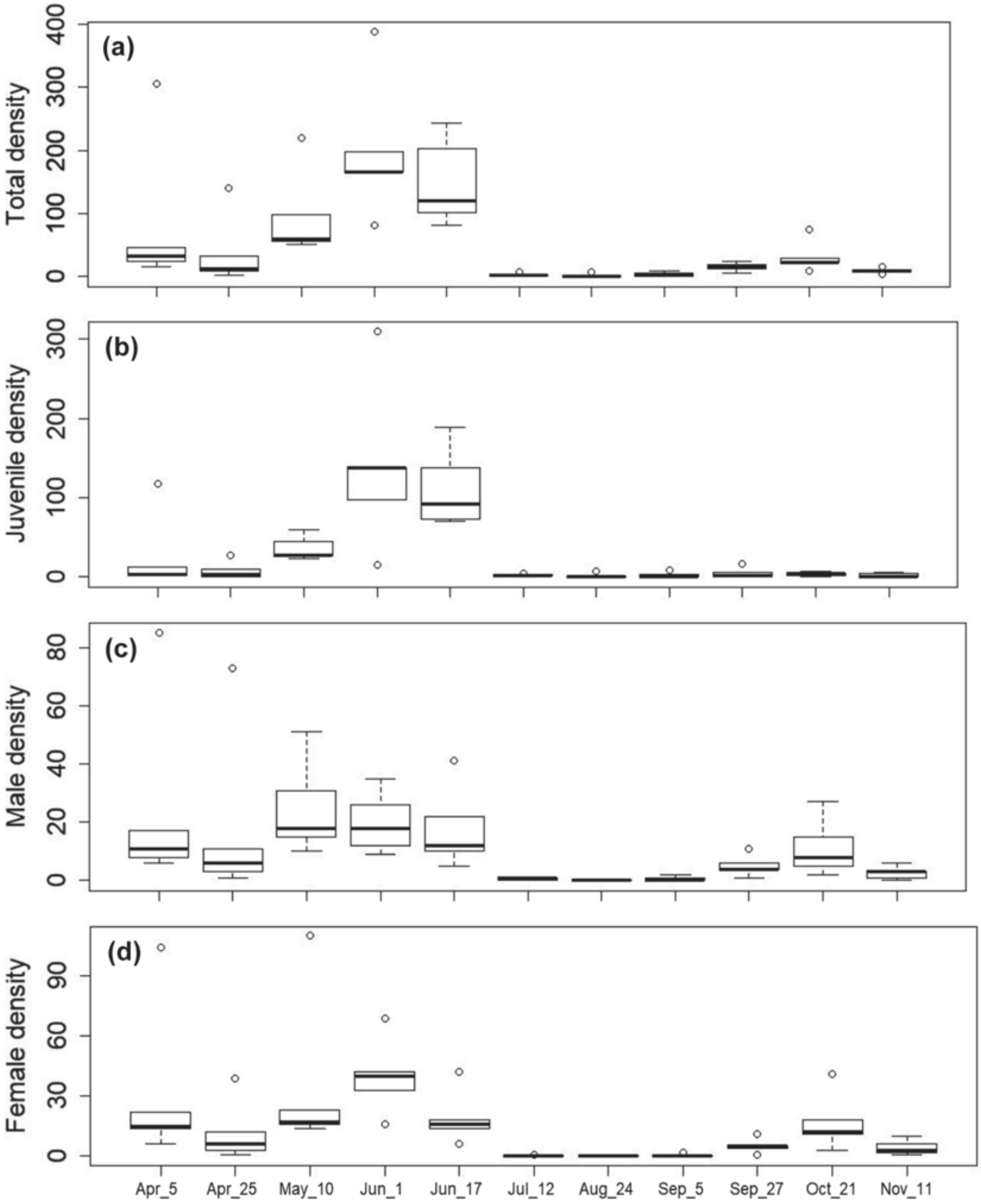

Fig. 3. **(a)** Total, **(b)** juvenile, **(c)** male and **(d)** female *Gammarus fossarum* densities (0.1 m^2) in samples collected from benthic sediments on five dates pre-drying (Apr 5 to Jun 17), one date between dry phases (Jul 12), and five dates after flow resumed (Aug 24 to Nov 11) in Gránický stream. The box area indicates the first and third quartiles, the central line indicates the median, whiskers represent 95 % confidence intervals, and circles indicate outliers; $n = 5$ for each date.

lowest on the first post-drying date (Table 2; Fig. 3). Total gammarid densities differed among phases and were 10-fold lower post-drying (10.8 ± 2.7 individuals 0.1 m^{-2}) compared to pre-drying (114 ± 20 individuals 0.1 m^{-2}; LMM, $t = -4.95$, $p < 0.01$), reflecting reduced densities of all population groups (all $p < 0.05$; H1). Total benthic densities declined with increasing discharge (LMM, $t = -2.56$, $p < 0.05$), and juvenile densities increased with water depth ($t = 2.97$, $p < 0.01$) and decreased with discharge ($t = -3.07$, $p < 0.05$), whereas female and male densities did not vary in response to hydrological variables ($p > 0.05$; H1).

Juvenile gammarid densities increased with declining %SHA (LMM, $t = -3.69$, $p < 0.05$), whereas total, male and female densities did not vary ($p > 0.07$; Table 2; H1[i]).

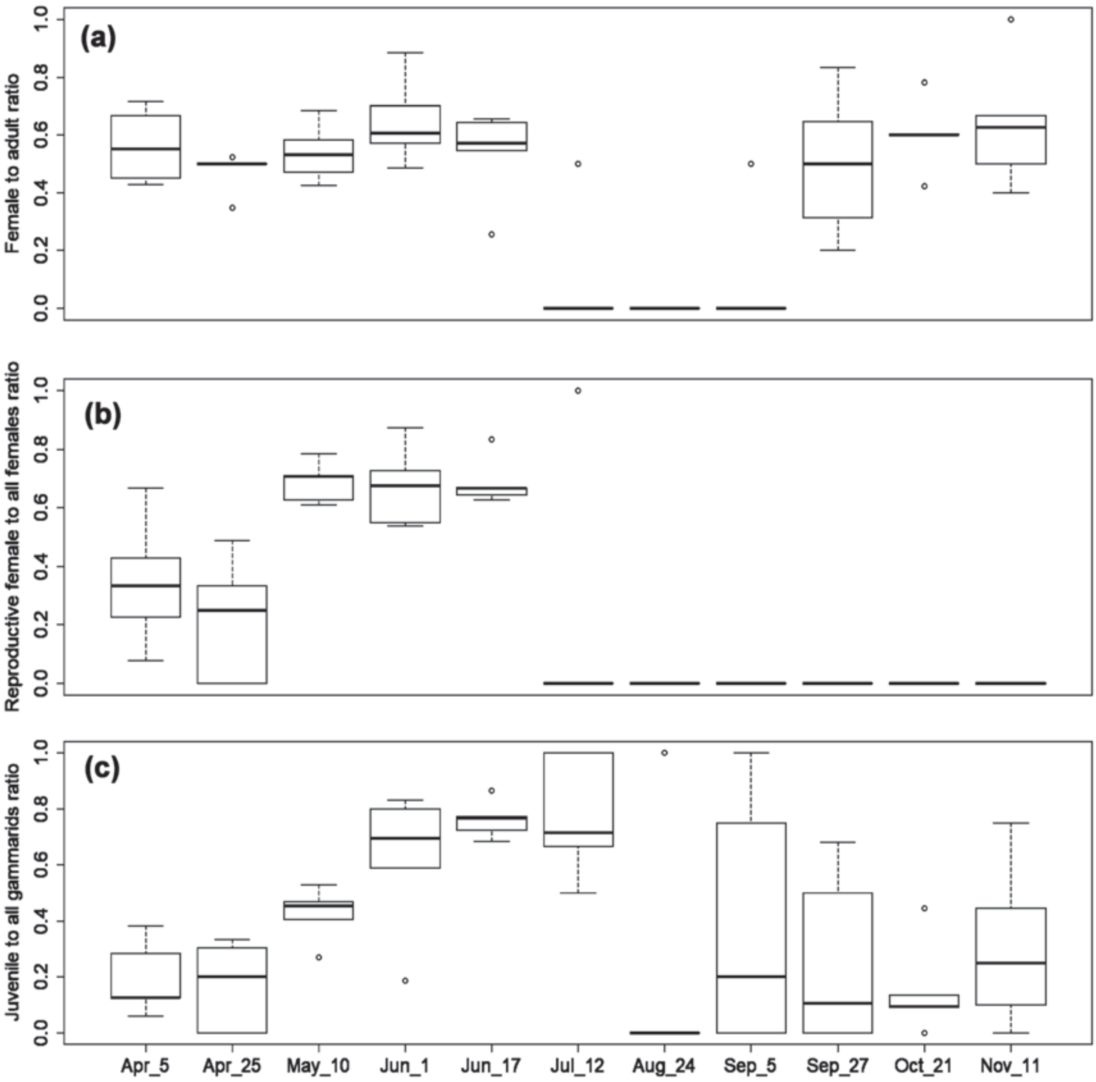

Fig. 4. The proportion of **(a)** females compared to all adults, **(b)** reproductive females compared to all females, and (c) juveniles compared to all gammarids in benthic samples collected on five dates pre-drying (Apr 5 to Jun 17), one date between dry phases (Jul 12), and five dates after flow resumed (Aug 24 to Nov 11) in Gránický stream. The box area indicates the first and third quartiles, the central line indicates the median, whiskers represent 95 % confidence intervals, and circles indicate outliers; $n = 5$ for each date, except as stated in the Table 2 title.

Mean total population densities were substantially higher in isolated pools (2723 ± 1306 individuals 0.1 m^{-2}) compared to flowing-phase populations in riffle, glide and marginal habitats (< 73 ± 30 individuals 0.1 m^{-2}), and also greatly exceeded peak densities in these other habitat types (387 individuals 0.1 m^{-2}; H1[i]; Table 2).

Very few gammarids were observed in dry sediments during dry-1 (4 ± 2 individuals 0.1 m^{-2}; $n = 3$), and none in samples collected during dry-2 ($n = 3$; H1[ii]). In freeze cores, densities of juveniles (which dominated the population, see below) were higher pre-drying (28 ± 13 individuals per layer) and in particular during dry-2 (91 ± 71 individuals per layer) compared to post-drying (1.0 ± 0.6 individuals per layer; one-way ANOVA, $p < 0.05$); comparable differences in total densities between pre-drying, dry-2 and post-drying cores were not significant ($p > 0.05$; H1[ii]). Total and juvenile densities (357 and 218 individuals) were almost 10-fold higher in the 0–10 cm layer of the dry-2 pool core than in other layers, with higher water levels observed in this habitat (< 5 cm below the sediment surface) compared to others (approx. 15 cm). Total benthic densities increased from the first to the fourth date after flow resumed, reflecting changes in male and female densities (LMM, all $p < 0.05$), whereas temporal changes in juvenile densities were not significant ($p > 0.05$; H1[iii]; Table 2).

Hypothesis 2: the response of gammarid population structure to hydrological changes

Considering all gammarids in the 55 benthic samples, the proportion of juveniles (compared to all gammarids), females (compared to all adults) and reproductive females (compared to all females) all differed among dates (RM ANOVA, all $F > 4.11$, all $p < 0.001$; Fig. 4; Table 2; H2). The proportion of juveniles increased with depth (LMM, $t = 2.49$, $p < 0.05$) and decreased with increasing discharge ($t = -3.00$, $p < 0.05$); neither the proportion of females nor of reproductive female responded to any hydrological variable (all $p > 0.08$).

The proportion of juveniles was higher during the pre-drying phase compared to post-drying (LMM, $t = -2.40$, $p < 0.05$; Table 2; H2), and increased from pre-drying dates 2 to 5 in relation to decreasing %SHA ($t = -6.48$, $p < 0.01$; H2[i]). Juveniles dominated the population during the between-dry phase (LMM, $t = 2.80$, $p < 0.05$; Fig. 4c; Table 2) and in isolated pools, where they accounted for 92 % of all gammarids, with females and males comprising the remaining 6 % and 2 %, respectively. The proportion of females compared to all adults did not differ between pre-drying and post-drying phases (LMM, $p > 0.05$) but increased with the time since flow resumption (LMM: $t = 4.79$, $p < 0.05$; Fig. 4a; Table 2; H2[iii]). The proportion of reproductive females compared to all females was higher pre-drying than post-drying (LMM, $t = -6.40$, $p < 0.001$): only one (reproductive) female was sampled during the between-dry phase, and of 135 females sampled across the five post-drying dates, none were reproductive (Table 2; Fig. 4b; H2).

Substrate composition varied among freeze cores and with depth into the bed. Cores from the glide and marginal habitats had an organic-rich surface layer. The 0–20 cm layers comprised larger gravel particles (mean grain size 60 mm) in the riffle core, whereas sand and fine gravel (35 mm) dominated the run core, and marginal core sediments spanned these size classes (50 mm declining to 20 mm). A low-permeability clay layer occurred at a depth of approx. 30–40 cm in the pool and riffle cores, below which mean grain size was lower in the glide core (10 mm) than in other cores (40 mm). Considering densities standardized to core volume, juveniles accounted for 61 % of 131, 80 % of 429, and 56 % of 13 gammarids recorded pre-drying, during dry-2, and post-drying, respectively. Body length was comparable in freeze cores taken pre-drying (4.5 ± 2.0 mm), during dry-2 (5.2 ± 1.9 mm), and post-drying (11 ± 4.3 mm; one-way ANOVA, $p > 0.05$; H2[ii]). Total gammarid densities ($r_s = -0.64$, $p < 0.05$) and maximum body length ($r_s = -0.47$, $p < 0.05$) decreased with depth; no other significant relationships were observed between gammarid response variables and sediment characteristics (H2[ii]).

Discussion

Our examination of the population structure of the ecologically important amphipod *Gammarus fossarum*, including measurement and sexing of 5967 individuals, provides new insight into population-level responses to drying in central European streams, complementing recent community-level studies (Pastuchová 2006; Řezníčková et al. 2007; Řezníčková et al. 2010; Řezníčková et al. 2013). Our results show that short-term drying events severely reduce amphipod population densities, with the loss of juveniles and absence of reproductive females in the recolonizing assemblage potentially affecting long-term population integrity. By encompassing populations across habitats including isolated pools, dry benthic sediments and saturated hyporheic sediments, our results enable exploration of how refuge use differs between adult and juvenile members of a population.

Response of gammarid population densities to hydrological changes and drying

Our first hypothesis, that gammarid population densities would vary over time in relation to hydrological variability, was generally supported by our data. Contraction of the submerged habitat area, which H1(i) inferred as driving increasing benthic densities, did occur before the first dry phase (dry-1). Juvenile gammarid densities increased as habitat availability declined, which apparently supported H1(i) but is also likely to reflect seasonal increases in juvenile abundance. However, flowing-phase densities of all population groups peaked at 75 % SHA on the fourth date, *not* at 54 % SHA on the fifth and final pre-drying date. A notable decline in discharge and velocity occurred between these dates, which (along with intensifying biotic interactions) may have initiated voluntary drift, facilitating entrance into downstream perennial refuges (Meijering 1977; Elliott 2002; Vander Vorste et al. 2016a). Although males may be particularly common in the drift (MacNeil et al. 2003), our data suggest that all gammarids exhibit this behaviour, including females, with benthic densities of females declining by 52 % as discharge fell between dates 4 (200 individuals) and 5 (96 individuals). In addition, gammarids are positively rheotactic (Hultin 1971) and our counts of gammarid movements, recorded on video, document greater upstream migration during late flow recession than during baseflow (P. Pařil, unpublished observations).

Gammarids may also respond to submerged habitat contraction by migrating at the sediment surface ahead of a receding water line (Poznańska et al. 2013). Compared to drift, this may be a lower-risk strategy that promotes use of wet habitats. However, in temporary streams, such migrations can concentrate invertebrates within submerged habitats that contract into isolated pools (Bogan & Lytle 2011; Hill & Milner 2018). Pool inhabitants may be exposed to intense biotic interactions including predation (Berezina 2009) and declining water quality (Boulton 2003, Labaude et al. 2017), and we recorded very low minimum oxygen concentrations. Despite such metabolic stressors, we observed peak densities in isolated pools during dry phases, supporting H1(i). If water levels fall below the substrate surface, as observed in Gránický stream, pool inhabitants may be stranded on drying sediments (Extence 1981; Stubbington et al. 2009), or may migrate vertically into saturated subsurface interstices (Poznańska et al. 2013; Vadher et al. 2017). Gammarid densities were three-fold higher in freeze cores during the dry phase compared to wet phases, and were notably high in the 0–10 cm core layer in an area in which water levels had dropped < 5 cm below the bed. Although we have no statistical support for H1(ii), these observations highlight the importance of perennial subsurface refuges in promoting in-situ survival (Boulton 1989; Bogan et al. 2017).

Gammarids have limited desiccation tolerance and persist for up to a few days after free water is lost (Stubbington et al. 2009; Poznańska et al. 2013). Accordingly, benthic densities declined in Gránický stream by 98–99 % after 10-day and 20-day dry phases compared to the final pre-drying date, supporting H1(ii) and previous studies (Meyer et al. 2004; Řezníčková et al. 2007). Such reductions involved all population groups, but juveniles were more persistent than adults, accounting for 13 of 17 and all of seven individuals recorded after dry-1 and dry-2, possibly because their smaller body size enabled their migration into, persistence within, and rapid recolonization from moist subsurface interstices (McGrath et al. 2007). However, we observed very low post-drying densities of all gammarids within freeze cores, suggesting that subsurface sediments may be a graveyard for many individuals (Boulton & Stanley 1995; Young et al. 2011), especially if the water table drops below the level to which gammarids can move through interstices (Vadher et al. 2015). As such, the hyporheic zone *may* be a major recolonist source, but only where organisms are small enough to enter the interstitial environment and persist there in a viable state until surface flow resumes (Stubbington 2012; Vander Vorste et al. 2016a).

No adult gammarids were recorded on the first date post-drying (11 days after continuous flow resumed), and adults were far less abundant than juveniles in the dry-phase freeze cores, indicating that few adults persisted within the dry reach. However, after flow resumed, increasing gammarid densities reflected adult male and female but not juvenile densities, partially supporting H1(iii). Along with the high proportion of male compared to female adults on post-drying date 2 (discussed below), our observations indicate that male gammarids are important early contributors to population recovery, their ability to recolonize from upstream and downstream perennial surface waters being promoted by their strong swimming ability (Lehmann 1967; Adams & Greenwood 1983; Elliott 2005). In contrast, juvenile abundance remained low post-drying, indicating that drying events may have altered recruitment and therefore population structure, as discussed below.

Response of gammarid population structure to hydrological variability and drying

We hypothesized that differential responses of different gammarid population groups to hydrological drivers would alter population structure, with decreasing juvenile representation as discharge declined reflecting increasing predation of smaller individuals by larger individuals in a contracting submerged habitat area (Bogan & Lytle 2007; Stubbington et al. 2011). In direct contrast to this hypothesized pattern, the proportion of juveniles increased in relation to %SHA (H2[i]). This result, as well as the 92 % contribution of juveniles to isolated pool populations, suggests that seasonal reproductive activity and subsequent increases in juvenile abundance offset any increase in predation by both larger conspecifics and other predators, even though vertebrates such as salamanders (which occur in Gránický stream) also discriminate between gammarid prey on the basis of size (Ruff & Maier 2000).

We hypothesized that the proportion of juveniles in subsurface sediments would be lower during the dry phase compared to wet phases due to predation being a size-dependent trigger of vertical migration during wet phases, whereas dry-phase water loss provides size-independent impetus to migrate (H2[ii]). However, we hypothesized that this dry-phase adult influx would only occur if interstitial pathways accommodated larger individuals, and we explored this hypothesis by relating body size to substrate characteristics. Although mean body size was two-fold higher post-drying (11 mm) compared to pre-drying and during dry-2 (< 5.2 mm), very few specimens were recorded post-drying and we found no statistical support for H2(ii). Thus, the smaller size of most sediment inhabitants suggests that interstitial pathway dimensions may have physically restricted vertical movements of larger individuals in Gránický stream (McGrath et al. 2007), regardless of their impetus to migrate. Low replication, low abundance, and the influence of seasonal variability mean that we have insufficient evidence to reject H2(ii); population responses are likely to vary in relation to environmental drivers including sediment characteristics, as previously documented in the field (Descloux et al. 2013) and confirmed by laboratory experiments (Vadher et al. 2015; Vadher et al. 2017; Vadher et al. 2018b).

We hypothesized that the proportion of females compared to all adults would be lower after dry phases (H2[iii]) due to faster male recolonization (Lehmann 1967; Adams & Greenwood 1983, Elliot 2005). Although the proportional representation of females in the population was comparable pre- and post-drying, we found support for our hypothesis: the proportion of females was lowest during the between-dry phase and increased with time after flow resumption, with increasing female densities equalling or exceeding those of male gammarids from the third post-drying date. Our results suggest that, as the initial dominance of larger males with greater dispersal ability declines, females become increasingly important contributors to population resilience after flow resumes. Experimental field studies that characterize recolonization pathways (e.g. Vander Vorste et al. 2016a) are needed to determine whether male and female (and juvenile) resilience is facilitated by comparable or distinct mechanisms.

We did not formulate hypotheses regarding the abundance or proportion of reproductive females, due to the limited evidence available to inform such hypotheses. Our results are nonetheless noteworthy: whereas females with eggs or embryos were more common pre-drying, such females were absent after flow resumed. In perennial streams, *G. fossarum* reproduction continues until late September (Pöckl et al. 2003), whereas exposure to a drying event (which individuals may survive by inhabiting metabolically stressful habitats such as poorly oxygenated pools and subsurface sediments; Stubbington et al. 2011; Bogan et al. 2017) may have caused early onset of reproductive diapause, as observed in response to other stressors (Ladewig et al. 2006). Previous studies have also suggested that streambed drying, in particular associated with unpredictable drought disturbances, has long-term ecological effects due to reduced recruitment (Boulton & Lake 1992; Resh 1992). An increasing occurrence of drying may gradually reduce population integrity in temporary streams, with impacts rarely characterized due to the community focus of relevant research (Lake 2003).

Conclusions

Most ecological studies of temporary streams explore the effects of drying on communities, leaving population-level effects poorly characterized (Boulton 2003; Lake 2003; Lancaster & Ledger 2015). We show that drying changes population structure, with differential impacts on juveniles and adults skewing populations towards adult dominance. Impacts may be severe if repeated drying events occur, and if high-velocity flow resumptions displace those seeking refuge in subsurface sediments (Stubbington et al. 2016). We observed the absence of reproductive females and severe reduction in juveniles in the first months after flow resumed, highlighting the need to characterize longer-term impacts on population structure. If ecologically important taxa such as gammarids decline in abundance, reduced CPOM processing as well as energy transfer through predation and consumption may alter ecosystem function (Monroy et al. 2016), with changes to food webs potentially spanning multiple trophic levels across aquatic and terrestrial habitats, at spatial scales that reflect the extent of drying (Ledger et al. 2013). Management actions may need to target particular population groups, for example sediment manipulation to enhance the refuge potential of subsurface sediments (Boulton 2007), to promote persistence of juveniles. With changing precipitation patterns interacting with other stressors to increase the extent of intermittence in central Europe (Kadlec 2001; Laaha et al. 2017) and other regions, we call for sensitive management strategies that recognize the value of natural intermittence while supporting populations as they adapt to altered, harsher flow regimes.

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REVIEW ARTICLE

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Parallels and contrasts between intermittently freezing and drying streams: From individual adaptations to biodiversity variation

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Abstract

1. Intermittently freezing and drying lotic systems are common in many parts of the world. These ecosystems provide dynamic habitats for biota, as both freezing and drying processes result in the loss of water flow along stream and river channels. However, research into the ecological effects of intermittent freezing on stream biota has remained relatively scarce compared to the recent increase in studies exploring the ecology of intermittent streams that dry.
2. Climate change is predicted to alter the distribution and dynamics of intermittently freezing stream types. A better understanding of the responses of stream biota to intermittent freezing would inform predictions of the effects of ongoing climate change on these ecosystems.
3. In this review, we compare the effects of both freezing and drying events on stream biota, ranging from individual-level adaptations to population effects, community-level biodiversity variation and food webs.
4. Despite major contrasts in environmental conditions (e.g. the drying stream channels are exposed to a direct interaction with the riparian zone during the dry phases and the freezing stream channels are enclosed during the freezing phases), we show that both freezing and drying phenomena have pervasive effects on the structure and dynamics of aquatic stream biota.
5. At the individual organism level, aquatic taxa use a range of physiological, morphological, life-history, and behavioural responses to persist despite changing habitat conditions in both freezing and drying streams. The variety of organisms' responses can result in both biodiversity increases and decreases, which may affect population and community dynamics in both ecosystem types.
6. Future research should further explore the population and community-level effects of freezing in stream ecosystems. Due to the ecological parallels between intermittently drying and freezing streams, approaches developed in research into drying streams may also be applied in freezing stream ecosystems. This could improve our understanding of climate change effects on freezing stream ecosystems.

KEYWORDS
ecological similarity, environmental harshness, ice, intermittency, lotic systems

1 | INTRODUCTION

Intermittent rivers and ephemeral streams (IRES; see Table 1 for further explanation of terms used in the review) that result from periodic drying of stream channels covers over 50% of the length of the global river network and are common in arid, semiarid, and Mediterranean climate regions (Datry, Larned, & Tockner, 2014). Past research on IRES has mainly focused on drying streams (Figure 1a), although, parallel to IRES, there are rivers and streams that sometimes freeze completely (Figure 1c). This phenomenon, in which all liquid water in a stream channel freezes to solid ice that reaches the streambed can be conceptualised as equivalent to the loss of surface water in drying IRES.

These freezing IRES (FIRES) are governed by seasonal freeze–thaw cycles (Allard, Buffin-Bélanger, & Bergeron, 2011). For example, it is estimated that 60% of river basins in the Northern Hemisphere experience some kind of freeze–thaw cycles during the winter (Allard et al., 2011), and the severity of these processes varies depending on changes in hydrological conditions, snowfall, and temperature (Shen, 2016). Whereas glacial streams are formed by channelised accumulation of water on, in, or under a glacier (Bennett & Glasser, 2010), FIRES can occur in unglaciated landscapes. The changing ice conditions in FIRES have direct and indirect effects on physical, chemical, and biological processes in freezing stream ecosystems (Prowse et al., 2006). However, based on extensive literature searches and personal observations by the authors, studies exploring the ecological effects of ice formation on freshwater ecosystems and their biodiversity are scarce when considering the generality of freezing processes at higher latitudes and altitudes in both hemispheres. In particular, only few papers (Olsson, 1981; Parker & Huryn, 2013) have examined streams that freeze totally down to the streambed. This lack of research on FIRES probably results from logistic difficulties in conducting research in streams during winter (e.g. due to low temperatures) and because of their remote geographical locations (e.g. in alpine environments and at high latitudes). However, a better understanding of the effects of freezing on stream biodiversity is urgently needed, because ongoing climate change is increasing temperatures, especially at high latitudes (Cohen et al., 2014), with the most pronounced changes expected to occur during winters (IPCC, 2013). These changes will alter ice conditions, such as the thickness and duration of ice cover, in stream ecosystems (IPCC, 2013; Stickler, Alfredsen, Linnansaari, & Fjeldstad, 2010). Evidence of postponed freezing and earlier thawing of ice cover has already been observed in high-latitude streams (Lind & Nilsson, 2015).

Because the loss of free water in both IRES and FIRES alters the connectivity and availability of suitable habitats for aquatic species, FIRES may experience similar ecological consequences to IRES that dry out completely (Figure 2). In recent years, an increasing body of literature has improved our knowledge of the ecology of streams that dry, helping researchers to predict biotic responses of IRES communities to drying (Datry, Bonada, & Boulton, 2017; Leigh et al., 2016). However, FIRES remain a research challenge. Therefore, understanding the potential ecological similarities between intermittently freezing and drying streams could encourage researchers to apply approaches developed in IRES research to FIRES, and hence increase our knowledge of these freezing ecosystems.

In this review, we explore the parallels and contrasts between aquatic communities in FIRES and IRES ecosystems. We compare the effects of total freezing and total drying of stream channels on stream biota, ranging from individual organism-level adaptations to population dynamics, community-level biodiversity variation and food webs. We also highlight important FIRES research priorities.

2 | AQUATIC SPECIES HAVE DIVERSE ADAPTATIONS TO FREEZING AND DRYING

A range of physiological, morphological, life-history, and behavioural traits have been documented among aquatic species living in either freezing or drying streams. These traits can be divided into resistance (i.e. tolerating freezing or drying in situ) and resilience (i.e. avoiding freezing or drying by moving into a refuge or by recolonising the disturbed sites from refuges) responses, which allow individuals, populations, and communities to recover after a freezing or drying phase (Fritz & Dodds, 2004; Hershkovitz & Gasith, 2013; Strachan, Chester, & Robson, 2015; Townsend & Hildrew, 1994).

2.1 | Organism-level adaptations in cyanobacteria, algae, and plants

Streams that experience complete freezing or drying are occupied by species that have the appropriate traits and adaptations to prevail or avoid the extreme environmental conditions (Cavalli, Baattrup-Pedersen, & Riis, 2014; Southwood, 1977). Freshwater algae including chlorophytes and bacteria such as cyanophytes can survive periods of freezing due to resistant vegetative cells with thick cell walls, accumulation of reserve products within their cytoplasm, and lowered molecular weight solutes that lower their freezing point and prevent intracellular ice crystallisation (Hawes, 1990; Sheath, Vis, Hambrook, & Cole, 1996; Vincent & Howard-Williams, 1986). Some algal species also remain dormant in streambeds while waiting for the ice to thaw (Howard-Williams, Vincent, Broady, & Vincent, 1986). For example, Antarctic stream communities dominated by taxa adapted to severe freezing include cyanophytes such as *Phormidium*, *Oscillatoria*, *Nostoc*, and *Gloeocapsa*; chlorophytes such as *Binuclearia*, *Prasiola*, and *Tribonema*; and the pennate diatoms *Navicula* and *Hantzschia* (Howard-Williams et al., 1986).

Similar adaptations can be found in algae in drying streams, with algae such as the chlorophyte *Oedogonium* growing thickened cell walls and cystic cells to resist desiccation (Timoner, Buchaca, Acuña, & Sabater, 2014). Algal species can also have different growth forms in dry conditions, for example *Cymbella* and *Gomphonema* diatoms can form tubes or stalks that place cells within desiccation-tolerant filaments (Sabater et al., 2017). Some algae can also remain in the

TABLE 1 Glossary of the main terms used in the review

Term	Definition	References
Abbreviations		
IRES	Intermittent rivers and ephemeral streams: lotic systems that periodically cease to flow and may experience partial or complete streambed drying	Datry et al. (2017)
FIRES	Freezing intermittent rivers and ephemeral streams: lotic systems that periodically experience freezing of whole water column	
Other terms		
Anhydrobiosis	A state of reduced metabolism in which an organism loses most of its body water and produces substances that protect cells from desiccation-related damage. Normal metabolism resumes when favourable environmental conditions are restored	Guidetti and Jönsson (2002)
Antifreeze proteins	Substances that prevent the freezing of body fluids or tissues in sub-zero temperatures	Duman (2015)
Cryoprotectant	A substance that protects biological tissues and cells from the detrimental effects of freezing, mainly by preventing ice crystal formation	Karow (1969)
Diapause	A dormant state that arthropods enter in response to a predictable environmental stress. Metabolism slows and development ceases in response to external environmental signals and an internal biological clock. Development resumes when diapause is broken by an external stimulator such as increasing day length or specific temperatures	Gill, Goyal, and Chahil (2017)
Freeze-avoidant species	Species that avoid freezing behaviourally or by preventing their body fluids from freezing by using cryoprotectants and antifreeze proteins and by removing ice-nucleating agents through gut clearing	Frisbie and Lee (1997)
Freeze-tolerance species	Species that survive freezing of their body fluids by controlling extracellular ice formation by using ice-nucleating agents in their haemolymph, which promote freezing at relatively high sub-zero temperatures	Frisbie and Lee (1997)
Hyporheic zone	The sediments beneath and alongside a streambed, where shallow groundwater and surface water mix	Orghidan (2010)
Ice-nucleating agents	Minute solid particles or large molecules and proteins that promote formation of ice crystals by binding to water molecules	Duman (1982)
Inoculative freezing	Internal ice formation	Frisbie and Lee (1997)
Littoral	Shallow water zone near the edge of a river channel	Olsson (1981)
Quiescence	A response of an organism to an unpredictable environmental stress in which metabolism is reduced and development ceases	Gill et al. (2017)
Refuge	A place where a species can avoid detrimental environmental conditions	Bogan et al. (2015) and Huryn et al. (2005)
Reserve products	Components of algal cells such as starch and lipids, which support cell growth and survival by buffering variation in energy and carbon supplies	Vitova, Bisova, Kawano, and Zachleder (2015)
Supercooling	A process in which the temperature of liquids or gases lower below their freezing point without turning solid	Ashton (1983)

moist streambed sediments while waiting for water flow to resume (McKew, Taylor, McGenity, & Underwood, 2011).

To remain in their preferred habitat, some aquatic plants, such as pondweed (*Potamogeton*) species, can avoid being displaced by scouring ice through the formation of overwintering organs such as frost-tolerant or cold-resistant tubers, turions, and buds, or dormant apices and stems (Lind, Nilsson, Polvi, & Weber, 2014; Renman, 1989). In addition, vegetative growth and reproduction help plants to survive in frozen reaches (Lind et al., 2014; Nichols, Schloesser, & Hudson, 1989). Some vascular plants can even survive being enclosed in ice, as long as the basal parts of their rosettes remain unfrozen in the substrate (Renman, 1989).

In dry channels, plant persistence can also be facilitated by organs below the streambed surface. These plants then continue to grow from below-ground buds after flow resumes, even if their above-ground biomass decays during the dry period. Some aquatic plants further rely on diaspores and seed banks that begin to develop after water returns (Sabater et al., 2017). Physiological changes also promote resistance to drying in vascular plants. For instance, forget-me-nots (*Myosotis scorpioides*) can increase their leaf thickness (Germ & Gaberščik, 2003), and water lobelias (*Lobelia dortmanna*) can decrease the water content in their tissues to better withstand gravity changes in drying streams (Pedersen & Sand-Jensen, 1992; Sabater et al., 2017). Some plants, such as yellow water buttercup

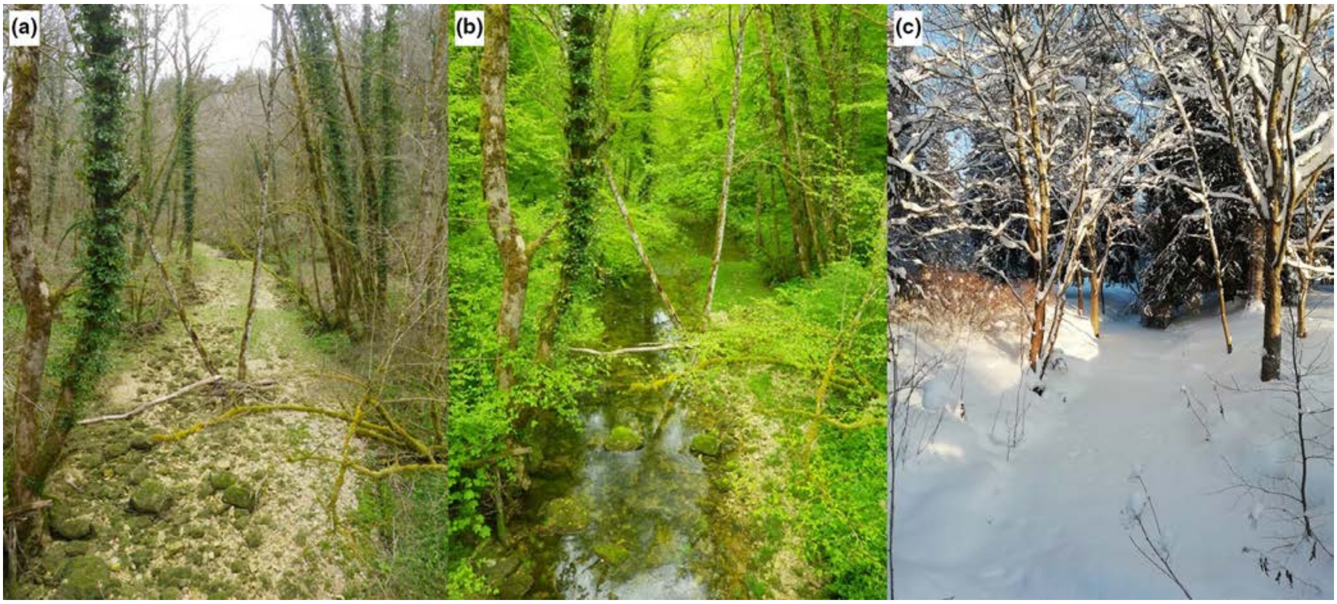

FIGURE 1 Intermittently freezing and drying streams in different phases: the Audeux River, an intermittent stream during a dry phase (a) and a flowing phase (b); and a frozen forest stream under snow and ice cover (c). Photos a and b are from the Doubs, France; Credit: B. Launay. The frozen forest stream (c) is located in Oulu, Finland (Credit: K.E. Tolonen) [Colour figure can be viewed at wileyonlinelibrary.com]

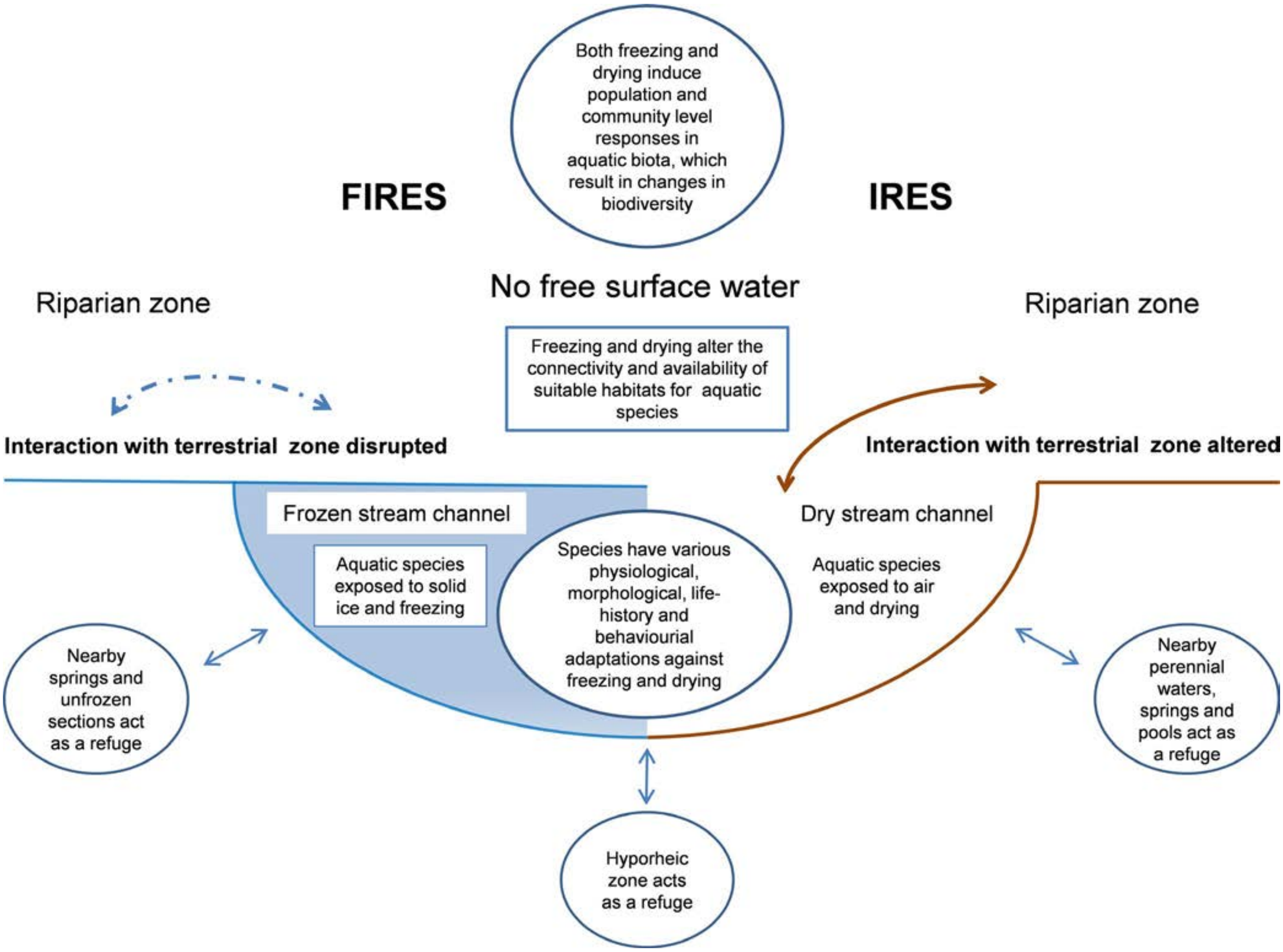

FIGURE 2 A schematic figure comparing how habitat connectivity and biota are altered by intermittency in freezing intermittent and ephemeral stream (FIRES) and in drying intermittent and ephemeral stream (IRES) channels [Colour figure can be viewed at wileyonlinelibrary.com]

(*Ranunculus flabellaris*) and the pondweed *Potamogeton wrightii*, can increase the thickness of their leaf cuticle or their stomatal density to regulate water and gas exchange (Sabater et al., 2017).

2.2 | Organism-level adaptations in animals

Like plants, animals living in either FIRES or IRES rely on various strategies to survive in their continuously changing habitats (Poff, 1997; Southwood, 1977). Physiological adaptations, such as freeze-avoidance and freeze-tolerance promote the resistance of organisms to ice, and often explain the ability of aquatic invertebrates to tolerate freezing conditions (Block, 1982; Danks, 2007; Irons, Miller, & Oswood, 1993; Oliver, 1968; Walters, Sformo, Barnes, & Duman, 2009). Macroinvertebrate survival is strongly influenced by direct contact with external ice, which induces faster and thus more adverse inoculative freezing, or avoidance of direct ice contact when supercooling enables them to withstand lower temperatures (Frisbie & Lee, 1997). Freeze-avoiding macroinvertebrates protect themselves by supercooling and maintaining their body fluids in a liquid state even below their natural freezing point. Species can lower their supercooling point by synthesising cryoprotectant and antifreeze proteins in the haemolymph, and by removing ice-nucleating agents through gut clearing (Lencioni, 2004). Freeze-tolerant species (e.g. the chironomids *Pseudodiamesa branickii* and *Diamesa cinerella*; Lencioni, Jousson, Guella, & Bernabò, 2015) can also tolerate extracellular ice formation through production of ice-nucleating agents in their haemolymph, which helps to control ice-crystal formation in their body fluids (Lee, 1989). This controlled extracellular ice formation helps to prevent cellular damage and provides time for metabolic adjustments to freezing (Block, Baust, Franks, Johnston, & Bale, 1990). For instance, the stonefly *Nemoura arctica* can survive being enclosed in ice by producing both glycerol and ice-binding factors (Walters et al., 2009). These molecular mechanisms, combined with an ability to migrate into the unfrozen hyporheic zone (Boulton, 2000; Orghidan, 2010), can even allow some macroinvertebrates to stay active in refuge areas during winter without entering a quiescent or diapausing state (Lencioni et al., 2015; Silveri, Tierno de Figueroa, & Maiolini, 2008).

It is also common for invertebrates to have life-history traits such as long life cycles, quiescence and diapause, which enable their development to extend over, and beyond, a cold season (Brittain, 1982; Danks, 2007). During quiescence or diapause, development is temporarily suspended, to tolerate adverse environmental conditions. Another frequently employed life-history adaptation that promotes resilience in cold environments is a reduction in the number of generations per year, and univoltinism or bivoltinism are favourable strategies in environments where the breeding season is very short (Danks, 1999; Lencioni, 2004).

For macroinvertebrate species that have adapted to withstand severe winter conditions enclosed in ice, the survival rates can be high (Mihalicz, 2015; Olsson, 1981; Walters et al., 2009). Olsson (1981) demonstrated a survival rate between 82 and 100% for individual macroinvertebrates enclosed in ice in the frozen substratum of a littoral zone of a river. Further, living individuals of various worms (Nematoda, Oligochaeta), snails (Gastropoda), fingernail clams (Sphaeriidae), leeches (Hirudinea), waterlice (Isopoda), caddisflies (Trichoptera), and non-biting midges (Chironomidae) were found in the frozen substratum (Olsson, 1981). Many of the enclosed species, such as the snail *Gyraulus acronicus*, the worm *Lumbriculus variegatus* and some chironomid midges, had formed resting cysts, cocoons, and epiphragms that are not found when species are in an active state (Olsson, 1981). These constructions may enable the species to endure the mechanical stress exerted by ice (Danks, 2007). Other macroinvertebrate taxa, such as some mayfly (Ephemeroptera: Baetidae), stonefly (Plecoptera: Perlodidae), and blackfly (Diptera: Simuliidae) species, can also survive stream freezing in their egg stage (Clifford, 1969; Kurtak, 1974; Lillehammer, Brittain, Saltveit, & Nielsen, 1989).

Overall, these mechanisms resemble those that protect macroinvertebrates in drying IRES from desiccation (Bogan et al., 2017; Strachan et al., 2015). Invertebrate species can, for example, form different dormant stages to withstand dry conditions (Cover, Seo, & Resh, 2015; López-Rodríguez, Tierno de Figueroa, Fenoglio, Bo, & Alba-Tercedor, 2009). Species belonging to families such as the Enchytraeidae (Oligochaeta) can make desiccation-tolerant cocoons (Maraldo, Ravn, Slotsbo, & Holmstrup, 2009), whereas some copepods form resting-stage eggs when exposed to drying (Strachan et al., 2015). Some stoneflies and caddisflies can form desiccation-tolerant eggs in dry conditions (Strachan et al., 2015). Some IRES species can further survive long dry periods by entering an anhydrobiotic state in which dehydrated membranes and other cellular structures are stabilised by molecules, such as proteins and non-reducing sugars, which prevent desiccation from causing lethal structural damage (Crowe, Hoekstra, & Crowe, 1992; Strachan et al., 2015). Invertebrate species in IRES can also have different life-history adaptations in response to drying (López-Rodríguez, Tierno de Figueroa, & Alba-Tercedor, 2009). For instance, the stonefly *Brachyptera vera cordubensis* shows a fast univoltine life cycle with egg dormancy during dry phases. In contrast, the stonefly genus *Tyrrhenoleuctra* includes species that have a univoltine life cycle including a long adult flight period and nymphal dormancy during the dry phase. *Tyrrhenoleuctra* also shows signs of ovoviviparism in IRES, as its eggs are fully developed when laid, and hatch rapidly after laying when the flow resumes (López-Rodríguez, Tierno de Figueroa, & Alba-Tercedor, 2009).

The main behavioural adaptation promoting species resilience to freezing in running waters is to withdraw to habitats that do not freeze. For example, mayflies, stoneflies, and amphipods (Amphipoda) can be abundant in river margins during summer, but are not present there during winter, indicating that individuals have migrated from the freezing shoreline to unfrozen sections of the river (Olsson, 1981). Species may also avoid the freezing streambed by moving to nearby springs, where water generally remains ice-free and water temperatures are more stable due to the thermal influence of groundwater (Jones, Kielland, & Hinzman, 2015). Springs also provide critical winter refuges, for example, for perlodid stoneflies that withstand freezing (Huryn et al., 2005). Insects may also migrate vertically into the hyporheic zone of the streambed (Irons et al., 1993; Orghidan, 2010).

These behavioural responses are comparable to those of fauna in drying streams (Strachan et al., 2015), where species escape drying through means of dispersal such as drift, crawling and swimming (Boersma & Lytle, 2014; Paltridge, Dostine, Humphrey, & Boulton, 1997; Pires, Beja, & Magalhães, 2014; Robson, Matthews, Lind, & Thomas, 2008). Species can also burrow into the saturated subsurface sediments of the hyporheic zone (Kawanishi, Inoue, Dohi, Fujii, & Miyake, 2013; Timoner, Borrego, Acuña, & Sabater, 2014), although the usage of the hyporheic zone as a refuge from drying has also been questioned (del Rosario & Resh, 2000), and the use of the hyporheic zone can depend on the characteristics of the subsurface sediments and on the taxon in question (Stubbington, 2012). Species can also seek refuge in nearby permanent pools or perennial upstream and downstream reaches (Bogan, Boersma, & Lytle, 2015; Paltridge et al., 1997; Pires et al., 2014), from which recolonists arrive after flow returns. In addition, some species spend the dry period as aerial terrestrial forms (Bogan & Boersma, 2012). For instance, some stonefly and caddisfly species have aerial adults that aestivate in humid caves (Dobat, 1973; Salavert, Zamora-Muñoz, Ruiz-Rodríguez, Fernández-Cortés, & Soler, 2008).

3 | THE EFFECTS OF FREEZING AND DRYING ON POPULATIONS AND COMMUNITIES

3.1 | Influences on populations

Freezing and drying in lotic ecosystems can be major factors affecting aquatic organisms at the population level (Borgstrøm & Museth, 2005). Complete freezing of streams can cause changes in population structure by affecting the recruitment of new individuals and by changing species distributions and abundances. Solid ice can limit the access of migratory fish to their spawning grounds in rivers (Bergeron, Roy, Chaumont, Mailhot, & Guay, 1998), or exclude fish from their summer habitats (Parker & Huryn, 2013). Streambed freezing can also affect the recruitment of young fish, such as brown trout (*Salmo trutta*), into local populations when small nursery streams with deposited eggs freeze completely (Borgstrøm & Museth, 2005). However, in some cases, small fish such as young salmonids can use the hyporheic zone as a refuge at least during ice formation (Heggenes, Bremset, & Brabrand, 2010).

Drying causes fragmentation of populations and complicates fish migrations by disrupting migration pathways (Marshall et al., 2016; Pires et al., 2014). The hyporheic zone can support population maintenance of aquatic organisms in drying streams, by acting as a refuge for benthic fish and invertebrates following surface drying in IRES (Kawanishi et al., 2013; Vander Vorste, Corti, Sagouis, & Datry, 2016; Vander Vorste, Malard, & Datry, 2016). Drying events can further affect population densities and population structure in IRES. For example, Pařil et al. (2019) demonstrated that short-term drying events severely reduce amphipod population densities and result in the loss of juveniles and reproductive females in the population in the first months after the flow resumed. The absence of juveniles was explained by their relatively weak dispersal capacities compared to faster and stronger swimming adults, which recolonised sites more quickly. The absence of reproductive females may reflect the physiological stress caused by drying, which could have caused early onset of reproductive diapause, as observed in response to other environmental stressors (Ladewig et al., 2006). Overall, such effects of drying could reduce long-term population persistence (Pařil et al., 2019).

3.2 | Influences on communities and biodiversity

Freezing and drying have both positive and negative effects on the biodiversity of stream communities. For instance, ice can serve directly as a habitat for algae and microbes during winter, hence increasing their total diversity (Frenette, Thibeault, Lapierre, & Hamilton, 2008). Further, ice-scouring can affect the abundance and distribution of aquatic plant and algae species by detaching them from the stream sediments (Rounick & Gregory, 1981; Scrimgeour, Prowse, Culp, & Chambers, 1994; Virtanen, Muotka, & Saksa, 2001), creating free habitat patches available for colonisation by additional species and thus increasing total species diversity (Lind et al., 2014; Virtanen et al., 2001). As a result, Virtanen et al. (2001) suggested that ice formation and scouring could be an important determinant of bryophyte community composition in northern streams, with frequent ice scouring preventing any species from becoming dominant and hence increasing local species diversity.

Substratum freezing can also affect the structure of communities (Parker & Huryn, 2011). When the streams freeze completely, communities can shift from a frost-sensitive to a frost-tolerant taxonomic composition (Erixon, 1979). Furthermore, Parker and Huryn (2011) compared the macroinvertebrate community structure of different stream types with different freezing propensities and identified distinct assemblages in different streams. In streams with evidence of substratum freezing, macroinvertebrate biomass mainly comprises species known to survive in frozen environments (Irons et al., 1993). In contrast, streams with perennial flow had the lowest proportion of biomass of freezing-tolerant taxa, whereas intermediate proportions of both types were found in streams where springs were present (Huryn et al., 2005). As a result, Huryn et al. (2005) suggested that the groundwater or hyporheic sources of the spring-fed streams may have provided a refuge from the freezing substratum (Orghidan, 2010).

In IRES, species richness and composition are also affected by temporal variability in the hydrological conditions (Dieterich & Anderson, 2000; Katz, Denslow, & Stromberg, 2012; Rolls, Heino, & Chessman, 2016). In general, drying has negative effects on local biodiversity (Datry, Larned, Fritz, et al., 2014; Datry, Larned, & Tockner, 2014; Timoner, Borrego, et al., 2014; Timoner, Buchaca, et al., 2014), and species in dry-phase aquatic communities are typically dominated by subsets of communities present in streams during wet phases (Datry, Larned, Fritz, et al., 2014; Zeglin et al., 2011). However, drying IRES can have higher overall biodiversity due to change over time. Drying can allow channels to be temporarily colonised by terrestrial species, as well as by lentic taxa if pools remain within a largely dry channel (Bogan & Lytle, 2007; Corti & Datry, 2016; Stubbington, England, Wood, & Sefton, 2017). IRES can therefore support higher total species diversity (i.e. β and γ diversity) than aquatic habitats with more stable water conditions, due to lotic, lentic and, terrestrial taxa *taking turns* to occupy a single location (Bogan & Lytle, 2007; Katz et al., 2012; Sabater et al., 2017). Drying also induces changes in the structure of stream communities. For instance, Santos and Stevenson (2011) showed how the different flow regimes (perennial, intermittent, and ephemeral) in headwater streams supported distinct invertebrate community types, with a transition in functional feeding groups from perennial to ephemeral sites (Santos & Stevenson, 2011).

3.3 | Influences on food webs

Freezing of streams also affects the structure of food webs. For instance, ice cover blocks the light that streambed algal and microbial communities need to grow, hence affecting the primary production and autotrophy (Frenette et al., 2008). Parker and Huryn (2013) further examined how disturbance imposed by freezing structures food webs in FIRES of Arctic Alaska. Their results indicated that disturbances caused by bed movements and freezing resulted in low-complexity food webs (i.e. lower linkage densities and connectance) compared to communities in more stable streams (Parker & Huryn, 2013). The absence of fish also resulted in shorter food chain lengths in these streams. It was suggested that fish were excluded from some streams because of winter freezing and because of a lack of hydrological connections after spring thaw, which prevented colonisation during summer. Freezing can hence act as an environmental filter that affects community and food web structures in streams (Parker & Huryn, 2013; Scrimgeour et al., 1994).

Intermittency by drying also influences food webs in streams. For instance, changes in microbial communities during dry phases change the way in which detritus is processed (Datry et al., 2018), and food webs usually sustained by autotrophy during flowing phases moves to heterotrophy with drying (Reid, Quinn, Lake, & Reich, 2008). Drying also reduces the linkages, chain lengths, and connectivity of food webs in streams (Ledger, Brown, Edwards, Milner, & Woodward, 2013). Bogan and Lytle (2007) reported increases in the proportion of predators from 25% during high flows to 75% during low flows, increasing the predator–prey ratios as well as predator densities in remnant pools (Walters & Post, 2011). Overall, IRES food webs are characterised by smaller-sized consumers as large-bodied top predators tend to disappear during complete drying (Bogan & Lytle, 2011). Thus, food chain length in IRES becomes shorter compared to neighbouring perennial rivers (McHugh, Thompson, Greig, Warburton, & McIntosh, 2015). However, in contrast to FIRES where the ice cover interrupts the connection between streams and land during winters, drying enables food webs to extend into terrestrial habitats (Corti & Datry, 2016; McIntosh et al., 2017). This gives riparian and terrestrial predators belonging to groups such as mammals, birds, lizards, and spiders the opportunity to eat aquatic organisms in drying pools (McIntosh et al., 2017). This increases the importance of terrestrial components in IRES food webs during dry phases (Boulton & Suter, 1986).

4 | SYNTHESIS AND AVENUES FOR FUTURE RESEARCH

In this review, we compare the effects of two types of surface water loss—drying and freezing—on individuals, populations, and communities of aquatic organisms in IRES and FIRES ecosystems (Table 2). Despite the fundamental environmental differences that distinguish drying streams, in which terrestrial habitats can extend into the channel, from freezing streams, in which ice creates a barrier between habitats, the loss of water affects the structure and dynamics of stream biota in both FIRES and IRES. Similar types of organism-level adaptations are used to ensure the long-term persistence of communities in both stream types. Responses are also observed at the population, community, and food-web levels. Although the mechanisms between the two steam types may differ, these different responses cause biodiversity to increase or decrease, affecting the dynamics of biota in both ecosystem types.

To date, ecological research on IRES has mainly focused on drying streams (Datry, Fritz, & Leigh, 2016; Leigh et al., 2016) and *freezing ecology* of aquatic environments in streams has not been thoroughly studied. In particular, studies on the ecological effects of total water column and bottom freezing in stream channels are scarce, making it difficult to make general conclusions about the effects of freezing on FIRES biodiversity. This lack of research in FIRES is concerning, as the percentage of river basins experiencing annual ice-processes in the Northern Hemisphere during winter is as high as 60% (Allard et al., 2011) and, just as drying affects ecosystem structure and functioning in IRES (von Schiller, Bernal, Dahm, & Martí, 2017), ice can have notable effects on physical and biological processes in FIRES (Allard et al., 2011; Prowse et al., 2006). Hence, more studies on the ecological effects of freezing on stream ecosystems should be implemented. This is even more urgent now that climate change-induced alterations in freezing patterns together with other anthropogenic stressors, such as increasing water abstraction (Rixen et al., 2011), will probably affect the winter habitat conditions for stream biota. For instance, intensive water abstraction for artificial snowmaking in ski resorts during natural low-flow periods in late autumn (de Jong, Lawler, & Essery, 2009) can lead to more frequent freezing–drying episodes. These changes could cause cascading and unpredictable effects throughout freezing aquatic ecosystems.

Changes in freezing–thawing cycles in FIRES due to climate change could alter their biodiversity, if species adapted to cope with certain ice conditions are faced with novel environments. Future climate scenarios predict that FIRES ecosystems will become less

TABLE 2 Summary table of the adaptations and responses of species, populations, communities, and food webs in freezing intermittent rivers and ephemeral streams (FIRES) and in drying intermittent rivers and ephemeral streams (IRES)

Level of organisation	Example organisms	Adaptations and responses: FIRES	Adaptations and responses: IRES	References
Organisms	Algae, bacteria	Vegetative or dormant cells with thickened cell walls	Thickened cell walls and cystic cells	FIRES: Hawes (1990), Howard-Williams et al. (1986), Sheath et al. (1996), and Vincent and Howard-Williams (1986)
		Accumulation of reserve products within cytoplasm	Dormant cells	IRES: McKew et al. (2011) and Sabater et al. (2017)
		Lowered molecular weight solutes	Different growth forms	
	Aquatic plants	Overwintering organs such as turions, buds, or dormant apices and stems	Below-ground buds	FIRES: Lind et al. (2014), Nichols et al. (1989), and Renman (1989)
		Vegetative growth and reproduction	Diaspores and seed banks	
			Changes in tissue water content	IRES: Germ and Gaberščik (2003), Pedersen and Sand-Jensen (1992), and Sabater et al. (2017)
			Changes in leaf thickness	
	Invertebrates	Inoculative freezing	Anhydrobiotic state	FIRES: Block (1982), Boulton (2000), Danks (2007), Irons et al. (1993), Lencioni (2004), Oliver (1968), Olsson (1981), and Walters et al. (2009)
		Avoidance of direct ice contact	Univoltinism	
		Supercooling	Long adult flight period	
		Controlled extracellular ice formation	Nymphal dormancy	
		Long life-cycles	Ovoviviparism	
		Quiescence and diapause	Desiccation-tolerant cocoons, resting-stage eggs and desiccation-tolerant eggs	IRES:Bogan et al. (2017), Cover et al. (2015), Crowe et al. (1992), López-Rodríguez, Tierno de Figueroa, Fenoglio, et al. (2009), López-Rodríguez, Tierno de Figueroa, and Alba-Tercedor (2009), Maraldo et al. (2009), and Strachan et al. (2015)
		Univoltinism and bivoltinism		
		Resting stages of cysts, cocoons and epiphragms		
	Macroinvertebrates, fish	Movement to refuges such as springs and the hyporheic zone	Escape drying through means of dispersal such as drift, crawling and swimming to remnant pools, springs, hyporheic zone and other perennial reaches	FIRES: Heggenes et al. (2010), Huryn et al. (2005), Irons et al. (1993), Olsson (1981), and Orghidan (2010)
			Aestivation in humid caves	IRES: Boersma and Lytle (2014), Bogan et al. (2015), Dobat (1973), Paltridge et al. (1997), Pires et al. (2014), Robson et al. (2008), and Salavert et al. (2008)
Populations	Fish	Changes in population structure by affecting the recruitment of new individuals	Difficulties for fish migrations	FIRES: Bergeron et al. (1998), Heggenes et al. (2010), and Parker and Huryn (2013)
		Difficulties for fish migrations	Fragmentation of populations	IRES: Kawanishi et al. (2013), Marshall et al. (2016), Pařil et al. (2019), Pires et al. (2014), Vander Vorste, Corti, et al. (2016), and Vander Vorste, Malard, and Datry (2016)
		Fragmentation of populations	Altered population densities and population structures	

(Continues)

TABLE 2 (Continued)

Level of organisation	Example organisms	Adaptations and responses: FIRES	Adaptations and responses: IRES	References
Communities	Algae, aquatic plants, macroinvertebrates	Both positive and negative effects on biodiversity	Both positive and negative effects on biodiversity	FIRES: Frenette et al. (2008), Huryn et al. (2005), Parker and Huryn (2011), Rounick and Gregory (1981), Scrimgeour et al. (1994), and Virtanen et al. (2001)
		Changes in community composition and structure	Changes in community composition and structure	IRES: Bogan and Lytle (2007), Datry, Larned, Fritz, et al. (2014), Dieterich and Anderson (2000), Katz et al. (2012), and Rolls et al. (2016)
				Timoner, Borrego, et al. (2014), Timoner, Buchaca, et al. (2014), and Sabater et al. (2017)
Food webs		Changes in primary production	Changes in primary production	FIRES: Frenette et al. (2008), Parker and Huryn (2013), and Scrimgeour et al. (1994)
		Lower levels of linkage densities and connections	Reduced linkage, length and connectivity of food webs	IRES: Ledger et al. (2013) and Reid et al. (2008)

fragmented and warmer, and, if biota cannot adapt to such changes, the structure of ecological communities in freezing streams could experience substantial alterations. Comparative studies of the potential effects of changing habitat dynamics on multiple biological groups in FIRES are needed. Because of the similarities between intermittently freezing and drying streams, applying approaches developed in IRES research, and comparing findings from IRES with those from FIRES could increase our knowledge of these overlooked freezing ecosystems. A better understanding of FIRES ecology will in turn help us to predict the effects of climate change on these ecosystems and manage them efficiently in the future.

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Review

Biomonitoring of intermittent rivers and ephemeral streams in Europe: Current practice and priorities to enhance ecological status assessments

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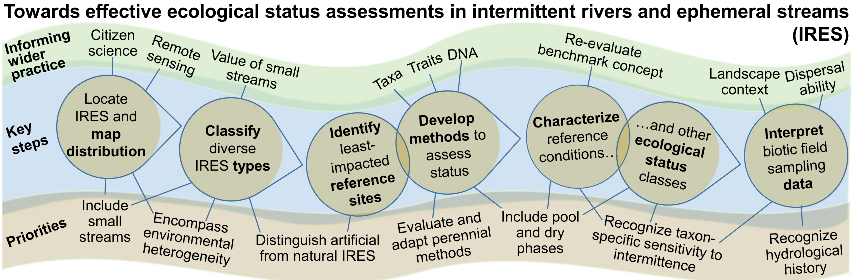
HIGHLIGHTS

- Flow intermittence creates challenges for ecological status assessments.
- Classifying ecologically relevant typologies will underpin future biomonitoring.
- Methods used in perennial rivers need evaluation before use in temporary rivers.
- Metrics may need adaptation due to taxon-specific sensitivity to intermittence.
- Novel biomonitoring tools (e.g. DNA, terrestrial biota) also require development.

GRAPHICAL ABSTRACT



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ABSTRACT

Intermittent rivers and ephemeral streams (IRES) are common across Europe and dominate some Mediterranean river networks. In all climate zones, IRES support high biodiversity and provide ecosystem services. As dynamic ecosystems that transition between flowing, pool, and dry states, IRES are typically poorly represented in biomonitoring programmes implemented to characterize EU Water Framework Directive ecological status. We report the results of a survey completed by representatives from 20 European countries to identify current challenges to IRES status assessment, examples of best practice, and priorities for future research. We identify five major barriers to effective ecological status classification in IRES: 1. the exclusion of IRES from Water

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Abbreviations: BQE, biological quality element; COST, European Cooperation in Science and Technology; EU, European Union; GES, good ecological status; GIG, Geographical Intercalibration Group; IRES, intermittent rivers and ephemeral streams; RBD, River Basin District; SMIRES, Science and Management of Intermittent Rivers and Ephemeral Streams; WFD, Water Framework Directive.

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Framework Directive biomonitoring based on their small catchment size; 2. the lack of river typologies that distinguish between contrasting IRES; 3. difficulties in defining the 'reference conditions' that represent unimpacted dynamic ecosystems; 4. classification of IRES ecological status based on lotic communities sampled using methods developed for perennial rivers; and 5. a reliance on taxonomic characterization of local communities. Despite these challenges, we recognize examples of innovative practice that can inform modification of current biomonitoring activity to promote effective IRES status classification. Priorities for future research include reconceptualization of the reference condition approach to accommodate spatiotemporal fluctuations in community composition, and modification of indices of ecosystem health to recognize both taxon-specific sensitivities to intermittence and dispersal abilities, within a landscape context.

Contents

1. Introduction

Temporary rivers and streams, which are defined by periodic flow cessation and often experience loss of some or all surface water (Datry et al., 2014a; Leigh et al., 2015), are common in oceanic and continental temperate regions (Snelder et al., 2013; Stubbington et al., 2017), and can dominate mediterranean-climate and semi-arid river networks (Skoulikidis et al., 2011, 2017; Tockner et al., 2009). These ecosystems are often classified as intermittent rivers or ephemeral streams (IRES), with intermittent rivers typically conceptualized as systems with relatively long, seasonal flowing phases, compared to precipitation-driven hydrological unpredictability in ephemeral streams. However, the intermittent-ephemeral boundary is indistinct and encompasses only two of many possible intermittence regimes, from near-perennial to episodic flow (Cid et al., 2017; Datry et al., 2017a; Uys and O'Keeffe, 1997). Despite this variability, for simplicity, we use *IRES*, *temporary*, and *intermittence* as terms encompassing *all* lotic ecosystems that experience flow cessation and/or drying, and for clarity, we provide descriptive detail in each instance where a particular type of intermittence is considered.

IRES flowing-phase communities can be diverse in multiple aquatic groups including diatoms (Tornés and Ruhí, 2013), macrophytes (Westwood et al., 2006), invertebrates (Bonada et al., 2007; Datry, 2012), and fish (Pires et al., 1999). Although local-scale taxonomic diversity typically declines with increasing intermittence (Davey and Kelly, 2007; Datry et al., 2014b; Tornés and Ruhí, 2013), diversity among sites (i.e. spatial β-diversity) can be higher in IRES compared to perennial systems due to habitat heterogeneity (Schriever and Lytle, 2016; Tornés and Ruhí, 2013; Westwood et al., 2006; but see Datry et al., 2016a) and dispersal limitation, in particular in isolated headwaters (Brown and Swan, 2010; Sarremejane et al., 2017). Equally, taxonomic diversity among times (i.e. temporal β-diversity) can be enhanced in temporary compared with perennial systems due to fluctuations in community composition between lotic, lentic, and terrestrial phases (Bogan and Lytle, 2007; Corti and Datry, 2015; Ruhí et al., 2017). These diversity contributions can result in higher regional-scale biodiversity in networks that include temporary reaches (Katz et al., 2012; Larned et al., 2010; Stubbington et al., 2017).

Recognition of IRES biodiversity and ecosystem service provision across all hydrological phases (Datry et al., 2017b) coincides with increasing anthropogenic alteration of intermittence regimes, with considerable water resource pressures in regions dominated by urban and agricultural land uses (Acuña et al., 2017; Kummu et al., 2016). Increases in the spatiotemporal extent of intermittence reflect over-abstraction (Boix et al., 2010; Jaeger et al., 2014; Mainstone et al., 1999), whereas effluent discharge, water diversions, and releases from impoundments can cause artificial perennialization (Hendriks et al., 2014; Luthy et al., 2015; Morais et al., 2004). These pressures interact within a changing climate that features more extreme events, including hydrological droughts (i.e. surface water deficits; Forzieri et al., 2014; Ledger and Milner, 2015). Anthropogenic activities typically reduce biodiversity by eliminating sensitive taxa, particularly where intermittence increases (Benejam et al., 2010; Datry et al., 2014b; Garcia et al., 2016).

The European Union (EU) Water Framework Directive 2000/60/EC (WFD; EC, 2000) requires EU Member States and other participating countries to achieve at least 'good' ecological status (GES) or 'good ecological potential' in all surface waters (EC, 2003a). Ecological status (hereafter, *status*) is assessed through comparison of 'biological quality elements' (BQEs, for example benthic invertebrate assemblages) with 'reference conditions', i.e. the communities indicative of undisturbed or minimally disturbed sites. Progress towards GES has been limited (EC, 2012; Voulvoulis et al., 2017), and one significant challenge is to incorporate IRES into biomonitoring programmes (Reyjol et al., 2014; Skoulikidis et al., 2017). Other legislative drivers for biomonitoring include the EU Habitats Directive 92/43/EEC (EC, 1992), under which IRES may fall within Special Areas of Conservation monitored to assess the conservation status of habitats and/or species (see Section 4.1). The EU Biodiversity Strategy also provides impetus for the monitoring and protection of ecosystems supporting high biodiversity and robust ecosystem service provision (EC, 2011). In addition, national status assessments have identified rare IRES inhabitants including specialist species (Macadam, 2016), providing impetus for population monitoring and legislative protection (e.g. Službeni list RCG br. 76/06, 2006 in Montenegro).

The EU COST Action CA15113 (*Science and Management of Intermittent Rivers and Ephemeral Streams*; SMIRES; COST, 2015) is seeking to translate increasing understanding of IRES into tangible tools for ecosystem management. The SMIRES Working Group 4: *Community Ecology and Biomonitoring in IRES* (WG4; SMIRES, 2016) is tasked with adapting current biomonitoring methods and developing novel tools to promote effective IRES status assessments. In this paper, we use information gathered from WG4 members to review current practice in IRES biomonitoring. We identify challenges to effective status assessment and, as a result, we establish the need to develop new, specifically designed tools to enable status characterization in IRES across and beyond Europe. To this end, we highlight best practice in IRES status characterization, identify opportunities for its wider adoption, and suggest priorities for future research.

2. Ecological status assessments in European rivers: collation of information

SMIRES WG4 comprises >90 members based in 25 'COST Member Countries' (COST, 2016), including academic researchers, and stakeholders from national 'competent authorities' with responsibility for WFD compliance. WG4 members were asked to collaborate to provide written accounts of national biomonitoring activity. Specifically, information was requested about the biomonitoring conducted to assess status in both IRES and perennial rivers, including the biotic groups used as bioindicators (Appendix A). In addition, qualitative descriptions of perceived issues surrounding, and limitations of, current IRES biomonitoring were sought. This initial survey was supplemented by a second survey focussing on national implementation of WFD stages including "water body" designation; classification of river typologies; reference site identification; characterization of reference conditions; use of perennial status assessment methods; and development of alternative methods for IRES (Appendix B). Information provided in written responses to the initial and second surveys was supplemented by discussion with respondents, both at WG4 meetings and via email.

Representatives from 20 countries contributed biomonitoring information in the initial survey (Fig. 1); in addition, Romania provided limited comments. Of the 20 represented countries, 15 are EU Member States, 13 of which had adopted all of their second WFD 'River Basin Management Plans' by early 2017; the two exceptions are Greece and the archipelago-specific 'River Basin District' (RBD) of the Canary Islands, Spain (EC, 2016). Of the non-Member State respondents, Iceland is also committed to WFD implementation as part of the European Economic Area; Switzerland has set targets comparable to those in the WFD as part of trade agreements; and Macedonia, Montenegro, and Serbia are being supported in implementing the WFD as candidate countries seeking accession to the EU. Mediterranean and other temperate-climate regions were well-represented in both Western and Central Europe, with Finland and Iceland representing northern latitudes. Germany, Norway, and Sweden were the most significant omissions in terms of land area, and regionally, Eastern Europe was poorly represented. All river 'Geographical Intercalibration Groups' (GIGs; formed to promote status benchmarking across countries implementing the WFD; Van de Bund, 2009) were represented, by 3 of 5 *Alpine*; 8 of 18 *Central/Baltic*; 5 of 9 *Eastern Continental*; 8 of 9 *Mediterranean*; and 2 of 5 *Northern* countries, respectively. Representatives from 13 countries (Croatia, Cyprus, Czech Republic, Greece, Hungary, Macedonia, Montenegro, Netherlands, Serbia, Slovakia, Spain, Switzerland, UK) spanning 4 GIGs (Alpine, Central/Baltic, Eastern Continental, Mediterranean) also responded to the second survey.

3. Ecological status assessments in European rivers: results

Considering all lotic ecosystems, 18 of 20 surveyed countries have established status assessment protocols for perennial rivers, all of which are based on biota characterized at the community level using a taxonomic approach; methods remain in development in Malta and Montenegro (Table 1). Common bioindicator groups comprise the BQEs *benthic invertebrates* (18 countries), *phytobenthos* (17), *macrophytes* (16), *fish* (15), and *phytoplankton* (6); as such, benthic invertebrates are the only BQE used across all surveyed countries with established protocols. This information agrees with and can be supplemented by the comprehensive review of Birk et al. (2012), who reported freshwater monitoring methods used in 28 European countries: those surveyed here, with five exceptions (Iceland, Macedonia, Montenegro, Serbia, Switzerland) and 13 additions (Austria, Belgium, Denmark, Estonia, Germany, Ireland, Latvia, Lithuania, Luxembourg, Norway, Romania, Slovenia, Sweden). We direct readers to Birk et al. (2012) for further information on methods used in perennial freshwater ecosystems, and to Dallas (2013) for information specific to mediterranean-climate rivers; here, we focus on IRES biomonitoring.

Of the 18 surveyed countries with established biomonitoring programmes, 16 (and Romania) include IRES (typically larger systems with predictable intermittence) within sampling networks, whereas perennial rivers are prioritized in Finland and Macedonia (Table 1). In many countries, including Croatia, Czech Republic, Iceland, Poland, Slovakia, Switzerland, and the UK, respondents noted that a 'small' proportion of biomonitoring sites were temporary; poor understanding of the extent of IRES within river networks typically prevented quantification this proportion in relation to IRES occurrence. However, in the Netherlands, the proportion has been quantified as 266 of 6460 sites (i.e. 4%) in total and in the upper reaches, where an estimated two-thirds of the network is temporary, these sites poorly represent IRES compared to 1665 perennial sites. In contrast, in Hungary, an estimated 35% of the WFD-monitored river length is temporary, which effectively represents intermittence at 291 of 923 (i.e. 31%) of monitored water bodies (but see Section 4.1).

Temporary reaches account for a much higher proportion of the total length of river networks in Mediterranean-Basin RBDs, but IRES sometimes remain underrepresented by water body-based biomonitoring networks. For example, >80% of the network length is temporary in the *Algarve*, *Guadiana*, and *Sado and Mira* RBDs of southern Portugal, but only 40% of biomonitoring sites are within IRES. In contrast, in the 16,438 km^2 *Catalan* RBD in north-east Spain, 64% of 248 water bodies (and therefore biomonitoring sites) are temporary, comprising 51% larger rivers with seasonal intermittence and 13% smaller streams with ephemeral flow; this proportion of water bodies exceeds the

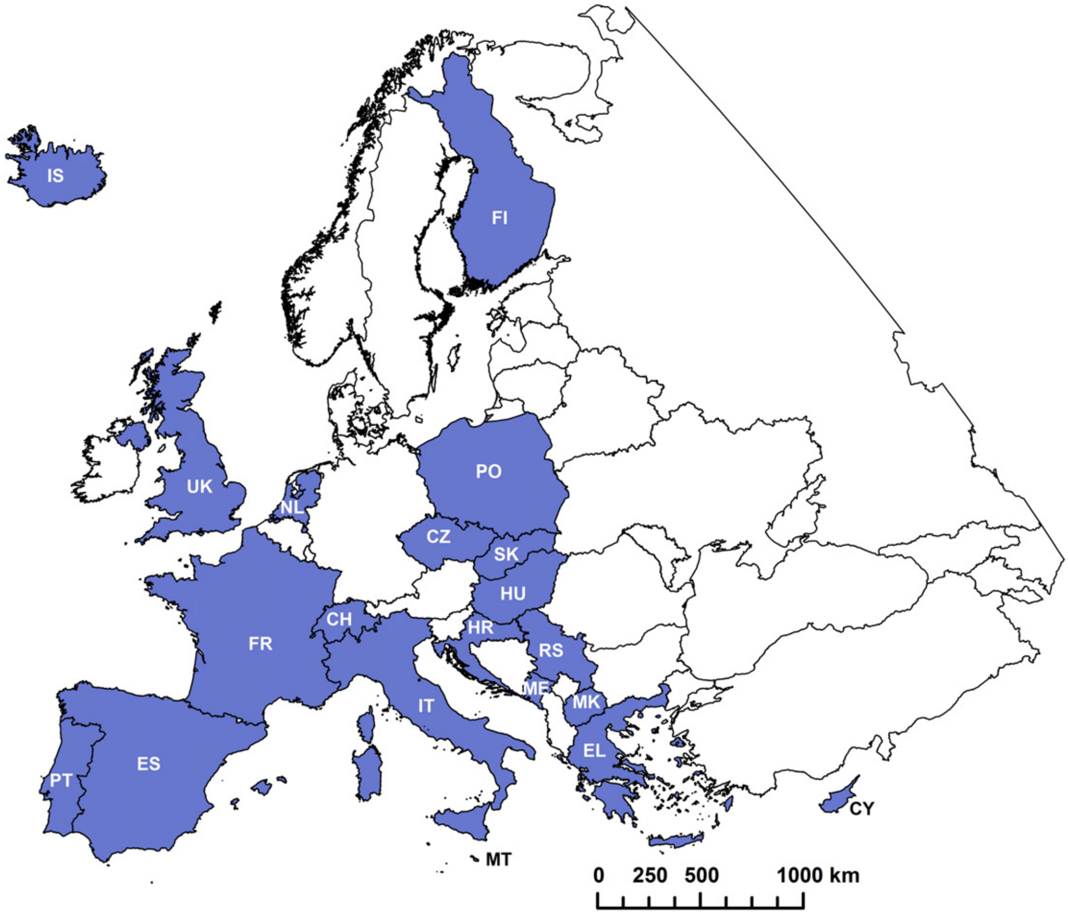

Fig. 1. European countries providing information about national biomonitoring activity in river ecosystems. Abbreviations: CH, Switzerland; CY, Cyprus; CZ, Czech Republic; EL, Greece; ES, Spain; FI, Finland; FR, France; HR, Croatia; HU, Hungary; IS, Iceland; IT, Italy; ME, Montenegro; MK, Republic of Macedonia; MT, Malta; NL, Netherlands; PO, Poland; PT, Portugal; RS, Serbia; SK, Slovakia; UK, United Kingdom.

estimated 58% temporary river length, comprising 50% seasonal, intermittent rivers and 8% ephemeral streams. Similarly, in Cyprus, 87% of water bodies classified for WFD monitoring are IRES, exceeding the 85% estimated temporary river length.

Survey respondents' descriptions of issues surrounding (and limitations of) current IRES biomonitoring, and their responses to the second survey of WFD-focussed questions, are explored in Section 4.

4. Challenges to effective IRES ecological status assessment

Currently, status assessments are routinely conducted for IRES in the Mediterranean regions in which they dominate; elsewhere in Europe, poor recognition of their extent has left IRES excluded from or poorly represented in biomonitoring programmes, which prevents identification of degraded ecosystems that require restoration or management actions. Even where biomonitoring is conducted, poor understanding of IRES may limit its effectiveness. The ongoing challenges of status assessment in IRES have recently been examined by Dallas (2013), Reyjol et al. (2014), Cid et al. (2017), and Skoulikidis et al. (2017); these Mediterranean-focussed reviews raise issues of wider interest, complementing our broader consideration of IRES across European climate zones.

4.1. Legislative definitions may not recognize IRES

The WFD aims to protect “all bodies of surface water”, but alongside perennial streams, many IRES (and in particular ephemeral streams) are within catchments that fall below the lowest WFD size typology (10–100 km^2), leaving them without legal recognition as “water bodies”. These small systems are typically excluded from biomonitoring networks, or their status is classified based on contiguous perennial reaches. For example, in Hungary, only 1031 of 9800 nationally registered watercourses have catchments >10 km^2, leaving 89.5% of watercourses (equating to 74% of the network length) beyond the remit of WFD-related monitoring. Given the contribution that IRES headwaters make to biodiversity, their inhabitation by rare and endemic IRES specialists (e.g. Macadam, 2016; Matono et al., 2012), and their provision of wider ecosystem services (Datry et al., 2017b), this exclusion is at odds with national and EU-wide commitments to ecosystem protection (EC, 2011), especially in regions where small IRES are numerous (Lazaridou et al., 2016). Skoulikidis et al. (2017) and Stubbington et al. (2017) explore this issue in relation to Mediterranean-region and oceanic-climate IRES, respectively.

Small IRES may fulfil other nationally determined criteria for designation as a WFD water body. For example, some IRES in Cyprus are designated because they support native fish populations of conservation interest. In addition, two IRES types are Habitats Directive Annex I habitats: “water courses of plain to montane levels with the *Ranunculion fluitantis* and *Callitricho-Batrachion* vegetation”, which include the ‘winterbourne’ headwaters of UK chalk IRES; and “intermittently flowing Mediterranean rivers of the *Paspalo-Agrostidion*”, which occur across six Mediterranean countries. Certain UK chalk streams are designated as water bodies regardless of size, because they fulfil a national criterion requiring maintenance and improvement of Special Areas of Conservation (UKTAG, 2003). In contrast, in Spain, many intermittently flowing rivers of the *Paspalo-Agrostidion* remain excluded from WFD consideration because of their small catchment size. For example, in the Catalan RBD, 143 of 248 designated water bodies (equating to

Table 1
Summary of survey responses received from 20 European countries reporting national biomonitoring activity in rivers including intermittent rivers and ephemeral streams (IRES).

Description of national biomonitoring activity	*n* (%) agreeing	Additional information on exceptions
Protocols are established for ecological status assessment in perennial rivers	18 of 20 (90)	No established protocols in: • Malta • Montenegro
Biota are characterized at the community level using a taxonomic approach	18 of 18 (100)	No exceptions; no routine biomonitoring: • is at non-community levels • uses functional approaches • uses metabarcoding of DNA
IRES are included within biomonitoring networks	16 of 18 (89)	IRES not included in networks in: • Finland • Macedonia
Intermittence is recognized in WFD river typologies	9 of 18 (50)	Recognized in: • All 8 Mediterranean GIG countries • Mediterranean regions of Croatia
Status assessment is only done to characterize flowing phases	16 of 16 (100)	No exceptions; no routine biomonitoring: • to characterize pool-phase status • to characterize dry-phase status
IRES status is assessed using protocols developed for perennial rivers	12 of 16 (81)	IRES-specific protocols used in: • Cyprus • Portugal • Spain IRES-evaluated method used in Greece

GIG, Geographical Intercalibration Group; RBD, River Basin District; WFD, Water Framework Directive.

1059 km of the 3808 km river length, and encompassing all flow permanence regimes) are also ‘Natura 2000’ protected areas and are monitored, but an additional 7176 km of small systems, including 1750 km located within the Natura 2000 network, are excluded based on their catchment size.

The Habitats Directive provides alternative impetus to assess the conservation status of IRES in designated sites (Fritz et al., 2017). However, different authorities may be responsible for WFD and Habitats Directive implementation, and collaboration is needed to ensure that IRES are not excluded from monitoring programmes. For example, the Environment Agency of England may conduct WFD status assessments in downstream perennial reaches of chalk streams in which the winterbourne headwaters are also protected under the Habitats Directive. A second public body, Natural England, has Habitats Directive responsibility and conducts complementary monitoring that encompasses winterbourne reaches; their assessment of conservation status is also informed by Environment Agency data. Habitat protection may also be afforded by other international- to local-scale site-specific designations, but such legal protection leaves most IRES excluded from monitoring activity.

4.2. Typologies that distinguish between contrasting IRES require development

WFD status assessments are based on robust surface water typologies, and incorporation of IRES into biomonitoring programmes therefore requires classification of IRES types. IRES are recognized by the WFD, which classifies a “temporary” river type that occurs in five Mediterranean countries (Van de Bund, 2009). This classification of one temporary category overlooks profound variability among IRES (Belmar et al., 2011; Cid et al., 2017), variability which is reflected by IRES communities, most strikingly between the fundamental ‘intermittent’ and ‘ephemeral’ IRES types (e.g. Argyroudi et al., 2009; Bonada et al., 2007; Stubbington et al., 2009; but see Delgado et al., 2012). This variability (and associated differences in boundaries between status classes) has necessitated flexibility, rather than consistency, in the biomonitoring methods and indices developed for and used within Mediterranean “temporary” rivers (Fritz et al., 2017).

Accordingly, national river classifications in few Mediterranean countries adequately represent IRES. For example, Spain has 37 classified river typologies based on natural variables including climate, geology, geomorphology, and altitude as well as river discharge, with several typologies including but not distinguishing between IRES and perennial systems (BOE, 2015). Similarly, in Portugal, eight of 15 national river classifications encompass both perennial and temporary water bodies. Exceptions include Cyprus, Greece, and Italy, where contrasting intermittence regimes are distinguished (using variable terminology) by the duration and predictability of flowing phases (Lazaridou et al., 2016; Skoulikidis et al., 2017); in Cyprus, this hydrological classification uses the ‘temporary stream regime’ tool developed by Gallart et al. (2012). In addition, five intermittent river types have been defined in the Mediterranean-climate Dinaric ecoregion of Croatia, spanning small and medium catchment sizes; upland and lowland altitudinal classes; and three sub-ecoregions with climates characterized by year-round precipitation (NN, 2013). These exceptions highlight EU-wide inconsistency in IRES subtype recognition, and a common failure to make even fundamental distinctions, notably between IRES that seasonally recede to chains of pools and those that experience unpredictable periods of complete drying (Argyroudi et al., 2009); these issues are explored by Skoulikidis et al. (2017) and have been addressed by the MIRAGE project (CORDIS, 2015; Nikolaidis et al., 2013; see Section 5.1).

Of the 13 non-Mediterranean surveyed countries in which WFD status assessment methods are established, none recognize flow permanence in river typologies. For example, the UK defined 18 river typologies using WFD “System A”, i.e. using prescribed altitude, catchment area, and geology categories; no hydrological parameters inform this classification system (UKTAG, 2003). Discharge categories do, however, inform UK model predictions of community composition across, for example, 43 benthic invertebrate assemblage end groups, but the lowest discharge category is $<0.31\ m^3\ s^{-1}$ i.e. intermittence is not recognized (UKTAG, 2008). Other countries have used WFD “System B” to define typologies, which allows classification to be informed by additional, optional factors including a “river discharge [flow] category”. Despite this, System B classification categories may be reminiscent of those in System A, for example Hungary used slope, catchment area, and geochemistry (comparable to altitude, catchment area, and geology) along with sediment size. In Switzerland, a non-WFD classification system is used, but despite the inclusion of one hydrological category (average annual discharge) along with biogeographical region, altitude, average slope, and geology, none of 54 river typologies are IRES (OFEV, 2015). However, national examples of good practice exist. In particular, the temporary reaches of slow-flowing, sand-bed streams which lose surface water for <10 weeks annually are classified as a type monitored in the Netherlands, but this is in addition to WFD-related activity, because these streams are too small to be deemed “water bodies”. Romanian regulatory agencies also distinguish between three intermittence classes based on drying frequency. Similarly, to inform future improvements in regulatory biomonitoring, the Czech BIODROUGHT project (see Section 5.3) distinguishes IRES with annual drying of >1 km for >7 days from near-perennial and perennial systems.

Classification of sufficient IRES typologies to characterize their heterogeneity is needed at the national scales at which regulators operate, with international collaboration desirable from the outset to share best practice and harmonize national typologies. Ideally, quantitative hydrological metrics should be developed to inform classification of ecologically relevant IRES types, with zero-flow periods recognized as primary sources of variation in community composition (Leigh and Datry, 2017; Oueslati et al., 2015). Metrics should incorporate the temporal characteristics of intermittence: the frequency, magnitude, duration, rate of change, timing, and predictability of lotic, lentic, and terrestrial phases (Costigan et al., 2017; Leigh and Datry, 2017; Poff et al., 1997).

Development of quantitative hydrological metrics is hampered by poor IRES representation in gauging station networks, with few continuous hydrological monitoring points located in temporary reaches (Costigan et al., 2017; Leigh and Datry, 2017; Snelder et al., 2013), for example, <5% of the network in Portugal; <1% in Spain and Switzerland; and none in countries including Greece, Hungary, Macedonia, Montenegro, the Netherlands, Poland, and Slovakia. Fewer still coincide with biomonitoring sites, for example, of >5000 gauging stations in France, 123 are in IRES, and at two of these, biotic and hydrological data can be linked – despite intermittence characterizing an estimated 39% of the river network (Snelder et al., 2013). Exceptions to this poor representation include Cyprus, where hydrological monitoring encompasses perennial to episodic systems, with 80 of 104 gauging stations on rivers with intermittent or ephemeral flow, 30 of which correspond with biomonitoring sites; Croatia, where ≥60 of 440 gauging stations are on IRES and hydrological data are collected at nine of 41 IRES biomonitoring points; and Serbia, where 218 flow gauging sites include 54 on IRES, of which 19 are also biomonitored. Even when hydrological data are available, the characteristics of an intermittence regime can be unclear due to difficulties in distinguishing between lentic and dry zero-flow states. To address this limitation of gauging station data, Cypriot authorities also make qualitative observations of aquatic states, following Gallart et al. (2012).

The scarcity of hydrological data necessitates use of other intermittence indicators. Aquatic communities including macroinvertebrates can indicate intermittence, but to date, have only proved able to differentiate between broad, antecedent states i.e. flowing or disconnected pool states (Cid et al., 2016; Řezníčková et al., 2013). Other informative qualitative methods encompass remote sensing using aerial or satellite photography or deployed sensors (González-Ferreras and Barquín, 2017; Spence and Mengistu, 2016) and citizen science initiatives (Datry et al., 2016b; Gallart et al., 2016; Turner and Richter, 2011), and perennial-dominated gauging-station data may allow modelling of IRES distribution (Snelder et al., 2013) and characterization of spatial patterns of intermittence (Larned et al., 2011). Qualitative description of intermittence may enable ecologically relevant classification, with Gallart et al. (2012) defining Mediterranean 'intermittent pool', 'intermittent-dry', and 'episodic or ephemeral' types (Nikolaidis et al., 2013). Equally, Delgado et al. (2012) assigned 60 Mediterranean island 'temporary streams' with overlapping intermittence regimes using non-hydrological criteria, namely altitude, slope, and other geomorphological and topographic discriminators. Beyond the Mediterranean region, broad IRES types such as UK *winterbourne chalk streams* (Westwood et al., 2006) and Dutch *slow-flowing, sand-bed upper reaches* (Van der Molen et al., 2013) have well-characterized environmental characteristics that encompass geomorphological variability, representing a starting point for classification.

4.3. Determination of reference conditions is challenging in dynamic IRES

Only once IRES typologies are classified can undisturbed or minimally disturbed typology-specific reference sites be identified, and their communities then characterized. Accordingly, few surveyed countries have identified IRES-specific reference sites, with most exceptions coming from IRES-rich Mediterranean regions. Relevant research initiatives include the GUADALMED (Sánchez-Montoya et al., 2009; see Dallas, 2013) and MIRAGE projects (Prat et al., 2014; see Section 5.1), which evaluated reference site selection criteria, informed by WFD guidance to use information about anthropogenic pressures to screen potential sites (EC, 2003b). For example, Prat et al. (2014) used threshold values for 37 attributes (relating to catchment land uses, morphological alterations, invasive species, and flow regime modification) to identify pressures and therefore potential reference sites. Similarly, Delgado et al. (2012) used land cover percentages and the absence of other anthropogenic pressures (point sources of pollution, hydromorphological alteration, and significant flow regulation) to select potential IRES reference sites in Spain's Mediterranean Balearic Islands. In addition, predictive models built using perennial reference conditions (e.g. the UK RIVPACS/RICT approach; UKTAG, 2008) warrant exploration to evaluate their applicability to IRES. These broad screening procedures require validation by expert judgement of habitat quality, as well as supplementation by quantitative analysis of chemical variables and biotic assemblages (Chaves et al., 2006; Lunde et al., 2013).

Distinguishing natural from artificial IRES during the site selection process is crucial to inform ecological target-setting based on the natural flow regime (Reyjol et al., 2014) or an alternate desired state (Acreman et al., 2014; Dallas, 2013), but scarcity of hydrological data hampers such differentiation. In response, and building on Richter et al.'s (1996) perennial-focussed 'indicators of hydrologic alteration', the 'MIRAGE toolbox' (Prat et al., 2014) and 'Temporary Rivers Ecological and Hydrological Status' (TREHS) software (Gallart et al., 2017) outline a means of distinguishing natural from artificial IRES (see Section 5.1). Guidance developed for perennial systems (EC, 2003b) may also inform identification of reference sites and subsequent definition of reference conditions in and beyond Mediterranean IRES.

Identifying undisturbed reference sites may be unfeasible across much of Europe, due to widespread, long-term human influences encompassing land use change, river regulation, and physical habitat modification (EC, 2003b), and in IRES, the problem of reference site identification may be compounded by difficulties in characterizing the natural intermittence regime. Across river ecosystems, 'best available' sites (i.e. those representing the least disturbed conditions; Feio et al., 2014), which may be located across international borders (Nijboer et al., 2004), may provide alternatives. For example, data from pristine Polish sites were used to characterize reference conditions for Dutch communities, due to the common occurrence of most indicator taxa (Nijboer et al., 2006). However, the notion that *any* site remains 'pristine' is questionable, especially due to climate change: the truly pristine benchmark against which to compare sampled sites is shifting (Hawkins et al., 2010), with climate-driven increases in drying exacerbating deviations in IRES (Datry et al., 2014a), especially in Mediterranean regions (Schneider et al., 2013).

Characterization of reference conditions at validated sites is complicated by IRES spatial heterogeneity, with Munné and Prat (2009, p. 659) describing benthic invertebrate assemblages from Catalan IRES reference sites as lacking "a unique typological aggregation". The composition of unimpacted communities partly reflects the spatial arrangement of temporary and perennial reaches (Datry et al., 2016c), and where intermittence varies longitudinally, corresponding variability in community composition may complicate selection of representative sites (Westwood et al., 2006). Community composition also varies between systems within a typology due to environmental heterogeneity among sites with equivalent spatial positions (Schriever and Lytle, 2016) and comparable instream habitats, reflecting the longitudinal, lateral and vertical dimensions of the landscape context in which individual sites are situated (Ward et al., 2002; see Section 4.5.1). Data from multiple sites may therefore require integration to adequately characterize assemblages (Jyrkänkallio-Mikkola et al., 2016; Passy and Blanchet, 2007).

As well as these practical challenges, reference conditions present conceptual difficulties in IRES: they are viewed as a single benchmark against which other states are compared (Hawkins et al., 2010; Stoddard et al., 2006), whereas IRES are ecosystems characterized by spatiotemporal variability (Bonada et al., 2007; Cid et al., 2017; Datry et al., 2016c). Increasing the spatial resolution of sampling networks and/or the temporal resolution of sample collection may therefore be necessary to characterize variability within each classified river type (Dallas, 2013). However, even if such increased biomonitoring activity can be resourced and seasonal variation accounted for (Munné and Prat, 2011), description of one assemblage that characterizes unimpacted conditions may still be compromised by unpredictable variation in assemblage composition (Bunn and Davies, 2000; Datry et al., 2016c). Such variability as well as low taxa richness compared to perennial systems may mean that the aquatic groups defined as WFD BQEs (Van de Bund, 2009) are inappropriate, in particular to represent systems with ephemeral or episodic flow (e.g. Cazemier et al., 2011). Debate is therefore needed to either: (a) select a single state in which status can be adequately represented using an established bioindicator (e.g. the benthic invertebrate BQE sampled late in a flowing phase in a predictable, near-perennial river; Sánchez-Montoya et al., 2012); (b) select a single state in which status may be effectively characterized by a novel bioindicator (e.g. terrestrial biota in an IRES with long, uninterrupted dry phases; see Section 4.4.2); or (c) integrate aquatic and terrestrial assemblage data collected across flowing, pool, and dry phases to provide a holistic picture of ecosystem health.

4.4. Ecological status classification is based on perennial reaches, flowing phases, and perennial indices

4.4.1. Status classification is based on perennial reaches

Small catchment size may result in IRES status classification being based on perennial sites within a contiguous WFD “water body”, a practice which assumes that sites with contrasting flow permanence have comparable ecological responses to equivalent anthropogenic pressures. However, contrasting environmental conditions and biotas may render this assumption invalid, and in all situations where one status class does not represent contiguous perennial and temporary reaches, designation as one water body may contravene WFD guidance (EC, 2003c) and monitoring networks may require expansion. For example, IRES may be less impacted by non-native invasive species than perennial rivers, if invaders cannot tolerate flow cessation and/or drying, or more impacted, if invaders are highly adaptable (Larson et al., 2009; Stoffels et al., 2017; Stromberg et al., 2007). Equally, abstraction at a given rate may have minimal ecological impacts in perennial rivers that decrease in depth; moderate impacts in IRES that experience longer dry phases; and severe impacts when perennial flow shifts to a temporary regime that includes dry phases (Skoulikidis et al., 2011).

Polluting effluents can have greater impacts when discharged into IRES. Firstly, an effluent which artificially sustains perennial flow in a natural IRES inherently causes the hydrological regime to fall below GES (Fig. 2). In such situations, water quality is also unlikely to meet WFD physico-chemical status targets, in particular as natural contributions to streamflow decline during dry seasons, reducing effluent dilution (Fig. 2; David et al., 2013; Morais et al., 2004). Biotic communities will respond to fluctuations in physico-chemical water quality and hydrological regime (Fig. 2), but attempting to characterize status using aquatic BQEs is inappropriate during artificial flowing phases. Instead, novel dry-phase biomonitors require development (see Section 4.4.2).

4.4.2. Status classification is based on flowing phases

Where status assessments are conducted in IRES (i.e. in 16 surveyed countries), all restrict biomonitoring activity to wet phases. Sampling is timed to maximize the likelihood of capturing a predictable and stable lotic community that has had sufficient time to establish since the last flow resumption. Such scheduling is primarily feasible in IRES with long, predictable flowing phases, whereas systems with short, unpredictable flowing phases pose considerable challenges, because characterization of an unknown stage in community succession prevents accurate status assessment. Across IRES, if lentic conditions are encountered, samples are typically collected in suboptimal conditions, which may lead to inaccurate status classification (Argyroudi et al., 2009; Buffagni et al., 2009); if a channel is dry, samples are not collected.

Reliance on flowing-phase sampling views ecological quality as temporally stable, but fluctuations between lotic, lentic, and terrestrial habitats are accompanied by changes in biotic communities and so potentially in responses to anthropogenic pressures. A singular focus on lotic assemblages may therefore provide an incomplete characterization of ecosystem health, in particular as lentic and dry phase durations increase. Although acceptable in a WFD-driven context, policy drivers including the EU Biodiversity Strategy (EC, 2011) provide impetus for comprehensive health assessments that encompass lentic and dry phases, to ensure that IRES habitats favour persistence of all life stages of associated rare species, and to verify robust ecosystem service provision. For example, specialist IRES insects may be present as juveniles during flowing phases and as dormant eggs in dry channels, with their persistence depending on environmental conditions during both wet and dry phases (Armitage and Bass, 2013); use of riparian zones by adults also necessitates appropriate habitat availability beyond an IRES channel.

Novel bioindicators may be required to supplement flowing-phase classification if comprehensive assessments encompassing temporal variability in status across lotic, lentic, and dry phases are pursued

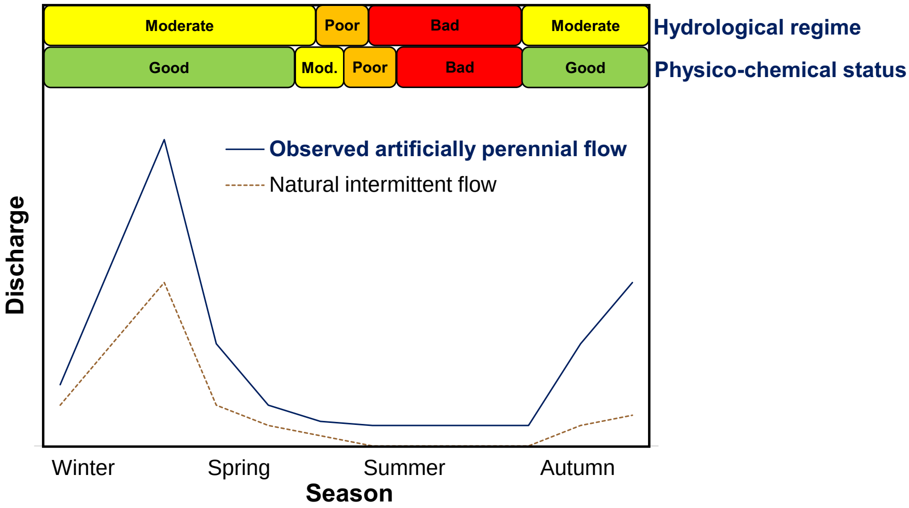

Fig. 2. Hypothesized annual changes in non-biological aspects of ecological status (i.e. physico-chemical quality and hydrological regime) in relation to seasonal changes in discharge in an artificially perennial reach downstream of a sewage treatment plant. The natural, seasonally intermittent flow regime is also indicated. Temporal variation in discharge is informed in part by Morais et al. (2004) and is likely to vary among years and among rivers. Terminology follows Water Framework Directive Section 1.2.1 (EC, 2000). Mod., moderate.

(Skoulikidis et al., 2017). Identification of effective dry-phase biomonitors is a priority, and biotic groups including terrestrial invertebrate communities (Gerlach et al., 2013; Hodkinson and Jackson, 2005), terrestrial carabid beetles (Rainio and Niemelä, 2003), aquatic invertebrate 'seedbanks' (Stubbington and Datry, 2013), dried biofilms including diatoms (Barthès et al., 2015) and/or bacteria (Romaní et al., 2013), and instream vegetation (Westwood et al., 2006) may have the potential to distinguish between sites of contrasting status; different biotic groups will be needed to characterize responses to various hydromorphological and physico-chemical pressures.

4.4.3. Status classification is based on perennial indices

In total, 13 of 16 surveyed countries that characterize IRES flowing-phase status do so using indices developed exclusively for perennial systems (Table 1; Table 2 footnote, plus France and Greece), which assumes the suitability of these approaches for IRES. Although sometimes evaluated and found to be appropriate (e.g. Prat et al., 2014; Table 2; see Section 5.1), the accuracy with which perennial indices characterize IRES flowing-phase status varies (Argyroudi et al., 2009; Morais et al., 2004; Munné and Prat, 2011). For example, Munné and Prat (2011) noted interannual variability in perennial index values calculated to characterize benthic invertebrate communities at temporary reference sites in north-east Spain, reflecting taxonomic differences between high- and low-discharge years. Equally, a perennial macrophyte index (evaluated as appropriate in other Mediterranean countries) is considered unsuitable in IRES in Cyprus due to taxonomic differences in community composition (Papastergiadou and Manolaki, 2012); in contrast, standard benthic invertebrate and phytobenthos indices are effective in Cypriot rivers with seasonal intermittent flow, but not in unpredictable ephemeral streams (Buffagni et al., 2012; Montesantou et al., 2008). In response to evaluation of perennial metrics as inappropriate for IRES, three Mediterranean survey respondent countries (Cyprus, Portugal, and Spain) have developed indices for status classification specifically for IRES, as explored in Section 5. In addition, Greece has developed 'HESY-2', an index evaluated as suitable for the assessment of benthic invertebrate assemblages in both temporary and perennial national river types (Lazaridou et al., 2016).

Spatial and temporal characteristics of IRES flow regimes interact to influence the suitability of perennial indices for flowing-phase status assessments (Fig. 3). Spatially, as distance from a temporary reach to upstream and/or downstream perennial recolonist sources increases, differences between temporary and perennial lotic communities become more pronounced (Datry, 2012; Pavićević and Pešić, 2012) and perennial index suitability may decline (Fig. 3, b–d). The spatial arrangement of temporary reaches in relation to perennial reaches also influences IRES community composition; for example, upstream perennial reaches promote recolonization by drifting aquatic taxa (Fritz and Dodds, 2004; Fig. 3, a,f). Time is a crucial modifier of the resultant compositional differences between perennial and temporary communities: as flowing phase duration increases, differences decline then disappear, as taxa with varying dispersal abilities form a recovering assemblage originating from catchment-wide recolonist sources (Datry et al., 2014b; Leigh et al., 2016). Therefore, as spatial isolation increases, the period in which perennial metrics are suitable for IRES status assessments may decrease (Fig. 3, a–d). Sampled assemblages should therefore be explored in a landscape context and informed by taxon-specific dispersal abilities (see Section 4.5.1).

Broad-scale ecohydrological analyses of macroinvertebrate biomonitoring data have identified drying events as a primary determinant of community composition in IRES, with taxon-specific responses to intermittence reflecting trait variation (Leigh and Datry, 2017). Biotic sensitivity to anthropogenic stressors and to flow intermittence often covary (Hughes et al., 2009), meaning that a taxon's absence from IRES may reflect either deviation from GES or an antecedent flow-cessation or drying event. For example, many mayfly and stonefly juveniles are sensitive to both environmental degradation (Paisley et al., 2014) and intermittence (Boulton and Lake, 2008; Chadd et al., 2017; see Section 5.3). As a result, indices developed to assess the status of perennial rivers may perform poorly in IRES, and require adaptation to recognize the influence of taxon-specific sensitivities to flow cessation and drying on the occurrence and abundance of individual taxa. With short-term deviation from GES permissible under the WFD if deterioration reflects natural events such as hydrological drought, such adapted indices are needed to prevent inaccurate claims that the absence of intermittence-sensitive taxa indicate a legislative breach.

4.5. All biomonitoring is restricted to community-level, taxonomic characterization

4.5.1. Metacommunity dynamics require recognition

Biomonitoring reported by all 20 survey respondents uses a taxonomic approach to characterize biota at a local community level in *all* water bodies, including both temporary and perennial systems. This indicates EU-wide collection, analysis, and interpretation of biomonitoring data according to the 'species sorting' perspective, which assumes that taxa differ in their responses to environmental variation, and that

Table 2

Evaluation of biotic indices developed for perennial rivers to determine their suitability for intermittent rivers characterized by long, seasonal flowing phases[a]. Only 'replacements' that are being tested by or are in use by WFD competent authorities are listed. Indices have not been evaluated in other surveyed countries in which IRES biomonitoring is conducted[b].

WFD GIG	Country	Index	Biotic group	Suitable?	Replacement	Source
Eastern Continental	Croatia	IBMWP, SI_{HR}	MIV	Yes		Mihaljević et al. (2011)
		IPS	Diatoms	No	TDI	Mihaljević et al. (2011)
Mediterranean[c]	Cyprus	STAR_ICMi	MIV	Yes		Buffagni et al. (2012)
		IPS	Diatoms	Yes		Montesantou et al. (2008)
		IBMR	Macrophytes	No	MMI	Papastergiadou and Manolaki (2012)
	Greece	HESY-2	MIV	Yes		Lazaridou et al. (2016)
	Portugal	IPtIS, IPtIN	MIV	Yes		INAG (2009)
		IPS	Diatoms	Yes		EC (2012), in Skoulikidis et al. (2017)
	Spain – Catalan RBD	IBMWP	MIV	No	IMMi-T; IMMi-L	Munné and Prat (2011)
		IPS	Diatoms	Yes		Burfeid et al. (2017)
Northern; Central/Baltic	France	I_2M_2	MIV	No	Required	Pelte et al. (2012, 2014)

[a] No indices have been evaluated as suitable for IRES with short, unpredictable (i.e. ephemeral or episodic) flowing phases; caveats to use in IRES with seasonal intermittence may apply.

[b] I.e. Croatia, Czech Republic, Hungary, Iceland, Italy, the Netherlands (using an adapted species list), Poland, Serbia, Slovakia, Switzerland, and the UK.

[c] Spain is also in the Central/Baltic GIG but examples are relevant only to the Mediterranean GIG.

Abbreviations: HESY-2, Hellenic Evaluation System-2; I_2M_2, French macroinvertebrate multimetric index (Mondy et al., 2012); IBMR, L'Indice Biologique Macrophytique en Rivière; IBMWP, Iberian Biological Monitoring Working Party; IMMi-L, Iberian Mediterranean Multimetric Index (IMMi) – qualitative; IMMi-T, IMMi – quantitative; IPS, Specific Polluosensitivity Index; IPtIN, Invertebrate Index for northern Portugal; IPtIS, Invertebrate Index for southern Portugal; MIV, macroinvertebrates; MMI, Multimetric Macrophyte Index; RBD, River Basin District; SI_{HR}, Croatian index; STAR_ICMi, Standardization of River Classifications Intercalibration Common Metric index; TDI, Trophic Diatom Index.

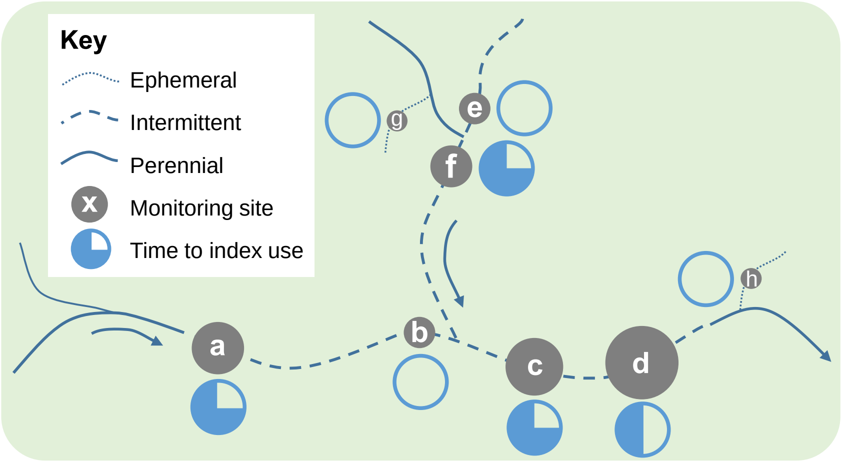

Fig. 3. Plan view of a hypothetical river network, indicating spatial and temporal influences on the suitability of a perennial biotic index for use at temporary sites. Size of symbols *a* to *h* is proportional to index 'suitability', where suitability is based on similarity in community composition between perennial and temporary sites during periods of peak diversity. Fill of partial circles indicates the period of continuous flow needed before the index becomes suitable: in *intermittent* reaches with seasonal, predictable flow cessation and drying, site *d* and sites *a*, *c* and *f* require 6 and 9 months of continuous flow, respectively, before index use is valid; and at sites *b* and *e*, differences in community composition persist throughout an annual cycle, meaning that the perennial index is unsuitable and requires modification to recognize taxon-specific intermittence sensitivities. Perennial indices are unlikely to be suitable at sites *g* and *h* within an annual cycle, since *ephemeral* reaches experience flowing phases that are unpredictable and often short.

local abiotic conditions are the primary determinants of community composition (Leibold et al., 2004). However, local communities connected by dispersal form metacommunities structured by the wider processes that influence dispersal (Heino, 2013; Sarremejane et al., 2017). Ignoring metacommunity dynamics may impede accurate status classification; for example, due to dispersal limitation, sites isolated by distance or by natural landscape features may support fewer taxa (a, Fig. 4) than sites of equivalent status that are longitudinally, laterally, and vertically connected to many recolonist sources (b, Fig. 4). A metacommunity perspective is particularly important in IRES, where transitions between flowing, pool, and dry phases necessitate repeated recolonization from instream, riparian, and more distant refuges (Cañedo-Argüelles et al., 2015; Datry et al., 2016c).

Taxon-specific dispersal abilities require consideration within a landscape framework that recognizes the spatial arrangement of temporary reaches (Fig. 4): (1) longitudinally, in particular the occurrence of and distance to connected perennial upstream and downstream refuges (Larned et al., 2011); (2) laterally, including the availability of terrestrial habitat to support aerial adult insects (Stubbington et al., 2016) and the distance to other recolonist sources (White et al., 2017); and (3) vertically, including the quality of subsurface sediments as a refuge for recolonists, potentially including saturated hyporheic sediments (Stubbington, 2012; Vander Vorste et al., 2016a) and/or drying sediments that support an invertebrate 'seedbank' (Stubbington and Datry, 2013). This landscape influence requires interpretation of sampled assemblages in a context of temporal variability: local communities will achieve peak diversity more quickly after a hydrological transition (i.e. pool formation, drying, or flow resumption) where distance to recolonist sources is lower.

4.5.2. *Functional approaches may complement taxonomic community characterization*

Taxon-specific responses to environmental changes make taxonomy-based indices of environmental degradation spatially and temporally

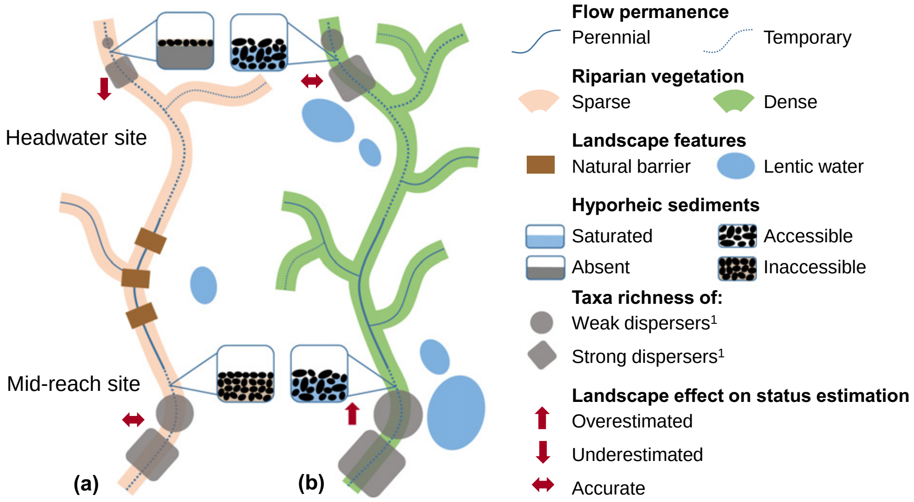

Fig. 4. Longitudinal, lateral, and vertical dimensions of the landscape influence lotic community taxa richness, which can result in overestimation or underestimation of ecological status at temporary river sites of equivalent status. Two possible scenarios are shown: plan view of (a) a catchment with sparse riparian vegetation, natural barriers (e.g. waterfalls), few lentic surface waters, a low drainage density, and fine-sediment-clogged (light brown) or absent (dark grey) hyporheic sediments; here, status may be underestimated in the headwaters; (b) a catchment with dense riparian vegetation, no natural barriers, abundant lentic surface waters, a high drainage density, and saturated, accessible hyporheic sediments; here, status may be overestimated in the mid-reaches. [1]Symbol size is proportional to richness; differences between weak and strong dispersers will decrease as flowing phase duration increases (temporal changes not shown).

variable, which can impede accurate status assessment (Bady et al., 2005). Functional approaches can complement taxonomic community characterization by exploring the biological and ecological traits possessed by a sampled assemblage. Despite limitations of biological trait databases (Wilkes et al., 2017), measures of functional diversity can be reliable indicators of human impacts in perennial rivers (Charvet et al., 2000; Dolédec et al., 1999), and Bruno et al. (2016) found functional redundancy (i.e. the number of taxa performing a comparable ecosystem function) of woody riparian vegetation lining seasonal Mediterranean intermittent rivers to be a sensitive indicator of anthropogenic alteration that remained stable among sites of equivalent status. In Hungarian rivers, metric screening during development of new macroinvertebrate-based multimetric indices identified a combination of ecological traits and taxonomic descriptors as the most effective discriminator among status classes (Várbíró et al., 2011, 2015).

Traits of aquatic biota can also complement taxonomic descriptors to indicate intermittence regimes, distinguishing among perennial, intermittent, and ephemeral systems (Bonada et al., 2007; Giam et al., 2017), between flowing and pool conditions (Cid et al., 2016), and identifying antecedent dry phases (Pařil et al., 2015). By acting as a proxy for hydrological data in IRES, functional characterization of aquatic communities can inform description of IRES reference conditions (Cid et al., 2016). However, differences among functional assemblages from sites with contrasting flow regimes can be obscured by high variability within a regime (Leigh et al., 2016) and by high functional redundancy (Vander Vorste et al., 2016b), and as for taxonomic assemblages, compositional differences among temporary and perennial sites decline then disappear as flowing phase duration increases (see Section 4.4.3; Vander Vorste et al., 2016b).

Where development of new IRES-specific status assessment methods is required, functional approaches warrant consideration alongside taxonomic approaches, their potential to distinguish between sites exposed to different natural and anthropogenic stressors having been demonstrated in IRES for aquatic biota including macroinvertebrates (Bonada et al., 2007; Cid et al., 2016; Mondy et al., 2016; Suárez et al., 2017) and biofilms (including bacteria, algae, and diatoms; Acuña et al., 2015; Amalfitano et al., 2008; Romaní et al., 2013). Functional aspects of terrestrial biota remain poorly characterized in dry channels (but see Corti and Datry, 2014; McCluney and Sabo, 2012) but evidence from other habitats indicates their potential, with the functional traits of riparian arthropod assemblages demonstrated as sensitive to habitat conditions including flooding regime (Lambeets et al., 2009).

4.5.3. Molecular approaches may provide an integrated picture of IRES ecological health

Molecular characterization of sampled assemblages may also have advantages over morphology-based taxonomic approaches, with genetic tools receiving increasing attention by academic researchers (Leese et al., 2016) and regulatory agencies (Vasselon et al., 2017). DNA sequences act as species-level 'barcodes' (Hebert et al., 2003), and metabarcoding allows automated identification of the species in bulk samples that comprise assemblages of whole organisms, or from degraded environmental DNA in water or sediment samples (Baird and Hajibabaei, 2012; Elbrecht et al., 2017; Taberlet et al., 2012). Metabarcoding and other molecular approaches overcome the taxonomic limitations of standard biomonitoring in which reliance on morphology can achieve too coarse an identification level to characterize responses to environmental drivers (Cardoso et al., 2011; Macher et al., 2016; Pešić et al., 2017). For example, identification of Chironomidae (Diptera) to family and *Deleatidium* (Ephemeroptera) to genus prevents quantification of their biodiversity contributions and obscures species-level responses to environmental variability (Cañedo-Argüelles et al., 2016; Macher et al., 2016; Stubbington et al., 2016). Species-level identification makes DNA-based tools particularly promising for application to such taxonomically demanding IRES biota, especially in regulatory biomonitoring programmes where financial constraints promote uptake of cost-effective solutions (Elbrecht et al., 2017). Through its integration of freshwater and terrestrial data from multiple sites, metabarcoding may prove particularly appropriate for catchment-scale characterization of IRES biodiversity (Deiner et al., 2016).

Although metabarcoding is time-effective in its concurrent identification of most common taxa, it may fail to identify rare species (i.e. those occurring at low abundance, which may also be of conservation interest), even in bulk samples (Hajibabaei et al., 2011). Molecular approaches may therefore complement but should not replace traditional biomonitoring activity (Hajibabaei et al., 2016). Other molecular tools with potential to characterize biotic responses to status variability include transcriptomics, proteomics, and metabolomics (Colin et al., 2016). In particular, transcriptomics (i.e. characterization of gene expression using RNA transcripts) may complement taxonomic biomonitoring by determining the metabolic activity (and therefore, potentially, the physiological health) of IRES communities – as well as presenting novel opportunities to explore ecological quality using the expression of genes responsible for important ecosystem processes (Poretsky et al., 2005; von Schiller et al., 2017).

5. Best practice in IRES ecological status assessment

Despite the described challenges of IRES status characterization, innovative examples of recent work to improve practice exist, notably in Mediterranean countries where IRES dominate lotic networks and are consequently central to routine biomonitoring. The wider applicability of this best practice requires exploration across regions with different climates and therefore, potentially, contrasting IRES typologies.

5.1. The MIRAGE and LIFE+ TRivers projects: defining a sampling time window

Within the Mediterranean Basin, advances have been made to improve biomonitoring of intermittent rivers with long seasonal flowing phases, through adaptation of methods developed for perennial rivers. The *Mediterranean Intermittent River ManAGEment* project (MIRAGE; CORDIS, 2015) developed an integrated 'toolbox' to improve IRES biomonitoring, with classification of the hydrological regime (including identification of artificial IRES) emphasized as a pre-requisite for effective status assessment (Gallart et al., 2016; Prat et al., 2014). The MIRAGE project tested macroinvertebrate-based methods developed for perennial rivers in IRES, demonstrating their suitability for some IRES, if flowing phases are long-lasting and sampling is conducted long enough after flow resumption for aquatic diversity to peak (García-Roger et al., 2011; Prat et al., 2014); Mazor et al. (2014) reached comparable conclusions in Californian mediterranean-climate IRES. Equally, Burfeid et al. (2017) determined that status can be assessed in IRES using indices developed for perennial-river diatom assemblages, but only during flowing phases (Table 2); once flow between connected pools ceases, index performance declines considerably.

To implement MIRAGE recommendations, the *LIFE+ TRivers* project (TRivers, 2014) has developed TREHS software, which uses quantitative gauging station data and qualitative information from interviews, aerial photographs, and site visits to characterize temporary flow regimes, including anthropogenic alteration of natural regimes and differentiation of natural and artificial IRES (Gallart et al., 2017). The resultant flow regime classification can inform selection of the most appropriate time window for collection of lotic biomonitoring samples and also identifies systems in which perennial sampling protocols are inappropriate. TREHS is currently (in 2016–17) being tested by the Catalan (http://aca-web.gencat.cat/aca) and Júcar River Basin (http://www.chj.es) Water Agencies in Spain, and this evaluation will inform improvements in hydrological regime classification, environmental objective setting, and status assessments in Mediterranean IRES and more widely.

5.2. Development of new biotic indices for intermittent Mediterranean rivers

Evaluation of biotic indices designed for perennial rivers as unsuitable for IRES has provided impetus for index adaptation and for the development of new approaches, most commonly for lotic macroinvertebrate communities in seasonally intermittent rivers. For example, the IRES-specific multimetric indices IMMi-T and IMMi-L outperform the standard perennial-river IBMWP metric in their detection of environment impacts in Spanish IRES (Munné and Prat, 2009; Sánchez-Montoya et al., 2010; also see Dallas, 2013), leading to their incorporation into regulatory biomonitoring in the Catalan region (Munné et al., 2016). Similarly, García et al. (2014) developed the multimetric INVMIB index for Balearic Island IRES and demonstrated its ability to distinguish between status classes using macroinvertebrate assemblage data; this index has been adopted by regulators. Finally, Morais et al. (2004) and Pinto et al. (2004) established that, compared to perennial metrics, a specifically designed multimetric macroinvertebrate-community index was more sensitive to organic pollution and more robust to seasonal variability in siliceous catchments of IRES-dominated southern Portugal, contributing to the subsequent development of official indices (Table 2).

In Cyprus, the Multimetric Macrophyte Index (MMI) was developed to assess water quality in intermittent rivers, after Papastergiadou and Manolaki (2012) showed no response of a standard, perennial index to the pressure gradient characterized for the Mediterranean "temporary" river type (Van de Bund, 2009; Table 2); testing is underway to ensure national reliability of this index.

5.3. Recognizing and characterizing responses to intermittence

Beyond the Mediterranean Basin, research initiatives examining variation in IRES biota have focussed on responses to intermittence, rather than to ecological quality, in oceanic-climate temperate regions. For example, the Czech Republic BIODROUGHT project (Polášek, 2013a) used taxonomic and functional analyses to develop a suite of 370 macroinvertebrate indicator taxa (primarily species) that can be used to calculate the probability that a stream (sampled to represent peak lotic diversity) has experienced no drying, a < 7 day drying event, or a longer dry period in the preceding year (Polášek, 2013b; Pařil et al., 2015). Quantifying such initiatives, a system developed in the Netherlands has determined the sensitivity of 2236 invertebrate species to drying (Verberk et al., 2012). 'Affinity scores' from 0 (none) to 10 (very high) are assigned to species according to their association with one perennial class and each of four intermittence classes (that describe IRES with typical annual dry periods of <6 weeks to >5 months); for example, *Gammarus pulex* (Amphipoda) scores 8 in the *perennial* class, 2 in the *<6 weeks intermittence* class, and 0 in other classes. A comparable Dutch system that assigns scores based on species-specific affinities for lotic and lentic habitats (Verberk et al., 2012) can characterize invertebrate community responses to flow cessation.

A complementary index has been developed to describe macroinvertebrate community responses to hydrological drought disturbances in near-perennial UK IRES (Chadd et al., 2017). Building on work in Australia (Boulton and Lake, 2008), 92 families/genera have been assigned to six habitat types based on their occurrence (but not abundance) and scored to reflect their sensitivity to loss of this habitat: 1. flow reduction (scores 10, 9); 2. loss of lateral connectivity (8, 7); 3. flow cessation (6, 5); 4. disconnected pool formation (4, 3); 5. disconnected pool contraction (2, 1); and 6. complete surface drying (0). The resultant Drought Effect of Habitat Loss on Invertebrates (DEHLI) index is calculated as the average score per taxon (Chadd et al., 2017).

National assignment of taxon-specific scores indicating sensitivity to flow cessation and drying has considerable potential to enhance IRES biomonitoring, and development of these national initiatives at the EU level is a priority of SMIRES WG4 (SMIRES, 2016). Firstly, the presence and/or abundance of scored taxa could be integrated within an index indicating a site's natural drying or flow cessation regime to inform classification of river typologies. Scores could then inform expectations of community composition during reference condition characterization and subsequent status assessments, including identification of deviations from GES caused by anthropogenic alteration of natural flow regimes. In addition, interpretation of environmental degradation indices in light of intermittence sensitivity scores may demonstrate that a short-term status deterioration does not constitute a legislative breach, for example in near-perennial rivers during drought disturbances.

Community-level approaches are complemented by recent exploration of specific taxa as potential status indicators, in particular, mites (Acari, which are recognized as perennial-river bioindicators; Goldschmidt, 2016) in Mediterranean regions of the Balkan Peninsula. Firstly, the ratio of Oribatida (Acari) to Ostracoda (Crustacea) can be used to differentiate perennial from temporary streams (Pešić, unpublished). Secondly, the '$\mathrm{PTH_{fam}}$' index (informed by Plecoptera, Trichoptera, and Hydrachnidia [Acari] families; Miccoli et al., 2013) may be a sensitive status indicator in IRES compared to other taxa combinations (Pozojević and Pešić, unpublished). Research is ongoing to evaluate and test the $\mathrm{PTH_{fam}}$ index, prior to potential recommendation for wider use.

Community-level and taxon-specific intermittence sensitivity scoring can be informed by research initiatives compiling IRES biodiversity information. Notably, the Intermittent River Biodiversity Synthesis (IRBAS) project (www.irbas.cesab.org/irbas) has created an international, open-access database of hydrological and biological data, primarily reporting aquatic invertebrate assemblages sampled from IRES during flowing phases (Leigh et al., 2017). The database currently comprises >2500 samples from European and other countries, and is expected to grow considerably due to input from ongoing initiatives including the SMIRES consortium (COST, 2015).

6. Conclusions: priorities to address the challenges to IRES ecological status assessment

A fundamental first step to enable establishment of IRES biomonitoring programmes is to map IRES occurrence across Europe (1, Fig. 5; 1, Table 3; SMIRES, 2016). Next, typologies that represent IRES natural heterogeneity (in particular, their contrasting intermittence regimes) and that recognize anthropogenic hydrologic alterations (in particular, discrimination of natural and artificial IRES) need classification; exclusion of typologies with small catchments is unlikely to be justifiable (2, Fig. 5; 2–3, Table 3). Ideally, classification should be informed by quantitative, long-term hydrological data that encompass natural inter-annual variability (Dallas, 2013), although qualitative, expert description of intermittence regimes may provide an informative preliminary classification (2, Fig. 5; 3, Table 3).

Following typology classification, reference sites require identification and validation during field visits to candidate sites identified by pressure-based screening (3a, Fig. 5; 4, Table 3; EC, 2003b). Characterization of reference site communities for each typology is the next step towards status assessment, although the benchmark definition of the reference condition approach may require adaptation to provide a more flexible view of unimpacted IRES (4, Fig. 5; 6, Table 3) and to encompass lentic and dry as well as flowing phases (5a, Table 3). To characterize reference conditions (and lower status class boundaries) effectively, status assessments methods require development, starting with evaluation of those used in perennial rivers (3b, Fig. 5; 5b, Table 3). Where these methods need adaptation or replacement, the accuracy of modified or novel approaches will be enhanced by recognizing taxon-specific intermittence sensitivities and dispersal abilities (5, Fig. 5; 5c, Table 3). Mediterranean regions have spearheaded adaptation of indices, and new, IRES-specific methods (e.g. García et al., 2014; Munné

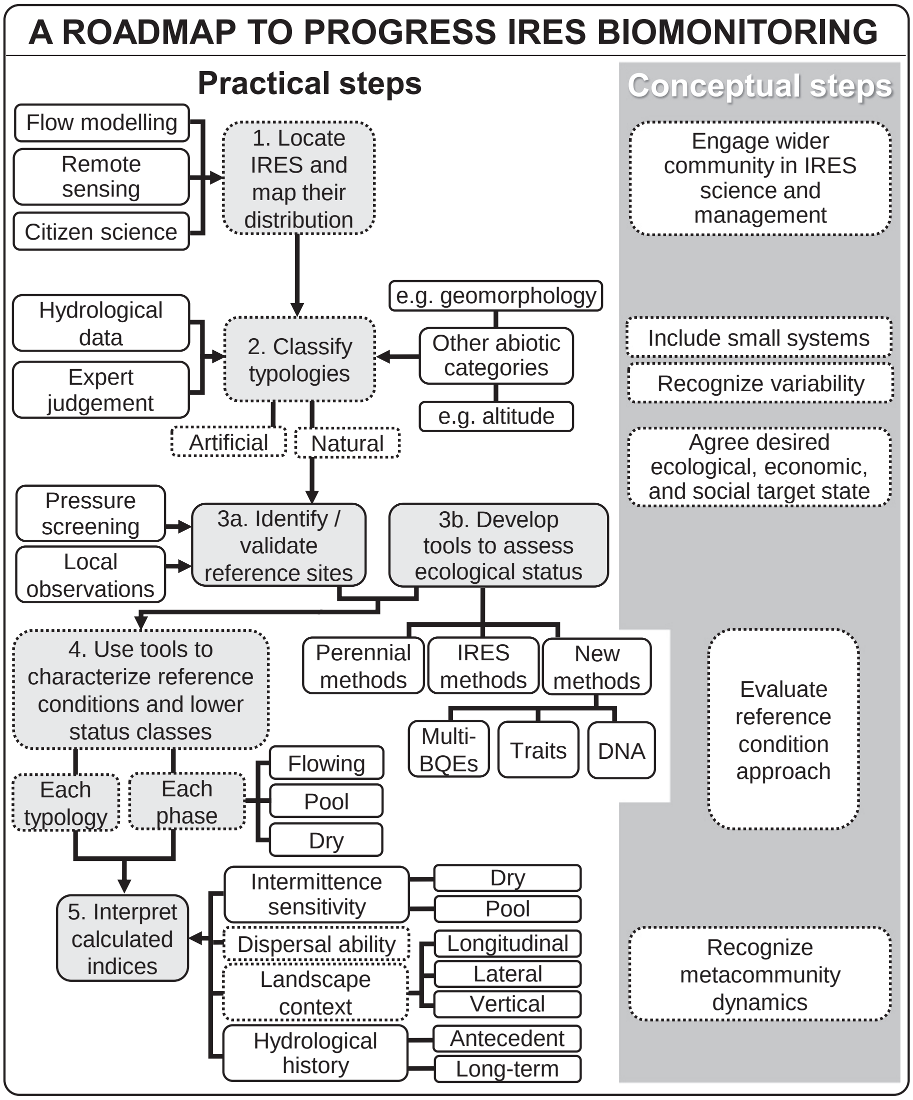

Fig. 5. A roadmap for the development of effective ecological status assessments in intermittent rivers and ephemeral streams (IRES). Filled boxes indicate key stages; vertical arrows navigate between stages; horizontal arrows indicate aspects informing each stage; dotted lines in the *Conceptual steps* box align vertically with related *Practical steps*. BQE, biological quality element (EC, 2000).

and Prat, 2009; 5b, Table 3) warrant evaluation to establish their potential for wider adoption, or to inform flexible development of comparable tools elsewhere.

In interpreting sampled assemblages, regulators should recognize a site's spatiotemporal context: its hydrological history, and the availability of recolonists from the wider metacommunity (Fig. 4; 5, Fig. 5; 5c, Table 3). Beyond the taxonomic classification of site-specific communities, integration of structural and functional aspects of multiple biotic groups could promote sensitive characterization of ecological responses to interacting anthropogenic stressors (Hughes et al., 2009; 5d, Table 3). In addition, molecular approaches including DNA metabarcoding and transcriptomics could transform biomonitoring by integrating taxonomic, functional, and phylogenetic diversity information (Yu et al., 2012) from the lotic, lentic, and terrestrial assemblages (Deiner et al., 2016; 5d, Table 3) that collectively respond to interacting anthropogenic pressures. Newly developed methods – potentially spanning characterization of dry-phase biota and quantification of ecological processes, using traditional and molecular approaches (von Schiller et al., 2017) – will require rigorous testing and validation to inform implementation of standardized protocols by regulatory agencies, and these protocols must be efficient, to facilitate uptake. Where novel biotic groups that fall outside of WFD BQEs prove informative, debate will be needed to justify their use (Jeppesen et al., 2011).

Collaboration is key to transforming IRES status assessment: between academics and river managers, to promote implementation of research recommendations; between countries, to disseminate best practice and enact new practices in a way that balances standardization with flexibility; between aquatic and terrestrial ecologists, to develop suites of bioindicators that represent both wet and dry phases; and between ecologists and geneticists (including DNAqua-Net COST Action members; Leese et al., 2016), to promote incorporation of IRES into emerging metabarcoding approaches from an early stage. The goal of these collaborations is to explicitly recognize the extent and value of IRES; to develop effective status assessment methods for use in biomonitoring schemes that adequately represent IRES and their diverse communities; and ultimately, to promote protection of IRES as they become an increasingly common landscape feature.

Table 3

The current state of biomonitoring in intermittent rivers and ephemeral streams (IRES) across Europe, including challenges limiting progress; highlights to date (i.e. best practice examples); overarching priority actions; and steps towards achieving these actions. Numbered points reflect a logical sequence of priority actions, with scope for concurrent progress to be made towards multiple actions; in particular, characterization of reference conditions and development of status assessment methods are complementary activities. Numbered citations are provided separately for each row.

	Current state	Highlights to date	Priority actions	Steps towards action completion	Sources
1	Mapping of IRES distribution hampered by poor representation in gauging station networks, in particular for small, headwater IRES.	Citizen scientists mapped >4000 km of flow states in France[1]; these data have been explored by the IRBAS project[2], leading to a national citizen science network[3].	Locate and map distribution of European IRES.	Develop a citizen science network to supplement hydrological monitoring data[4] and support Europe-wide mapping.	[1]Datry et al. (2016c); [2]Leigh et al. (2017); [3]ONEMA (2017); [4]SMIRES (2016).
2	IRES with catchments <10 km^2 are too small to be defined as WFD "water bodies" that require status assessment[1]; resources often restrict biomonitoring to "water bodies".	Non-WFD biomonitoring done by national agencies e.g. in slow-flowing sand-bed Dutch IRES[2]. National criteria define some small IRES as WFD water bodies e.g. in the UK[3].	Expand biomonitoring networks to encompass small streams including IRES, motivated by non-WFD drivers e.g.[4].	Identify national priorities to start expansion of biomonitoring networks.	[1]EC (2000); [2]Van der Molen et al. (2013); [3]UKTAG (2003); [4]EC (2011).
3	National IRES typologies are non-existent or fail to reflect IRES dynamism and heterogeneity. Hydrological data to inform development of IRES typologies are scarce.	Five WFD typologies distinguished in Croatia[1]. Official distinction of contrasting IRES in Cyprus, Greece, and Italy[2–3]. Classification of reach-scale Mediterranean IRES flow regimes[4–6].	Classify national typologies, including distinction between IRES with flowing phases that contrast in their predictability and duration.	Build on qualitative IRES descriptions in local use. Evaluate ability of new tools to inform typology classification more widely, including differentiation of natural and artificial IRES[4–7].	[1]NN (2013); [2]Skoulikidis et al. (2017); [3]Lazaridou et al. (2016); [4]Gallart et al. (2012); [5]Prat et al. (2014); [6]Gallart et al. (2017); [7]Polášek (2013a).
4	Reference sites identified in few countries. Absence of unimpacted sites in some countries.	Pressure criteria to screen sites developed for perennial rivers[1] and for Mediterranean IRES[2–4]. MIRAGE toolbox[4] and TREHS software[5] identify natural IRES. Expert judgement used to identify reference sites for 3 Croatian WFD typologies[6].	Screen and validate reference sites for each classified typology (see point 3), using sufficient sites to represent spatial variability[7].	Explore wider use of pressure criteria used to screen Mediterranean IRES and perennial rivers. Validate screened sites using expert judgement of field conditions.	[1]Wallin et al. (2003); [2]Sánchez-Montoya et al. (2009); [3]Delgado et al. (2012); [4]Prat et al. (2014); [5]Gallart et al. (2017); [6]NN (2013); [7]Munné and Prat (2009).
5	If assessed, IRES status is classified:				
	(a) during only wet, typically flowing, phases, whereas dry phases are excluded	Initial exploration of dry-phase communities[1]. Characterization of pool-phase communities[2].	Characterize variability in status among phases. Develop tools to assess status during each phase. Define a time window in which each tool is effective.	Evaluate potential of dry-phase biotas to indicate status. Evaluate ability of DNA-based tools to integrate catchment-wide biotic information from all phases[3].	[1]Corti and Datry (2015); [2]Cid et al. (2016); [3]Deiner et al. (2016).
	(b) using (often unevaluated) perennial methods	Perennial methods validated for assessment of flowing phases in IRES with long, predictable flowing phases[1–2]. New IRES-specific indices developed for flowing phases[3–6] when perennial methods have been deemed unsuitable[7].	Evaluate suitability of perennial methods for flowing phase assessments across Europe. Adapt or replace unsuitable methods, recognizing covarying biotic responses to degradation and intermittence. Develop new tools for IRES with short/unpredictable flowing phases.	Assign taxon-specific intermittence sensitivity scores[8–9] to inform expectations of community composition in IRES with different flow permanence regimes.	[1]Prat et al. (2014); [2]Morais et al. (2004); [3]Munné and Prat (2011); [4]Papastergiadou and Manolaki (2012); [5]Sánchez-Montoya et al. (2010); [6]García et al. (2014); [7]Munné et al. (2016); [8]Verberk et al. (2012); [9]Chadd et al., 2017.
	(c) using only community-level methods	Recognition that biomonitoring across freshwaters should incorporate metacommunity dynamics[1–3].	Interpret communities in a landscape context that recognizes the spatial arrangement of perennial and temporary reaches and wider recolonist sources.	Assign taxon-specific dispersal weightings, to inform expectations of community composition in IRES in different landscape contexts.	[1]Heino (2013); [2]Datry et al. (2016c); [3]Ruhí et al. (2017).
	(d) using only taxonomic methods.	Trait-based communities more stable than taxonomic communities within status classes[1] (but see[2–3]).	Explore functional approaches (trait-based and ecosystem services[4]) to status assessment.	Explore the potential of molecular approaches e.g. DNA metabarcoding and transcriptomics to overcome taxonomic limitations of traditional biomonitoring[5].	[1]Bruno et al. (2016); [2]Leigh et al. (2016); [3]Vander Vorste et al. (2016b); [4]von Schiller et al. (2017); [5]Leese et al. (2016).
6	Reference conditions characterized in few countries. Single benchmark concept	Diatom[4] and invertebrate[5] assemblages characterized in Mediterranean IRES with long, predictable flowing phases.	Develop conceptual alternatives to defining a single benchmark in dynamic ecosystems.	Recognize influence of metacommunity dynamics and landscape context on reference conditions.	[1]Bunn and Davies (2000); [2]Datry et al. (2016c); [3]Ruhí et al. (2017); [4]Delgado et al. (2012); [5]Munné and Prat (2009); [6]NN (2013).

Table 3 (*continued*)

Current state	Highlights to date	Priority actions	Steps towards action completion	Sources
inappropriate for dynamic ecosystems with unpredictable taxonomic communities[1–3].	'Best' and 'worst' values calculated for macrophytes, macroinvertebrates, and phytobenthos in Croatia[6].		Characterize reference conditions and class boundaries for each typology, informed by development of status classification methods.	

WFD, Water Framework Directive (EC, 2000).

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Appendix A. Initial survey used to request information about national biomonitoring activity from Working Group 4 members in the COST Action CA15113 Science and Management of Intermittent Rivers and Ephemeral Streams

SECTION 1 – RESPONDENT DETAILS

Your **name:**
Other Action participants involved in preparing this document**:**
Non-Action participants involved in preparing this document**:**
Country you represent**:**

SECTION 2 – BIOINDICATORS AND BIOMONITORING PROTOCOLS IN YOUR COUNTRY

**Numbers reflect the order of tasks listed in CA15113 Memorandum of Understanding.*

WG4 task 1*: *"List the different bioindicators and protocols used in the participating countries to assess the ecological status of rivers, including IRES when relevant"*

2.1 State the biotic groups used as bioindicators/biomonitors (including those used in **perennial** systems).
2.2 For each group listed in (2.1), describe the protocols used, *and/or* provide links to online descriptions, *and/or* provide protocols as attachments.

WG4 task 3*: *"identify issues and limitations [of current bioindicators and biomonitoring protocols]".*

2.3 Briefly note any issues and limitations for further discussion at WG meetings (optional section, leave blank if you have no comments at this stage).

SECTION 3 – RESEARCH ACTIVITY* INVOLVING YOUR COUNTRY

**Including MSc, PhD, post-doc and other projects or activities, and including collaborations with other countries*

3.1 Give details of any research activity relating to WG4 activities currently being conducted.
[Hyperlink to online shared spreadsheet]
3.2 Give details of any planned future research activity relating to WG4 activities.
[Hyperlink to online shared spreadsheet]

Appendix B. Second survey used to request further information about national biomonitoring activity from Working Group 4 members in the COST Action CA15113 Science and Management of Intermittent Rivers and Ephemeral Streams

In your country:

1. Have WFD typologies been developed that distinguish between different types of IRES? If "yes", provide details of classification criteria/each IRES type. If "no", state any descriptions that are in common use (e.g. in the UK, 'winterbourne chalk streams/rivers').

2. Are river typologies (including perennial typologies) classified using WFD System A or B (see WFD Annex II, Sections 1.1 and 1.2.1)? If System B is used, which "optional factors" are used in addition to the obligatory factors?

3. Have reference sites been identified for IRES typologies? If so, provide details of the site identification process (e.g. pressure criteria for site screening, modelling, expert judgement).

4. Have reference conditions (and boundaries between ecological status classes) been described for IRES typologies? If so, give details of classification criteria (e.g. quality elements considered).

5. Are *any* flow gauging stations located on IRES? If so, how many are on IRES and of these, how many match biomonitoring sites? What % of the *gauging station network* is located on IRES - and how does this relate to the % of the *river network* that is intermittent?

6. Are river flows (or pool/dry states) characterized by regulatory agencies using any method other than hydrological monitoring at gauging stations (e.g. citizen science initiatives, models)?
7. If perennial methods are used to assess IRES ecological status, has their suitability been evaluated? If so, state the methods/metrics tested and the results of the evaluation (suitable/not suitable).

[Responses used to inform Table 1]

8. If specific methods/indices have been developed for IRES (or IRs; e.g. because perennial methods were evaluated and found to be unsuitable), provide details.

[Responses used to inform Table 1]

9. Are any non-taxonomic (e.g. DNA metabarcoding, trait-based) approaches used to assess the ecological status of any rivers (including perennial rivers)?

10. Do any IRES that are too small to be designated as WFD water bodies based on catchment size fulfil *other* criteria for designation as a WFD water body?

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Ecological values of intermittent rivers for terrestrial vertebrate fauna

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HIGHLIGHTS

- Intermittent streams exhibited higher abundance and species richness than perennial streams.
- Shorter dry periods in intermittent streams boosted abundance and species richness.
- Hydrological variables, recreational and grazing uses, and riparian slope were the main factors explaining abundances and species richness.
- Dry channels were frequently used, mainly as corridors and as source of food.
- Intermittent rivers should be considered target ecosystems for conserving wildlife.

GRAPHICAL ABSTRACT

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ABSTRACT

Rivers are generally considered critical habitats for biodiversity; however, this often ignores the fact that many rivers may run dry and support terrestrial as well as aquatic fauna. Here, we investigated the ecological value of intermittent rivers for terrestrial vertebrates by installing camera traps along rivers subject to varying dry periods in two contrasting European climatic zones. We then analysed i) species presence and behaviours (as a proxy of ecological functions) on perennial and intermittent streams; ii) environmental (hydrological and geomorphological) and anthropogenic factors affecting the frequency of occurrence and number of species recorded; and iii) the importance of hydrological factors as regards ecological functioning. In both study areas, we recorded a higher number of species and individuals along intermittent streams than perennial streams, with highest values in intermittent reaches exhibiting shorter dry periods. Both abundance and species richness were strongly affected by hydrological factors in both study areas, including not only the occurrence but also the duration of the dry period. Dry channels played a key role as migration corridors and as a source of food, being used more frequently than riparian habitats when the river ran dry. Our findings indicate that terrestrial vertebrate fauna benefit from dry phases in rivers. Intermittent rivers, supporting a high abundance and diversity of fauna, should be considered as target ecosystems for wildlife conservation. Not doing so will jeopardise urgently needed conservation strategies in the face of accelerating global climate change.

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1. Introduction

Freshwater ecosystems are considered critical habitats for biodiversity worldwide as they provide vital ecological functions for fauna (Baron et al., 2002). Moreover, they harbour a disproportionately large fraction of the world's total biodiversity (Dudgeon et al., 2006; Balian et al., 2008). Nevertheless, the ecological importance of riverine habitats for fauna may have been underestimated (Sánchez-Montoya et al., 2017) as most assessments tend to ignore the fact that a large proportion of rivers undergo a dry phase that could potentially have positive effects on river-wide biodiversity (Datry et al., 2014; Sánchez-Montoya et al., 2020a, 2020b).

In rivers, both biodiversity and ecological function (e.g. corridor, refuge, food and water supply) tend to be considered under a classical conception that envisions such ecosystems as having water perennially in space and time (Sánchez-Montoya et al., 2017). Under this view, flowing channels provide habitats and functions for aquatic and semi-aquatic species (Bernhardt and Palmer, 2011) and account solely toward freshwater biodiversity (Vörösmarty et al., 2010). Under the same framework, riparian zones have been identified as key habitats for terrestrial fauna, providing important ecological functions such as migration corridors or food supply (e.g. Sabo et al., 2005; Hilty et al., 2006). This traditional perception is strongly biased as almost half the total length of global rivers may naturally cease to flow and eventually dry up (hereafter “intermittent rivers”; Datry et al., 2014). In recent decades, climate change and human activities have caused the proportion of intermittent streams to increase, even in more humid temperate climates outside traditionally dry regions, e.g. in Central Europe (Pařil et al., 2019; Straka et al., 2021). Ignoring the presence of dry phases in rivers, and their potential effects on wildlife, may limit decision-making as regards conservation strategies (Sánchez-Montoya et al., 2017).

Intermittent rivers begin to be understood as shifting mosaics of aquatic (lotic and lentic channels) and terrestrial (dry channels) habitats during alternating wet and dry phases (Datry et al., 2016). In this framework, a new river paradigm has emerged that highlights the concept of wet and dry river phases contributing substantially to overall biodiversity (Corti and Datry, 2016; Bunting et al., 2021) and ecological functions (Sánchez-Montoya et al., 2017). In particular, dry channels have emerged as pivotal landscape elements (Steward et al., 2012) for both invertebrate (e.g. Steward et al., 2011, 2017; Sánchez-Montoya et al., 2016a, 2020a) and vertebrate fauna (Sánchez-Montoya et al., 2016b, 2017).

The extent to which dry phases in intermittent rivers benefit terrestrial vertebrates is still not fully understood, despite the growing evidence of high diversity and ecological functions supporting by intermittent rivers (Sánchez-Montoya et al., 2017). A recent study, for example, identified dry channels as preferential movement corridors for terrestrial vertebrates rather than fringe habitats such as riparian zones and upland areas (Sánchez-Montoya et al., 2016b). This corridor function is of major interest for policymakers and environmental managers when advancing and adapting conservation and restoration strategies (Clerici and Vogt, 2013). Other essential functions have also been reported for rivers during the dry phase, including water and food resources, breeding and nesting sites, migration stopovers and resting and shelter areas (Sánchez-Montoya et al., 2017). In this sense, however, the importance of intermittent rivers for terrestrial fauna may be climate dependent. In arid and semi-arid zones, for example, intermittent streams may play a more important role than in temperate areas as they could provide habitats with higher humidity and more abundant vegetation than the surrounding landscape (Soykan et al., 2012).

Furthermore, little is known about how the duration of a dry period influences the presence and activity of terrestrial vertebrates. Dry periods can be highly variable in rivers, with shorter, predictable period restricted to the dry season in temperate areas (Pařil et al., 2019) and longer, less predictable periods stretching beyond the summer period in arid and semi-arid regions (Sánchez-Montoya et al., 2018).

In addition to hydrological factors, geomorphological features, such as riparian slopes that affect terrain ruggedness (e.g. McKinney et al., 2003; Sappington et al., 2007), and anthropogenic activities (Boyle and Samson, 1985), such as recreational use and animal grazing (e.g. Vavra, 2005, Ewers and Didham, 2006), may be important drivers adversely impacting the availability of habitats and the ability to use river beds as movement corridors (Leslie and Douglas, 1979).

In this study, we investigated the ecological value of intermittent rivers for terrestrial vertebrates by analysing their presence and behaviour (as a proxy for ecological functions), based on data gathered using camera traps during both wet and dry phases of perennial and intermittent rivers in the two contrasting climatic zones of Spain (Mediterranean) and the Czech Republic (temperate). In doing so, we i) estimated species abundance and richness in perennial and intermittent streams subject to differing dry periods and evaluated potential environmental factors affecting frequency of occurrence (abundance) and number of species recorded (species richness), and ii) assessed how different hydrological factors affect fauna behaviours in channels and riparian habitats, and the predominant behaviours in the study rivers as a whole. We hypothesised that i) in agreement with Sánchez-Montoya et al. (2017), who recorded multiple ecological functions along channel habitats during both wet and dry phases, intermittent rivers would prove to be as valuable as perennial rivers for terrestrial vertebrates in terms of abundance and species richness; ii) that, given the limited tolerance to flow intermittence potentially shown by some wildlife (Sánchez-Montoya et al., 2017), duration of dry period, in addition to its occurrence, would affect both the presence and activity of terrestrial vertebrates; and iii) that dry channels would provide key ecological functions for terrestrial fauna. To the best of our knowledge, this is the first study to assess the ecological importance of intermittent rivers for a wide range of terrestrial vertebrate fauna.

2. Methods

2.1. Study area and sampling sites

This study took place in perennial and intermittent streams in two contrasting European climatic areas, i.e. the Taibilla (370 km^2) and Alhárabe (345 km^2) river catchments in southeastern Spain, and in the Moravian Karst and Drahany highland areas (total area ca 200 km2) in southeastern Czech Republic (Fig. 1). Both study catchments are relatively undisturbed, with natural land cover >90% and large areas of forest. In Spain, riparian habitats consist of helophytes (*Scirpus holoschoenus*), silver poplar (*Populus alba*) and willow trees (*Salix* spp.), with uplands covered in shrubs and pines (*Pinus* spp.), whereas in the Czech Republic, riparian habitats consist of alder (*Alnus glutinosa*), ash (*Fraxinus excelsior*) and hazel (*Corylus avellana*), with uplands covered mainly in oak (*Quercus* spp.), hornbeam (*Carpinus betulus*) and Scots pine (*Pinus sylvestris*).

According to the Köppen-Geiger system (Peel et al., 2007), the Spanish study area exhibits a warm temperate climate (Csa; mean annual temperature = 14.3 °C; mean annual rainfall = 583 mm) with hot and dry summers, while the Czech Republic has a continental humid climate type (Dfb; mean annual temperature = 8.7 °C; mean annual rainfall = 508 mm) with warm summers. Both areas have perennial and intermittent streams characterised by spatially and temporally intermittent flow regimes that tend to be more predictable in the Czech Republic (Sánchez-Montoya et al., 2018; Pařil et al., 2019). In Spain, the dry period commonly starts in June/July and ends in November/December, though longer dry periods may occur in some years (Sánchez-Montoya et al., 2018), while the dry period is usually short and restricted to summer (July to August/September) in the Czech Republic, though there have been exceptional supra-seasonal droughts (e.g. 2014–2019) that caused many streams to run dry until winter and

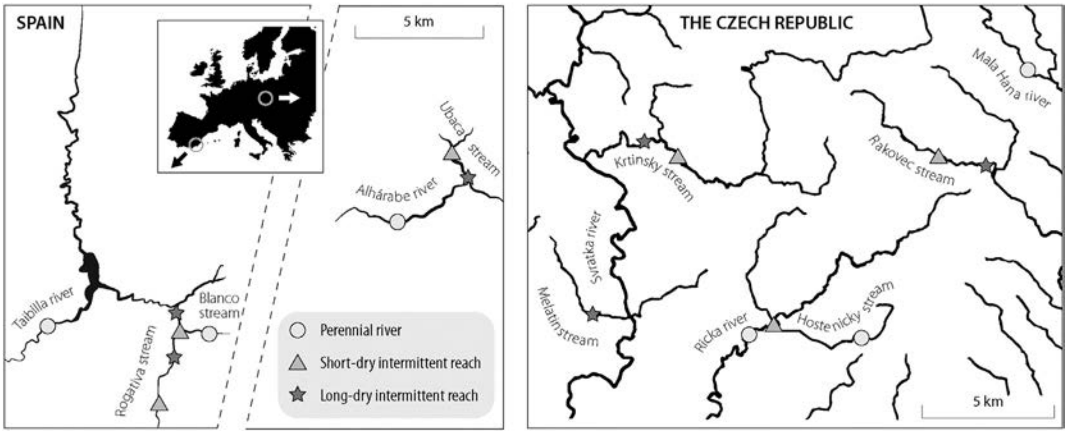

Fig. 1. Hydrological type and location of the 18 study reaches in the two study areas (Spain and the Czech Republic).

previously perennial to become intermittent streams (Boergens et al., 2020; Mozny et al., 2020).

For the purposes of this study, we selected nine reaches in each area characterised by similar substrate, mean channel width (Spain = 1.5–9.0 m; Czech Republic = 1.5–5.0 m) and micro-topography. Of the nine reaches, three were located on perennial streams and six on intermittent streams, with three of the latter in intermittent reaches undergoing a short-dry period (hereafter short-dry intermittent; mainly flowing channels that had dry channels and/or isolated pools in the previous year), and the other three in reaches with a long-dry period (hereafter long-dry intermittent; mainly dry riverbeds and/or isolated pools during the study period; see Table S1).

2.2. Data collection

A total of 18 camera traps were installed (nine in each country) in order to record species presence and the various behaviours (functions) displayed. In Spain, Trophy®Cam Essential HD E3 cameras traps (Bushnell, USA) with a 0.3 s trigger speed, a 900 nm flash and 100 ft. (30 m) motion activated PIR sensors were used, while Cuddeback X-Change Triple Flash camera traps (Cuddeback, USA) with a 0.2 s trigger speed, an 850 nm flash and 100 ft. (30 m) motion activated IR sensors were used in the Czech Republic. Each camera trap was attached to a tree in the riparian zone at a height of ca. 2–3.5 above the channel, and positioned such that both channel and riparian habitats were included in the frame (Figs. S1 and S2). Each camera operated for 24 h/day and recorded a 10 s video each time movement was detected, the videos being saved onto a memory card. In Spain, the traps were active for 11 months (August 2017 to July 2018), resulting in 334 sampling days for all reaches except three perennial streams with 303, 244 and 212 days, and one long-dry intermittent reaches with 305 days. In the Czech Republic, the traps were active for eight months (November–December 2017 and May–October 2018, i.e. excluding winter months when streams are typically frozen), with 254 sampling days for each camera. Each camera trap was visited every two months or less in order to replace batteries and/or memory cards and to download videos onto a computer.

All video recordings were analysed to register the number of individuals, species, date, time, river location (channel, riparian and both), and the hydrological channel state of channels (wet, isolated pools and dry; Table S1). Species were recorded as rodents, lagomorphs, carnivores, ungulates or birds. Also, videos with people, pets (dogs) and livestock (sheep and goats) were analysed as a proxy of recreational and grazing uses in each study reach. A temporal buffer of 1 min was used to distinguish between consecutive videos at a camera trapping station. In addition, we recorded the behaviour of each animal that corresponded with an ecological function provided by the channel or riparian habitat (Table 1 and Fig. 2). Ecological functions recorded were: water resource, food resource, hygiene, corridor, pathway and occupancy. The same trained observer conducted the entire behavioural coding process in each study area, using previously established criteria, in order to avoid subjectivity and observer bias. At each camera trap station, we determined the percentage of riparian cover and the slope of the riparian zone by visual estimation. In addition, we estimated the amount of recreational and grazing use by calculating the percentage of records with people, pets or livestock. Finally, we calculated percentage natural cover within two delimited 50 m and 250 m circular buffer zones centred on the camera station, using Corine Land Cover 2012 in Spain (Scale 1:100.000, IGN) and a detailed map server (www.mapy.cz; Scale 1:10.000) in the Czech Republic.

2.3. Statistical analysis

Cramér's V (Cramér, 1946), implemented within the R package *oii* (Hale et al., 2017), was used to identify significant correlations in contingency tables relating the frequency of occurrence of each hydrological and fixed factor pair. Cramér's V index is based on χ^2 and describes the association between factors ranging from 0 (no association) and 1 (perfect association).

Generalised linear mixed models (GLMMs; Zuur et al., 2009) were used to identify factors affecting the total abundance and species richness of the fauna recorded, and abundances of the two predominant species in each study area, in order to assess the consistency of general patterns. Response variables were defined as the number of individuals and number of different species per camera trap (nine in each study

Table 1
Ecological functions provided by the two habitats in the study reaches, according to different behaviours performed by terrestrial vertebrates.

River function	Habitat	Behaviour description
Water resource	Channel	When an animal was drinking
Food resource	Channel and riparian	When an animal was feeding on vegetation, searching food and eventually feed, or hunting other animals
Hygiene	Channel	When an animal was performing cleaning behaviour
Corridor	Channel and riparian	When the animal performed longitudinal displacement along
Pathway	No habitat distinction	When the animal moved from one riparian side to another across the channel
Occupancy	Channel and riparian	When the animal just standing on

Fig. 2. Images taken from videos of A) *Cervus elaphus* and B) *Capra pyrenaica* drinking water in wet channels (water resource function). C) *Sus scrofa* feeding in a dry channel and D) *Capra pyrenaica* feeding in the riparian zone (food resource function). E) *Dendroscopos* sp. cleaning in a wet channel (hygiene function). F) *Lutra lutra* moving along a wet channel and G) *Meles meles*, H) *Genneta genneta* and I) *Vulpes vulpes* moving along dry channels (corridor function). J) *Vulpes vulpes* crossing a wet channel (pathway function). K) *Cervus elaphus* standing in a dry channel, and L) *Capra pyrenaica* standing in the riparian zone (occupancy function).

area) and month (11 in Spain and eight in the Czech Republic). Data were analysed separately for each study area. The models were fitted using the *glmer.nb* function in the R package *lme4* (Bates et al., 2015). Given the relatively low number of sampling sites, we used a reduced set of univariate mixed models as candidate models, with the above described variables as fixed factors and camera ID as a random effect, thereby guarding against the risk of overfitting and finding spurious relationships (Johnson and Omland, 2004). Slope, recreation/grazing uses, riparian vegetation cover and natural cover (at the 50 m and 250 m buffers) and four hydrological factors (i.e. flow regimen type, flow regimen subtype, channel stage and hydrological type) were considered as fixed factors (Table S1). These fixed factors were then sorted into categorical groups (see Table S1 for a description of categories), with the number of categories varying in each study area. Hydrological type assessment, based on presence/absence of water in the channel in different flow regimen subtypes, resulted in three categories in Spain and seven in the Czech Republic. Model selection was based on the corrected version of the Akaike Information Criterion (AICc), using the R package MuMIn. This approach identifies the most parsimonious model (lowest AICc) and ranks the remaining models. ΔAICc was calculated as the difference in AICc between each model and the best model in the set. Akaike weights (w_i) were used to evaluate the strength of evidence among competing models.

Multinomial regression analysis (Ripley and Venables, 2011) was performed to examine the significance of the four potential hydrological explanatory factors (Table S1; flow regimen types and subtypes, channel stage and hydrological types) affecting ecological functions (Table 1 and Fig. 2). Multinomial regression is a simple extension of binary logistic regression that allows for more than two categories of dependent variable, and is considered suitable for analysing multiway contingency tables. Every table row showed each observed animal performing only one behaviour regardless the number of animals and type of behaviours occurring in each video recorded. The analysis was performed using the R packages *nnet* (Ripley and Venables, 2011) and effects (Fox et al., 2019). All analyses were performed in R v.3.6.3 (R Core Team, 2020).

3. Results

3.1. Terrestrial species abundance and richness

In Spain, the nine cameras recorded 1222 videos with wildlife, livestock, pets and humans (7303 individuals). Of these, 67% were of wildlife (1317 individuals), 31% livestock (sheep and goats; 5963 ind.) and 2% humans and/or pets (24 ind.). In the Czech Republic, the nine cameras recorded 1615 videos with wildlife, pets and humans (3561 individuals). Of these, 95% were of wildlife (2639 ind.) and 4% humans and/or pets (110 ind.). No livestock were recorded at Czech sites.

In both study areas, short-dry intermittent reaches exhibited a higher abundance than either perennial reaches in perennial streams and long-dry intermittent reaches in both study areas (Table 2). In Spain, abundance was similar between perennial and long-dry intermittent reaches, while abundances in both long-dry and short-dry intermittent reaches of intermittent streams were double those in perennial streams in the Czech Republic (Table 2).

A total of 16 spp. were recorded in Spain and 10 spp. in the Czech Republic (Table 2). Carnivores and birds were the most diverse groups in Spain (5 spp.), while ungulates were the predominant group in the Czech Republic (4 spp.). In both study areas, wild boar (*Sus scrofa*) accounted for the majority of individuals recorded (Spain 55%, Czech Republic 70%), followed by red fox (*Vulpes vulpes*, 19%) in Spain and roe deer (*Capreolus capreolus*, 21%) in the Czech Republic. As with abundance, more species were recorded in intermittent streams than perennial strams, with short-dry intermittent reaches having highest values in both study areas (Table 2).

3.2. Factors affecting abundance and species richness

In both study areas, Cramér's V values (Table S2 and Table S3) indicated that associations between hydrological and the rest of study factors were mainly moderate (0.25 < V < 0.75; Spain 73%, Czech Republic 55%) or weak (V < 0.25; Spain 13%, Czech Republic 30%).

For abundance, models that included hydrological variables (channel stage and flow regimen type) were among the three most parsimonious models (lowest AICc; Table 3) in both study areas, with Spanish sites also including recreational and grazing uses and Czech sites including riparian slope. In both study areas, more individuals were recorded in intermittent streams than perennial streams (Fig. 3). While abundances were slightly higher in wet (flowing) channels than dry channels in Spain, conversely higher abundances were recorded in pools and dry channels than in wet channels in the Czech Republic. Finally, in Spain abundance was higher in those reaches undisturbed by recreation or livestock use compared to most disturbed reaches, and in the Cezch Republic flatter riparian slope reaches displayed higher abundances than those with steeper slopes (Fig. 3).

Regarding species richness, recreational use was selected as one of the three most parsimonious models in both study areas (Table 4), along with natural cover (50 m buffer area) and riparian slope in Spain and flow regime type and subtype in the Czech Republic (Table 4). In Spain, areas with lower disturbance (higher natural cover and absence of humans or livestock) and flatter riparian slopes had a highest number of species. In the Czech Republic, more species were recorded in intermittent than perennial streams, with highest values along short-dry reaches, and in no recreational reaches compared to their disturbed counterparts (Fig. 3).

Table 2
Total abundance of terrestrial vertebrate and abundance per camera and month (in parenthesis) and species richness, recorded in perennial, short dry intermittent and long dry intermittent reaches in Spain and the Czech Republic. The IUCN category (EN, endangered; VU, vulnerable; NT, near threatened; LC, lesser concern) is shown for the recorded species.

		Spain			The Czech Republic		
	IUCN Category	Perennial	Short-dry intermittent	Long-dry intermittent	Perennial	Short-dry intermittent	Long-dry intermittent
Rodents							
*Sciurus vulgaris**	LC	2 (0.08)	6 (0.18)	2 (0.06)	0 (0.00)	0 (0.00)	0 (0.00)
Lagomorphs							
*Lepus europaeus***	LC	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	11 (0.45)	3 (0.12)
*Lepus granatensis**	LC	0 (0.00)	13 (0.39)	1 (0.03)	0 (0.00)	0 (0.00)	0 (0.00)
*Oryctolagus cuniculus**	EN	0 (0.00)	5 (0.15)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Carnivores							
*Genneta genneta**	LC	0 (0.00)	2 (0.06)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
*Lutra lutra**	NT	1 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
*Martes foina**	LC	1 (0.04)	6 (0.18)	1 (0.03)	0 (0.00)	0 (0.00)	0 (0.00)
*Meles meles****	LC	0 (0.00)	0 (0.00)	5 (0.16)	0 (0.00)	0 (0.00)	3 (0.12)
*Vulpes vulpes****	LC	12 (0.48)	153 (4.64)	87 (2.72)	0 (0.00)	14 (0.58)	0 (0.00)
Ungulates							
*Capreolus capreolus***	LC	0 (0.00)	0 (0.00)	0 (0.00)	30 (1.25)	332 (13.83)	192 (8.0)
*Capra pirenaica**	LC	62 (2.48)	107 (3.24)	69 (2.16)	0 (0.00)	0 (0.00)	0 (0.00)
*Cervus elaphus****	LC	0 (0.00)	9 (0.27)	1 (0.03)	0 (0.00)	88 (3.66)	97 (4.04)
*Ovis orientalis musimon***	VU	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.900)	0 (0.00)	5 (0.21)
*Sus scrofa****	LC	218 (8.72)	397 (12.03)	109 (3.41)	430 (17.91)	761 (31.71)	670 (27.91)
Birds							
*Aquila fasciata**	LC	0 (0.00)	1 (0.03)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
*Anas platyrhynchos**	LC	1 (0.12)	4 (0.12)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
*Ardea cinérea**	LC	1 (0.04)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
*Columba palumbus****	LC	3 (0.04)	23 (0.70)	5 (0.16)	0 (0.00)	1 (0.04)	0 (0.00)
Corvidae***	–	0 (0.00)	10 (0.30)	0 (0.00)	0 (0.00)	1 (0.04)	0 (0.00)
Dendroscopos sp. **	–	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.04)	0 (0.00)	0 (0.00)
Total abundance		301 (12.04)	736 (22.30)	280 (8.75)	461 (19.21)	1208 (50.33)	970 (40.41)
Species richness		9	12	9	3	7	6

* Species that occur exclusively in study areas in Spain.
** Species that occur exclusively in study areas in the Czech Republic.
*** Species that occur in both study areas (Spain and the Czech Republic).

Table 3

Summary of abundance model selection results for terrestrial vertebrates in Spain and the Czech Republic (K = number of parameters; AICc = Akaike's information criteria; ΔAICc = change in AICc, relative to the highest ranked model; wi = Akaike weight). Models are ranked according to Akaike's information criteria. (FlowReg = Flow regimen type; FlowReg_Sub: Flow regimen subtype; ChannelStage = Channel stage; Hydrol = Hydrological type; Slope = Slope of riparian zone; RecreaGrazUse = Recreational and/or grazing uses; RipCover = Riparian vegetation cover; NC50 = Natural cover at 50 m buffer; NC250 = Natural cover at 250 m buffer; see supplementary material for description of factors and their categories).

Spain					The Czech Republic				
Models	*K*	AICc	ΔAICc	*wi*	Models	*K*	AICc	ΔAICc	*wi*
RecreaGrazUse	5	627.6	0.00	0.179	Slope	5	655.7	0.00	0.547
ChannelStage	4	627.9	0.26	0.157	ChannelStage	5	658.7	2.94	0.126
FlowReg	4	628.2	0.56	0.135	FlowReg	5	658.9	3.22	0.109
Month	4	628.3	0.66	0.128	Month	4	660.2	4.44	0.059
NC250	4	628.3	0.69	0.126	RecreaGrazUse	4	660.8	5.04	0.044
Slope	5	628.7	1.05	0.106	RipCover	4	661.0	5.29	0.039
NC50	5	629.4	1.80	0.073	NC50	4	661.9	6.19	0.025
FlowReg_Sub = Hydrol	5	630.1	2.51	0.051	NC250	5	662.0	6.28	0.024
RipCover	5	630.4	2.75	0.045	FlowReg_Sub	5	662.0	6.31	0.023
Null	1	2146.8	1519.21	0.000	Hydrol	9	665.4	9.68	0.004
					Null	1	3082.8	2427.03	0.000

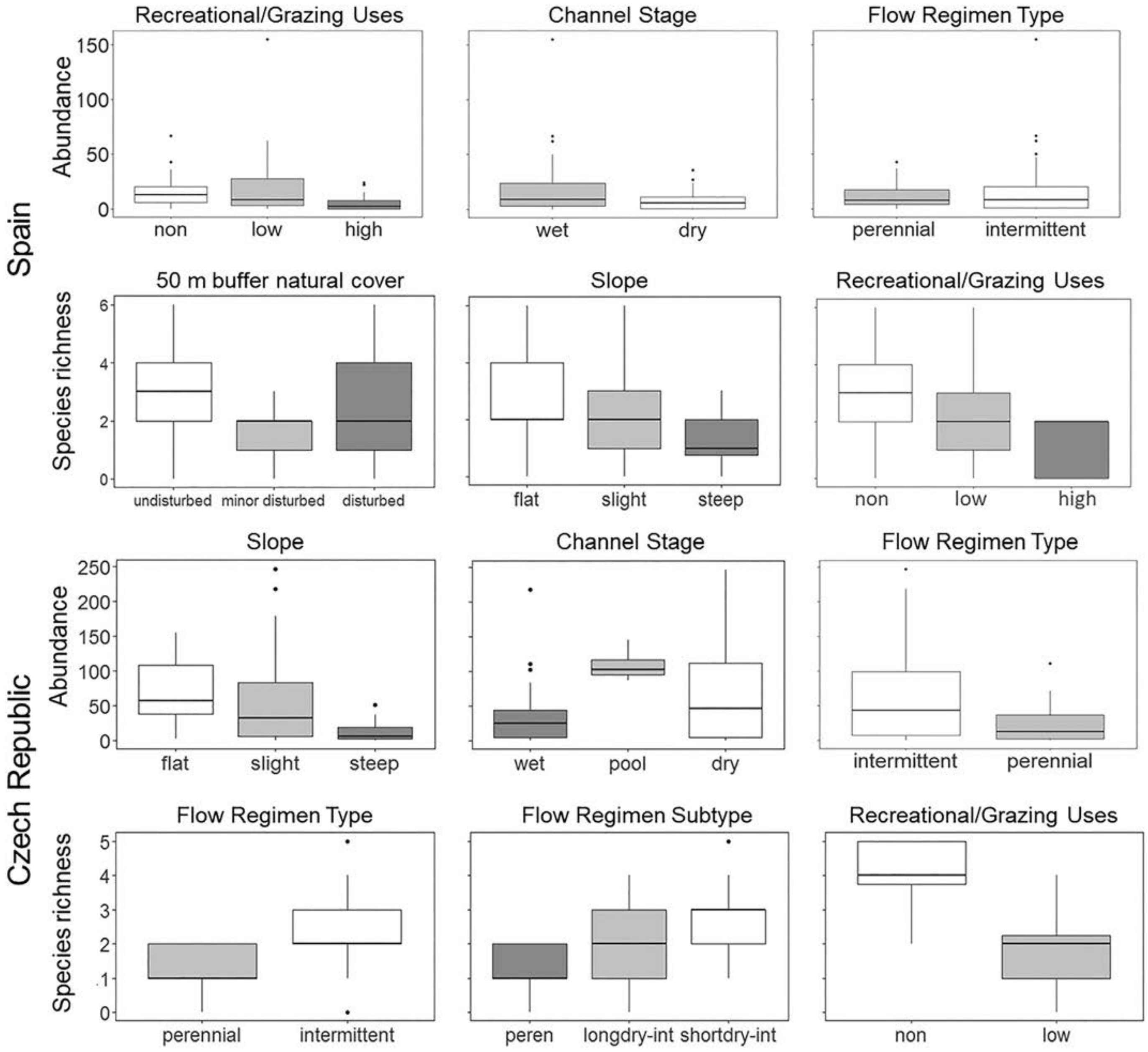

Fig. 3. Box-plots of abundance and species richness for the three main factors resulting from GLMMs in Spain and the Czech Republic. Boxes represent interquartile ranges (25th to 75th percentile), range bars show maximum and minimum values and lines represent medians.

Table 4
Summary of species richness recorded model selection results for terrestrial vertebrates in Spain and the Czech Republic (K = number of parameters; AICc = Akaike's information criteria; ΔAICc = change in AICc, relative to the highest ranked model; wi = Akaike weight). Models are ranked according to Akaike's information criteria. (FlowReg = Flow regimen type; FlowReg_Sub: Flow regimen subtype; ChannelStage = Channel stage; Hydrol = Hydrological type; Slope = Slope of riparian zone; RecreaGrazUse = Recreational/Grazing uses; RipCover = Riparian vegetation cover; NC50 = Natural cover at 50 m buffer; NC250 = Natural cover at 250 m buffer; see supplementary material for description of factors and their categories).

Spain					The Czech Republic				
Models	K	AICc	ΔAICc	Wi	Models	K	AICc	ΔAICc	wi
NC50	5	300.0	0.00	0.194	RecreaGrazUse	4	222.8	0.00	0.280
Slope	5	300.4	0.34	0.163	FlowReg	4	222.9	0.04	0.275
RecreaGrazUse	5	300.5	0.48	0.153	FlowReg_Sub	5	223.4	0.54	0.214
FlowReg	4	301.1	1.09	0.112	NC250	5	224.4	1.58	0.127
NC250	4	301.1	1.12	0.111	Slope	5	227.1	4.29	0.033
ChannelStage	4	301.5	1.51	0.091	Month	4	227.3	4.49	0.030
Month	5	301.5	1.51	0.091	NC50	4	227.8	4.92	0.024
FlowReg_Sub = Hydrol	5	302.9	2.87	0.046	ChannelStage	5	229.9	7.07	0.008
RipCover	5	303.3	3.22	0.039	Null	1	230.8	7.95	0.005
Null	1	330.4	30.33	0.000	Hydrol	9	231.9	9.05	0.003
					RipCover	5	630.4	407.51	0.000

For wild boar abundance (the predominant species in both study areas) hydrological variables in both study areas were including in the three most parsimonious models, followed by recreational use and natural cover (250 m buffer) in Spain, and riparian slope in the Czech Republic (Tables S4 and S5). Regarding red fox abundance, the second most abundant species in Spain, hydrological variables (channel stage and flow regimen type) and riparian slope were including in the most parsimonious models (Table S4). Finally, in the Czech Republic, the abundance of roe deer, the second most abundant species, was mainly modulated by hydrological factors (flow regimen and flow regimen subtype) and recreational use (Table S5). For each factor, abundance patterns for predominant species followed a similar trend to those for total abundance in both study areas (Figs. S3 and S4).

3.3. Ecological function and factors affecting fauna behaviour

Multinomial analysis detected that the hydrology type was the factor most affecting ecological functions observed in river reaches in both study areas (Table 5). In Spain, riparian habitats in perennial streams were more often used as food resource, occupancy and corridor than in intermittent streams (Fig. 4). Dry channels of long-dry intermittent reaches were more frequently used as a food resource, occupancy and corridor than perennial and short-dry intermittent reaches. Finally, wet channels of short-dry intermittent reaches were more used as pathways and water resource than their counterparts. Similarly, in the Czech Republic riparian habitats of perennial reaches were more often used as food resource, and dry channels of long-dry intermittent reaches as corridors than the rest of hydrological types (Fig. 5). In addition, dry channels of short-dry intermittent reaches were notable for being used as food resources. Finally, dry channels in both short and long-dry intermittent reaches and pools of long-dry intermittent reaches were frequently used as corridors, similarly to riparian habitats in both type of intermittent reaches, but only in the case that channels were flowing.

Table 5
Summary of the multinomial regression models used to explain observed behaviours (ecological functions) in the two study areas. Models are ranked according to Akaike's information criteria. (FlowReg = Flow regimen type; FlowReg_Sub: Flow regimen subtype; Hydrol = Hydrological type; see supplementary material for description of factors and their categories).

Models	df	logLik	AICc	ΔAICc	wi
Spain					
FlowReg_Sub = Hydrol	21	−2272.438	4587.5	0.00	1
FlowReg	14	−2371.888	4772.1	184.54	0
ChannelStage	14	−2468.715	4965.7	378.20	0
Null	7	−2620.345	5254.8	667.24	0
The Czech Republic					
Hydrol	56	−5538.009	11,189.9	0.00	1
FlowReg_Sub	24	−5652.145	11,352.6	162.75	0
ChannelStage	24	−5907.219	11,862.8	672.90	0
FlowReg	16	−6016.681	12,065.5	875.63	0
Null	8	−6206.969	12,430.0	1240.09	0

4. Discussion

In this study, we analysed the ecological value of intermittent streams relative to perennial streams based on the presence of a wide range of terrestrial vertebrates, and the functional role they provide for fauna. Main findings allowed to detect that different hydrological stream types accumulated distinct levels of abundance and species richness, and specific ecological functions provided by those reach types (perennial reaches, and intermittent reaches undergoing different dry periods) and habitats (flowing and dry channels, isolated pools and riparian zones).

4.1. Abundance, species richness and contributing factors

In both Spain and the Czech Republic, our findings indicated that intermittent streams harbour higher abundance and species richness of terrestrial vertebrates than their perennial counterparts. These findings reinforce previous evidence for the key role played by dry phases in supporting wildlife diversity and the important role played by intermittent rivers in providing ecological value not only for aquatic but also for terrestrial biota (Sánchez-Montoya et al., 2017). These results support also the notion, until now only validated for invertebrate communities (Corti and Datry, 2016; Bunting et al., 2021), that both aquatic and terrestrial biota should be considered when characterising the total biodiversity of intermittent rivers. Such findings should help change the current public perception that intermittent rivers are unable to provide ecosystem services and habitats for biodiversity, and thus are less valuable and less worthy of conservation than perennial rivers (Rodríguez-Lozano et al., 2020).

More specifically, we found that intermittent reaches with shorter dry periods supported more individuals (both study areas) and more species (the Czech Republic) than perennial reaches and intermittent reaches exhibiting longer dry periods. Consistently, hydrological factors in both study areas had a strong effect on both abundance and species richness in both study areas. Further, it was not just the occurrence of a dry period but also its duration that modulated the presence of fauna, with more recurrent wet-dry cycles appearing to benefit terrestrial fauna. This reinforces the idea that terrestrial vertebrates benefit from the alternance of both aquatic (flowing channels and pools) and terrestrial (dry riverbeds) habitats in intermittent rivers (Sánchez-Montoya et al., 2017). That implies that the natural flooding and drying patterns in intermittent rivers are essential to warrant the proper

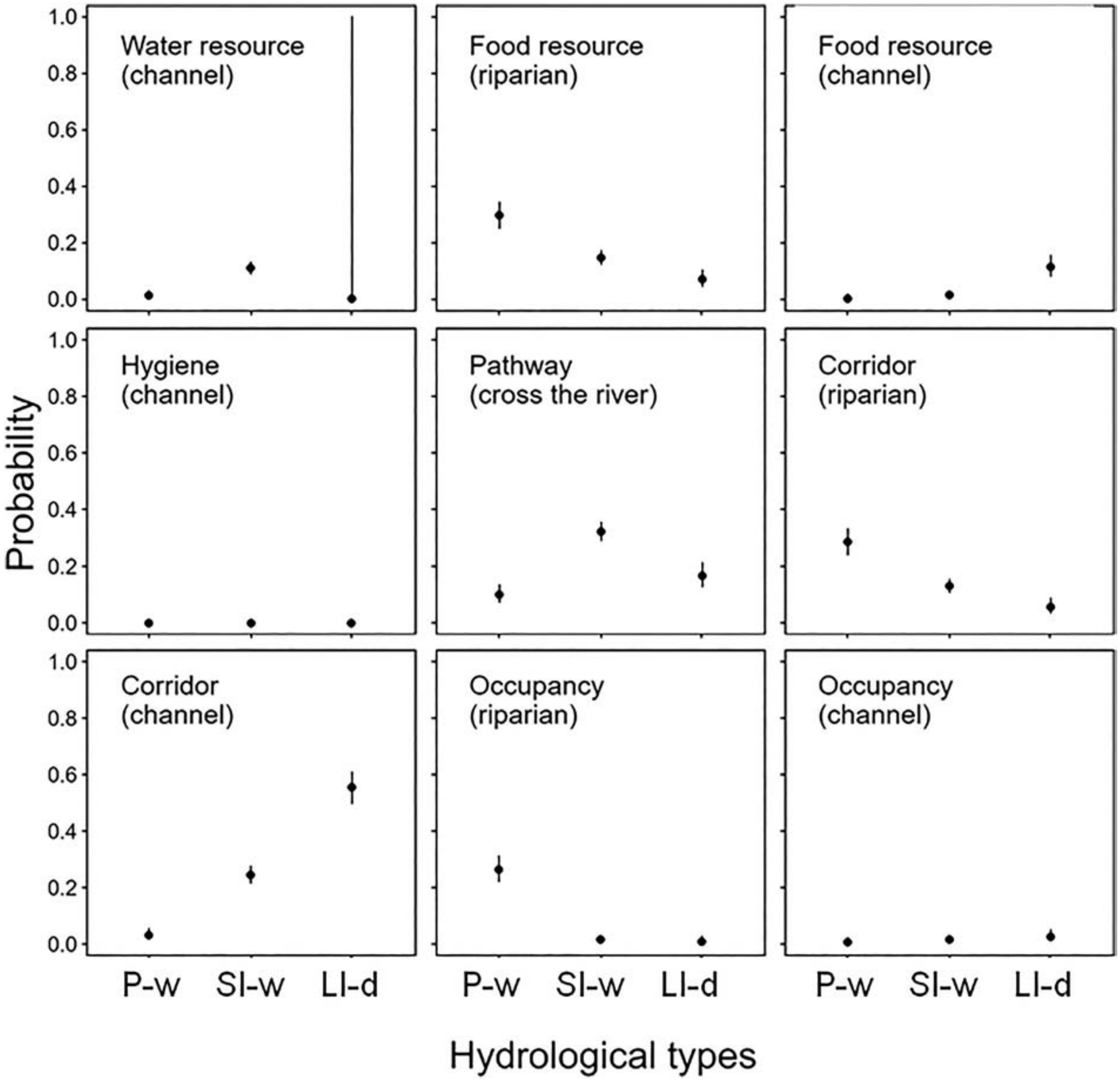

Fig. 4. Estimated probability of recording ecological functions provided by the three hydrological types in Spain (P-w = wet channels in perennial streams, SI-w = wet channel in short-dry intermittent reaches, LI-d = dry channels in long-dry intermittent reaches). Vertical lines represent 95% confidence intervals.

management of these habitats that demand connectivity among them and the surrounding landscape (Sheldon et al., 2010; Robson et al., 2013). Likewise, these results support the previous notion based on invertebrate aquatic communities, that increasing dry periods may decrease abundances and species richness (Leigh and Datry, 2016; Sánchez-Montoya et al., 2018). Therefore, current findings expand our knowledge that the duration of the dry period may also modulate terrestrial vertebrate communities.

In our study streams, abundance and species richness of terrestrial vertebrates were adversely affected by land uses, as in terrestrial ecosystems, given that commonly this type of perturbation results in habitat degradation and/or fragmentation that negatively affect fauna (Boyle and Samson, 1985). In addition, reaches subject to recreational uses (walking, cycling, mountain biking or off-road driving) and livestock grazing had lower species richness than undisturbed stream reaches in both study areas, as commonly found in terrestrial ecosystems (Marzano and Dandy, 2012). Similarly, the slope of riparian zones proved to be an important influencing factor for vertebrate presence in both study areas, with reaches with flatter slopes having more individual and species than reaches with steeper slopes, as result probably of easier access to, and passage across, the stream channel.

In this study, we detected similar affecting factors and patterns were detected for abundances of total community and predominant species in both study areas, which may reflect the consistency of general patterns in fluvial ecosystems with communities dominated by mammals.

4.2. Ecological functions and contributing factors

While terrestrial (riparian) and aquatic (flowing channel) habitats along permanent rivers and streams have long been recognised as pivotal ecosystems for terrestrial fauna, the role of dry channels in intermittent rivers has largely been ignored until now. In our study, intermittent rivers provided multiple ecological functions similarly to perennial rivers for a wide range of terrestrial vertebrates along channels and riparian habitats during both wet and dry phases, acknowledging previous evidences (Sánchez-Montoya et al., 2017). Therefore, the present study extends our view of the importance of rivers for terrestrial fauna by integrating their dry phase. In agreement with that detected for

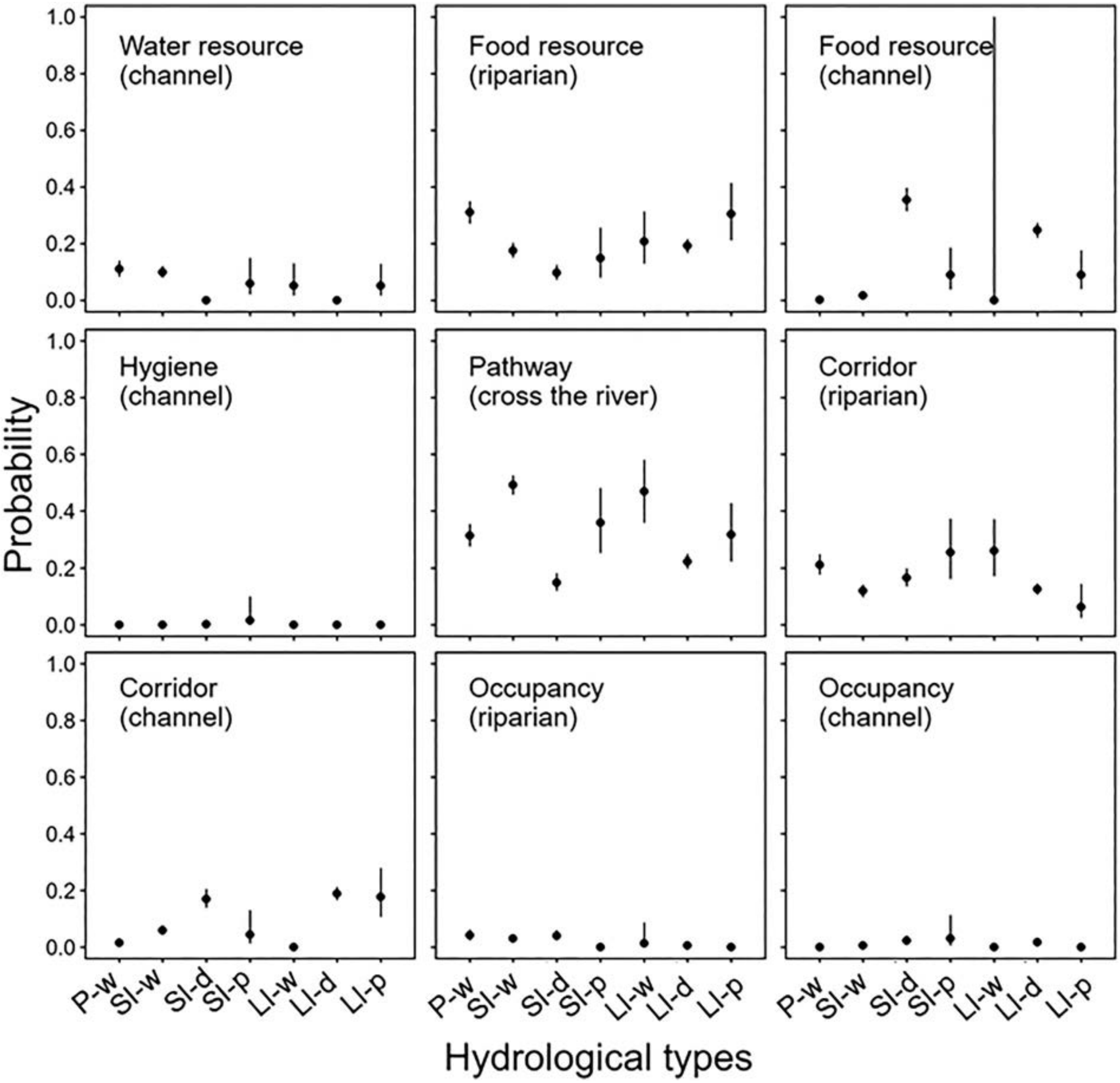

Fig. 5. Estimated probability of recording ecological functions provided by the seven hydrological reach types in the Czech Republic (P-w = wet channels in perennial streams; SI-w = wet channels in short-dry intermittent reaches; SI-d = dry channels in short-dry intermittent reaches; SI-p = pool channels in short-dry intermittent reaches; LI-w = wet channels in long-dry intermittent reaches; LI-d = dry channels in long-dry intermittent reaches; LI-p = pool channels in long-dry intermittent reaches). Vertical lines represent 95% confidence intervals.

abundance and richness species models, the duration of the dry period rather than its presence explaining fauna behaviours and therefore river functionality.

As might be expected, our study found that flowing channels in both perennial and intermittent streams were major suppliers of water (Nilsson and Dynesius, 1994), and that riparian habitats in perennial rivers acted mainly as terrestrial corridors (Doyle, 1990; Sabo et al., 2005; Hilty et al., 2006) and food suppliers (Oakley et al., 1985). In addition, we noted that wet (flowing) channels of short-dry intermittent reaches acted as important pathways for crossing streams, thereby facilitating increased landscape permeability for the movement of wildlife, in contrast to wet channels of perennial streams characterised by deeper waters.

In addition, this study also showed that dry channels provided essential ecological functions for wildlife. In fact, dry channels were frequently used as migration corridors, even more that their fringing riparian zones, supporting that reported by a recent study which identified for first time dry channels as corridors for terrestrial vertebrate that act as key landscape elements, enhancing natural connectivity both along rivers and within the catchment (Sánchez-Montoya et al., 2016b). Dry channels were also important as direct and indirect suppliers of food, and again, more often so than the adjacent riparian zone. Our video recordings clearly showed that dry channels also provided terrestrial vegetation, which colonise from adjacent terrestrial habitats (Tooth and Nanson, 1999), for herbivores and ungulates, and additionally live prey for carnivores.During the initial phase of drying, for example, omnivorous wild boars may be attracted to the river bed by the large numbers of aquatic macroinvertebrates concentrated in shrinking pools along the riverbed. This appeared to be a widespread feeding behaviour and was frequently observed on video recordings and backed up by the presence of wild boar footprints in wet sediment. This predatory pressure on macroinvertebrates may be especially intense (Motta et al., 2020) in areas now overpopulated by wild boar or in temperate areas where intermittent streams were relatively uncommon before the onset of global warming (Crabot et al., 2020). This new finding could be used to provide better protection to threatened dry river networks (e.g. through control of wild boar populations; Risch et al., 2021), particularly as regards specific biota (e.g.

macroinvertebrates) that are increasingly being threatened by the joint impacts of climate change and human activities (Stubbington et al., 2018).

5. Conclusions

This study revealed that intermittent rivers and streams represent valuable ecosystems for terrestrial vertebrates, given both the high abundance and diversity of fauna recorded and the multiple ecological functions provided by this type of freshwater ecosystem. This is in general contrast to the public's perception of intermittent rivers as barren ecosystems. This, combined with the lack of legal protection given to intermittent rivers worldwide (Acuña et al., 2014), may place them among the most threatened of freshwater ecosystems. This study provided new information on the mechanisms linking intermittent rivers and terrestrial vertebrate fauna. The neglection of this existing interaction jeopardizes a complete understanding of the functioning of freshwater ecosystems, and in turn limiting conservation strategies which are especially urgent given the current scenario of climate change and increasing human pressures (Schneider et al., 2013). As such, we believe that intermittent rivers and streams should now be considered as priority ecosystems for wildlife conservation.

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Data and material availability

Available upon request from M.M.S.M and P.P.

CRediT authorship contribution statement

P.P. and M.M.S.M. conceived and designed the study with inputs from R.G. P.P., M.M.S.M., R.G. and T.B. collected the data; M.M.S.M. and J.F.C. analysed the data; M.M.S.M. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.151308.

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Fundam. Appl. Limnol. 193/1 (2019), 39–49 **Article**

Sediment characteristics influence benthic macroinvertebrate vertical migrations and survival under experimental water loss conditions

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With 5 figures and 4 tables

Abstract: Subsurface riverine sediments can provide an instream refuge for benthic macroinvertebrates during drying events in intermittent streams. The permeability of sediments may strongly influence the ability of fauna to migrate vertically through benthic and subsurface substrates. We examined the vertical movement of four common taxa with different levels of mobility (*Gammarus fossarum*, *Ephemera danica*, *Pisidium* spp. and Tubificinae spp.) in a laboratory experiment using substrates composed of three different grain sizes (2–7 mm, 7–10 mm and 20−32 mm). Experimental substrates were placed in perforated mesocosms so that the deeper layers of the substrate remained saturated while the upper layers were subject to gradual drying. The mesocosms were inspected on five occasions over 32 days to determine the number of live specimens. We hypothesized that substrates comprising smaller particles (with smaller interstitial spaces) would be more difficult for macroinvertebrates to move through than substrates with larger interstices. We observed significant differences in the vertical distribution among substrate treatments for all taxa. We also hypothesized that the deeper saturated sediment layer would facilitate higher survival rates than the upper layers with reduced moisture content. The results indicated the substrate moisture content had a significant effect on the survival of *G. fossarum* and *E. danica*, but the effect was less clear for *Pisidium* spp. and Tubificinae spp. Our study demonstrates that sediment characteristics influence the availability of stream bed refuge for benthic macroinvertebrates and that their use depends on taxon specific abilities to access subsurface habitats.

Keywords: macrozoobenthos; drying; streambed; porosity; moisture; substrate water content; refugia

Introduction

Intermittent streams are common globally and make up a significant proportion of the river network, particularly in Mediterranean, semi-arid and arid climate regions. There has been a growing research interest centred on intermittent stream hydrology and ecology in recent years (e.g. Gasith & Resh 1999; Bonada et al. 2007a; Bogan et al. 2013; Bogan et al. 2014), partly in response to the hypothesised transformation of some currently perennial streams into intermittent systems due to future climate change (Mantyka-pringle et al. 2012; van Vliet et al. 2013). These hypothesised effects extend beyond those areas traditionally associated with intermittent streams and even include humid continental temperate regions such as Central Europe (see Peel et al. 2007). Given that stream drying is not common or widespread in temperate humid continental regions, stream biota may not be adapted to drying and may display different responses to those fauna adapted to regular drying in semi-arid and Mediterranean regions (Bonada et al. 2007b). Research focused on intermittent streams has recently highlighted their ecological complexity and diversity (Larned et al. 2010; Datry et

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al. 2014; Datry et al. 2016a; Datry et al. 2016b; Leigh et al. 2016) and the need to develop specific biomonitoring tools to characterise their ecological status (Acuña et al. 2014; Stubbington et al. 2018); especially in relation to the implementation of the EU Water Frame Directive (WFD; European Commission 2000).

Stream flow reduction is typically accompanied by decreasing surface water levels, which may result in zero discharge and streambed drying. Many benthic taxa avoid the effects of gradual stream drying by emerging as aerial adults (Dieterich & Anderson 1995; Lytle 2008), actively drifting downstream to perennial reaches or adjacent residual pools (Boulton 2003; Dewson et al. 2007) or seeking refuge in moist habitats such as the hyporheic zone (Wood et al. 2010; Young et al. 2011; Stubbington 2012; Vander Vorste et al. 2016). The hyporheic zone represents the saturated sediments and interstices beneath the riverbed and may extend laterally in to the stream banks and floodplain (White 1993). This zone is a dynamic ecotone between surface and groundwater systems, and plays an important role in the functioning of lotic ecosystems (Bo et al. 2006; Boulton et al. 2010). The surface layers of the hyporheic zone are widely considered to be used by benthic invertebrates as a refuge during flow cessation (Malard et al. 2001; Wood et al. 2010; Stubbington 2012; Lencioni & Spitale 2015), and as a ‘seedbank’ for recolonisation after flow resumption (Stubbington & Datry 2013; Stubbington et al. 2016; Vander Vorste et al. 2016).

The moist and saturated subsurface sediments provide benthic invertebrates suitable environmental conditions for survival during drying events. For example, they provide abiotic stability (compared with the surface of the riverbed), allowing aquatic fauna to avoid extreme or lethal temperatures and desiccation during dry periods (Wood et al. 2010; Dole-Olivier 2011; Stubbington & Wood 2013). The invertebrate communities of benthic and subsurface habitats are primarily determined by sediment porosity and substrate stability (Lencioni & Spitale 2015). The composition of streambed sediments plays a key role in the recolonisation process following stream drying (Gayraud & Phillipe 2003; Bo et al. 2007), when the benthic community may be partially derived from the ‘invertebrate seedbank’ (Stubbington & Datry 2013; Stubbington et al. 2016). Compared with sand dominated substrates, which are primarily colonised by sand-dwelling specialists, coarse grained hyporheic sediments typically facilitate the development of abundant and species-rich invertebrate assemblages within the larger interstitial spaces (Dole-Olivier 2011).

Interstitial spaces within the riverbed represent a unique habitat for many organisms because of the high permeability (Bo et al. 2006; Stubbington & Datry 2013). Many studies indicate that the migration of organisms into deeper layers of the substrate depends on the availability of interconnected interstitial habitat (e.g. Gayraud & Philippe 2003; Bo et al. 2006; Stubbington et al. 2011; Vadher et al. 2017). Although there is an evidence of benthic invertebrate use of interstitial habitat to a depth of several meters in the hyporheic zone (Mermillod-Blondin et al. 2015), most benthic taxa occur up to 20–30 cm below the stream bed (Williams & Hynes 1974; Mermillod-Blondin et al. 2003; Bo et al. 2006), especially in the headwater streams (Wagner et al. 1993) that maintain sufficient moisture (Bogan et al. 2014). The proportion of fine sediment (grain sizes < 2 mm) plays a crucial role in determining the availability of interstitial pore spaces and their connectivity (Wood & Armitage 1997; Stubbington et al. 2011). An increase in fine sediment infiltration and deposition has the potential to reduce substrate porosity (Packman & MacKay 2003) – small particles may clog interstices and may become impassable for migrating invertebrates (Claret et al. 1999; Boulton 2003; Extence et al. 2013; Vadher et al. 2015). This may also lead to a reduction in interstitial flow, dissolved oxygen and nutrient exchange between benthic and hyporheic habitats (Strayer et al. 1997; Wooster et al. 2008; Mathers & Wood 2016).

Survival in the hyporheic zone following flow cessation is primarily associated with the presence of free water, sediment permeability, the disturbance severity and resistance of individual taxa (Dole-Olivier 2011). During unfavourable conditions in the benthic zone, some invertebrates may migrate vertically into the hyporheic zone using it as a refuge (Montalto & Marchese 2005; Stubbington 2012; Stubbington & Wood 2013; Verdonschot et al. 2015).

The body size of organisms may be closely related to the availability of interstitial spaces; even large-bodied species may be able to burrow into fine sediments and physically enlarge pore sizes (Claret et al. 1999). The ideal body shape for movement through interstitial spaces is: i) long and thin with a flexible body to allow easy passage between substrate particles; or ii) small and rounded shape with a hard-protective shell or exoskeleton to withstand the pressure of the surrounding sediment (Williams & Hynes 1974). Hyporheic refuge use is therefore taxon-specific (James et al. 2008; Stubbington et al. 2011; Vadher et al. 2017), dependent on substrate porosity (Chester & Robson 2011; Robson et al. 2011) and taxa adaptations (Franken et al. 2006; Dole-Olivier 2011).

In the current study we hypothesized that substrates comprised of smaller particles (with smaller interstitial spaces) would be more difficult for organisms to migrate through than substrates comprised of larger particles (with larger interstitial spaces). This would theoretically result in different patterns of vertical distribution of individuals among substrates comprised of different grain sizes. Second, we hypothesized that the deeper saturated sediment layers would facilitate higher relative survival than the unsaturated upper layers with reduced moisture content. We postulated that the retention of moisture within the sediments would be crucial for the persistence of fauna under experimental water loss conditions.

Material and methods

Test organisms

We collected 1200 individuals of four common aquatic macroinvertebrate taxa from streams near Brno, Czech Republic. The four organisms considered were: i) freshwater shrimp – *Gammarus fossarum* Koch in Panzer, 1835; ii) burrowing mayfly larvae – *Ephemera danica* Müller, 1764; iii) *Pisidium* spp.; and iv) oligochaetes from the subfamily Tubificinae (sensu Erséus et al. 2008). We selected these taxa based on our previous research (BIODROUGHT project, www.biodrought.cz, Straka et al. 2019) related to invertebrate resistance to streambed drying. After the experiment the individuals were examined to confirm the identification of the latter two taxa. This process indicated the bivalves comprised 60 % *Pisidium casertanum* (Poli, 1791) and 40 % *Pisidium personatum* Malm, 1855 respectively and 75 % *Tubifex tubifex* (O.F.Müller, 1774) and 25 % *Limnodrilus hoffmeisteri* Claparède, 1862. After the experiment we measured the body diameter of 20 randomly selected specimens of each taxon to evaluate their ability to penetrate and migrate through the experimental substrates. The measured value was b-axis of the specimen (without extremities) i.e. the length of the axis that is perpendicular to both the longest axis and the shortest axis of the body. The body diameter (b-axis) was used to determine whether the taxa was able to pass through a pore space of a given size (Table 1).

Experimental conditions

We sieved sediment through a set of sieves to obtain 3 grain sizes for 3 different treatments: i) sandy-gravel 2–7 mm diameter; ii) gravel 7–10 mm diameter; and iii) coarse-gravel 20–32 mm diameter. No other particle sizes were included (e.g. fine sediment). Before sieving, the sediment was dried at 105 °C for 24 h to ensure no other invertebrates were present. For each substrate a theoretical pore size (interstitial spaces) was calculated. Each sediment treatment was considered to comprises spherical objects with diameters of 7, 10 and 32 mm respectively and the resulting pore size was calculated based on the circular space between three touching spheres (Table 1).

We placed the sieved sediment and water in experimental mesocosms. Stream water from the sample sites was used in the experiments with the following characteristics measured after 2 days acclimation in laboratory: temperature (18.2 °C), pH (8.32), conductivity (588 µS cm^{-1}), dissolved oxygen (9.12 mg l^{-1}) and oxygen saturation (100.60 %). Each experimental mesocosm had a height of 14.5 cm and 0.5 l volume (Fig. 1). The bottom of the mesocosm was filled with sand (grain size < 0.5 mm) to a depth of 3 cm. The sand layer prevented the invertebrates

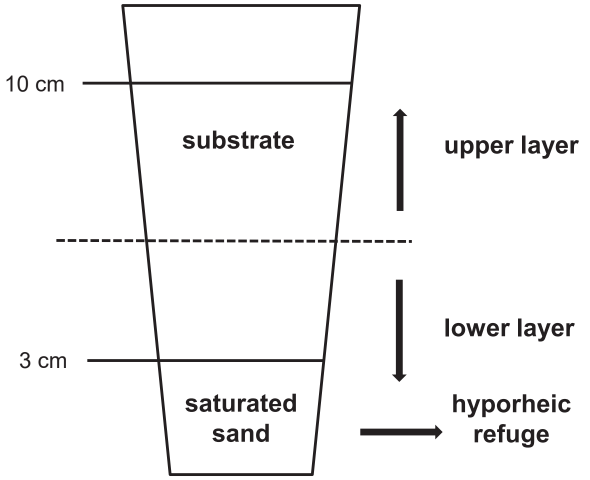

Fig. 1. Representation of an experimental mesocosm: the saturated sand layer represents the hyporheic refuge. The treatment layers were divided to lower (0–6 cm) and upper (6–10 cm) levels.

Table 1. Sediment treatments used in the experiment: pore size was calculated according to the sediment grain size. Average b-axis for all studied taxa was determined based on 20 individuals.

	sandy-gravel substrate	gravel substrate	coarse-gravel substrate
Grain size [mm]	2–7	7–10	20–32
Pore size [mm]	1.1	1.5	5.0
Gammarus fossarum [mm]	1.6	1.6	1.6
Ephemera danica [mm]	2.6	2.6	2.6
Pisidium spp. [mm]	1.7	1.7	1.7
Tubificinae spp. [mm]	0.4	0.4	0.4

emigrating from the mesocosm via perforations in the base and enabled capillary action to raise water from the tray into the experimental sediments (see below). Above 3 cm of sand a 7 cm layer of one of three sediment treatments was used. For experimental purposes we considered the mesocosm to be comprised of two different layers – i) upper (4 cm below sediment surface) and ii) lower (4–7 cm below the sediment surface) (Fig. 1). At the beginning of experiment all mesocosms were filled with water to a height of 12.5 cm (so that there was 2.5 cm of free water above the upper sediment layer).

The experiment commenced after 48 h of stabilisation within a controlled climate cabinet. During the acclimation and for the whole experimental duration a photoperiod of 16 h light/8 h dark and a constant temperature of 18 °C was maintained. After the stabilisation period, 10 shrimp and bivalve individuals were placed into half of the prepared mesocosms. For mayflies and tubificid worms, 7 individuals were released into the other half due to lower densities of the taxa in the source populations. After the release of invertebrates, we left all individuals for 1-hr acclimation. The bottom of each mesocosm was perforated with a needle and left for 1-hr to allow dewatering of the sediment to take place and the test organisms to gradually react to the water level reduction (e.g. by burrowing into sediment). The mesocosms were placed in shallow trays with continuously aerated water, so that the water level in the mesocosms was kept constant at 3 cm saturating the sandy layer and simulating a hyporheic refuge. The mesocosms were covered with 1 mm mesh netting to prevent emergence of any invertebrates. Mean air temperature and humidity provided within the controlled climate cabinet was 17.7 °C (range 16.4–18 °C) and 96.9 % (range 74.3–100 %), respectively.

Given the potential predatory behaviour of gammarids (Dick & Platvoet 2000; McGrath et al. 2007; Leuven et al. 2009) we placed them in the same mesocosms as the *Pisidium* spp. to reduce/prevent predation. The second set of mesocosms contained mayfly larvae and tubificid worms. We checked the vertical position of taxa in the drying substratum five times –4, 11, 18, 25 and 32 days following drying. The substrate was inspected under a stereo microscope in 1-cm layers and individual invertebrates extracted. The depth of live individuals observed in the substrate was recorded and the specimens preserved in 4 % formaldehyde or in 70 % ethanol in case of bivalves. On each of the five dates 12 mesocosms were inspected – two replicates for each of three substrate types (with each replicate comprising separate mesocosms for *G. fossarum* and *Pisidium* spp. and for *E. danica* and tubificid worms respectively). In total we obtained data for 60 experimental mesocosms following the five inspection points (Fig. 2).

To characterise variability in substrate moisture occurred, soil moisture probes (MicroLog V3A) were placed in three animal-free mesocosms. In each mesocosm one probe was placed in upper sediment layer and the second in the lower sediment layer (see Fig. 1) to provide a continuous record of substrate moisture.

Data analysis

Previous experimental laboratory studies of vertical migration have showed rapid reaction (within minutes to hours) to water level reduction (Vadher et al. 2015; Vadher et al. 2017). Thus, we anticipated that individual invertebrates would migrate immediately during water drawdown and prior to the first inspection (4 days) and would not migrate further through the sediment during the experiment. We therefore combined data from all individual trials to examine the final depth recorded for live individuals at the end of the experiment. Differences among treatments were identified using the nonparametric Kruskal-Wallis test and multiple comparisons of dispersion (post hoc test). Further we calculated the time over which 50 % mortality (LT50) occurred for each taxon using probit regression with a Schneider-Orelli correction for treatment (Finney 1971), and 95 % confidence intervals (CI_{95}) to detect differences in mean survival time among sediment treatments. We considered non-overlapping CI_{95} as statistically significant results.

Substrate moisture varied little and did not decrease over time due to saturation from the trays below. The only difference recorded was the higher moisture content in lower layers of all treatments compared with upper layers (Fig. 3). Given that the substrate moisture differed between the upper and lower layers for all treatments we combined the data for all individuals of

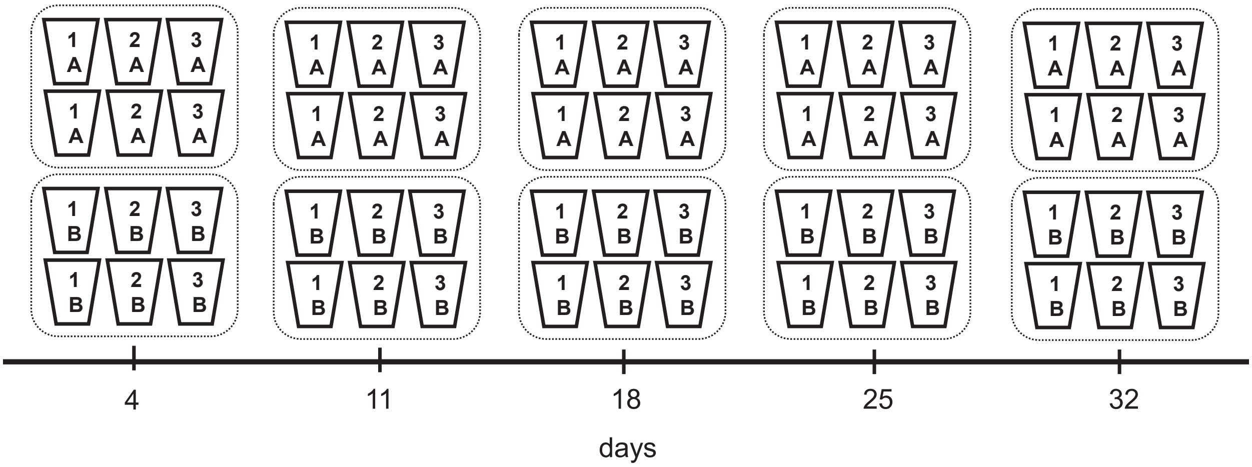

Fig. 2. Experimental design: mesocosms for **A** – *G. fossarum* + *Pisidium* spp., **B** – *E. danica* + Tubificinae spp. and different sediment treatments (1, 2, 3) with two replicates (dotted areas) for each inspection date (4–32 days).

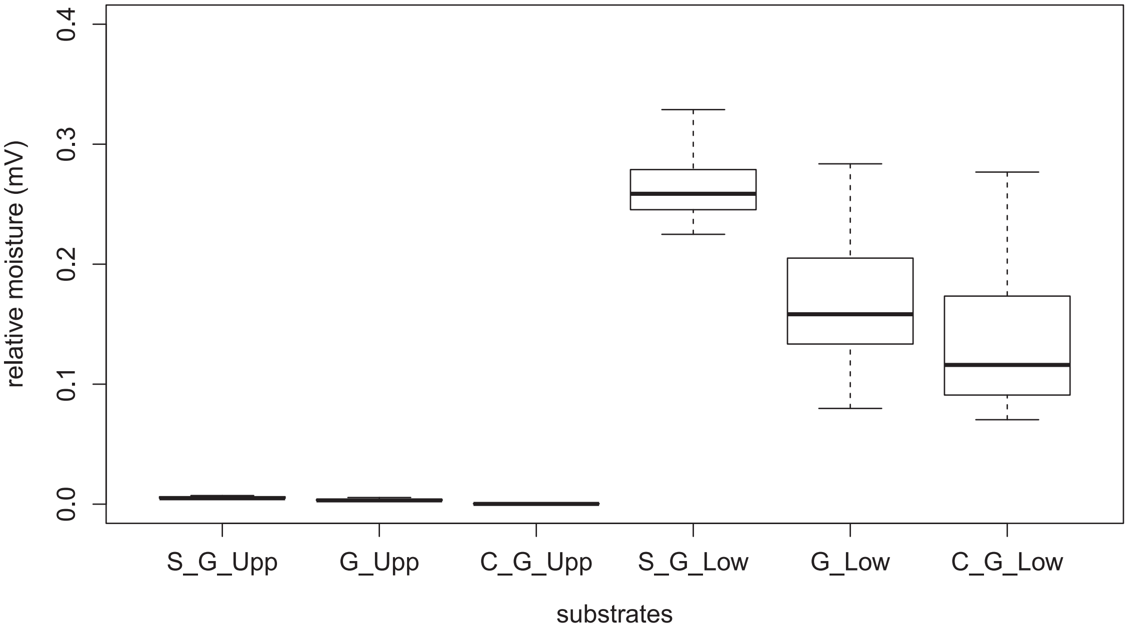

Fig. 3. Moisture content (represented by voltage in mV) during whole experiment in each treatment for the upper (Upp) and lower (Low) layers of the mesocosms. The boxes represent the 50 % inter-quartile range of measured values with the median value (thick horizontal line), whiskers represent measured values outside the middle 50 %; S_G = sandy-gravel, G = gravel, C_G = coarse-gravel substrate.

Table 2. Mutual comparison of dispersion (post hoc test of Kruskal-Wallis nonparametric one-way ANOVA) for all treatments. Differences in dispersion are represented by p values; ns = non significant result.

	Gammarus fossarum			***Ephemera danica***			***Pisidium* spp.**			**Tubificinae spp.**		
	sandy-gravel	**gravel**	**coarse-gravel**	**sandy-gravel**	**gravel**	**coarse-gravel**	**sandy-gravel**	**gravel**	**coarse-gravel**	**sandy-gravel**	**gravel**	**coarse-gravel**
sandy-gravel		$p<0.001$	$p<0.001$		ns	$p<0.001$		$p<0.001$	$p<0.001$		ns	$p<0.001$
gravel			$p<0.05$			$p<0.001$			$p<0.001$			$p<0.001$

each taxon which survived from the upper and lower layer for all treatments to calculate middle survival / survivorship rates. Survival rates represent the distribution of individuals which survived during the experiment and allow differences between the upper and lower layers to be explored. The differences recorded were tested using a nonparametric Mann-Whitney U test. Statistical analyses were processed in STATISTICA12 (StatSoft Inc. 2013) and the R statistical computing environment (R Development Core Team 2018).

Results

Vertical distribution

We observed significant differences in the vertical distribution for each taxon among substrates (Kruskal-Wallis test: $p<0.001$) (Table 2). In the sandy-gravel, *G. fossarum* individuals were exclusively recorded in the upper layer (Fig. 4a) whereas individuals occurred primarily in the lower layer of the coarse-gravel treatment and were spread across both layers in the gravel treatment.

The vertical distribution of *E. danica* was significantly different in the coarse-gravel compared with the sandy-gravel and gravel treatments (Table 2). Individuals were recorded in the lower layer during the whole experiment for the coarse-gravel treatment (Fig. 4b). We observed no significant differences in the vertical distribution of *E. danica* between the sandy-gravel and gravel substrate treatment (Table 2), where individuals occurred exclusively in the upper layer (Fig. 4b).

Pisidium spp. displayed very different vertical distribution patterns across the three treatments (Table 2). With increasing interstitial spaces individuals utilised lower layers of the mesocosms (Fig. 4c). Tubificinae spp. did not display any significant differences in vertical distribution in sandy-gravel and gravel sediment treatments (Table 2), where most individuals were recorded in the lower layer (Fig. 4d). In the coarse-

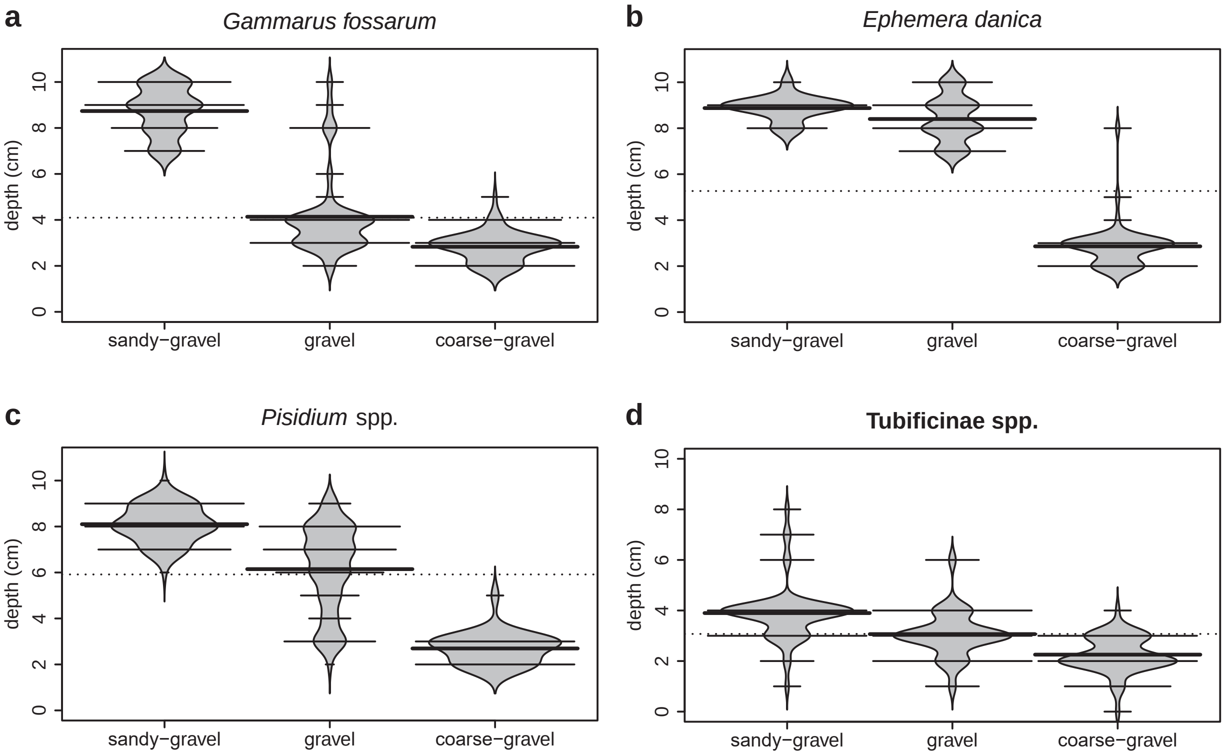

Fig. 4. Bean plots represent proportion of individuals observed at different depths during the entire experiment for the three sediment treatments for **a)** *G. fossarum*, **b)** *E. danica*, **c)** *Pisidium* spp. and **d)** Tubificinae spp. Bean-lines show relative proportion of individuals, thick bean-lines represent mean values for each treatment, dotted line represent mean values for all treatments.

Table 3. Results for LT50: values represent days when 50 % mortality occurred, based on the presence of taxa in different substrate types ± 95 % confidence intervals (CI_{95}) – lower, upper. Non-overlapping intervals indicate significant differences in mortality among substrate treatments.

	LT50 (day)	CI_{95}	
		lower	upper
Gammarus fossarum			
sandy-gravel	6.3	4.68	8.44
gravel	12.9	8.99	18.61
coarse-gravel	24.8	20.81	26.67
Ephemera danica			
sandy-gravel	5.5	3.45	8.91
gravel	7.7	5.17	11.50
coarse-gravel	30.6	17.66	53.23

gravel treatment individuals were almost exclusively recorded in the 3 cm saturated sand layer at the bottom of the mesocosms (Fig. 4d).

Survival rates

We observed significant differences in LT50 between tested treatments for *G. fossarum* and *E. danica* (Table 3). Both taxa experienced 50 % mortality first in the sandy-gravel, then in gravel and last in coarse-gravel treatments during the experiment period. The longest survival period for *G. fossarum* and *E. danica* was recorded in the coarse-gravel treatment, which significantly differed from the other treatments (Table 3). *G. fossarum* displayed significant differences in mortality between the sandy-gravel and gravel sediment treatments. Abundances of *Pisidium* spp. and Tubificinae spp. decreased little during the experiment and their LT50 was thus not determined.

We also observed significant differences in survivorship within the upper and lower layer for *G. fossarum* and *E. danica* (Table 4; Fig. 5a, b). In both instances, the middle survival duration in the upper layer was 4 days compared with 11 days in lower layer. Unlike *G. fossarum* and *E. danica*, the location of individuals in the upper or lower layer of the mesocosm did not affect *Pisidium* spp. survivorship. Bivalve individuals had similar survivorship for both substrate layers, with a middle survival duration of 18 days (Table 4). We observed similar patterns of survivorship for both substrate layers – with a slightly higher proportion in the upper layer (Fig. 5c). Mean Tubificinae

Table 4. Results of Mann-Whitney U test: comparison of observed abundances in the two mesocosm layers (upper and lower) during the experiment for all taxa. Differences in survival rate are indicated by p values, median value represents middle survival rate; ns = non significant result.

	Middle survival rate (days)		**Mann-Whitney U test**
	upper layer	**lower layer**	***p*-value**
Gammarus fossarum	4	11	< 0.001
Ephemera danica	4	11	< 0.001
Pisidium spp.	18	18	ns
Tubificinae spp.	25	18	ns

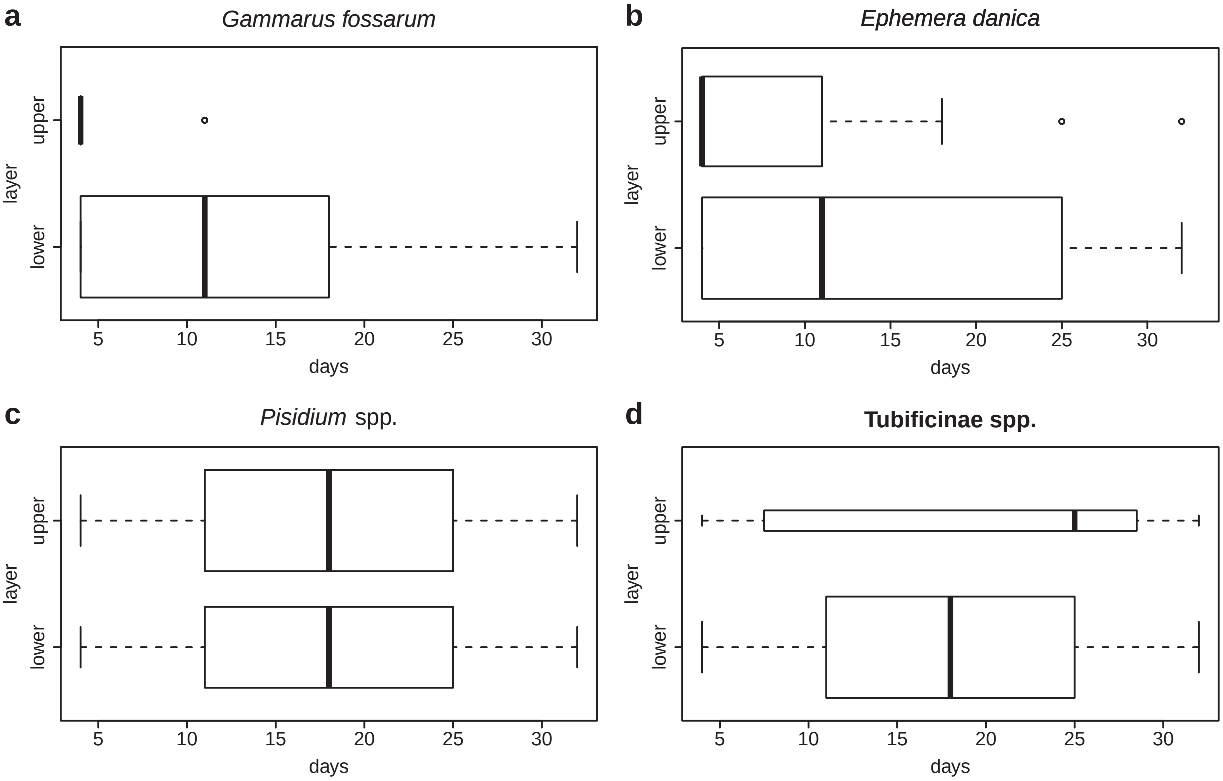

Fig. 5. Box plots indicating the presence of individuals surviving the experiment in upper and lower substrate layer of the mesocosms and their middle survivorship rate (vertical thick line – median) for **a)** *G. fossarum*, **b)** *E. danica*, **c)** *Pisidium* spp. and **d)** Tubificinae spp. The boxes are drawn with widths proportional to the square-roots of the number of observations in the treatments. The boxes represent 50 % inter-quartile range of measured values, whiskers represent measured values outside the middle 50 % and points represent outlier values.

spp. survivorship was higher (25 days) in the upper layer than the lower layer (18 days), but differences were not statistically significant (Table 4). Almost all tubificids migrated to the lower layer in all treatments and very low numbers of individuals were recorded in the upper layer (Fig. 5d).

Discussion

We found support for our hypothesis that substrates comprised of smaller particles would be harder for organisms to migrate through than substrates comprised of larger particles (and larger interstices). The size of available interstitial spaces in substrates not affected by sedimentation primarily reflects grain size and depth of available habitat (Bo et al. 2007). We observed significant differences in the patterns of vertical distribution for each of the taxa considered among the three treatments comprised of different sediment grain sizes. In our experiment, the ability to migrate into and through the sediment was related to body size. When interstitial pores were larger than the body diameter (Table 1) almost all individuals migrated through the substrate in to the lower moist refuge layer. This was the case for Tubificinae spp. for all sediment treatments and for *G. fossarum*, *E. danica* and *Pisidium* spp. for the coarse-gravel treatment. When body diameter was approximately the same as the pore size, as was the case of *G. fossarum* and *Pisidium* spp. for the gravel-substrate, individuals were located throughout the upper and lower substrate layers, indicating that some individuals could migrate vertically whereas others became stranded.

When body diameter exceeded interstitial pore size, such as for *G. fossarum* and *Pisidium* spp. in the sandy-gravel treatment, the distribution of individuals was confined to the upper layer of the mesocosm. This corresponds with field observations in which gammarids were absent from all hyporheic samples with a high proportion of interstitial fine sediment and were almost exclusively recorded from clean coarse-grained sediments (e.g. Stubbington et al. 2011; Mermillod-Blondin et al. 2015). A coarse gravel sediment structure provided suitable large interstices, whereas a fine-grained sandy sediment structure and sedimentation limited the suitability of habitat for macroinvertebrates (Strayer et al. 1997). However, Dunscombe et al. (2018) suggest that even large bodied invertebrates can migrate into bed sediments if heterogeneous sediments occur. In our experiment, *E. danica,* a strong burrowing species (Svensson 1977; López-Rodríguez et al. 2009) did not migrate through sediments with interstices smaller than its body diameter (i.e. sandy-gravel and gravel substrate). Our results indicate that the ability to migrate into the subsurface is more likely for small-bodied and thin taxa. Omesová et al. (2008) noted the body diameter for successful colonisation of subsurface streambed sediments as less than 0.5 mm, which corresponds with our results for Tubificinae spp. (Table 1). However, other studies have not demonstrated a connection between substrate size, vertical migration and body size of macroinvertebrates (e.g. Poznańska et al. 2017; Vadher et al. 2017).

Our results also support the hypothesis that moisture retention in subsurface sediments is critical for their use as a refuge in drying streams. The burrowing of macroinvertebrates into moist substrates to avoid surface sediment drying has been documented (e.g. Imhof & Harrison 1981; Poznańska et al. 2013; Poznańska et al. 2015; Verdonschot et al. 2015). We observed higher survival rates in the lower layer of the mesocosms for *G. fossarum* and *E. danica*. This supports observations of live individuals in deeper substrate layers for *Nemoura cambrica* Stephens, 1836, *Asellus aquaticus* (Linnaeus, 1758), *Gammarus pulex* (Linnaeus, 1758), *Hydropsyche siltalai* Döhler, 1964 and *Heptagenia sulphurea* (Müller, 1776) during experimental sediment drying (Vadher et al. 2017). Similarly, Poznańska et al. (2017) and Poznańska-Kakareko et al. (2017) demonstrated the influence of substrate moisture content on the mortality of the chironomid larvae *Stictochironomus sticticus* (Fabricius, 1781) and oligochaetes *Potamothrix moldaviensis* Vejdovský & Mrázek, 1903 both in the laboratory and under field conditions. In contrast, *Pisidium* spp. appears to be less affected by reduced sediment moisture in our experiment (Fig. 5c) and similar results were reported by Poznańska-Kakareko et al. (2017). This may be due to their thick shell providing protection against water loss (Williams & Hynes 1974). In our experiment the subsurface sediment moisture content was sufficient for *Pisidium* spp. to persist in the upper layers of the mesocosms. Similarly, the absence of vertical migration has been observed in streams where subsurface conditions remain homogenous throughout the flow reduction period (James & Suren 2009). Under these conditions, invertebrates, such as *Pisidium* spp. may not expend energy on migration if a more suitable refuge is not present.

Our experimental study highlights the importance of streambed permeability and the availability of interstitial spaces as refuge habitat during stream drying. This function of the streambed (benthic and hyporheic zone) has been demonstrated in a number of studies (e.g. Stubbington 2012; Storey & Quinn 2013; Stubbington & Datry 2013; Vander Vorste et al. 2016) and its importance is likely to increase in streams which may become intermittent as a result of climate change and water resource pressures (Davies 2010; Mantyka-pringle et al. 2012). However, the negative effects of fine sediment deposition on stream ecosystem functioning (Richards & Bacon 1994; Wood & Armitage 1997; Mermillod-Blondin et al. 2015) and clogging of subsurface habitats (Schälchi 1992; Packman & MacKay 2003; Bo et al. 2007) may compound these pressures in intermittent streams, where alternative refuges, such as surface water pools and adjacent perennial river reaches may be absent.

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ORIGINAL ARTICLE

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An unexpected source of invertebrate community recovery in intermittent streams from a humid continental climate

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Abstract

1. Intermittent streams are naturally dominant landscape features of Mediterranean and arid regions, but also occur more and more in humid climates, such as in the Czech Republic. Organism abilities to cope with drying (i.e. resistance forms) have been quantified in naturally intermittent streams from Mediterranean and arid regions, in which long-term flow intermittence patterns have promoted physiological adaptations. In contrast, the capacity of aquatic communities to persist without requiring resistance adaptations under recent intermittent flow regimes in more humid climates is virtually unexplored, along with its contribution to community recovery upon rewetting.
2. Here, we addressed the ability of aquatic invertebrates devoid of specific desiccation-resistance forms (e.g. cysts or eggs) to cope with climate change-induced flow intermittence from rivers in continental climate. Owing to the high relative air humidity, the importance of riparian cover and the short duration of drying events, we expected taxonomically and functionally rich communities to persist in the dry streambed during phases with no surface water and to contribute to community recovery upon flow resumption.
3. To explore these ideas, we collected invertebrate communities in the riverbeds before, during and after drying from 10 intermittent rivers in the Czech Republic. We quantified the pool of organisms remaining alive in the dry riverbeds and determined its taxonomic and functional contribution to the recovery of benthic communities upon rewetting.
4. Of the organisms collected, 83%, belonging to 22 taxa, were able to survive during the dry phase without producing desiccation-resistance forms. This pool of organisms contributed substantially both taxonomically and functionally to the quick recovery of benthic communities. The exponential decrease in taxonomic and functional richness implies an initial quick loss of sensitive taxa, while a pool of less sensitive taxa persisted long in the dry riverbeds, hence contributing to the fast community recovery upon rewetting.
5. Survival rates of active aquatic macroinvertebrates devoid of specific desiccation-resistance adaptations have rarely been quantified. Probably promoted by benign environmental conditions prevailing on dry riverbeds in this study, their survival

demonstrated the need to protect communities in dry riverbed and their adjacent riparian environments so that assemblages can recover rapidly from short periods without river flow. Riparian forest management along intermittent streams from continental climates could help conserve the value of dry streambeds as a source of resilience for riverine communities.

KEYWORDS
drying, global change, similarity, temporary rivers, tolerance

1 | INTRODUCTION

Intermittent streams are naturally prevalent in river networks all over the globe (Datry et al., 2014; Larned, Datry, Arscott, & Tockner, 2010). While they are a dominant landscape feature of Mediterranean and arid regions, they also occur abundantly in more humid climates (Buttle et al., 2012; Stubbington et al., 2017). For example, a large proportion of western, humid France is composed of intermittent streams (Snelder et al., 2013). In response to climate change and increased needs for water, many perennial rivers are becoming intermittent (Döll & Schmied, 2012; Gudmundsson, Seneviratne, & Zhang, 2017; Laaha et al., 2016). This is particularly true in areas where intermittent rivers were uncommon in the past such as the Czech Republic (CR). Prone to a continental, humid climate (Peel, Finlayson, & McMahon, 2007), the stream network of CR was dominantly composed of perennial waters until recently when the proportion of intermittent streams has grown considerably: in the past decade, many streams have started to dry for the very first time and today, 50% of stream length in the country is at risk of drying (Benčoková, Krám, & Hruška, 2011; Van Lanen et al., 2016; Zahrádková et al., 2015).

Naturally intermittent streams are characterised by a high variability of habitats, alternating in time and space between aquatic lotic, lentic to terrestrial conditions which accommodate rich and diverse biotic communities (Datry et al., 2014). This dynamism has triggered physiological adaptations for many aquatic species to cope with flow cessation and drying events (Bogan et al., 2017; Strachan, Chester, & Robson, 2015; Williams, 2006). Some species can, for example, persist weeks or even months *in-situ* during drying as desiccation-resistance forms in dry streambed sediments (Storey & Quinn, 2013; Stubbington & Datry, 2013). Others are able to escape drying reaches and quickly recolonise upon flow resumption from nearby perennial refuges, such as perennial reaches in the network (Bogan, Boersma, & Lytle, 2013) or the underlying hyporheic zone (Vander Vorste, Malard, & Datry, 2016). Others may to persist in moist interstices in dry streambeds (Boulton, Stanley, Fisher, & Lake, 1992; Strachan et al., 2015), although the importance of this pool of taxa to contribute to community recovery upon rewetting has not been quantified to date. Invertebrate resistance strategies to drying have been well quantified in naturally intermittent streams from Mediterranean and arid regions (Bogan et al., 2017; Boulton et al., 1992; Robson, Chester, & Austin, 2011; Stubbington & Datry, 2013), whereas the capacity of aquatic communities to persist under novel intermittent flow regimes is underexplored (Datry, Bonada, & Boulton, 2017; Datry, Vander Vorste, et al., 2017). In humid areas where most rivers and streams have been historically perennial, the resilience of aquatic communities to on-going drying trends is unclear. In the CR, small streams (up to 4th Strahler order) dominate (90% of total stream length) and have become prone to frequent drying events in the last decade as a result of climate change and increased water abstraction for agriculture (Trnka et al., 2016; Van Lanen et al., 2016; Zahrádková et al., 2015). How resilient are the communities from these *novel* ecosystems (sensu Hobbs et al., 2014) affected by anthropogenic flow intermittence is an open question. Drying events in humid areas are unpredictable and rather short, typically <1 month (Pařil et al., 2019; Řezníčková, Pařil, & Zahrádková, 2007; Řezníčková, Soldán, Pařil, & Zahrádková, 2010). The lack of adaptation of species that have not experienced long-term flow intermittence in the past could make them highly vulnerable to anthropogenic flow intermittence induced by direct (e.g. water abstraction, channel modification) and indirect (e.g. climate change) causes. Conversely, the high relative humidity, promoted by the dense riparian cover and short dry periods could allow species to tolerate desiccation and persist despite anthropogenic flow intermittence.

In this study, we addressed the ability of aquatic invertebrates from continental humid climates to cope with direct and indirect anthropogenically-induced flow intermittence. We sampled invertebrate communities before, during and after several drying events, in 10 near-natural streams of the CR with minimal direct human impact. Owing to the high relative air humidity, substantial riparian cover and the short duration of drying events, we expected taxonomically and functionally rich communities to persist in the dry streambeds during phases with no surface water without requiring desiccation-resistance forms. We also expected predictable patterns of β-diversity and its components of these communities based on recent studies (Schriever et al., 2015; Vander Vorste et al., 2016). We first predicted both taxonomic and functional richness of communities in dry streambeds to decrease with increasing duration of the dry phase to increase again upon flow resumption. This is because dry phase duration is known to be a primary control of invertebrate communities (Stubbington & Datry, 2013), while benthic communities recover gradually upon flow resumption (Leigh et al., 2016). Secondly, we predicted a dominance of nestedness for functional β-diversity and a dominance of turnover for the taxonomic β-diversity of dry streambed sediments. This is due to the functional redundancy of intermittent stream communities, in which different taxa share identical functional trait combinations (Datry, Bonada, & Boulton, 2017; Datry, Vander Vorste, et al., 2017; Schriever et al., 2015; Vander Vorste et al., 2016). Thirdly, we predicted that a substantial proportion of river communities will be supported upon rewetting by the pool of tolerant species, both structurally and functionally, indicating that this species pool could promote the recovery of communities upon rewetting.

2 | METHODS

2.1 | Study sites

Ten river reaches were selected throughout the CR (range of latitude 48.8°–50.5°, range of longitude 13.9°–17.6°, Figure 1) and within the Dfb Köppen class (Peel et al., 2007), indicating a humid continental climate. The selection of the reaches was based on preliminary streambed temperature and drying observations through photo-traps and temperature loggers, and was intended to span a wide gradient of drying duration. Selected reaches ranged in altitude from 250 to 480 m, in a region receiving 640 mm of annual rainfall with a mean temperature of 7.9°C (Tolasz, Míková, Valeriánová, & Voženílek, 2007) and placed proportionally on siliceous (five sites) and calcareous (five sites) bedrock. For these 2–4th Strahler order streams, distance to the source ranged from 2.7 to 10.9 km (mean 5.8 km). Reaches were all pristine, without influence of contamination, channel modification, or water abstraction, while the upstream catchments were dominated by forest (55%), extensive agriculture (43%) and <5% urbanisation (CORINE Land Cover 2017, Table S1).

2.2 | Study design

Our study design was as follows: first, we sampled invertebrate communities before, during and after several drying events in the 10 intermittent streams of the CR. Second, we explored the taxonomic and functional diversity independently for each of these three phases and related them with drying and flowing patterns. Third, we quantified the similarities among the three phases and particularly explored that of post-drying samples with during-drying samples using presence–absence data to quantify the potential contribution of communities of the dry period to the recovery of benthic communities (both from taxonomic and functional perspectives) upon rewetting. This design was based on (Datry, Corti, & Philippe, 2012; Datry, Bonada, & Boulton, 2017; Datry, Vander Vorste, et al., 2017).

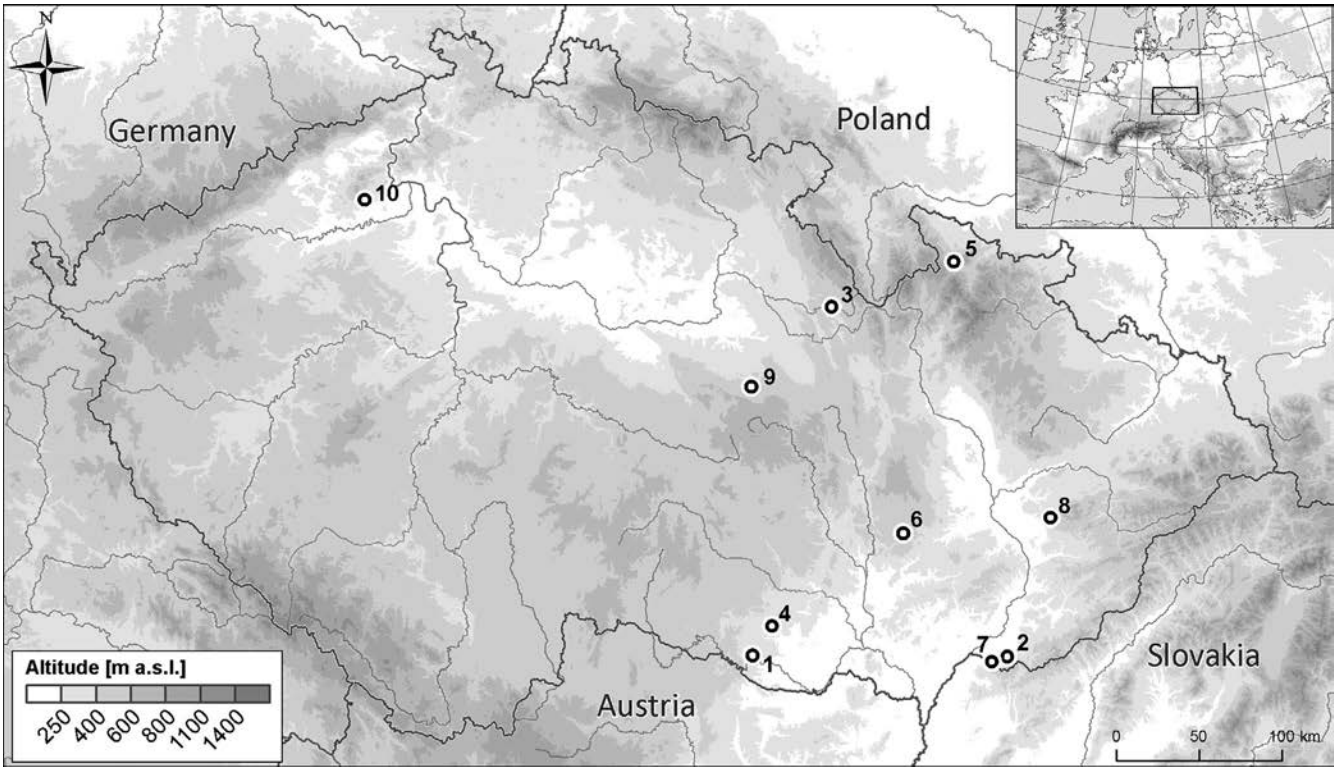


FIGURE 1 Map showing the sampling sites within the 10 intermittent streams of the Czech Republic. Stream name and sampling site: 1. Granicky p.—Znojmo, 2. Jarkovec—Lucina, 3. Kamenicna—Zamberk, 4. Krepicka—Horni Dunajovice, 5. Obloucnik—Vapenna, 6. Rakovec—Bukovinka, 7. Sudomericky p.—Radejov, 8. Zhrta—Jankovice, 9. Zejbro—Dolivka, 10. Zejdlik—Trebivlice. Site characteristics are detailed in Table S1

2.3 | Drying duration and frequency

To quantify the drying phase characteristics, one temperature logger (HOBO Onset Pro v2 U22-001, Onset Computer Corporation), one water level logger (Solinst Levelogger Edge, Solinst Canada Ltd.), and one photo-trap (Acorn 5310MG, Zhuhai Ltl Acorn Electronics Co., Ltd.) were installed at each reach. Water level loggers were placed in the deepest section of the streambed to account for the persistence of surface water in residual pools, while temperature loggers were placed in shallower section of the streambed to detect flow cessation and record temperatures prevailing on dry riverbeds. The photo-traps were installed in trees in the adjacent riparian zone (at 3 m height) and were facing a graduated scale to measure the water level in the streambed, where temperature and water level loggers were placed. Data were collected every 30 min on loggers and each 4 hr on photo-trap.

Based on all collected data, the extent of surface water drying with one-day resolution was recorded for all sampled streambeds. The number of days with no flow prior to sampling date in sampling year and number of days after rewetting prior to sampling date were used in subsequent analyses as explanatory variables.

2.4 | Macroinvertebrate sampling

We sampled macroinvertebrates from each reach during flowing phases before and after dry periods and during the dry period, for 3 years: 2013 (six reaches), 2014 (seven reaches), and 2015 (five reaches). Altogether, 54 samples were collected from the 10 stream reaches.

2.4.1 | During dry phase

Dry sediments (DRY) were sampled during summer between 26 July and 28 August, 1–152 days following drying phase onset. At each reach, an area of 20 × 20 cm was randomly chosen in the lowest point of one riffle and one pool and pooled together. At each area, about 5 L of sediments were collected to a depth of 12.5 cm with a shovel, placed in plastic bags and transported to laboratory in cool boxes. In the laboratory, samples were processed within 24 hr. Samples were washed through 500-μm nets and macroinvertebrates sorted under a stereomicroscope with 50× magnification. Dead and alive organisms were distinguished and separated based on apparent living signs, including movement of organisms. Stages <0.5 mm, such as eggs or cysts were disregarded and not considered here, as our aim was not to focus on desiccation-resistance stages, largely explored by other studies (Storey & Quinn, 2013; Stubbington & Datry, 2013).

The key environmental variables known to influence the survival of organisms in intermittent rivers (Strachan, Chester, & Robson, 2016; Stubbington & Datry, 2013) were quantified. Basic characteristics of dried bed sediment were identified by granulometric analysis on consecutive sieves (mesh sizes [cm]: 6.3, 3.15, 1.6, 0.8, 0.4, 0.2, 0.1, 0.05, 0.025, 0.0125, 0.0063) and each fraction was weighed. Sediment moisture was measured for 70 randomly selected samples by collecting during the dry phase 1 L of sediments in the first 12.5 cm of the streambeds and in the vicinity (<50 cm) of invertebrate sampling areas. Stored in sealed plastic bags, the samples were brought to the laboratory within 24 hr, were weighed wet, dried at 105°C to a constant mass and reweighed. Moisture was determined from differences between wet and dry mass.

2.4.2 | During flowing phase

Macroinvertebrates were sampled before and after the dry periods using the Czech national standardised protocol *PERLA* (Kokeš et al., 2006). It is based on a proportional multihabitat 3-min kick sampling using a pond net (frame 25 × 25 cm; mesh size 500 μm; 1-m long mesh). Samples were pre-sorted in the field by experienced limnologists for 20 min to ensure detection of rare taxa. Pre-picked macroinvertebrates and remaining samples were then separately preserved in 4% formaldehyde and further processed in the laboratory using stereomicroscopes.

Pre-dry period samples (PRE) were collected in spring between 28 March and 29 April, whereas post-dry period samples (POST) were collected in autumn between 27 September and 21 November. POST samples were collected typically 1 month upon flow resumption (mean = 38 days). Due to severe drought in 2015, two reaches were sampled only 2 and 3 days after flow resumption at the end of October to avoid sampling during winter when streams were frozen.

2.5 | Invertebrate identification and species traits

All macroinvertebrate taxa were identified to the lowest taxonomic resolution according to obligatory identification level stated in the Czech IS ARROW database (http://hydro.chmi.cz/isarrow/); (Czech Hydrometeorological Institute 2018) using current literature predominantly to species (71%) and genus (20%) levels (only 19% to family of higher taxonomic units) in PRE and POST samples (but due to a less precise level of identification in dry sediment, the whole dataset was adjusted according to DRY samples—see Data analysis).

The trait database assembled during the BIODROUGHT project (www.biodrought.eu), which contains information about 35 different species traits related to ecology, life history, physiology, and morphology of Central European freshwater macroinvertebrate taxa, was used for general characterisation of the dry streambed community. This database integrates information from >500 published articles and other resources (such as the database freshwaterecology.info and Tachet, Richoux, Bournard, & Usseglio-Polatera, 2010). Altogether, 12 biological traits with 53 trait categories, which have been shown to respond to flow intermittence (Aspin et al., 2019; Datry et al., 2014) were selected (Table 1). All traits are fuzzy-coded in range 0–1. Using 53 trait categories, the majority of taxa possessed at least slightly different combination of traits and therefore, almost every taxon represented different functional units. Because of that, we defined the functional diversity as the number of general functional groups within a sample and estimated the functional diversity following the approach presented in Aspin et al. (2019). We defined the trait profile groups, i.e. groups

TABLE 1 List of species traits used for cluster analysis to separate taxa into 18 trait profile groups

No.	Trait	Category	No.	Trait	Category
1.	Feeding	Specialist	8.	Locomotion	Swimming/scatting
		Generalist			Swimming/diving
2.	Temperature preference	Very cold			Burrowing/boring
		Cold			Sprawling/walking
		Moderate			(Semi)sessil
		Warm			Other
		Eurytherm	9.	Life cycle type	Semivoltine
3.	Armouring	Soft			Univoltine
		Softly sclerotised			Bivoltine
		Sclerotised			Trivoltine
		Armoured			Multivoltine
4.	Resistance to drying	No drought resistance			Flexible
		Egg diapause	10.	Reproduction	Ovovivipar
		Larvae diapause			Free isolated eggs
		Adult diapause			Cemented isolated eggs
		Unknown resistance type			Fixed clutches
5.	Fecundity	<100 eggs in hatch			Free clutches
		100–300 eggs in hatch			Clutches in vegetation
		300–500 eggs in hatch			Terrestrial clutches
		500–1,500 eggs in hatch			Asexual
		1,500–3,000 eggs in hatch			Parasitic
		>3,000 eggs in hatch	11.	Body flexibility	None
6.	Dissemination strategy	Aquatic passive			Low
		Aquatic active			High
		Aerial passive	12.	Respiration	Tegument
		Aerial active			Gill
7.	Dissemination potential	<10 m			Plastron
		10–100 m			Spiracle (aerial)
		100–1,000 m			Hydrostatic vesicle (aerial)
		>1,000 m			Tapping of air stores of aquatic plants
					Extension/excursion to surface

Note: Traits were extracted from the Biodrought database. Numerical values of traits except first categorical trait *feeding* vary between 0 and 1. Trait 11. Body flexibility is related to capability of substrate interstices penetration and increasing with higher flexibility. Trait 6. Dissemination strategy is related to dispersal mode which enable successful recolonisation of intermittent streams and increasing from poor *aquatic passive* to most efficient *aerial active* dispersal. Detailed definition of traits 1, 2, 4, 6, 7, 8, 9, 10, and 12 are available on frehwaterecology.info (Schmidt-Kloiber & Hering, 2015).

of functionally similar (or equal) species and used such groups as units for functional diversity quantification. To find most homogeneous groups of taxa according to their trait, the Gower dissimilarity matrix was calculated from species × traits matrix and Ward's hierarchical grouping method was used as clustering method (Aspin et al., 2019). The optimal number of the most homogenous (i.e. with lowest average cluster width) clusters has been defined using cluster width criterion using R package cluster (Maechler, Rousseeuw, Struyf, Hubert, & Hornik, 2017).

All species belonging to same cluster constitute the same functional unit. Two different matrices were therefore used for subsequent analyses: species × samples matrix and functional units × samples matrix.

2.6 | Data analysis

First, accumulation curves were plotted against the number of samples to compare the sampling effort among the three type of samples (PRE, DRY, POST) and ensure similar sampling effort (Figure S1).

To ensure consistent taxonomic resolution among sample types (PRE, DRY, POST), we used the resolution of DRY samples, which contained small and sometimes indistinguishable juvenile and partly damaged specimens. In general, the species level was reached for 57%, genus level for 38%, and family and higher level for 5% of all taxa. All singletons (taxa occurring only once in the whole dataset) and taxa observed exclusively in compared samples from DRY sediments (and therefore not contributing to flowing-phase community) were removed prior to subsequent analyses.

2.6.1 | Taxonomic and functional richness

Taxonomic (i.e. number of taxa) and functional richness (i.e. number of functional groups—for details see Bonada, Doledec, & Statzner, 2007) and total abundance were compared among sample types using one-way analysis of variance (ANOVA). We used trait profile groups as functionally non-redundant units for functional diversity estimation and defined the functional diversity as number of functionally highly similar groups of taxa (see Aspin et al., 2019). To test our first prediction, the relationships between taxonomic and functional richness in DRY samples and the dry phase duration was explored by linear regression, as for the relationship between taxonomic and functional richness of POST samples and the duration since flow resumption. The differences in the relationships between duration of drying and both taxonomic and functional diversity were assessed using an analysis of covariance (ANCOVA, exploring the significance of the factor interaction). Similarly, the differences in the relationships between the duration of the flowing phase and both taxonomic and functional diversity were assessed using an ANCOVA. The normality of data was visually checked using quantile–quantile plots, the homogeneity of variances was tested using Bartlett tests and variables were log-transformed to meet homoscedasticity.

2.6.2 | Taxonomic and functional β-diversity partitioning

To explore the β-diversity of DRY samples, we calculated (1) turnover component, (2) nestedness component, and (3) total Bray–Curtis dissimilarity for both (taxonomic and functional) types of dry streambed communities following Baselga (2013), for all pairs of DRY samples within the same year. The three β-diversity measures were then compared between DRY and POST samples using non-parametric Kruskal–Wallis H tests.

2.6.3 | Dry streambed community contribution to flowing-phase communities

We calculated the potential contribution of DRY samples to community composition in POST samples during recovery in two different ways. We first compared the number of total, shared and unique taxa and functional units among DRY, PRE, and POST samples (abundance data were not used due to the discrepancy in sampling methods among DRY and PRE/POST samples). The ratio of the number of shared taxa by the total number of taxa in the flowing phase community defined the contribution in terms of shared species. Similarly, the ratio of the number of shared functional units by the total number of functional units in the flowing phase community defined the contribution in terms of functional diversity. Secondly, we quantified taxonomic and functional community dissimilarity among sample types for each site using the Chao index calculated on presence–absence data, which is particularly suitable when sample size and taxonomic richness differ among groups (Anderson & Millar, 2004; Cañedo Argüelles et al., 2015; Chao, Chazdon, Colwell, & Shen, 2005). Both taxonomic and functional community dissimilarities were then plotted against the duration since flow resumption.

All statistical analyses were carried out using R software (R Development Core Team, 2017) and functions in the package Vegan (Oksanen et al., 2017) and CommEcol (Melo, 2017). Graphics were produced by ggplot2 package for R (Wickham, 2009). For all tests, $p < 0.05$ indicated statistical significance.

3 | RESULTS

3.1 | Dry riverbed environmental characteristics

The average temperature at the surface of the riverbed sediments was 15 ± 5.6°C, while the average sediment moisture during dry phases was 11.9 ± 8.9% (Table 2). The mean ± *SD* grain size (in mm) of whole dataset of 25th, 50th, and 75th percentiles was 3.1 ± 3.3 mm, 19.4 ± 10.1 mm, and 47.9 ± 15.5 mm, respectively, while mean proportion (%) of small particles < 1 mm was 29.0 ± 10.3% (Table 2).

3.2 | Characteristics of the sampled communities

Taxonomic and functional richness, and total abundance were lowest in DRY, followed by POST and highest in PRE samples (Figure 2a–c and Table S2). DRY samples harboured, on average, fewer species (22 ± 11) than PRE samples and POST samples (60 ± 15 and 45 + 14, respectively; $F_{2,51} = 36.7$, $p < 0.001$) but the loss of functional traits was less pronounced (10 ± 3 in DRY, 16 ± 2 in PRE, and 14 ± 2 in POST; $F_{2,51} = 29.8$, $p < 0.001$). The same pattern followed the log-transformed total abundance being lowest in DRY (5.2 ± 0.9) and higher in PRE and POST (8.3 ± 0.7 and 6.8 ± 0.9, respectively; $F_{2,51} = 59.6$, $p < 0.001$).

PRE samples were dominated in abundance by Diptera 44% (Chironomidae prevailed), and Ephemeroptera, Oligochaeta, and Plecoptera represented >10%. On average, 22 taxa were found alive in DRY samples (Table S2 and Video S1), representing a proportion of 83% of all recorded taxa (the remaining being collected dead). In terms of abundance, a total of 69% of all organisms were found alive (i.e. 4,961 organisms alive and 2,228 dead). DRY samples were dominated by Oligochaeta (25%), Coleoptera, (23%), and Diptera (20%), and similarly, POST samples were dominated by Oligochaeta (24%), Diptera (18%), and Coleoptera (14%) accompanied with Plecoptera (16%; Table S2).

3.3 | Influence of dry and flowing phases on taxonomic and functional richness

In DRY samples, taxonomic and functional richness decreased exponentially with increasing dry phase duration ($F_{1,16}$ = 16.6, $p < 0.001$ for taxonomic richness and $F_{1,16}$ = 23.8, $p < 0.001$ for functional richness, respectively). In general, doubling the duration of the dry phase (an increase of dry phase duration by 100%) resulted on average in the loss of 3.15 taxa and 1.4 functional units (Figure 3). The decrease was lower for functional richness than for taxonomic richness (TR; $F_{1,32}$ = 7.97, p = 0.008).

After flow resumption, both taxonomic and functional richness increased exponentially with duration of the flowing phase (Figure 4, $F_{1,16}$ = 20.1, $p < 0.001$ for taxonomic and $F_{1,16}$ = 7.1 p = 0.017 for functional richness, respectively). The increase in taxonomic richness was higher than that in functional richness (FR; $F_{1,32}$ = 23.95, $p < 0.001$). In general, doubling the duration of the flowing phase (an increase of flowing phase by 100%) resulted in an increase of 15.4 taxa and 1.8 functional units in POST samples in average. Even after several months of rewetting, the number of species in POST samples was still largely below that of PRE samples ($F_{1,34}$ = 10.2, $p <$ 0.001, Figure 2a, compare with Figure S2 for perennial sites where the number of taxa is more stable).

TABLE 2 Environmental characteristics of the studied dry riverbeds

Variables	Mean	*SD*	Min.	Max.
Temperature on the dry riverbed (°C)				
Total	15.0	5.6	−11.1	56.6
Day	16.1	5.8	−10.2	56.6
Night	13.3	4.8	−11.1	29.3
Sediment moisture (% water content)	11.9	8.9	0.4	50.0
Grain size (mm)				
25% percentile	3.1	3.3	0.1	13.3
50% percentile	19.4	10.1	1.0	40.2
75% percentile	47.9	15.5	18.6	87.9
Particles < 1 mm (%)	29.0	10.3	13.4	55.2

3.4 | Nestedness and turnover components of taxonomic and functional β-diversity

The total β-diversity was higher for taxonomic than for functional composition for all sample types (PRE, DRY and POST, Kruskal–Wallis $p < 0.001$). Overall, total β-diversity was highest in DRY samples and lowest in PRE samples (Figure 5). For both taxonomic and functional β-diversity, the turnover component was dominant across all sample types, ranging from 5 to 100% of the total β-diversity with a mean of 82.7% (Figure 5). In contrast, the nestedness component was marginal for each sample type (Figure 5).

3.5 | Contribution of communities surviving during dry phases to benthic community recovery

In terms of number of shared taxa, the contribution of DRY samples to POST samples was as high as 43% just 10 days upon rewetting (with 12 taxa in common), but gradually decreased with the duration of flowing period (Figure 6a, $F_{1,12}$ = 20.1, R^2 = 0.59, $p < 0.001$). After 60 days of flowing conditions, this contribution was <15% with only nine taxa in common (Figure 6a). With respect to community composition, the similarity between DRY and POST samples was about 60% during the early phases of flow resumption (i.e. 10 days) and also decreased gradually with the duration of flowing period (Figure 6b, $F_{1,12}$ = 6.2, R^2 = 0.28, p = 0.029). After 60 days of flowing conditions, this contribution was <25% (Figure 6b).

Rather similar patterns were found for functional diversity. In terms of number of shared traits, the contribution of DRY samples to POST samples was about 75% 10 days upon rewetting (with 9 trait units in

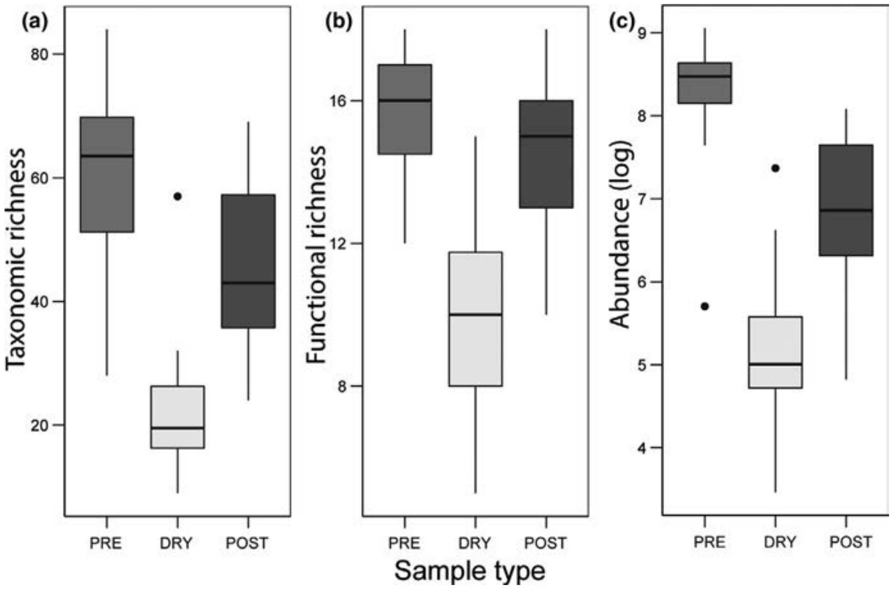


FIGURE 2 Differences in (a) taxonomic and (b) functional richness, and (c) abundance of invertebrates among PRE, DRY and POST samples

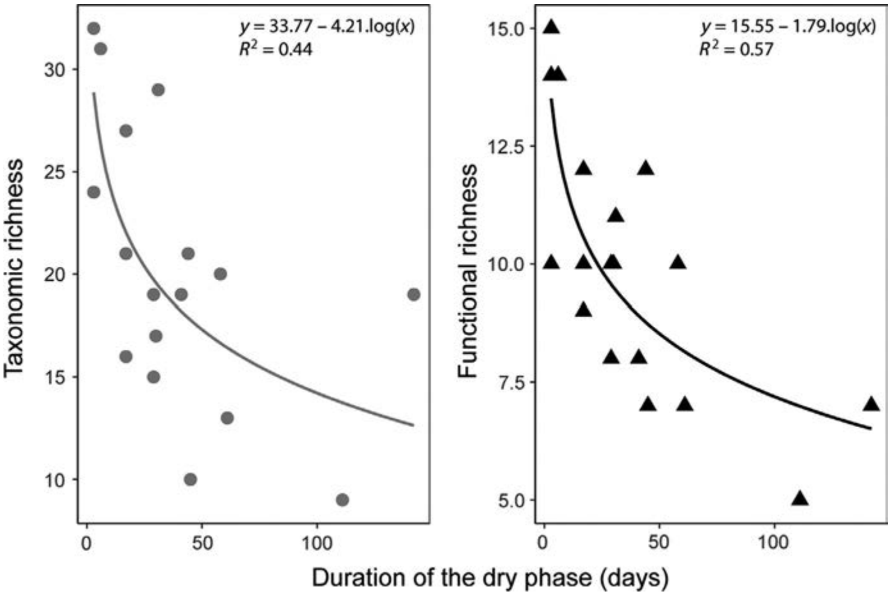


FIGURE 3 Relationships between taxonomic (grey circles) and functional (black triangles) richness of dry riverbed invertebrate communities and the duration of dry phases

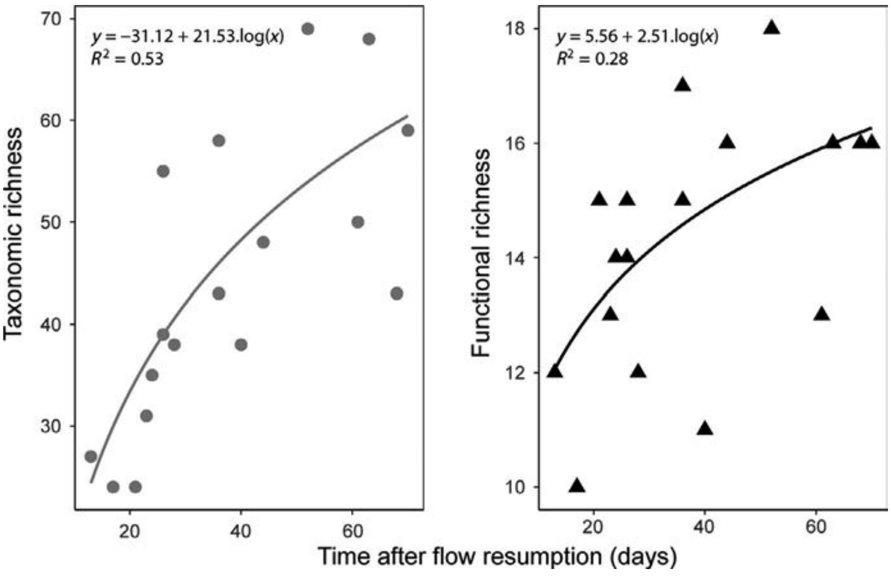


FIGURE 4 Relationships between taxonomic (grey circles) and functional (black triangles) richness of invertebrate communities during flowing phases and the time after flow resumption

common), but gradually decreased with the duration of flowing period (Figure 6c, $F_{1,11}$ = 7.8, R^2 = 0.36, p = 0.020). After 60 days of flowing conditions, this contribution was *c.* 57% with only seven traits in common (Figure 6c). With respect to functional composition, the similarity between DRY and POST samples was *c.* 80% during the early phases of flow resumption (i.e. 10 days) but no significant decrease with the duration of flowing period was found (Figure 6d). After 60 days of flowing conditions, this contribution was approximately 76% (Figure 6d).

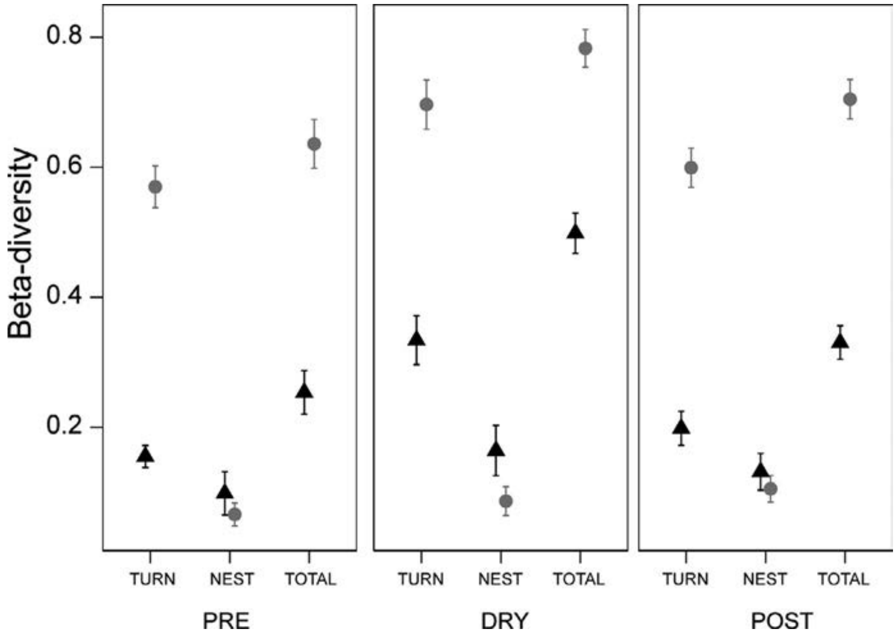
4 | DISCUSSION

We explored the ability of aquatic invertebrates without resistance adaptations from continental humid climates to cope with anthropogenically-induced (i.e. water abstraction, land-use alterations, climate change) flow intermittence. Contrary to what is reported from mesic climates, where natural flow intermittence promoted a development of physiological resistance to desiccation (Strachan et al., 2015, 2016; Stubbington & Datry, 2013), and owing to the high relative air humidity, riparian cover, and the short duration of drying events, we expected taxonomically and functionally rich communities to persist in the dry streambed during phases with no surface water. This prediction was supported, showing how stream invertebrate communities may survive through periods of anthropogenically-induced flow intermittence, in continental regions of Europe where increasing numbers of streams are affected by anthropogenic flow intermittence.

4.1 | Communities of invertebrates on dry riverbed sediments

On average, 22 taxa (83% of the total number of taxa collected during the dry periods) were found alive in the sampled sediments,

FIGURE 5 Beta-diversity components (turnover, TURN; nestedness, NEST; and total) for functional and taxonomic richness for PRE, DRY, and POST samples. Mean and 95% confidence interval are plotted for each sample type separately



which is as high as previous studies have found emerging from dry sediments that were artificially rewetted for 3 weeks (e.g. 22 taxa in Boulton, 1989; 24 taxa in Datry et al., 2012) and which included eggs and other desiccation-resistant forms. The pool of taxa surviving moist conditions during the dry phase in the 10 riverbeds of this study is richer than previously expected (Datry et al., 2012; Strachan et al., 2015, 2016; Stubbington, Gunn, Little, Worrall, & Wood, 2016), indicating that it could be an important source of resilience in continental areas, as suggested previously (Strachan et al., 2016). On average, only 30% of organisms were found dead during the dry period, indicating that their large proportion was able to cope with the temporary lack of surface water. Dry riverbed communities were dominated by Oligochaeta (mostly semi-terrestrial Lumbricidae and Enchytraeidae), Coleoptera (Hydraenidae and Elmidae), and Diptera (mostly Chironomidae), which are frequently reported from rewetted sediments (Datry et al., 2012; Stubbington & Datry, 2013). Their proportions were much higher in dry riverbeds compared to samples collected during flowing phases, which suggests that they are tolerant to drying. In contrast, several taxa found abundantly during the flowing phases such as Plecoptera (Nemouridae, Taeniopterygidae, and Capniidae), Ephemeroptera (Leptophlebiidae, Baetidae), and Amphipoda (*Gammarus fossarum*) were only occasionally found alive in dry sediment samples for short drying durations, indicating a low tolerance of these specific stages to drying. In terms of functional aspects, the number of functional units in DRY samples (10 in average) were half of that in POST (14) or PRE (16) samples, yet DRY samples seemed to contribute substantially to the recovery of functional diversity of stream communities upon rewetting, with up to 63% of shared traits on average. However, this contribution also varied with duration of drying and time since rewetting.

This high survival of organisms on these streams during the dry phase can be explained by the mild environmental conditions prevailing in the dry riverbeds. During sampling, temperature at the surface of the dry sediments was 16°C on average during daylight, while mean sediment moisture stay 11.9% relatively high, even after 4 months of drying. In packs of leaf litter deposited over dry sediments, moisture content could be as high as 50% and mean air moisture ranged from 80% at the surface to 96% in the top 5 cm below the surface (Havelka, 2018). These conditions are much more benign than those reported from Mediterranean (Steward et al., 2011), oceanic temperate (Datry et al., 2012), or arid (Stanley, Buschman, Boulton, Grimm, & Fisher, 1994) dry riverbeds, where temperature can exceed 60°C at the bed surface for the most of the daylight. The high (90–100%) riparian cover at most sites probably protected dry riverbeds from the high temperatures and decrease of air humidity. This emphasises a need to couple the management of rivers and their riparian zones, which is rarely done in practice (Storey & Quinn, 2013).

4.2 | Effects of drying duration on invertebrate communities

As predicted, both taxonomic and functional richness of dry riverbed communities decreased with increasing duration of the dry phases. While the duration of the dry phase was shown previously to be a key determinant of invertebrate seedbank composition (Larned, Datry, & Robinson, 2007; Storey & Quinn, 2013; Stubbington & Datry, 2013) and globally of stream community persistence (Bogan et al., 2013; Datry, 2012; Leigh & Datry, 2017) in arid and Mediterranean intermittent streams, our results indicate it also controls the number of taxa and traits able to survive throughout the dry phases in continental regions. Several studies reported decreased invertebrate richness with increasing dry period duration when analysing the invertebrate seedbank (Datry et al., 2012; Larned et al., 2007; Stubbington & Datry, 2013), that is, the pool of species producing

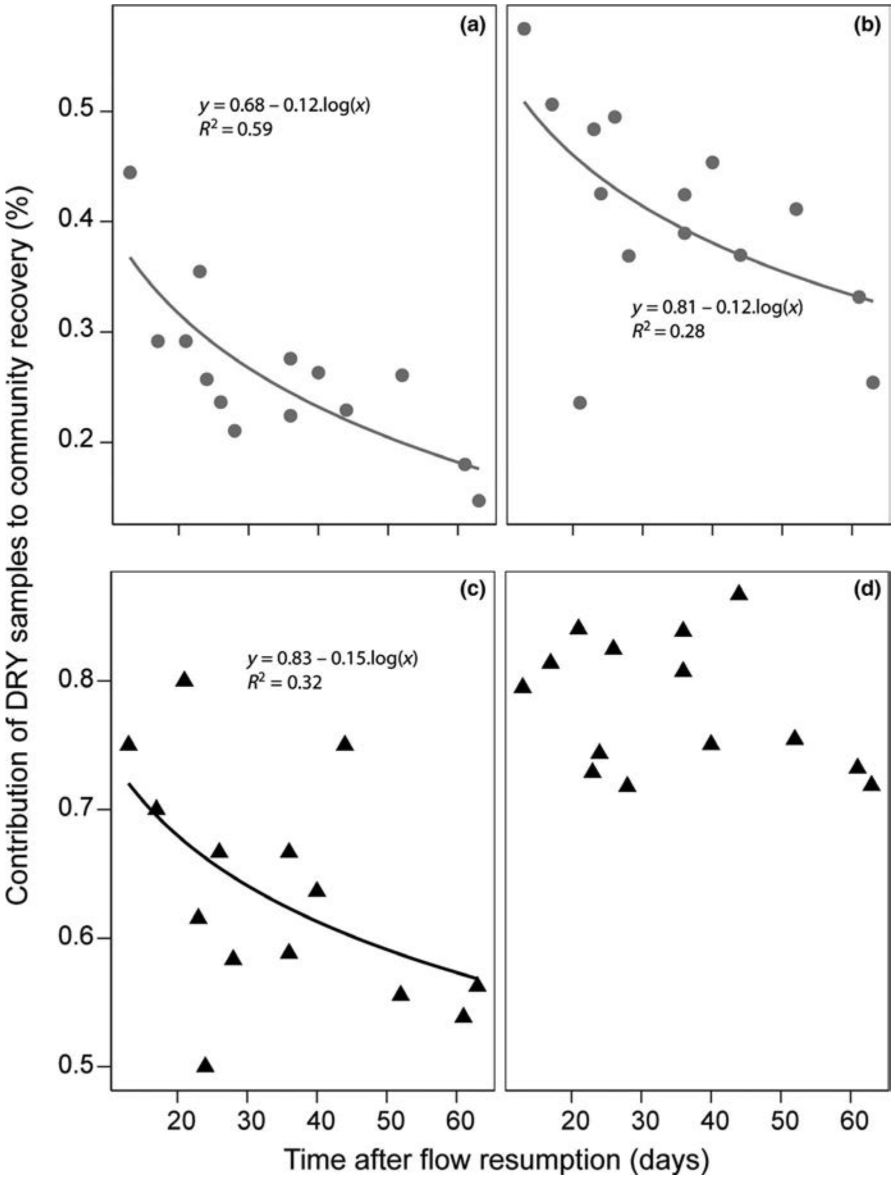


FIGURE 6 Contribution of DRY sample communities to benthic community recovery as % in terms of (a) shared taxa, (b) community composition, (c) shared biological traits, and (d) functional composition

desiccation-resistant forms to cope with dry events. However, to our knowledge, this is the first time that such relationship is documented for the invertebrates surviving as active forms (i.e. not as resistant forms) in dry riverbeds in continental streams.

Contrary to what has been reported for the invertebrate seedbank (Larned et al., 2007; Stubbington & Datry, 2013), the decrease of taxonomic richness (and functional richness) with increasing dry period duration was not linear but exponential. This could be due to the high number of taxa collected here in the first days of the dry phase (on average *c.* 30 taxa), which then exponentially decreased, while for the invertebrate seedbank, the richness of the pool of resistant species was always <30 and generally <20 taxa per sample. This indicates that there is a pool of invertebrates that cannot survive long without surface water, inducing a sharp decrease during the first 3–4 weeks of dry period, beyond which the decrease became more linear probably because the remaining taxa are the most tolerant to desiccation. Accordingly, drying acts as a ramp disturbance (sensu Lake, 2000) for the organisms that are not able to escape to perennial refuges (i.e. perennial reaches, adjacent ponds and wetlands, underneath hyporheic zone). However, even after 100 days of drying, *c.* 20 taxa were still found alive indicating they can tolerate very long dry events. These taxa were Oligochaeta (earthworm *Eiseniella tetraedra* and Enchytraeidae as *Henlea*/*Fridericia* spp.), Nematoda and Diptera (Ceratopogonidae- *Bezzia* sp., Stratiomyidae- *Oxycera* sp. and Chironomidae-*Chaetocladius piger*-Gr, *Smittia* sp.) which are mostly semi-terrestrial forms and common inhabitants of intermittent rivers worldwide and are also considered as desiccation resistant (Arscott, Larned, Scarsbrook, & Lambert, 2010; Bogan et al., 2013, 2017; Datry, Bonada, & Boulton, 2017; Datry, Vander Vorste, et al., 2017). Our results confirm these previous findings but suggest that this be due to the tolerance of dry conditions, without necessarily requiring the use of desiccation-resistant stages such as eggs, dormant, or quiescent stages (Strachan et al., 2015; Stubbington et al., 2017). Documenting such quantitative relationships will help predict the effects of increasing stream drying in the CR (Benčoková et al., 2011; Van Lanen et al., 2016; Zahrádková et al., 2015) and potentially in other places with similar climates, as a response to global change and guiding conservation and adaptation strategies.

Both taxonomic and to a lesser extent, functional β-diversity of the communities from dry riverbeds was dominated by turnover, not by nestedness. This pattern was probably due to (1) the high stochasticity of drying in streams from the CR; (2) the lack of adaptation of communities to drying as opposed to what is general observed in more arid climates; and (3) the relatively mild conditions prevailing during dry phases as discussed above. These three factors alone or combined could explain the removal of taxa from the communities with increasing dry phase duration did not follow a consistent pattern leading to nestedness, as often reported along flow intermittence gradients (Datry et al., 2014). In contrast, site-specific responses might have induced turnover, i.e. the taxa responded to drying differently across the studied sites. This context dependency could be due to contrasted local habitat conditions, the variability in biotic interactions (such as the presence of predators and scavengers in the streams or adjacent riparian zones, Steward et al., 2011) and/or other variables not measured in this study. This outcome implies that it might be challenging to predict the effects of flow intermittence in streams recently prone to drying and calls for comparing the responses of communities to natural and artificial flow intermittence.

4.3 | Recovery upon rewetting and community resilience

Although limited in terms of number of taxa, communities collected from dry sediments seem to have an important role in the recovery of benthic communities upon rewetting. About 2 weeks after rewetting, almost half of benthic taxonomic richness was composed of taxa also collected in the dry sediments—this was also true in terms of community composition (i.e. based on the relative abundance of each taxon in the community). This proportion is substantial and exceeds by far the contribution of the invertebrate seedbank found previously in temperate, arid, and neotropical environments (Datry, Bonada, & Boulton, 2017; Datry, Vander Vorste, et al., 2017; Hay, Jenkins, & Kingsford, 2018). The contribution to recovery in terms of functional diversity, based on the traits coded here, was very high. Besides, this contribution did not increase with flow duration as the taxonomic contribution did, indicating that the pool of species on dry sediments allowed a very quick and constant functional recovery. However, additional work is needed to better understand how drying influences the functional traits of river communities (Aspin et al., 2019; Cid et al., 2016; Vander Vorste et al., 2016). Aquatic invertebrates alive in dry riverbeds can thus be a significant source of resilience for benthic communities in these continental streams, despite the intense predation pressure to which dry riverbeds are usually prone (Corti & Datry, 2016; McCluney & Sabo, 2016; Steward et al., 2011).

After 1.5 months of flow resumption, only 20% of the taxa from benthic communities were in common with dry riverbed communities, reflecting the fast ecological successions documented from IRES (Bogan et al., 2013; Leigh et al., 2016). Taxa from perennial reaches (Arscott et al., 2010; Bogan et al., 2013), underlying hyporheic zones (Datry, 2012), and other refuges located in the catchment colonise gradually previously dry reaches, supporting our initial expectation and explaining why the contribution of dry riverbed communities decreased with time. Such increase in taxonomic and functional richness with time since flow resumption is a common feature of IRES—this study indicates that the initial phases of community recovery are largely driven by the pool of invertebrates surviving over the dry sediments. However, compared with Mediterranean regions (Bonada et al., 2007; Vander Vorste et al., 2016), the recovery seems to be much slower in these continental streams as even 3 months following rewetting, communities were still not fully recovered. Such patterns, often found in intermittent rivers (Datry, 2012; Datry et al., 2014; Datry, Moya, Zubieta, & Oberdorff, 2016; Leigh et al., 2016) were confirmed by the fact that taxonomic richness from 8 adjacent perennial sites showed no differences between pre- and post-drying sampling periods. While this probably reflects the few adaptations shown by species to cope with drying for periods as long as in the Mediterranean region, it provides evidence supporting the need to conserve and protect dry riverbed communities from anthropogenic activities (Steward et al., 2011) to promote community and ecosystem resilience in stream exposed to global change and increased stream network fragmentation by drying.

5 | CONCLUSIONS

Our results indicate that a large pool of invertebrate species can cope with drying without requiring specific adaptations, at least when benign conditions prevail in streambeds during the dry phase. In turn, we quantified for the very first time how this pool of taxa could contribute to community recovery upon rewetting in these forested intermittent streams. In the context of climate change and increasing stream drying, our results show the need for joint management of drying streams and their adjacent riparian zones to promote benign environmental condition allow aquatic invertebrates to survive for weeks or months without surface water. Riparian forest management along intermittent streams from continental climates, like these from the CR, could help conserve the value of dry streambeds as source of resilience for riverine communities.

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SUPPORTING INFORMATION

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PRIMARY RESEARCH PAPER

Macroinvertebrate seedbank survival in pristine and nutrient-enriched intermittent streams and its contribution to flow phase communities

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Abstract Intermittent stream research focuses mostly on pristine streams. The impacts of nutrient enrichment on invertebrate communities in these systems thus remains underexplored, especially in temperate climates. This study aimed to compare taxa survival during stream drying within pristine and nutrient-enriched sediment streambeds and to evaluate seedbank contribution for community recovery. Ten dry sediment subsamples were each collected from three streams of each nutrient status type. These were rehydrated for 60 days in laboratory with persisting and hatching invertebrates being recorded. Oligochaetes and molluscs were the most abundant; stoneflies and mayflies were common; caddisflies were represented by only a few specimens. The seedbank comprised of around one-third of taxa recorded during flow phases with slightly higher taxa numbers in nutrient-enriched streams. Moisture content positively affected seedbank taxa richness, while dry period duration decreased it. Taxa richness has a unimodal response to nutrient enrichment, with fine sediment proportion revealing no significant effect. Our results show a richer macroinvertebrate seedbank in drying sediments under moist conditions with mild nutrient enrichment, while raised nutrient levels limited macroinvertebrate survival. These results highlight the crucial role of seedbanks in community recovery of intermittent temperate streams, which will become more common with climate change.

Keywords Rehydration · Moisture · Dry period duration · Fine sediment · Resistance · Community recovery

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Introduction

Intermittent streams are dominant water bodies in Mediterranean, semi-arid, and arid landscapes (Datry et al., 2014a; Larned et al., 2010; Leigh et al., 2016). In humid temperate regions, especially Central Europe (see Peel et al., 2007), they are less common. However, they are expected to become increasingly frequent (Crabot et al., 2020; Ionita et al., 2017) due to the combined effects of climate change and increasing water demands (Mantyka-Pringle et al., 2012; van Vliet et al., 2013). Invertebrates inhabiting repeatedly drying stream reaches possess various mechanisms to persist and recover *in situ* following disturbance (Chester & Robson, 2011; Datry et al., 2017; Vander Vorste et al., 2016; Williams, 2006). Desiccation-resistant stages persisting in dry sediments is referred to as the 'seedbank' (Stubbington & Datry, 2013; Stubbington et al., 2016), and includes both active forms and dormant life stages that activate only after rewetting (Brock et al., 2003; Stubbington & Datry, 2013). While dormant invertebrate stages must undergo a type of transformation before becoming active (Strachan et al., 2015; Tronstad et al., 2005; Williams, 2006), active invertebrates can quickly revive after inundation. The seedbank of dormant invertebrates has been widely documented from dry streambeds, and the seedbank itself was recognised as an important resistance and recovery mechanism (Larned et al., 2007; Storey & Quinn, 2013; Strachan et al., 2015; Stubbington & Datry, 2013). However, there is still a lack of information about aquatic invertebrate seedbanks from humid continental climate regions (Pařil et al., 2019b), especially in nutrient-enriched intermittent streams.

We focus here on the ability of aquatic macroinvertebrates to persist within disturbed and repeatedly drying reaches in pristine and nutrient-enriched streams via active (sensu Pařil et al., 2019b) and dormant stages. Even though there is a growing research interest in streams that have recently changed from permanent to intermittent (Datry et al., 2017), most attention has been paid to pristine streams with stream drying being seen as the principle ecological driver shaping community assemblage (Pařil et al., 2019b; Straka et al., 2019). Other potential stressors are often omitted. Little is known, for instance, about the intermittency influence on communities in nutrient-enriched streams (Datry et al., 2014b; Steward et al., 2012). Different community responses to drying may occur in places where the local biota is exposed to raised nutrient levels originating from anthropogenic pollution (Stubbington et al., 2019). These, for example, may change primary consumer community structure and increase food supply (Dang et al., 2009; Gafner & Robinson, 2007). Or they may lead to eutrophication, oxygen depletion and associated habitat degradation (Davis et al., 2018) resulting in reduction of taxonomic diversity, which is common to nutrient-enriched streams (Everall et al., 2019; Gafner & Robinson, 2007). The ability to thrive in nutrient-enriched streams is considered to be taxon-specific (Dang et al., 2009). The impact of nutrient enrichment on the aquatic invertebrate richness of intermittent streams remains largely undescribed (Beermann et al., 2018; Gafner & Robinson, 2007; Sabater et al., 2011).

Abiotic factors that influence invertebrates' survival capability in drying streambeds includes streambed moisture, sediment structure, duration of the dry period, or temperature correlated with canopy cover (Piggott et al., 2012; Storey & Quinn, 2013; Stubbington & Datry, 2013; Stubbington et al., 2016). Moist sediments provide suitable survival conditions for invertebrates during the desiccation (Stubbington & Wood, 2013; Verdonschot et al., 2015). Thus, a reduction in moisture content can lower their survival rates. This impact has been documented in experimental tests (Loskotová et al., 2019; Poznańska et al., 2015, 2017). Moisture is considered as one of the main factors enabling macroinvertebrates to survive in situ during the dry episodes in Central Europe (Pařil et al., 2019b). Furthermore, habitat heterogeneity, related mostly to mesohabitat substrate variation, increases invertebrates' survival success, because it can provide interconnected microhabitat refugia within a disturbed site (Beisel et al., 1998; Brown, 2007). The deposition of fine sediment (particle diameter < 2 mm) into streams thus leads to a clogging of streambed interstitial spaces (Extence et al., 2013; Packman & MacKay, 2003), reduced habitat variability (Davis et al., 2018) and sediment permeability, thereby limiting invertebrate survival (Loskotová et al., 2019; Vadher et al., 2015, 2017).

In this study, we tested the impact of four selected environmental factors—substrate moisture content, dry period duration, nutrient enrichment, and fine sediment (< 2 mm) proportion—on macroinvertebrate species richness. Our aims were to (i) find taxa that can survive stream drying within a streambed, (ii) investigate effects of selected environmental factors on survival, and (iii) evaluate the importance of the seedbank on invertebrate community recovery in pristine and nutrient-enriched streams. We tested for community composition and richness effects among three different mesohabitats (riffle, pool, and marginal). We predicted that increasing moisture content would enhance invertebrate survival, while increasing dry period duration, nutrient enrichment, and fine sediment would reduce taxa richness within the dry streambed. We also predicted differences in seedbank taxa composition and richness among the three mesohabitat types since they might differ in environmental conditions. Finally, we predicted substantial seedbank contribution to community composition during flow phases and its importance for community recovery at both pristine and nutrient-enriched sites.

Materials and methods

Dry streambed sampling and processing

We chose three naturally pristine (PRIST) and three nutrient-enriched (NUTEN) sites in small intermittent streams (2nd–4th Strahler order). All were of comparable altitude, size, geology, and hydrology, and were located within the same basin of the Morava river in the White Carpathians (Czech Republic). Pristine streams were exposed to minimal anthropogenic impact while nutrient-enriched sites were located downstream of wastewater sewage effluents. All sampling sites were continuously monitored using scouting cameras (Acorn 5310MG, Zhuhai Ltl Acorn Electronics Co., Ltd.) and temperature loggers (HOBO Onset Pro v2 U22-001, Onset Computer Corporation) to determine dry period duration for different mesohabitats with hours precision. All samples were collected during a two-day period in August 2018. In total 60 dry sediment samples were obtained: from each site, representing 25 m of stream reach, three different mesohabitats were included: four samples from riffles, four from pools, and two from marginal habitats. Each sample was collected using a trowel from a 20 × 20 cm area 15 cm in depth. The sample was immediately weighed, placed in a plastic box, and transported to the laboratory.

In the laboratory, samples were split into thirds. The first third was dried and sieved through a set of meshes to obtain the grain structure and proportion of fine substrate (< 2 mm). Simultaneously, 100 g of the fine substrate was separated for chemical and 100 g for moisture content analyses, determined by comparing weight before and after oven-drying for 24 h at 105 °C. The second third was immediately fixed in 4% formaldehyde, invertebrates were sorted under stereomicroscope, with these data being used to characterize the surviving invertebrate (i.e., the active form) community composition before rehydration (subsample DRY). Finally, the last third was rehydrated in the laboratory under controlled conditions (subsample REHYD) for 60 days. Together, we gained four different treatments comprising macroinvertebrate communities before and after rehydration (DRY and REHYD), and from pristine and nutrient-enriched sites (PRIST and NUTEN), and their mutual combinations (i.e., DRY-PRIST, DRY-NUTEN, REHYD-PRIST and REHYD-NUTEN).

Subsample rehydration

Each REHYD subsample was placed into an 8 l experimental polyethylene mesocosm of 30 × 20 cm area and 14 cm height (see Fig. 1). Each sample was rehydrated with dechlorinated tap water. The following characteristics were measured after 2 days of laboratory acclimation: temperature (17.7 °C), pH (7.76), conductivity (510.5 μS cm^{-1}), dissolved oxygen (8.76 mg l^{-1}), and oxygen saturation (96.9%). Each mesocosm was covered with a lid. To support air circulation and increase an area suitable for emerging adults, a dome-shaped strainer and 1 mm mesh netting was attached to the lid, see Fig. 1. Mesocosms were continuously aerated by aquarium air curtains. Each mesocosm was inspected weekly; visible living individuals (larvae and adults) were continuously collected to reduce predation and competition. Temperature, conductivity, pH, and dissolved oxygen in each mesocosm was measured. For the whole duration of the experiment, a constant air temperature of 16.5 °C and photoperiod 15-h light/9-h dark was maintained, simulating light conditions during the sampling period. After 60 days of inundation, all REHYD subsamples were washed through a 500-μm mesh-size net, fixed (4% formaldehyde), and invertebrates were sorted out and identified to the lowest possible taxonomic level (predominantly species or genus). Terrestrial organisms were excluded.

Sampling during flow phases

To evaluate seedbank proportion in the source community and its potential contribution to community recovery after flow resumption, we compared experimentally rehydrated seedbank composition to

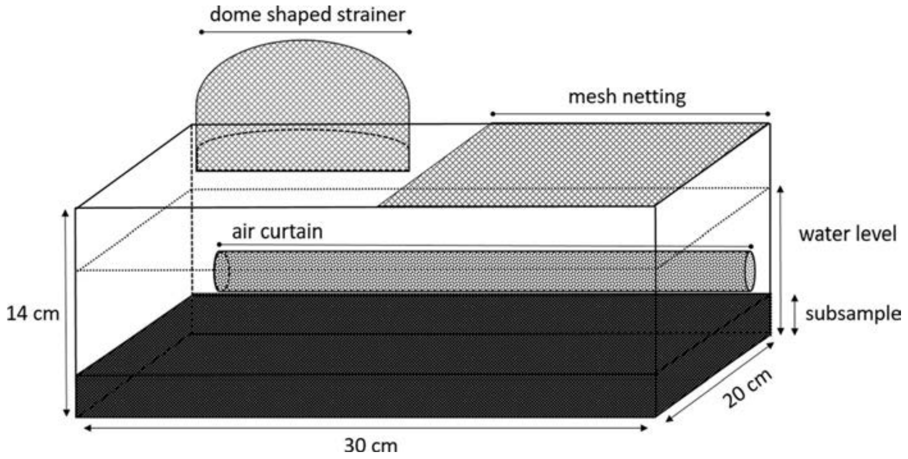


Fig. 1 Scheme of used mesocosm: subsample was rehydrated and aerated by aquarium air curtain. The box was covered with a dome-shaped strainer and with 1 mm mesh netting for emerging adults attached to the lid

invertebrate samples from the spring (pre-drying) and autumn (post-drying) seasons. Invertebrates were sampled according to the standardised PERLA protocol (Kokeš et al., 2006) based on proportional multihabitat 3-min kick sampling using a 500-µm mesh-size net (frame 25 × 25 cm). All samples were preserved in 4% formaldehyde and processed under a stereomicroscope. Spring samples were taken 147–241 days (mean 184 ± 31 days) after flow resumption in the preceding year and 63–90 days (mean 74 ± 11 days) before drying, whereas autumn samples were taken 7–75 days (mean 32 ± 23 days) after flow resumption in the year of sampling.

Data analyses

Principal component analysis (PCA) ordination was used to simplify measured physicochemical factors—conductivity, the concentration of P-PO_4^{3-}, N-NH_4^+, N-NO_2^-, N-NO_3^-, Cl^-, IC (inorganic carbon) and TOC (total organic carbon)—into two main general nutrient enrichment gradients which were used as a nutrient enrichment proxy in all subsequent analyses.

Taxa accumulation curves were produced to visually check sampling efficiency. Taxa richness was calculated for: (i) each separate mesocosm, (ii) summed mesohabitat types (riffle, pool, and marginal), and (iii) summed sampling sites. These results were compared between PRIST and NUTEN sites using Generalized estimating equations (GEE) using a Poisson distribution with differences being tested by defined contrasts. The same approach (GEE with Poisson distribution) was also used to test for significant impact of sediment moisture, duration of the dry period before seedbank sampling, both PCA proxy nutrient enrichment gradients, and the fine sediment proportion on seedbank richness using sampling site as a grouping factor. We applied backward selection to choose the most parsimonious model. For simplicity, each variable was also tested separately with the regressions for each significant predictor being plotted.

Non-metric multidimensional scaling (NMDS) was used to describe seedbank community structure using Bray–Curtis distances on taxa data aggregated at the mesohabitat level. Differences between PRIST and NUTEN sites were tested using permutational analysis of variance (PERMANOVA). Differences in the numbers of unique and shared taxa were calculated between seedbank communities of each site type and tested by defining the contrasts in GEE with the Poisson distribution.

The overall contribution of the seedbank to flow phase communities at different site types, expressed as a percentage of flow phase community taxa observed in seedbank, was visually compared using boxplots. Differences in seedbank contribution between PRIST and NUTEN site categories were tested by generalised linear model (GLM) with binomial distribution and defined contrasts.

Statistical analyses were processed in the R statistical computing environment (R Core Team, 2019) and functions in the packages 'vegan' (Oksanen et al., 2019), 'nlme' (Pinheiro et al., 2020), 'geepack' (Hojsgaard et al., 2006) and 'multcomp' (Hothorn et al., 2008). Graphics were produced by the 'ggplot2' package for R (Wickham, 2016). For all tests, a *P*-value < 0.05 indicates statistical significance.

Results

Environmental factors

Minimum, maximum, mean, and standard deviations of all measured physicochemical factors for both site-groups are presented in Table 1. The PCA revealed two main gradients covering 67% of total variability within environmental data. The first axis, represented by nutrient enrichment by nitrogen and phosphorus, explained 36.6% of the variability; the second axis, represented by carbon amounts, explained 30.4% of the total variability (see Fig. 2). The PCA loadings for two main PCA axes are presented in Table 2.

Taxonomic composition of dry streambed samples

Accumulation curves indicated that the number of samples taken in the field was sufficient to represent site assemblages (see Online Resource 1). In dry streambed subsamples (DRY) 1499 individuals from 54 taxa were present, while 3776 individuals from 79 taxa were present in laboratory rehydration subsamples (REHYD). Together 42 taxa representing 1266 individuals were exclusively present in REHYD samples. The mean number of REHYD taxa in each mesocosm was 8.3 ± 5 for PRIST sites and 9.8 ± 4.2 for NUTEN sites (Fig. 3) with no significant difference between the site types ($P = 0.990$). The mean number of observed taxa aggregated by mesohabitat type was 16.1 ± 10 and 18.3 ± 7.5 at PRIST and NUTEN sites, respectively (Fig. 3), again with no significant difference between the site types ($P = 0.980$). Finally, the mean number of REHYD taxa at each site was 28.3 ± 10.1 and 34 ± 8.9 at PRIST and NUTEN sites, respectively (Fig. 3), also with no differences between the site types ($P = 0.950$).

Oligochaetes and molluscs were the most abundant taxonomic groups in all four treatments. Caddisflies were almost absent, except for a few specimens found only in PRIST samples, while flatworms and leeches occurred only in NUTEN samples. Stonefly larvae (*Nemoura cinerea* (Retzius, 1783) and juveniles of *Isoperla* sp. and *Zwicknia* sp.) were exclusively found in REHYD subsamples. The majority of mayflies had higher numbers of emerging adults than stoneflies. The only mayfly species observed exclusively in DRY subsamples was *Centroptilum luteolum* (Müller, 1776). However, in REHYD subsamples, we observed several species that differed from those found in DRY

Table 1 Environmental factors and nutrient characteristics measured for all mesocosms in pristine (PRIST) and nutrient-enriched (NUTEN) sites

Environmental factor	PRIST				NUTEN			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Moisture content (%)	3.4	34.2	12.74	6.8	0.4	50	10.04	10.31
Fine sediment proportion (%)	2.47	26.57	12.46	6.23	1.81	24.8	13.33	6.53
Dry period (days)	18	74	51.78	17.87	7	70	38.56	23.93
Conductivity	124	282	166.5	30.33	138	495	225.07	99.85
$P\text{-}PO_4^{3-}$	0.02	0.2	0.09	0.06	0.25	1.8	0.94	0.37
$N\text{-}NH_4^+$	0.01	0.96	0.45	0.21	0.11	1.9	0.62	0.52
$N\text{-}NO_2^-$	0.02	0.3	0.1	0.07	0.02	1.26	0.33	0.39
$N\text{-}NO_3^-$	1.2	15.11	5.72	4.31	2.28	31.42	9.72	7.34
Cl^-	0.49	1.66	0.58	0.23	0.5	2.9	0.88	0.7
IC	9.19	32.81	17.45	5.35	6.34	12.42	9.28	1.38
TOC	5.41	103.54	48.26	29.67	4.22	66.34	25.14	17.1

IC inorganic carbon, *TOC* total organic carbon

Fig. 2 PCA ordination on selected measured physicochemical factors among the samples (NUTEN and PRIST) represented by the first two axes (PC1, PC2) with highest explanatory variation; *cond.* conductivity, *IC* inorganic carbon, *TOC* total organic carbon

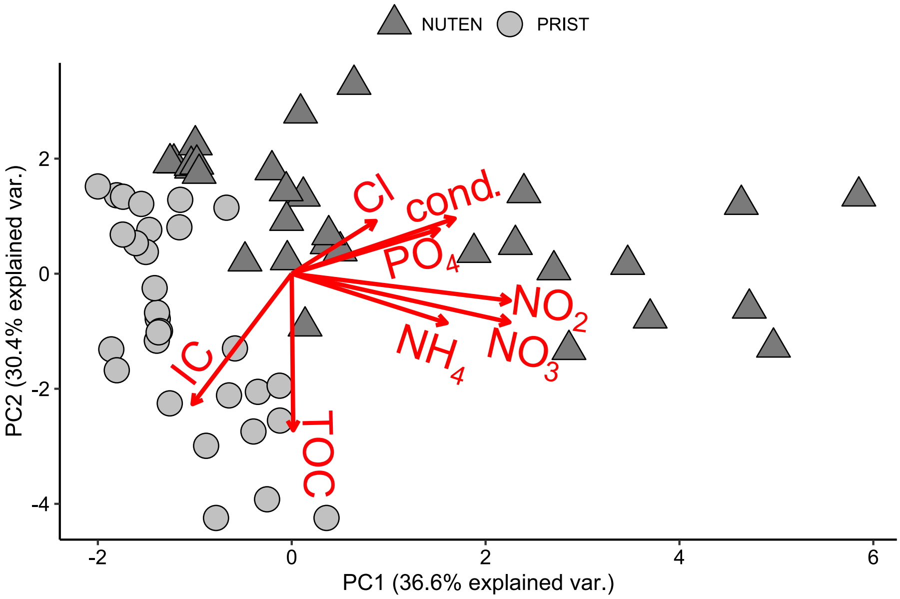


Table 2 The loadings of all measured physicochemical factors for two main PCA axes (PC1, PC2)

Factor	PC1	PC2
P-PO_4^{3-}	0.3035	0.1538
N-NH_4^+	0.3196	− 0.1712
N-NO_2^-	0.4501	− 0.0937
N-NO_3^-	0.4492	− 0.1687
Cl^-	0.1731	0.1833
IC	− 0.2046	− 0.4534
TOC	0.0031	− 0.5443

IC inorganic carbon, *TOC* total organic carbon

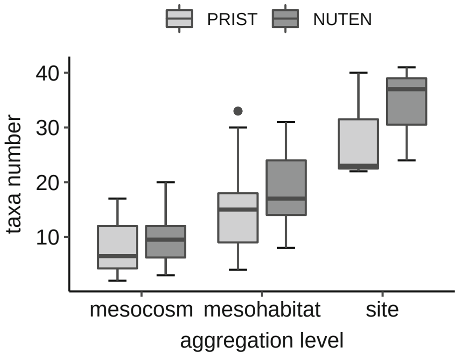


Fig. 3 Boxplots of taxa numbers at different aggregation levels for mesocosms separately, summed for mesohabitat types, and summed for sampling sites at PRIST and NUTEN sites

treatments, namely *Baetis libenauae* Keffermüller, 1974, *Baetis vernus* Curtis, 1834, *Electrogena ujhelyii* (Sowa, 1981), *Habrophlebia fusca* (Curtis, 1834), *Habrophlebia lauta* Eaton, 1884, and *Paraleptophlebia submarginata* (Stephens, 1835). Flatworms were only represented by *Dugesia polychroa* (Schmidt, 1861), which was found exclusively in REHYD subsamples. Crustaceans were represented by two species, namely *Asellus aquaticus* (Linnaeus, 1758) observed in both types of NUTEN treatments, and *Proasellus coxalis* (Dollfus, 1892) observed in all treatments. All observed taxa and their subsample/site abundances are listed in Online Resource 2; taxa frequencies and densities are listed in Online Resource 3.

Effects of selected environmental factors on seedbank taxa richness

The most parsimonious GEE model revealed significant effects of sediment moisture content, dry period duration, and first PCA axis (PC1) of nutrient enrichment on seedbank taxa richness (Table 3). Those factors also revealed their significance when tested separately. While the relationship between taxa number and dry period duration was logarithmic negative (Fig. 4a), the relationship between taxa number and moisture content was logarithmically rising (Fig. 4b).

Table 3 The most parsimonious GEE model's coefficients with the Poisson distribution of analysed environmental factors

	Estimate	Std.err	Wald	*Pr*(>|*W*|)
Intercept	2.4821	0.1883	173.8	< 0.0001***
PC1	0.1040	0.0628	2.75	0.0975
$PC1^2$	− 0.0318	0.0102	9.82	0.0017**
Moisture content	0.0202	0.0033	37.32	< 0.0001***
Dry period	− 0.0097	0.0021	20.68	< 0.0001***

Finally, the relationship between taxa numbers and nutrient enrichment (PC1) was unimodal (Fig. 4c).

NMDS ordination revealed different taxonomic composition among studied sites and mesohabitat types (final stress = 0.167). The results showed that NUTEN sites harbour communities that are consistent among different mesohabitats but differ among sites. In contrast, PRIST sites overlap in ordination space, indicating more homogenous taxa composition but reveal distinct differences among the mesohabitat types (Fig. 5). Regardless of apparent slight overlap of both groups in multidimensional space, we detected clear differences between PRIST and NUTEN site groups in taxonomic composition (PERMANOVA, $F_{1,16} = 2.25$, $P = 0.020$). Comparing the unique and shared taxa of PRIST and NUTEN sites (Fig. 6), no significant differences were found between numbers of shared and unique taxa at PRIST ($P = 1.000$) and NUTEN ($P = 0.270$) sites same as between numbers of unique taxa at PRIST and NUTEN sites ($P = 0.810$).

Contribution of seedbank to flow phase communities

During flow phases, 28,666 individuals from 123 taxa were recorded in spring (pre-dry) season, and 5659 individuals from 95 taxa were recorded in autumn (post-dry) season. Mean taxa number observed during three sampled seasons for both site types are visualised in Fig. 7a. Comparisons of seedbank taxa richness with the flow phases community richness revealed seedbank contribution to spring source communities as 25.2 ± 11.5% and 28.7 ± 1.2% at PRIST and NUTEN sites, respectively, and to autumn recovered communities as 34.1 ± 6.5% at PRIST sites and 35.1 ± 7.5% at NUTEN sites (Fig. 7b). However, the

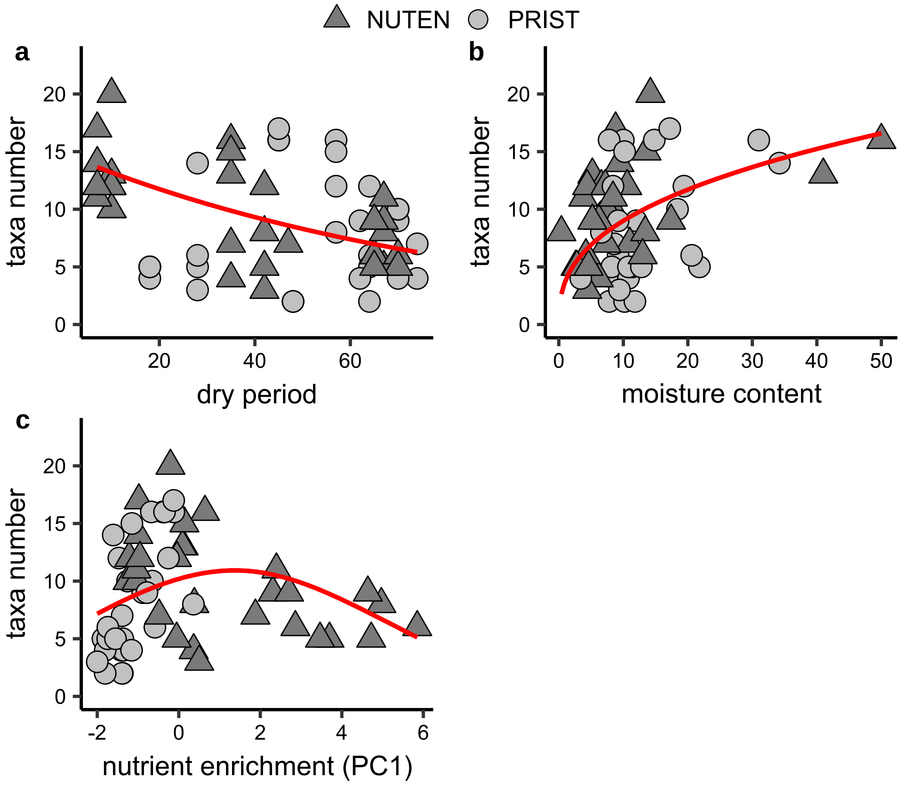
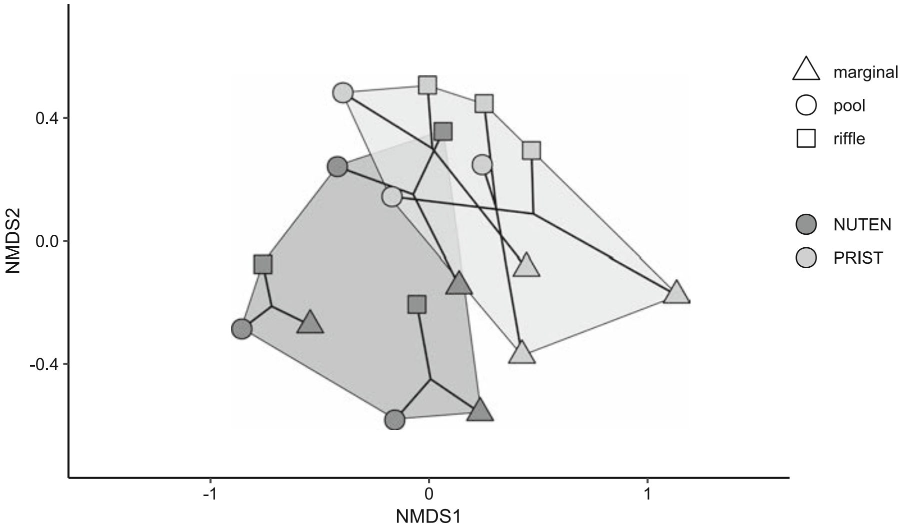


Fig. 4 Relationship between seedbank taxa number and the **a** dry period duration, **b** moisture content, and **c** nutrient enrichment represented by PC1 for PRIST and NUTEN sites

Fig. 5 NMDS ordination on taxa richness in REHYD subsamples from pristine (PRIST) and nutrient-enriched (NUTEN) sites linked with envelopes delimiting PRIST and NUTEN sites in ordination space (shaded areas). Communities are aggregated by mesohabitat types (riffle, pool, and marginal)



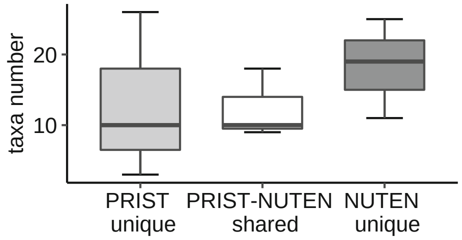


Fig. 6 Boxplots representing the number of unique taxa at pristine sites (PRIST unique), number of shared taxa at both site types (PRIST-NUTEN shared), and number of unique taxa at nutrient-enriched sites (NUTEN unique)

differences between PRIST and NUTEN site contributions were insignificant both in the spring (P = 0.920) and autumn (P = 1.000) season.

Discussion

Seedbank taxa composition

We confirmed previous observations from various climatic zones (Bohle, 2000; Philipp & Forster, 2000; Robson et al., 2011; Strachan et al., 2015) that some Ephemeroptera and Plecoptera species can survive streambed drying in the form of dormant desiccation-resistant life stages such as eggs or even juvenile larvae for stoneflies. The only mayfly larvae we observed in both dry (DRY) and rehydrated (REHYD) subsamples was *C. luteolum*. Other mayfly and stonefly larvae and adults were found exclusively in REHYD samples from both pristine (PRIST) and nutrient-enriched (NUTEN) sites. This resistance strategy to survive *in situ* drying might be especially beneficial for taxa with low dispersal abilities (Stubbington & Datry, 2013), such as Ephemeroptera and Plecoptera whose adults are weak fliers (Churchel & Batzer, 2006). Exclusively in REHYD subsamples we observed the tricladid *D. polychroa*, which confirms the presence of dormant eggs or resistant cysts of some flatworm species in sediments (Strachan et al., 2015; Young, 1974). Consistently, tricladid taxa are frequently observed in rehydrated dry riverbed sediments (Stubbington & Datry, 2013). Cyst formation is also known for some oligochaetes species (Kaster & Bushnell, 1981; Montalto & Marchese, 2005), but we observed oligochaetes specimens throughout all the treatments. Thus, we cannot unequivocally confirm the formation of cysts since they might survive in an active form. Crustaceans were represented only by *A. aquaticus* and *P. coxalis*. We did not observe any *Gammarus fossarum* Koch in Panzer, 1835 specimens, even though they abundantly inhabit these streams during flow phases. This observation confirms its expected limited ability to survive in drying sediments (Loskotová et al., 2019; Pařil et al., 2019a) and strengthens the case that it persists drying by upstream migration or downstream drift (Pařil et al., 2019a; Poznańska et al., 2013; Vander Vorste et al., 2016). Even though we observed different taxonomic composition in the seedbank of PRIST and NUTEN sites, we did not detect any significant differences between

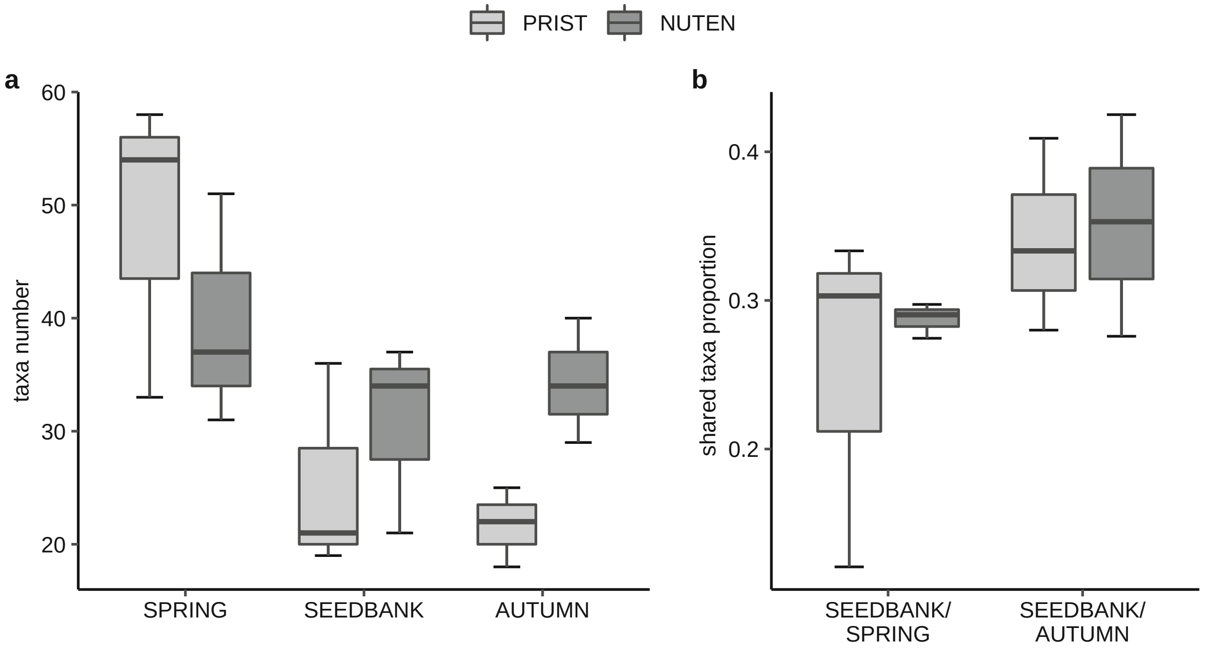


Fig. 7 **a** Mean taxa number in spring flow phase (SPRING), dry phase (SEEDBANK), and autumn flow phase (AUTUMN) community, and **b** the contribution of seedbank to the flow phase community expressed as shared taxa proportion in seedbank and spring (SEEDBANK/SPRING) and autumn (SEEDBANK/AUTUMN) flow phase community for PRIST and NUTEN sites

taxa richness indicating similar overall diversity on a local scale. The difference between PRIST and NUTEN sites is evident on the smaller scale of a single mesohabitat reach, with different NUTEN mesohabitats hosting a similar species pool, and PRIST sites having more heterogeneous taxonomic composition among mesohabitats. We suggest the homogenising factor between NUTEN mesohabitats to be higher nutrient loads which equalizes their conditions (Beisel et al., 1998). The higher species differentiation between mesohabitats in PRIST sites is more related to spatial heterogeneity within the sampled reach (Downes et al., 1998; O'Connor, 1991; Resh et al., 1988). Such findings highlight the urgency of mesohabitat diversity protection via conservation and restoration of the hydro-morphological diversity that serves as the key structures for biodiversity maintenance during the dry phases.

Environmental factors effects on seedbank taxa composition

We observed almost no effect of fine sediment proportion (see its range in Table 1) on seedbank richness, which is in contrary to previous observations (Loskotová et al., 2019; Vadher et al., 2015, 2017) which showed that fine sediment clogged the streambed interstitial spaces used as refugia or refugial interconnections paths (Williams, 2006). An increase in fine sediment may negatively affect sensitive taxa (e.g., EPT taxa; Bruen et al., 2017), and otherwise positively affect taxa favouring fine sediment-rich habitats (Bo et al., 2007; Matthaei et al., 2006; Piggott et al., 2012) as are some oligochaetes (e.g., *Eiseniella tetraedra* (Savigny, 1826), *Nais* spp., *Henlea ventriculosa/perpusilla* gr.) or even molluscs (e.g., *Pisidium* spp.) frequently observed in the experiment. In our study, the proportion of fine sediment was low (see Table 1) and did not exceed 27%. In contrast to other studies (Bo et al., 2007; Davis et al., 2018; Piggott et al., 2012), where fine sediment was far beyond 50% of total substrate, the low levels of fine sediment in our experiment might explain its marginal effect on invertebrates' survival.

Elevated nutrient levels also impacted seedbank taxa richness. While slight nutrient enrichment enhanced and increased taxa richness, taxa richness decreased with excess nutrient loading after a threshold was reached. Some previous studies (Matthaei et al., 2010; Piggott et al., 2012) observed a similar unimodal response of richness to nutrient addition. Richness increases due to primary production elevation with low nutrient loading (Niyogi et al., 2007). However, at high nutrient loading rates only a few highly competitive taxa persist (Elbrecht et al., 2016). High nutrient loading rates from sewage effluents and treatment plants will only be exacerbated when climate change leads to lower water flow and dilution rates.

As seen in previous studies (Larned et al., 2007; Storey & Quinn, 2013; Stubbington & Datry, 2013), we recorded a significant decline in seedbank richness as dry period duration lengthened. This effect is more pronounced in moderate climates with higher relative humidity, more frequent precipitation, and denser riparian cover (Pařil et al., 2019b; Verdonschot et al., 2015) reaching over 66% of the open canopy at our sites as compared to harsher Mediterranean or arid regions (Bonada et al., 2007). Even though extended dry periods (60–100 days) lead to an exponential decrease of taxa richness in these climates (Pařil et al., 2019b), the dry period in our current study never exceed two months, thus the decrease rate was less pronounced.

Higher moisture retention clearly increases seedbank survival ability and is therefore of crucial importance for invertebrate resistance to drying streambeds (e.g., Poznańska-Kakareko et al., 2017; Stubbington & Datry, 2013; Tronstad et al., 2005; Verdonschot et al., 2015). Our study recorded well-developed seedbank community in streambed sediments over ca 10% of water content with taxa richness culminating at approximately 50% moisture. To summarise, the analysed factors (except for fine sediment proportion) significantly influence seedbank taxa richness, making each of them quite important for community composition with the leading effect being moisture content followed by the dry period duration and nutrient enrichment.

Seedbank contribution to flow phase source and recovered community

According to a comparative study (Stubbington & Datry, 2013), invertebrate communities inhabiting harsher regions (e.g., Mediterranean, semi-arid, or arid) tend to have more distinct rehydrated dry phase and recovered flow phase communities with, for example, only 5% taxa in common (Chester & Robson, 2011). In the more benign climate of our study region, we observed more than 25% overlap between the seedbank and spring source community and around 35% between the seedbank and autumn recovered community in both PRIST and NUTEN sites. This suggests comparable seedbank contributions to community recovery in both studied stream types and with the observed constant proportion of shared taxa, indicates high turnover between rehydrated and *in situ* samples.

Conclusion

The invertebrate seedbank is a substantial component of the community recovery process in the post drying period of both pristine and nutrient-enriched streams, where some sensitive taxa could survive in the form of dormant-resistant stages. Since the invertebrate community in nutrient-enriched streams is partly adapted to a harsher environment, its seedbank can withstand drying more than the seedbank from pristine streams. Contrary to nutrient-enriched streams, mesohabitat diversity plays a crucial role in undisturbed sites, where various habitat conditions enable survival of different taxonomic groups adapted to persist in dry refugia with specific physical and chemical conditions. However, moisture content is still the leading factor enhancing seedbank taxonomic diversity and survival in all intermittent streams, followed by negative effects of the dry period duration and high nutrient enrichment. Management strategies and restoration measures should focus primarily on protection of mesohabitat diversity and providing enough sediment moisture retention (e.g., preservation of riparian vegetation for shading), mitigation of the dry period (e.g., reduction in water abstractions), and reduction in high nutrient input (e.g., agricultural run-off, wastewater outflows) to promote invertebrate persistence and diversity on intermittent streams during climate change, which intensifies negative impacts of human activities.

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RESEARCH ARTICLE

Disentangling responses to natural stressor and human impact gradients in river ecosystems across Europe

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Abstract

1. Rivers are dynamic ecosystems in which both human impacts and climate-driven drying events are increasingly common. These anthropogenic and natural stressors interact to influence the biodiversity and functioning of river ecosystems. Disentangling ecological responses to these interacting stressors is necessary to guide management actions that support ecosystems adapting to global change.
2. We analysed the independent and interactive effects of human impacts and natural drying on aquatic invertebrate communities—a key biotic group used to assess the health of European freshwaters. We calculated biological response metrics representing communities from 406 rivers in eight European countries: taxonomic richness, functional richness and redundancy, and biomonitoring indices that indicate ecological status. We analysed metrics based on the whole community and on a group of taxa with traits promoting resistance and/or resilience ('high RR') to drying. We also examined how responses vary across Europe in relation to climatic aridity.
3. Most community metrics decreased independently in response to impacts and drying. A richness-independent biomonitoring index (the average score per taxon; ASPT) showed particular potential for use in biomonitoring, and should be considered alongside new metrics representing high RR diversity, to promote accurate assessment of ecological status.
4. High RR taxonomic richness responded only to impacts, not drying. However, these predictors explained little variance in richness and other high RR metrics,

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potentially due to low taxonomic richness. Metric responsiveness could thus be enhanced by developing region-specific high RR groups comprising sufficient taxa with sufficiently variable impact sensitivities to indicate ecological status.

5. *Synthesis and applications*. Metrics are needed to assess the ecological status of dynamic river ecosystems—including those that sometimes dry—and thus to identify priority sites requiring action to tackle the causes of environmental degradation. Our results inform recommendations guiding the development of such metrics. We propose concurrent use of richness-independent 'average score per taxon' indices and metrics that characterize the richness of resistant and resilient taxa. We observed interactions between aridity, impacts and drying, highlighting that these new metrics should be region specific, river type specific and adaptable, promoting their ability to inform management actions that protect biodiversity in river ecosystems responding to climate change.

KEYWORDS
bioassessment, biomonitoring, flow intermittence, intermittent river, macroinvertebrate, multiple stressors, resistance and resilience, temporary stream

1 | INTRODUCTION

Biodiversity is declining at unprecedented rates as interactions between anthropogenic activities and climatic extremes increase at local to global scales (Pereira et al., 2010). Declines are particularly severe in freshwater ecosystems, in which multiple interacting anthropogenic pressures (e.g. land-use change, pollution and climate change) result in stressors that are altering communities characterized by high biodiversity (Reid et al., 2019). Rivers experience these human impacts alongside natural flow extremes: seasonal or unpredictable disturbances in which natural stressors such as the absence of water (during drying) and fast flows (during floods) disrupt ecosystem structure and function (White & Pickett, 1985). Rivers are thus suitable ecosystems in which to explore the concurrent, independent and interacting responses of ecological communities to human impacts and natural stressors (Gutiérrez-Cánovas et al., 2015). These responses may vary across climatic gradients, due to the contrasting environmental conditions in which communities have evolved (Birk et al., 2020; Bonada et al., 2007).

The communities inhabiting river ecosystems comprise taxa which vary in their responses to human impacts. Community responses can be summarized by taxonomic and functional metrics, including biomonitoring indices that use the impact sensitivities of taxa in a community to assess ecological status. Ideal biomonitoring indices respond only to human impacts (i.e. a single response), but the concurrent effects of impacts and natural stressors on these metrics can be equal to (additive), greater than (synergistic) or less than (antagonistic) the sum of their individual effects and stressors can act in opposing directions (Côté et al., 2016), hampering the capacity of indices to assess ecological status (Stubbington et al., 2018). In particular, anthropogenic and natural stressors (such as drying in river ecosystems) can eliminate a comparable range of sensitive taxa. As a result, metrics based on taxonomic richness—including biomonitoring index totals—can experience antagonistic decreases, whereby responses to impacts weaken as community exposure to natural stressors increases (Soria et al., 2020; Figure 1a), an interactive effect which may be exacerbated by environmental harshness (e.g. climatic aridity; Piggott et al., 2015; Figure 1). In contrast to index totals, 'average score per taxon' (ASPT) indices describe community sensitivity to impacts independent of taxonomic richness, and may thus experience less pronounced decreases in response to stressors (Wilding et al., 2018; Figure 1b). However, research is needed to inform the development of metrics that respond independently to impacts and natural stressors (Gutiérrez-Cánovas et al., 2015), and thus to enable effective biomonitoring in ecosystems responding to environmental change (Nõges et al., 2016).

Taxa with common responses to environmental variability can be defined using functional traits (Suding et al., 2008). Many traits promote resistance and/or resilience to drying in freshwater ecosystems; for example, desiccation tolerance enables survival after a river dries, and strong dispersal facilitates rapid recovery after water returns (Bogan et al., 2017). The composition of community subsets comprising drying-adapted taxa may thus reflect responses to human impacts but not river drying, enabling identification of impacted conditions, including in rivers that sometimes dry (i.e. temporary rivers). Assemblages of resistant and resilient taxa can be diverse in rivers experiencing frequent, predictable drying (Bonada et al., 2007), but no large-scale studies have documented spatial variability in their responses to co-occurring natural and anthropogenic stressors.

Metrics based on the functional traits of the taxa comprising an assemblage enable comparison of regions with contrasting species

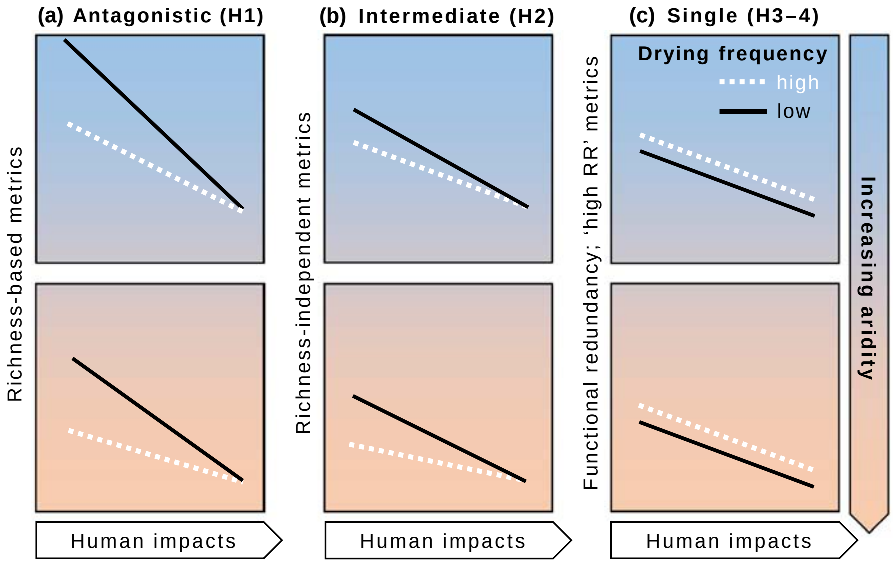

FIGURE 1 Hypothesized responses of invertebrate-based metrics to human impacts at river sites with high and low drying frequencies across a climatic gradient. Hypotheses (H) 1–4 are described in the text; (a) antagonistic, (b) intermediate and (c) single responses are sensu Côté et al. (2016)

pools (Suding et al., 2008). Different functional metrics, such as richness and redundancy, can have contrasting responses to co-occurring stressors. For example, functional richness (which quantifies the trait space filled by an assemblage) can decrease along both natural and anthropogenic stress gradients as stress-sensitive traits are lost (Gutiérrez-Cánovas et al., 2015). In contrast, functional redundancy (which indicates the number of taxa making similar contributions to ecosystem functioning) can be unresponsive to natural stressors such as river drying until high disturbance intensities are reached (Aspin et al., 2019), but declines with human impacts if drying-tolerant taxa vary in both their traits and their impact sensitivities (Soria et al., 2020). This single negative response of functional redundancy to impacts (Figure 1c) may facilitate its use in biomonitoring of temporary rivers (Bruno et al., 2016).

We characterized freshwater invertebrate community responses to human impact and natural drying gradients in European rivers. Established biomonitoring indices and the availability of trait information (Sarremejane et al., 2020; Tachet et al., 2010) make freshwater invertebrates an effective group with which to disentangle taxonomic and functional responses to multiple stressors (Statzner & Bêche, 2010). Our aim was to identify metrics that distinguish between responses to impacts and drying. Specifically, we identified metrics with single responses to impacts and those with independent or interactive responses to both impacts and drying.

We hypothesized antagonistic decreases in response to human impacts and drying for richness-based metrics (H1; Figure 1a); less pronounced decreases for richness-independent ASPT indices (H2; Figure 1b); and single negative responses to impacts for functional redundancy (H3) and metrics describing a 'high RR' group comprising taxa with traits promoting resistance and/or resilience to drying (H4; Figure 1c). We examined how responses vary across Europe in relation to variability in climatic aridity (Figure 1). As global change increases the spatial and temporal extent of river drying, our goal is to describe general principles that inform development of metrics to assess the ecological status of dynamic rivers.

2 | MATERIALS AND METHODS

2.1 | Datasets and their assignment to groups

We compiled 15 datasets describing invertebrate assemblages, flow permanence and human impacts in 406 rivers in eight European countries (Figure 2; see Appendix S4, Table S1). We analysed all datasets in one *all-region* dataset, which identified interactions between aridity, impacts and river drying, and we therefore also analysed three *regional* datasets (based on biogeographical classifications; EEA, 2002, 2009; Figure S1). We used nonmetric multidimensional scaling ordinations to visualize assemblage composition (Figure S2) and to define coherent regional groups, removing one dataset characterized by exceptional compositional variability. Three datasets (GB_NE, GB_SE and GB_SW) represent Great Britain within the Atlantic biogeographical region (hereafter, *Atlantic*). Five *West Mediterranean* datasets (ES_E, ES_NE1, ES_NE2, ES_S and PT) represent the Ibero-Macronesian ecoregion of the Mediterranean biogeographical region. Three *East Mediterranean* datasets (CY, GR_NW and GR_SE) are from western Balkan ecoregions within the Mediterranean biogeographical region and Cyprus, which had comparable communities (Figure S2c). Three datasets were not assigned to regional groups due to insufficient replication and/or impact gradient length (Figure S1; Table S1). We also analysed four *individual* datasets (CY, ES_NE1, ES_NE2 and ES_S), which represented all datasets with sufficient impact and drying gradients to warrant individual analysis (Feld et al., 2016; Figure S1).

All samples were collected during wet phases using standard quantitative or semi-quantitative methods designed to capture the taxa present in all habitat types (Table S1). We excluded samples collected during non-flowing wet phases because flow cessation alters community composition (Chadd et al., 2017; Appendix S1.1). We retained flowing-phase samples from all months to characterize responses to variability in environmental conditions across time, and explored seasonal differences in supporting analyses

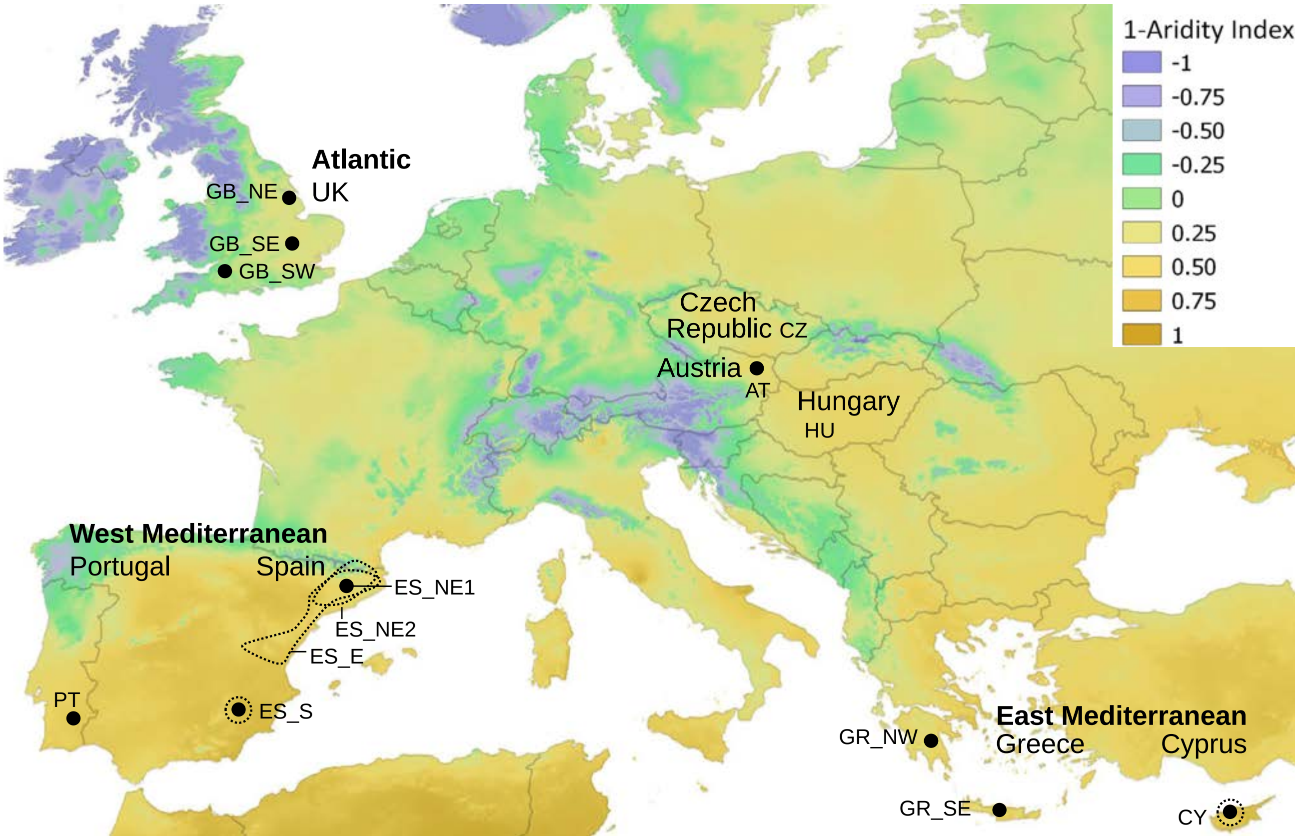

FIGURE 2 Study areas characterized by the 14 datasets used in all-region analyses, three regional analyses (bold text) and four individual analyses (dotted lines); freeform lines enclose larger study areas; CZ and HU datasets are nationwide. Datasets are labelled using ISO two-letter country codes, with abbreviated cardinal points describing the location relative to other national datasets (Table S1). 1–Aridity index is the inverted ratio of precipitation to potential evapotranspiration; values increase with aridity

(Appendix S1.2). We retained samples from perennial sites to encompass a full drying frequency gradient (0–1), and validated this approach in supporting analyses (Appendix S1.3).

We harmonized biological datasets to family level, which all datasets achieved for most taxa. We excluded Oligochaeta, which were typically identified to this coarser resolution, and excluded meiofauna and semi-aquatic taxa, which were recorded inconsistently. Family-level data can effectively characterize taxonomic and functional responses to human impacts and drying (Datry et al., 2014; Gayraud et al., 2003), and we validated this approach by comparison with genus-level analyses (Appendix S1.4). We converted abundance data to presence–absence, to facilitate comparison of samples collected using different field methods (Gayraud et al., 2003; Table S1), and validated this approach in supporting analyses (Appendix S1.5).

2.2 | Characterization of human impact, river drying and climatic gradients

To provide a broad measure summarizing overall site-specific human impact levels (i.e. general degradation, sensu Poikane et al., 2020), we adapted criteria proposed by Sánchez-Montoya et al. (2009). We calculated the number of impacts per site from a maximum of 21 human influences relating to riparian vegetation, invasive species, pollution, land use, river morphology and hydrological alteration (Table S2), then converted numbers to proportions, where 0 indicates unimpacted sites. We described site-specific flow permanence using three candidate variables, with field observations confirming that no-flow conditions equated to a dry bed with or without disconnected pools (Table S3). We used four candidate variables to describe climate (Table S3), including an aridity index (AI, i.e. precipitation/potential evapotranspiration). AI was calculated using WorldClim data (http://www.worldclim.org) then inverted (1–AI) so that values increase with aridity.

2.3 | Functional trait assignment

To identify traits promoting resistance and/or resilience to drying, we considered biological response traits in Tachet et al. (2010), plus female wing size and type, which describe the dispersal potential of aerial adult insects which have aquatic juveniles (Sarremejane et al., 2020). Informed by literature and expert opinion, we selected 22 trait categories across nine traits as responsive to drying

(Tables S4–S5). We weighted each category from 1 to 4, with higher scores indicating greater resistance and/or resilience to drying (Table S4). For each genus, traits were coded using a fuzzy approach (Chevenet et al., 1994), in which affinities between 0 (no affinity) and 4 (strong affinity) were assigned to each trait category. Affinities were then converted to percentage affinities within each trait and averaged across all genera with each family. We calculated family-specific resistance/resilience (RR) scores as the sum of each trait affinity multiplied by the corresponding trait category weight (Table S4). Family scores were then ranked to identify the high RR group for each dataset, comprising its top 33% of families (Table S6).

2.4 | Calculation of biological response metrics

We used five metrics to characterize the responses of the whole community and the high RR group. For both groups, we calculated three family-level taxonomic metrics: richness (*FamRich*) and two biomonitoring indices of ecological status. In all-region models, we used the Whalley, Hawkes, Paisley and Trigg (WHPT) and WHPT-ASPT indices (Paisley et al., 2014). WHPT updates the widely used BMWP index (Armitage et al., 1983), which was developed for UK rivers and has been demonstrated as responsive to environmental degradation across and beyond Europe (e.g. Mustow, 2002). We validated its capacity to represent all-region responses in supporting analyses (Appendix S1.6). We used region-specific indices in regional and individual models, that is, WHPT in the Atlantic region, the Iberian BMWP (IBMWP; Alba-Tercedor et al., 2002) in the West Mediterranean region and STAR-ICMi (Buffagni et al., 2006) in the East Mediterranean region.

We calculated two functional metrics, redundancy (*FuncRed*) and richness (*FuncRich*), as described in Appendix S2. In brief, FuncRed was calculated as the difference between taxonomic diversity and functional diversity and represents the extent to which an assemblage is 'saturated' by taxa with comparable traits (de Bello et al., 2007). FuncRich was calculated as the multidimensional trait space representing each assemblage (Villéger et al., 2008). We also calculated functional metrics and taxonomic richness at genus level for selected datasets (Atlantic, ES_E, ES_S), and calculated FuncRed and FuncRich at a mixed subfamily level for the all-region dataset (Appendix S1.4).

2.5 | Modelling

We analysed the whole community and the high RR group for each of the eight (one all-region, three regional, four individual) datasets, that is, 16 models (Figure S1). Following Zuur et al. (2010), we used variance inflation factors (VIF) to identify collinearity among candidate predictor variables representing river drying and, in all-region models, climate (Table S3). We retained three predictor variables with VIF <2: aridity (as 1–AI), drying frequency and the proportion of human impacts (Table S3), with their non-collinearity evidencing the limited influence of hydrological alteration on the human impact gradient. We calculated skewness values to assess the distribution of each response variable, then used square-root or log transformations to reduce values >0.5.

We ran linear mixed-effects models to characterize metric responses to the three predictor variables and their pairwise interactions. To account for the non-independence of samples from the same site and dataset, we included *site* nested within *dataset* as random factors in all-region and regional models and *site* as a random factor in individual models. We used a multi-model inference approach to quantify the size and significance of metric responses (Anderson & Burnham, 2002). We assessed model performance using Akaike information criteria (AIC), considered models with a ΔAIC <2 as equally good, and averaged these models (Anderson & Burnham, 2002). We partitioned the variance explained by predictors and their interactions in the top all-region models. We used marginal and conditional goodness-of-fit statistics (R^2_m and R^2_c, respectively) to evaluate model performance (Mac Nally et al., 2018). Independent (single, additive) and interactive (antagonistic, opposing, synergistic) response types were classified using the sign and significance of responses to predictors and their interactions (Feld et al., 2016). We used significance levels of $p < 0.01$ and <0.001 for response variables violating one or both of the assumptions of normality and homoscedasticity, respectively.

Analyses were conducted in R (R Core Team, 2019) using the packages *biomonitoR* (Laini et al., 2020), *lme4* (Bates et al., 2015), *MuMIn* (Bartoń, 2019), *usdm* (Naimi et al., 2014) and *variancePartition* (Hoffman & Schadt, 2016). Our study did not require ethical approval.

3 | RESULTS

The environmental and biological characteristics of each dataset are described in Appendix S3 and Table S7.

3.1 | Community responses to human impacts and river drying

In the all-region model, all community metrics decreased independently in response to both human impacts and drying (i.e. additive responses; Figure 3; Table 1h). The proportion of variance explained by impacts was highest for the two biomonitoring indices (4.0%–4.6%) and particularly low for functional redundancy (<1%; Table 1a; Table S15). Plotted slopes indicated the greater independence of WHPT-ASPT compared to the WHPT index total (Figure 3b,c), and effect sizes (Table 1b,c) and explained variance (Table S15c,d) were higher for impacts compared to drying for WHPT-ASPT but not the WHPT total. Responses were largely comparable at family and mixed subfamily taxonomic levels (Appendix S1.4).

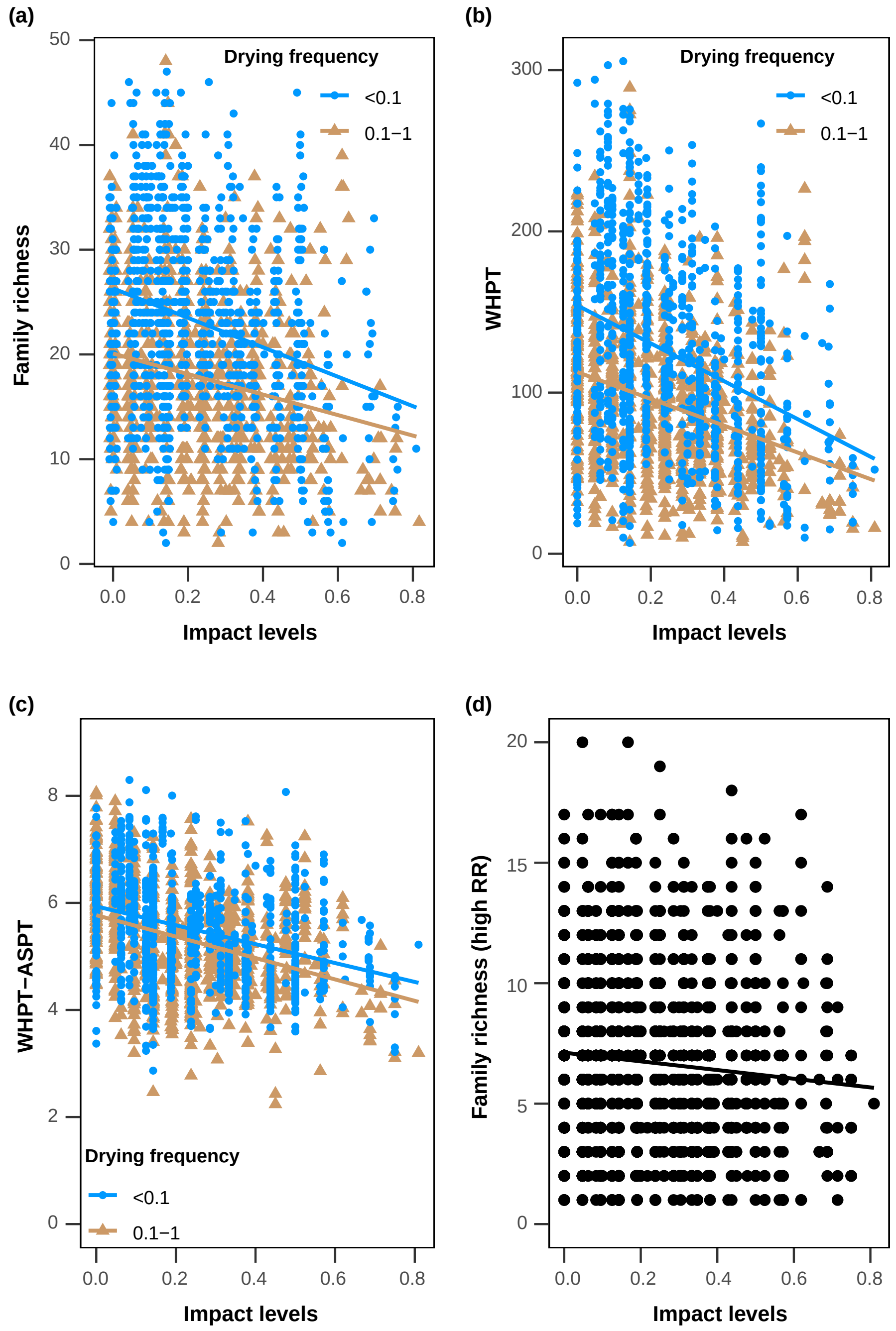

FIGURE 3 Metric responses to human impact levels: community (a) family-level taxonomic richness (FamRich); (b) WHPT; and (c) WHPT-ASPT at high and low drying frequencies; (d) high RR FamRich

Of the regional models, the two Mediterranean models included most independent (i.e. single or additive) responses (Tables S9e–S10e): all community metrics decreased with impacts, and most variance was explained for biomonitoring indices (Tables S9a–S10a). In the Atlantic region, FamRich and the WHPT total experienced antagonistic declines in response to impacts and drying (Table S8), and responses were largely comparable at family and genus levels (Table S16a). Of the individual models (Tables S11–S14), a particularly high proportion of variance (34%–52%) was explained for taxonomic metrics in the ES_S model, in which FamRich declined only in response to impacts (i.e. a single response), and IASPT and IBMWP both decreased independently with impacts and drying (i.e. additive responses; Table S11). Genus- and family-level responses were comparable in both the ES_E and ES_S models (Table S16a). Response types for all models are summarized in Table S17.

3.2 | High RR responses to human impacts and river drying

In the all-region high RR model, FamRich decreased only in response to human impacts (i.e. a single response; Figure 3d; Table S18h), and both biomonitoring indices declined independently in response to

TABLE 1 Linear mixed-effects model results: (a) goodness-of-fit (marginal R^2, R^2_m; conditional R^2, R^2_c); (b–g) significance (*p*) and effect size (ES) of (b) human impacts, (c) river drying, (d) aridity and (e–g) their interactions for response metrics summarizing the all-region community dataset (*n* = 1,763); and (h–j) the independent (additive) and interactive (antagonistic, synergistic) response types

				Individual effects						Interactions						Response types		
	Intercept ($p < 0.001$)	(a) Goodness-of-fit		(b) Impacts		(c) Drying		(d) Aridity		(e) Impacts × drying		(f) Aridity × impacts		(g) Aridity × drying		(h) Impacts and drying	(i) Aridity and impacts	(j) Aridity and drying
Response metric	ES	R^2_m	R^2_c	*p*	ES	*p*	ES	*p*	ES	*p*	ES	*p*	ES	*p*	ES			
Family richness	19.8	0.236	0.795	<0.001	−2.02	<0.001	−2.63	<0.001	−3.30	0.385	0.214	0.051	−0.712	<0.001	1.25	Additive	Additive	Antagonistic
Functional redundancy	0.296	0.190	0.566	<0.001	−0.005	0.008	−0.004	<0.001	−0.019	0.628	−0.001	0.388	−0.002	<0.001	0.006	Additive	Additive	Antagonistic
Functional richness	0.247	0.103	0.677	<0.001	−0.024	<0.001	−0.018	0.006	−0.031	0.069	0.007	0.026	−0.013	0.095	0.007	Additive	Synergistic	Additive
WHPT	10.1	0.339	0.869	<0.001	−0.778	<0.001	−0.866	<0.001	−1.66	0.596	0.041	0.040	−0.234	<0.001	0.417	Additive	Synergistic	Antagonistic
WHPT-ASPT	5.59	0.370	0.913	<0.001	−0.283	<0.001	−0.240	<0.001	−0.765	0.175	−0.034	0.666	−0.017	0.126	0.042	Additive	Additive	Additive

impacts and drying (i.e. additive responses; Table S18h). The proportion of variance explained by impacts was very low, exceeding 1.0% only for WHPT and WHPT-ASPT (Table S15). In all regional and individual models, most variance in impact-responsive high RR metrics was explained for biomonitoring indices (Tables S8a–S14a). Genus-level analysis of the ES_S high RR assemblage increased the response strength (R^2_m) of all metrics, and produced a single impact-driven decline in FuncRed (Table S16).

3.3 | Effects of aridity on all-region responses to impacts and drying

In the all-region community model, all metrics declined with aridity (Table 1d), which explained between 4.9% (FuncRich) and 30% (WHPT-ASPT) of variance in metric responses (Table S15). Impact- and aridity-driven decreases in FamRich and WHPT-ASPT were independent (i.e. additive responses; Figure 4a), whereas an impact-related decrease in the WHPT total increased with aridity (i.e. a synergistic response, Table 1i), but this interaction explained negligible variance (Table S15a). In contrast, aridity reduced the strength of some drying-driven decreases (i.e. antagonistic responses; Table 1j), reflecting metric stability at arid sites (Figure 4b). In the all-region high RR model, interactions between impacts and aridity explained negligible variance (Table S15), whereas interactions between aridity and drying frequencies were antagonistic for FamRich and WHPT (Table S18i,j). WHPT-ASPT was the only metric for which decreases in response to aridity, impacts and drying did not interact, in either the community or the high RR model (Tables 1h–j and S18h–j).

4 | DISCUSSION

Taxon absences caused by natural disturbances can compromise interpretation of biomonitoring data collected to inform management actions that protect biodiversity. This challenge is particularly pronounced in dynamic ecosystems including temporary rivers, which fluctuate between wet and dry states. Despite this dynamism, we identified metrics representing invertebrate communities that had independent, negative responses to drying and human impacts, and these responses were strongest for biomonitoring indices. However, limited variance was explained in our Europe-wide analyses, likely reflecting context-dependent metric responses to environmental variability among rivers. In addition, the taxonomic richness of families with traits promoting resistance and/or resilience to drying (our 'high RR' group) responded only to impacts—not drying—but this response was weak, due to low taxonomic richness. As rivers experience increasing climate-driven drying (Tramblay et al., 2021), our results highlight the need to develop region-specific indices for use in ecological status assessments. By identifying priority sites for further investigation, such assessments can inform management actions that support biodiversity within dynamic river ecosystems.

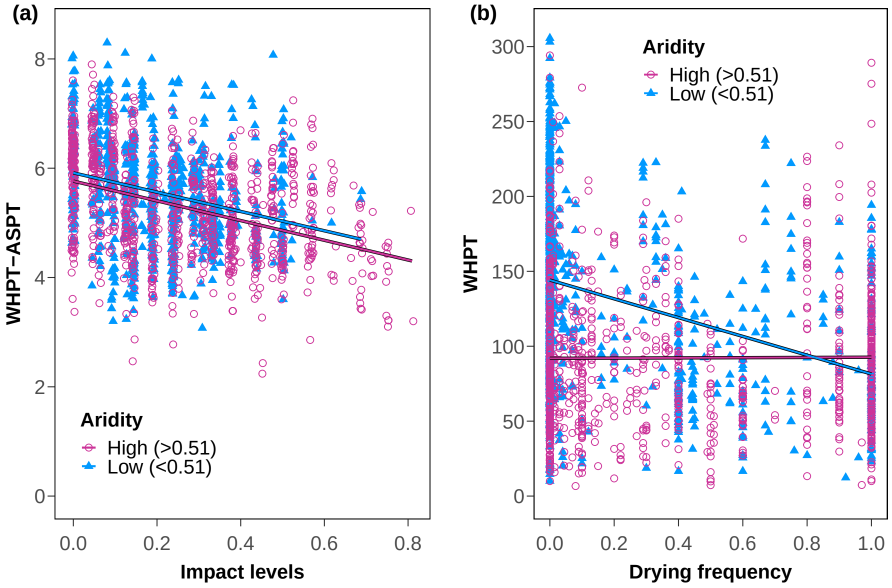

FIGURE 4 Community metric responses in relation to aridity: (a) WHPT-ASPT response to human impacts and (b) WHPT response to drying frequency

4.1 | Community metrics decreased in response to human impacts and river drying

Our first hypothesis was that richness-based metrics including biomonitoring index totals would experience antagonistic declines in response to increasing human impacts and drying frequencies, because drying-driven reductions in impact-sensitive taxa can limit community responses to impacts (Datry et al., 2014; Gutiérrez-Cánovas et al., 2015). In contrast to this hypothesis, all community-based metrics experienced independent, additive declines with impacts and drying in the all-region model. As such, we found limited support for our second hypothesis, that decreases would be less pronounced for richness-independent ASPT indices, although in the all-region model, impacts explained more variance than drying in WHPT-ASPT but not the index total. ASPT indices assess assemblages based on the impact sensitivities of their constituent families independently of taxonomic richness, thus facilitating impact discrimination in both temporary rivers (Wilding et al., 2018) and perennial rivers that experience seasonal flow variability (Álvarez-Cabria et al., 2010).

Metrics with additive responses can be used in ecological status assessments if values are adapted to recognize the communities expected at unimpacted sites exposed to a natural stressor, in our case, drying (Soria et al., 2020). Identifying taxa which are associated with perennial flow—and which are therefore absent from temporary rivers regardless of their impact levels (Chadd et al., 2017)—could guide expectations of community composition and thus interpretation of metrics such as ASPT indices. However, ASPT indices can overestimate ecological status if fewer taxa than expected contribute to index values (Clarke et al., 2003). An ASPT should thus be considered alongside a measure of taxonomic richness—or potentially alongside a functional metric such as redundancy, which, despite our third hypothesis, responded to both impacts and drying in this study. As such, we recommend the development of richness metrics based on taxa representative of specific river types, a process which could be enabled by our high RR group—and also by genus- or species-level characterization, to recognize within-family variability in responses to both impacts and drying (see Section 4.2; Bonada et al., 2004; Chadd et al., 2017).

The limited variance in biological response metrics explained by human impacts and drying in the all-region models likely reflects variability introduced by the spatiotemporal breadth of our pan-European analysis. Accordingly, our goodness-of-fit statistics (and specifically, conditional R^2) indicate that site-specific habitat conditions explained considerable variance in metric responses. Furthermore, we analysed uncalibrated biomonitoring indices, which failed to represent variability among the communities expected in different river types (Clarke et al., 2003). Index calibration was not possible because only one (Mediterranean) temporary river type is officially classified in European biomonitoring (van de Bund, 2009). This situation overlooks the considerable environmental and biological variability among temporary river types and hampers characterization of distinctive, type-specific communities indicative of unimpacted reference conditions and of deviations from such conditions (Cid et al., 2020; Stubbington et al., 2018). Characterization of distinctive regional river types and their associated communities is a priority to underpin improvements in temporary river biomonitoring and management (Clarke et al., 2003; Stubbington et al., 2018). In addition, our capacity to detect biological responses to impacts was hampered by a short impact gradient in the Atlantic region (Feld et al., 2016), highlighting the need to collect data representing the full range of impact levels experienced across the breadth of European temporary rivers.

4.2 | Resistant and resilient taxa responded mainly, but weakly, to impacts

Our fourth hypothesis, that metrics characterizing assemblages of taxa with traits promoting resistance and/or resilience to drying would respond only to human impacts, was partly supported. First, in all models in which high RR taxonomic richness responded significantly to impacts, it decreased only in response to increasing impacts—not drying. However, such responses reflected only West Mediterranean assemblages, in which taxonomic richness was relatively high, reflecting the greater range of adapted taxa that occur in temporary rivers in which disconnected pools persist during seasonal dry phases (Bonada et al., 2007). In addition, impacts consistently explained little variance in metrics representing the high RR group, likely because—for metrics including WHPT-ASPT—values varied little among some taxon-poor assemblages. Ensuring representation of sufficient taxa with sufficiently variable impact sensitivities (Hering et al., 2006) within high RR groups defined for specific regional river types (van de Bund, 2009) could thus strengthen metric responsiveness, using the approaches outlined below.

First, to improve representation of impact-sensitive high RR taxa within region-specific groups, temporary river specialists could be better represented. For example, the traits conferring resistance to drying on specialist insect species associated with small, lowland, temporary rivers (Armitage & Bass, 2013) in our Atlantic dataset were obscured by the traits of dominant generalists in our family-level assignment. As also indicated by our genus-level ES_S analyses, such examples highlight that finer-resolution taxonomic identification can enhance characterization of biological responses to natural stressors (Chadd et al., 2017; England et al., 2019), and thus the selection of taxa to include in high RR metrics. In addition, although beyond our scope, novel metrics based on the abundance of specialist taxa may be more responsive to impact levels than presence–absence-based metrics (Gutiérrez-Cánovas et al., 2019).

Second, representation of high RR families—and genera and species—within certain high-potential taxonomic groups could be expanded. For example, human impact detection by indices developed for small temporary Mediterranean rivers is enhanced by inclusion of true fly and beetle families alongside selected mayflies, stoneflies and caddisflies (Munné & Prat, 2009). True flies, which dominated our high RR group, have diverse environmental preferences (Paisley et al., 2014) and can increase in relative abundance with drying duration in temporary rivers (Datry et al., 2014), due to their prevalent resistance traits (Tachet et al., 2010). We also identified beetles and true bugs as common high RR families, reflecting resilience traits including strong aerial dispersal (Sarremejane et al., 2020). In particular, the single, strong responses to human impacts identified for the beetle and bug-rich ES_S dataset from semi-arid Spain evidences the potential use of these taxa in temporary river biomonitoring (Bilton et al., 2006). However, metric performance is constrained by representation of sufficient taxa with sufficiently variable sensitivities to impacts, restricting the potential of these groups to regions with adequate taxonomic richness (Gutiérrez-Cánovas et al., 2019).

Third, the performance of high RR metrics could be improved by recognizing that traits conferring resistance and/or resilience to drying vary among river types. For example, the 'interstitial' 'substrate relation' trait (which reflects use of wet subsurface sediments; Tachet et al., 2010) only promotes persistence at sites with permeable sediments; the resilience conferred by dispersal traits depends on site-specific connectivity to colonist sources (Cid et al., 2020); and traits may encompass irrelevant subtypes such as resistance forms that confer cold tolerance (Ditrich & Papáček, 2009).

Finally, representation of high RR taxa could be maximized by using field methods designed to promote consistent, comprehensive sampling of temporary river taxa, including those inhabiting marginal habitats (England et al., 2019). Collectively, these recommendations could enable characterization of taxonomic assemblages specific to distinctive river types, informing the development of richness metrics that enhance ecological status assessments in temporary rivers.

5 | CONCLUSIONS

We outline principles upon which to develop biomonitoring schemes that recognize variability among river types—and in particular, among highly diverse temporary rivers (Stubbington et al., 2018). Our community-level results suggest that biomonitoring indices can identify responses to human impacts despite concurrent responses to drying, but require adaptation to reflect the values representative of rivers with contrasting flow permanence (Soria et al., 2020). Richness-independent ASPT indices show promise as indicators of impact levels, but considered alone, could overestimate ecological status. An ASPT should thus be considered alongside a metric representing the richness of the assemblages of resistant and/or resilient taxa characteristic of specific regional river types. Our high RR group provides a basis for adaptation and development of such responsive, region-specific richness metrics. Finally, our all-region models identified interactions with aridity and drying that influenced metric responses to impacts (Piggott et al., 2015), suggesting that climate-driven shifts in river flow regimes may concurrently alter ecosystems and our capacity to manage them effectively. Flexible approaches are therefore needed to monitor, manage and protect river ecosystems as they respond to global change.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to report. Thibault Datry is an Associate Editor of the *Journal of Applied Ecology*, but took no part in the peer review and decision-making processes for this paper.

AUTHORS' CONTRIBUTIONS

R.St., R.Sa., N.C., T.D. and A.L. conceived the ideas and designed the methodology; all authors except A.L., C.G.-C. and R.Sa. acquired the data; R.St., R.Sa., A.L., I.T., M.P. and Z.C. analysed the data; R.St. led the manuscript writing; A.L., A.M., C.G.-C., D.B., D.S.-F., N.B., N.C., J.W., P.P., R.Sa., T.A., T.D. and Z.C. contributed to drafts; and all authors gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data are available via the Dryad Digital Repository https://doi.org/10.5061/dryad.08kprr53j (Stubbington et al., 2021).

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SUPPORTING INFORMATION

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Part II: Spatiotemporal variation in aquatic macroinvertebrate distribution driven by a changing climate, anthropogenic activities and taxa dispersal

Paper 15: Pařil, P., Bojková, J., Špaček, J. & Helešic, J. (2008). Ecology of *Leuctra geniculata* (Plecoptera: Leuctridae), an AtlantoMediterranean species on the north–eastern border of its area. Biologia 63, 574–581. DOI: 10.2478/s11756–008–0087–y

Paper 16: Straka, M., Špaček, J., & **Pařil**, P. (2015). First record of the invasive polychaete *Hypania invalida* (Grube, 1960) in the Czech Republic. BioInvasions Records, 4, 87–90. DOI: 10.3391/bir.2015.4.2.03

Paper 17: Schenkova, J., **Pařil**, P., Petřivalská, K., & Bojková, J. (2010). Aquatic oligochaetes (Annelida: Clitellata) of the Czech Republic: check–list, new records, and ecological remarks. Zootaxa, 2676, 29–44. DOI: 10.11646/zootaxa.2676.1.2

Paper 18: Sarremejane, R., Puey, N. C., Datry, T., Stubbington, R., Alp, M., Canedo–Arguelles, Cordero–Rivera A., Csabai Z., Gutiérrez–Cánovas C., Heino J., Forcellini M., Millán A., Paillex A., **Pařil** P., Polášek M., Tierno de Figueroa J. M., Usseglio–Polatera P., Zamora–Muñoz C. & Bonada, N. (2021). DISPERSE, a trait database to assess the dispersal potential of European aquatic macroinvertebrates. Scientific data, 7, 386. DOI: 10.1101/2020.02.21.953737

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Ecology of *Leuctra geniculata* (Plecoptera: Leuctridae), an Atlantomediterranean species on the north-eastern border of its area

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Abstract: The first records of *Leuctra geniculata* Stephens, 1836 in the north-eastern border of its area (the Czech Republic) are presented and an overview of references, synonyms and distribution of the species is given. The ecological preferences of the species, supported by chemical and hydromorphological parameters, are defined. Probable dissemination paths into the Czech Republic and the supposed life cycle of the species are discussed. Photographs of morphological characters, SEM photos of eggs, associated macroinvertebrate assemblages (EPT taxa) and maps of distribution are included.

Key words: Plecoptera; *Leuctra geniculata*; ecology; first records; Czech Republic

Introduction

Some rare stonefly species have been found during detailed monitoring of the ecological quality of streams in the Czech Republic. *Capnopsis schilleri* (Rostock, 1892) (Špaček et al. 1999; Helešic et al. 2001) in S Bohemia and *Agnetina elegantula* (Klapálek, 1905) in E Bohemia (Špaček 1998; Špaček et al. 2003) have been recorded. *Leuctra geniculata* Stephens, 1836, a new species for the fauna of the Czech Republic, has been found within various monitoring programmes (PERLA system monitoring, saprobiological monitoring, water authorities' monitoring).

Leuctra geniculata was originally described in Great Britain (terra typica) by Stephens (1836) as *Nemoura geniculata*. By the designation of the International Commission on Zoological Nomenclature, the genus name was changed to *Leuctra* (Stephens, 1835) in 1968. Illies (1966) established a new monotypic genus *Euleuctra* for the species but Consiglio (1975) synonymized this genus back to *Leuctra*.

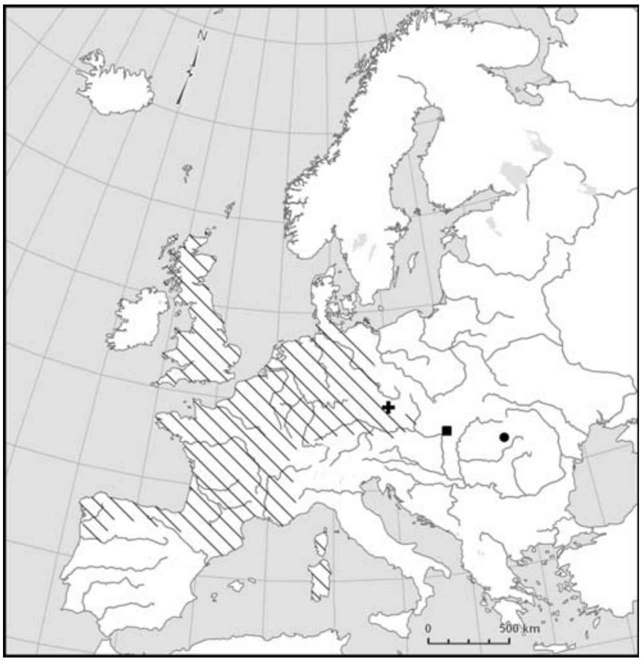

Fig. 1. The occurrence of *Leuctra geniculata* in Europe with marks for findings outside the conjunctive area: + the first record in the Czech Republic in the river Střela, year: 2003; historical findings in ■ Hungary, year: 1914 and ● Transylvania (Romania), year: 1912.

The species was later recorded in the basins of major rivers (the Rhine and its tributaries) in W Europe (Müller-Liebenau 1961; Zwick 1973; Sartori & Ruffieux 1996) and is presently listed in Fauna Europaea (Fochetti & Figueroa 2004) from Spain, Portugal, Switzerland, Austria, Great Britain, Belgium, Luxembourg, Germany, France, Hungary and Romania (records from the last two listed countries are doubtful – see comments given below), although in some countries it was considered extinct, e.g., the Netherlands (Mol 1984). It was also found in Sardinia, Corsica (Consiglio 1975) and N Africa (Aubert 1959). The border of its area lies in the eastern part of the Rhine basin, as well as in the western pre-Alp region (Illies 1955). It was originally classified into the eastern–western group of plecopterans (Illies 1953) and later as an Atlantomediterranean species (Illies 1978). However, there are historical records from Transylvania in Romania (Szilády 1912; Kis 1974) and from the Hungarian plains (Pongrácz 1914). The disjunctive occurrence outside the conjunctive area (Fig. 1) was considered to result from climatic changes after the Pleistocene (Raušer 1962). On the other hand, *L. geniculata* is not mentioned in the checklist of Slovakia (Krno 2003) and the historical records eastwards from Vienna, in Hungary (Pongrácz 1914) and Romania (Kis 1974), were not confirmed by recent records and seem to be doubtful (Raušer 1957; Kis 1974; Illies 1978). In the last few years, Graf (1999) recorded this species in Lower and Upper Austria, and Reusch & Weinzierl (1999) listed it from Germany (Baden-Württemberg, Bavaria, the Rhine basin, the North Rhine-Westfalia region). The Isar River in Munich (Bavaria, Germany) is one of its well-documented sites close to the Czech Republic (Dorn & Weinzierl 1999). Although Raušer (1980) did not specify any site, he anticipated the occurrence of the species on the territory of the Czech Republic. However, a later detailed stonefly monitoring programme between 1994 and 1996 at 149 sites evenly distributed throughout the whole country did not confirm its occurrence (Soldán et al. 1998).

Larvae of *L. geniculata* prefer greater lowland streams and rivers with slower current and a cobble-gravel bottom from hyporhitral to epipotamal zones (Hynes 1941; Berthélemy 1966; Illies 1978; Grauvogl 1992). They were found predominantly at altitudes up to 500 m a.s.l., despite Aubert (1959) mentioning a maximum altitude of 1,000 m a.s.l. or Despax (1951) and Illies (1955) even as high as 1,500 m a.s.l.; e.g., in Sardinia it lives in small springs (Consiglio 1975). Small specimens live among stones and gravel while bigger larvae (over 6 mm) are able to burrow into the sediment (Hynes 1941). According to Elliott (1987), *L. geniculata* is clearly univoltine. Studies by Hynes (1941) and Neveu et al. (1979) have also shown this species to be univoltine, although Hynes (1977) supposed a two-year cycle. The eggs, representing an overwintering stage, hatch in early spring and the last instars occur from June to July. Adult emergence is known to take place from August to November, depending on the region and altitude. Imagos are sporadically found in April and May (Hynes 1977), which may support the possibility of a two-year cycle. According to Graf et al. (2002), the larvae are very probably detritivorous shredders and grazers with a higher preference for algae than other species of the *Leuctra* genus (Hynes 1941).

This paper presents new information on ecological preferences, probable ways of dissemination, associated macroinvertebrate assemblages (Ephemeroptera, Plecoptera and Trichoptera) and the supposed life cycle of *L. geniculata* on the north-eastern border of its conjunctive area.

Material and methods

The larvae of *L. geniculata* were collected using the PERLA method (Kokeš et al. 2006), i.e., semiquantitative three minute multihabitat kick sampling with use of a pond net (500 μm mesh size) followed by sorting of the material in laboratory. The rest of samples was taken by the qualitative saprobiological method, i.e., predominant sampling in riffle stretches with only sorting of alive material in the field. Specimens' lengths, maturation stages, dates of records, site characteristics and used sampling methods are presented in Table 1.

Scanning electron microscope photos were taken by SEM JEOL JSM-6300 (critical point dried, gold-coated).

Results and discussion

Morphology

The species is classified into the *L. geniculata* group (Aubert 1954, 1959; Raušer 1962), which is presently considered monotypic (Ravizza 2002). The male of this group is characterized by a single sclerotized process on tergum VI and larvae are, analogously to the *L. braueri* group, characterized by antennae with a whorl of hairs around the apex of each antennal segment (Ravizza 2002). Critical distinguishing characters of *L. geniculata* larvae are as follows: broad, densely haired and with a flattened head (equal in width or wider than the thorax), with pronounced outgrowths on the basal antennal segments 5–18 (Fig. 2) and sparsely haired wing pads. The legs and cerci are densely haired (Fig. 2). The last instar larvae are robust, 8 to 11 mm in body length, strongly haired with a grey-brown colour pattern (Hynes 1977; Raušer 1980; Elliott 1987). Due to the different habitus (robustness, dense hairs) and antennal projections, later larval stages (over 4 mm) are easily distinguishable and confusion with other *Leuctra* species is not likely. Scanning electron microscope photos of ovarioles and the egg capsule with the external chorionic sculpture of *L. geniculata* are presented in Fig. 3. López-Rodríguez et al. (2004) published only optic microscope photos of *L. geniculata* eggs and Ubero-Pascal et al. (2001) took SEM photos of selected chorionic sculptures but did not find specific attachment structures enabling them to be easily distinguished from the eggs of the other species.

Description and ecological characteristics of the sites in the Czech Republic

The first record (2003) on the territory of the Czech Republic is from the Střela River (Fig. 4), a left-side tributary of the Berounka River downstream from the city of Plzeň (Pilsen). This earliest seasonal record dates from 27 May when six larvae were found downstream of the weir in the village of Nebřeziny, in a relatively undisturbed stretch of the river (see Table 1 for the site characteristics). More than 30 taxa of macroinvertebrates with a rich EPT (Ephemeroptera, Plecoptera, Trichoptera) fauna (14 taxa) were detected at this site in 2003 (Table 2), including species preferring the hyporhitral zone, such as *Isoperla oxylepis,* or species preferring the epipotamal zone (*Perla burmeisteriana*, *Heptagenia sulphurea*, *Potamanthus luteus*). On 4 September 2004, one female was found at the same site, together with

Table 1. Characteristics of *Leuctra geniculata* finding sites in the Czech Republic ("/" = no data).

Finding site (no.)	1		2		3	4	5	6	7	Total	
Water course	Střela River		Malše River		Zbirožský Brook	Ohře River	Libocký Brook	Ohře River	Labe River		
Site	Nebřeziny		Roudné		Čilá	Louny	Libočany	Tvršice	Děčín		
River basin	Berounka		Vltava		Berounka	Ohře	Ohře	Ohře	Labe		
Latitude (N)	49°55′14″		48°56′01″		49°56′19″	50°21′28″	50°19′57″	50°20′26″	50°46′48″		
Longitude (E)	13°24′58″		14°28′59″		13°44′29″	13°48′13″	13°30′50″	13°35′11″	14°12′28″		
Collected	Horák	Špaček	Lanková	Potužák	Pařil	Skála	Skála	Skála	Špaček		
Determination	Pařil	Špaček	Pařil	Kolářová	Špaček	Skála	Skála	Skála	Špaček		
	2003	2004	2004	2005	2004	2004	2004	2004	2005		
Abiotic parameters										Min.	Max.
Altitude (m a.s.l.)	318		390		325	174	208	194	124	124	390
Strahler order	5		6		5	6	5	6	8	5	8
Q_a ($m^3\ s^{-1}$)	2.9		6.9		0.6	35.7	1.7	/	309.0	0.6	309.0
Average stream depth (m)	0.4		0.3		0.2	0.4	0.3	0.5	1.5	0.2	1.5
Average stream width (m)	14		19		6.5	16	5.5	20	75	5.5	75
Chemical parameters										Min.	Max.
pH											
Annual average	/	8.1	7.6	7.6	8.2	7.8	8.2	7.3	7.6		
Min.–max.	/	7.6–8.8	7.3–7.9	7.4–7.7	8.1–8.3	7.6–8.1	7.5–8.5	7.0–7.6	7.4–9.0	7.0	9.0
Conductivity (mS m^{-1})											
Annual average	/	41	17	16	44	51	66	42	37		
Min.–max.	/	26–55	15–20	13–19	41–48	42–57	36–91	35–52	29–43	15	91
Water temperature (℃)											
Annual average	/	8.4	8.0	/	/	10.6	9.5	8.9	12.8		
Max.	/	20.0	16.6	/	16.9	16.4	21.6	16.9	22.7	/	22.7
BOD (mg L^{-1})											
Annual average	/	2.2	2.4	2.7	/	2.1	3.4	1.7	3.6		
Min.–max.	/	1.1–5.0	1.3–5.1	1.3–5.3	/	1.2–3.8	1.5–13.0	1.2–2.3	1.8–6.7	1.1	13.0
Dissolved oxygen (mg L^{-1})											
Annual average	/	11.9	11.5	11.2	/	/	/	12.0	10.8		
Min.–max.	/	8.6–14.4	14.0–9.4	8.7–13.4	8.2–11.4	/	/	9.6–13.7	8.0–12.0	8.0	14.4
Biological characteristics										Min.	Max.
Sampling method	saprobiological method			PERLA	PERLA	saprobiological method		saprobiological method			
Saprobic Index (Czech Nat. St.)	2.0	2.0	1.8	1.7	2.0	1.9	1.7	1.9	2.1	1.7	2.1
Number of EPT taxa	14	16	14	37	23	7	10	9	15		
Total number of taxa	30	33	24	77	58	21	19	30	/		
Specimens' characteristics											
Year	2003	2004	2004	2005	2004	2004	2004	2005	2005		
Date of record	27 May	4 Sept.	19 July	12 Sept.	27 June	19 August	20 August	15 Sept.	20 July		
Number of specimens	6	1♀	1	1	1	1	1	2	3		
Length of specimens (mm)	4.2–5.1	11.7	4.6	8.5	8.3	9.5	13.0	7.5, 9.0	9.8, 9.9, 10.3		
Development of wing pads	5 sps. none 6th insinuated		none	develop.	short	short	developed	1st short 2nd develop.	all short		

33 taxa of other macroinvertebrate larvae, including 16 EPT species.

The easternmost site within the Czech Republic (390 m a.s.l.) is the epipotamal stretch of the Malše River in the village of Roudné (Fig. 4). Twenty-four additional taxa, including fourteen EPTs (e.g., potamal species *Heptagenia sulphurea* and *Oligoneuriella rhenana*) were found in mid-July 2004. The following year, the occurrence of *L. geniculata* was confirmed there by the more detailed PERLA method and a very rich EPT community was detected as well (37 EPT taxa including potamal species, such as *Perla burmeisteriana*, *Caenis luctuosa*, *Ecdyonurus insignis* and *Potamanthus luteus*).

In the Zbirožský potok brook (Fig. 4), a left tributary of the Berounka River, 4 km upstream from the village of Čilá, 57 taxa coexist with *L. geniculata* (23 EPT taxa). However, only *Perla burmeisteriana* was recorded amongst the typical epipotamal EPT taxa. This brook differs from other sites (Table 2) because it belongs to a metarhitral/hyporhitral transition zone. Only 4.5 km downstream, it joins the Berounka River, which has an epipotamal character; therefore, an upstream migration of *L. geniculata* is likely.

In the north-western part of the Czech Republic, close to the border with Germany, three sites with *L. geniculata* were found (20 kilometres from each other). Two sites are situated on the epipotamal stretch of the

Table 2. Ephemeroptera, Plecoptera and Trichoptera (EPT taxa) collected together with *Leuctra geniculata* ("+" = presence of the species).

Finding sites/Number of site	1		2		3	4	5	6	7
Water course	Střela		Malše		Zbirožský Brook	Ohře	Libocký Brook	Ohře	Labe
Site	Nebřeziny		Roudné		Čilá	Louny	Libočany	Tvršice	Děčín
Year	2003	2004	2004	2005	2004	2004	2004	2005	2005
Ephemeroptera									
Baetis buceratus Eaton, 1870		+							
Baetis fuscatus (L., 1761)	+	+	+	+	+	+	+	+	+
Baetis lutheri Müller-Liebenau, 1967				+			+		
Baetis muticus (L., 1758)					+				
Baetis rhodani (Pictet, 1843–1845)	+	+	+	+			+	+	+
Baetis scambus Eaton, 1870	+		+	+					
Baetis cf. *vardarensis* Ikonomov, 1962								+	
Baetis vernus Curtis, 1834	+			+				+	
Caenis luctuosa (Burmeister, 1839)				+					+
Caenis macrura Stephens, 1835					+	+			
Caenis pseudorivulorum Keffermüller, 1960		+				+			
Centroptilum luteolum (Müller, 1776)				+	+				
Ecdyonurus insignis (Eaton, 1870)			+	+					
Ecdyonurus torrentis Kimmins, 1942				+					
Ephemera danica Müller,1764				+	+				
Ephemerella ignita (Poda, 1761)	+	+	+	+	+	+		+	+
Habrophlebia lauta Eaton, 1884					+				
Heptagenia coerulans Rostock, 1877	+								+
Heptagenia sp. juv.									
Heptagenia sulphurea (Müller, 1776)	+		+						+
Oligoneuriella rhenana (Imhoff, 1852)			+						+
Paraleptophlebia sp.				+					
Potamanthus luteus (L., 1767)		+		+		+			+
Rhithrogena semicolorata (Curtis, 1834)				+					
Plecoptera									
Isoperla oxylepis (Despax, 1936)	+								
Isoperla sp.				+					
Leuctra albida Kempny, 1899			+		+				
Leuctra fusca (L., 1758)		+		+					
Leuctra sp. juv.	+						+		
Nemoura sp.				+					
Perla burmeisteriana Claassen, 1836	+		+	+	+				
Trichoptera									
Anabolia furcata Brauer, 1857					+				
Annitella obscurata (McLachlan, 1876)					+				
Athripsodes cinereus (Curtis, 1834)				+					+
Brachycentrus subnubilus Curtis, 1834			+	+					
Ceraclea annulicornis (Stephens, 1836)				+	+				
Ceraclea dissimilis (Stephens, 1836)									+
Halesus sp.					+		+		+
Hydropsyche bulbifera McLachlan, 1878							+		
Hydropsyche contubernalis McLachlan, 1865									+
Hydropsyche incognita Pitsch, 1993			+	+				+	+
Hydropsyche instabilis (Curtis, 1834)	+	+							
Hydropsyche pellucidula (Curtis, 1834)	+	+		+	+		+		+
Hydropsyche siltalai Döhler, 1963	+			+					
Hydropsyche sp.			+	+	+				
Hydroptila sp.		+						+	
Chaetopteryx major McLachlan, 1876				+					
Chaetopteryx sp.			+						
Chaetopteryx villosa (F., 1798)				+	+				
Cheumatopsyche lepida (Pictet, 1834)		+		+					
Lepidostoma hirtum (F., 1775)				+	+				
Lype reducta (Hagen, 1868)					+				
Mystacides azurea (L., 1761)				+	+	+			
Mystacides longicornis (L., 1758)		+							
Notidobia ciliaris (L., 1761)				+					
Odontocerum albicorne (Scopoli, 1763)					+				
Oecetis furva (Rambur, 1842)				+					
Polycentropus flavomaculatus (Pictet, 1834)		+		+	+	+	+	+	
Psychomyia pusilla (F., 1781)		+		+			+		
Rhyacophila dorsalis (Curtis, 1834)			+	+					

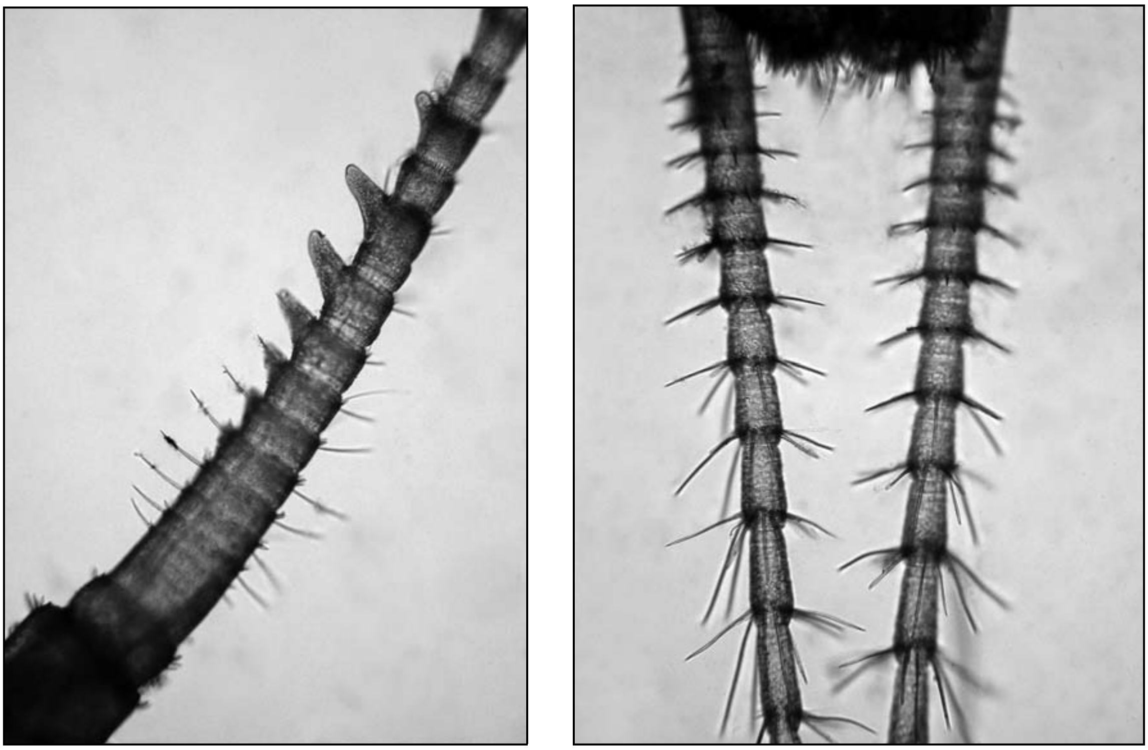

Fig. 2. Basal antennal segment (left) and cerci (right) of a *Leuctra geniculata* larva (river Střela in Nebřeziny, 27 May 2003).

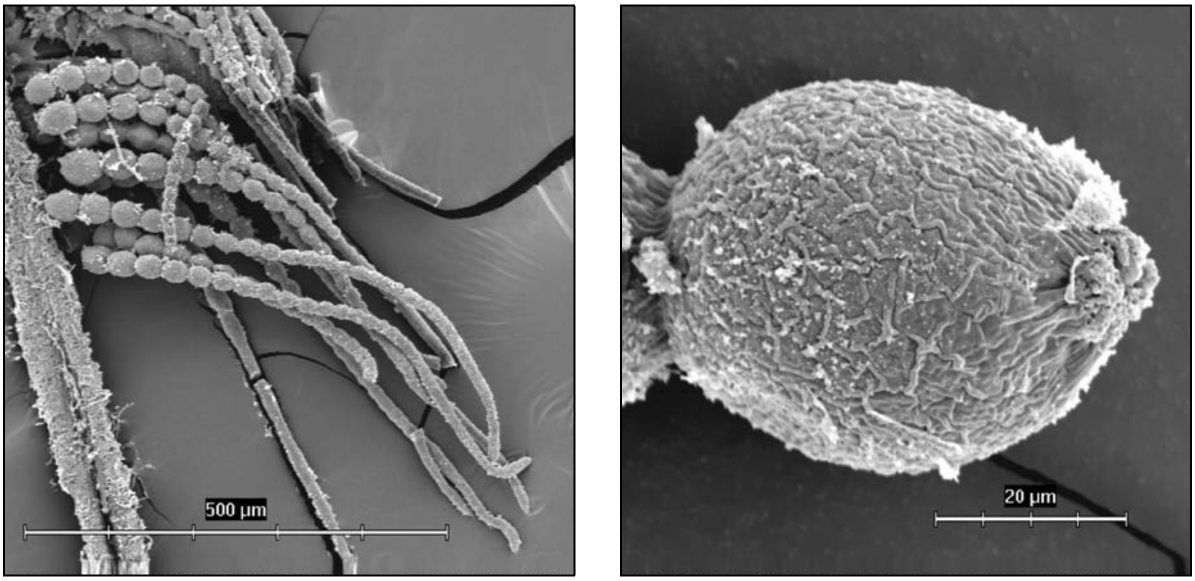

Fig. 3. Scanning electron microscope photos of ovarioles attached to the lateral oviduct (left) and single egg capsule with external chorionic sculpture (right) of *Leuctra geniculata* (river Střela in Nebřeziny, 4 September 2004).

Ohře River near the town of Louny, and the third (Libocký potok brook) on its left tributary, 300 m upstream from the Ohře River main channel (Fig. 4). The latest date of larval capture was 15 September 2005, near the village of Tvršice. The number of species identified at the three sites in the Ohře River basin varied between 19 and 30 taxa, or 7 and 10 EPT taxa, respectively (the lower number of taxa is the result of a rough saprobiological sampling method). Species with potamal preferences, *Potamanthus luteus* and *Baetis* cf. *vardarensis* were recorded in the Ohře River.

The last site (Bojková & Špaček 2006) is located only 10 km upstream from the German border in the Labe (Elbe) River, in the town of Děčín (Fig. 4), with the lowest altitude (124 m a.s.l.). This stretch is at the lower end of an epipotamal segment of the Labe River. For the details about the finding sites, see Table 1.

Notes on the ecology of the species in the Czech Republic

Habitats of *L. geniculata* in the Czech Republic are situated in the western and southern parts of the Bo-

Table 2. (continued)

Finding sites/Number of site	1		2		3	4	5	6	7
Water course	Střela		Malše		Zbirožský Brook	Ohře	Libocký Brook	Ohře	Labe
Site	Nebřeziny		Roudné		Čilá	Louny	Libočany	Tvršice	Děčín
Year	2003	2004	2004	2005	2004	2004	2004	2005	2005
Rhyacophila evoluta McLachlan, 1879		+							
Rhyacophila vulgaris gr.				+					
Rhyacophila nubila (Zetterstedt, 1840)	+				+				+
Rhyacophila sp.		+					+	+	
Sericostoma sp.					+				

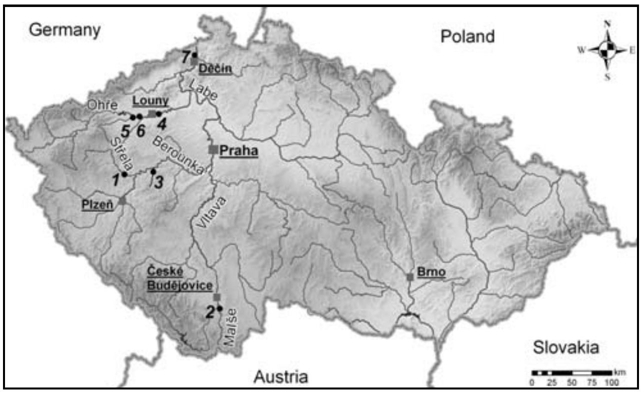

Fig. 4. Map of the Czech Republic with records of *Leuctra geniculata* (●) numbered in order of collection date (1. river Střela in Nebřeziny, 2. river Malše in Roudné, 3. Zbirožský potok Brook near Čilá, 4. river Ohře in Louny, 5. Libocký potok Brook in Libočany, 6. river Ohře in Tvršice, 7. river Labe in Děčín).

hemian massive (Hercynian) in the Labe basin. All sites were expectedly close to the German border (not further than 85 km from the border), considering that the conjunctive area of the species distribution is in western and southern Europe.

For the physiochemical data, see Table 1. The average width of the watercourses at the sites of occurrence varied from 5.5 to 75 m, the average depth from 0.2 m to 1.5 m, the altitude between 124 and 390 m a.s.l. and the average annual discharge Q_{a} varied from 0.6 to 309 m^3. Due to these characteristics, we assume that in the Czech Republic this species prefers mostly lowland and upland mid-sized rivers in stream orders of 5–8, according to Strahler (1957), and can occasionally colonize smaller tributaries. Most of the river stretches consisted predominantly of cobble-gravel-sand substrates and, in some cases, *L. geniculata* inhabited fine deposits near the river bank. The adult female on the Střela River was found in a typical habitat: alder branches hanging down into the stream (López-Rodríguez et al. 2004).

The calculated Saprobic Index of the macroinvertebrate community according to the Czech National Standard (CSN 75 7716 1998) varied at the seven sites from 1.7 to 2.1 and did not fully respond to the individual saprobic valence in the Czech saprobic norm of *L. geniculata* ($S_{\text{valence}} = 1.4$; $I_{\text{weight}} = 3$), but corresponds better with the Austrian saprobic valences ($S_{\text{valence}} = 2.0$; $\mathrm{I}_{\text{weight}}=3$) proposed by Graf et al. (2002).

Because the appropriate chemical parameters are not available for all sites, only incidental data have been used to illustrate species preferences, e.g., besides our data, those published by the Czech Hydrometeorological Institute (CHMI; http://www.chmi.cz/) and the Ministry of Agriculture of the Czech Republic (http://www.voda.mze.cz/). The CHMI data comprise the annual average values (usually one measurement per month throughout a year), as well as the maximum and minimum values of the year when the occurrence of *L. geniculata* was recorded. From the pH range (7.0 to 9.0) it follows that the species prefers neutral or slightly alkaline waters. The maximum summer temperature was observed in the Labe River (22.7°C) and the average annual water temperature at all sites varied from 8.0 to 12.8°C. The Czech maximum temperature corresponds with the maximum temperature of 24°C from Spain (Sánchez-Ortega et al. 2003). Conductivity ranges from 15 to 91 mS m^{-1} and the annual average at one of the sites reached up to 66 mS m^{-1}, which can indicate tolerance of the species to this parameter, often connected with slight or moderate pollution. It also corresponds with BOD$_5$ values that varied from 1.1 to 13 mg L^{-1} and the annual average of the particular site varied in a narrow band from 1.7 to 3.6 mg L^{-1}. In accordance with the Czech National Standard (ČSN 75 7221 1998), the sites were predominantly classified in the better part of the scale from quality class I to III (the norm defines the five classes from the best (I) to the worst (V) in reverse order, in accordance with the Water Framework Directive (European Parliament & Council 2000). The range of O$_2$ concentration from 8.0 to 14.4 mg L^{-1} indicates a higher oxygen demand of the species, which confirms the average O$_2$ saturation at collecting sites from 82 to 102%.

L. geniculata belongs to late summer–autumn species (Despax 1951; Kis 1974; Elliott 1987; Sánchez-Ortega et al. 2003) and, accordingly, early instar larvae (up to 5 mm in body length) without or with small wing pads were found on 27 May and 19 July. The larvae (8.3 to 10.3 mm in body length) in samples from 27 June, 20 July and 19 August had only short wing pads. Records of last larval instars (9 to 13 mm in body length) with well-developed wing pads on 20 August, 12 September and 15 September (the second of two specimens), as well as one adult on 4 September, respectively, can indicate the univoltine life cycle of this species in the

Czech Republic, as expected by the forenamed authors.

In half of the samples, Plecoptera were represented by larvae of *Perla burmeisteriana* and *Leuctra fusca*, which prefer transition between the rhitral and potamal zones. Most of the Ephemeroptera and Trichoptera larvae found together with *L. geniculata* also preferred predominantly potamal stretches (for the details see Table 2). Preferences of the species mentioned above fully correspond with the longitudinal distribution within river zones supposed by Graf et al. (2002), who proposed preferences from the epirhitral to the metapotamal zone, with its main occurrence in epipotamal stretches. The altitudinal preferences in Central Europe markedly differ from the situation in Mediterranean populations, e.g., in Sardinia, Corsica and the Pyrenees, where the species is often found in the crenal zone and springs at altitudes above 1,000 m a.s.l. (Consiglio 1975; Sánchez-Ortega et al. 2003).

Contrary to the scarce distribution of *L. geniculata*, the *Leuctra* species of similar dimensions, morphology (hair and shape of body), food demands (shredders) and larval development as the species *Leuctra braueri* Kempny, 1898 and *Leuctra nigra* (Olivier, 1811) are widely distributed in the Czech Republic. They have different habitat preferences and prefer to inhabit smaller streams from the epirhitral zone up to the springs at altitudes from 220 to 1100 m a.s.l. (Soldán et al. 1998).

The presented data on *L. geniculata* give basic information about its preferences on the north-eastern border of its area. The majority of our ecological data corresponds with the results and assumptions of the aforementioned authors (Grauvogl 1992; Graf et al. 2002; Sánchez-Ortega et al. 2003; López-Rodríguez et al. 2004), although the species was also found in watercourses with lower water quality.

Possible ways of species dissemination

All sites where *L. geniculata* has been recorded recently in the Czech Republic were investigated in detail between 1955 and 1960 (Křelinová 1962) and partially also between 1994 and 1996 (Soldán et al. 1998). However, *L. geniculata* was not recorded there until 2003; therefore, we suppose its dissemination to the Czech Republic took place around the turn of the century. The Labe River probably could not serve as a corridor for the species expansion to the Czech Republic because the chronology of findings was the opposite (from upper stretches to lower parts of the Labe basin – see Fig. 4) and there are no findings of *L. geniculata* in the Labe basin in Germany. Another possible way of expansion is more likely, regarding the chronology of records in the Czech Republic. It is a dissemination from the Danube basin via the south-east to the Vltava catchment (including Malše and Berounka) and/or the Ohře catchment and then faster downstream into the Labe basin. It can be expected that *L. geniculata* will disseminate eastwards to other Czech rivers and streams up to the altitude of 500 m a.s.l. (limitation by the continental climate) in both the Elbe and the Danube basins (in the latter case via the Morava River from Austria and Slovakia), respectively. The present dissemination of the species is probably related to the marked improvement of water quality in Czech rivers during the last 15 years, particularly in the epipotamal stretches that are preferred by the species. The northwards expansion of this Atlantomediterranean species could also be associated with the growth of average temperatures in the Czech Republic (Pišoft et al. 2004) related to global climate changes, but sufficient data are not available for the validation of this hypothesis.

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Rapid Communication

First record of the invasive polychaete *Hypania invalida* (Grube, 1960) in the Czech Republic

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Abstract

Hypania invalida is a freshwater polychaete of Ponto-Caspian origin that has recently colonised many large European rivers. Here we report the first record of this species in the territory of the Czech Republic. It was found at four sites in the Elbe River close to the Czech-German border in 2014. We presume that the most probable vector was the shipping industry.

Key words: Labe, Elbe, Ponto-Caspian, non-indigenous species, aquatic, neozoa

Introduction

Large rivers are very sensitive to the invasion by non-indigenous aquatic species. They can serve as natural pathways stretching over large distances. Most large rivers are anthropogenically disturbed and often exploited as navigation channels. For these reasons, large rivers are under special threat, which is reflected by large numbers of non-indigenous species (e.g. Leprieur et al. 2008; Leuven et al. 2009). Currently, there is only one Czech river (the Elbe) that is used as a navigation channel connected to large European navigation routes. A couple of non-native aquatic invertebrate species have recently invaded the Elbe River in the Czech Republic: *Corbicula fluminea* (O. F. Müller, 1774), *Dikerogammarus villosus* (Sowinsky, 1894), *Hemimysis anomala* (Sars, 1907), *Atyaephyra desmarestii* (Millet, 1831), and *Jaera istrii* Veuille, 1979 (Beran 2000; Špaček et al. 2003; Horecký et al. 2005; Straka and Špaček 2009).

Hypania invalida (Grube, 1960) is one of the few polychaetes occurring in fresh waters (Glasby and Timm 2008). From its original distribution in the Ponto-Caspian region, it started to spread through European inland waters in the 1950s and 1960s (e.g. Woźniczka et al. 2011; Zorić et al. 2011; Vanden Bossche et al. 2001). Its invasion of the Elbe River occurred through the Southern corridor (Bij de Vaate et al. 2002). First, it invaded the Danube River, then the Main-Danube Canal, subsequently the Rhine River, and through the Mittelland Canal it invaded the Elbe River (Tittizer et al. 2000). It was first found in the Elbe River in 2007 near Wittenberg, Germany (Eggers and Anlauf 2008) c. 440 km away from the German-Czech border. Here we report the first record of this species in the Czech stretch of the Elbe River.

Methods

Benthic invertebrate samples were collected by a multi-habitat kick sampling method using a standardised net comprising of a square frame (25 × 25 cm) and a mesh with a pore size of 500 µm. A few sites close to the Czech-German border were sampled to evaluate the impact of ship navigation and for routine environmental monitoring.

Table 1. Records of *Hypania invalida* in the Czech stretch of the Elbe River.

Site No.	Location	Habitat	Number of specimens	Record coordinates		Record date
				Latitude	Longitude	
1	Děčín	natural bank	2	50°50′10″N	14°13′34″E	19 November 2014
2	Děčín	river groyne	9	50°49′40″N	14°13′36″E	19 November 2014
3	Děčín	river groyne	2	50°49′27″N	14°13′29″E	19 November 2014
3	Děčín	river groyne	1	50°49′27″N	14°13′29″E	19 June 2014
4	Lovosice	port	76	50°31′01″N	14°03′28″E	21 June 2014

Figure 1. Elbe River at site 3, on 19th June 2014. Man-made river groyne near Děčín. Photograph by M. Straka.

Figure 2. Four specimens of *Hypania invalida* collected at site 2. Photograph by M. Straka.

Samples were taken in spring, autumn and summer. Samples were preserved in 4% formaldehyde and all invertebrates were sorted out and identified in the laboratory.

Hypania invalida was identified at four sites (Table 1). Site 1 is located on the left bank of the Elbe River (Labe in Czech) close to the Czech-German border. The river bank at this site is anthropogenically unmodified and natural with pebbles and cobbles dominating the substrates. Sites 2 and 3 are artificial river groynes made for shipping purposes (Figure 1). The dominant substrate is pebbles and cobbles, with some quarry stones. Site 4 is a port for recreational craft and has a ferry located 1 km downstream of a weir with a lock chamber. The river banks are composed of quarry stones and paving adjustments. Gravel and stones with muddy sediment dominate the bottom substrate here. River width varies between 100 and 150 m at all sites.

Results and discussion

Hypania invalida was found for the first time in the territory of the Czech Republic in a sample from the 19th of June 2014 at site 3 near Děčín (Figure 1). On the 21st of June 2014, 76 specimens were recorded in one sample at site 4 at the Lovosice city port near the ferry. This site is 50 km distant from the previous one, suggesting that the species is widely distributed in the river, and that it is likely that the species also occurs at other localities in the Czech stretch of the Elbe River. Establishment of the species was confirmed in autumn 2014 at two other sites near Děčín (Table 1). However, observed abundances were relatively low and contributed to approximately 1% of the entire macroinvertebrate assemblage at the Děčín sites and to 20% at the Lovosice site.

Hypania invalida is gonochoric with an exclusively sexual mode of reproduction. The males discharge their sperm into the water column and fertilise eggs within the female dwelling tubes. Later, small juveniles disperse in the water and can be easily transported through the ballast water of ships (Norf et al. 2010). Downstream dispersal of this polychaete is enhanced by simple drifting, especially during floods (Woźniczka et al. 2011). Since the ship dock is located approximately 4 km upstream of collection sites 1–3 and site 4 is within a port, we conclude that the most probable vector of colonisation of the Czech Elbe River was the shipping industry, followed by downstream drift dispersion.

The species is very characteristic, with a conically elongated segmented body (Figure 2). On the sides there are two rows of tufts of long bristles and near the mouth there are conspicuous tentacles. These are partly retracted in fixed material but are still readily visible. The species can therefore be easily identified and so far cannot be mistaken with other species already occurring in the Elbe River.

The preferred habitat is a muddy bottom with reduced flow velocity (Woźniczka et al. 2011); however, Šporka (1998) found this species to be dominant in a gravel bottom of the main river channel. It is interesting that *Hypania invalida* has a strong preference for the druses of the mollusc *Dreissena polymorpha* (Pallas, 1771) (Šporka and Nagy 1998; Yakovlev and Yakovleva 2010). *Hypania invalida* finds shelter and food among *Dreissena polymorpha* shells. Since we sampled with a multi-habitat method, we cannot link species occurrence to a specific substrate type. Nevertheless, soft sediment is a common substrate among the boulders of the Elbe River riprap zone, and *Dreissena polymorpha* is widespread in the studied part of the Elbe River.

Due to its preferences for soft bottom patches, Zorić et al. (2011) considered *Hypania invalida* to have only a limited influence on the overall benthic community. Devin et al. (2006) also stated that *Hypania invalida* has a low competitive value. However, its population densities can reach very high numbers (> 10 000 ind./m^2) (Vanden Bossche et al. 2001; Woźniczka et al. 2011) and at least a partial impact on river ecosystems cannot be excluded.

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Aquatic oligochaetes (Annelida: Clitellata) of the Czech Republic: check-list, new records, and ecological remarks

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Abstract

This study contributes to the knowledge of central European clitellates by creating a check-list of Oligochaeta (sensu oligochaetous Clitellata; Erséus 2005) of the Czech Republic, exclusive of taxa in the family Enchytraeidae. In total, 95 aquatic oligochaete species representing 43 genera are reported for the Czech Republic. Rare species are highlighted and associated with the categories for threatened species as outlined by the International Union for the Conservation of Nature. The first records of *Trichodrilus strandi* Hrabě, 1936, *Pristina jenkinae* (Stephenson, 1931), *Pristina osborni* (Walton, 1906), *Rhyacodrilus subterraneus* Hrabě, 1963, *Aulodrilus limnobius* Bretscher, 1899, and *Aulodrilus pigueti* Kowalewski, 1914 in the Czech Republic are presented. Their ecology, morphology, and distribution are discussed.

Key words: Oligochaeta, *Trichodrilus strandi*, *Pristina jenkinae*, *Pristina osborni*, *Rhyacodrilus subterraneus*, *Aulodrilus limnobius*, *Aulodrilus pigueti*, habitat preferences, first records, red list, alien species

Introduction

Aquatic Oligochaeta (sensu oligochaetous Clitellata; Erséus 2005), are typical inhabitants of bottom sediments, forming communities whose species composition is a relevant information source for water quality assessment (Brinkhurst & Cook 1974; Chapman 2001; Verdonschot 2006). Hence, reliable species identification and a complete knowledge of their ecology are essential. Since the turn of the 20th century many phylogenetic changes and taxonomic shifts in Oligochaeta have been made. Therefore, the monographs published by renowned Czech oligochaetologists in the 20th century have become insufficient for valid identification. The list of oligochaete species of the Czech Republic is scattered among old monographs, mostly written in Czech, and recent papers that have documented distributional records for particular species. Furthermore, the territory of the present Czech Republic was previously a part of several different countries, so its records of oligochaete fauna formed a subset of data published on larger territories.

Taxonomic surveys on aquatic oligochaetes have a long tradition in the Czech Republic. Many world-famous taxonomists, whose names have been assigned by patronymy to oligochaete species and genera, came from the Czech territory. At the end of the 19th century, when the Czech Republic was a part of Austria-Hungary, František Vejdovský (1849–1939) and Antonín Štolc (1863–1917) published numerous works dealing with the classifications and morphology of Oligochaeta (e.g. Vejdovský 1876, 1883, 1884, 1892; Štolc 1886, 1888). At the beginning of the 20th century, Alois Mrázek (1868–1923) and Lev Černosvitov (1902–1946, born in Russia) continued their works with studies on anatomy and reproduction (e.g. Mrázek 1913a, 1913b), and morphology and faunistics (e.g. Černosvitov 1928, 1930, 1935). Fundamental research on this group was made by Sergěj Hrabě (1899–1984), who was born in Russia where he lived with his Czech parents until the October Revolution. After the revolution, during the civil war and under difficult circumstances, he moved back to what was then Czechoslovakia. He published an admirable volume of almost 100 scientific papers and monographs, in which he (and other authors), among others, described 11 new species to science

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from Czechoslovakia and 15 genera new for science and 77 new species of oligochaeta from other countries. Both fundamental monographs by Hrabě (1954, 1981) still serve as the list of Czech oligochaetes and remain the major source of information on the morphology of many species. These works were published prior to 1992, when the former Czechoslovakia was split into the Czech Republic and Slovakia, and therefore include species from both countries irrespective of the new political boundaries. This list was supplemented by species documented by Eva Lišková (1964, 1976), and more recently by species documented in faunistic studies (e.g. Wohlgemuth & Schenková 1999) and ecological studies (e.g. Uzunov *et al.* 1988; Schenková *et al.* 2001a, 2001b; Schenková 2005; Schenková & Kroča 2007). Aquatic and semiaquatic species of the family Lumbricidae were treated in the monograph by Pižl (2002), which dealt with predominantly terrestrial species. Up to now, aquatic species of the family Enchytraeidae have been investigated insufficiently (cf. Hrabě 1954) or even omitted (cf. Hrabě 1981). Extensive studies focusing on the terrestrial and semiaquatic Enchytraeidae occurring in terrestrial habitats were published by Chalupský (1988, 1991, 1994). New records for the Czech Republic were recently reported by Jiří Schlaghamerský (e.g. Schlaghamerský 2007; Schlaghamerský & Kobetičová 2005, 2006; Šídová & Schlaghamerský 2007; Schlaghamerský & Pižl 2009). With respect to taxonomic development, the distribution of the family Enchytraeidae in the Czech Republic needs detailed investigation of both freshwater and terrestrial habitats, and a revision of the published historical data.

The objectives of this study are (i) to summarise and update the check-list of aquatic oligochaete species for the Czech Republic, (ii) to evaluate the frequency of all species occurrences in the Czech Republic and update the Red list and (iii) to discuss new records with extended information on their distribution, preferred habitats, ecological requirements, and morphological characteristics. Six oligochaete species reported herein as new for the Czech Republic—*Trichodrilus strandi* (Lumbriculidae), *Pristina jenkinae* and *Pristina osborni* (Naididae: Pristininae), *Rhyacodrilus subterraneus* (Naididae: Rhyacodrilinae), and *Aulodrilus limnobius* and *Aulodrilus pigueti* (Naididae: Tubificinae)—were studied in detail.

Material and methods

The check-list of aquatic oligochaetes (exclusive of taxa in the family Enchytraeidae) was compiled from published records (Hrabě 1954, 1981; Schenková 2005; Schenková & Kroča 2007; Pižl 2002) and from unpublished material determined and/or revised by the authors. Semiaquatic species of the family Lumbricidae were selected based on the data published in the monograph by Pižl (2002), after personal communication with the author. Nomenclature for species discussed in this paper follows Erséus *et al.* (2008) and a synonymy of species (according to Timm 2009) is presented for the six species reported as new records for the Czech Republic.

Each species discussed herein was given a category for its frequency of occurrence in the Czech Republic based upon published data and unpublished records. The unpublished records were compiled from those discussed in running water quality assessments and other projects in which the authors have participated since 1996. This dataset includes approximately 1300 sites across the whole of the Czech Republic. Species were sorted into four categories: F1—scarce (up to 10 sites), F2—medium frequent (10–100 sites), F3—very frequent (more than 100 sites) and A—absent (i.e., not recorded from the Czech Republic since 1996). For scarce or rare species, a category of threat in the Czech Republic according to the International Union for Conservation of Nature 1994 was recommended. As we applied the IUCN criteria at national level, we used guidelines prepared by the IUCN/SSC Regional Applications Working Group (Gärdenfors *et al.* 2001, IUCN 2003, Miller *et al.* 2007). A national and regional category for a particular species may not be the same as its global category, because at global level the whole range of a species is evaluated.

Because many oligochaete species dwell in different freshwater habitats, they were sampled by various methods. The conservation and processing of samples and species identification were the same.

Trichodrilus strandi was collected quantitatively using a metal frame of 25 × 25 cm^2 in spring fen habitats. Vegetation and upper bottom layer were gathered to the depth of 5 cm and this substrate was elutriated through a 250 μm mesh net.

Pristina jenkinae, *Rhyacodrilus subterraneus*, and *Aulodrilus limnobius* were collected in reaches of the river (20–100 m in length) using a three-minute kick-sampling of all visually distinguishable habitats, which were sampled proportionally to their occurrences in the reach of the river using a hand net (25 × 25 cm^2, mesh size 500 µm) (Kokeš *et al.* 2006).

Aulodrilus pigueti was collected using the same type of hand net (25 × 25 cm^2, mesh size 500 µm) in the bottom substrate of a fishpond and in submerged littoral vegetation.

Pristina osborni was collected in a large river from a depth up to 3 m using an "air lift" vacuum sampler designed for sampling in large, fast-flowing rivers (Pehofer 1998) and the grab "van Veen" (Lie & Pamatmat 1965). The samples were elutriated through the 250 µm mesh net.

Samples obtained by all these methods were fixed by 4% formaldehyde in the field and then sorted under a stereomicroscope in the laboratory. Oligochaetes were picked up, permanently mounted in Canada balsam and identified under a light microscope using the keys by Hrabě (1954, 1981), Timm & Veldhijzen van Zanten (2002) and Timm (2009).

New records of selected species included the following information associated with specimens: name of the water body, name of the settlement (town, village, city), closest site from which the specimen(s) was/were collected, latitude and longitude coordinates for the collecting site, name(s) of the person(s) who collected and/or identified the species and date of collection. Abbreviations for the names of collectors and persons who identified the species are presented below: IS—Ivan Skála, JB—Jindřiška Bojková, JK—Jiří Kokeš, JSc—Jana Schenková, JSy—Jan Sychra, JZ—Jiří Zahrádka, KB—Karel Brabec, KP—Karla Petřivalská, LO—Libuše Opatřilová, PH—Pavel Horák, PP—Petr Pařil. Data on habitat characteristics - abiotic and chemical parameters, water saprobity (Kolkwitz & Marsson 1909), ecology, morphology (if new or untypical features were recorded) and distribution of species are noted.

Results and discussion

The check-list of 95 aquatic/semiaquatic oligochaete species for the Czech Republic comprises the families Lumbriculidae (12 species), Naididae sensu Erséus *et al.* (2008) (a total of 72 species)—which includes the Naidinae (36 species), Pristininae (eight species), Tubificinae (22 species), and Rhyacodrilinae (six species), Propappidae (one species), Criodrilidae (one species), Haplotaxidae (one species) and Lumbricidae (8 species) (Table 1).

The majority of the evaluated species (44) were moderately or widely distributed (F2 and F3) in the Czech Republic. They are mostly ubiquitous and euryecious species (e.g., several species of Tubificinae inhabiting running and stagnant waters) and many species can tolerate high organic matter content and pollution. There were also two species, *Potamothrix moldaviensis* Vejdovský et Mrázek, 1903 and *Potamothrix bavaricus* (Oschman, 1913) (both in the subfamily Tubificinae), that are considered alien species in Europe (Drake 2009). However, their non-indigenous status in central Europe is questionable because they were recorded historically from areas within the border of today's Czech Republic. *P. moldaviensis* has previously been described in the Vltava (Moldau) River (the Elbe basin) in the beginning of 20th century and *P. bavaricus* was recorded in the Elbe basin in the 1930s (Hrabě 1939). Both species recently occur in moderately polluted rivers—*P. moldaviensis* prefers larger rivers, while *P. bavaricus* prefers smaller streams.

Thirty-eight species were considered rare (F1). Some of these species are not necessarily endangered, since they can be locally abundant in either restricted areas or particular habitats. For instance, *Stylodrilus lemani* (Grube, 1879) is abundant in a few eutrophic streams in southern Moravia (located in the south-eastern part of the Czech Republic) and *Haemonais waldovogeli* Bretscher, 1900 is dominant in the bottom fauna of small fishponds in the Czech-Moravian Highland Mountains. By contrast, one species, *Branchiura sowerbyi* Beddard, 1892, is a non-indigenous thermophilic species which has been spreading throughout Europe, most likely via water transport (Lišková 1964; Gruszka 1999). However, its invasion is very slow because of a lack of suitable habitats (warmer waters). We have documented four records of *B. sowerbyi* since 1964, when it was found (Lišková 1964) for the first time in Czechoslovakia. Two other species, *Psammoryctides moravicus*

(Hrabě, 1934) and *Paranais frici* Hrabě, 1941—both considered aliens of Ponto-Caspian origin in Europe (Drake 2009)—were recently found sporadically in the Labe and Morava Rivers that serve as immigration gateways into Czech waters.

TABLE 1. Aquatic Oligochaeta of the Czech Republic. Lumbriculidae, Naididae (Naidinae, Pristininae, Rhyacodrilinae, Tubificinae), Propappidae, Haplotaxidae, Criodrilidae, and Lumbricidae. Frequency of occurrence since 1996: F1—scarce (up to 10 sites), F2—medium frequent (10–100 sites), F3—very frequent (more than 100 sites) and A—absent. Lumbricidae, affinity to wet habitats and frequency of occurrence refers to Pižl (2002, and personal communication).

Taxon	Author	Reference	Frequency
Lumbriculidae			
Lamprodrilus mrazeki	Hrabě, 1929	Hrabě 1954, 1981	A
Lumbriculus variegatus	(Müller, 1774)	Hrabě 1954, 1981	F3
Rhynchelmis limosella	Hoffmeister, 1843	Hrabě 1954, 1981	F2
Stylodrilus absoloni	(Hrabě, 1970)	Hrabě 1981	A
Stylodrilus brachystylus	Hrabě, 1929	Hrabě 1954, 1981	F3
Stylodrilus heringianus	Claparède, 1862	Hrabě 1954, 1981	F3
Stylodrilus lemani	(Grube, 1879)	Hrabě 1954, 1981	F1
Stylodrilus parvus	(Hrabě & Černosvitov, 1927)	Hrabě 1954, 1981	F1
Trichodrilus allobrogum	Claparède, 1862	Hrabě 1954, 1981	F1
Trichodrilus moravicus	Hrabě, 1937	Hrabě 1954, 1981	F1
Trichodrilus pragensis	Vejdovský, 1876	Hrabě 1954, 1981	A
Trichodrilus strandi	Hrabě, 1936	**new record**	F1
Naididae: Naidinae			
Amphichaeta leydigi	Tauber, 1879	Hrabě 1954, 1981	F1
Arcteonais lomondi	(Martin, 1907)	Hrabě 1954, 1981	F1
Aulophorus furcatus	(Müller, 1773)	Hrabě 1954, 1981	F1
Chaetogaster cristallinus	Vejdovský, 1884	Hrabě 1954, 1981	F1
Chaetogaster diaphanus	(Gruithuisen, 1828)	Hrabě 1954, 1981	F2
Chaetogaster diastrophus	(Gruithuisen, 1828)	Hrabě 1954, 1981	F2
Chaetogaster langi	Bretscher, 1896	Hrabě 1954, 1981	F2
Chaetogaster limnaei	Baer, 1827	Hrabě 1954, 1981	A
Chaetogaster setosus	Světlov, 1925	Hrabě 1954	A
Dero digitata	(Müller, 1774)	Hrabě 1954, 1981	F2
Dero dorsalis	Ferronière, 1899	Hrabě 1954, 1981	A
Dero nivea	Aiyer, 1929	Hrabě 1954	A
Dero obtusa	Udekem, 1855	Hrabě 1954, 1981	A
Haemonais waldvogeli	Bretscher, 1900	Hrabě 1954, 1981	F1
Nais alpina	Sperber, 1948	Hrabě 1954, 1981	F3
Nais barbata	Müller, 1774	Hrabě 1954, 1981	F2
Nais behningi	Michaelsen, 1923	Hrabě 1954, 1981	F1
Nais bretscheri	Michaelsen, 1899	Hrabě 1954, 1981	F2
Nais christinae	Kasprzak, 1973	Hrabě 1981	F1
Nais communis	Piguet, 1906	Hrabě 1954, 1981	F2

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Taxon	Author	Reference	Frequency
Nais elinguis	Müller, 1774	Hrabě 1954, 1981	F3
Nais pardalis	Piguet, 1906	Hrabě 1954, 1981	F2
Nais pseudobtusa	Piguet, 1906	Hrabě 1954, 1981	F1
Nais simplex	Piguet, 1906	Hrabě 1954, 1981	F2
Nais stolci	Hrabě, 1981	Hrabě 1954	F1
Nais variabilis	Piguet, 1906	Hrabě 1954, 1981	F2
Ophidonais serpentina	(Müller, 1774)	Hrabě 1954, 1981	F2
Paranais frici	Hrabě, 1941	Hrabě 1954, 1981	F1
Piguetiella blanci	(Piguet, 1906)	Schenková 2005	F1
Ripistes parasita	(Schmidt, 1847)	Hrabě 1954, 1981	F1
Slavina appendiculata	(Udekem, 1855)	Hrabě 1954, 1981	F2
Specaria josinae	(Vejdovský, 1884)	Hrabě 1954, 1981	F1
Stylaria lacustris	(Linnaeus, 1767)	Hrabě 1954, 1981	F3
Uncinais uncinata	(Øersted, 1842)	Hrabě 1954, 1981	F1
Vejdovskyella comata	(Vejdovský, 1884)	Hrabě 1954, 1981	F2
Vejdovskyella intermedia	(Bretscher, 1896)	Hrabě 1954, 1981	F1
Naididae: Pristininae			
Pristina aequiseta	Bourne, 1891	Hrabě 1954, 1981	F1
Pristina amphibiotica	Lastočkin, 1927	Hrabě 1954	A
Pristina bilobata	(Bretscher, 1903)	Schenková & Kroča 2007	F1
Pristina jenkinae	(Stephenson, 1931)	**new record**	F1
Pristina longiseta	Ehrenberg, 1828	Hrabě 1954, 1981	F1
Pristina menoni	(Aiyer, 1930)	Hrabě 1954	F1
Pristina osborni	(Walton, 1906)	**new record**	F1
Pristina rosea	(Piguet, 1906)	Hrabě 1954, 1981	F2
Naididae: Rhyacodrilinae			
Bothrioneurum vejdovskyanum	Štolc, 1886	Hrabě 1954, 1981	F3
Branchiura sowerbyi	Beddard, 1892	Hrabě 1981	F1
Epirodrilus pygmaeus	(Hrabě, 1935)	Hrabě 1954, 1981	F2
Rhyacodrilus coccineus	(Vejdovský, 1875)	Hrabě 1954, 1981	F3
Rhyacodrilus falciformis	Bretscher, 1901	Hrabě 1954, 1981	F2
Rhyacodrilus subterraneus	Hrabě, 1963	**new record**	F1
Naididae: Tubificinae			
Aulodrilus japonicus	Yamaguchi, 1953	Hrabě 1954, 1981	F2
Aulodrilus limnobius	Bretscher, 1899	**new record**	F1
Aulodrilus pigueti	Kowalewski, 1914	**new record**	F1
Aulodrilus pluriseta	(Piguet, 1906)	Hrabě 1954, 1981	F2
Haber speciosus	(Hrabě, 1931)	Hrabě 1954, 1981	A
Ilyodrilus templetoni	(Southern, 1909)	Hrabě 1954, 1981	F2
Limnodrilus claparedeanus	Ratzel, 1868	Hrabě 1954, 1981	F3
Limnodrilus hoffmeisteri	Claparède, 1862	Hrabě 1954, 1981	F3

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Taxon	Author	Reference	Frequency
Limnodrilus profundicola	(Verrill, 1871)	Hrabě 1981	F1
Limnodrilus udekemianus	Claparède, 1862	Hrabě 1954, 1981	F2
Peipsidrilus pusillus	Timm, 1977	Hrabě 1981	A
Potamothrix bavaricus	(Oschmann, 1913)	Hrabě 1954, 1981	F2
Potamothrix bedoti	(Piguet, 1913)	Hrabě 1954, 1981	A
Potamothrix hammoniensis	(Michaelsen, 1901)	Hrabě 1954, 1981	F2
Potamothrix moldaviensis	Vejdovský & Mrázek, 1903	Hrabě 1954, 1981	F2
Psammoryctides albicola	(Michaelsen, 1901)	Hrabě 1954, 1981	F2
Psammoryctides barbatus	(Grube, 1861)	Hrabě 1954, 1981	F3
Psammoryctides moravicus	(Hrabě, 1934)	Hrabě 1954, 1981	F1
Spirosperma ferox	Eisen, 1879	Hrabě 1954, 1981	F2
Tubifex ignotus	(Štolc, 1886)	Hrabě 1954, 1981	F2
Tubifex nerthus	Michaelsen, 1908	Hrabě 1981	A
Tubifex tubifex	(Müller, 1774)	Hrabě 1954, 1981	F3
Propappidae			
Propappus volki	Michaelsen, 1916	Hrabě 1954	F2
Haplotaxidae			
Haplotaxis gordioides	(Hartmann, 1821)	Hrabě 1954, 1981	F2
Criodrilidae			
Criodrilus lacuum	Hoffmeister, 1845	Hrabě 1954	F1
Lumbricidae			
Aporrectodea georgii	(Michaelsen, 1890)	Pižl 2002	F1
Aporrectodea handlirschi	(Rosa, 1879)	Pižl 2002	F1
Aporrectodea limicola	(Michaelsen, 1890)	Pižl 2002	F1
Eisenia fetida	(Savigny, 1826)	Pižl 2002	F2
Eisenia spelaea	(Rosa, 1909)	Pižl 2002	F1
Eiseniella tetraedra	(Savigny, 1826)	Pižl 2002	F3
Helodrilus oculatus	Hoffmeister, 1845	Pižl 2002	F1
Octoclasion tyrtaeum	(Savigny, 1826)	Pižl 2002	F2

Thirteen species have not been recorded in studies conducted in the Czech Republic since 1996. Their absence from more recent collections could be caused by various reasons. Firstly, particularly unique habitats were not studied, thus species limited in distribution to these areas where not recorded—e.g., a cave species *Stylodrilus absoloni* (Hrabě, 1970), a groundwater species *Trichodrilus pragensis* Vejdovský, 1876, and a snail parasite *Chaetogaster limnaei* Baer, 1827. Secondly, most of the material discussed in this paper was collected in running water habitats; stagnant waters were studied to a lesser extent. This could be the reason for the absence of the temporal pool specialist *Lamprodilus mrazeki* Hrabě, 1929 and stagnant water specialists such as *Dero dorsalis* Ferronière, 1899, *Dero nivea* Aiyer, 1929, *Dero obtusa* Udekem, 1855, and *Chaetogaster setosus* Světlov, 1925. Other species that have not been reported since 1996 include *Pristina amphibiotica* Lastočkin, 1927, *Haber speciosus* (Hrabě, 1931), *Peipsidrilus pusillus* Timm, 1977, *Potamothrix bedoti* (Piguet, 1913), and *Tubifex nerthus* Michaelsen, 1908—each of these had previously been reported only once in the country, and we consider them to be either very rare or regionally extinct in the Czech Republic.

TABLE 2. Proposal of the Red list of aquatic Oligochaeta of the Czech Republic. Two species from the family Lumbricidae according to Pižl (2005) are included.

endangered (EN)		**vulnerable (VU)**	
Arcteonais lomondi	(Martin, 1907)	*Amphichaeta leydigi*	Tauber, 1879
Criodrilus lacuum	Hoffmeister, 1845	*Aporrectodea limicola*	(Michaelsen, 1890)
Haber speciosus	(Hrabě, 1931)	*Pristina aequiseta*	Bourne, 1891
Lamprodrilus mrazeki	Hrabě, 1929	*Pristina bilobata*	(Bretscher, 1903)
Peipsidrilus pusillus	Timm, 1977	*Pristina jenkinae*	(Stephenson, 1931)
Piguetiella blanci	(Piguet, 1906)	*Pristina longiseta*	Ehrenberg, 1828
Stylodrilus absoloni	(Hrabě, 1970)	*Pristina osborni*	(Walton, 1906)
Trichodrilus pragensis	Vejdovský, 1876	*Nais behningi*	Michaelsen, 1923
Trichodrilus strandi	Hrabě, 1936	*Nais christinae*	Kasprzak, 1973
		Nais stolci	Hrabě, 1981
		Rhyacodrilus subterraneus	Hrabě, 1963
		Specaria josinae	(Vejdovský, 1884)
near threatened (NT)		*Stylodrilus lemani*	(Grube, 1879)
Apporrectodea georgii	(Michaelsen, 1890)	*Uncinais uncinata*	(Ørsted, 1842)

The Red List of aquatic oligochaetes of the Czech Republic (Schenková 2005, Pižl 2005) was modified on the basis of the new extensive data by excluding *Aulodrilus japonicus* Yamaguchi, 1953, *Ilyodrilus templetoni* (Southern, 1909), *Epirodrilus pygmaeus* (Hrabě, 1935), *Psammoryctides albicola* (Michaelsen, 1901), and *Slavina appendiculata* (Udekem, 1855), which had been formerly considered endangered. The number of recent records and distribution of these species within the Czech Republic indicate the stability of their populations. On the basis of those new data, we changed the protection status of another three species—*Pristina bilobata* (Bretscher, 1903), *Pristina longiseta* Ehrenberg, 1828, and *Uncinais uncinata* (Ørsted, 1842)—from endangered (EN) to vulnerable (VU). However, we now suggest that the status of endangered species (EN) to be allocated to *Trichodrilus strandi*, *Haber speciosus*, *Lamprodrilus mrazeki*, *Peipsidrilus pusillus*, and *Stylodrilus absoloni* with respect to their very restricted occurrence—not only in the Czech Republic, but also in neighbouring countries. Vulnerable protection status (VU) was proposed for *Amphichaeta leydigi* Tauber, 1879, *Nais behningi* Michaelsen, 1923, *Nais christinae* Kasparzak, 1973, *Nais stolci* Hrabě, 1981, *Pristina aequiseta* Bourne, 1891, *P. osborni*, *P. jenkinae*, *R. subterraneus*, *Specaria josinae* (Vejdovský, 1884), *S. lemani* and *Vejdovskyella intermedia* (Bretscher, 1896), based on their rare occurrence and scattered distributions. The new Red list of Czech aquatic oligochaete species with suggested changes is given in Table 2.

Six species collected during this present study are reported here as new records for the Czech Republic: *Trichodrilus strandi*, *Pristina jenkinae*, *P. osborni*, *Rhyacodrilus subterraneus*, *Aulodrilus limnobius* and *A. pigueti*. The extent of their physical and chemical characteristics of finding sites is summarised in Table 3.

Species accounts

Family: Lumbriculidae

Trichodrilus strandi Hrabě, 1936

Records. Bílé Potoky Spring Fen, Valašské Klobouky, *49°06'52"N / 18°01'40"E*, lgt. JB, det. JSc (2006); Hrubý Mechnáč Spring Fen, Lopeník, *48°56'27"N / 17°47'51"E*, lgt. JB, det. JSc (2006); Kalábová Spring

Fen, Březová, *48°56'22"N / 17°44'39"E*, lgt. JB, det. JSc (2006); Chmelinec Spring Fen, Vyškovec, *48°56'22"N / 17°51'20"E*, lgt. JB, det JSc (2006); Hutě Spring Fen, Žítková, *48°59'27"N / 17°54'33"E*, lgt. JB, det. JSc (2006).

Characteristics of sites. *T. strandi* was recorded in 2006 in the Western Carpathian spring fens, which are situated in the easternmost part of the Czech Republic, on the boundary with Slovakia (Fig. 1). We have collected this species from altitudes 350–642 m a.s.l. in extremely mineral-rich spring fens with precipitation of cold water travertine (tufa). The bottom substrate of spring fens was dominated by inorganic material (gravel and sand) and coarse particulate organic matter (leaves and wood). Tufa precipitated on all submerged surfaces forms either small incrustations or strong layers.

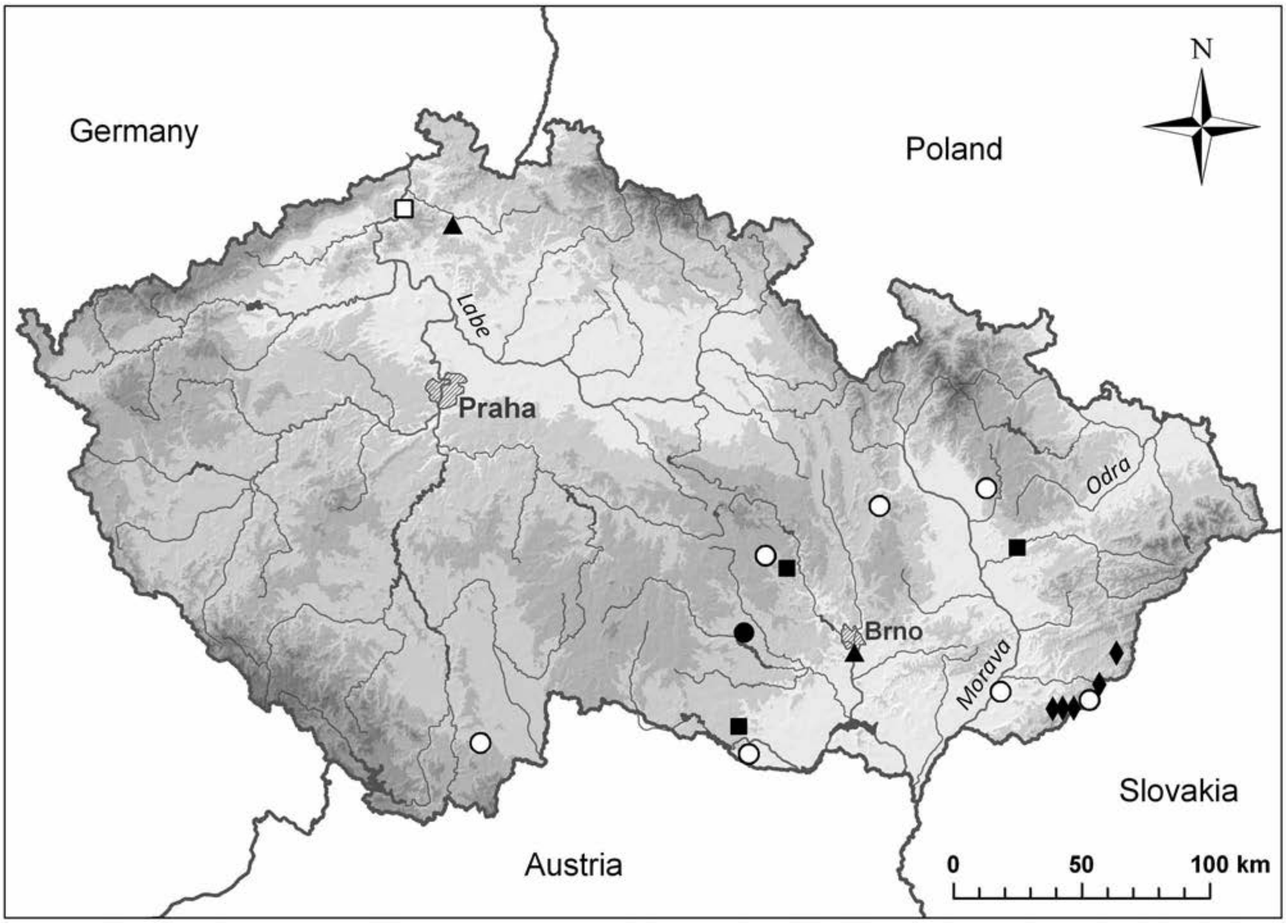

FIGURE 1. The distribution of ♦ *Trichodrilus strandi*, ■ *Pristina jenkinae*, ☐ *Pristina osborni*, ▲ *Rhyacodrilus subterraneus*, ○ *Aulodrilus limnobius* and ● *A. pigueti* in the Czech Republic.

Ecology. *T. strandi* is well adapted for this rather hostile environment because of a tiny flexible body that enables it to utilise small interstitial spaces, particularly those in tufa-covered substrates. In studied sites, it represented the eudominant species followed by 16 additional oligochaete taxa, mostly taxa in the family Enchytraeidae. Its main adaptation can be feeding on specific kinds of bacteria that colonise surfaces in mineral-rich waters (Tarmo Timm pers. com.). Although the environment in spring fens is rather stable with small temperature fluctuations, we recorded seasonal variations in the ratio between juveniles and adults from 50% of juveniles in spring to their prevalence (80%) in autumn. *T. strandi* is a monocyclic gatherer-collector burrowing in sediments of xenosaprobic streams, and it is considered a K-strategist (Šporka 2003).

Morphology. This species can be distinguished from other taxa in that all chaetae are bifid with tiny upper teeth, the male pores are located on conical porophores in IX, and spermathecal pores are present in X (Fig. 2). The positions of sexual pores in *T. strandi* (porophores in X, spermathecal pores in IX) are opposite of those in the genus *Stylodrilus*, with which young *T. strandi* can be confused. We have recorded variability in the spermathecae position of 200 individuals: 36% had spermathecae in X, 38% in between X and XI (crossing the septa) and 26% in XI. We did not observe any lateral wings in the genital region, but we identified only fixed individuals.

Distribution. The nearest known localities for *T. strandi* are in the Valča Brook—a tributary of the Turiec River in the Malá Fatra Mts. (Slovakia) (Šporka 1996a), which is approximately 80 km from our sites (Šporka 1996a), and the spring outflow of an ice cave in the Tatra Mts.—approximately 150 km from our sites (Hrabě 1942). European distribution is highly scattered with records from karstic or flysh areas in France (Giani 1979; Juget & Dumnicka 1986), Croatia (Karaman 1987) and Italy (Brinkhurst 1963). According to Fauna Europaea (Timm & Giani 2004), the species was found in the Czech Republic, but unfortunately no additional information for this report was provided, nor is available. These data most likely represent records reported from areas in what is now known as Slovakia prior to its split from the former Czechoslovakia. *T. strandi* as the stygophilous species, representative of the Lumbriculidae, is more limited in global distribution (Martin *et al.* 2008). This species is worth further investigation since its populations seem to be geographically separated and there is even the question if they represent the same species.

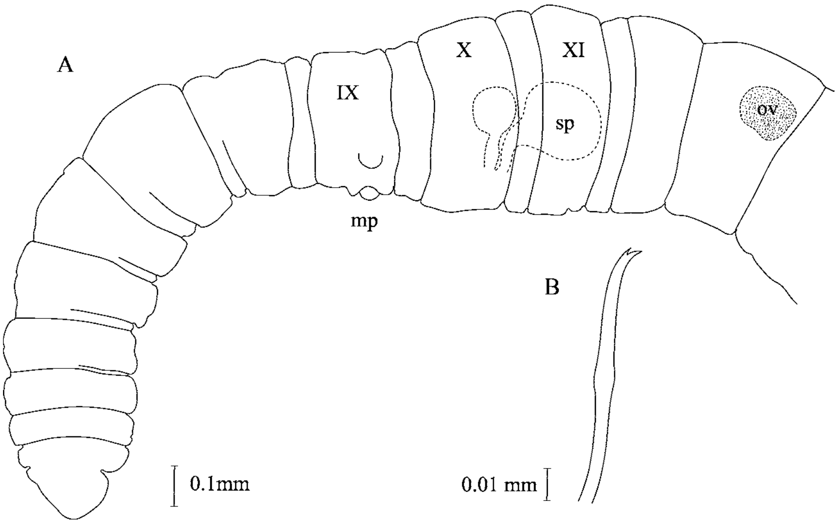

FIGURE 2. *Trichodrilus strandi*. A: Schematic drawing of reproductive organs in IX, X, XI segment, lateral view. mp: male pore on conical porophorus, sp: spermatheca, ov: ovarium. B: dorsal chaeta.

Family: Naididae

Subfamily: Pristininae

Pristina jenkinae (Stephenson, 1931)

Synonyms: *Naidium jenkinae* Stephenson, 1931; *Pristina idrensis* Sperber, 1948; *Pristinella jenkinae* (Stephenson, 1931)

Records. Teplá Brook, Věžná, *49°26'42"N / 16°16'33"E*, lgt. PP, det. PP (2003); Bečva River, Lipník-Osek, *49°29'48"N / 17°31'06"E*, lgt. KB, det. PP (2004); Gránický Brook, Znojmo, *48°51'60"N / 16°01'33"E*, lgt. PP, det. PP (2005); all specimens were immature.

Characteristics of sites. The species was found in two different habitats: epirhithral and hyporhithral streams. The epirhithral habitat was represented by two small brooks (2nd and 3rd Strahler order) with dominating stony-gravel substrates, natural channel morphology and forested catchments (catchment area up to 20 km^2). The hyporhithral habitat was a 30 m wide shallow reach of the river of 7th Strahler order (catchment area 1,526 km^2) with cobble-pebble substrate, which flows through extensively used farmland. A natural self-restoration of this reach of the river resulted from a big flood in 1997.

Ecology. *Pristina jenkinae*, a detritophagous oligochaete, was recorded in different freshwater habitats (Timm & Veldhijzen van Zanten 2002), including hyporheic zones (Strayer & Bannon-O'Donnell 1988; Giani *et al.* 2001; Wetzel & Taylor 2001), and in wet soil of a tropical rainforest (Collado & Schmelz 2001). In central Europe, it was reported from rhithral and potamal zones, with preferences from xeno- to beta-mesosaprobity (Hörner *et al.* 2002). The absence of any historic records of this species from the Czech Republic are likely attributed to difficulties in its identification (ecophenotypic variation in its chaetae, and status as a valid taxon) and its rare to occasional occurrence (Wetzel & Taylor 2001).

Morphology. This species can be distinguished from related European *Pristina* species by none or slight shortening of the upper teeth in ventral chaetae from the anterior to posterior end, parallel teeth of dorsal needles with upper teeth slightly (anterior most) or distinctly (posterior bundles) shorter and thinner (from 1/2 to 2/3), smooth hair chaetae, and gradual intestinal dilatation from ½ VI to VII (Collado & Schmelz 2001; Timm & Veldhijzen van Zanten 2002). A single spermathecal chaeta is present in VII in mature specimens, but we unfortunately did not find any sexually active individuals during this present study.

Distribution. *Pristina jenkinae* has a cosmopolitan distribution (Timm & Veldhijzen van Zanten 2002) and it has been reported from many other European countries: Germany, Poland, Slovakia, Moldova, Romania, Italy, France, Spain, Portugal, Norway, Sweden, The Netherlands, the UK, Greece, and Finland (Timm & Giani 2004). However, the absence of records in other European countries can be connected with identification difficulties and the unclear taxonomical status of this taxon. *P. jenkinae* was at first synonymised with *P. idrensis* by Kathman (1985) and recently redescribed and discussed as probably distinct species by Collado & Schmelz (2001).

Pristina osborni **(Walton, 1906)**

Synonyms: *Naidium osborni* Walton, 1906; *Naidium minutum* Stephenson, 1914; *Pristinella osborni* (Walton, 1906); *Pristina minuta* (Stephenson, 1914)

Records. Labe (Elbe) River, Děčín *50°42'34"N / 14°11'44"E*, lgt. JK, det. PP (2008); all specimens were immature.

Characteristics of site. The species was found in a single site, in the Labe (Elbe) River approximately 20 km far from the Czech/German boundary near Dečín (Tetschen). This river is the largest in the Czech Republic (8th Strahler order) and it is extensively used for shipping to the German port of Hamburg. The mean depth of this reach of the river was 2.4 m, the bottom substrate was predominantly sand (45%) and gravel (55%) and water quality is classified according to BOD (biochemical oxygen demand) in the beta-mesosaprobity (Table 3).

Ecology. *Pristina osborni* inhabits freshwaters including hyporheic waters and caves (Giani *et al.* 2001) and wet soil (Stout 1958). Its ability to inhabit also semi-terrestrial environments enhances the opportunity for freshwater populations of this species to persist in intermittent wetlands (Montalto & Marchese 2005). In tropical regions, *P. osborni* prefers areas with higher calcium content (Alves *et al.* 2008). Asexual reproduction occurs most commonly via paratomy (Timm & Veldhijzen van Zanten 2002). The species is not usually abundant within the oligochaete community (Giani *et al.* 2001; Alves *et al.* 2008).

Morphology. The species can be distinguished by the typical shape of its dorsal bifid chaetae, with the teeth diverting in a wide angle and the presence of only one needle and one smooth hair seta in the dorsal bundles. The budding zone begins from XII, and the stomach dilatation begins abruptly in segments VII–VIII. The morphology of the genital organs was described in detail by Erséus and Grimm (1998).

Distribution. *Pristina osborni* has a cosmopolitan distribution (Timm & Veldhijzen van Zanten 2002), and is absent only in the eastern Palaearctic region (Timm & Giani 2004). European records are limited to southern and western countries (Portugal, Spain, Italy, France, The Netherlands, and Germany; Timm & Giani 2004). The Labe (Elbe) River, which flows through north-eastern Germany to the Baltic Sea, likely serving as a migration corridor for this species—from its original distribution area in western Europe (as well as it was documented for aliens) into the Czech Republic. *P. osborni* could spread into this reach of the river, probably from a lower part of the watershed via upstream migration or dissemination by shipping. However, downstream dissemination into the Labe River—proven to be an alternative pathway of spreading of epipotamal species (Pařil *et al*. 2008)—is rather improbable for *P. osborni*.

TABLE 3. Summary of the characteristics of the aquatic oligochaete species *Trichodrilus strandi*, *Pristina jenkinae*, *Pristina osborni*, *Rhyacodrilus subterraneus*, *Aulodrilus limnobius* and *A. pigueti* sites in the Czech Republic.

Species	*Trichodrilus strandi*	*Pristina jenkinae*	*Pristina osborni*	*Rhyacodrilus subterraneus*	*Aulodrilus limnobius*	*Aulodrilus pigueti*
Sites:						
No of sites	5	3	1	2	7	1
No of records	15	3	1	2	8	1
River basin	Morava	Morava	Labe	Morava, Labe	Morava, Dunaj, Labe	Morava
Abundance	>10%	1–2%	1–2%	1–2%	2.1–5%	1–2%
Water body	spring brook/ helocrene	brook/river	river	brook/river	brook/river	fish-pond
Abiotic parameters						
Min.-max.:						
Altitude [m a.s.l.]	350–642	225–420	120	190–240	165–485	440
Strahler order	1	2–7	8	5–6	3–4	–
Ann. average discharge [$m^3 s^{-1}$]	–	0.001–15.3	309	1.2–5.2	0.01–0.8	–
Average stream width [m]	–	0.9–33.0	100	7.2–12.0	1.9–5.0	–
Chemical parameters						
Min.- max.:						
pH	7.3–8.2	6.0–9.0	8.8	7.3–8.5	6.0–9.1	9.5–9.8
Conductivity [$mS m^{-1}$]	406–599	91–1180	427	333–737	118–921	414–434
Ann. average water temp. [°C]	7.0–14.5	10.6–10.7	14.3	10.2–10.9	8.1–11.8	–
BOD [$mg O_2 l^{-1}$]	–	1.0–6.6	4	1.2–20.8	0.8–9.4	–
Dissolved oxygen [$mg l^{-1}$]	2.4–12.7	7.1–14.7	8.9	6.9–14.1	5.4–13.7	6.9–15.4

Subfamily: Rhyacodrilinae

Rhyacodrilus subterraneus Hrabě, 1963

Records. Robečský Brook, Česká Lípa, *50°40'05"N / 14°30'27"E*, lgt. IS, det. JS/PP (2007), immature specimens; Svitava River, Brno, *49°08'43"N / 16°37'48"E*, lgt. PH, det. PP (2006); one mature specimen.

Characteristics of sites. Although most published records refer to groundwater and hyporheic habitats (Erséus *et al.* 1999; Wetzel & Taylor 2001), we found *R. subterraneus* in surface waters. These surface habitats, however, likely receive groundwater influences. The Robečský Brook is situated in the north-western part of the Czech Republic in semi-natural landscape with numerous wetlands and fishponds. The sample was taken downstream of the Peklo Natural Reserve, where a brook flows through a narrow sandstone gorge with substrate formed of mud (40%), sand (30%) and gravel (30%). On the contrary, the second sampling site is situated downstream of Brno, the second largest city in the country, in the straightened and dyked Svitava River stretch (substrate: 20% mud, 20% sand, 30% gravel and 30% stones), which also serves as a recipient of sewage water from urban settlements.

Ecology. *Rhyacodrilus subterraneus* occurs in fresh and brackish water (Erséus *et al.* 1999); in reference to its name, especially in interstitial hyporheic zones and wet soil. Many records were reported from deeper hyporheic habitats (Strayer 2001) and caves (Wetzel & Taylor 2001; Ferreira *et al.* 2007); nevertheless, it was also found in species-rich oligochaete assemblages in eutrophic streams (Timm *et al.* 1997). Reproduction in this species is sexual (Timm & Veldhijzen van Zanten 2002), but in some regions (United States) only immature specimens have been found (Wetzel & Taylor 2001).

Morphology. *Rhyacodrilus subterraneus* can be distinguished from its congeners by straighter and two times longer upper tooth of anterior dorsal chaetae with intermediate teeth, and two times longer upper tooth in anterior ventral chaetae. Penial chaetae near male pores in XI segment of mature specimens are only single with a simple curved tip (Timm & Giani 2004).

Distribution. Prior to records collected during this present study, this rare Holarctic species had been found only in western European countries (France, Germany, Norway, Sweden, Spain, and The Netherlands; Timm & Giani 2004), and in the eastern and Midwestern United States (Strayer & Bannon-O'Donnell 1988; Strayer 2001; Wetzel & Taylor 2001; Kathman & Brinkhurst 1998).

Subfamily: Tubificinae

Aulodrilus limnobius Bretscher, 1899

Records. Gránický Brook, Znojmo, *48°51'60"N / 16°01'33"E*, lgt. PP, det. PP. (2005); Drietomice River, Starý Hrozenkov, *48°57'13"N / 17°52'29"E*, lgt. PP, det. PP (2005); Trusovický Brook, Jívová, *49°42'27"N / 17°21'40"E*, lgt. KB, det. PP; Olešná River, Zvole, *49°29'26"N / 16°09'44"E*, lgt. KB, det. PP (2002); Nectava River, Březinky, *49°39'24"N / 16°46'52"E*, lgt. KB, det. PP (2002); Okluky River, Uherský Ostroh, *48°59'38"N / 17°24'07"E*, lgt. H, det. PP (2002); Farský Brook, Trhové Sviny, *48°50'24"N / 14°37'55"E*, lgt. JZ, det. PP (2002); all specimens were immature.

Characteristics of sites. All records were from small and middle-sized brooks (3rd and 4th Strahler order, river width up to 5 m) with a bottom substrate dominated by sand, gravel, and stones. Most of these highland stretches had a natural morphology (with buffer strips) and extensively used (cropland, grassland), partially forested catchments. The brooks have good water quality (from oligo- to beta-mesosaprobity), with one exception of lowland stream (Okluky River, alpha-mesosaprobity).

Ecology. Being a detritophagous species, *A. limnobius* occurs from hyporhithral to potamal, and also has been collected from standing water areas including deeper, profundal habitats (Hörner *et al.* 2002; Šporka 2003; Alves *et al.* 2008). It can tolerate intermediate eutrophication (Verdonschot 2006) and organic pollution from oligo- to alpha-mesosaprobity (Hörner *et al.* 2002). Microhabitat preferences of fine substrates rich in organic material (pelal, psamal and argylal) have been reported (Šporka 2003; Alves *et al.* 2008). Among oligochaetes, *A. limnobius* belongs to K-strategists (Šporka 2003) with one reproductive cycle per year, mostly realised by asexual reproduction (architomy); mature specimens are rare (Timm & Veldhijzen van Zanten 2002). Worms burrow in sediment, where they build reinforced tubes of silt (Timm & Veldhijzen van Zanten 2002).

Morphology. *Aulodrilus limnobius* has characteristic bifid crotchets, with up to 10 chaetae per bundle (all with shorter upper teeth, and occasionally with wing-like dilations of the distal ends of the chaetae in posterior segments), and an unsegmented tail, serving as a respiratory organ—typical for this genus. It can be distinguished from its congeners known to occur in the Czech Republic—*A. pigueti, A japonicus*, and *A. pluriseta*—by a lack of hair chaetae (Timm & Veldhijzen van Zanten 2002; van den Hoek & Verdonschot 2005).

Distribution. This cosmopolitan species has been recorded in most of European countries, absent only in Austria, the UK, Croatia, Denmark, Greece, Slovenia, and Portugal (Timm & Giani 2004). According to Fauna Europaea (Timm & Giani 2004), the species was found also in the Czech Republic, but unfortunately, the source of this data is not available.

Aulodrilus pigueti Kowalewski, 1914

Records. Štěpánek Pond, Pozďatín, *49°13'22"N / 16°02'25"E*, lgt. JS, det. KP (2007); the single specimen collected during this study was immature.

Characteristics of sites. *Aulodrilus pigueti* was found at a single locality, the Štěpánek fishpond in the Czech-Moravian Highland Mountains (Českomoravská vrchovina), which is situated in the middle part of the Czech Republic. This fishpond is a small shallow pond (2.2 ha) exploited by intensive fish farming. The management of the fish stock composed of *Cyprinus carpio* Linnaeus and *Tinca tinca* Linnaeus involved supplementary feeding and application of manure, which caused nutrient enrichment. Macrophytes, mostly *Glyceria maxima* (Hartman) and *Typha latifolia* Linnaeus, covered only 5% of the littoral zone.

Ecology. *Aulodrilus pigueti* burrows in sediments where it forms tubes from detritus (Timm & Veldhijzen van Zanten 2002). Finogenova and Arkhipova (1994), Schloesser *et al.* (1995) and Šporka (1996b) reported that *A. pigueti* inhabits various freshwater environments, such as lakes, rivers, and marshes. It prefers water quality from oligo- to alpha-mesosaprobic and substrate pelal (Šporka 2003), and tolerates a lower amount of dissolved oxygen and acidification (Orciari & Hummon 1975; report EPA/600/3-90/073). The sample from which this single specimen of *A. pigueti* was identified was dominated by *Dero digitata* (Müller, 1774), more species in the genus *Limnodrilus*, and other Tubificinae.

Morphology. *Aulodrilus pigueti* can be distinguished from all other taxa by distal dilatations of bifid crotchets with shorter upper teeth (typical for the genus *Aulodrilus*). Dorsal crotchets beginning from VI–IX are replaced by oar-shaped pectinate chaetae with a rounded or sometimes slightly bifid tip. It is possible to distinguish it from congeners and all other Tubificinae by the presence of oar-shaped chaetae in dorsal bundles in its middle segments.

Distribution. *Aulodrilus pigueti* is a cosmopolitan species, often found in tropical countries (Timm & Veldhijzen van Zanten 2002; Arslan & Sahin 2003). The nearest record to the Czech Republic is from backwaters of the Morava River in Slovakia (Šporka 1996b); it has also been recorded from the neighbouring countries of Germany and Poland (Timm & Giani 2004). While *A. pigueti* is widely distributed in many other European countries it has not been reported from Austria, Hungary, Romania, Belgium, the UK, Denmark, Latvia, Switzerland, and Slovenia (Timm & Giani 2004).

Conclusions

This updated check-list of aquatic oligochaetes of the Czech Republic, based on historical and recent records, includes 95 species: Lumbriculidae (12 species), Naidinae (36 species), Pristininae (eight species), Tubificinae (22 species), Rhyacodrilinae (six species), Propappidae (one species), Criodrilidae (one species), Haplotaxidae (one species), and Lumbricidae (8 species). The Red list of Czech aquatic oligochaete species has been corrected and updated, and now includes nine endangered species (EN), 14 vulnerable species (VU), and one near threatened (NT). Special attention should be given to the protection and conservation of unique habitats from which these endangered and vulnerable species have been reported. In addition, it is important to establish and support monitoring programs for all aquatic habitats to document introduced and invasive species that are known to be spreading throughout Europe and elsewhere worldwide.

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DATA DESCRIPTOR

DISPERSE, a trait database to assess the dispersal potential of European aquatic macroinvertebrates

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Dispersal is an essential process in population and community dynamics, but is difficult to measure in the field. In freshwater ecosystems, information on biological traits related to organisms' morphology, life history and behaviour provides useful dispersal proxies, but information remains scattered or unpublished for many taxa. We compiled information on multiple dispersal-related biological traits of European aquatic macroinvertebrates in a unique resource, the DISPERSE database. DISPERSE includes nine dispersal-related traits subdivided into 39 trait categories for 480 taxa, including Annelida, Mollusca, Platyhelminthes, and Arthropoda such as Crustacea and Insecta, generally at the genus level. Information within DISPERSE can be used to address fundamental research questions in metapopulation ecology, metacommunity ecology, macroecology and evolutionary ecology. Information on dispersal proxies can be applied to improve predictions of ecological responses to global change, and to inform improvements to biomonitoring, conservation and management strategies. The diverse sources used in DISPERSE complement existing trait databases by providing new information on dispersal traits, most of which would not otherwise be accessible to the scientific community.

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Background & Summary

Dispersal is a fundamental ecological process that affects the organization of biological diversity at multiple temporal and spatial scales[1,2]. Dispersal strongly influences metapopulation and metacommunity dynamics through the movement of individuals and species, respectively[3]. A better understanding of dispersal processes can inform biodiversity management practices[4,5]. However, dispersal is difficult to measure directly, particularly for small organisms, including most invertebrates[6]. Typically, dispersal is measured for single species[7,8] or combinations of few species within one taxonomic group[9–11] using methods based on mark and recapture, stable isotopes, or population genetics[5,12]. Such methods can directly assess dispersal events but are expensive, time-consuming, and thus impractical for studies conducted at the community level or at large spatial scales. In this context, taxon-specific biological traits represent a cost-effective alternative that may serve as proxies for dispersal[5,6,13,14]. These traits interact with landscape structure to determine patterns of effective dispersal[15,16].

Aquatic macroinvertebrates inhabiting freshwater ecosystems include taxa with diverse dispersal modes and abilities (Fig. 1). For species with complex life cycles, such as some insects, this diversity is enhanced by life stages with different dispersal strategies. For example, aquatic juveniles of many insects disperse actively and/or passively in water whereas adults fly over land[17]. In all cases, dispersal is affected by multiple traits relating to the morphology[6,12], life history and behaviour[2] of different life stages.

We compiled and the harmonized information on dispersal-related traits of freshwater macroinvertebrates from across Europe, including both aquatic and aerial (i.e. flying) stages. Although information on some dispersal-related traits such as body size, reproduction, locomotion and dispersal mode is available in online databases for European[18–20] and North American taxa[21], other relevant information is scattered across published literature and unpublished data. Informed by the input of 19 experts, we built a comprehensive database containing nine dispersal-related traits subdivided into 39 trait categories for 480 European taxa. Dispersal-related traits were selected and their trait categories fuzzy-coded[22] following an approach comparable to that used to develop existing databases[23]. Our aim was to provide a single resource facilitating the incorporation of dispersal into ecological research, and to create the basis for a global dispersal database.

Methods

Dispersal-related trait selection criteria.

We defined dispersal as the unidirectional movement of individuals from one location to another[1], assuming that population-level dispersal rates depend on both the number of dispersing propagules and dispersers' ability to move across a landscape[11,24].

We selected nine dispersal-related morphological, behavioural and life-history traits (Online-only Table 1). Selected morphological traits were maximum body size, female wing length and wing pair type, the latter two relating only to flying adult insects. Maximum body size influences organisms' dispersal[6], especially for active dispersers[25], with larger animals more capable of active dispersal over longer distances (e.g. flying adult dragonflies[6], Fig. 1). Wing morphology, and in particular wing length, is related to the dispersal of flying adult insects[6,26]. Female wing length was selected because females connect and sustain populations through oviposition, thus representing adult insects' colonization capacity[27]. Females with larger wings are likely to oviposit farther from their source population[6,10,28]. We also described insect wing morphology as wing pair types, i.e. one or two pairs of wings, and the presence of halters, elytra or hemielytra, or small hind wings[12] (Fig. 1). Selected life-history traits were adult life span, life-cycle duration, annual number of reproductive cycles and lifelong fecundity. Adult life span and life-cycle duration respectively reflect the adult (i.e. reproductive) and total life duration, with longer-lived animals typically having more dispersal opportunities[13]. The annual number of reproductive cycles and lifelong fecundity assess dispersal capacity based on potential propagule production, with multiple reproductive cycles and abundant eggs typically increasing the number of dispersal events[6]. Dispersal behaviour was represented by a taxon's predominant dispersal mode (passive and/or active, aquatic and/or aerial), and by its propensity to drift, which indicates the frequency of flow-mediated passive downstream dispersal events.

Data acquisition and compilation.

A taxa list was generated based on the taxonomies used in existing European aquatic invertebrate databases[18,20]. Trait information was sourced primarily from the literature using Google Scholar searches of keywords including trait names, synonyms and taxon names (Supplementary File 1, Table S1), and by searching in existing databases[18,21]. Altogether, >300 peer-reviewed articles and book chapters were consulted. When no European studies were available, we considered information from other continents only if experts considered traits as comparable across regions. When published information was lacking, traits were coded based on authors' expert knowledge and direct measurements. Specifically, for 139 species in 69 genera of Coleoptera and Heteroptera, female wing lengths were characterized using measurements of 538 individuals in experts' reference collections, comprising organisms sampled in Finland, Greece and Hungary. The number of species measured within a genus varied between 1 and 10 in relation to the number of European species within each genus. For example, for the most species-rich genera, both common and rare species from northern and southern latitudes were included.

Fuzzy-coding approach and taxonomic resolution.

Traits were coded using a 'fuzzy' approach, in which a value given to each trait category indicates if the taxon has no (0), weak (1), moderate (2) or strong (3) affinity with the category[22]. Affinities were determined based on the proportion of observations (i.e. taxon-specific information from the literature or measurements) or expert opinions that fell within each category for each trait[29]. Fuzzy coding can incorporate intra-taxon variability when trait profiles differ among e.g. species within a genus, early and late instars of one species, or individuals of one species in different environments[29]. Most traits were coded at genus level, but some Diptera and Annelida were coded at family, sub-family or tribe level because of their complex taxonomy, identification difficulties and the scarcity of reliable information about their traits.

Fig. 1 The dispersal-related trait diversity of aquatic macroinvertebrates. Taxa that disperse in water include the *crustacean* genera *Potamon* (**a**) and *Asellus* (arrow in **b**), planarians (**b**), the bivalve mollusc genus *Unio* (**c**), insect larvae such as the Diptera genus *Simulium* (**d**) and Plecoptera genus *Leuctra* (**e**), and adult Coleoptera including the dytiscid genus *Cybister* (**f**). Such aquatic dispersers may move passively in the drift (**c**,**d**) and/or actively crawl or swim (**a**,**b**,**e**,**f**). Most adult insects have wings and can fly overland (**f**–**n**). Wings are morphologically diverse and include various types: one wing pair, as in Diptera such as the syrphid genus *Eristalis* (**g**); one pair of wings with elytra for Coleoptera including the genus *Enochrus* (**h**) or with hemielytra for Heteroptera such as the genus *Hesperocorixa* (**i**); two wing pairs including one pair of small hind wings for Ephemeroptera including the genus *Ephemera* (**j**); and two pairs of similar-sized wings for the Trichoptera genus *Polycentropus* (**k**), the Megaloptera genus *Sialis* (*i*) and the Odonata genera *Ischnura* (**m**) and *Crocothemis* (**n**). Wings range in size from a few mm in some Diptera (**g**) up to more than 3 cm (**l**–**n**), with the Odonata exemplifying the large morphologies. Taxa vary in the number of eggs produced per female, ranging from tens per reproductive cycle for most Coleoptera and Heteroptera such as the genus *Sigara* (**o**) to several hundreds in the egg masses of most Ephemeroptera and Trichoptera, such as those of the genus *Hydropsyche* (**p**). Credits: Adolfo Cordero-Rivera (**a**–**g**,**i**,**k**–**n**), Jesús Arribas (**h**), Pere Bonada (**j**), José Antonio Carbonell (**o**) and Maria Alp (**p**).

Data Records

DISPERSE can be downloaded as an Excel spreadsheet from the Intermittent River Biodiversity Analysis and Synthesis (IRBAS) webpage (irbas.inrae.fr) and the data repository Figshare[30].

The database comprises three sheets: DataKey, Data and Reference list. The "Datakey" sheet summarizes the content of each column in the "Data" sheet. The "Data" sheet includes the fuzzy-coded trait categories and cites the sources used to code each trait. The first six columns list the taxa and their taxonomy (group; family; tribe/sub-family or genus [depending on the level coded]; genus synonyms; lowest taxonomic resolution achieved) to allow users to sort and compile information. Sources are cited in chronological order by the surname of the first author and the year of publication. Expert evaluations are reported as "Unpublished" followed by the name of the expert providing the information. Direct measurements are reported as "Direct measurement from" followed by the expert's name. The "Reference list" sheet contains the references cited in the "Data" sheet, organized in alphabetical order and then by date.

In total, the database contains nine dispersal-related traits divided into 39 trait categories for 480 taxa. Most (78%) taxa are insects, principally Coleoptera and Trichoptera, as these are, together with Diptera, the most diverse orders in freshwater ecosystems[31]. DISPERSE provides complete trait information for 61% of taxa, with

Trait	Categories
Maximum body size (cm)	<0.25
	≥0.25–0.5
	≥0.5–1
	≥1–2
	≥2–4
	≥4–8
	≥8
Female wing length (insects only) (mm)	<5
	≥5–10
	≥10–15
	≥15–20
	≥20–30
	≥30–40
	≥40–50
	≥50
Wing pair type (insects only)	1 pair + halters
	1 pair + elytra or hemielytra
	1 pair + small hind wings
	2 similar-sized pairs
Life-cycle duration	≤1 year
	>1 year
Adult life span	<1 week
	≥1 week–1 month
	≥1 month–1 year
	≥1 year
Lifelong fecundity (number of eggs per female)	<100
	≥100–1000
	≥1000–3000
	≥3000
Potential number of reproductive cycles per year	<1
	1
	>1
Dispersal strategy	Aquatic active
	Aquatic passive
	Aerial active
	Aerial passive
Propensity to drift	Rare/catastrophic
	Occasional
	Frequent

Table 1. Dispersal-related aquatic macroinvertebrate traits included in the DISPERSE database.

1–2 traits being incomplete for the 39% remaining taxa (Table 2, Fig. 2). The traits with the highest percentage of information across taxa were wing pair type and maximum body size, followed by dispersal strategy, life-cycle duration, potential number of reproductive cycles per year, and female wing length (Table 2). The percentage of completed information was lower for two life-history traits: adult life span and lifelong fecundity (Table 2).

Technical Validation

Most of the trait information (88%) originated from published literature (Supplementary File 1) and the remaining traits were coded based on expert knowledge (9%) and direct measurements (3%) (Table 2). The database states information sources for each trait and taxon, allowing users to evaluate data quality. Most traits were coded using multiple sources representing multiple species within a genus. When only one study was available, we supplemented this information with expert knowledge, to ensure that trait codes represented potential variability in the taxon.

Using insects as an example, we performed a fuzzy correspondence analysis (FCA)[22] to visualize variability in trait composition among taxa (Fig. 3). Insect orders were clearly distinguished based on their dispersal-related traits, with 32% of the variation explained by the first two FCA axes. Wing pair type and lifelong fecundity had the highest correlation with axis A1 (coefficient 0.87 and 0.63, respectively). Female wing length (0.73) and maximum body size (0.55) were most strongly correlated with axis A2 (Fig. 3 and 4). For example, female Coleoptera typically produce few eggs and have intermediate maximum body sizes and wing lengths, Odonata produce an

Trait	Taxa completed (%)	Source of information (%)		
		Literature	Expert	Measured
Maximum body size	99	100		
Female wing length	95	57	12	31
Wing pair type	100	100		
Life-cycle duration	98	100		
Adult life span	79	65	35	
Lifelong fecundity	75	77	23	
Potential number of reproductive cycles per year	98	100		
Dispersal strategy	98	100		
Propensity to drift	80	90	10	
All traits	61	88	9	3

Table 2. Percentage of taxa completed and relative contribution of different sources of information (i.e. literature, expert knowledge, direct measurement) used to build the DISPERSE database.

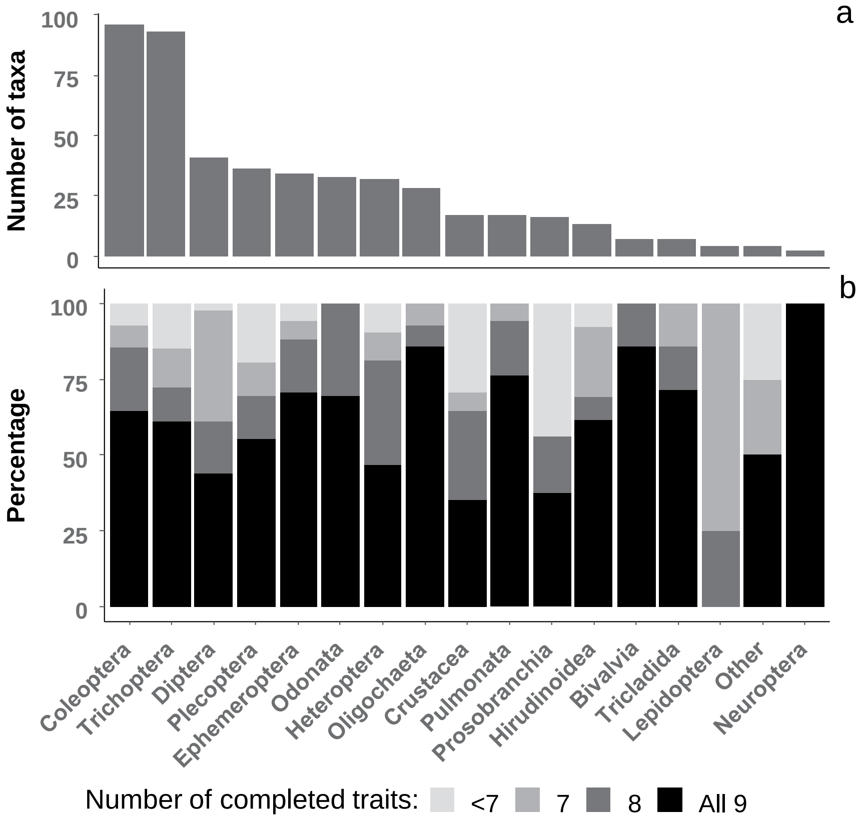

Fig. 2 Total number of taxa and percentage of the nine traits completed in each insect order and macroinvertebrate phylum, sub-phylum, class or sub-class. "Other" includes Hydrozoa, Hymenoptera, Megaloptera and Porifera, for which the database includes only one genus each.

intermediate number of eggs and have long wings, and Ephemeroptera produce many eggs and have short wings (Fig. 1 and 4).

The database currently represents a Europe-wide resource which can be updated and expanded as new information becomes available, to include more taxa and traits from across and beyond Europe. For example, additional information could be collected on other measures of wing morphology[10,14] and functionality or descriptors of exogenous dispersal vectors such as wind and animals[32]. New data can be contributed by contacting the corresponding author or by completing the contact form on the IRBAS website (http://irbas.inrae.fr/contact), and the online database will be updated accordingly. DISPERSE lays the foundations for a global dispersal trait database, the lack of which is recognized as limiting research progress across multiple disciplines[33].

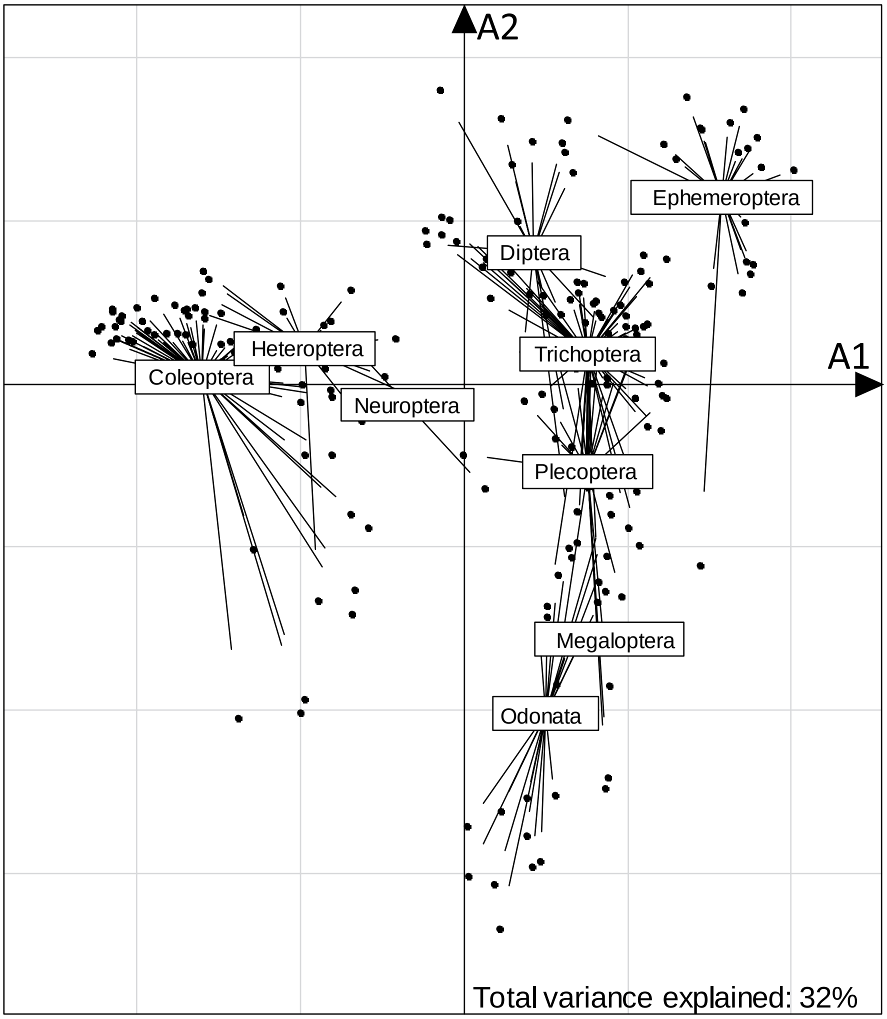

Fig. 3 Variability in the dispersal-related trait composition of all insect orders with complete trait profiles along fuzzy correspondence analysis axes A1 and A2. Dots indicate taxa and lines converge to the centroid of each order to depict within-group dispersion.

Usage Notes

DISPERSE is the first publicly available database describing the dispersal traits of aquatic macroinvertebrates and includes information on both aquatic and aerial (i.e. flying) life stages. It provides good coverage of macroinvertebrates at the genus level, which is generally considered as sufficient to capture biodiversity dynamics[34–37]. It will promote incorporation of dispersal proxies into fundamental and applied population and community ecology in freshwater ecosystems[5]. In particular, metacommunity ecology may benefit from the use of dispersal traits[15,38], which enable classification of taxa according to their dispersal potential in greater detail. Such classification, used in combination with, for example, spatial distance measurements[39,40], could advance our understanding of the effects of regional dispersal processes on community assembly and biodiversity patterns. Improved knowledge of taxon-specific dispersal abilities may also inform the design of more effective management practices. For example, recognizing dispersal abilities in biomonitoring methods could inform enhancements to catchment-scale management strategies that support ecosystems adapting to global change[41,42]. DISPERSE could also inform conservation strategies by establishing different priorities depending on organisms' dispersal capacities in relation to spatial connectivity[43].

DISPERSE could also improve species distribution models (SDMs), in which dispersal has rarely been considered due to insufficient data[13], limiting the accuracy of model predictions[44,45]. Recent trait-based approaches have begun to integrate dispersal into SDMs[45], and information from DISPERSE could increase model accuracy[46,47]. Including dispersal in SDMs is especially relevant to assessments of biodiversity loss and species vulnerability to climate change[46,48,49]. DISPERSE could also advance understanding of eco-evolutionary relationships and biogeographical phenomena. In an evolutionary context, groups with lower dispersal abilities should be genetically and taxonomically richer due to long-term isolation[50,51]. From a biogeographical perspective, regions affected by glaciations should have species with greater dispersal abilities, enabling postglacial recolonization[52].

By capturing different dispersal-related biological traits, DISPERSE provides information on organisms' potential ability to move between localities as well as on reproduction and recruitment[15]. Traits also facilitate

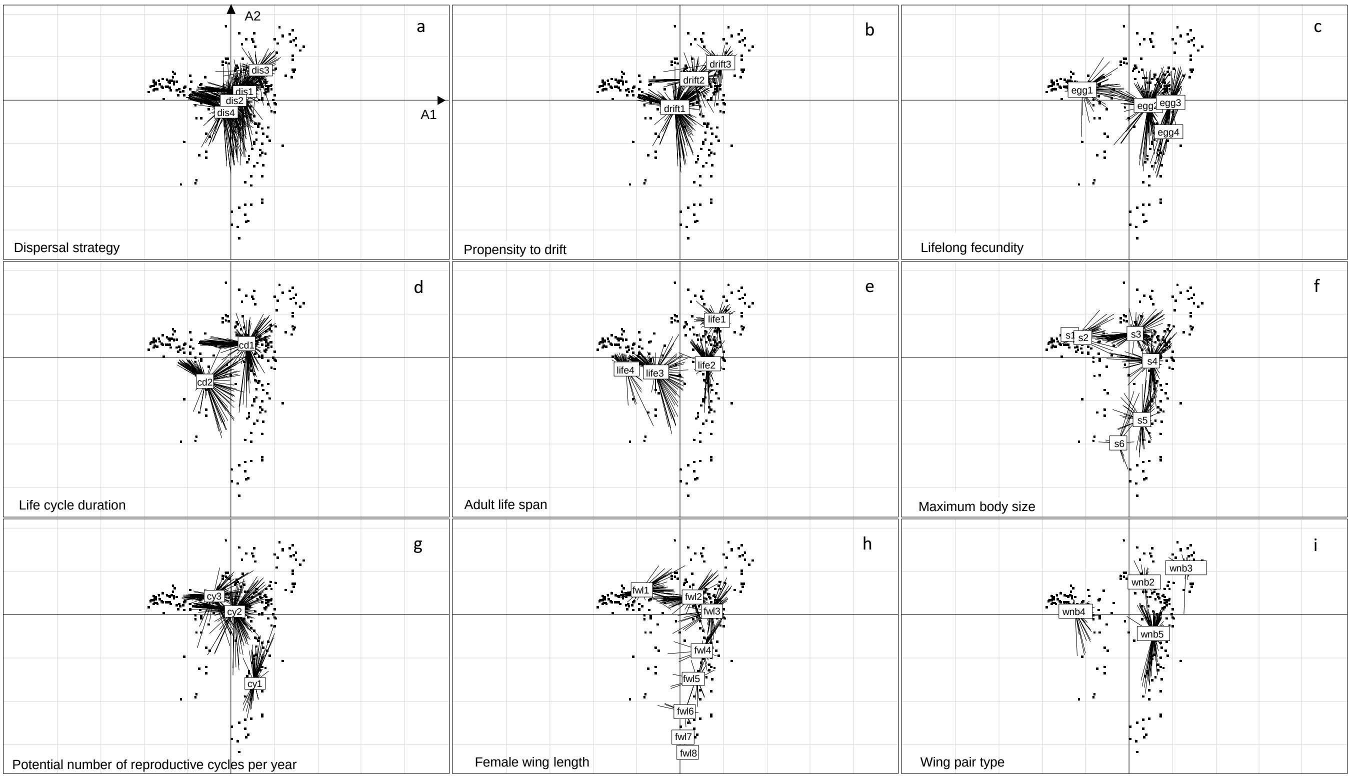

Fig. 4 Trait category locations in the fuzzy correspondence analysis ordination space for each trait of all insect orders with complete trait profiles: (**a**) Dispersal strategy = **dis1**: aquatic passive, **dis2**: aquatic active, **dis3**: aerial passive, **dis4**: aerial active; (**b**) Propensity to drift = **drift1**: rare/catastrophic, **drift2**: occasional, **drift3**: frequent; (**c**) Fecundity = **egg1**: < 100 eggs, **egg2**: ≥ 100–1000 eggs, **egg3**: 1000–3000 eggs, **egg4**: ≥ 3000 eggs; (**d**) Life-cycle duration = **cd1**: ≤ 1 year, **cd2**: > 1 year; (**e**) Adult life span = **life1**: < 1 week, **life2**: ≥ 1 week – 1 month, **life3**: ≥ 1 month – 1 year, **life4**: > 1 year; (**f**) Maximum body size (cm) = **s1**: < 0.25, **s2**: ≥ 0.25–0.5, **s3**: ≥ 0.5–1, **s4**: ≥ 1–2; **s5**: ≥ 2–4, **s6**: ≥ 4–8; (**g**) Potential number of reproductive cycles per year = **cy1**: < 1, **cy2**: 1, **cy3**: > 1; (**h**) Female wing length (mm) = **fwl1**: < 5, **fwl2**: ≥ 5–10, **fwl3**: ≥ 10–15, **fwl4**: ≥ 15–20, **fwl5**: ≥ 20–30, **fwl6**: ≥ 30–40, **fwl7**: ≥ 40–50, **fwl8**: ≥ 50; (**i**) Wing pair type = **wnb2**: 1 pair + halters, **wnb3**: 1 pair + 1 pair of small hind wings, **wnb4**: 1 pair + 1 pair of elytra or hemielytra, **wnb5**: 2 similar-sized pairs.

comparison of taxa with different dispersal strategies, which could inform studies conducted at large spatial scales, independent of taxonomy[53].

Users should note that the dispersal-related traits included in DISPERSE represent an indirect measure of dispersal, not effective dispersal. Therefore, the database is not intended to substitute population-level studies related to dispersal, but to act as a repository that collates and summarizes information from such studies. As freshwater biodiversity declines at unprecedented rates[54,55], collecting, harmonizing and sharing dispersal-related data on freshwater organisms will underpin evidence-informed initiatives that seek to support the resilience of ecosystems adapting to global change.

Code availability

Analyses were conducted and figures were produced using the R environment[56]including the package ade4[57]. Scripts are available at Figshare[30].

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Author contributions

N.B., N.C. and R.Sa. developed the idea and data collection framework. R.Sa. compiled most of the dispersal-related trait data and structured the database. All authors contributed to the addition and checking of information included in the database, and Z.C. provided direct measurements of several taxa. A.C.-R. designed Fig. 1. M.A., M.C.-A., C.G.-C. and M.F. helped to finalize the database reference list. N.C. wrote the first draft of the manuscript, and all authors contributed to its finalization. R.St. proofread the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Part III: Reconstruction of past climate development in the Western Carpathians using recent and paleo‑communities

Paper 19: Hájková, P., **Pařil**, P., Petr, L., Chattová, B., Grygar, T. M., & Heiri, O. (2016). A first chironomid–based summer temperature reconstruction (13–5 ka BP) around 49 N in inland Europe compared with local lake development. Quaternary Science Reviews, 141, 94–111. DOI: 10.1016/j.quascirev.2016.04.001

Paper 20: Šolcová, A., Jamrichová, E., Horsák, M., **Pařil**, P., Petr, L., Heiri, O., Květoň, J., Křížek, M., Hartvich, F., Hájek, M. & Hájková, P. (2020). Abrupt vegetation and environmental change since the MIS 2: A unique paleorecord from Slovakia (Central Europe). Quaternary Science Reviews, 230: 106170. DOI: 10.1016/j.quascirev.2020.106170

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A first chironomid-based summer temperature reconstruction (13–5 ka BP) around 49°N in inland Europe compared with local lake development

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ABSTRACT:

Temperature reconstructions for the end of the Pleistocene and the first half of the Holocene based on biotic proxies are rare for inland Europe around 49°N. We analysed a 7 m long sequence of lake deposits in the Vihorlat Mts in eastern Slovakia (820 m a.s.l.). Chironomid head capsules were used to reconstruct mean July temperature (T_{July}), other proxies (diatoms, green algae, pollen, geochemistry) were used to reconstruct local environmental changes that might have affected the climate reconstruction, such as epilimnetic total phosphorus concentrations (TP), lake level changes and development of surrounding vegetation. During the Younger Dryas (YD), temperature fluctuated between 7 and 11 °C, with distinct, decadal to centennial scale variations, that agree with other palaeoclimate records in Europe such as $\delta^{18}O$ content in stalagmites or Greenland ice cores. The results indicate that the site was somewhat colder than expected from the general south-to-north YD temperature gradient within Europe, possibly because of north-facing exposition. The warmer phases of the YD were characterised by low water level or even complete desiccation of the lake (12,200–12,400 cal yr BP). At the Late-Glacial/Holocene transition T_{July} steeply increased from from 11 to 15.5 °C (11,700–11,400 cal yr BP) – the highest T_{July} for entire sequence. This rapid climate change was reflected by all proxies as a compositional change and increasing species diversity. The open woodlands of *Pinus*, *Betula*, *Larix* and *Picea* were replaced by broad-leaved temperate forests dominated by *Betula*, later by *Ulmus* and finally by *Corylus* (ca 9700 cal yr BP). At the same time, input of eroded coarse-grained material into the lake decreased and organic matter (LOI) and biogenic silica increased. The Early-Holocene climate was rather stable till 8700 cal yr BP, with temporary decrease in T_{July} around 11,200 cal yr BP. The lake was productive with a well-developed littoral, as indicated by both diatoms and chironomids. A distinct decline of T_{July} to 10 °C between 8700 and 8000 cal yr BP was associated with decreasing chironomid diversity and increasing climate moistening indicated by pollen. Tychoplanktonic and phosphorus-demanding diatoms increased which might be explained by hydrological and land-cover changes. Later, a gradual warming started after 7000 cal yr BP and representation of macrophytes, periphytic diatoms and littoral chironomids increased. Our results suggest that the Holocene thermal maximum was taking place unusually early in the Holocene at our study site, but its timing might be affected by topography and mesoclimate. We further demonstrated that temperature changes had coincided with variations in local hydrology.

1. Introduction

Recent climate changes have stimulated an intense research on past climatic variations and their impact on both biotic and abiotic ecosystem processes. Quaternary climate changes have been reconstructed using isotope composition in long ice-core or marine

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Abbreviations: LOI, loss-on-ignition; LG, Late Glacial; T_{July}, mean July temperature; TP, epilimnetic total phosphorus; YD, Younger Dryas.

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sequences (e.g. Blockley et al., 2012; Lowe et al., 2008), or, for Europe, by climate model runs driven by changes in past climate forcing factors such as variations in the North Atlantic thermohaline circulation (e.g. Renssen et al., 2012). Climatic changes, recent or pre-historic, are, however, never uniform across different regions (e.g. Heiri et al., 2014a) and spatial variability in climate dynamics may affect large-scale edaphic processes and species distribution. Regional and local climate can substantially deviate from the global models (Mayewski et al., 2004; Feurdean et al., 2014) because of specific topography and landscape settings. Fossil remains of different organisms like pollen, macrofossils, diatoms or chironomids are often used as climate proxies in local and regional reconstructions (e.g. Davis et al., 2003; Buczkó et al., 2013; Heiri et al., 2014a; Väliranta et al., 2014). Generally, they have shown that the Holocene (since ca 11,650 cal yr BP, Walker et al., 2009) is a warm period with relatively stable climatic conditions compared to Pleistocene. At the end of the Late Glacial (LG), summer temperature in Europe increased, partially as a consequence of orbitally-forced summer insolation, which in the northern Hemisphere was the highest in the Early Holocene (Laskar et al., 2004), partially due to changes in other climate forcing and amplifying factors such as greenhouse gas concentrations, ocean current changes and melting of large continental ice sheets (e.g. Clark et al., 2001; Renssen and Isarin, 2001; Menviel et al., 2011). Nevertheless, in Europe there was some variation in climate during the Holocene, even if with lower amplitude than observed in the late Pleistocene. A review of 50 globally-distributed palaeoclimatical records has shown that Holocene climate variations have been larger and more frequent than is commonly recognized (Mayewski et al., 2004). Several periods of rapid climate change (RCC) were revealed, from which two took place in the Early and Middle Holocene (9000–8000 cal yr BP, 6000–5000 cal yr BP). Most of the climate change events in these globally distributed records were characterised by polar cooling, tropical aridity, and major atmospheric circulation changes. Several abrupt short-term oscillations during the Holocene were also recorded by both, oxygen isotopes in ice-sheet cores (Blockley et al., 2012) and biotic proxies (e.g. Magny et al., 2003; Rosén et al., 2001; Davis et al., 2003; Tóth et al., 2012, 2015). The so called 8.2 ka event was the most pronounced temperature change within the Early and Middle Holocene, which was reflected by a decrease in *Corylus* pollen in the fossil record of North Europe (Seppä et al., 2005; Rasmussen et al., 2008) and less frequently also in Central Europe (Tinner and Lotter, 2001; Dudová et al., 2014). Contrary, chironomid-based reconstructions captured this event rarely (Płóciennik et al., 2011; but see Seppä et al., 2007 and Heiri et al., 2003). It is hence likely that this short-term North Atlantic cooling triggered by Laurentide ice-sheet collapse (Wiersma and Renssen, 2006) influenced regional and local summer temperatures and some types of ecosystems only locally and moreover, it appears that some biotic proxies do not consistently reflect this short-term climate oscillation.

A widely used biotic proxy for temperature reconstruction are fossil chironomids in lake sediment records. Chironomids have a rather short life-cycle and relatively high dissemination ability and therefore show a rapid response to changing environment (Brooks et al., 2007). There are numerous stenotopic species within the chironomids which can provide reliable reconstructions of the past environment. Identification is usually possible at the level of genera or species morphotypes, often with known ecological preferences. In the last 15 years, several calibration data-sets were developed for July air temperature (T_{July}) reconstruction in Eurasia (e.g. Brooks and Birks, 2001; Nazarova et al., 2011; Holmes et al., 2011; Heiri et al., 2011, 2014a). In East-Central Europe, there is a gap in knowledge on chironomid-inferred climate from the LG and Holocene periods. Further, even if chironomids are very good indicators of changes in July temperatures, some autogenic processes not triggered by climate can influence chironomid species turnover and thus distort the climate reconstruction. Typically, there is a general positive correlation between temperature and productivity, but lake productivity can increase independently of temperature because of changing nutrient concentrations and it may be difficult to separate these two influences (Velle et al., 2010). Interpretation of quantitative reconstructions should be therefore done with caution and other biotic or abiotic proxies can help to separate potential independent effects of productivity, oxygen and water level changes from climate influence (e.g. Heiri and Lotter, 2005). Geochemical analyses may serve as a proxy for catchment erosion and diatoms and green algae as reliable proxies of trophic conditions (Battarbee et al., 2001). Regional vegetation composition, reconstructed by means of fossil pollen, can characterise lake catchments in terms of potential intensity of erosion, hydrology or biogeochemistry and in addition may indicate coarse-scale climatic changes as well (Davis et al., 2003; Mauri et al., 2015). In this study we covered all these proxies to provide the first chironomid-based temperature reconstruction for inland Europe around 49°N, covering the end of the Pleistocene and the first half of the Holocene, and to compare it with reconstructed local development of the sedimentary environment.

The study site in the Vihorlat Mts (49°N) is situated between a more southerly located site with a chironomid inferred temperature reconstruction in the Eastern Carpathians (Retezat Mts., 45°N; Tóth et al., 2012, 2015) and a more northerly located site in the Polish lowland (52°N; Płóciennik et al., 2011). According to a review by Heiri et al. (2014a), there is a rather high number of sites where July air temperatures are reconstructed based on chironomids in the Alps, British islands and NW Europe, but data are almost missing for the latitude 47–52°N in East-Central Europe. Tátosová et al. (2006) and Hošek et al. (2014) provided some data on fossil chironomid assemblages, but without quantitative T_{July} reconstruction. Thus, this study fills a gap in our knowledge about past climate in East-Central Europe. Moreover, the position of the study site is transitional between oceanic and continental climate influences and thus shifts in atmospheric circulation and pressure changes, e.g. associated with variations in the predominance of North Atlantic oscillation states, may substantially have influence local climate. Combining different proxies, we aim to separate the influence of past climate changes in the study region from independent local processes like autogenic changes in productivity and lake depth. The main aims of our study were: 1) to reconstruct mean July temperatures (T_{July}) based on chironomid assemblages; 2) to reconstruct local environmental conditions and processes like lake productivity and water level changes using diatoms and green algae to control for undesired local effects in climate reconstruction and 3) to reconstruct changes in the lake surrounding using pollen and geochemical methods to detect influence of changing vegetation cover and extent of erosion.

2. Material and methods

2.1. Study site and sediment sampling

The study site named Hypkaňa is located in the westernmost part of the Eastern Carpathians, in the Vihorlat Mts in eastern Slovakia (East-Central Europe; 820 m a.s.l.; 48°54.787′ N, 22°09.814′ E; see Fig. 1). The geological bedrock is formed by neogenic andezite. The recent climate of the region is characterised by mean annual temperatures of 4–6 °C (mean in January −5–−6 °C, mean in July 14–16 °C) and mean annual precipitation of 1000–1200 mm (http://geo.enviroportal.sk/atlassr). The daily mean temperature (long time series of air temperature, 1961–1990)

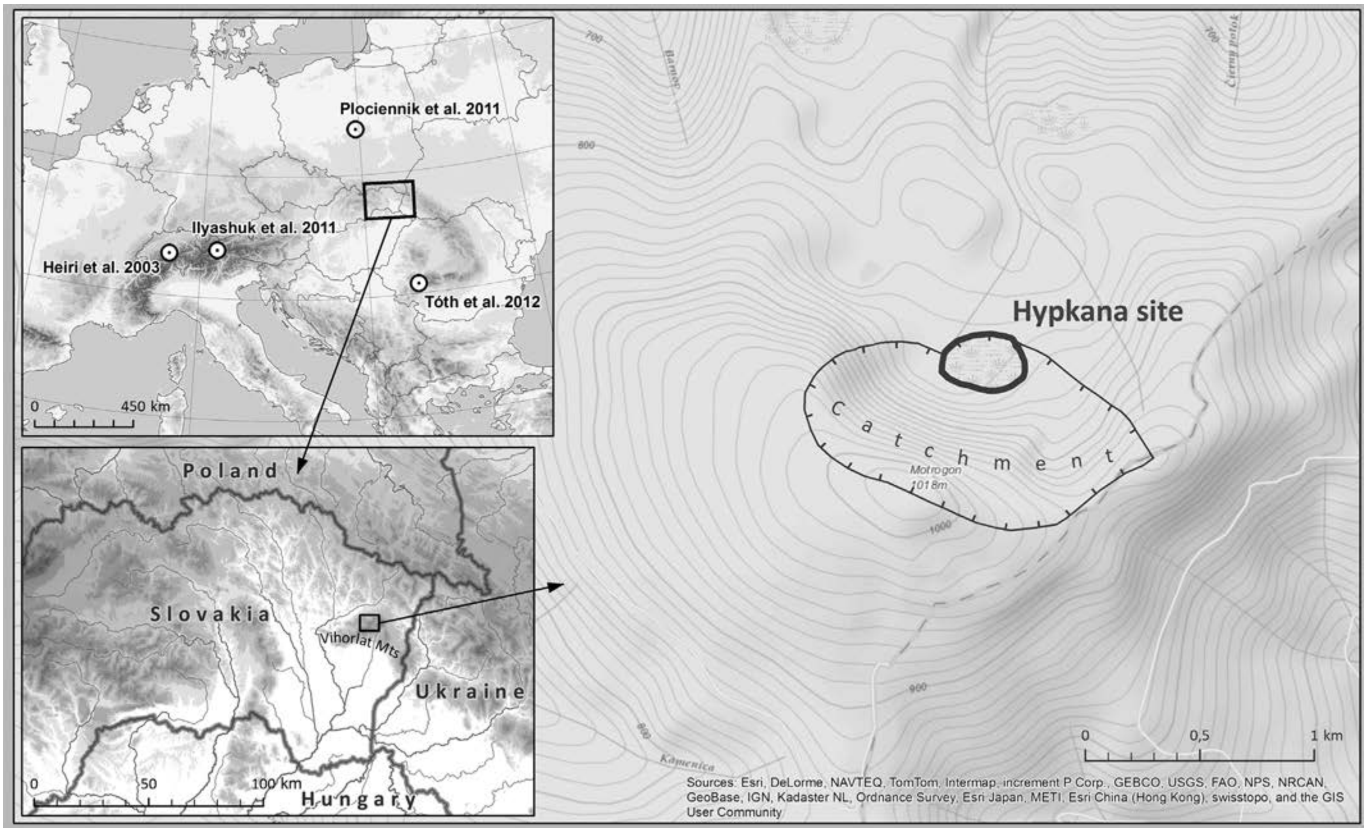

Fig. 1. Position of the study site in Europe and the Carpathian Mts and its geomorphological features and catchment size. The position of some of the sites with T_{July} reconstructions based on chironomids that are discussed in the text are indicated.

in the nearest meteorological station Kamenica nad Cirochou (178 m a.s.l., northern foothills of the Vihorlat Mts) is 18.5 °C, which corrected to 820 m a.s.l. based on a July temperature lapse rate of 0.6 °C/100 m of altitude would be equivalent to 14.7 °C. However, the real temperature of the study site is probably lower because of the northern slope position of the Motrogon Mt (1018 m a. s. l.). The study site has a small catchment area and the present-day mire of ca 2.1 ha has been a Nature Reserve since 1980. The recent vegetation is dominated by *Eriophorum vaginatum, Oxycoccus palustris, Vaccinium myrtillus* and *Molinia caerulea* in the herbaceous layer and *Sphagnum recurvum* agg., *S. magellanicum* and *Polytrichum commune* in the bryophyte layer. The surrounding landscape is overgrown by beech forests and the nearest village Zemplínske Hámre is situated 3.5 km northwards at 400 m a.s.l. The whole profile was 11.1 m deep, of which almost 8 m consisted of lake sediments suitable for chironomid and diatom analyses. The profile was obtained from the central part of the mire in the beginning of May 2012 using combination of a single gouge auger (6 cm diameter, 100 cm length) for the upper slightly decomposed peat sequence and a chamber corer (5 cm diameter, 50 cm length) for limnic sediments analysed in this study. We have sampled two parallel overlapping cores to avoid incomplete recovery.

2.2. Dating and age-depth modelling

Selected macrofossils of terrestrial plants (seeds of taxa specified in Table 1, spindles of *Eriophorum*, bryophytes, *Picea* needles) and ephippia of Cladocera were sent for AMS dating to the Centre for Applied Isotope Studies, University of Georgia, Athens, USA. The IntCal13 calibration curve was used for calibration of ^{14}C dates (Reimer et al., 2013). We obtained altogether 14 radiocarbon dates, from which 11 were used for the depth-age modelling of the entire core including the upper peat layer (see Table 1). Two dates (UG-15694, UG-15690) were excluded because they caused an age reversal and decreased the quality of the model to zero. One date (UG-15689) did not disagree with other ages, but excluding of this date was important for obtaining a reliable Bayesian model with an agreement value at least around 60% (the recommended level). The upper two excluded ^{14}C dates were obtained from macrofossils of mire vegetation (seeds of *Carex rostrata* and *Menyanthes trifoliata*) found in the lake sediment. Likely these macrofossils were transported from the upper layers (by coring or by bioturbation processes) and therefore their ^{14}C date was younger than expected. An age-depth model (Fig. 2) with 1 cm resolution based on a *P_Sequence* function with the k parameter equal to 0.5 cm^{-1} and log10(k/k0) equal to 0.3 was calculated using OxCal 4.2.4. (Bronk Ramsey, 2009). To incorporate potential changes in the sedimentation rate (e.g., contact of different types of deposits), the command *Boundary* was applied. The boundaries were placed at 955 cm (grey gyttja/brown gyttja) and at 323 cm (gyttja/peat). In the text below we use mean values of modelled data in the range of 95.4% and we rounded them to the nearest 50 year step. For the formal subdivision of the Holocene we followed Walker et al. (2012) with the Early-Middle Holocene boundary at 8200 cal yr BP and the Middle-Late Holocene boundary at 4200 cal yr BP.

2.3. Biotic proxies

Samples for pollen analysis (0–800 cm: 1 cm^3, 800–1110 cm: 0.5 cm^3) were treated by acetolysis (Faegri and Iversen, 1989). A minimum of 500 terrestrial pollen grains were counted and determined using pollen keys (Beug, 2004; Reille, 1992). The algae

Table 1

Results of ^{14}C dating (AMS method) from the sediment profile studied. The calibrated ages are median values and intervals of the calibrated 2σ range BP. Dates assigned by asterisk are excluded from the age-depth model.

Samples	Depth (cm)	Dating method	^{14}C age in uncal. BP	Cal yr BP (interval)	Cal yr BP (median)	Material
UG-17161	54–56	AMS	630 ± 25	553–662	597	Bryophytes (*Sphagnum* leaves)
UG-17162	104–106	AMS	2450 ± 25	2361–2701	2526	Spindles (*Eriophorum vaginatum*) + sphagna
UG-17163	174–176	AMS	2770 ± 25	2789–2943	2862	Spindles (*Eriophorum vaginatum*)
UG-19965	214–216	AMS	2870 ± 20	2925–3066	2988	Needles, spindles, bryophytes
UG-15688	265–270	AMS	3380 ± 25	3570–3692	3631	Seeds (*Carex rostrata*)
UG-15689*	320–325	AMS	3510 ± 25	3700–3855	3778	Seeds (*C. rostrata, Menyanthes trifoliata*)
UG-15690*	455–460	AMS	3650 ± 30	3888–4084	3986	Seeds (*Carex rostrata*)
UG-19966	540–545	AMS	6970 ± 50	7689–7930	7802	Ephippia (Cladocera)
UG-15691	700–705	AMS	7930 ± 30	8635–8978	8807	Seed (*Acer* cf. *campestre*)
UG-19968	800–805	AMS	8530 ± 35	9480–9545	9518	Ephippia (Cladocera)
UG-15692	835–840	AMS	8830 ± 30	9709–10,147	9928	Seed (*Picea abies*)
UG-15693	930–935	AMS	9980 ± 45	11,259–11,695	11,477	Ephippia (Cladocera)
UG-15694*	1035–1040	AMS	9780 ± 30	11,181–11,241	11,211	Needles (*Pinus*), seeds (*Betula, Carex* sp.)
UG-15695	1090–1095	AMS	11,020 ± 40	12,749–13,010	12,880	Needles (*Pinus*), ephippia (Cladocera)

of the genus *Pediastrum* and other chlorococcal algae were identified according to Komárek and Jankovská (2001). The nomenclature of all identified pollen types follows Beug (2004). Percentage pollen diagrams were constructed using the total sum (TS) comprising arboreal and non-arboreal pollen. Aquatic and local wetlands plants (including Cyperaceae and *Alnus*), algae and other non-pollen palynomorphs were excluded from the TS. Using *Lycopodium* tablets as a marker we calculated pollen and microcharcoal (fraction 0.01–0.1 mm) concentration and finally pollen and microcharcoal influx.

Sediment samples for chironomid analysis (2–3.5 g of wet weight; 0.8–2.8 g of dry weight) were deflocculated for 20 min in 10% KOH solution (60–75 °C) and then passed through 250 and 100 μm sieves. The chironomid capsules were hand sorted under a stereomicroscope (20–40× magnification) and only specimens consisting of more than half of the mentum were counted. The wet sediments were dried to a constant weight and the number of chironomid remains was calculated to 1 g of dry sediment and identified using Brooks et al. (2007). In all sorted layers (excluding depth 1030–1035 cm) the number of 50 head capsules was reached, which is recommended as a minimum count for the calculation of temperature reconstructions (Heiri and Lotter, 2001). Thus this single layer was excluded from T_{July} reconstruction. Reconstructed T_{July} was re-calculated to T_{July} in 0 m a.s.l. for better comparison with other reconstructions in the literature based on July temperature lapse rates of 0.6 °C/100 m (see e.g. Heiri et al., 2014a). Selected chironomid taxa were classified into ecological categories according to demands on trophic status, bathymetric distribution and preference of macrophytes using relevant literature (Wiederholm, 1983; Brooks et al., 2007; see also Appendix A).

Diatom samples were prepared following the method described in van der Werf (1955). Small quantities of the samples were cleaned by adding 37% H_2O_2 and heating to 80 °C for about 1 h. The reaction was completed by addition of $KMnO_4$. Following digestion and centrifugation, the resulting clean material was diluted with distilled water to avoid excessive concentrations of diatom valves that may hinder reliable observations. Known quantities of *Lycopodium* spores were added to estimate diatom concentrations. Cleaned diatom valves were mounted in Naphrax$^{®}$, a high-refractive index medium. In each sample, 400 diatom valves were identified and enumerated on random transects at 1,000× magnification using an Olympus B×50 microscope equipped with Differential Interference Contrast (Nomarski) optics. Further, diatoms were classified into five groups according their life form (see also Buczkó et al., 2013 and Appendix B): aerophytic (in subaerial and terrestrial habitats), benthic (at the bottom and shore of the lake), planktonic and tychoplanktonic (in the water column) and periphytic (attached to surfaces).

2.4. Geochemical analyses, LOI, MS

The weight percentage of organic matter was determined by means of loss-on-ignition (LOI) according to Heiri et al. (2001) and Holliday (2004) in each sample. The samples were dried at 105 °C for 24 h, and the combustion at 550 °C took 3 h. Magnetic susceptibility (MS) was determined using a Kappabridge KLY-2 device (Agico, Czech Republic). The results were normalized to get mass-specific magnetic susceptibility in $m^3 \cdot kg^{-1}.10^{-9}$. Magnetic susceptibility provides information about input of eroded clastic sediments (e.g. Shakesby et al., 2007). X-ray fluorescence analysis (EDXRF) of geochemical properties of rocks and soils was carried out using a PANalytical MiniPal4.0 spectrometer with a Peltier-cooled silicon drift energy-dispersive detector. The samples were powdered by agate pestle and mortar and put into measuring cells with a Mylar foil bottom without any further pre-treatment. The analyses were not calibrated and signal counts per second (c.p.s.) of individual elements were evaluated (Grygar et al., 2010). The XRF analytical signal is proportional to element concentrations (Matys Grygar et al., 2014), however, the calibration of this simple XRF setup was not performed: it would depend on matrix effects (element composition, grain size, mean organic matter content), especially in the case of light elements (Al and Si). Matrix effects for such non-destructive XRF analyses are best corrected by using ratios of element signals, such as Zr/Rb or Al/Si.

Because of variable and mostly very high biogenic silica content, the Al/Si ratio, otherwise a versatile proxy of sediment grain size (Grygar et al., 2010; Bouchez et al., 2011) could not be used as it reflects contributions of biogenic silica as well as the grain size trends. The Zr/Rb ratio, another grain size proxy (Jones et al., 2012), can be used to evaluate relative proportions of coarse silt or the finest sand (the typical grain size of zircons, Bouchez et al., 2011) relative to other clastic components, but with negligible influence by autochthonous components. Because in clastic components Zr and Si usually correlate due to their prevalence in coarser size fractions (zircons and quartz), we used Si/Zr as proxy for the relative ratio of biogenic silica to detritic clastics with little lithogenic influence. The Rb/K ratio was used as a proxy for the intensity of chemical weathering, because although both elements are mobilized by chemical weathering, Rb is more strongly retained in clay minerals (illite and smectite) and, hence, in surface sediments it is enriched by chemical weathering (Hu and Gao, 2008).

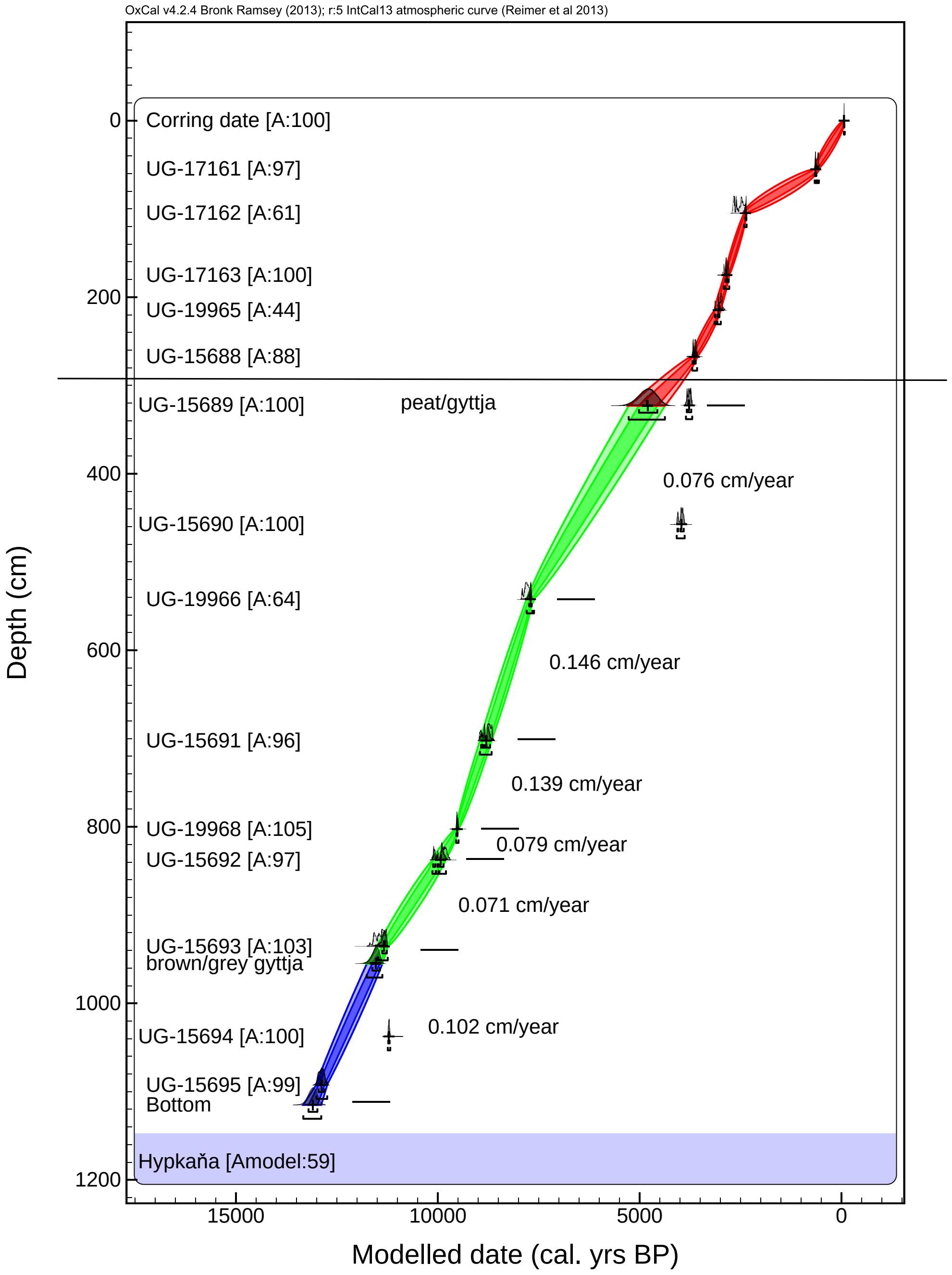

Fig. 2. Age-depth model based on 11 radiocarbon dates. Three dates were excluded from the final model to obtain a reliable Bayesian model with the maximal possible agreement value, which reached 59% between calibrated and modelled dates. The command *Boundary* was used to incorporate sedimentary boundaries, which might change sedimentation rate. Sedimentation rate values are given on the right site of the age-depth curve. The horizontal line indicates the gyttja/peat boundary.

2.5. Data analyses

Species stratigraphic diagrams of chironomids and diatoms as well as a diagram of chemical sediment composition were created using the C2 software (Juggins, 2007). Both, diatom and chironomid counts were converted to percentage data. The pollen percentage diagram was plotted using the Tilia v. 1.7.16 (Grimm, 2011) software. The zonation in pollen, diatom and chironomid diagrams is a result of Coniss cluster analyses with square root transformation of data. To analyse changes in the total species composition of pollen, diatoms and chironomids we used detrended correspondence analyses (DCA) in the Canoco software (ter Braak and Šmilauer, 2002) with down-weighting of rare species, logarithmic transformation of species data and detrending by segments. The length of gradient was 2.14 standard deviation units (SD) for diatoms, 2.08 SD for chironomids and 2.22 by SD for pollen. The variation explained by the first axis was 17.2% for diatoms, 16.1% for chironomids and 28.6% for pollen.

We used an inference model for chironomid-based temperature reconstruction calculated from a Swiss-Norwegian chironomid calibration dataset (Heiri et al., 2011) in order to infer T_{July}. This calibration dataset has been formed by amalgamating two local calibration datasets from Switzerland (Heiri and Lotter, 2010) and Norway (Brooks and Birks, 2001). Altogether, 60 chironomid taxa from the fossil data were used for T_{July} reconstruction. Cold-demanding *Derotanypus* sp. was abundant in some Late-Glacial layers of the sediment record. In the Swiss calibration dataset head capsules of *Derotanypus* have not been differentiated from other Tanypodinae larvae belonging to *Macropelopia*, *Apsectrotanypus* and *Psectrotanypus*, since all these groups were very rare (max. abundance 2.6%) and often missed glossae, paraglossae and other diagnostic features. Therefore these taxa are also grouped in the Swiss-Norwegian calibration dataset (Heiri et al., 2011). In the absence of other options we assigned *Derotanypus* in the Hypkaňa record to the category *Apsectrotanypus/Derotanypus/Macropelopia/Psectrotanypus* in the calibration data. T_{July} estimates were based on weighted averaging partial least squares (WA-PLS) regression and calibration of square-root- transformed chironomid percentage data. A bootstrapped (cross-validated) root mean squared error of prediction (RMSEP) was 1.4 °C and r^2 0.87 (Heiri et al., 2011).

125 diatom species from the fossil data, which were present also in the calibration datasets, were used to infer epilimnetic total phosphorus (TP) concentrations using diatom-water chemistry transfer functions (Juggins, 2001) based on a combined European diatom data-base (EDDI; http://craticula.ncl.ac.uk/Eddi/jsp). The modern diatom calibration set consists of 477 samples and covers a range of 2–1189 μg TP L^{-1}. The weighted averaging method (WA) and log transformed TP values were used for reconstruction. A jackknifed RMSEP was 0.33 log TP, r^2 0.64, mean bias 0.002 and maximum bias 0.72 log TP. For reconstruction, we used square-root-transformed diatom percentage data.

3. Results and interpretations

3.1. Chronology and sediment description

Using 11 radiocarbon dates we obtained a reliable depth-age model (Fig. 2), which reached the agreement value of 59% between calibrated and modelled values. The sedimentation rate in the lake part of the profile was relatively stable and linear, ranging between 0.07 cm yr^{-1} (in the depth of 935–802 cm and 542–323 cm) and 0.15 cm yr^{-1} (in the depth of 702–542 cm). The error values varied mostly between 50 and 100 years, being only higher (120–220 years) in the depth of 280–500 cm.

The lake sediment (gyttja) accumulated from the LG (ca. 13,000 cal yr BP) up to ca. 4800 cal yr BP. The bottom layer (1115–1037 cm) consisted of light greyish-brown gyttja, the layer 1037–1031 cm of light grey gyttja with admixture of sand, the layer 1031–1002 cm of greyish-brown gyttja with small inorganic admixture with exception of 1025–1022 cm, which was more dark and organic. The layer 1002–955 cm was built up by light greyish-brown gyttja. At 955 cm there was a gradual transition from grey to brown gyttja. The zone 955–920 cm was characterised by alternation of dark and light brown layers. The layer 920–720 cm was built by dark brown gyttja and between 720 and 705 cm there was a gradual transition to brown gyttja (705–531 cm) and light brown gyttja (531–323 cm). For more details see Appendix C.

3.2. Reconstruction of mean July temperature

Pollen analysis confirmed the age depth model for the site and suggested that the record encompassed the entire Younger Dryas (YD) period. The Allerød/YD transition is characterised by a *Betula* pollen decrease, whereas the YD/Early Holocene transition is very clearly distinguished by distinct increase of *Betula* pollen and steep decrease of *Pinus* pollen. Based on the Swiss-Norwegian calibration dataset and inference model for chironomid-based temperature reconstruction, the reconstructed T_{July} oscillated between 7 and 11 °C in the LG (11.8–15.9 °C if corrected to modern sea level; Fig. 3). The lowest T_{July} values were reconstructed at the end of the Allerød period (ca. 13,050–12,950 cal yr BP; 6.9–7.3 °C), at ca. 12,500 cal yr BP (8.5 °C) and at ca. 12,000 cal yr BP (7.6 °C, last cooling). Periods of relatively high temperatures were reconstructed for 12,850–12,600 (9.2–11 °C) and 12,200–12,400 cal yr BP (10.1–10.9 °C). At the end of the YD before the LG/Holocene transition, the first warming up to 10.8 °C was reconstructed (dated to ca. 11,900 cal yr BP in our record). The next warming can be already attributed to the LG/Holocene transition. At 11,600 cal yr BP T_{July} increased to 13.7 °C and at 11,400 cal yr BP to 15.5 °C, which was the highest reconstructed value of T_{July} within the whole Early and Middle Holocene in the study site, although large sections of the interval 11,000–8700 cal yr BP were characterised by very similar temperature values. These temperatures were also higher than recent (1961–1990) mean daily July temperature at the elevation of the study site (14.7 °C). Comparing the course of Holocene temperatures, a distinctly cooler phase was reconstructed between 8700 and 8000 cal yr BP (from 13.9 °C to 10.1–11.1 °C) and at about 7000 cal yr BP (from 12.2 °C to 10.2 °C).

3.3. Lake development in the Late Glacial

Radiocarbon dating suggests that the lake originated at the end of the Allerød interstadial due to landslide activity, which created a dam on the small brook discharging on the hill slopes. Higher abundance of *Betula, Ulmus* and *Quercus* pollen rather confirm this age of origin, however, the rest of pollen spectra do not differ substantially from that typical for YD vegetation. Therefore it may also be possible that the oldest sediment layers originate from the earliest section of the YD interval. The Greenland ice core records indicate that the YD interval started around 12850 cal yr BP which overlaps with our ^{14}C age, but the accuracy of age-depth model in this section is only ca 100 years. In the LG, the lake was a shallow pond as is indicated by chironomids and diatoms (Figs. 4 and 5, zones Hd-1a, 1110–1085 cm, Hch-1a, 1115–1080 cm; 13,100–12,750 cal yr BP). Chironomid assemblages were composed of oligotrophic and cold-demanding taxa such as *Tanytarsus lugens*-type and *Derotanypus* sp., pioneering taxa such as *Corynocera ambigua-type*, semiterrestrial taxa (*Limnophyes* sp.) and chironomids from nearby streams and terrestrial environments (*Georthocladius* sp., *Pseudoorthocladius* sp.). Diatoms were represented by small benthic forms of the genera *Staurosira, Pseudostaurosira and Staurosirella,* which are pioneer species typical for cold oligotrophic lakes with frequent ice cover. Planktonic species (*Asterionella formosa*, *Fragilaria crotonensis*, *Stephanodiscus dubius*) were continually increasing in this developmental zone (Figs. 5 and 6) possibly reflecting increasing water level (cf. also increasing *Tanytarsus lugens*-type, *Chironomus anthracinus*-type). Oligotrophic algae taxa such as *Pediastrum integrum* and *Pediastrum kawraiskyi* were typical for the initial zone (Fig. 6). Around the shallow lake, wetland vegetation dominated by Cyperaceae and *Sphagnum* species developed (Fig. 7, Hp-1). In the next zone (Hd-1b, 1080–1040 cm; Hch-1b; 1075–1040 cm; 12,700–12,400 cal yr BP) evidence suggests that the water depth was continually decreasing and planktonic diatoms were again substituted by small benthic species (*Pseudostaurosira brevistriata*, *Opephora mutabilis*, *Staurosira construens* var. *venter*). At about 1040 cm terrestrial species like *Pinnularia obscura*, *Pinnularia borealis*, *P. schoenfelderii* and *Microcostatus* cf. *kraskei* indicate dry conditions. Cold-demanding species with high demands for oxygen which can also colonize running waters, occupied (but in low abundances) probably the shallow water of the cold lake or nearby streams (*Heterotrissocladius marcidus-type, Stempellinella* sp.). The next sediment layer (zones Hd-2 + Hch-2, samples in 1030 cm and 1030–1035 cm, respectively, ca 12,300 cal yr BP) suggests total lake desiccation which is indicated by a sole presence of (semi)terrestrial *Limnophyes* sp. with a single head capsule, a species-poor terrestrial

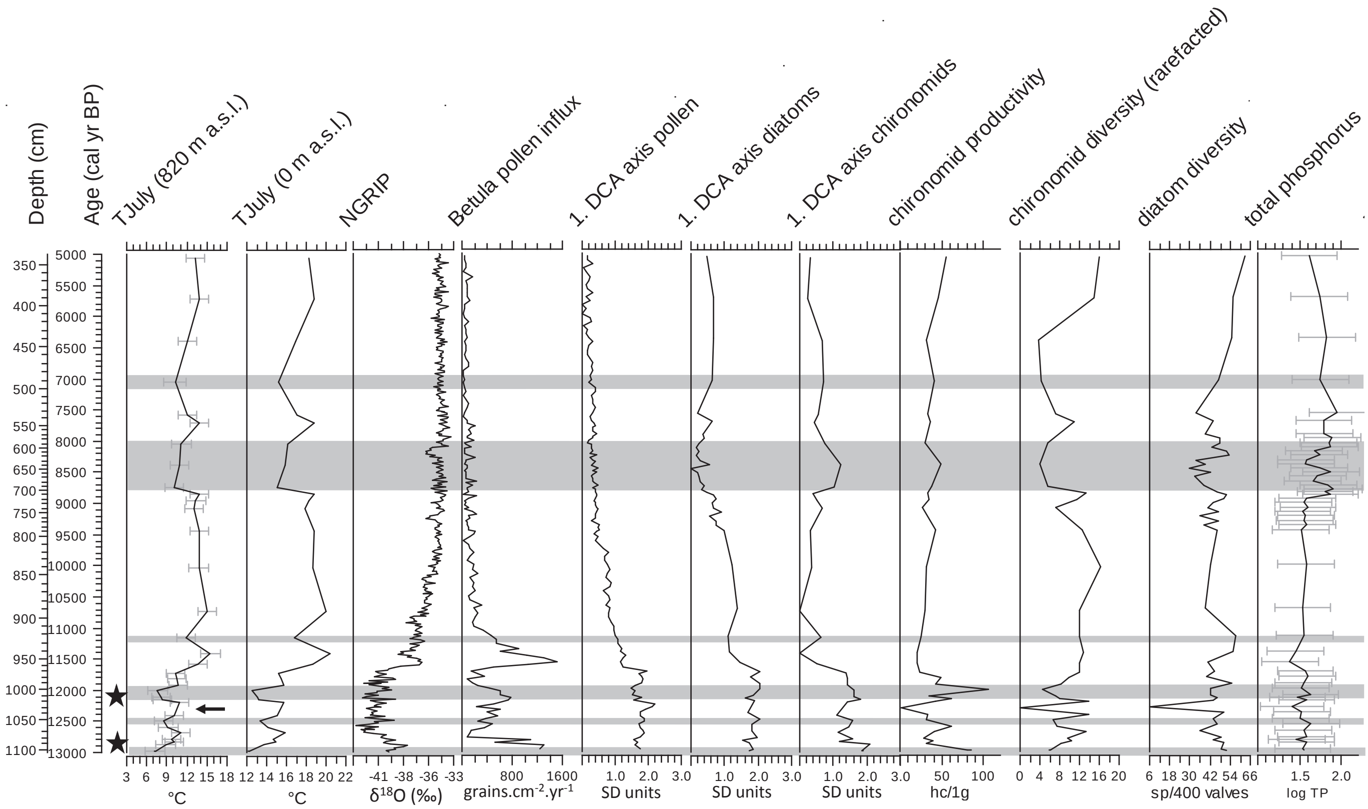

Fig. 3. Results of the chironomid-based T_{July} reconstruction (at 820 m a.s.l. and adjusted to modern sea level using lapse rates of 0.6 °C/100 m), the NGRIP $\delta^{18}O$ record, *Betula* pollen influx, 1st DCA axis of pollen, diatoms and chironomids, chironomid productivity (head capsules per 1 g of dry sediment), chironomid diversity (rarefacted number of taxa), diatom diversity (species per 400 counted valves) and diatom-based epilimnetic total phosphorus. The asterisks indicate layers with *Derotanypus* presence and the arrow indicates the dry layer. The colder periods discussed in the text (low reconstructed T_{July}) are indicated by grey shadings. Data about ^{18}O concentrations from Greenland ice core were obtain from http://www.iceandclimate.nbi.ku.dk/data/ This data file accompanies the following two papers: Seierstad et al. (2014) and Rasmussen et al. (2014).

diatom assemblage (*Hantzschia abundans, H. amphioxys, Pinnularia borealis*) and the almost total absence of green algae (Fig. 6). Hence, the T_{July} reconstruction from this layer was biased and therefore not used. Later (zones Hd-3a + b, Hch-3, 1020–970 cm, 12,200–11,700 cal yr BP), the succession typical for a shallow pond started again with terrestrial chironomid groups (*Smittia* sp.), species typical of cold oligotrophic lakes (*Corynocera ambigua-type*) and taxa tolerating low temperatures (*Derotanypus* sp., *Hydrobaenus* sp., *Stempelinella* sp.). Chironomid assemblages suggest that the water level was gradually increasing, but the lake was probably still shallower than before desiccation. Diatoms indicate gradual restoration of the lake-environment as well as a development of phytoplankton. From ca 1000 cm upwards (12,000 cal yr BP), phytoplankton strongly decreased and it was substituted by small flagilaroid species (*Staurosirella pinnata, Staurosira construents* var. *venter, Opephora mutabilis*). The presence of aerophytic and limnoterrestrial diatoms (*Chamaepinnularia aerophila, Caloneis aerophila*) might indicate either water level decrease, development of a shallow littoral environment or erosion from the surroundings. An increase of macrophytes in the littoral area is also suggested by chironomids (*Cricotopus intersectus*-type, *Tanytarsus pallidicornis*-type).

3.4. Lake development in the Early Holocene

The very beginning of the Holocene (from ca 11,600 cal yr BP onwards) was characterised by the onset or increase of chironomids requiring higher temperatures and higher trophic states (e.g. *Microtendipes pedellus*-type, *Paratanytarsus penicillatus*-type). Groups adapted to cold conditions decreased or even disappeared (*Corynocera ambigua-type*, *Derotanypus* sp., *Zavrelimyia* type A). *Tanytarsus lugens*-type also disappeared for a short time, since the lake had not yet developed an increased water table and deep profundal. Also phytophilic chironomid groups (e.g. *Glyptotendipes pallens*-type, several *Cricotopus* types) occurred in this zone (Hch-4a; 960–890 cm, 11,600–10,700 cal yr BP) and overall chironomid diversity increased steeply (Fig. 3). The green algae assemblage (Fig. 6) is characterised by the disappearance of cold-demanding *Pediastrum kawraiskyi*, decrease of oligotrophic *P. integrum* and steep increase of planktonic *Tetraedron minimum* and *Scenedesmus* sp. Diatom assemblages were characterised by the dominance of planktonic species as well *(Asterionella formosa, Stephanodiscus dubius*). *Stauroneis smithii* was substituted by *S. gracilior.* Local wetland vegetation around the lake consisted of *Alnus* (ca 25% of terrestrial pollen sum) and Cyperaceae, which reached lower abundance than in the previous zone (Fig. 7; Hp-2; 965–925 cm; ca 11,700–11,200 cal yr BP). The presence of macrophytes was indicated by epiphytic diatom taxa (e.g. *Cocconeis pediculus, Cocconeis placentula, Epithemia goeppertiana, E. andata*). Running water taxa, probably coming from a small stream nearby, were present within both, chironomids (*Epoicocladius* sp., *Chaetocladius* sp.) and diatoms (*Planothidium frequentissimum, P. lanceolatum, Reimeria sinnuata*). Even though delimitation of zone 4 is similar for both proxies (960–705 cm for chironomids and 955–720 cm for diatoms; ca 11,600–8950–(8800) cal yr BP), the subzones are positioned differently. Chironomids indicate an earlier change (between 890 and 840 cm), characterised by an increase in abundance of *Cladotanytarsus mancus*-type and *Tanytarsus lugens*-type. The highest

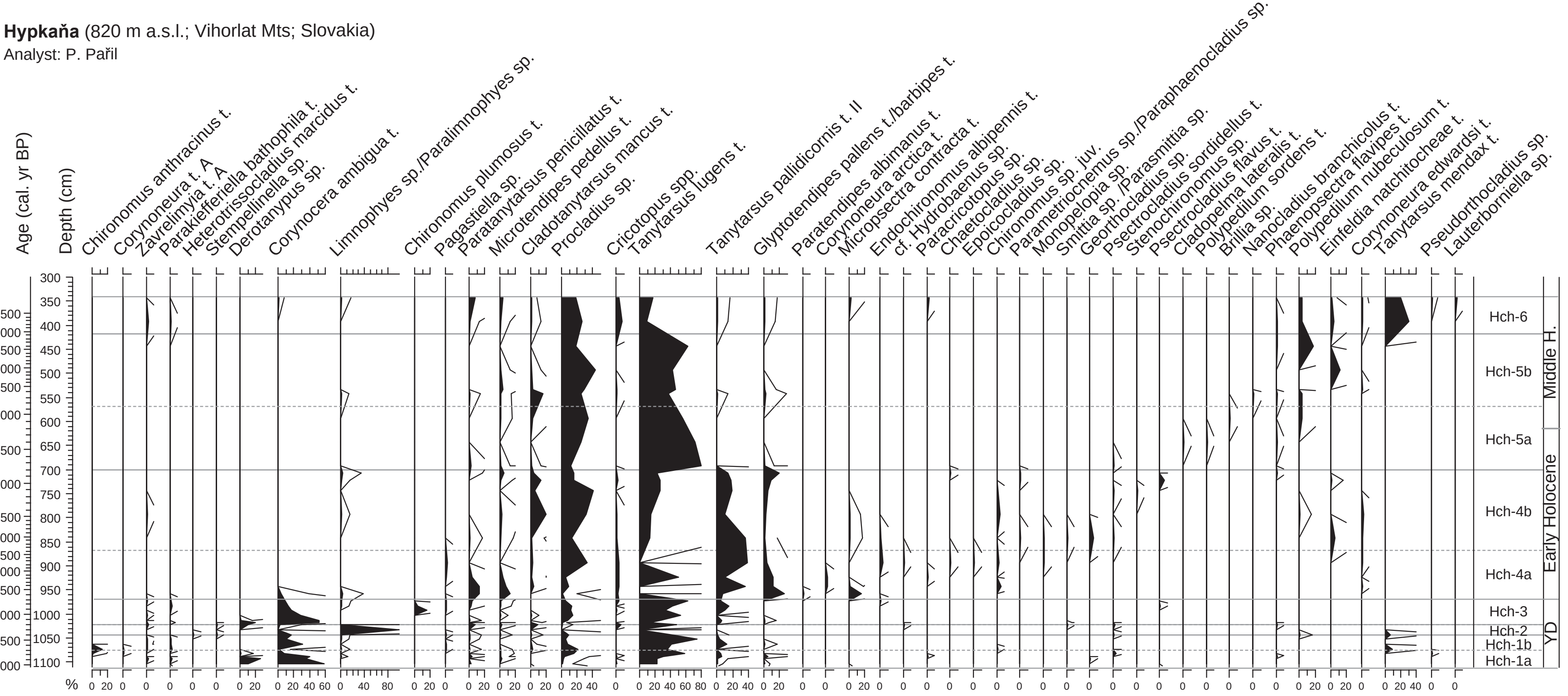

Fig. 4. Stratigraphical diagram of the most important and abundant chironomid morphotypes. The taxa are given in percentages. Rare groups were merged into wider species complexes or to genus level for illustration purposes (but not for quantitative reconstruction). The local chironomid zones are based on results of Coniss cluster analyses with square root transformation of fossil data. Chironomids were identified by P. Pařil (t. = type according Brooks et al., 2007).

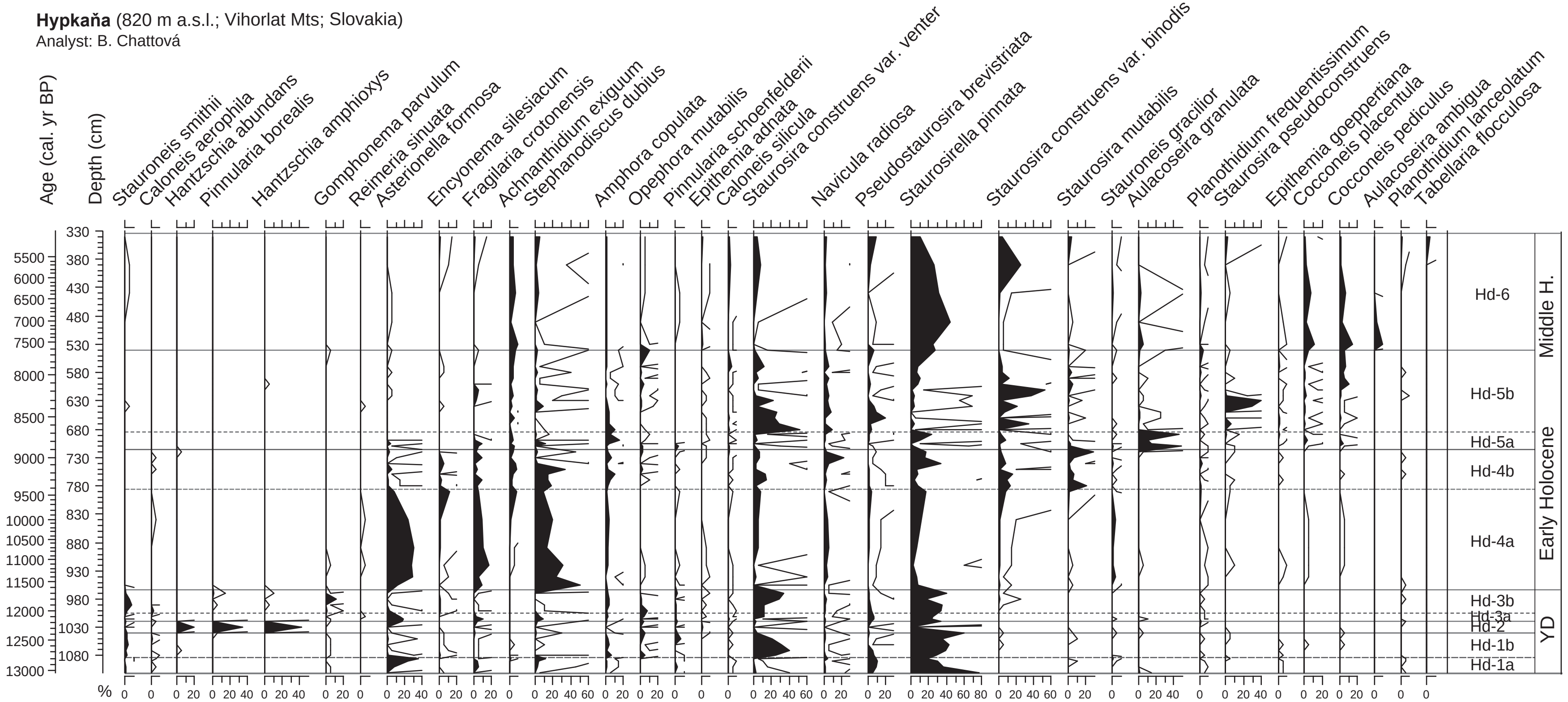

Fig. 5. Stratigraphical diagram of the most important and abundant diatom species. Species are given in percentages. The local diatom zones are based on results of Coniss cluster analyses with square root transformation of fossil data. Diatoms were identified by B. Chattová.

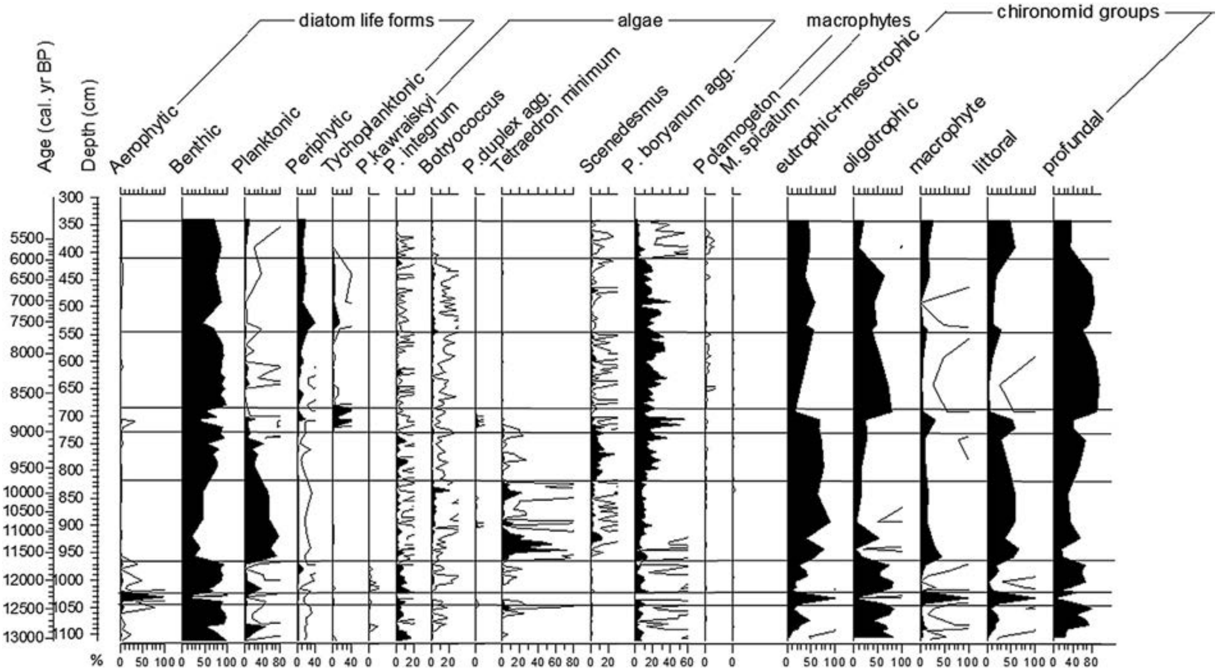

Fig. 6. Summarizing diagram showing representation of different diatom life forms, species of green algae, pollen of macrophytes and chironomid ecological groups. For information about taxa included into particular categories see supplementary material S1 (chironomids) and S2 (diatoms).

diversity of chironomid taxa within the whole profile was recorded at the beginning of the subzone Hch-4b (at about 10,000 cal yr BP). In contrast, a peak in diatom diversity appeared already at the beginning of the subzone Hd-4a (at about 11,500–11,000 cal yr BP), with diversity decreasing thereafter. Diatom species composition indicates distinct change at 790–780 cm (Hd-4a/4b; ca 9400 cal yr BP), where planktonic species (*Asterionella formosa*, *Fragilaria crotonensis*, *Stephanodiscus dubius*) gradually decreased in abundance (Fig. 6) and mesotrophic *Encyonema silesiacum* and *Achnathidium exiiguum* contrarily increased. Simultaneously, the composition of planktonic green algae also changed (at 800 cm; ca 9600 cal yr BP) from an assemblage dominated by *Tetraedron minimum* to dominance of *Scenedesmus*. Abundances of *Pediastrum boryanum* agg. increased. At the end of the subzone Hd-4b, planktonic species almost disappeared and benthic species started to dominate again.

A distinct change in all proxies was apparent at ca 710 cm. In the local terrestrial vegetation, pollen abundance of *Alnus* again increased (Hp-5; 710–430 cm; 8850–6250 cal yr BP). The diatom assemblages of the zone Hd-5a (710–690 cm; 8850–8750 cal yr BP) were characterised by dominance of tychoplanktonic species of the genus *Aulacoseira*, mostly *Aulacoseira granulata.* This species creates hard silicified frustules and therefore it requires an increased degree of turbulence in order to stay in suspension (Saunders et al., 2008). Thus, it indicates turbulent, unstable conditions such as those caused by mixing of water layers by wind (cf. Buczkó et al., 2013). Increased turbulence is almost always associated with increased nutrient flux from the hypolimnion (Stone et al., 2011) which corresponds well with the diatom-inferred TP increase at 710 cm indicating increased trophic state and productivity of autotrophic organisms (diatoms, algae *Pediastrum boryanum* agg., macrophytes). Also chironomids indicate a distinct change in the depth between 710 and 690 cm, even if the reaction to increased phosphorus was apparently not so distinct and the representation of eutrophic (including mesotrophic) and oligotrophic taxa was similar. From the depth of 690 cm onwards, the taxonomic diversity was very low and cold-demanding and profundal-preferring taxa such as *Tanytarsus lugens*-type and *Procladius* sp. started to dominate in the record. Taxa requiring coarse-grained substratum (*Brillia* sp., *Microtendipes pedellus*-type) also occurred (subzone Hch-5a; 695–590 cm; 8750–8050 cal yr BP). Planktonic green algae disappeared (*Tetraedron minimum*) or decreased (*Scenedesmus*; Fig. 6). Also diatom assemblages (subzone Hd-5b; 680–550 cm; 8650–7750 cal yr BP) were characterised by the disappearance of (tycho)-planktonic species and dominance of benthic species (*Amphora copulata, Navicula radiosa*, *Pseudostaurosira brevistriata, Staurosira pseudoconstruens, Staurosira construens* var. *binodis*).

3.5. Lake development in the Middle Holocene

In the chironomid record (subzone Hch-5b; 545–440 cm; 7750–6350 cal yr BP), the more warm-demanding littoral taxa (*Cladotanytarsus mancus*-type) and phytophilic taxa (*Cricotopus* spp., *Glyptotendipes pallens*-type) appeared at the beginning of the Middle Holocene (the beginning of the zone Hch-5b; ca 8000–7600 cal yr BP), but later their abundances again decreased. Towards the end of this zone the abundance of taxa which can colonize sediment or aquatic vegetation (e.g, *Polypedilum nubeculosum*-type) increased, which suggest that the lake may have been shallower and probably over-grown by macrophytes and wetland vegetation (at about 450 cm; 6500 cal yr BP). This is supported also by an increase of *Potamogeton* and Cyperaceae pollen and *Sphagnum* spores (Figs. 6 and 7). In diatom assemblages (lower part of the zone Hd-6; ca 540–430 cm; 7700–6250 cal yr BP), benthic species dominated (e.g. *Staurosirella pinnata*) and were represented at a high diversity. Total diatom species diversity steeply increased (Fig. 3). There was also a higher representation of periphytic (*Cocconeis pediculus*, *Cocconeis placentula*, *Gomphonema* spp.) and tychoplanktonic species (*Aulacoseira ambigua*, *Aulacoseira granulata*) as compared to the previous zone. Finally, the last phase before the complete lake terrestrialization (Hch-6, 395–340 cm, 5750–5050 cal yr BP; Hd-6 – upper part; 390–340 cm; 5700–5050 cal yr BP) was characterised by a decrease in green algae (*Pediastrum boryanum* agg., Fig. 6) and chironomids typical of

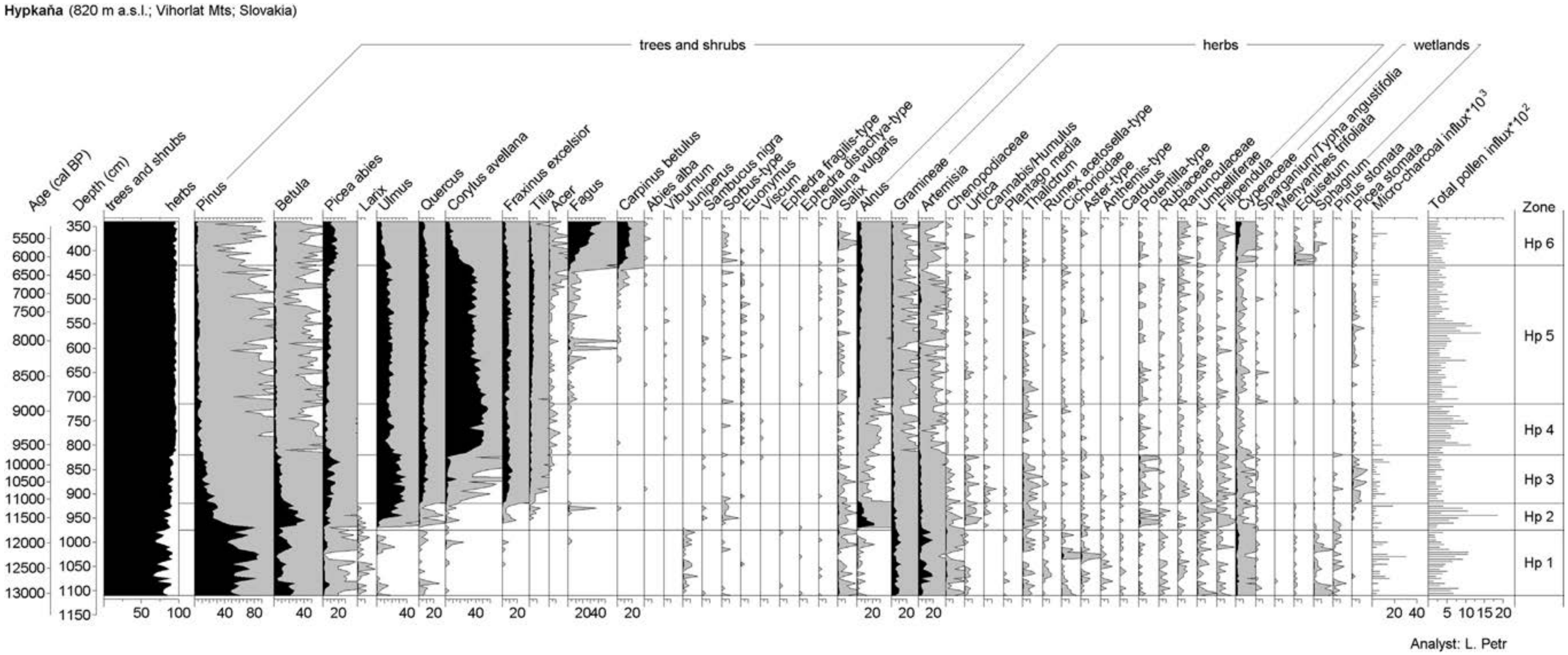

Fig. 7. Pollen percentage diagram with time scale in calibrated years BP (before 1950). At the end of the diagram, microcharcoal particles influx and total pollen influx are given. The local pollen zones are based on results of Coniss cluster analyses with square root transformation of fossil data.

cooler and oligo-to mesotrophic lakes (e.g. *Tanytarsus lugen*-type). Diversity of chironomids and diatoms distinctly increased (Fig. 3). In chironomid assemblages, littoral species (*Polypedilum nubeculosum*-type, *Einfeldia natchitocheae*-type) and groups often associated with vegetation (*Cricotopus* spp.) increased in abundance and ubiquistic groups such as *Tanytarsus mendax*-type started to dominate. In diatom assemblages, species of epibryon (*Tabellaria flocculosa*) and acidophilous species (of the genera *Eunotia, Delicata, Stauroneis, Neidium*) appeared. In the pollen record (Hp-6, 425–345 cm, 6150–5100 cal yr BP), the increase of Cyperaceae pollen and re-appearance of *Sphagnum* spores also indicate spreading of local wetland and mire vegetation.

3.6. Regional vegetation development

The LG forests near the lake were mostly composed of *Pinus* (40–90%) and *Betula* (5–30%) according to fossil pollen spectra and also according to needles and seeds used for AMS dating (Fig. 7; Hp-1; 1110–975 cm; ca 13,050–11,750 cal yr BP). Pollen of other trees and shrubs including the temperate ones (*Picea, Larix, Ulmus* and *Quercus*) were present in lower or very low abundances (*Fagus, Fraxinus* and *Corylus*) and came probably from the lower altitude. *Juniperus*, and *Salix* pollen suggested that in the shrub layer these taxa may have occurred. *Artemisia* and Gramineae were dominant pollen taxa suggesting open steppe or tundra vegetation in the vicinity of the lake, less common were Chenopodiaceae, *Thalictrum, Filipendula, Urtica, Rumex acetosella* t. (=type) and steppe species *Ephedra fragilis* t. and *E. distachya* t. The AP/NAP ratio fluctuated between 65 and 90%. Microcharcoal was relatively frequent. After the warming, at the transition between the LG and Early Holocene (Hp-2; 970–920 cm; ca 11,700–11,150 cal yr BP), the pollen percentages of *Pinus* strongly decreased (to 30%), whereas *Betula* (up to 30–40%) and *Alnus* (10–20%) increased. At the end of the zone *Picea* and *Ulmus* pollen also increased, whereas other temperate trees expanded to a lesser extent (*Quercus, Tilia, Fraxinus, Corylus* and *Fagus*). The AP/NAP ratio was very high, around 95%. *Larix* and *Juniperus* pollen almost disappeared together with *Ephedra*. Open-landscape pollen taxa, such as *Artemisia* and Gramineae, remained dominant among the herbs but their abundances decreased along with Chenopodiaceae. Microcharcoal also decreased. In contrast, pollen of *Cannabis*/*Humulus* t. appeared. The next zone (Hp-3; 915–820 cm, 11,050–9750 cal yr BP) was characterised by the onset of temperate deciduous forests in the landscape. Pollen of *Ulmus* (30–40%) was dominant, pollen of *Quercus* (5–10%), *Fraxinus* (5–10%), *Tilia* (<5%) and *Picea* (5–20%) increased substantially, whereas pollen of *Betula* and *Alnus* decreased. This zone is also characterised by higher abundance of *Picea* stomata indicating the presence of this tree near the lake. The AP/NAP ratio was still high. The zone Hp-4 (815–715 cm; 9700–8900 cal yr BP) was characterised by a distinct decrease of *Ulmus* (from ca 30 to 10%) and steep increase of *Corylus* pollen (up to 50–55%) indicating rather dry climate. Pollen of *Pinus, Picea* and *Betula* decreased, whereas *Acer* and *Fagus* pollen started to increase even if at low abundances. The AP/NAP ratio stabilised around 95%. *Artemisia* and Gramineae reached their lowest values, but their curves remained continual and uninterrupted indicating presence of treeless vegetation somewhere in a wide region, probably at lower altitudes. In the next zone (Hp-5; 710–430 cm; 8850–6250 cal yr BP), *Corylus* decreased slowly (down to 35%), but still remained dominant, whereas *Picea* pollen and stomata increased slightly. *Fagus* and *Acer* pollen curves were already continual (closed curves) and, at the end of the zone, pollen of *Carpinus* also appeared. Microcharcoal influx had decreasing values during this zone. Finally, zone Hp-6 (425–345 cm; 6150–5100 cal yr BP), the last zone of the pollen record in the lake sediments, was characterised by a steep increase in *Fagus* (up to 40%) and *Carpinus* (about 15%) pollen. Pollen of other tree taxa (*Ulmus, Quercus, Tilia, Fraxinus*) decreased slightly (to 5–10%) and pollen of *Corylus* decreased steeply (to 5%). The first pollen grains of *Abies* appeared and AP/NAP remained high.

3.7. Results of geochemical analyses

According to geochemical composition, the profile was divided into six zones (Fig. 8). While the sediments were composed of mainly mineral components in the zone HG-1, autochthonous components like organic matter (represented by LOI) and biogenic silica (Si or Si/Zr in XRF analysis, microscopic observation of diatom frustules) were present in substantial amounts in all other zones. In zone HG-1 there are several cycles of changing input of mineral matter. In the minima of these cycles (minima of lithogenic elements) there are the first maxima of Si/Zr representing the biogenic silica to mineral matter ratio (1010–1005 cm; ca 12.100–12.050 cal yr BP). In the zone HG-2 the Si/Zr ratio is much increased, whereas Zr/Rb and Zr/Ti decreased slightly, which we interpret as fining of mineral grains. The change between zones HG-1 and HG-2 (LG/Early Holocene transition) was very abrupt. From zone HG-1 up to zone HG-4 the element ratios show increasing weathering intensity, K/Ti decreases, while Rb/K increases. In zone HG-3, only 10 cm thick but very distinct, there is a maximum of mineral matter components (increase of all lithogenic elements and MS) at the expense of biogenic silica, with only a minor change in the lithogenic element ratios. This suggests that the nature of the mineral matter did not change much but its input was enhanced, or, alternatively, productivity suddenly dropped. Zone HG-3 is an interruption of the system evolution. Subsequent zone HG-4 is not much different geochemically than zone HG-2. The Si/Zr ratio in zone HG-4 is even higher than in zone HG-2 indicating the highest proportion of the biogenic silica in the sediment. The boundary of zones HG-4/5 is abrupt, the transient strata are only 20 cm thick. Zone HG-5 resembles zone HG-3. The amount of clastics increases at the expense of the biogenic silica with the clastics characterised by enhanced proportion of silt/finest sand content (higher Zr/Rb). Simultaneously MS increases nearly to the level of the prevalently clastic zone HG-1. Zone HG-5 is terminated abruptly by a return to enhanced biogenic silica content at the expense of mineral matter components (beginning of the zone HG-6). Contrarily to the zones HG-2 and HG-4, MS is enhanced in the zone HG-5 and 6, although total Fe content remains more or less the same. Generally, higher MS in zones HG-5 and HG-6 is not proportional to the rather weakly enhanced Zr/Rb ratio. This, together with the nearly constant content of total Fe, suggests that these higher values are probably not related to a higher amount of coarser lithogenic magnetite grains or other magnetic mineral grains. Either large amounts of diamagnetic biogenic opal in underlying zones (high Si/Zr) or post-depositional destruction of magnetic minerals decreased MS in the zones with high productivity.

4. Discussion

4.1. Potential effects of productivity and water table changes on the reconstruction

The T_{July} reconstruction based on chironomids corresponds well with results of other climate proxies in East-Central and Eastern Europe (pollen: Feurdean et al., 2008a,b; chironomids: Tóth et al., 2012, 2015; Płóciennik et al., 2011; stalagmites: Tamaş and Causse, 2001, Demény et al., 2013). It was recently illustrated that co-varying factors in calibration datasets may influence reconstructions based on biotic proxy-indicators (Velle et al., 2010;

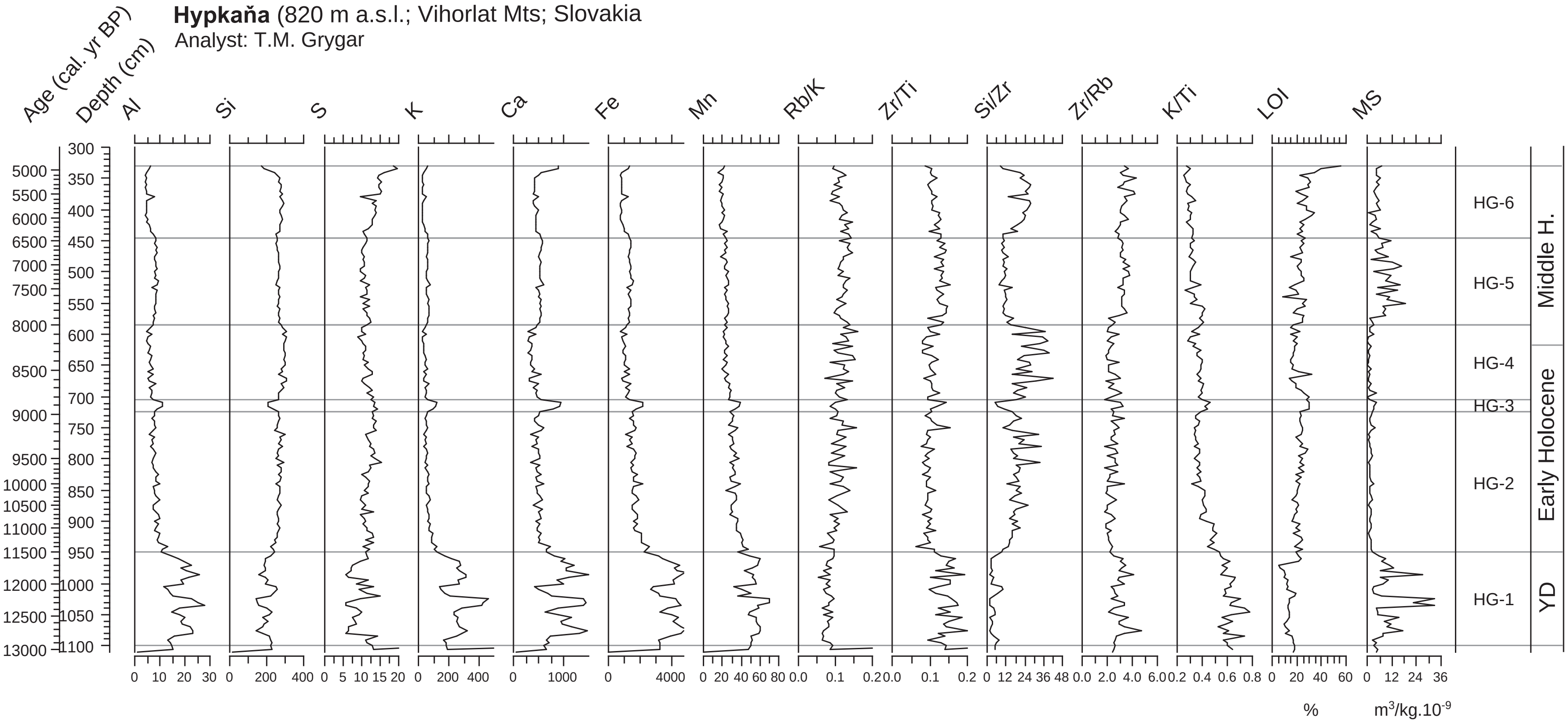

Fig. 8. Results of geochemical analyses, magnetic susceptibility (MS) and loss-on-ignition (LOI at 550 °C). Also ratios of some elements are shown.

Juggins, 2013). In the case of reconstructions based on chironomids, Brodersen and Anderson (2002) demonstrated that taxa with high temperature optima occur mostly in eutrophic conditions and taxa with low temperature optima occur in oligotrophic conditions. This positive correlation between temperature and trophic status was documented to some extent in most of the published calibration datasets (Brooks and Birks, 2001; Heiri and Lotter, 2005; Heiri et al., 2011). If these two environmental factors develop independently, the T_{July} reconstructions may be biased. Therefore we reconstructed trophic status using diatom-inferred TP to assess whether past variations in TP may have led to potential problems in the T_{July} reconstruction. Diatom-inferred TP stayed relatively constant in the profile varying between 23 μg L^{-1} (in 955 cm, 11.500 cal yr BP) and 80 μg L^{-1} (in 700 cm, 8800 cal yr BP). However, an increase (from 39 μg L^{-1} to 79 μg L^{-1}) is registered at 8850 cal yr BP (from 710 cm up), with fluctuating values and only few short-term decreases thereafter (most distinct in 640–630 cm; 8300–8400 cal yr BP). In contrast, chironomid-inferred T_{July} decreased at 8700 cal yr BP and with the exception of 7600–7700 cal yr BP stayed lower than in the Early Holocene (Fig. 3). As the relationship between temperature and trophic status in this part of the profile is negative, not positive, we exclude a bias in T_{July} reconstruction caused by a positive interaction. Further, also minimum values during the YD (at ca 12,000 and 12,500 cal yr BP) were characterised by moderate increases in diatom-inferred TP (Fig. 3).

Another factor, which might influence T_{July} reconstruction is the changing depth of the lake as water in the shallow lake can be strongly warmed during the summer, whereas deep stratified lakes are characterised by profundal environments with optimal conditions for cold-demanding chironomid taxa (Velle et al., 2010). A deepening of the lake could therefore, in principle lead to a decrease in reconstructed Holocene temperatures. However, the most pronounced decrease in the Holocene part of the record at 8700 cal yr BP was probably associated with a decrease of the lake depth probably due to terrestrialization, as suggested by a decrease in planktonic diatoms and algae and by high abundance of *Aulacoseira* species (Saunders et al., 2008). An opposite situation was observed within the YD period. Here, higher reconstructed T_{July} values are related to the lower inferred lake depth (Fig. 3). Thus in this section of the record the reconstructed T_{July} values could be slightly biased (increased) by lake level variations, although they still remained in the range of the YD temperatures and were clearly cooler than reconstructed Holocene temperatures. Finally, the reconstructed increase of T_{July} after 7000 cal yr BP could be called into question, because the lake started to terrestrialize up to ca 5000 cal yr BP, when the open water surface completely disappeared and thus the depth of lake was decreasing.

4.2. *Younger Dryas T_{July} fluctuations and the LG/Holocene transition*

Focusing on the reconstructed T_{July} for the YD, we recorded very variable temperatures between 7.2 and 10.8 °C at 820 m a.s.l. (i.e. ca 12.1–15.7 °C adjusted to modern sea level, ca 49°N). These values are considerably lower (about ca 4.5 °C) than those reconstructed at more southerly locations in the eastern and central parts of Southern Europe (42–44.5°N) or the Alpine region (ca 46°N), but about 3 °C higher than in the Baltic region (56.5–58°N) or British Isles (54–55.5°N) situated more to the north (Heiri et al., 2014a). It seems that reconstructed values reflect the latitude, because reconstructed T_{July} is intermediate. As the study site is geographically closer to the Alps than to the Baltic region, the reconstructed values are likely slightly lower then would be expected according to latitude, which might be attributed to the exposition of the study site (northern slopes). The comparison with the record from central Poland (almost 52°N, 13–16 °C corrected to 0 m a.s.l.; Płóciennik et al., 2011) also suggests slightly lower T_{July} values at our study site than would be expected according to latitude. The rather distinct climatic fluctuations during the YD resemble minor variations recorded in the oxygen isotope records of the Greenland ice sheet (e.g. Lowe et al., 2008; Rasmussen et al., 2014) and also by other non-quantitative climate proxies in Europe (Von Grafenstein et al., 1999). However, Younger Dryas summer temperatures reconstructed in other chironomid records (e.g. Tóth et al., 2012; Ilyashuk et al., 2009; Brooks and Birks, 2001) are much more stable and other YD reconstructions from Europe also do not show such minor centennial-scale oscillations (e.g. Lauterbach et al., 2011).

Some previous studies have suggested that YD climate was unstable. For example, it has been proposed that rapid alternations between glacial growth and melting may have affected sea-ice cover and the influx of warm salty water in the Nordic sea (Bakke et al., 2009). Schwark et al. (2002) suggested a middle Younger Dryas warming (MYDE; 12,200–12,300 cal yr BP) in southwestern Germany by increased pollen productivity of *Betula* and increased content of nC_{27}-alkanes in the lake sediment which correspond with increased input of *Betula* litter to the lake. Such short-term warmings are also apparent in the NGRIP and GRIP ice core data (Rasmussen et al., 2014), which show several short positive excursions of $\delta^{18}O$, although the amplitude of these changes is small compared with the centennial scale shifts between the YD and adjacent climate periods. High YD fluctuations were recorded also in the Eastern Carpathians by means of variability in ^{18}O content in stalagmites (Tamaş and Causse, 2001). Sediment composition from the YD period also varied considerably in our record, confirming that local environmental conditions were affected by climatic changes at our study site (Fig. 8). In the chironomid-inferred T_{July} record, there are two warmer periods dated to 12,850–12,600 cal yr BP and, more distinctly, at 12,400–12,200 cal yr BP within the YD. These warmer phases coincide with distinct water level declines which are indicated in our record by gradual displacement of aquatic taxa by terrestrial ones and by changing input of mineral matter into the lake (Fig. 8). Low lake level episodes have been recorded also in Central Europe during the YD and were accompanied by changes in sediment composition as well (cf. Karasiewicz et al., 2013). Since temperature variations inferred by chironomids within the YD co-vary with apparent changes in water table, this may have influenced the results. Furthermore, inferred temperature variations within the YD are within the prediction error of the age-depth model (Fig. 3). We conclude that our results suggest that July air temperatures may have shown centennial-scale variations at the study site, but that these changes coincided, and may have been amplified, by local hydrological changes. Additional evidence (e.g. additional YD temperature reconstructions for the study region) will have to be developed to resolve whether these variations represent real air temperature changes or chironomid response to other environmental variations (e.g. changes in water table).

The LG/Holocene transition was characterised by a rapid increase of the reconstructed T_{July}. The beginning of the warming in our record is dated to between ca. 11,700 and 11,600 cal yr BP (from 10.3 to 13.7 °C), which agrees with the onset of Holocene assessed as Global Stratotype section in NGRIP (11,700 b2k, Walker et al., 2009). In the next sample temperatures rise by another 1.8 °C (from 13.7 to 15.5 °C) to reach a first maximum at ca 11,400 cal yr BP. Thus in ca. 300 years the T_{July} has apparently increased by ca. 5 °C. According to the review of Heiri et al. (2014a), a similar steep increase of reconstructed T_{July} has been recorded in some sites in Alpine region (by ca 4 °C) but especially also at higher latitudes (British islands by ca 5 °C, Norway 4–5 °C). Much lower and slower warming occurred at low latitudes (SW Europe, E and CS Europe) and altitudes (Baltic region, Heiri et al., 2014a; almost no change in

Holocene onset in middle Poland, Płóciennik et al., 2011). The only chironomid-based T_{July} reconstruction from the whole Carpathians (45°N, Retezat Mts; Tóth et al., 2012) has also shown a delayed warming at the beginning of the Holocene by about 2.5–3 °C. At the YD to Holocene transition no major changes in summer temperature was detected in this record, but summer temperatures then increased in two steps and reached 12.0–13.3 °C during the Preboreal. The comparison of pollen data from the Western Carpathians and the adjacent areas indicates that during the LG the pollen spectrum of the study site was similar to the sites located in steppe-tundra landscapes of the Inner-Carpathian basins (e.g., Hájková et al., 2015), while just after the Holocene onset it became similar to samples from Western Slovakia which are rich in temperate trees (Petr et al., 2013; Hájková et al., 2013). Such a conspicuous development towards a temperate landscape is exceptional within East-Central Europe as is indicated also by gradient analysis of available pollen samples from the LG and Holocene onset (Jamrichová et al. unpublished data) and might be caused by the geographic position of the study site on the boundary between oceanic and continental climate influences. The LG/Holocene transition was apparently accompanied by a steep increase of lake depth indicated by increased representation of planktonic diatoms and algae (*Tetraedron minimum*), by a decrease of coarse-clastic input to the lake sediment and a steep decline of magnetic susceptibility. These changes could be attributed to the climate moistening which followed climate warming (e.g. Feurdean et al., 2008a). This climate improvement triggered rapid spread of deciduous trees, firstly *Betula*, which has probably spread into the semi-open forest-tundra above the lake, then *Alnus,* which has occupied wet places near the lake and forest springs (ca 11,600 cal yr BP) and later also *Ulmus* (11,300–11,000 cal yr BP). The rapid reaction of *Betula* to climate improvement was recorded also by macrofossils in the Eastern Carpathians (at ca 11,500 cal yr BP; Feurdean et al., 2008b) and by pollen and lipid biomarkers in Central Europe (Schwark et al., 2002).

4.3. Holocene thermal maximum

Another often studied and discussed topic is the timing and duration of the Holocene thermal maximum (HTM) in summer temperature, which is mostly positioned in the large interval of 11 and 5 ka BP (Renssen et al., 2009, 2012). Based on global atmosphere-ocean-vegetation model runs, Renssen et al. (2012) have revealed that in large sections of Europe the timing of HTM is expected to be between 7 and 6 ka BP, which agrees with some proxy-based reconstructions (e.g. Heiri et al., 2014b; Renssen et al., 2009). For example, based on pollen assemblages Davis et al. (2003) reconstructed maximum summer temperatures around 6 ka for Northern Europe and Western Central Europe and around 7–8 ka in central eastern Europe. However, reconstruction based on other proxy records placed the HTM to different sections of this interval, some of them also to the Early Holocene (9500–9100 cal yr BP (Tóth et al., 2015) or 10,000–8600 cal yr BP (Ilyashuk et al., 2011)). In our study site, the sample with the highest reconstructed T_{July} was at the beginning of the Holocene (15.5 °C at 11,400 cal yr BP and 15.0 °C at 10,750 cal yr BP), but the whole period 11,600–8850 cal yr BP was relatively warm with temperatures being slightly higher, the same or slightly lower than today (13.7–15.5 °C; today temperature for the altitude of the lake estimated to 14.7 °C). Temperature was slowly decreasing from ca. 10,500 cal yr BP onwards toward the Early/Middle Holocene transition with an exception of short cooling event at about 11,200 cal yr BP (11.9 °C). A possible reason for the discrepancy between the different records is that local orography and the effects of mountain ranges may have influenced local climates, leading to earlier Holocene temperature maxima in regions which are downwind from mountain ranges and, e.g., shielded from the influence of North Atlantic air masses. In these situations the high summer insolation values of the Early Holocene may have led to higher temperatures than later in the Holocene period. Alternatively, changes in water tables discussed above may have to some extent affected chironomid assemblages and influenced the trend in chironomid-inferred temperatures. However, since an early HTM has also been reported from other Holocene records from European mountain lakes, we consider orographic effects or regional differences in the timing of the HTM across Europe the more likely explanation.

4.4. Early/Middle Holocene cooling

We recorded a distinct T_{July} decrease (from 13.9 to 10.1 °C) between 8850 and 8750 cal yr BP, which lasted to ca 8000 cal yr BP (Fig. 3). A distinct cooling event at 8200 yr BP lasting for 100–200 years has been reported from the Greenland ice core oxygen isotope records but also many other climate records from around the North Atlantic (Alley and Ágústsdóttir, 2005). Mayewski et al. (2004) showed that this cooling event was embedded, or formed part of, a longer lasting period of climate variations they named the first Holocene rapid climate change (RCC) placed to 9000–8000 cal yr BP. Short lived cooling events within this period have been reported from several other chironomid records. Heiri et al. (2003) described a period of cooler summer temperatures (ca. 8200–7800 cal yr BP) which coincided with changing meltwater flux from the American continent to the North Atlantic (Heiri et al., 2004). A decrease of chironomid-inferred T_{July} was also recorded in the central Poland (Płóciennik et al., 2011: 8700–8000 cal yr BP) and from the eastern central Alps (Ilyashuk et al., 2011: 8200–8000 cal yr BP). A temperature decrease was also recorded by oxygen isotope composition of diatoms in the Eastern Carpathians (Magyari et al., 2013) and by oxygen isotope data from a Hungarian speleothem (Demény et al., 2013: 9000–8000 yr BP). Based on pollen-inferred temperatures, Feurdean et al. (2008a) detected a decrease of winter temperatures (in the coldest month) between 8200 and 8000 cal yr BP, whereas summer temperatures did not change. Total pollen influx in our record (mostly of *Ulmus, Quercus, Fraxinus* and *Corylus*; data not shown) decreased at ca 8700 cal yr BP probably due to decreased pollen productivity as a reaction to climate deterioration (Fig. 7, Andersen, 1980; Sjögren et al., 2006). At the study site, simultaneously other analysed parameters and proxies also changed at about 8700 yr BP. The duration of the cold episode at 8700–8000 cal yr BP is distinctly longer in our record than reported for the 8.2 ka event (e.g. Wiersma and Renssen, 2006). However, since we have only a limited number of radiocarbon dates in this section of the record (Fig. 2, Table 1) we cannot rule out that this event is shorter than dated in our records. Alternatively, the cooling could be related to the longer first Holocene RCC described by Mayewski et al. (2004).

Geochemical analyses have shown that the cool period in our core came after an abrupt short-term input of mineral matter into the lake indicated by an increase of all lithogenic elements and MS and decrease of biogenic silica proxy (cf. zone HG-3, Fig. 8), which was reflected by dominance of tychoplanktonic diatoms in the sediment (Fig. 6). Both proxies could indicate increased precipitation resulting in higher erosion and more intense water column mixing in the lake, such as due to enhanced wind. Enhanced mixing usually creates an upward flux of nutrients and silica from the hypolimnion (Stone et al., 2011), which would agree with increased diatom-inferred TP in our record. Also increased *Alnus* pollen (both percentages and influx) might indicate higher wetness and development of wet alder carr in the terrestrialized littoral or expanding wetlands in the surrounding landscape. Moreover, *Alnus* was revealed to have higher pollen productivity under wetter climate in the research of pollen traps (Van der Knaap et al., 2010). The climate moistening in this period (8700–8600 cal yr BP) is also documented by a conspicuous expansion of temperate forests in our record (decline of *Corylus*, slight increase of *Ulmus*, *Fagus* and *Picea*) and also in other records from the deciduous-forest zone of the Carpathians (Feurdean et al., 2015; Hájek et al., 2016). Increased precipitation in Central and East-Central Europe was reconstructed also by testate amoebae (higher water level at 8300–8000 cal yr BP, Schnitchen et al., 2006), by increased water level of lakes (8300–8000 and 7500–7000 cal yr BP, Magny, 2004; 8400 cal yr BP, Buczkó et al., 2013) or climate-mediated decline of fire activity expressed by decrease of charcoal influx at about 8500 cal yr BP (Feurdean et al., 2012, 2015). Even if the timing in these records is not completely the same as in our study, the differences are not so high to clearly exclude synchronicity and might be caused by some inaccuracy in age-depth models. Nevertheless, the timing of climate moistening could be also affected by local and regional factors like position on mountain ranges, distance from the Atlantic or prevailing wind direction. According to Magny et al. (2003), mid-latitudes between ca 50° and 43° responded to the cooling by wetter climate, whereas Southern and Northern Europe had contrarily drier climate. The extent of this wet mid-European zone could reflect the strength of the Atlantic Westerly Jet in relation to the thermal gradient between low and high latitudes (Magny et al., 2003). Moreover, climate moistening was probably associated with increased cloudiness, which could have prevented warming of the lake water in summer and thus locally amplified the effect of cooling and decreased values of reconstructed T_{July}.

It is remarkable that indicators of erosion and turbulent water conditions were present only before the reconstructed cold period and later no input of eroded inorganic matter from the surrounding landscape to the lake was detected by geochemical analyses (Fig. 8). Likely the development of terrestrial vegetation in the lake surrounding triggered by climate moistening prevented further erosion, as suggested by increase of tree pollen (*Tilia, Fagus, Picea*) and decrease of *Corylus* pollen. This period is also characterised by increased diatom-inferred total phosphorus content (Fig. 3). Increased trophic state and nutrient availability for autotrophic organisms in the lake is indicated also by increased abundance of nutrient-demanding algae *Pediastrum boryanum* agg. (Fig. 6). There are several possible mechanisms of nutrient enrichment of lake water. It could have been caused by increased input of litter from nearby trees, especially *Alnus*. Another explanation could be flux of nutrients from the hypolimnion due to enhanced wind activity (e.g. Stone et al., 2011). Input of phosphorus through the erosion from the surrounding catchment may have been possible only at the beginning of the cold phase, later such an increased nutrient input is not supported by geochemical analyses, as the proxies for clastic input (Zr/Rb, Zr/Ti or Rb/K) did not change. Another possible mechanism for changes in nutrient availability in the lake could have been changes in seasonality connected with cooling of climate. The prolonged winters could have caused longer ice cover and thus stronger stratification in the lake, anoxia in the hypolimnion and consequent internal phosphorus loading (Kirilova et al., 2009). Released phosphorus would then become available for organisms after spring mixing of hypo- and epilimnetic layers. However, increased phosphorus concentrations were not so distinctly reflected in the species composition of chironomids since the proportion of eutrophic and oligotrophic taxa was rather equal in this zone (Fig. 6) and oligotrophic taxa distinctly increased their abundances compared to the previous zone. It was documented that the reaction of heterotrophic chironomids to changes in lake water nutrients is not as predictable as the reaction of autotrophic organisms because chironomids are influenced by increased productivity indirectly through the food availability (macrophytes, algae, but also other invertebrates and organic detritus) and oxygen concentrations (Brodersen and Lindegaard, 1999; Brodersen and Quinlan, 2006), which could be influenced also by other factors than phosphorus availability. Moreover, fossil chironomid records from the central part of small lakes are composed by species from both, the littoral and the profundal, where different environmental conditions can occur (Van Hardenbroek et al., 2011). Jeppesen et al. (1997) demonstrated that littoral environments can host more oligotrophic taxa than the profundal and their input to deepest part of lakes can shift the chironomid signal to more oligotrophic conditions. Also oxygen availability in the profundal might be an important factor. If for some reasons (e.g. cold climatic conditions) the oxygen amount remains high in the profundal, the chironomid species composition may stay the same (cold-demanding oligotrophic taxa) and may not react on the increased nutrient input (Brooks and Birks, 2001; Little et al., 2000).

5. Conclusions

From the analysis of an almost 7 m thick layer of lake sediments from East-Central Europe (Carpathian Mts.), we inferred considerable variation in environmental conditions and in particularly mean July temperatures reconstructed from chironomids during the latest Glacial and Early Holocene. In the cold YD, T_{July} was on average around 9.5 °C with lowest values about 7 °C. Considerable temperature variability within the YD was inferred, with two warmer events (12,850–12,600 and 12,200–12,400 cal yr BP) which may have been related to instability of climate recorded in other records (e.g. the Nordic Seas). Other chironomid records from Europe typically do not feature within YD temperature variability of this amplitude. However other proxies (geochemistry, diatoms) support climatic variations, particularly hydrological changes, at the study site during this interval and suggest that warmer conditions were accompanied by a water table decrease. The YD was followed by distinct warming of up to 5 °C between 11,600–11,400 cal yr BP. The warmest Holocene temperatures (15–15.5 °C) were recorded in the earlier part of the Holocene between 11,400 and 8850 cal yr BP. This agrees with some other chironomid records from European mountain ranges (Carpathians, central Alps) but contrasts with other reconstructions from different parts of Europe. Possibly this difference is related to local topographic effects on local climates or regional differences in Holocene climate development within Europe. During the course of the Holocene, a distinct but short-lived cooling was reconstructed around 11,200 cal yr BP, possibly corresponding with the 11.4 ka event and a more pronounced cooling event between 8700 and 8000 cal yr BP. The timing of the latter cooling coincides with cool episodes recorded in other palaeoclimate records from Europe and the North Atlantic region. The cooling is longer than reported for the 8.2 ka event, but similar in duration as the longer lasting cooling episode referred to as the first Holocene RCC by Mayewski et al. (2004) that the 8.2 ka event is embedded in. At Hypkaňa geochemical evidence and the composition of diatom assemblage supported a shift to wet and cold conditions during this interval.

Author contributions

PH and LP conceived the research, LP identified pollen and green algae, prepared samples for geochemical analyses and measured LOI and MS, PP identified chironomids and prepared data for quantitative reconstruction of T_{July}, BCh identified diatoms and prepared data for quantitative reconstruction of TP and TMG carried out geochemical analyses. PH created age-depth model and prepared samples for AMS dating. OH calculated chironomid-inferred T_{July}. All authors provided ecological interpretations of results and participated on the preparing of manuscript, which was leaded by PH.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.quascirev.2016.04.001.

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Abrupt vegetation and environmental change since the MIS 2: A unique paleorecord from Slovakia (Central Europe)

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ABSTRACT

Research on past abrupt climate change and linked biotic response is essential for understanding of the future development of biota under changing climatic conditions, which, in turn, is necessary for adequate progress in ecosystem management and nature conservation. The present study presents the first comprehensive reconstruction of local and regional environment at the Western Carpathian/Pannonian Basin border, including a first chironomid-based paleoclimate reconstruction and $\delta^{18}O$ and $\delta^{13}C$ records from travertine, to investigate abrupt biota and climate shifts since the Marine Isotope Stage (MIS) 2. A range of biotic and abiotic proxy data in organic-calcareous sediment sequence were analysed using a multi-proxy approach to produce a detailed reconstruction of past ecosystem conditions. The results illustrate that the most prominent abrupt change in the local environment occurred directly at the MIS 2/MIS 1 transition at 14,560 cal BP as a consequence of increased precipitation and an increase in reconstructed mean July temperature by ~2.2 °C. Abrupt changes in local environment during the early Holocene were closely linked to travertine precipitation rate around thermal springs and thus indirectly to the climate until the arrival of the Late Neolithics around 6400 cal BP. Regional vegetation response (derived from pollen data) to the climatic fluctuations lagged, with the most prominent changes around 14,410 cal BP and 10,140 cal BP. Our data suggest the presence of a steppe-tundra ecosystem with evidence for low amounts of temperate broadleaf trees during the MIS 2, indicating close proximity to their northern glacial refugium. We demonstrate the ability of $\delta^{18}O$ and $\delta^{13}C$ stable isotope record from travertine to reflect abrupt climatic and environmental changes. The study provides evidence about benefits using travertine deposits coupled with high-resolution paleoecological data to investigate past biotic and abiotic responses to abrupt climate change.

1. Introduction

Biotic and particularly vegetation responses to past climate change is a frequently studied paleoecological research topic, ranging from investigating the magnitude, speed and direction of ecosystem change, to the resilience and resistance of various ecosystem components to changing climatic conditions (e.g. Feurdean et al., 2014; Randsalu-Wendrup et al., 2012; Camill and Clark, 2000). Information on these aspects of ecosystem change is crucial for modelling future responses to climate development and related nature conservation strategies (Willis and Birks, 2006). In the Quaternary, major vegetation change took place particularly at

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the interglacial/glacial transitions, where complete replacement of species communities often took place as a consequence of abrupt climate shifts. Pronounced shorter term climatic changes also occurred within interglacial and glacial periods. During the Last Glacial period (114,000–11,700 cal BP), ca 25 abrupt climatic fluctuations known as Dansgaard-Oeschger events occurred (Dansgaard et al., 1993; Rasmussen et al., 2014), during which atmospheric and ocean conditions alternated between relatively mild (interstadial) and cold (stadial) conditions. Unfortunately, continental biotic paleoecological records (e.g. fossil pollen, plant macrofossils) dated back to the Last Glacial or previous Interglacial are only found in exceptional circumstances (e.g. Le Grande Pile, De Beaulieu and Reille, 1992), making it difficult to know how abrupt climatic events impacted terrestrial and aquatic ecosystems.

In the Panonnian Basin (Central-Eastern Europe), loess accumulations are often studied to understand past glacial conditions (Feurdean et al., 2014; Hošek et al., 2017; Sümegi et al., 2012), however, loess accumulations are typically characterised by low pollen and macrofossil preservation making them less than ideal environments to study terrestrial and aquatic ecosystems. Wet environments (lakes, bogs, fens, etc.), which are more suitable for plant fossil preservation and thus more appropriate for environment and vegetation reconstructions, were rare during the MIS 2 (29,000–14,600 cal BP) in the Pannonian Basin (but see e.g. Magyari et al., 1999; Sümegi et al., 2011, 2013) because of cold and dry conditions. In the Pannonian Basin, late Quaternary biotic paleorecords typically span the past 14,600 years (MIS 1) (e.g. Hájková et al., 2013, 2015; Jamrichová et al., 2014; Magyari et al., 2001, 2008; 2010; Petr et al., 2013; Šolcová et al., 2018), which unfortunately do not provide information regarding terrestrial and aquatic ecosystem responses during the MIS 2/MIS 1 transition. Moreover, in the north-western part of the Pannonian Basin, on the Western Carpathian/Pannonian Basin border, older biotic records from MIS 2 are completely missing. It has been suggested that the border between the Western Carpathians and Pannonian Basin provided northern local glacial refugia for temperate biota, as well as provided a relevant migration route (Juřičková et al., 2014, 2018; Sümegi and Náfrádi, 2015; Willis et al., 1995). Detailed studies of past ecosystem change from this region are therefore crucial for better constraining the timing and extent of temperate biota migration after the Last Glacial.

Several paleoclimatological reconstructions from chironomids (Hájková et al., 2016) and calcareous tufa (Juřičková et al., 2018; Dabkowski et al., 2019) have been published from the Western Carpathian/Pannonian Basin border, however, these sites are from mid-to-high elevations (>400 m asl). Currently, no detailed paleoclimatological reconstruction from fossil records exist from lowland sites (below 400 m asl), where precipitation and temperatures would be different in comparison with the mountainous part of the Western Carpathians. Precipitation and the overall water availability for plants were probably the most important environmental drivers determining the distribution of glacial temperate-tree refugia (Willis et al., 2000), as well as determining whether sparse steppe-tundra or hemiboreal (taiga) forests prevailed during MIS 2 in this region. Although the influence of climate on geothermal-related carbonate precipitation (travertine) is considered to be generally less obvious compared with karstic-related carbonate precipitation (calcareous tufa) due to the more complex system of deep hydrothermal water circulation (Capezzuoli et al., 2014), they still provide potential for multidisciplinary studies of past climate changes, associated vegetation development and the evolution of human societies (Šolcová et al., 2018, Prado-Pérez et al., 2013). In the Danubian Lowland (NW part of the Pannonian Basin), numerous thermal springs occur, with a high density near Santovka village (Fig. 1). The first paleoecological investigation of travertine deposits in this region was recently reported (Santovka-village profile; see Šolcová et al., 2018), however this study was chronologically limited to the Holocene. We therefore decided to focus on a second location, Santovka-Pramene Budzgov, situated only ~2 km away from the Santovka-village profile, to provide the first paleoclimatological and paleoecological record dating back to the MIS 2. We applied a multiproxy approach using terrestrial and aquatic proxies (geochemistry, magnetic susceptibility, loss on ignition, pollen, plant macrofossils, chironomids and molluscs) to reconstruct ecosystem function and change over time. Since chironomid assemblages react sensitively to temperature change (Eggermont and Heiri, 2012) chironomid analysis also provides insights on past temperature development at the study site. Stable carbon and oxygen isotopes from travertine carbonates were used to reconstruct whether the basin was influenced by deep or shallow circulation waters, and to constrain the source of CO_2 and detect past climatic fluctuations. In addition, we conducted an electrical resistivity tomography survey to document sub-surface material to better understand the extent of the sedimentation basin. The research objectives of this study are to; 1) reconstruct the regional and local vegetation history during MIS 2; 2) reconstruct temperature variability during the MIS 2/MIS 1 transition at 14,560 cal BP, and investigate the subsequent vegetation response; 3) reveal major abrupt vegetation and environmental changes during the Holocene and determine their causes; and 4) reconstruct deep or shallow circulation waters of calcareous springs and assess the possibility of developing paleoclimate reconstructions from travertine carbonates.

2. Study area

Santovka-Pramene Budzgov (Santovka-PB) is located between the villages of Santovka and Bory near the stream Búr (48°10′12.3″N, 18°45′23.1″E; 150 m asl) (Fig. 1). The study area is formed by andesitic sandstones, overlaid by loess deposits, various deluvial covers and alluvial deposits in the bottom of the valley. The epiclastic andesitic sandstones contain re-deposited tuffs and other fine-grained insets, which are flyschoid in character with interbeddings of pellitic sediment (Nagy et al., 1998). Locally, calcium carbonate-rich springs form travertine mounds (Fig. 3). These springs extract calcium carbonate from the bedrock that is also built by Upper Triassic limestones. The local climate is characterised as warm (8–9 °C mean annual temperature) and dry (550–600 mm mean annual precipitation) (http://geo.enviroportal.sk/atlassr/).

The first permanent human settlement near Santovka village is dated to the Middle Neolithic period (Želiezovce group; 7,000–6,700 cal BP) (Janek, 1972). Human occupation continued during the Late Neolithic (Lengyel I-II culture; ~6,700–6,000 cal BP), with evidence of a large settled area (~25 ha) situated between Santovka and Domadice villages (Budinský-Krička, 1941; Ambros, 1977; Jakab, 1977; Pavúk, 1977, 1987; 1994, 1997; Bača, 1990).

3. Material and methods

3.1. Coring of the paleoecological profile

In May 2015, a 620 cm long profile (333–950 cm from the surface) was obtained from Santovka-PB using a percussion drilling set. The upper 333 cm (0–333 cm) contained clayey flushes from adjacent slopes which were not analysed. During profile coring, it was necessary to break through a consolidated travertine layer present between ~643 and 591 cm, which resulted in missing sediments at these depths. Once obtained, sediment cores were then stored in plastic tubes in the fridge and then sampled at the Department of Botany and Zoology, Masaryk University in Brno.

Fig. 1. Location of the Santovka-Pramene Budzgov study site located in southwest Slovakia (Central-Eastern Europe) on the Western Carpathian/Pannonian Basin border. Locations of other study sites discussed in the text are indicated.

3.2. *Electrical resistivity tomography survey*

In April 2015, Electrical Resistivity Tomography (ERT) survey was performed at Santovka-PB. ERT is a geophysical subsurface-imaging technique, widely applied in various geoscience studies. Interpretations of results were based on knowledge of the local geology (Nagy et al., 1998), supported by documented profile sedimentary records, and knowledge of geological and geomorphological formations in the area. Altogether, 6 profiles (Figs. 3 and 4) were measured in the area of the infilled paleolake. All ERT profiles used a Wenner-Schlumberger array, which is suitable for expected subhorizontal layering (e.g. Loke, 1997; Engel et al., 2017; Hošek et al., 2019). Profile 1 was lead along the valley axis, crossing the main travertine mound (accumulation), where the paleolake was dammed (Figs. 3 and 4). Profile 2 crossed the valley perpendicular to profile 1, across the dam. Profile 3 crossed the eastern side of the valley floor and the travertine dam. Profile 4 crossed the western side of the valley floor and another travertine mound situated on a mild slope west of the travertine dam. Two transversal profiles, 5 and 6, were taken 20 m and 100 m distance from the travertine dam. Profiles 2, 3, 5, and 6 intersected profile 1 at 85 m, 137 m, 192 m and 285 m (Fig. 3), respectively.

3.3. *^{14}C dating*

AMS ^{14}C dating was processed at the Center for Applied Isotope Studies at the University of Georgia, USA. For radiocarbon dating, 14 terrestrial plant macrofossils and 3 terrestrial mollusc shells were used (see Supplementary Information, Table 1). Pre-treatment and processing protocols for radiocarbon dating can be found on CAIS's webpage (http://cais.uga.edu/analysis_ams.html). Obtained ^{14}C dates were calibrated and an age-depth model was created using Oxcal 4.2.4 (Bronk Ramsey, 2009) and the IntCal13 atmospheric curve (Reimer et al., 2013).

3.4. *Plant macrofossil and mollusc analysis*

For plant macrofossil and mollusc analysis, sediments were sub-sampled at 3 cm (volume 40 mL) resolution, and rinsed through a 200 μm mesh. Subsequently, both plant and mollusc macrofossils were manually sorted from the residuum, identified and counted under a dissecting microscope at 12× magnification and higher. The Reference collection at the Department of Botany, Charles University was consulted for identification of plant remains together with several atlases and identification keys (Bojňanský and Fargašová, 2007; Cappers et al., 2006; Berggren, 1969; Katz et al., 1977; Velichkevich and Zastawniak, 2006, 2008). Plant nomenclature follows Kubát et al. (2002). Molluscs were identified using Ložek (1964), Horsák et al. (2013, 2019) and Nekola et al. (2018).

3.5. *Pollen analysis*

For pollen analysis, sediments were sub-sampled every 6 cm (volume 1 cm^3). The samples were later processed following standard techniques that use HF and HCl to eliminate mineral material from the samples (Faegri et al., 1989). A minimum of 500 terrestrial pollen grains were identified except between 947 and 793 cm (i.e. the inorganic basal part of profile) where a total of 300 pollen grains were counted. Pollen identification was aided by standard keys and photo collections (Beug, 2004; Reille, 1992; Punt and Clarke, 1984; Blackmore et al., 2003). Non-pollen palynomorphs were identified following Van Geel et al. (1980). Pollen counts were transformed into percentages using the sum of all terrestrial plants except *Alnus*, aquatic pollen and non-pollen palynomorphs. The excluded taxa were calculated as the proportion of the sum of all pollen grains counted in a sample.

3.6. *Chironomid analysis and mean July air temperature reconstruction*

For chironomid analysis, sediments were sub-sampled every 3 cm between depths 935–700 cm. Samples (1.9–18.4 g of wet weight; 1.2–13.9 g of dry weight) were deflocculated for 20 min in 10% KOH (60–75 °C) and then passed through 250 and 100 μm mesh sieves. Sediments with at least 6 g of dry weight were mostly processed. At depth 905 cm, the density of chironomid heads were <10 g^{-1}, thus a larger volume was sorted. Chironomid capsules were hand-sorted under a dissecting microscope (20–40× magnification), and only specimens consisting of more than half of the mentum were counted. Chironomid heads were standardized to 1 g of dry sediment (sediment was dried to a constant weight at 105 °C). Chironomids were identified to standardised types following Brooks et al. (2007). A minimum number of 35 head capsules were counted (with the exception of depth 811 cm, where 20 head capsules were counted), and the recommended count of 50 capsules was reached in more than 50% of the samples. This is recommended for calculating and reconstruction July mean air temperatures (T_{July}) (Heiri and Lotter, 2001; Quinlan and Smol, 2001). Selected chironomid taxa were classified into ecological categories according to requirements regarding lake trophic status, bathymetric distribution and preference for macrophytes (Wiederholm, 1983; Brooks et al., 2007). At depths 820–814 cm, 844–832 cm, and 935–883 cm, chironomid abundances were relatively low, thus samples were merged (taken as a one depth) to reach a sufficient number of head capsules necessary to reconstruct T_{July}. Three samples (depths 893 cm, 871 cm and 790 cm) were excluded due to the absence of head capsules. A total of 14 depths from 33 sorted samples were therefore used to reconstruct past T_{July}. For temperature reconstruction a chironomid-temperature inference model based on a Swiss-Norwegian chironomid calibration dataset and Weighted-Averaging/Partial-Least-Squares regression was used (Heiri et al., 2011). The model is characterized by a bootstrapped (cross-validated) root mean squared error of prediction (RMSEP) of 1.4 °C and r^2 of 0.87 (Heiri et al., 2011) when assessed within the calibration dataset. Inferred T_{July} values were adjusted to 0 m asl of modern sea level using a lapse rate of 0.6 °C/100 m (see e.g. Heiri et al., 2014a) for comparisons with other reconstructions (Heiri et al., 2014b). Percentages were square-root transformed before reconstruction. Chironomid-based palaeotemperature reconstructions in the northern hemisphere are conventionally calibrated to July or August temperature, which represent the warmest month of the year in large parts of the Northern Hemisphere, although chironomid assemblages can be expected to respond mainly to changes in absolute temperatures during the multi-week period of maximum temperatures during summer, rather than to temperatures during a particular month (Samartin et al., 2017). Reconstructed T_{july} can be expected to be closely correlated to past changes in such absolute summer temperature values and to be representative for past temperatures during July as the month is defined by Berger and Loutre (1991; i.e. a constant number of days from Spring equinox).

3.7. Geochemical and sedimentological analyses

Loss-on-ignition (LOI) was used to determine the amount of organic carbon and carbonates present in the sediments (Heiri et al., 2001). One cm^3 samples were dried at 105 °C for 24 h, ground up and weighed to calculate water content. Samples were then dried at 550 °C and 950 °C, each for 3 h, and then weighed to determine percent organic carbon and carbonate content.

Magnetic susceptibility (MS) provides information about the amount of transported clastic material caused by erosion (Karlén and Matthews, 1992; Shakesby et al., 2007), and was determined using a Kappabridge KLY-2 magnetic system (Agico, Czech Republic). The instrumental data were normalized to obtain mass-specific magnetic susceptibility in $m^3 \cdot kg^{-1} \cdot 10^{-9}$.

Samples for geochemical analysis were dried and ground up to reduce sediment heterogeneity. Geochemical composition of the sediments was determined using a pXRF Innov X Delta device with a 4W Rh tube and 25 mm 2 silicon drift detector in soil analysis mode (each measurement consists of two 30-s beams). Values below the detection limit were replaced by values equivalent to half of the instrumental detection limit. For results, see the Supplementary Information.

3.8. Stable isotope analyses

Stable carbon isotope analysis was performed on carbonate fragments manually collected from the wet-sieved sediment. If the carbonate sand was very fine and did not allow manual separation, the wet sieved sediment was dried and leached with 30% H_2O_2 to remove organic carbon from the sample. Isotope relative mass spectrometry (IRMS) was used to determine the abundance of carbon and oxygen stable isotopes using the same method as Juřičková et al. (2018). Briefly, carbon dioxide was released from carbonates with anhydrous phosphoric acid; then it entered a mass spectrometer (Deltaplus XL, Thermo Finnigan, Bremen, Germany) via an interface (GasBench II, Thermo Finnigan, Bremen, Germany). The relative abundances of $^{13}C/^{12}C$ and $^{18}O/^{16}O$ found within the released carbon dioxide molecules were expressed using a ‘δ’ notation. Standard deviations for both $^{13}C/^{12}C$ and $^{18}O/^{16}O$ were mostly <0.2‰. Final results were expressed with respect to the International Atomic Energy Agency (IAEA) standard VPDB (Vienna Pee Dee Belemnite; IAEA, Vienna, Austria).

3.9. Numerical analyses and data visualization

Summary diagrams containing geochemical, macrofossil, mollusc, pollen and chironomid data were created in Tilia v. 1.7.16 and TILIA.GRAPH (Grimm, 2011). Cluster analysis by sum-of-squares (Coniss analysis) was applied to identify the main zonation of the local (macrofossil, mollusc, chironomid and geochemical analysis) and regional (pollen) successions.

To explore temporal patterns within each of the datasets, principal components analysis (PCA) (geochemical composition, pollen data) and detrended correspondence analysis (DCA) (macrofossil and mollusc data) were performed using Canoco v 5.10. (Ter Braak and Šmilauer, 2012) with a down-weighting of rare species, logarithmic transformation of species data and detrending by segments. A gradient length of 1.8 standard deviation units (SD) was identified for pollen, 1.6 SD units for geochemical composition, 10.5 SD units for macrofossils and 7.8 SD units for molluscs. For pollen, 25.58% of the variation is explained by the first axis, 58.98% for geochemical composition, 9.96% for macrofossils and 17.64% for molluscs.

A combined diagram with both $\delta^{18}O$ (VPDB) and $\delta^{13}C$ (VPDB) values was created in Canoco v 5.10. (Ter Braak and Šmilauer, 2012).

4. Results

4.1. Sedimentological analysis

The bottom section of the sediment profile (947–783 cm) consisted predominantly of inorganic calcareous sediment often with rounded travertine pieces and sand. Sediment between 783 and 686 cm was more fine-grained with an admixture of organic limnic sediment (gyttja) and fine travertine pieces. Sediments alternated between dark brown organic layers and beige inorganic calcareous layers between depths 686 and 638 cm. Consolidated travertine was present between 638 and 590 cm. Highly organic material with fine travertine pieces gradually changed to light beige calcareous sediment between 590 and 560 cm. Organic sediment was again present between 406 and 400 cm. Inorganic sediment continued from depth 406 cm to the top of the profile (0 cm). For a more detailed description, see Table 2 in the Supplementary Information.

4.2. Radiocarbon dating

A reliable age-depth model was obtained using 13 AMS radiocarbon dates (SI Table 1) (Fig. 2), which reached an agreement value of 81% between calibrated and modelled values. The margin of error of the modelled ages (95% confidence interval) varied mostly between 89 and 384 years (935–838 cm), 76 and 150 years (837–688 cm), 200 and 274 years (590–582 cm) and 52 and 200 years (582–401 cm). Four radiocarbon dates were excluded from the age-depth model (SI Table 1); samples UGAMS 24859 and UGAMS 29463 were much younger than neighbouring data indicating contamination by younger material from the upper section of the profile between 686 and 643 cm. This was probably caused by breaking through the consolidated travertine layer (between 643 and 590 cm) during coring; and UGAMS 29465 and UGAMS 29466 were excluded as they are present in clayey flushes (333–0 cm) with evident sediment mixing where superposition of layers was not maintained.

4.3. ERT survey

Profile 1 (the longitudinal profile; 0–555 m length, almost 100 m depth) had four distinct resistivity zones (Fig. 4). Clearly identifiable was the travertine body located approximately 100–150 m from the start of the profile, reaching a thickness of approximately 18–20 m. The travertine deposit appeared to be represented by sharply limited zones of high resistivity (mostly 200–800 Ωm with extremes above 1000 Ωm). Approximately 150 m from the start of the profile, a 10–12 m thick surficial zone of medium resistivity (10–30 Ωm) was observable along the whole length of the profile, which was interpreted as travertine enriched lake sediment or lake/swamp sediments. A zone of low resistivity (3–10 Ωm) dominated between 5–10 m and 25–30 m below the surface along the whole profile, interpreted to be the original floodplain deposits. The bottom 30–100 m of the profile was formed by distinct zone of about 30–80 Ωm interpreted as a bedrock. Between 170 and 180 m from the start of the profile 1 at a depth 30–45 m was a significant vertical step (~10 m) in two distinct resistivity zones, indicating presence of a tectonic fault transversal to the valley. This is supported by the presence of travertine mounds, formed by springs, which are found roughly in line of the presumed faultline. The top 10–15 m of profile 2 were dominated by medium resistivity (Fig. 4), with distinct isolated islands of high resistivity (200–500 Ωm) interpreted as travertine bodies. Below 15 m from the surface, a low resistivity zone (3–10 Ωm) interpreted to be the original floodplain extends to a depth of 35–45 m below the surface. Bedrock exists below the original floodplain and had a medium resistivity (30–80 Ωm). According to profile 3 (Fig. 4), a 40 m-long, 40 m-wide and approximately 18 m tall compact travertine dam occurred, running east to west. Under the high-resistivity travertine body, a low resistivity zone existed until the bottom of the profile. The western end of profile 4 (approximately 50 m from the start of the profile) was formed by high-resistivity travertines, while the eastern side had medium resistivity of 30–50 Ωm (Fig. 3). The resistivity of two transversal profiles 5 and 6 was practically identical (Fig. 4). The top 5–10 m was formed by a medium resistivity (15–30 Ωm) layer, with a low (3–10 Ωm) resistivity zone below 10 m extending to approximately 30–35 m where the resistivity again increased to 20–60 Ωm.

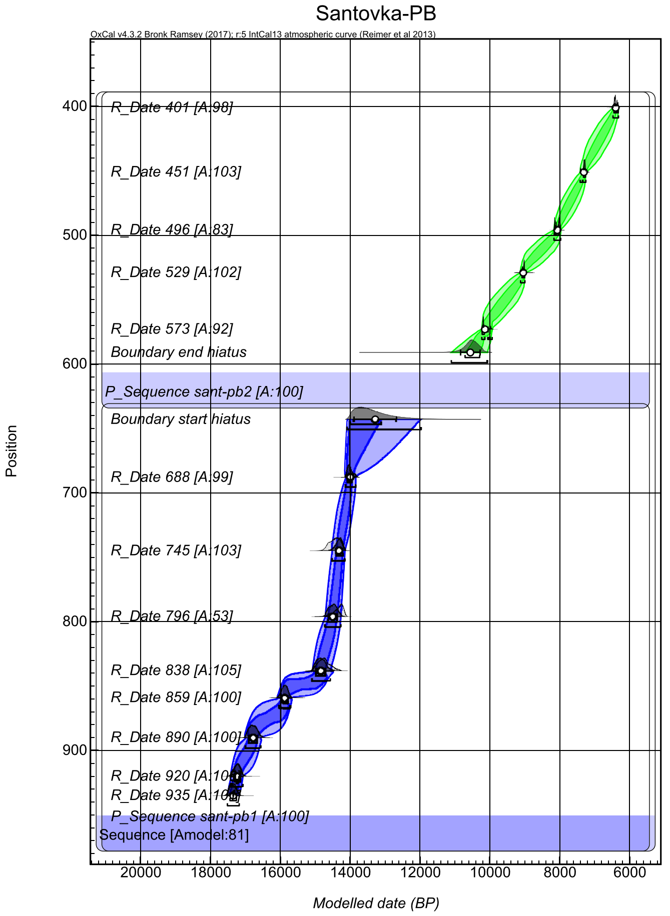

Fig. 2. Age-depth model of Santovka-Pramene Budzgov based on 13 ^{14}C dates. Highest posterior density ranges of 95.4% (light green colour) and 68.2% (dark blue colour) are displayed. Between 638 and 590 cm a temporal hiatus in fossil record was present. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4.4. Stable isotopes

δ^{13}C values that span the end of MIS 2 (depth 947–803 cm; 17,540–14,560) varied dramatically (ranging between −7.642‰ and +7.947‰), likely due to high erosion rates and the accumulation of diverse sedimentary material from the surrounding vicinity. δ^{18}O values ranged from −8.544 to −5.573 during the MIS 2. All Lateglacial samples (depth 803–686 cm; 14,560–13,950 cal BP) and six samples of early Holocene age (depth 591–550 cm; 10,560–9570 cal BP) had very high values of δ^{13}C (range from +0.2 to +7.1), which coincide well with isotope data from Slovakian travertines analysed by Gradziński et al. (2008), where δ^{13}C values range from +0.9 to +8.4‰ (Fig. 5). Lateglacial δ^{18}O values ranged from −10.739 to −6.913. The remaining samples of Holocene age (depth 547–472; 9500–7660 cal BP) showed distinctly lower δ^{13}C values (range from 0‰ to −2‰), while δ^{18}O showed a slight shift to higher values ranging from −7.632‰ to −6.254‰.

4.5. Macrofossils

Zone SA-mal, the bottom-most section of the sediment profile (depth 947–876 cm; 17,540–16,360 cal BP) was marked by a mixture of dwarf shrubs and herbs (*Betula nana*, *B. humilis*, Poaceae, *Selaginella selaginoides*), which indicate the presence of dwarf-birch tundra vegetation around the coring site (Fig. 6). Fish remains, *Zannichellia palustris* and *Cenococcum geophilum* (indicator of soil erosion in lake sediments) point to the existence of a stream at the coring site, which was also confirmed by higher values of MS. Zone SA-ma2 (depth 876–803 cm; 16,360–14,560 cal BP) was

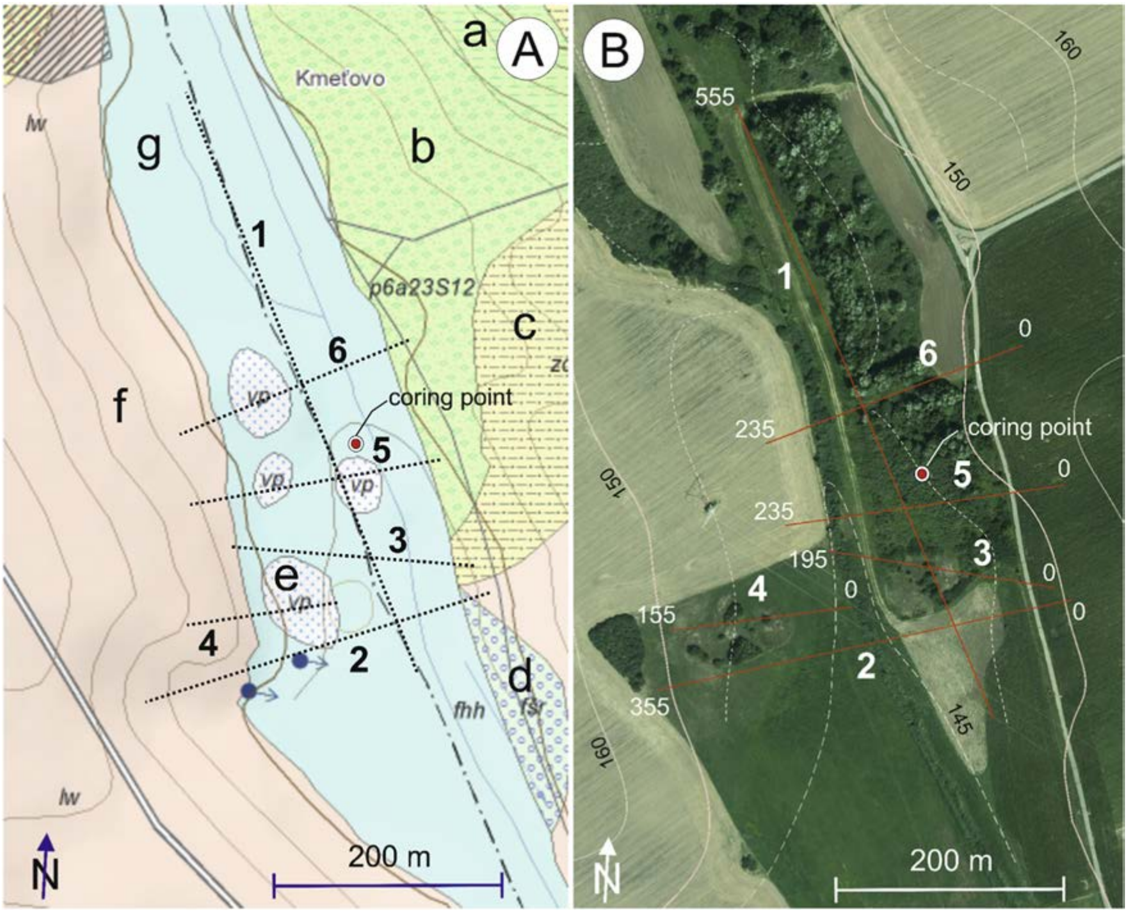

Fig. 3. Position of the Electrical Resistivity Tomography profiles (1–6) measured in the study area. A: Geological basemap 1:50 000 (Nagy et al., 1998); a – tuffitic sandstones and andezite conglomerates; b – epiclastic andesitic sandstones; c – eluvial-deluvial sediments; d – fluvial sediments of fluvial terrace; e – travertine; f – aeolian sediments (loess, loess loam); g – fluvial sediments of floodplain. B: Aerial photo of study area with contourlines (black numbers). White numbers indicate the number (large) and length (small) of each of the 6 profiles, with 0 delineating the start of each profile. Red dot indicates location of the coring point. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

characterized by an increase in species richness of aquatic macrophytes (e.g. *Stuckenia filiformis*, *Hippuris vulgaris*, *Potamogeton pussilus* agg.), which may indicate the transition from a wild stream to a permanent and calmer stream flow. Terrestrial vegetation was still predominately birch-dwarf tundra vegetation but with a higher representation of herbs (e.g. *Chenopodium glacum/rubrum*, *Polygonum aviculare*, *Veronica/Pseudolysimachion*), and less grasses in comparison to the previous zone. In Zone SA-ma3 (depth 803–686 cm; 14,560–13,950 cal BP), numerous new plant taxa rapidly appeared, especially wetland (e.g. *Carex caespitosa*, *Cicuta virosa*, *Sparganium erectum*, *Schoenoplectus tabernaemontani*) and aquatic species (*Potamogeton crispus*, *Chara* sp.), indicating the development of a calcareous, nutrient rich water reservoir with stagnant or slowly flowing water. The reservoir had to be deep enough to allow the presence of fish stock. Dwarf shrub vegetation still dominated with an admixture of *Pinus sylvestris/mugo*. Between 686 and 591 cm, a hiatus in fossil record was present. Above the hiatus in Zone SA-ma4 (depth 591–550 cm; 10,560–9570 cal BP), relatively few macrofossils were found, except *Schoenoplectus tabernaemontani* and *Chenopodium glaucum/rubrum*. Fish remains and *Chara* sp. oogonia together with low MS values and high organic and carbonate content indicate the presence of a shallow calcareous pool. In the upper zone of SA-ma5 (depth 550–480 cm; 9570–7800 cal BP) only a few macrofossils were preserved as a result of high carbonate precipitation rates of travertine deposits. Contrary, Zone SA-ma6 (depth 480–400 cm; 7800–6400 cal BP) was marked by a high abundance of macrofossils from various environmental groups. The common presence of *Chara* sp., *Najas marina*, *Ranunculus* subgen. *Batrachium*, *Schoenoplectus tabernaemontani*, *Typha latifolia/angustifolia*, *Cladium mariscus*, *Rumex maritimus*, fish remains and *Daphnia magna* ephippia point to the existence of a permanent calcareous pool surrounded by species-rich wetland vegetation. Preservation of macrofossils in the uppermost zone, SA-ma7 (depth 400–335 cm; 6400–? cal BP) was strongly influenced by increased erosional processes from the adjacent loess slopes. The presence of field weeds *Hyoscamus niger* and *Fumaria officinalis* in the profile was most likely linked with agricultural activities of Neolithic people, who may have cultivated crops on adjacent loess slopes, resulting in higher erosion rates.

4.6. Molluscs

The mollusc record covers the time interval between 14,560 and ~6000 cal BP as the bottom section of the profile (SA-mo1; depth 947–803 cm; 17,540–14,560 cal BP) was not suitable for shell preservation (Fig. 7). In general, mollusc record was very rich, consisting of 63 species identified among 7686 analysed shells. Only a single shell of the land snail *Vertigo genesii*, a cold adapted calcareous spring specialist, was found at a depth 945 cm (~17,500 cal BP). This characteristic species of the Late Glacial has

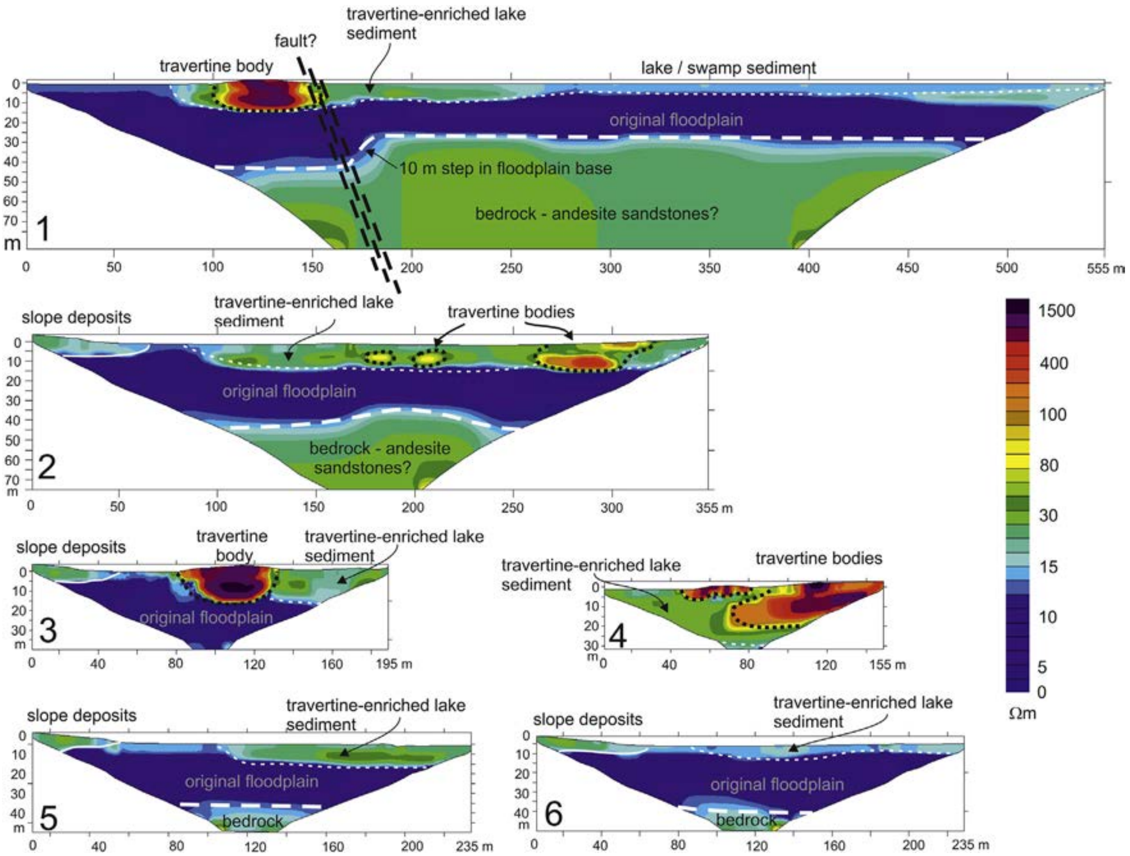

Fig. 4. Geomorphological and geological features of the Santovka-Pramene Budzgov valley based on interpreted Electrical Resistivity Tomography profiles (1–6). 1) is the longitudinal profile; 2–6) are the transversal profiles.

only rarely been reported from the full glacial layers. During Zone SA-mo2 (depth 803–686 cm; 14,560–13,950 cal BP), steppe snail species *Granaria frumentum* and *Helicopsis striata*, and the characteristic steppe-tundra snail *Vallonia tenuilabris* that became extinct in Europe during Early Holocene clearly indicate cold steppe conditions. Spring fen species (*Pupilla alpicola* and *Vertigo geyeri*) suggest the presence of fen habitats, likely developed along spring brooks. Except several species of shallow pools, running-water mollusc species were common, including *Pisidium tenuilineatum* and *P. pulchellum*; the latter represents the first record in Slovakia from both past and present Quaternary. Between depths 686–591 cm (13,950–10,560 cal BP), a 3000-year long hiatus was recorded in which the site became notably drier, without any running water habitats. During Zone SA-mo3 (depth 591–550 cm; 10,560–9570 cal BP), *Granaria frumentum* and *Pupilla muscorum* indicate an open, dry steppe environment, while the fen snails *Vertigo angustior* and *Pupilla alpicola*, and aquatic snails *Anisus vorticulus* and *Bathyomphalus contortus* show combination of fen wetland with shallow pools. A clear developmental shift to a typical semi-dry fen without any aquatic habitats, but also any steppe habitats in the surroundings, was observed at the beginning of Zone SA-mo4 (depth 550–480 cm; 9570–7800 cal BP). In contrast, a strong moistening happened at the beginning of Zone SA-mo5 (depth 480–400 cm; 7800–6400 cal BP) as most several land snail species disappeared (e.g. *Vertigo pygmaea*) or sharply reduced their abundances (e.g. *Vallonia pulchella*). These were replaced by a rich assemblage of aquatic molluscs requiring stable standing waters (e.g. *Anisus vorticulus* and *Lymnaea stagnalis*). Two wetland species, *Vertigo antivertigo* and *V. moulinsiana*, were abundant also in the wettest period (i.e. around 6400 cal BP) as they can climb up the vegetation and thus survive even long periods of habitat overflooding. In Zone SA-mo-6 (depth 400–335 cm; 6400–? cal BP), only few mollusc shells were found.

4.7. Pollen

Vegetation in Zone SA-p1 (depth 947–885 cm; 17,540–16,600 cal BP) was typical of cold, glacial conditions, dominated by non-forest vegetation (e.g., Poaceae, *Artemisia*, *Helianthemum nummularium* t.) and open stands of undemanding trees and shrubs such as *Pinus sylvestris* t., *P. cembra*, *Larix*, *Juniperus*, *Betula pubescens* t., and *B. nana* t. Pollen of deciduous trees (*Quercus*, *Corylus avellana*, *Tilia*, *Ulmus*) sporadically occurred as well (Fig. 8). Pollen grains of a tertiary species, *Carya*, were found in depths 929 (2 grains) and 935 (1 grains) cm. At the onset of Zone SA-p2 (depth 885–772 cm; 16,600–14,410 cal BP), *Pinus sylvestris* t. expanded synchronously with a decline in Poaceae and slight increase in *Quercus*, *Corylus avellana*, *Sparganium* and Cyperaceae indicating a change in local hydrological conditions. The composition of forest vegetation remained the same until the beginning of Zone SA-p3a (depth 772–686 cm; 14,410–13,950 cal BP), when cold-adapted vegetation disappeared (*Pinus cembra*, *Dryas* sp.) and/or gradually declined (*Larix*, *Betula nana* t.), and was replaced by the rapidly increasing *Pinus sylvestris* t. Aquatic and wetland plants (*Sparganium/Typha angustifolia*, *Typha latifolia* t., *Potamogeton/Triglochin*) also expanded during this zone indicating a change in hydrological conditions. Between 686 and 591 cm, a sediment hiatus is present. After the hiatus, in Zone SA-p3b (depth 591–573 cm; 10,520–10,140 cal BP), the pollen record remained the same as in the previous zone. The most prominent change in vegetation cover

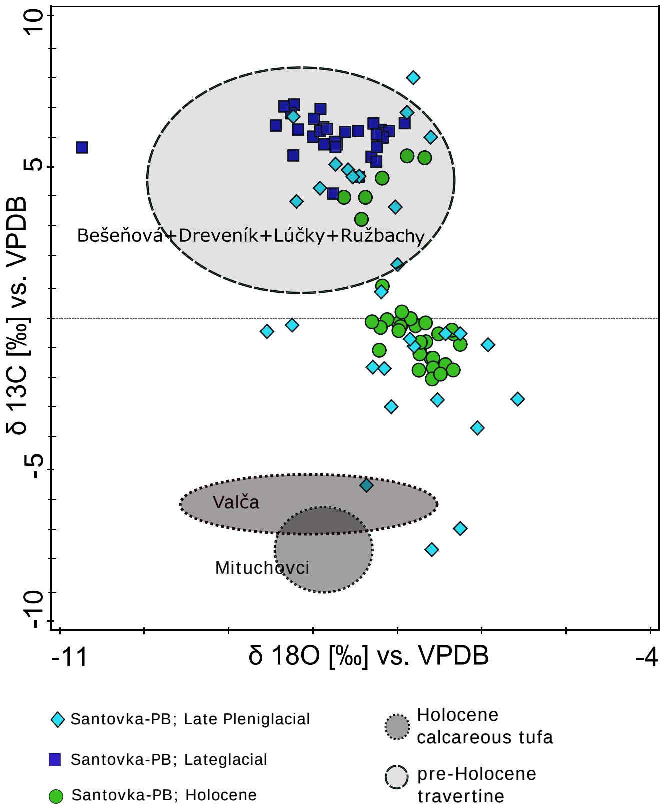

Fig. 5. Comparison of stable $\delta^{13}C$ and $\delta^{18}O$ isotopes derived from carbonate samples from Santovka-Pramene Budzgov and other sites from Slovakia (their locations indicated in Fig. 1). Data sources: Bešeňová+Dreveník + Lúčky + Ružbachy (Gradziński et al., 2008); Valča (Juřičková et al., 2018); Mituchovci (Dabkowski et al., 2019).

occurred at the onset of Zone SA-p4 (573–480 cm; 10,140–7800 cal BP) when deciduous trees (*Quercus*, *Corylus avellana*, *Tilia*, *Ulmus*) rapidly expanded, as did *Picea abies*. In Zone SA-p5 (480–400 cm; 7800–6400 cal BP), late-successional trees (*Fagus sylvatica, Abies alba*, *Carpinus betulus*) continuously occurred in the pollen record, and wetland taxa (*Typha latifolia* t. *Pediastrum boryanum* agg., *P. integrum*) increased indicating a change in local hydrological conditions. Additionally, crops (*Triticum* t.) and ruderals (*Polygonum aviculare* t.) first appeared ~7260 cal BP during this zone which overlaps with the Middle Neolithic period. Vegetation in Zone SA-p6 (depth 400–0 cm; 6400–? cal BP) is characterized by an increase in non-forest vegetation (e.g. Chenopodiaceae (Amaranthaceae), Poaceae) and the continuous occurrence of crops and ruderals, indicating increasing agricultural areas.

4.8. Chironomids

Zone SA-chi1 (depth 935–883 cm; 17,360–16,560 cal BP) was characterised by very low chironomid abundances (see chironomid productivity – i.e. head capsules per 1 g of dry sediment, Fig. 9). Phytophilic and epilithic chironomids (*Paratanytarsus* sp., *Cricotopus intersectus/laricomalis* t., *Orthocladius* sp.), as well as semi-terrestrial genera (*Limnophyes/Paralimnophyes*, *Pseudosmittia* sp.) were present, which are all indicative of a shallow lake with macrophytes in the littoral. In Zone SA-chi 2 (depth 883–844 cm; 16,560–15,150 cal BP), where low densities of chironomids continue, semi-terrestrial *Pseudosmittia* sp. again occurred together with several phytophilic and epilithic taxa, which were also recorded in the Zone SA-chi1. However, the appearance of *Odontomesa fulva* t., a cold stenotherm indicative of running water, and taxa preferring deep profundal zones (*Micropsectra contracta* t., *Zalutschia* sp.) suggest the input of cold running water from a nearby stream and increasing water depths. Zone SA-ch3 (depth 844–832 cm; 15,150–14,780 cal BP) taxa (*Chironomus plumosus* t.,

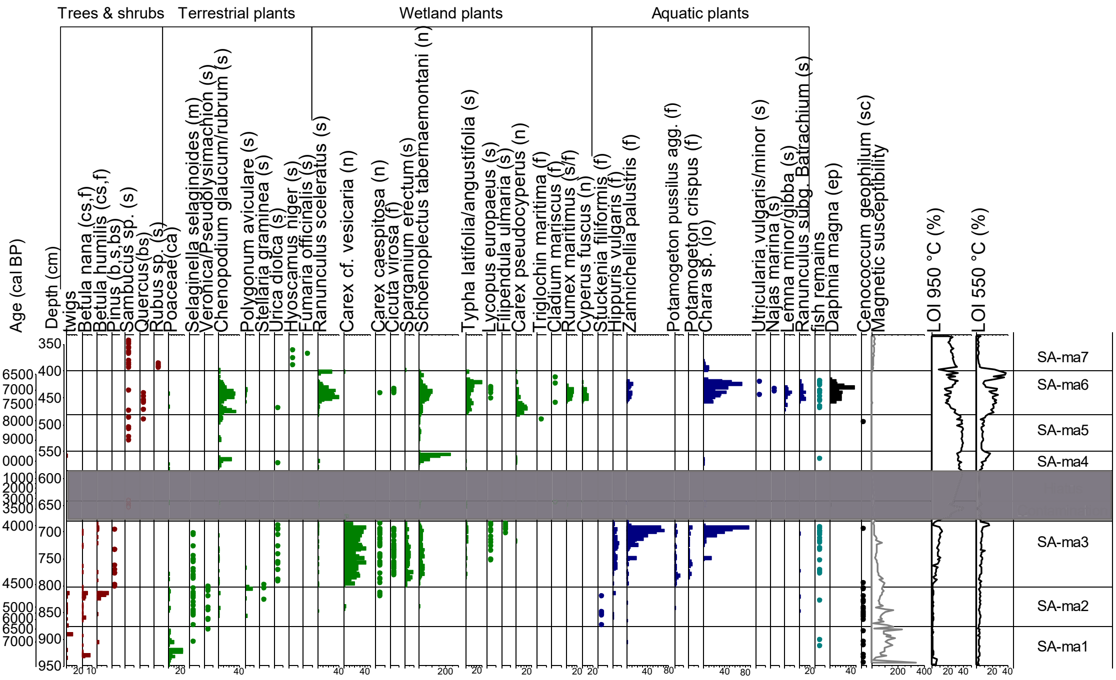

Fig. 6. Simplified macrofossil concentration diagram with loss on ignition and magnetic susceptibility from Santovka-Pramene Budzgov plotted on depth (cm) and calibrated age scales (years Before Present). Concentrations are represented by 40 cm^3 of sediment. b = bud; bs = bud scale; cs = catkin scale; ep = ephippium; f = fruit; n = nutlet; s = seed; sc = sclerotium. Colours define major vegetation groups (i.e. trees and shrubs, terrestrial plants, wetland plants, and aquatic plants). Grey box indicates the presence of a sediment hiatus. Analyst A. Šolcová. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

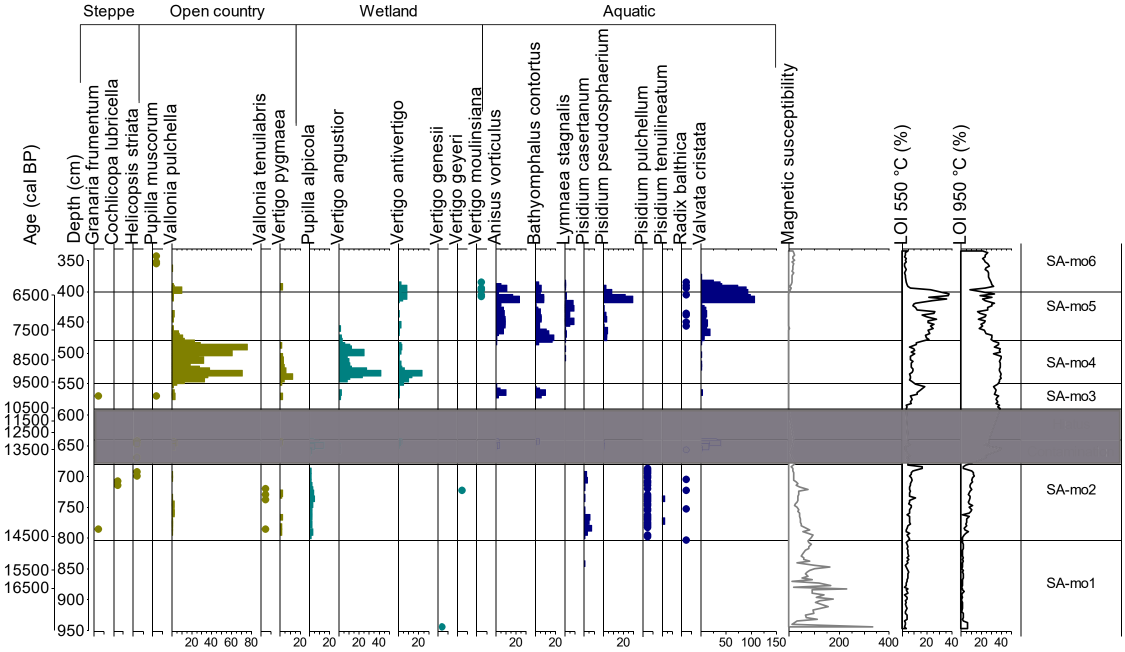

Fig. 7. Simplified mollusc concentration diagram with loss on ignition and magnetic susceptibility from Santovka-Pramene Budzgov plotted versus depth (cm) and calibrated age (years Before Present). Concentrations are represented by 40 cm^3 of sediment. Colours define dominant mollusc groups (i.e. steppe taxa, open country taxa, wetland taxa, and aquatic taxa). Grey box indicates the presence of a sediment hiatus. Analyst M. Horsák. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

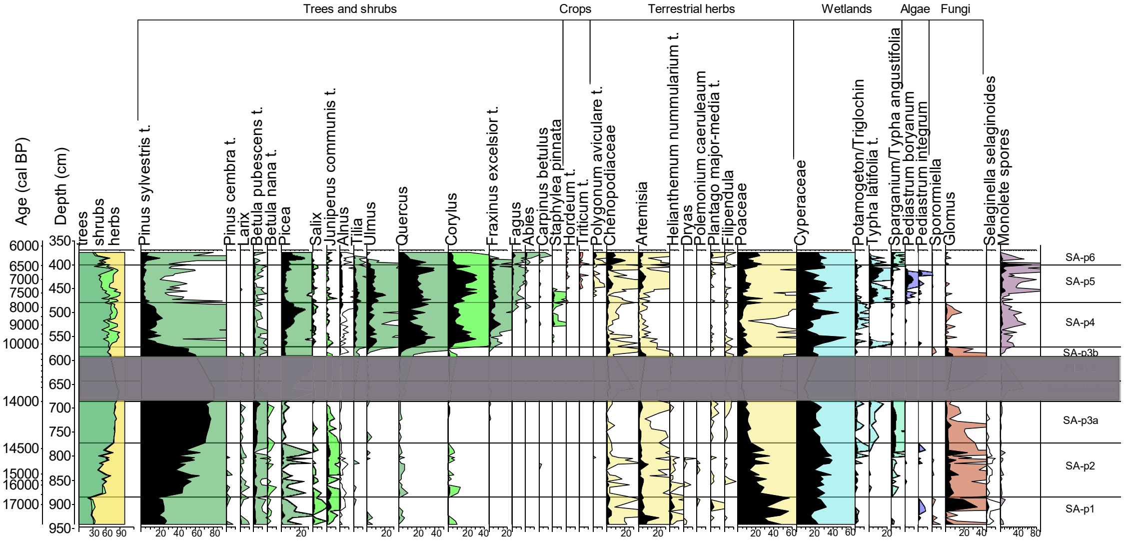

Fig. 8. Simplified percentage pollen diagram from Santovka-Pramene Budzgov plotted on depth (cm) and calibrated age scales (years Before Present). Colours represent major vegetation groups (i.e. trees and shrubs, crops, terrestrial herbs, wetland taxa, algae and fungi). Grey box indicates the presence of a sediment hiatus. Analyst E. Jamrichová. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Microtendipes pedellus t.) are indicative of higher trophic status and finer substrates. The input of flowing water was reflected by typical lotic taxa (*Parametriocnemus*/*Paraphenocladius* sp. or *Odontomesa fulva* t.), but the occurrence of profundal taxa such as *Micropsectra contracta* t. and *M. insignilobus* t. (sensu lato) could indicate higher water depth. Zone SA-chi 4 (depth 832-802 cm; 14,780–14,550 cal BP) was characterised by the presence of typical taxa of running water (*Odontomesa fulva* t. and *Parametriocnemus*/*Paraphenocladius* sp.), as well as semiterrestrial (*Pseudosmittia* sp., *Limnophyes*/*Paralimnophyes* sp.) and phytophilic/epilithic taxa (*Orthocladius* sp., *Cricotopus cylindraceus* t.). Cold stenotherm species (*Micropsectra radialis* t.) and oligostenotherms species (*Apsectrotanypus* sp.) were found in this zone, signalling the coldest conditions of the entire profile. Zone SA-ch5 (depth 802–700 cm; 14,550–14,070 cal BP) was characterised by an increase in chironomid density and taxonomic diversity, indicating an increase in lake productivity. Taxa preferring moderate to high temperatures, higher trophic states and macrophytes were frequent in this zone (*Cricotopus* sp., *Phaenopsectra flavipes* t., *Chironomus plumosus* t.). Increase of temperature was probably accompanied by a higher water table (higher representation of taxa that can colonize deeper lakes such as *Micropsectra contracta* t., *M. insignilobus* t.).

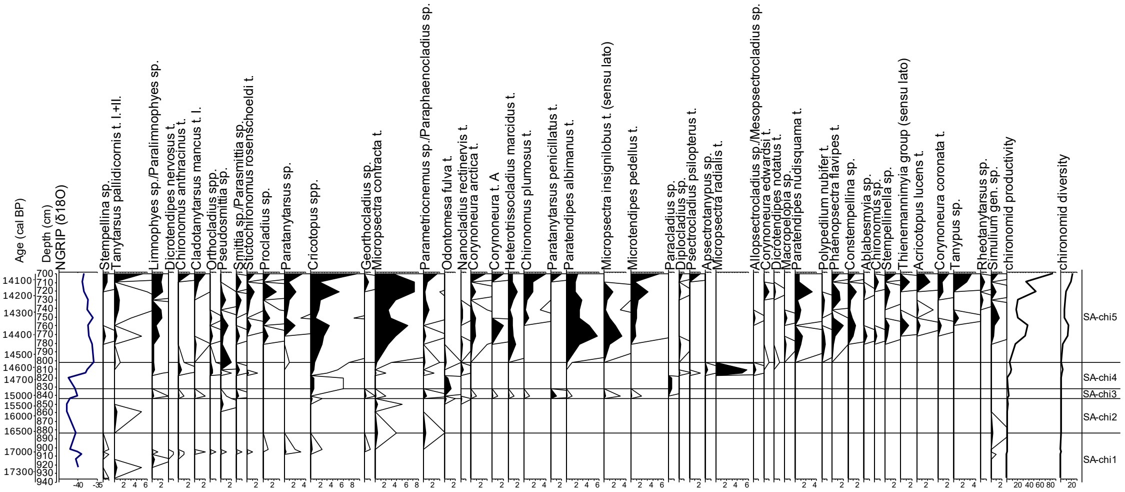

Fig. 9. Simplified chironomid concentration diagram of the most important and abundant chironomid morphotypes from Santovka-Pramene Budzgov plotted on a depth (cm) and the calibrated age scale (years Before Present). Concentration values are represented by their density per gram of dry weight. Some rare groups were merged into wider species complexes or to genus level for illustration purposes, but not for the quantitative T_{July} reconstruction. NGRIP $\delta^{18}O$ data (NGRIP dating group, 2006) were downloaded from ftp://ftp.ncdc.noaa.gov/pub/data/paleo/icecore/greenland/summit/ngrip/gicc05-20yr.txt. Chironomid productivity (head capsules per 1 g of dry sediment) together with chironomid diversity (number of taxa per 1 g of dry sediment) are shown on the right side of the diagram. Analyst P. Pařil.

4.9. Chironomid based temperature reconstruction

During the end of MIS 2 (~17,040–15,250 cal BP), T_{july} temperatures ranged between 15.7 °C and 15.3 °C (adjusted to modern sea level), based on merging 7 samples between 935 and 883 cm and 5 samples between 859 and 832 cm (Fig. 10). However, these two merged samples should be interpreted with caution as these reconstructions did not meet the 35 head capsule minimum. A more reliable reconstruction of T_{july} began ~14,660 cal BP (by merging depths 814 and 820 cm), which indicates a temperature of 13.7 °C. At 14,620 cal BP, temperatures were 12.7 °C (811 cm) but this sample was based on 19 specimens and thus should be interpreted with caution. From 14,620 to 14,440 cal BP (depths 811 cm and 781 cm), we observed a rapid increase in T_{july} by more than 3.8 °C, from 12.7 °C to 16.5 °C. Temperatures in the remaining profile (depth 14,410–14,070 cal BP; 772–700 cm) oscillated between 14.1 and 17 °C with changes generally within the model error of 1.4 °C.

5. Discussion

5.1. Ecosystem state during MIS 2: Steppe-tundra or taiga forest?

The surrounding vegetation at Santovka-PB was dominated by grasses (more than 50%) followed by *Pinus sylvestris* (around 20%)

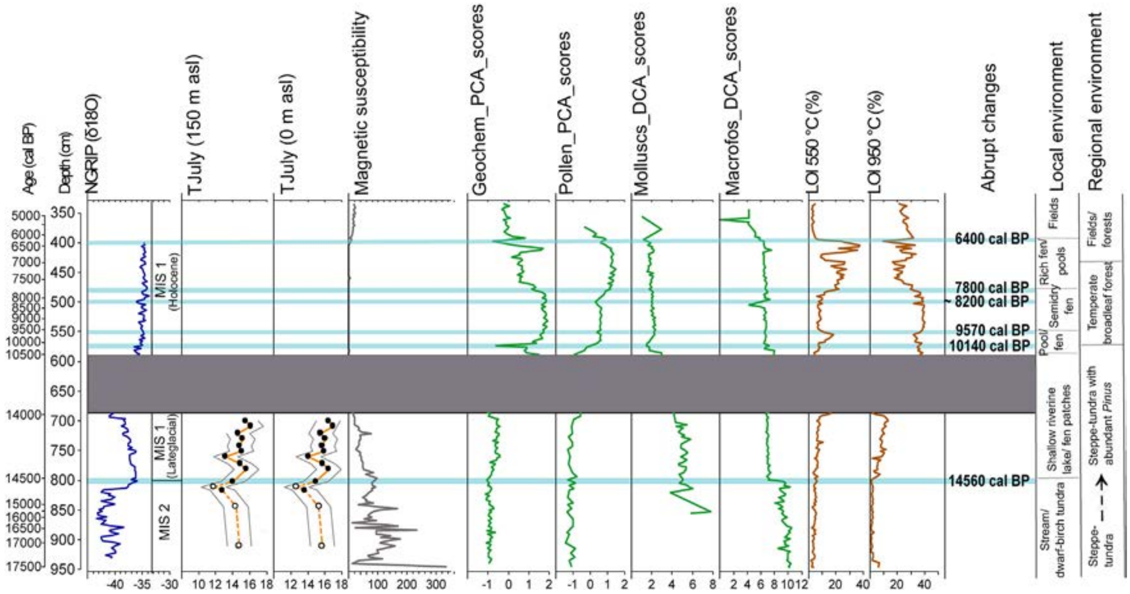

Fig. 10. Summary diagram of selected biotic and abiotic proxy data from Santovka-Pramene Budzgov (North Greenland Ice Core Project $\delta^{18}O$ values, mean July temperature (T_{July}) reconstruction based on the chironomid record, Magnetic susceptibility, PCA (Principal Components Analysis) and DCA (Detrended Correspondence Analysis) results of biotic proxy data and Loss on ignition). Displayed data were selected to demonstrate temporal changes in the local and regional environment. NGRIP $\delta^{18}O$ data (NGRIP dating group, 2006) were downloaded from ftp://ftp.ncdc.noaa.gov/pub/data/paleo/icecore/greenland/summit/ngrip/gicc05-20yr.txt. Empty circles in the T_{July} curve indicate limited validity of the reconstruction due to a low number of chironomids. Full circles display more reliable results in the T_{July} reconstruction, and are based on more than 35 individuals and a maximum of two adjoining depths merged together. Abrupt changes in the fossil record are highlighted by blue horizontal lines. Grey box indicates the presence of a sediment hiatus. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and with the increased admixture of open-landscape taxa (e.g. *Artemisia* and Amaranthaceae; incl. former Chenopodiaceae) between 17,550 and 16,500 cal BP (Fig. 8), suggesting an open steppe-tundra ecosystem in the vicinity of the site. According to Chytrý et al. (2019), Central European full-glacial steppe-tundra ecosystem with loess deposition was comprised of an admixture of dry environments (i.e. mosaics of desert or typical steppe), and more mesic/wetter environments on north-facing slopes, or along streams. In our case, on the left side of the valley where loess had been deposited (Fig. 3A), a typical steppe environment dominated by perennial clumped grasses probably prevailed and a sparse tree layer of *Pinus sylvestris* may have grown on more mesic places around Santovka-PB during MIS 2. Macrofossil analysis further revealed the presence of dwarf-birch tundra, which likely existed at the bottom of the valley. Abundant macrofossils of grasses may also indicate the local proximity of wet grasslands occurring along the local stream (Fig. 6) (see SI Fig. 2 for modern analogue). Paleoecological investigations from the Pannonian Basin indicate similar results with the predominance of *Pinus* (>50% pollen) and grasses (Sümegi et al., 2011, 2013; Kustár et al., 2016), with sporadic yet significant admixtures of temperate trees (Sümegi et al., 2013). Our fossil pollen record also captured small, but continuous amounts of temperate tree pollen (*Quercus*, *Corylus avellana*, *Tilia*, *Alnus*) between 17,550 and 14,600 cal BP (Fig. 8), indicating either these species were in close proximity to Santovka-PB, or long-distance pollen dispersion. The possibility of redeposition of temperate tree pollen seems negligible in this case, as only three pollen grains of tertiary species (*Carya*) were found in the bottommost section of the profile (17,500–17,300 cal BP). Local presence of temperate trees could have occurred in gallery forests along rivers and around lakes where higher soil and air moisture aided their survival (Willis and Van Andel, 2004). Despite of presence of temperate tree pollen, we lack undeniable evidence (i.e. macrofossil, wood, charcoal) about the presence of local temperate trees during MIS 2, necessary to prove the presence of a local refugium (Tzedakis et al., 2013). In contrast to lowland sites in the Pannonian Basin, mid-elevation sites (~350–600 m asl) from the Western Carpathians indicate the presence of hemiboreal (taiga) forest (AP>80%) dominated by *Picea abies*, *Pinus* and *Betula* with an admixture of temperate trees during MIS 2 (Jankovská and Pokorný, 2008). It is hypothesized that northern temperate forest microrefugia were located there, as a result of wetter climatic conditions due to orographical precipitation and relatively higher air humidity (Ložek, 2006; Jankovská and Pokorný, 2008; Juřičková et al., 2014).

Around 16,500 cal BP (880 cm), *Pinus sylvestris* pollen increased (up to ~60%) at the expense of grasses and other open-landscape taxa, indicating the advancement of pine forests with an admixture of other conifer taxa and temperate trees (e.g. *Quercus*, *Corylus avellana*). Despite the increase in *Pinus sylvestris* pollen, the steppe-tundra ecosystem persisted. Simultaneous with the advancement of pine forests was the first appearance of aquatic macrophyte taxa (e.g. *Stuckenia filiformis*, *Hippuris vulgaris*) (Fig. 6), suggesting wetter conditions. According to Nádor et al. (2007), mean July air temperatures decreased to ~12–15 °C and dry loess steppe started to expand in the Pannonian Basin around 16,500 cal BP as humidity decreased, which is contrary to our results. Such discrepancies could be explained by local climate deviations possibly linked to the proximity of the Western Carpathians. Moreover, a short-term substantial humid pulse and/or overall climate amelioration was recorded between 15,000 and 14,800 cal BP, as indicated by *Potamogeton pussilus* agg., *Schoenoplectus tabernaemontani* and several chironomid taxa (SA-chi 3, Fig. 9) all indicating a higher water table (Fig. 6).

5.2. Biotic response to abrupt climate change at MIS 2/MIS 1 transition

An abrupt and persistent climate amelioration occurred at the MIS 2/MIS 1 transition (corresponding to the GS-2a/GI-1e transition (Lowe et al., 2008; Rasmussen et al., 2014)) around 14,640 ± 186 cal BP (GICC05 timescale). This amelioration event is well documented in the NGRIP (North Greenland Ice Core Project) oxygen-isotope record as a rapid increase in $\delta^{18}O$ indicating rising temperatures over the Greenland ice sheet (Rasmussen et al., 2006; Lowe et al., 2008). In Central Europe, this transition is generally considered to coincide with the Oldest Dryas to Bølling-Allerød transition apparent in many lacustrine sediment records (e.g. Van Raden et al., 2013). This climatic event was captured at Santovka-PB, based on chironomid and macrofossil record, and is dated to 14,560 ± 130 cal BP (803 cm), which is within the margin of error of the GICC05 timescale model given by Rasmussen et al. (2006) and/or within the margin of error in our age-depth model, so we believed that this climate transition occurred synchronously at Santovka-PB and in Greenland.

At the MIS 2/MIS 1 transition, chironomid-based July temperature reconstructions from other mainland sites in Europe increased between 3 and 4 °C in the northern Alps (Larocque-Tobler et al., 2010), northern Italy (Heiri et al., 2007), and Jura, France (Heiri and Millet, 2005). Temperatures also increased between 2.7 and 4.0 °C in the southern Swiss Alps (Samartin et al., 2012), 2.8 °C in the southern Carpathians (Tóth et al., 2012), and 3.5–5.0 °C in Poland (Płóciennik et al., 2011). Our study site documented a 2.2 °C temperature increase at the MIS 2/MIS 1 transition, from ~11.8 ± 1.5 °C (811; sample age 14,620 cal BP) to 14.0 ± 1.4 °C (802 cm; sample age 14,550 cal BP) (Fig. 9). However, three of the four reconstructed MIS 2 temperatures must be interpreted with caution (including sample age 14,620 cal BP) due to low chironomid densities and/or because several samples had to be merged to reach minimum counts suitable for the climate reconstruction. When adjusted to 0 m asl for the end of the MIS 2 (13.7 °C - 14,660 cal BP and 12.7 °C - 14,620 cal BP), our T_{July} temperature reconstruction is ~2 °C lower than expected based on other chironomid reconstructions for 48° N in Europe (Heiri et al., 2014a). However, our coldest reconstructed T_{July} from MIS 2 overlaps with the dominance of cold stenotherm *Micropsectra radialis*-type chironomids (Fig. 9). Similarly, T_{july} are 1–2 °C lower (14.9 °C) at 14,550 cal BP during the earliest Lateglacial interstadial, although this value is only constrained by a single chironomid sample. Overall colder reconstructed temperatures in the Lateglacial interstadial may be due to a significant input of cold oxygen saturated river water when study site was likely a very shallow riverine lake possibly as shallow as 1–2 m. Such running water conditions likely enabled the persistence of more cold adapted taxa (which do not typically occur in fully lentic lake environments traditionally used for temperature reconstructions), thus giving a lower than expected temperature signal at Santovka-PB.

As a consequence of increases in both temperature and precipitation at the MIS 2/MIS 1 transition, local vegetation at Santovka-PB responded abruptly resulting in significant vegetation turnover (see macrofossil DCA scores, Fig. 10). For example, cold tolerant and rather oligotrophic aquatic species such as *Stuckenia filiformis,* which is a frequent lateglacial aquatic plant (Gałka and Sznel, 2013; Jamrichová et al., 2014; Petr et al., 2013), were replaced by more nutrient and warm-demanding species such as *Potamogeton crispus* and *Zannichellia palustris*. These warm-demanding taxa are commonly present in Holocene macrofossil records across Central Europe (e.g. Potůčková et al., 2018; Gałka et al., 2014), hence their early presence here is very striking. Their presence at Santovka-PB during the Lateglacial was probably enabled by less stream input/impact (represented by low magnetic susceptibility values in the record) as a result of the creation of a shallow lake where gyttja (organic lake sediment with high abundance of diatom frustules) started to accumulate. The creation of a lake, which based on ERT measurements was at least 415 m in length and 130–200 m in width, could be linked with increased accumulation of travertine deposits (Fig. 4), which may have dammed the stream. Remains of *Simulium* gen. sp. (black fly) larvae (Fig. 9) and shells of the clams *Pisidium tenuilineatum* and *P. pulchellum* (Fig. 7) in the fossil record indicate that the lake had at least slow flowing water. Around the lake, species-rich and warm and nutrient-demanding wetland vegetation expanded, dominated by *Schoenoplectus tabernaemontani*, *Cicuta virosa*, *Carex caespitosa*, *Sparganium erectum*, *Lycopus europaeus*, while steppe-tundra elements *Selaginella selaginoides*, *Betula nana* and *B. humilis* were present in the surrounding landscape (Fig. 6).

Regionally, large-scale vegetation change was not evident at the MIS 2/MIS 1 transition (Fig. 8), except for the disappearance of several open-landscape taxa (e.g. *Helianthemum nummularium* t., *Dryas*, *Polemonium caeruleum*), and a slight decline in Amaranthaceae (incl. former Chenopodiaceae). Rather, a large change in the pollen record took place ~150 years later around 14,410 cal BP, when *Pinus sylvestris* pollen increased to >70% and grasses decreased to <15%. Delayed reaction of regional vegetation to rapid climate changes is seen elsewhere as a consequence of various biotic and abiotic factors, such as migrational lags (Pennington, 1986; Hoek, 2001), competition with other trees (Birks, 1986), other climatic factors such as windiness or dryness (Paus, 2010), and local edaphic conditions and abiotic landscape factors (Hoek, 2001; Ruszkiczay-Rüdiger and Kern, 2016). Short-distance migrational lag is most likely the cause of the 150-year lag at our site as the expansion of trees is a relatively slow process which may take hundreds of years (Birks, 1986). From Pannonian Basin, there is no similarly detailed paleoecological investigation suitable for a comprehensive comparison of vegetation change at the MIS 2/MIS 1 transition with our results, making our study very exceptional.

5.3. Abrupt Holocene vegetation change linked with climate fluctuations and human impact

Regionally, two Early Holocene abrupt climatic events caused changes in regional vegetation at 10,140 and ~6400 cal BP (Figs. 8 and 10). The first change around 10,140 cal BP was the result of climate amelioration at the onset of the Holocene (Fig. 8), and is characterised by a switch from open pine forests with steppe patches, to temperate broadleaf forests dominated by *Quercus*, *Corylus avellana* and *Ulmus*. Across the Danubian Lowland, this regional vegetation change was recorded within a ~3500 year interval (between 11,000 and 7500 cal BP) depending on local soil substrates, orographical and climatological conditions, and distance from refugial population from which trees could spread (Jamrichová et al., 2014, 2017; Potůčková et al., 2018; Šolcová et al., 2018). The second event occurred at 6400 cal BP when anthropogenic indicators (e.g. *Triticum*, *Polygonum aviculare*) and open-landscape taxa (e.g. Amaranthaceae, *Artemisia*) started to increase at the expense of temperate broadleaf forest taxa as a result of Late Neolithic activities. This regional event is recorded also at Santovka-village (Šolcová et al., 2018), which is ~2 km from Santovka-PB, where anthropogenic indicators and the AP/NAP ratio records a sudden decrease at around 6650 cal. BP.

Locally, abrupt changes occurred at 9,570, 8,200, 7800 and 6400 cal BP resulting in various ecological changes at Santovka-PB (Figs. 6, 7 and 10). The first abrupt change around 9570 cal BP is the result of environmental conditions changing from a shallow pool to a calcareous fen habitat, as indicated by mollusc and macrofossil results and an increase in the percentage of carbonates (Figs. 6, 7 and 10). The increase in carbonates around 9570 cal BP is interpreted as travertine precipitation enhancement, and is in congruence with results from Poland where calcareous tufa deposits peaked between 9700 and 9500 cal BP. Together, these data are indicative of a relatively humid period across the region (Starkel et al., 2012). The second local environmental change between ~8260 and 8020 cal BP (Fig. 10) is the result of travertine precipitation rates decreasing, as well as a nearly-absent macrofossil record between 8260 and 8170 cal BP. This abrupt change in the local environment is most likely linked with the cold 8.2 ka event which was recorded across Europe and in Greenland (Alley and Ágústsdóttir, 2005; LeGrande and Schmidt, 2008). The third environmental change around 7800 cal BP is likely the result of the development of a calcareous lake surrounded by rich fen taxa, as demonstrated by an abrupt decrease in percent carbonates and increase in aquatic and wetland taxa at Santovka-PB (Figs. 6 and 7). At Santovka-village (Šolcová et al., 2018), water levels also increased around 7950 cal BP indicating climate moistening. Pronounced regional climate moistening between 8000 and 7500 cal BP is also confirmed by other paleoecological data from the Western Carpathians (Dabkowski et al., 2019; Juřičková et al., 2018), and from Europe (e.g. Cvetkoska et al., 2014; Bešta et al., 2015; Kalis et al., 2003; Nádor et al., 2007; Starkel et al., 2012). The last abrupt change at 6400 cal BP is likely the result of Late Neolithic activities impacting the local environment, which generally lead to land degradation (Willis et al., 1998). Specifically, increased erosion and soil flushes from nearby fields likely led to the infilling of the lake. This is indicated by the presence of weed macrofossils (*Hyoscamus niger*, *Fumaria officinalis*) and crop pollen (*Triticum* t., *Hordeum* t.), and by increased magnetic susceptibility values (SI Fig. 1). Our results indicate that local Holocene environmental changes were closely linked to spring activity and carbonate precipitation rates, and thus indirectly to the climate, until the arrival of Late Neolithics.

5.4. Tufa or travertine deposits and their exploitation for paleoclimate reconstructions

Terrestrial limestones (or flowstones) are mainly deposited as calcite crusts by carbonate-rich waters flowing into subaerial settings, and originate either from karstic cool water springs (i.e. calcareous tufa) or from geothermal warm waters (i.e. travertine). Terrestrial limestone stable isotope ratios of carbon ($^{13}C/^{12}C$) and oxygen ($^{18}O/^{16}O$) depend mostly on the physico-chemical conditions of the water (e.g. temperature, salinity, CO_2 concentration) that promote isotopic fractionation. Fractionation, resulting from the loss due to evaporation, degassing or metabolic consumption (e.g. algae, aquatic plants) of lighter isotopes during the (bio) geochemical cycle, induces discrete changes of C and O ratios (Gandin and Capezzuoli, 2008). High values of $\delta^{13}C$ (PDB) are typical for stable isotopic data obtained from travertine deposits, range from −2‰ to +8‰, whereas $\delta^{18}O$ (PDB) values usually range from −30‰ to −4‰. For calcareous tufa, $\delta^{13}C$ and $\delta^{18}O$ values typically range from −11‰ to −5‰ and from −12‰ to −3‰, respectively (Gandin and Capezzuoli, 2008). $\delta^{13}C$ and $\delta^{18}O$ (VPDB) values at Santovka-PB ranged between −7.6‰ and +7.9‰ and −10.7‰ to −5.9‰, indicating that analysed carbonate material is travertine (Fig. 4). Together with the results from our ERT survey, our data illustrate the presence of a local tectonic fault in which hydrothermal water was able to reach the surface resulting in accumulation of travertine deposits.

Prado-Pérez et al. (2013) demonstrated that $\delta^{13}C$ values in travertine deposits are mainly controlled by both the dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) from the surrounding parent material, which in turn is influenced by the different C sources existing in the area. When vegetation cover develops and biomass increases, the isotopic signature evolves towards more negative values due to the greater contribution of organic C, which is −27‰ vs. VPDB (Deines, 1980), to the dissolved carbon pool. Furthermore, according to Capezzuoli et al. (2014) 'travertines are linked to the availability of water, being influenced indirectly by tectonically driven ground water flow changes, which directly reflects rainfall availability and an elevated ground water'. Specifically, high amount of the rainfall could be reflected by the decrease of δ^{13}C values due to the more intense leaching of organic C from biomass and its penetration to the hydrothermal circuit. We therefore hypothesize that both higher rainfall amount and higher biomass accumulation (linked with broadleaf temperate tree expansion around 10,140 cal BP) contributed to the gradual decrease of δ^{13}C values at the beginning of the Holocene at Santovka-PB.

Prado-Pérez et al. (2013) also demonstrated that δ^{18}O values from travertine deposits reflect paleoclimatic conditions, as it corresponds to changes in their parent waters. Specifically, the isotopic signature of water is much more negative during cold and wet periods then during warm and dry periods. While our δ^{18}O record was influenced by high erosion rates during MIS 2 and the Lateglacial, and thus must be interpreted with caution, we observed an abrupt decrease in δ^{18}O values at 14,350 cal BP, interpreted as a cold oscillation. This is in congruence with T_{July}, which also illustrates a temperature decline at that time (Fig. 11). δ^{18}O values also decreased around 10,100 cal BP, likely caused by a distinct disturbance of thermohaline circulation in the North Atlantic Ocean (Bond et al., 1997; Björck et al., 2001) resulting in a short cooling event beginning around 10,300 cal BP and lasting ~200 years. Lastly, our travertine δ^{18}O values decreased around 8200 cal BP as a response to cold 8.2 ka event (Alley and Ágústsdóttir, 2005; LeGrande and Schmidt, 2008).

This study therefore highlights the importance of geothermal-related travertine precipitation and derived δ^{13}C and δ^{18}O as methods to reconstruct past abrupt climate and environmental change. Most notably, this is the first study to our knowledge to investigate isotopic signatures from travertine deposits from a precisely dated profile, together with numerous biotic and abiotic paleoecological data, allowing the cross-validation of paleoclimate reconstructions from different proxy data. Although some authors stressed that stable isotope signatures from one vertical profile from consolidated travertine deposits cannot be simply used for paleoclimatological evaluation (Kele et al., 2006), our results provide compelling evidence that δ^{18}O results from porous (i.e. fine pieces) travertine deposits reflect short-lasting climatic oscillations. However, some limitations are worth noting. Although changes in δ^{18}O values appear to correlate well with climate shifts, to disprove the influence of other drivers to δ^{18}O in travertine, further research is appropriate.

6. Conclusions

- An open steppe-tundra ecosystem dominated by Poaceae and *Pinus sylvestris* characterised the regional vegetation during the MIS 2 (17,550–14,560 cal BP), although pollen of *Quercus*, *Corylus avellana*, *Tilia* and *Alnus* was also found in small abundances, indicating our study site was in close proximity to their northern glacial refugium. A small stream surrounded by wetland grasses and/or dwarf-birch tundra was found locally.
- Open steppe-tundra was still present regionally at the beginning of MIS 1 (14,560–13,950 cal BP), however *Pinus sylvestris* began to expand. Locally, a calcareous lake with macrophytes surrounded by rich wetland vegetation and fen patches developed at Santovka-PB, although dwarf-birch tundra still surrounded the local environment. Taxa preferring higher temperatures and higher trophic states predominated in the aquatic and wetland environment.
- Two abrupt changes in regional vegetation (10,140 and 6400 cal BP) and four in the local environment (9,570, 8,200, 7800 and 6400 cal BP) were recorded during the early and middle Holocene. Local vegetation changes were related indirectly to climatic events (10,140, 9,570, ~8,200, 7800 cal BP), until 6400 cal BP when Neolithic-caused deforestation directly influenced local erosion rates and vegetation.
- Mean T_{July} increased from ~12.7 ± 1.5 °C to ~14.9 ± 1.4 °C (adjusted to modern sea level) at the MIS 2/MIS 1 transition at Santovka-PB, with biotic and abiotic proxies indicating significant humidification during this transition. Our proxies suggest wetter climatic conditions around 9570 cal BP, and abrupt humidification after 7800 cal BP, which corroborate with other paleoenvironmental records throughout Central-Eastern Europe.
- The local environment was influenced by travertine deposition around thermal springs, which occurred along a tectonic fault. δ^{18}O and δ^{13}C isotopic signatures obtained from travertine deposits demonstrate their ability to reflect short-lasting climatic

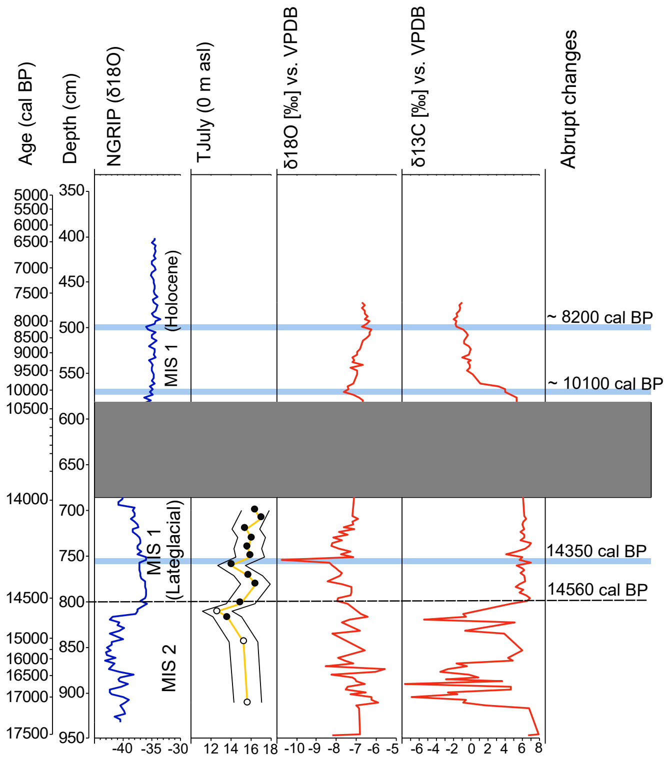

Fig. 11. δ^{13}C and δ^{18}O (Vienna Pee Dee Belemnite) from travertine deposits at Santovka-Pramene Budzgov, mean July temperature (T_{July}) and North Greenland Ice Core Project δ^{18}O data plotted on versus depth (cm) and calibrated ages (years Before Present). Displayed data were selected to demonstrate temporal changes in isotopic records together with reconstructed T_{July} and NGRIP δ^{18}O data. Abrupt changes in the fossil record are highlighted by blue horizontal lines. NGRIP δ^{18}O data (NGRIP dating group, 2006) were downloaded from ftp://ftp.ncdc.noaa.gov/pub/data/paleo/icecore/greenland/summit/ngrip/gicc05-20yr.txt. Empty circles in T_{July} curve indicate limited validity of reconstructed temperature due to a low number of individuals used in the reconstruction. Full circles display more reliable T_{July} results based on more than 35 individuals and a maximum of two adjoining depths merged together. Grey box indicates the presence of a sediment hiatus. Black dashed line indicates MIS 2/MIS 1 transition. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

events and major changes in the regional environment. Our results highlight the benefit of using travertine deposits, coupled with high-resolution paleoecological data, to investigate past biotic and abiotic responses to abrupt climate change.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.quascirev.2020.106170.

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Research Paper

Small-Scale Distribution of Aquatic Macroinvertebrates in Two Spring Fens with Different Groundwater Chemistry

key words: springs, fens, macroinvertebrate assemblages, within-site heterogeneity, seasonal variation

Abstract

We examined responses of macroinvertebrate assemblages to environmental and temporal variations along spring source-spring brook transects in two fen habitats, sharply differing in groundwater chemistry, and compared the patterns among individual taxonomical groups. We hypothesised a different importance of environmental heterogeneity and seasonal changes primarily linked to strong tufa precipitation, which causes stronger environmental filtering in the calcareous fen. In concordance, we observed that assemblages of the more homogenous calcareous fen primarily changed over time, due to seasonal shifts in source availability and favourable conditions. Their spatial distribution was determined by the amount of CPOM, tufa crusts and temperature variation, but a substantial part of the assemblage exhibited spatial uniformity (Plecoptera, Clitellata, and especially Trichoptera and Diptera). The assemblages of the more heterogeneous *Sphagnum*-fen were primarily driven by water pH and substrate and the season was a notably weaker predictor. We found that different macroinvertebrate groups can display various responses to the measured variables shaping the overall pattern obtained based on the whole community. Further, greater environmental heterogeneity can result in temporally stable species distribution patterns even at very small spatial scales within a single site.

1. Introduction

Small-scale distributions of macroinvertebrates in springs have been studied mainly in terms of the zonation patterns of assemblages along the springbrooks and spring source-springbrook gradients. These studies looked at different lengths of the spring fen gradient and different within-site heterogeneity and the composition of assemblages from different studies reflected different environmental factors such as discharge (Minshall, 1968; Chertoprud, 2006), temperature (Ward and Dufford, 1979; Resh, 1983; Von Fumetti *et al.*, 2007; Barquín and Death, 2011), and substrate (Ward and Dufford, 1979; Ferrington *et al.*, 1995; Ilmonen and Paasivirta, 2005). The distance from a spring-source was commonly

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interpreted as a proxy for rapid changes in the environment with decreasing influence of a stable supply of groundwater (*e.g.*, Von Fumetti *et al.*, 2007) and rapid changes of distributional patterns of macroinvertebrates at the species level and at higher taxonomic levels. These patterns have often been described by comparing the macroinvertebrate species occurring at different distances from the spring-source (*e.g.*, Resh, 1983; Williams and Hogg, 1988; Barquín and Death, 2011). Beside substrate, discharge and temperature regimes, groundwater chemistry may also influence macroinvertebrate distributions within a single spring site. It can be expected to be important in heterogeneous springs, where the input of groundwaters of different chemistry creates markedly different patches within a spring. Direct effects of pH, bases and metal content in water can alter the suitability of these microhabitats for macroinvertebrates since individual species and/or taxonomic groups are diversely sensitive to these factors, mainly due to physiological reasons (*cf.* Ford, 1989; Sutcliffe and Hildrew, 1989; Vuori, 1995). Glazier and Gooch (1987) and Glazier (1991) reported that alkalinity and pH had an important influence on spring assemblage compositions; similarly, Williams *et al.* (1997) found that water chemistry parameters, describing different levels of urbanisation, were important determinants of spring assemblages.

Benthic macroinvertebrate assemblages of springs are composed of species with different strengths of the linkage to the spring environment (Lindegaard *et al.*, 1998) and also with different biological and ecological traits (Williams, 1991; Wagner *et al.*, 1998). Therefore, the distribution of macroinvertebrates within a spring reflects not only the various habitat preferences of species (*e.g.*, Ilmonen and Paasivirta, 2005) and their requirements for different food sources (*e.g.*, McCabe and Sykora, 2000; Barquín and Death, 2006), but also their life histories (*cf.* Williams and Hogg, 1988; Barquín and Death, 2004). The responses of invertebrate orders and/or taxonomic groups to measured factors describing spring habitats can differ from one another (*e.g.*, Minshall, 1968; Resh, 1983; Williams and Hogg, 1988); thus, the whole macroinvertebrate assemblage may comprise taxa with different, even inverse, responses. However, there is a lack of studies directly comparing the responses of whole macroinvertebrate assemblages with responses expressed by the individual taxonomic groups comprising them.

In this study, we explored the within-site distribution of macroinvertebrates in two spring fens that significantly differ in water pH and mineral richness. The first site was a calcareous fen with differing degrees of tufa precipitation which provided an ecologically extreme habitat. The second site was a *Sphagnum*-fen characterised by different pH levels, mineral richness and different substrates. We aimed (i) to compare the spatial and temporal variations between the two complete macroinvertebrate assemblages and among individual taxonomic groups, namely Ephemeroptera, Plecoptera, Trichoptera, Diptera (except Chironomidae), Chironomidae, and Clitellata, and (ii) to analyse the influence of the measured variables on species composition of the assemblages. We hypothesised that temporal variation would be more important in the calcareous fen since precipitations of tufa can constrain the number of microhabitats and their diversity, while the spatial distribution of species would be more important in the environmentally more heterogeneous *Sphagnum*-fen and would be predominantly driven by gradients of pH and mineral richness.

2. Methods

2.1. Study Area and Sites

The studied spring fens are located in the Western Carpathian flysch zone, in the borderland between the Czech Republic and Slovakia. Flysch is characterised by a serial alternation of sandstone and claystone deposits with different calcium contents. Due to specific bedrock chemistry, spring fens with different mineral richness of groundwater occur in this area. Two spring fens representing the opposite ends of the pH/calcium gradient were selected for this study. The first site is a calcareous fen with strong tufa precipitation (the Bílé Potoky Nature Reserve; 49°06′47″ N, 18°01′24″ E) situated in the Bílé Karpaty Mountains; the second site is a *Sphagnum*-fen (the Obidová Nature Monument 49°31′03″ N, 18°31′24″ E) situated in the Moravskoslezské Beskydy Mountains. Both spring fens are composed of four helocrenes with springbrooks which drain the whole spring fen area and connect downstream. In each site, we chose a *ca.* 400 m long spring source-springbrook transect comprising three helocrenes of different groundwater chemistry and their springbrooks. The transect ran longitudinally through the spring fen and covered the whole site's heterogeneity.

The calcareous fen was situated on a steep meadow hillside which reaches an altitude between 380 and 500 m above sea level (m a.s.l.) and covers 8.8 ha. Great horsetail (*Equisetum telmateia* EHRH.) and butterbur (*Petasites hybridus* LINNÉ) were the dominant vascular plants; brown mosses (family Bryidae) dominated in the moss layer. However, no plants covered the bottom of the springbrooks. The pH of the water was alkaline (mean = 8.3 ± 0.2) and conductivity was very high (mean = 480 ± 43.0 µS cm^{-1}). The spring source-springbrook transect was characterised by an increasing discharge and an increasing variation in water temperature downstream (Table 1). An intense precipitation of calcium carbonate (tufa) formed strong crusts (mostly upstream) as well as small tufa grains (predominantly downstream). Beside tufa incrustations, the dominant types of bottom substrate were stones and dead leaves. Dead leaves dominated in the upper sections near the spring sources, while stones were mostly found in the lowermost section of the spring fen (Table 1).

The *Sphagnum*-fen was situated in a meadow with moderate slopes between 710 and 750 m a.s.l. and covered 7.3 ha. The spring source-springbrook transect was characterised by a sharp gradient in pH and mineral richness. The uppermost part of the spring fen was classified as a mineral-poor *Sphagnum*-fen characterised by an acidic pH (mean = 5.2 ± 0.6) and a high iron concentration (2620 µg l^{-1}). The remaining part of the *Sphagnum-fen* was classified as a mineral-rich *Sphagnum*-fen dominated by calcitolerant *Sphagna*. The mean pH was higher here than in the mineral-poor part of the *Sphagnum*-fen (pH 6.6 ± 0.7); the iron concentration remained very high in the middle part of the spring fen (mean = 2875 ± 650.9 µg l^{-1}) but this value decreased distinctly downstream (mean = 244 ± 72.7 µg l^{-1}). The conductivity was rather low (mean = 60 ± 14.2 µS cm^{-1}) along the whole *Sphagnum*-fen. The discharge was lower in the middle sections of the spring fen than in the upper and lower sections due to the high retention of water by peat in the middle sections (Table 1). The substrate characteristics changed considerably along the entire spring source-springbrook transect; fine detritus (FPOM) and *Sphagna* prevailed near the spring sources, whilst a coarse inorganic substrate (coarse gravel) dominated in the lower part of the *Sphagnum*-fen (Table 1).

2.2. *Field Sampling and Explanatory Variables*

Sampling was carried out three times: in spring (April–May), summer (July), and autumn (September) in 2006. Benthic samples were collected in five (calcareous fen) and six (*Sphagnum*-fen) sections of the springs which represented different abiotic conditions along the 400 m long transects from spring sources to the springbrooks draining each fen. The length of each section was approx. 50 m, and the distance between neighbouring sections was at least 20 m. In each section, a sample consisting of ten sampling plots (25 cm × 25 cm), which represented all of the habitats along the sampled spring section, was taken semiquantitatively with a hand net (mesh size 0.5 mm) by the kick-sampling method (FROST, 1971). After washing the sampled substrate several times in a bucket and removing the coarse inorganic substrate, the sample was elutriated through a net of a 0.5 mm mesh size and the remaining material was preserved in 4% formaldehyde. The samples were subsequently sorted and invertebrates were identified in the laboratory, mostly to the species and genus levels.

Water temperature, pH, conductivity, and dissolved oxygen were measured in the field using portable instruments (WTW Multi 340i/SET) in each sampling occasion. The annual variation of temperature of each section was calculated from individual measurements as the difference between maximal and minimal temperature values during the year. Discharge was estimated by sampling the spring water in a vessel for a defined period of time in each sampling. We recorded the percentage cover of stones, gravel, sand, coarse particulate organic matter (CPOM), fine particulate organic matter (FPOM), and tufa crusts in each section. For statistical analyses, the relative proportions of stones, gravel, and sand in each section were expressed by the phi coefficient (FURSE *et al.*, 1986). In autumn, water samples were collected and concentrations of soluble Na, K, Ca, Mg, Al, Fe, SO_4, NO_3, and PO_4 ions were measured

Table 1. Mean values of the physicochemical variables measured in the calcareous fen and the *Sphagnum*-fen in spring, summer, and autumn in 2006. The amount of inorganic substrate and FPOM did not change during the year, therefore concrete numbers of phi coefficient, tufa crusts, and FPOM are shown in the table instead of mean values. The samples are numbered from 1 to 5 and 1 to 6 and describe the increasing distance of the sampled sites away from the source of the fens. Var. in temperature – variation in temperature; CPOM – coarse particulate organic matter; FPOM – fine particulate organic matter.

	pH	Var. in temperature (°C)	Conductivity (µS cm^{-1})	Dissolved oxygen (mg l^{-1})	Discharge (ml s^{-1})	*phi*-coefficient	Tufa crusts (%)	CPOM (%)	FPOM (%)	Ca (mg l^{-1})	Fe (µg l^{-1})	Distance from helocrenes (m)
Calcareous-fen												
1	8.1 ± 0.12	3.7	523 ± 54.0	8.3 ± 1.9	377 ± 441	–3.2	30	43.3 ± 4.7	0	105	534	1
2	8.3 ± 0.05	4	474 ± 27.0	9.2 ± 2.3	377 ± 239	–1.9	30	53.3 ± 4.7	0	75.9	83	10
3	8.2 ± 0.12	4.8	497 ± 9.4	8.9 ± 2.2	767 ± 525	–3.1	20	33.3 ± 4.7	0	93.7	279	120
4	8.5 ± 0.08	7.4	495 ± 32.0	9.1 ± 2.5	933 ± 556	–0.775	20	30	0	92.9	130	220
5	8.4	7	483 ± 7.5	9.9 ± 3.2	933 ± 556	–2.95	20	10	0	82.5	164	300
Sphagnum-fen												
1	5.2 ± 0.6	6	55 ± 16.7	3.1 ± 3.0	233 ± 94	–1.41	0	0	60	9.3	2620	1
2	6.1 ± 0.6	5	74 ± 2.6	5.4 ± 1.3	63 ± 9	16.25	0	0	90	6.8	3660	1
3	6.5 ± 0.5	6	58 ± 4.3	6.8 ± 1.8	73 ± 61	–1.75	0	0	70	5.4	2090	10
4	6.6 ± 0.7	4	65 ± 16.2	7.5 ± 1.9	933 ± 634	–5.01	0	0	30	10	311	170
5	7.0 ± 0.3	5	47 ± 2.6	8.3 ± 2.0	300 ± 284	–3.55	0	0	40	6.3	143	200
6	6.8 ± 0.8	4	63 ± 14.5	7.8 ± 1.8	1067 ± 680	–4.83	0	0	5	10.4	278	300

in an accredited laboratory. These samples were collected in late autumn because of the relative stability of water chemistry in this period (*cf.* HÁJEK and HEKERA, 2004).

2.3. Statistical Analyses

We analysed responses of the entire benthic community first (*i.e.*, all taxa from all samples) and then from the whole array we chose the following taxonomic groups, which were the most species rich and abundant in studied sites: Ephemeroptera, Plecoptera, Trichoptera, Diptera except Chironomidae, and Clitellata. These datasets were analysed separately. The individual species-by-sample matrices were subjected to principal components analysis (PCA) due to the low species turnover within the studied transects. The length of the main gradient in the detrended correspondence analysis (DCA) did not exceed 3 units, indicating that ordination techniques based on the linear response model were most appropriate (LEPŠ and ŠMILAUER, 2003). The relationships between explanatory variables and site scores on the first four ordination axes of PCA were assessed using Spearman's rank correlations (r_s) for continuous variables and the Mann-Whitney U test for nominal variables. Partial redundancy analysis (pRDA) with the season as a covariable was used for testing the amount of variability explained by each physicochemical variable with a forward selection for each tested taxonomic group. The Monte-Carlo permutation test was used to test the significance of variables ($P = 0.01$; 1999 permutations). Prior to PCA and RDA, the species abundance data were log (x + 1) transformed to reduce the effect of dominant taxa. The variation partitioning approach (BORCARD *et al.*, 1992) was used to determine the relative amounts of variation in species data explained by two different constraining groups of variables (physicochemical variables and season) in separate RDAs. Since the explained variation was also influenced by the number of explanatory variables (PERES-NETO *et al.*, 2006), we only used the three most powerful predictors in each group of variables that were significant in the separate RDAs. The CANOCO 4.5 software package (TER BRAAK and ŠMILAUER, 2002) was used for computing the ordinations and STATISTICA 8 (HILL and LEWICKI, 2007) was used for the univariate analyses. Sequential Bonferroni corrections of the significance levels (HOLM, 1979) were applied for multiple comparisons of explanatory variables.

3. Results

3.1. Macroinvertebrate Diversity and Abundance

Altogether, 138 and 163 taxa were represented by 54,424 and 45,988 individuals recorded in the calcareous fen and in the *Sphagnum*-fen, respectively (Appendix 1). An abundance of non-insect taxa prevailed at both sites. The amphipod *Gammarus fossarum* KOCH accounted for 73% and 30% of all individuals in the calcareous fen and in the *Sphagnum*-fen, respectively. After *G. fossarum*, the clitellate *Trichodrilus strandi* HRABĚ was the most abundant species in the calcareous fen and the snail *Bythinella austriaca* (FRAUENFELD) was the most abundant species in the *Sphagnum*-fen (Fig. 1). In contrast, insect fauna was more taxon rich than non-insect fauna with a high dominance of Diptera, which was particularly rich in the calcareous fen and comprised nearly 65% of all taxa recorded there (Fig. 2). There were large proportions of other insect taxa in the *Sphagnum*-fen (Trichoptera and Plecoptera) and Clitellata were also characteristic for this site (Fig. 2).

3.2. Differences in the Proportions of Spatial and Temporal Variations between Spring Fens

The PCA and variation partitioning in partial RDAs revealed significant differences in the role of physicochemical variables and season for structuring of assemblages in the study of spring fens (Table 2, Fig. 3). When looking at the whole benthic assemblage in the calcare-

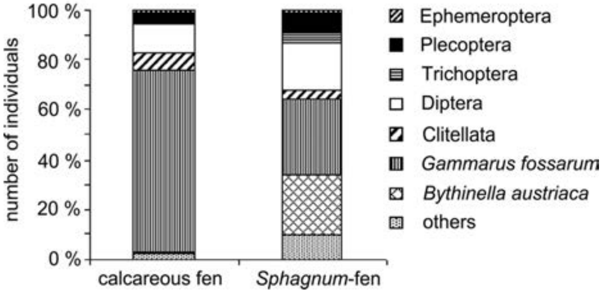

Figure 1. The relative abundance of macroinvertebrates at the studied sites.

ous fen, the first PCA axis (28% of the explained data variation) reflected the difference between spring, summer, and autumn samples, whereas the second ordination axis (18% of the explained data variation) expressed changes in physicochemical variables along the transect (Fig. 4). On the contrary, *Sphagnum*-fen assemblages revealed the opposite pattern; the first ordination axis (24.5% of the explained data variation) displayed the grouping of samples along the transect and the second PCA axis (17% of the explained data variation) was associated with the season (Fig. 5). The variation partitioning method revealed that physicochemical variables had a substantially greater influence (38%) than season (19%) in the *Sphagnum*-fen, while the difference in the variation explained by physicochemical variables and season was not so high in the calcareous fen (28.5% and 17%, respectively) when considering the whole assemblage (Fig. 3).

Furthermore, the differences in the effect of physicochemical variables and season between the individual taxonomic groups were also recorded. In the calcareous fen, the relative part of the species data variation explained by season was the highest for Trichoptera and Diptera (except Chironomidae) (32% and 28.5%, respectively), which corresponded well with the high significance of season in these groups in the PCA analysis (Table 2). Ephemeroptera was the group with the largest part of the variation explained by physicochemical variables in the calcareous fen (45.5%). In the *Sphagnum*-fen, the greatest influence of physicochemical variables (66%) and the lowest influence of season (3.7%) were characteristic for Ephemeroptera. The second highest proportion of the variation explained by physicochemical variables (43%) was recorded in the Trichoptera data set. Simultaneously, Ephemeroptera and Trichoptera were the only taxonomic groups in the *Sphagnum*-fen where the season was not significant on the first PCA axis (Table 2). In contrast, the smallest proportion of vari-

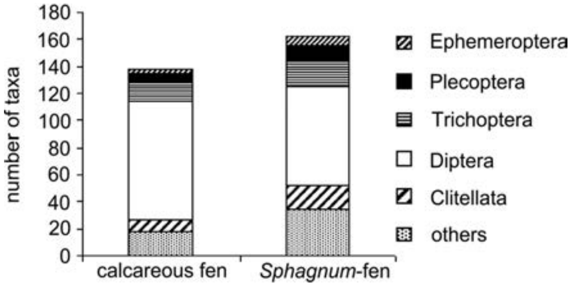

Figure 2. The taxonomic structure of macroinvertebrate assemblages at the studied sites.

Table 2. Relationships between PCA ordination site scores (first two axes), explanatory variables, and number of taxa and individuals in the calcareous fen and the *Sphagnum*-fen using Spearman's rank correlations (r_s) and the Mann-Whitney U test. Using Bonferroni corrections the current cut level for Spearman's correlations was $P < 0.003$ (in bold). Values at the significance level of $P < 0.01$ are in normal print. Diptera ex. Chir. – Diptera except Chironomidae; var. in temperature – variation in temperature; CPOM – coarse particulate organic matter; FPOM – fine particulate organic matter; ns – not significant.

calcareous fen	Ephemeroptera		Plecoptera		Trichoptera		Diptera exc. Chir.		Chironomidae		Clitellata		All taxa	
	1. axis	2. axis	1. axis	2. axis	1. axis	2. axis	1. axis	2. axis	1. axis	2. axis	1. axis	2. axis	1. axis	2. axis
Explained variance	0.798	0.157	0.364	0.291	0.301	0.211	0.309	0.240	0.306	0.235	0.531	0.210	0.279	0.178
	r_s		r_s		r_s		r_s		r_s		r_s		r_s	
pH	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Var. in temperature	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**0.775**
Conductivity	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Dissolved oxygen	ns	ns	**0.793**	ns	0.647	ns	ns	**0.832**	ns	**0.705**	ns	ns	ns	ns
Discharge	ns	ns	ns	ns	ns	ns	ns	ns	**–0.790**	ns	**–0.721**	ns	ns	ns
phi-coefficient	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Tufa crusts	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	–0.692
CPOM	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**–0.705**
Ca	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fe	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Distance from helocrenes	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**0.772**
Number of taxa	**0.885**	ns	–0.539	ns	ns	ns	0.686	ns	ns	ns	ns	ns	ns	ns
Number of individuals	**0.974**	ns	ns	**–0.75**	ns	ns	**0.9**	ns	**0.964**	ns	**0.985**	ns	**0.703**	ns
	P (U test)		*P* (U test)		*P* (U test)		*P* (U test)		*P* (U test)		*P* (U test)		*P* (U test)	
Season	0.03	ns	0.007	ns	0.005	ns	0.007	0.005	0.04	0.03	0.014	ns	0.002	ns
Sphagnum-fen	Ephemeroptera		Plecoptera		Trichoptera		Diptera exc. Chir.		Chironomidae		Clitellata		All taxa	
	1. axis	2. axis	1. axis	2. axis	1. axis	2. axis	1. axis	2. axis	1. axis	2. axis	1. axis	2. axis	1. axis	2. axis
Explained variance	0.606	0.248	0.358	0.197	0.259	0.207	0.393	0.151	0.305	0.201	0.324	0.227	0.245	0.171
	r_s		r_s		r_s		r_s		r_s		r_s		r_s	
pH	ns	ns	**0.733**	ns	ns	ns	ns	**0.662**	0.621	ns	ns	**0.788**	ns	ns
Var. in temperature	**–0.882**	ns	ns	ns	**–0.866**	ns	ns	ns	ns	**–0.64**	ns	ns	–0.629	ns
Conductivity	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Dissolved oxygen	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	–0.604	ns	ns	ns
Discharge	**0.739**	ns	ns	ns	**0.769**	ns	**0.679**	ns	ns	ns	ns	ns	0.634	ns
phi-coefficient	**–0.894**	ns	ns	ns	**–0.699**	ns	ns	ns	ns	**–0.642**	ns	–0.636	**–0.674**	ns
FPOM	**–0.914**	ns	ns	ns	**–0.825**	ns	ns	ns	–0.636	ns	ns	–0.605	**–0.887**	ns
Ca	**0.673**	ns	ns	ns	**0.712**	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fe	**–0.733**	ns	ns	ns	–0.636	ns	ns	**–0.843**	ns	ns	ns	**–0.724**	**–0.761**	ns
Distance from helocrenes	**0.886**	ns	ns	ns	**0.863**	ns	ns	**0.683**	ns	ns	ns	**0.723**	**0.889**	ns
Number of taxa	**0.968**	ns	ns	ns	ns	ns	ns	ns	ns	**0.770**	ns	ns	ns	–0.589
Number of individuals	**0.96**	ns	**–0.834**	ns	ns	0.595	**0.721**	ns	ns	**0.746**	ns	ns	0.607	ns
	P (U test)		*P* (U test)		*P* (U test)		*P* (U test)		*P* (U test)		*P* (U test)		*P* (U test)	
Season	ns	ns	0.02	0.05	ns	0.04	0.01	ns	0.008	ns	0.009	0.05	ns	0.03

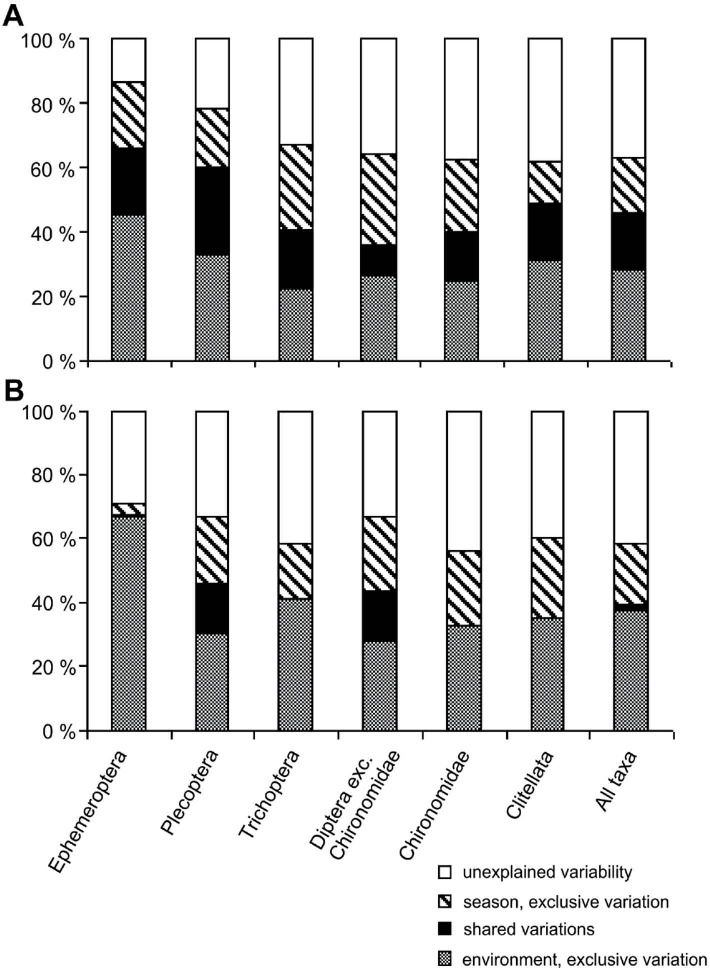

Figure 3. Comparison of the variance explained by physicochemical variables and season between the taxonomic groups of the calcareous fen (A) and the *Sphagnum*-fen (B) using the variation partitioning approach in separate RDAs.

ation explained by the physicochemical variables was detected in the Plecoptera (30%) and Diptera (28%) assemblages (Fig. 3).

3.3. Responses of Individual Taxonomic Groups to Environmental Gradients

Calcareous fen. According to the PCA, the main changes in the species composition of taxonomic groups were significantly associated with dissolved oxygen (Plecoptera and to a lesser degree Trichoptera) and discharge (Chironomidae and Clitellata) (Table 2). Numbers of individuals significantly correlated with the first PCA axes of all taxonomic groups (except Plecoptera and Trichoptera) (Table 2). Using the season as a covariable in partial RDA, variation in water temperature explained a large part of the variation in the whole

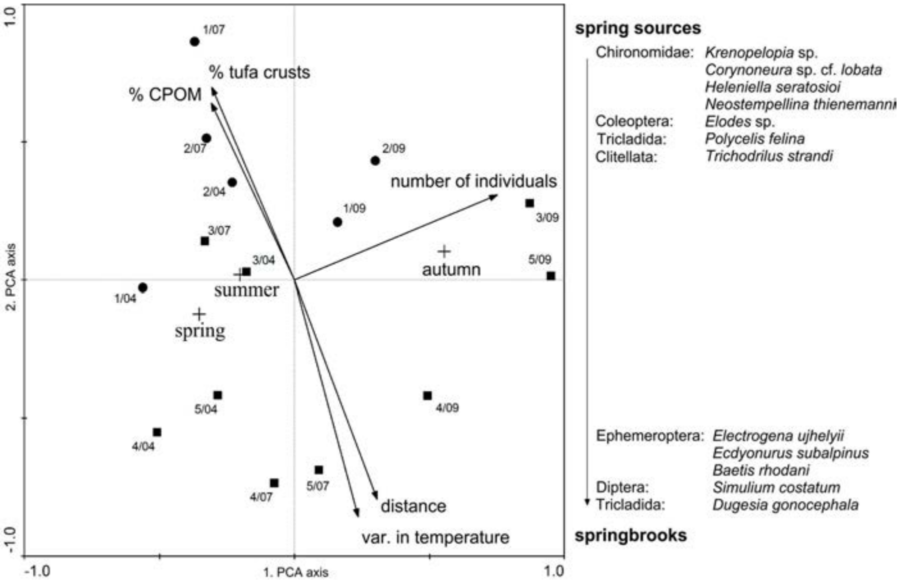

Figure 4. Principal component analysis (PCA) of all species data in the calcareous fen: ordination plot of sampled sites on the first two PCA axes with posteriori plotted abiotic variables. Only the variables significantly associated with the PCA axis at the level $P < 0.01$ are shown. The most important species which were characteristic of upstream and downstream sections are displayed. ● – springbrooks in the upper part of the fen connected to their spring sources; ■ – downstream springbrooks formed by the joining of upstream springbrooks. Numbers from 1 to 5 before the slash describe the increasing distance of the sampled sites from the spring source of the fen; numbers behind the slash describe the month of sampling. Var. in temperature – variation in temperature; CPOM – coarse particulate organic matter; distance – distance from helocrenes.

assemblage data set. Looking at the whole assemblage, the most significant factors in the individual taxonomic groups were the amount of CPOM (Ephemeroptera), variation in temperature and Ca concentration (Plecoptera), and tufa crusts (Chironomidae) (Table 3). In the Clitellata assemblage, the pRDA results confirmed the importance of discharge, indicating its significant influence on this taxonomic group. However, no significant predictor was found in the Diptera assemblage (which did not include Chironomidae) (Table 3).

Sphagnum-fen. Changes in physicochemical variables along the spring source-springbrook transect played a more important role in the structuring of macroinvertebrate assemblages in the *Sphagnum*-fen than in the calcareous fen. Changes along the longitudinal transect were best displayed by the first PCA axis of the Ephemeroptera and Trichoptera assemblages, which were significantly correlated with nearly all of the physicochemical variables, reflecting changes along the transect (Table 2). Water pH was the only physicochemical variable that correlated with the first PCA axis of Plecoptera, reflecting a seasonal variation in this assemblage. However, the remaining three taxonomic groups showed a different pattern; they were more closely related to the physicochemical variables obtained on the second PCA axis of each taxonomic group (Table 2). On the basis of partial RDA analysis, FPOM was identified as the most important predictor and pH as the second most important when looking at the whole assemblage (Table 3). Likewise, the amount of FPOM controlled a large part of the variation in the Ephemeroptera, Plecoptera, and Chironomidae data sets. The main changes in species composition of the remaining groups were explained by Fe and/

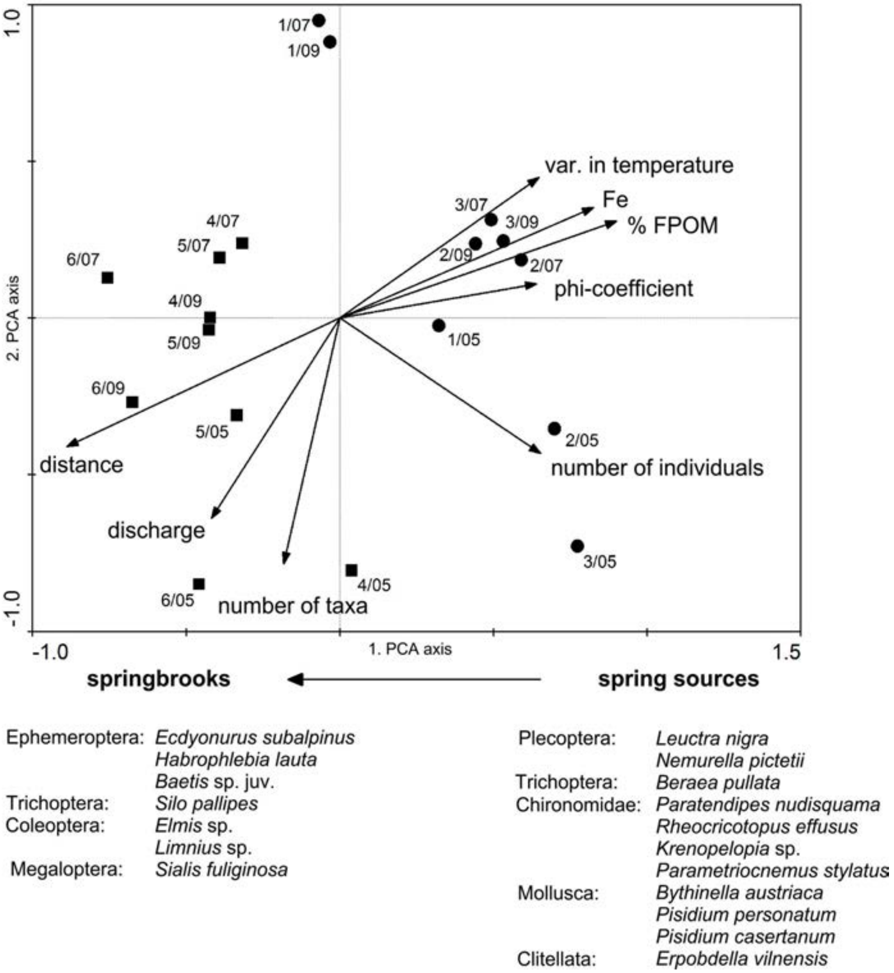

Figure 5. Principal component analysis (PCA) of all species data in the *Sphagnum*-fen: ordination plot of sampled sites on the first two PCA axes with posteriori plotted abiotic variables. Only the variables significantly associated with the PCA axis at the level $P < 0.01$ are shown. The most important species which were characteristic of upstream and downstream sections are displayed. • – springbrooks in the upper part of fen connected to their spring sources; ■ – downstream springbrooks formed by the joining of upstream springbrooks. Numbers from 1 to 6 before the slash describe the increasing distance of the sampled sites from the beginning of the fen; numbers behind the slash describe the month of sampling. Var. in temperature – variation in temperature; FPOM – fine particulate organic matter, distance – distance from helocrenes.

Table 3. Results of the forward selection procedure in pRDA using the season as a covariable for species data in the calcareous fen and the *Sphagnum*-fen. The numbers from 1 to 9 express the order of factors according to the proportion of explained variation in each taxonomic group. Variables significant at $P = 0.01$ (Monte-Carlo test) were included in the model; these variables are in bold. Significance levels: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$. Diptera ex. Chir. – Diptera except Chironomidae; var. in temperature – variation in temperature; CPOM – coarse particulate organic matter; FPOM – fine particulate organic matter.

calcareous fen	Ephemeroptera		Plecoptera		Trichoptera		Diptera exc. Chir.		Chironomidae		Clitellata		All taxa	
Var. in temperature	5	2%	**2**	**11%****	8	3%	2	7%	6	4%	5	5%	**1**	**16%****
Dissolved oxygen	**3**	**7%****	4	3%	6	5%	1	13%	7	4%	6	4%	3	6%
pH	6	2%	6	2%	7	4%	6	4%	4	6%	4	6%	5	5%
Conductivity	8	1%	3	5%*	3	8%	8	4%	3	6%	7	2%	2	7%
Discharge	4	3%	8	2%	4	6%	4	5%	2	6%	1	14%*	8	3%
phi-coefficient	9	0%	5	2%	9	0%	7	4%	8	4%	8	1%	6	5%
Tufa crusts	7	1%	7	2%	5	5%	5	4%	**1**	**13%****	2	11%	4	6%
CPOM	**1**	**30%*****	9	0%	2	9%	9	0%	9	0%	9	0%	7	4%
Ca	2	8%*	**1**	**19%****	1	10%	3	6%	9	0%	3	7%	9	0%
Fe	9	0%	9	0%	9	0%	9	0%	5	6%	9	0%	9	0%
Total variance		**54%**		**46%**		**50%**		**47%**		**49%**		**50%**		**52%**

Sphagnum-fen	Ephemeroptera		Plecoptera		Trichoptera		Diptera exc. Chir.		Chironomidae		Clitellata		All taxa	
FPOM	**1**	**50%*****	**1**	**17%*****	3	7%	8	3%	**1**	**20%*****	**7**	3%	**1**	**22%*****
Var. in temperature	4	7%*	3	7%*	**1**	**20%*****	5	4%	2	7%	**2**	**10%****	4	6%
pH	7	1%	2	8%*	**2**	**16%*****	9	1%	8	3%	4	5%	**2**	**11%****
Conductivity	9	0%	7	3%	8	3%	7	3%	7	4%	5	4%	8	3%
Dissolved oxygen	8	1%	4	5%	7	5%	4	4%*	9	2%	9	3%	7	3%
Discharge	**2**	**11%****	5	4%	9	2%	3	4%	6	5%	6	4%	9	3%
phi-coefficient	6	4%	9	2%	5	6%	6	3%	4	6%	3	6%*	**3**	**7%****
Ca	5	7%*	6	4%	6	6%*	**2**	**10%*****	3	7%*	8	3%	**5**	**6%****
Fe	**3**	**8%****	8	3%	4	7%*	**1**	**14%*****	5	6%	**1**	**19%*****	6	5%
Total variance		**89%**		**53%**		**72%**		**46%**		**60%**		**57%**		**66%**

or Ca concentrations (Diptera except Chironomidae and Clitellata), water pH (Trichoptera), and variation in water temperature (Trichoptera and Clitellata) (Table 3).

4. Discussion

4.1. Spatio-Temporal Variations in Macroinvertebrate Assemblages

In the calcareous fen, the assemblages primarily changed in response to the changing seasons (Fig. 4, Table 2), whereas in the *Sphagnum*-fen, they were primarily influenced by substrate and water chemistry (Fig. 5, Table 2). On one hand, this could be connected to the different within-site heterogeneity of environmental conditions between the two spring fens, and/or on the other hand to seasonal shifts in available sources and favourable conditions. Strong tufa precipitation in the calcareous fen created a rather uniform environment, thus the main microhabitat differentiation was caused by discharge and the presence of leaf packets, which varied seasonally. In contrast, the seasonal variation was overridden by a sharp gradient of physicochemical variables (substrate structure, pH and mineral richness) in the *Sphagnum*-fen. This demonstrates the importance of spatial heterogeneity for the distribution of macroinvertebrates in springs, which was also previously pointed out by Ward and Dufford (1979), who recorded a significant effect of substrate heterogeneity on macroinvertebrate diversity and abundance in a springbrook-pond system. The pronounced shared effect of physicochemical variables and season expressed in the calcareous fen (Fig. 3) can be attributed in particular to the seasonal variability in discharge and the amount of leaf packets (*cf.* significant correlations of physicochemical variables in the PCA analysis and their non-significant contribution after including the season as a covariable in pRDA; Tables 2 and 3). It also suggests an important role of seasonal shifts in the sources and periods of favourable environmental conditions in the calcareous fen. Harsh conditions in the calcareous fen may mean that aquatic fauna is strongly dependent on the allochthonous input of organic matter in the form of leaves and wood. A substantial part of the assemblages was formed by univoltine species (especially many plecopterans and dipterans) with early spring and spring emergence. This is obvious because the life cycles of many shredders in temperate regions are synchronised with the input of riparian leaves in the autumn (*e.g.*, Malicky, 1989; Williams, 1991). They showed a major growth period in late autumn and winter when they reached high abundance and species richness in the samples (Table 2). On the contrary, the *Sphagnum*-fen was not influenced by the seasonal input of organic matter since it was surrounded by coniferous forest and was rich in organic matter due to the continual strong formation of peat. We recorded equal numbers of individuals collected during the seasons and the numbers of taxa recorded even decreased towards autumn (Table 2).

4.2. Spatial Distribution of Macroinvertebrates in the Calcareous Fen

The spatial distribution of macroinvertebrates was primarily determined by factors linked with the distance from the helocrene spring sources: tufa precipitation, organic matter input and temperature variation (Tables 2 and 3). The sections directly following the helocrenes were inhabited by unique crenophilic species from all macroinvertebrate groups that were not present downstream, whereas some species occurred exclusively in downstream sections. The contribution by particular groups to the total composition of the macroinvertebrate assemblages differed. A relative spatial uniformity of species composition (and the dominant influence of seasonal variation) was particularly observed for Trichoptera, Diptera (except Chironomidae), Plecoptera, and Clitellata. The spatial uniformity of the Trichoptera assemblage was most likely attributable to the tufa substrate, which is extremely unsuitable for many species. It forms small particles similar to sand grains, which are known to be an unstable and nutrient-poor substrate for caddis flies (SOLUK, 1985; COBB and FLANNAGAN, 1990; PHILLIPS, 2003), and/or strong compact crusts which hinder access to the deeper parts of the bottom substrate (ZOLLHÖFER *et al.*, 2000). Moreover, Trichoptera assemblages, even if they included many species restricted to springs, did not show fixed zonation patterns in a spring source-springbrook gradient, but these patterns had a strong seasonal component (MCCABE and SYKORA, 2000). Similarly, spatially uniform Diptera (except Chironomidae) assemblages varied more distinctly during the season, most likely because they consisted of many eurytopic taxa which are able to colonise a wide range of habitats (*cf.* BRINKMANN, 1991; WAGNER *et al.*, 1998; DE JONG *et al.*, 2008). On the other hand, there were several Trichoptera and Diptera taxa dominating in tufa zones which either have mechanisms to cope with tufa deposition or may actually benefit from tufa formation (MARKS *et al.*, 2006). We found several calciphilous species of Trichoptera and Diptera (*e.g.*, *Rhyacophila pubescens* PICTET, *Plectrocnemia brevis* MCLACHLAN, *Pericoma calcilega* FEUERBORN, *Thaumastoptera calceata* MIK) which could favour these conditions. However, they were evenly distributed along the studied transect. For Plecoptera, we found an effect of Ca concentration, which was difficult to interpret. The species composition of the Plecoptera assemblage was similar along the studied transect, but their abundance was higher in sections with lower Ca concentrations. We observed strong calcium carbonate encrustations on the thorax and tergites of *Nemoura* and *Protonemura* larvae sampled in the places with the strongest tufa precipitation. These encrustations might have adverse effects similar to those of Fe precipitates which cause the inhibition of motion and the smothering of aquatic invertebrates in humic waters (VUORI, 1995). A similar situation was observed in clitellate assemblages which were locally negatively affected by discharge and the presence of strong tufa crusts, because they are sensitive to physical disturbances (BARQUÍN and DEATH, 2004) and the reduction of interstitial spaces in the stream bed caused by tufa deposition (*cf.* RUNDIO, 2009). Clitellates assemblages have been documented as not being as closely related to seasonal changes as insect fauna in springs (BARQUÍN and DEATH, 2009). However, in our study, the seasonal variation was distinctive owing to the seasonal dynamics of the dominant species *Trichodrilus strandi*, which is monocyclic with spring-summer copulation, lying cocoons in summer (ŠPORKA, 2003; SCHENKOVÁ *et al.*, 2010), with juveniles in autumn increasing the number of individuals in samples.

A comparably lower influence of seasonal variation was observed in Ephemeroptera and Chironomidae assemblages. Their species composition was significantly associated with coarse organic matter and tufa crusts. They formed discrete assemblages in places near helocrenes (Chironomidae) or in the springbrook (Ephemeroptera). This pattern resulted from the dominance of crenobiontic and madicolous Chironomidae such as *Neostempellina thienemanni* REISS, *Krenopelopia* sp., *Krenosmittia cf. camptophleps* (EDWARDS) and *Parachaetocladius abnobaeus* (WULKER) (LINDEGAARD, 1995) near the helocrenes and rheophilous mayflies (*Ecdyonurus subalpinus* KLAPÁLEK, *Electrogena ujhelyii* (SOWA)) dwelling predominantly in the springbrook section of the fen.

4.3. Spatial Distribution of Macroinvertebrates in the Sphagnum-*Fen*

The spatial distribution of macroinvertebrates along the transect was governed by a joint influence of pH/mineral richness and substrate. Particular taxa were more or less sensitive to variations in pH and iron concentrations along the transect and/or the preferred substrate of different volumes of fine detritus. Water chemistry varying along the studied transect influenced Clitellata and Diptera (except Chironomidae) assemblages by the effects of acidity and high iron concentrations (Table 3). Clitellates showed a shift from acidotolerant species-dominated assemblages (Enchytraeidae) to eurytopic aquatic species-dominated assemblages

(Naididae and Lumbriculidae). Diptera (except Chironomidae) were characterised by an increased abundance of acid-benefiting Ceratopogonidae (Sommer and Horwitz, 2001) and by a very rare occurrence of species from the families Tipulidae and Limoniidae, which were documented as being limited by pH and heavy metals (Sasaki *et al.*, 2005).

Two groups, Chironomidae and Plecoptera, were predominantly associated with substrate, regardless of acidity and the distance from the helocrenes (Table 3). Many Chironomidae species showed clear preferences for either spring sections with a dominance of FPOM or with a high amount of inorganic substrate. These results correspond well to the findings of Ferrington (1998), who emphasised the importance of a predominant substrate type for the chironomid assemblage composition in springs. A joint influence of pH/mineral richness and substrate was observed in the Ephemeroptera and Trichoptera assemblages. As the mayflies are the most acid-sensitive aquatic insects (Braukmann, 2001), they were completely missing in the most acidic parts of the fen. Under episodically slight acidic and circum-neutral conditions, they were less abundant in sections with organic substrate and abundant in sections with a higher discharge and coarse sediment. This response was caused by the different degrees of acid sensitivity of mayflies. *Baetis vernus* Curtis, an “acid-benefitting” species, which can even increase their densities with increasing acidity (Tixier *et al.*, 2009), and the “slightly acid-sensitive” species *Baetis niger* (Linnaeus), which tolerates episodically acid events (Braukmann, 2001; Petrin *et al.*, 2007), inhabited sections with circum-neutral conditions that were episodically weakly acidic. In sections with neutral conditions, *i.e.*, springbrooks, the number of mayflies significantly increased and the assemblage included “acid-sensitive” species. The distribution in these sections was governed by the substrate, namely by the proportions of FPOM (preferred by, *e.g.*, *Baetis niger*) and stones and gravel (occurrence of *Ecdyonurus subalpinus* and *Habrophlebia lauta* Eaton and higher abundance of *Baetis vernus*). Contrary to Ephemeroptera, the Trichoptera taxa richness and abundance remained constant along the *Sphagnum*-fen. However, some species occurred exclusively in acidic sections (*i.e.*, acid-tolerant and/or crenophilous species, according to Braukmann, 2001; Graf *et al.*, 2008) or in acid-neutral sections (*i.e.*, slightly acid-sensitive and acid-sensitive lotic species; Braukmann, 2001; Graf *et al.*, 2008). Spring-dwelling species are generally more tolerant to low pH levels than rhitrobiontic species (see also Braukmann, 2001; Sasaki *et al.*, 2005), which appears to be an adaptation to special crenal conditions (Hahn, 2000). Simultaneously, the Trichoptera assemblage dwelling in this site comprised many species with pronounced substrate preferences which were distributed in relation to the fine detritus/inorganic particle proportions in the bottom substrate (*e.g.*, *Silo pallipes*, *Odontocerum albicorne*, and *Hydropsyche saxonica*).

5. Conclusions

Spatial heterogeneity in environmental conditions can be an important factor that controls macroinvertebrate within-site distribution. Higher heterogeneity can result in temporally stable species distribution patterns even at very small spatial scales, contrary to environmentally more homogeneous habitats where seasonal changes are of higher importance. Environmentally more heterogeneous habitats also promote variation in individual taxa responses to the measured variables, shaping the overall pattern obtained based on the whole community. In the more heterogeneous habitat, the response of the whole assemblage was a complex of different group responses, contrary to analogical or non-significant responses in a less heterogeneous habitat. This study stresses the importance of segregating the responses of individual macroinvertebrate groups when studying species distribution patterns even within a single habitat. Responses of individual groups can be highly variable or even reverse at environmentally more heterogeneous sites.

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Appendix 1. The list of taxa collected in the calcareous fen and *Sphagnum*-fen in 2006.

		calcareous fen	*Sphagnum*-fen
Platyhelminthes			
	Dugesia gonocephala (Dugés, 1830)	×	
	Polycelis felina (Dalyell, 1814)	×	
Mollusca			
	Ancylus fluviatilis O. F. Müller, 1774		×
	Bythinella austriaca (Frauenfeld, 1857)	×	×
	Galba truncatula (O. F. Müller, 1774)	×	×
	Pisidium personatum Malm, 1855	×	×
	Pisidium casertanum (Poli, 1791)	×	×
	Radix peregra (O. F. Müller, 1774)		×
Annelida			
	Achaeta sp.		×
	Cernosvitoviella sp.		×
	Cognettia glandulosa (Michaelsen, 1888)		×
	Cognettia sphagnetorum (Vejdovský, 1878)		×
	Eiseniella tetraedra (Savigny, 1826)	×	×
	Enchytraeus sp.	×	×
	Erpobdella octoculata (Linnaeus, 1758)		×
	Erpobdella vilnensis Liskiewicz, 1927		×
	Fridericia sp.	×	×
	Haplotaxis gordioides (Hartmann, 1821)	×	
	Henlea cf. *perpusilla* Friend, 1911		×
	Limnodrilus hoffmeisteri Claparéde, 1862		×
	Lumbriculus variegatus (O. F. Müller, 1774)		×
	Marionina sp.		×
	Mesenchytraeus armatus (Levinsen, 1884)	×	×
	Nais communis Piguet, 1906		×
	Pristina rosea (Piguet, 1906)		×
	Rhyacodrilus falciformis Bretscher, 1901	×	×
	Stylodrilus heringianus Claparéde, 1862		×
	Tubifex tubifex (O. F. Müller, 1774)	×	×
	Trichodrilus strandi Hrabě, 1936	×	
Crustacea			
	Gammarus fossarum Koch, 1835	×	×
Ephemeroptera			
	Baetis niger (Linnaeus, 1761)		×
	Baetis rhodani (Pictet, 1845)	×	
	Baetis vernus Curtis, 1834		×
	Ecdyonurus subalpinus Klapálek, 1907	×	×
	Electrogena ujhelyii (Sowa, 1981)	×	
	Habrophlebia lauta Eaton, 1884		×
	Paraleptophlebia submarginata (Stephens, 1835)		×
	Rhithrogena sp.		×
Odonata			
	Aeshna cyanea (O. F. Müller, 1764)		×
	Cordulegaster boltonii (Donovan, 1807)	×	
	Pyrrhosoma nymphula (Sulzer, 1776)		×
Plecoptera			
	Amphinemura standfussi (Ris, 1902)		×
	Diura bicaudata (Linnaeus, 1758)		×
	Isoperla cf. *tripartita* Illies, 1954	×	

Appendix 1. (continued)

		calcareous fen	*Sphagnum*-fen
	Leuctra braueri Kempny, 1898	×	×
	Leuctra inermis Kempny, 1899		×
	Leuctra nigra (Olivier, 1811)		×
	Leuctra prima Kempny, 1899	×	
	Nemoura cf. *avicularis* Morton, 1894		×
	Nemoura cambrica Stephens, 1836	×	×
	Nemoura cinerea (Retzius, 1783)		×
	Nemoura marginata Pictet, 1835	×	×
	Nemoura sciurus Aubert, 1949		×
	Nemurella pictetii Klapálek, 1900		×
	Protonemura lateralis (Pictet, 1835)	×	×
Heteroptera			
	Velia caprai Tamanini, 1947		×
Megaloptera			
	Sialis fuliginosa Pictet, 1836		×
Neuroptera			
	Osmylus fulvicephalus (Scopoli, 1763)	×	
Coleoptera			
	Anacaena globulus Ad. (Paykull, 1798)	×	
	Anacaena sp. Lv.	×	×
	Agabus guttatus Ad. (Paykull, 1798)		×
	Agabus paludosus Ad. (Fabricius, 1801)		×
	Agabus sp. Lv.	×	
	Deronectes platynotus Ad. (Germar, 1834)		×
	Deronectes sp. Lv.		×
	Elmis aenea Ad. (O. F. Müller, 1806)		×
	Elmis maugetii Ad. (Latreille, 1798)		×
	Elmis sp. Lv.		×
	Elodes sp. Lv.	×	×
	Esolus angustatus Ad. (O. F. Müller, 1821)		×
	Eubria palustris Lv. Germar, 1818		×
	Hydraena gracilis Ad. Germar, 1824		×
	Hydraena morio Ad. Kiesenwetter, 1849	×	
	Hydraena nigrita Ad. Germar, 1824	×	
	Hydraena saga Ad. d'Orchymont, 1930	×	×
	Hydrobius fuscipes Lv. (Linnaeus, 1758)		×
	Laccobius striatulus Ad. (Fabricius, 1801)	×	
	Limnius perrisi Ad. (Dufour, 1843)		×
	Limnius perrisi Lv. (Dufour, 1843)		×
	Limnebius truncatellus (Thunberg, 1794)		×
	Oreodytes sanmarkii Ad. (C. R. Sahlberg, 1834)		×
	Platambus maculatus Ad. Linnaeus, 1758	×	×
	Platambus maculatus Lv. Linnaeus, 1758		×
Trichoptera			
	Beraea pullata (Curtis, 1834)		×
	Beraea sp.	×	
	Ernodes articularis (Pictet, 1834)		×
	Ernodes articularis/vicinus	×	
	Chaetopteryx fusca Brauer, 1857		×
	Chaetopteryx fusca/villosa	×	
	Chaetopteryx major McLachlan, 1876	×	×

Appendix 1. (continued)

		calcareous fen	*Sphagnum*-fen
	Crunoecia irrorata (Curtis, 1834)	×	
	Hydatophylax infumatus (McLachlan, 1865)		×
	Hydropsyche saxonica McLachlan, 1884		×
	Limnephilus cf. *ignavus* McLachlan, 1865		×
	Limnephilus cf. *sparsus* Curtis, 1834		×
	Notidobia ciliaris (Linnaeus, 1761)		×
	Odontocerum albicorne (Scopoli, 1763)		×
	Oligotricha striata (Linnaeus, 1758)		×
	Parachiona picicornis (Pictet, 1834)		×
	Plectrocnemia brevis McLachlan, 1871	×	
	Plectrocnemia conspersa (Curtis, 1834)	×	×
	Potamophylax nigricornis (Pictet, 1834)	×	×
	Rhyacophila philopotamoides McLachlan, 1879	×	
	Rhyacophila pubescens Pictet, 1834	×	
	Rhyacophila sp.	×	×
	Sericostoma personatum (Kirby *et* Spence, 1826)	×	×
	Silo pallipes (Fabricius, 1781)		×
	Wormaldia occipitalis (Pictet, 1834)	×	×
Diptera			
Tipulidae	*Nigrotipula nigra* (Linnaeus, 1758)	×	
	Tipula bosnica Strobl, 1898	×	
	Tipula fulvipennis De Geer, 1776	×	
	Tipula goriziensis Strobl, 1893	×	
	Tipula lateralis Meigen, 1804	×	
	Tipula maxima Poda, 1761	×	
	Tipula rufina Meigen, 1818	×	
	Tipula unca Wiedemann, 1817		×
Limoniidae	*Cheilotrichia* sp.	×	
	Eloeophila sp.	×	×
	Ellipteroides alboscutellatus (von Roser, 1840)	×	
	Gonomyia lucidula Meijere, 1920	×	
	Gonomyia sp.	×	
	Helius sp.		×
	Molophilus sp.	×	
	Neolimnomyia filata (Walker, 1856)	×	×
	Orimarga sp.	×	
	Scleroprocta sp.	×	×
	Thaumastoptera calceata Mik, 1866	×	
Pediciidae	*Dicranota* sp.	×	×
	Pedicia sp.	×	×
	Tricyphona sp.	×	×
Ptychopteridae	*Ptychoptera lacustris* Meigen, 1830	×	
Psychodidae	*Berdeniella* sp.	×	
	Jungiella sp.	×	
	Pericoma calcilega Feuerborn, 1923	×	
	Pneumia sp.	×	
	Psychoda sp.	×	
	Threticus sp.	×	
	Ulomyia sp.	×	×
Dixidae	*Dixa maculata* Meigen, 1818	×	×
	Dixa submaculata Edwards, 1920	×	

Appendix 1. (continued)

		calcareous fen	*Sphagnum*-fen
Thaumaleidae	*Thaumalea* sp.	×	×
Ceratopogonidae	*Atrichopogon* sp.	×	×
	Ceratopogoninae	×	×
	Dasyhelea sp.	×	×
Chironomidae	*Apsectrotanypus trifascipennis* (Zetterstedt, 1838)	×	×
	Brillia bifida (Kiefer, 1909)	×	×
	Chaetocladius piger Gr.	×	×
	Conchapelopia sp.	×	×
	Corynoneura cf. *lobata* Edwards, 1924	×	×
	Diamesa cf. *tonsa* (Haliday, 1856)	×	×
	Eukiefferiella cf. *brehmi* Gouin, 1943		×
	Eukiefferiella brevicalcar (Kieffer, 1911)		×
	Eukiefferiella cf. *fuldensis* Lehmann, 1972	×	×
	Heleniella serratosioi Ringe, 1976	×	×
	Heterotanytarsus apicalis (Kiefer, 1921)		×
	Heterotrissocladius marcidus (Walker, 1856)		×
	Krenopelopia sp.	×	×
	Krenosmittia cf. *camptophleps* (Edwards, 1929)	×	
	Limnophyes sp.	×	×
	Macropelopia cf. *nebulosa* (Meigen, 1804)		×
	Metriocnemus hygropetricus Gr.	×	×
	Metriocnemus fuscipes Gr.	×	×
	Micropsectra sp.	×	×
	Microtendipes chloris Gr.		×
	Nanocladius parvulus/rectinervis		×
	Natarsia sp.	×	×
	Neostempellina thienemanni Reiss, 1984	×	
	Orthocladius lignicola Kieffer, 1914	×	
	Orthocladius rivicola Gr.	×	×
	Orthocladius rivulorum Kiefer, 1909		×
	Paramerina sp.		×
	Parametriocnemus stylatus (Sparck, 1923)	×	×
	Paraphaenocladius sp.	×	×
	Paratendipes nudisquama (Edwards, 1929)		×
	Phaenopsectra sp.		×
	Polypedilum convictum Gr.	×	
	Polypedilum laetum Gr.		×
	Polypedilum scalaenum Gr.	×	×
	Prodiamesa olivacea (Meigen, 1818)	×	×
	Parachaetocladius abnobaeus (Wülker, 1959)	×	×
	Rheocricotopus atripes (Kiefer, 1913)	×	×
	Rheocricotopus effusus (Walker, 1856)	×	×
	Rheocricotopus fuscipes (Kiefer, 1909)	×	×
	Rheocricotopus sp.		×
	Smittia sp.	×	
	Stempellinella ciliaris/flavidula	×	×
	Synorthocladius semivirens (Kiefer, 1909)	×	×
	Tanytarsus sp.		×
	Thienemanniella clavicornis/vittata		×
	Trissopelopia sp.		×
	Tvetenia bavarica Gr.	×	×
	Zavrelimyia sp.	×	×

Appendix 1. (continued)

		calcareous fen	*Sphagnum*-fen
Simuliidae	*Simulium argyreatum* Meigen, 1838	×	
	Simulium cf. *carpathicum* (Knoz, 1961)	×	×
	Simulium costatum Friederichs, 1920	×	×
	Simulium ornatum Meigen, 1818	×	
	Simulium trifasciatum Curtis, 1839	×	
	Simulium vernum Gr.	×	×
Sciaridae		×	×
Cecidomyidae		×	×
Stratiomyidae	*Beris clavipes* (Linnaeus, 1767)	×	
	Beris vallata (Forster, 1771)		×
	Nemotelus pantherinus (Linnaeus, 1758)	×	
	Oxycera meigenii Staeger, 1844	×	
	Oxycera pardalina Meigen, 1822	×	
	Oxycera pygmaea (Fallen, 1817)	×	
Tabanidae	*Chrysops caecutiens* (Linnaeus, 1758)		×
	Hybomitra sp.		×
	Tabanus autumnalis Linnaeus, 1761		×
	Tabanus sp.	×	
Rhagionidae	*Chrysopilus* sp.	×	
Athericidae	*Ibisia marginata* (Fabricius, 1781)	×	
Empididae	*Chelifera* sp.	×	×
	Clinocera sp.	×	
	Dolichocephala sp.	×	
Dolichopodidae		×	
Syrphidae	*Melanogaster hirtella* (Loew, 1843)		×
Sciomyzidae	*Pherbellia* sp.	×	×
Drosophilidae	*Scaptomyza* sp.		×

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A global database of Holocene paleotemperature records

Darrell Kaufman *et al.*[#]

A comprehensive database of paleoclimate records is needed to place recent warming into the longer-term context of natural climate variability. We present a global compilation of quality-controlled, published, temperature-sensitive proxy records extending back 12,000 years through the Holocene. Data were compiled from 679 sites where time series cover at least 4000 years, are resolved at sub-millennial scale (median spacing of 400 years or finer) and have at least one age control point every 3000 years, with cut-off values slackened in data-sparse regions. The data derive from lake sediment (51%), marine sediment (31%), peat (11%), glacier ice (3%), and other natural archives. The database contains 1319 records, including 157 from the Southern Hemisphere. The multi-proxy database comprises paleotemperature time series based on ecological assemblages, as well as biophysical and geochemical indicators that reflect mean annual or seasonal temperatures, as encoded in the database. This database can be used to reconstruct the spatiotemporal evolution of Holocene temperature at global to regional scales, and is publicly available in Linked Paleo Data (LiPD) format.

Background & Summary

Placing recent global warming in the context of natural climate variability requires the long-term perspective afforded by paleoclimate proxy records. Reconstructing past global climatic changes relies on a variety of evidence from a large number of well-distributed sites. Previous syntheses of Holocene temperature records have typically focused on specific time horizons (mostly 6000 years ago), and are based nearly entirely on pollen assemblages from terrestrial archives[1–4], or are dominated by sea-surface temperatures[5] near continental margins. Few global datasets have been compiled based on evidence from a wide variety of proxy types, including ecological, geochemical and biophysical evidence from both marine and terrestrial archives. Understanding of paleoclimate is enriched by a multi-proxy approach. Using multiple proxy types can help expand geographic coverage while enabling an assessment of inherent proxy biases. However, assembling a comprehensive database of continuous (time-series instead of time-slice) Holocene paleotemperature proxy records supported by a uniform suite of metadata descriptors across a wide variety of proxy data types is challenging[6], and has not yet been attempted. In addition, a large portion of the data and metadata that form the basis of published paleoclimate studies have not been made available through public repositories, prior to this data synthesis.

This data descriptor presents version 1.0.0 of the Temperature 12k database (ref. [7], with additional supporting information and updates at: www.ncdc.noaa.gov/paleo/study/27330). It describes the methods used to assemble the database, including the criteria for record inclusion, and it describes each of the metadata fields that enable intelligent and automated reuse of the time-series data (Table 1). In addition, this data descriptor summarizes the major features of the records that comprise the database, including millennial-scale trends in Holocene temperature. The robustness of these major trends is explored by subdividing the dataset into various spatial, methodological, seasonal and other categories and visualizing the extent to which these data subsets represent the overall trends of the database both latitudinally, and through the past 12,000 years.

The data are useful for addressing a variety of paleoclimate research questions at global to regional scales. For example, they are needed to help understand how the ocean-atmosphere circulation has evolved along with past global climate changes. The database is designed for comparison with model-based simulations of climate, with the goal of evaluating model performance while gaining insights into the mechanisms and feedbacks associated with global climate change. Particular attention has been devoted to documenting the seasonality of temperature interpretations because climate forcing during the Holocene was dominated by orbitally controlled insolation changes that operated asymmetrically across the annual cycle[8].

[#] A full list of authors and their affiliations appears at the end of the paper.

This database complements the PAGES 2k Consortium[9] database of global paleotemperature records, which extends back 2000 years and is formatted similarly within the Linked PaleoData structure (LiPD[10]). The PAGES Iso2k[11] database, which focuses on water isotope records over the past 2000 years, is also being developed in the LiPD structure. These higher-resolution (mostly annual) time series of 2000-year-long records provide a bridge between the overall lower-resolution time series of this database and the highly detailed, but relatively brief instrumental-based record of climate.

Methods

The Temperature 12k database comprises paleotemperature records generated using a wide variety of techniques. It gathers data from previously published studies, each of which describes the methodologies used to generate the various data types, the scientific underpinnings of the techniques used to interpret the data in terms of past temperature change, and the important uncertainties. A major feature of this database is the integration of these complementary proxy data types and the harmonization of the metadata that describe them. The database is also quality-controlled; it comprises time-series data of relatively high resolution (sample spacing finer than 400 years; see below) and with a relatively well-established time scale, with the goal of creating a cohesive and uniform data product. This section describes the procedures used to assemble the database rather than the specific methods used to generate the individual data records that comprise the database.

Procedures - data sources. The Temperature 12k database builds on several published paleoclimate data compilations, including one recently used to reconstruct Holocene temperature gradients across the Northern Hemisphere[12], which itself drew from earlier compilations[5,13–15]. The majority of the pollen-based paleotemperature time series were obtained through the compilation of Marsicek *et al.*[16]. The majority of the marine records were gathered as part of the US-based Data Assimilation for Deep Time (DADT) project, and some were selected from the compilations of the German Climate Modeling Initiative (PalMod[17]). For these, we focused on the subset of proxy types that record (near) sea-surface temperature. In addition to culling data from previous paleoclimate data compilations, we searched the literature and public data repositories (PANGAEA and World Data Service for Paleoclimatology, NOAA) for appropriate records. The remainder of the datasets were obtained through either the supplements of publications, or from individual data generators, some of whom are co-authors on this data product. Several records were not available from the original data generators. To rescue such data, particularly where they fill geographic gaps, time series were digitized from the source publication (as noted in the metadata).

Most of the pollen-based paleotemperature time series, and data from marine and glacier-ice archives were previously available through public repositories. In contrast, of the 105 records based on chironomids and biomarkers from terrestrial deposits, only 37% were previously available through public repositories. In addition, this data product includes an expanded and harmonized suite of metadata for all of the records, including those that we obtained from data repositories. The database also includes the chronological data used to develop the age scale for the proxy time series. For a large portion of studies, this chronological information was not previously archived along with the proxy records *per se*.

Only data published in the peer-reviewed literature were considered. This restriction helps to assure that the data are high quality, intended for scientific reuse, and are supported by vetted, complete and often nuanced paleoclimate and geochronological interpretations.

Procedures - selection criteria. The Temperature 12k database was designed to address research questions involving temperature change over the Holocene at regional to global scales. Thousands of studies have been published that attest to Holocene temperature changes. These records have widely variable temporal resolution, duration, and chronological control. In order to provide a consistent, quality controlled data product, we selected records that meet specific, quantitative criteria. These criteria were designed for relatively broad inclusion, while concentrating on the highest-resolution and well-dated records. The criteria were adjusted for selected sites to fill major geographic gaps, or for other reasons as justified by authors in the '*QC Comments*' section of the metadata. The selection criteria were:

Temperature sensitivity. Only those proxy records that have a demonstrated relation with temperature were included. Because most of the paleotemperature time series are not sufficiently resolved temporally to meaningfully compare with instrumental-based observations, this demonstration of temperature sensitivity is typically based on accepted understanding of the proxy systems. The specific approach and the calibration data used for the resulting paleotemperature estimates are typically described in the original publication for each study site and are specified within the metadata for most records ('*Calibration Method*'). That a proxy type is sensitive to temperature does not preclude its sensitivity to other environmental variables, such as moisture availability or salinity. The extent to which a proxy indicator is temperature-dependent can vary among sites and through time. Proxy records with multiple simultaneous interpretations, such as those interpreted as a combination of changes in precipitation amount and temperature, were generally excluded from the database, unless the authors of the original studies identified temperature as the primary control on the proxy.

Proxy data do not have to be converted to units of degrees to be useful indicators of past temperature.

This database includes 43 relative temperature indicators that are reported in their native proxy variables (e.g., $\delta^{18}O$ of glacier ice). They are useful because (1) they attest to the timing and relative magnitude of change, which is sufficient for many statistical reconstruction methods, especially those that do not assume linearity between the proxy and the climate variable; (2) they are used in proxy-system modeling and in some cases can be compared directly to climate model output; and (3) they provide a more complete spatial coverage for the proxy network.

Name (Suppl. Table 1)	LiPD variable name	Essential?	Description
Data Set Name	dataSetName	yes	collection of proxy data and metadata
Site location			
Site Name	geo_siteName	yes	site name or marine core identification
Country Ocean	geo_countryOcean	auto	auto-generated based on NASA GCMD convention
Latitude	geo_latitude	yes	site latitude in decimal degrees (negative for Southern Hemisphere)
Longitude	geo_longitude	yes	site longitude in decimal degrees (negative for Western Hemisphere)
Elevation	geo_elevation	yes	site elevation in meters (negative for below sea level)
Source and attribution			
Publication 1	pub1_doi	yes	DOI of primary bibliographic reference; typically the original study that describes the data and authored by the data generator
Publication 2	pub2_doi	no	DOI of secondary bibliographic reference; typically a refinement of the original study including a new temperature calculation based on the original data
Original Data Citation	originalDataUrl	yes	persistent URL or DOI of original archived data file; data not previously deposited in open-source repository = “this compilation”
Neotoma ID	neotomaDatasetId	no	DOI or data identifier for pollen assemblage and other data stored in Neotoma Paleoecology Database
Proxy record			
Archive Type	archiveType	yes	major category of archive type (e.g., lake sediment)
Proxy General	paleoData_proxyGeneral	yes	major category of proxy type used to group records for plotting figures
Proxy Type	paleoData_proxy	yes	proxy type (e.g., pollen)
Proxy Detail	paleoData_proxyDetail	yes	specific type of material analyzed; can include species
Calibration Method	calibration_method	yes	statistical method used for calibration; “NA” for non-calibrated proxy types
Calibration Seasonality	calibration_seasonality	no	specific months used for calibration
Paleo Data Notes	paleoData_notes	no	information from original study; specific methods or interpretation that can help users understand the appropriate use and limitations of the proxy record
Climate interpretation			
Variable Name	paleoData_variableName	yes	“temperature” for calibrated records; “temperatureComposite” for auto-averaged; other variable names for non-calibrated records (e.g., d18O)
Units	paleoData_units	yes	°C for calibrated records; other variable units for non-calibrated proxies (e.g., permil)
Datum	paleoData_datum	yes	“abs” = absolute temperature; “anom” = temperature relative to a reference (anomaly); “SMOW” or “PDB” for d18O
Climate Variable	climateInterpretation1_variable	yes	primary climate variable sensed by proxy (“T” for this data product)
Climate Variable Detail	climateInterpretation1_variableDetail	yes	what environmental temperature is represented by the sensor and at what level (e.g., water@surface)?
Seasonality	climateInterpretation1_seasonality	yes	season represented by the climate variable; specific month number when available (e.g., annual = 1 2 3 4 5 6 7 8 9 10 11 12), otherwise generalized term (e.g., summer)
Season General	climateInterpretation1_seasonalityGeneral	yes	“summerOnly” = warm season with no annual record at site; “summer+“ = warm season with annual record available at site; “winterOnly” and “winter+“ = as above but for cold season; “annual” = annual record
Direction	climateInterpretation1_direction	yes	“positive” for proxy values that increase with temperature; “negative” for values that decrease with temperature
Time series			
Min Year	minYear	auto	youngest proxy sample; auto-generated from the time series data
Max Year	maxYear	auto	oldest proxy sample; auto-generated from the time series data
Resolution	paleoData_medianRes12k	auto	median spacing between consecutive samples over the past 12 ka
Ages Per kyr	agesPerKyr	auto	number of 14C, U/Th, and tephra ages per 1000 years over the past 12 ka
Quality control			
In Compilation	paleoData_inCompilation	yes	“Temp12k” for records that meet the selection criteria; “Tverse” for temperature-sensitive records that do not meet the criteria
QC Certification	paleoData_QCCertification	yes	initials of co-author(s) who certified that record meets selection criteria and added QC comments
QC Comments	paleoData_QCnotes	no	interpretative comments that help future users reuse the data intelligently; time-series data that were digitized from a published figure; are flagged; justification for retaining records that do not meet the selection criteria are provided.
Links to data			
Link to LiPDverse	lipdverseLink	auto	URL link for viewing, downloading, and editing the underlying LiPD file

Table 1. Brief description of selected metadata fields used in the Temperature 12k database and shown in Suppl. Table 1.

Duration and resolution. The database documents past temperature variability ranging in time-scale from multi-millennial trends to centennial excursions. In an effort to represent multi-millennial trends, while maintaining a relatively even temporal distribution of data coverage, we selected records that span a minimum continuous duration of 4000 years anytime within the past 12,000 years. To focus on records that can be used to resolve sub-millennial patterns, we selected time series with sample resolution ('*Resolution*') finer than 400 years (i.e., the median spacing between consecutive samples is less than 400 years over the past 12,000 years or over the full record length, if shorter). For some records, the selection cut-off values for duration and resolution were relaxed to improve the global coverage of the data network, especially in the Southern Hemisphere. These records are identified as such in '*QC Comments*.'

Chronological control. Age control is a fundamental variable underlying paleoclimate time series. We selected records that are supported by age control points with minimum spacing of 3000 years over the record duration or within the past 12,000 years. Records with gaps longer than 3000 years were accepted in data-poor regions, or for sequences that are supported by a relatively high frequency of ages (typically five or more over the Holocene). These exceptions are noted in '*QC comments*.' The chronological control points for almost all records were copied from the original articles, downloaded from data repositories, or obtained from the data generators. Unless they were unavailable, this database includes the chronological data and metadata necessary to generate age-depth models for proxy data from sediment and speleothem archive types. In some cases, where the original chronology was obsolete, such as for those originally reported on a radiocarbon time scale, we generated a new age model using 'Bacon'[18] and added a note in the '*QC Comments*.' Chronological data include depth, raw radiometric or other types of age controls, errors, and associated corrections when available. Other metadata such as material type analysed and sample identifiers were also included when available.

Data Records

This data descriptor presents version 1.0.0 of the Temperature 12k database (ref. [7], www.ncdc.noaa.gov/paleo/study/27330 and https://doi.org/10.25921/4RY2-G808).

Metadata. The database includes a large variety of metadata to facilitate subsampling, analysis, and intelligent reuse (described briefly in Table 1). The metadata (Suppl. Table 1) include essential information, with one entry (row) for each proxy record (time series), with a large portion of sites ('*Site Name*') represented by more than one record. These are based on different proxy types (e.g., alkenone and Mg/Ca from the same marine sediment sequence), or they represent different seasons based on a single proxy type, usually pollen. This database is a subset of a larger compilation of paleoclimate datasets that are configured in the same format, including the PAGES 2k Consortium[9] database. The 1319 records that comprise this data product are identified within this larger collection by '*In Compilation*' = 'Temp12k'. The final column in Suppl. Table 1, '*Link to LiPDverse*,' displays the URL that links from the metadata table to each dataset where the metadata and associated proxy time series and chronology data can be viewed and downloaded individually.

The specific metadata fields (Table 1; Suppl. Table 1) document information about the:

(1) site location, including: '*Site Name*,' '*Country Ocean*' (based on NASA GCMD location keywords), latitude ('*Latitude*'), longitude ('*Longitude*'), and '*Elevation*.' Geodetic data are in units of decimal degrees with respect to the WGS84 ellipsoid.

(2) bibliographic citations (DOI when available). '*Publication 1*' typically refers to the original study that describes the data and was authored by the data generator, whereas '*Publication 2*' typically refers to some refinement of the original study including subsequent paleotemperature analyses based on the original data. For most of the pollen records from North American and Europe, '*Publication 1*' is the first of the citations listed for the dataset as referenced in the Neotoma Paleoecology Database (hereafter, "Neotoma") and '*Publication 2*' is the synthesis study of Marsicek *et al.*[16], the most recent and comprehensive analysis of pollen data from this region.

(3) data source. '*Original Data Citation*' is the data citation (persistent identifier) used to locate the proxy data and paleotemperature values in a long-term and publicly accessible repository. Data from online supplements of articles are often behind paywalls and therefore not public, and some have been superseded by versions that were subsequently modified and stored in data repositories. Data transferred to a public repository for the first time as part of this data product are listed as '*Original Data Citation*' = 'this compilation.' Taxonomic assemblage data are beyond the scope of this paleoclimate-oriented database. For pollen, information is provided in the metadata to access the assemblage and other information in Neotoma. Namely, '*Neotoma ID*' is either the dataset identifier or the DOI for the landing page, which includes assemblage and other (meta) data for pollen records that are currently curated in Neotoma. For some marine microfossil records, '*Original Data Citation*' is a link to the assemblage data stored at PANGAEA and WDS-NOAA Paleoclimatology.

(4) bio-physical indicator and method used to infer past temperature, including: '*Archive Type*,' '*Proxy General*', and '*Proxy Type*,' '*Proxy Detail*.' The latter is particularly useful for proxy records that are based on isotope and geochemical analyses for which the specific sensor species or material type is essential information. '*Proxy General*' is used to group proxy types to simplify plotting of figures. The '*Calibration Method*' used to translate proxy data to temperature is stated for most calibrated proxy records, or is typically stated within the original publication. '*Paleo Data Notes*' provides additional pertinent information about the proxy record, including its limitations as represented by the original study.

(5) climate interpretation. All of the records included in this data product are temperature sensitive ('*Climate Variable*' = 'T'), and most are calibrated ('*Variable Name*' = 'temperature'), either as absolute temperature ('*Datum*' = 'abs') or as a temperature anomaly ('*Datum*' = 'anom'). '*Climate Variable Detail*' provides further information about what environmental temperature is represented by the sensor (air, surface water, subsurface water). '*Variable Name*' and '*Units*' are = 'temperature' and 'degC' for the calibrated records in this data product. Some proxy records are related to, but not calibrated to temperature ('*Variable Name*'

is not 'temperature' for these records). For these non-calibrated records, '*Variable Name*' and '*Units*' refer to the native proxy type, such as 'd18O,' which is expressed in units of 'permil.' '*Direction*' applies to the non-calibrated proxy types. It is 'positive' for proxy values that increase with increasing temperature and 'negative' for values that are inversely related to temperature.

In some cases, authors of original studies presented alternative interpretations of temperature for a particular season based on a single proxy type. We selected the interpretation that the author of the original study deemed superior, or when ambiguous, authors of this data product made the selection based on rationale noted in the '*QC Comments*' of the metadata. Preference was generally given to the highest resolution or most recent rendition of a proxy record. When there was no clear basis for selection, and different interpretations were based on the same proxy data (e.g., two different training sets were applied to the same assemblage data), the time series were combined, first by subtracting the record means to avoid artifacts related to combining time series of different lengths or number of samples, then by averaging to express temperatures as temperature anomalies ('*Datum*' = 'anom'). These composited temperature records are designated, '*Variable Name*' = 'temperatureComposite' and are noted in the '*QC comments*'. The paleotemperature time series used in the composites are retained in the database. Paleo temperatures from sites within the margins of former ice sheets were not corrected for the effect of isostatic rebound.

(6) time of year represented by the climate variable ('*Seasonality*'). When available, specific months are listed according to the corresponding calendar-month number. Because of the wide variety of specific seasonalities included in the database, '*Season General*' is used to generalize the seasonality as either annual, summer or winter. Several marine records represent transitional seasons; for these, spring was grouped with summer and fall with winter. Six or more months overlapping with June were categorized as annual. This field is also used to distinguish sites for which there are both summer and annual (= 'summer+' and 'annual') from sites where summer records are not paired with an annual counterpart (= 'summerOnly'), with an equivalent formulation for 'winter+' and 'winterOnly.' This enables easy filtering of the database to select sites with either seasonal or annual time series, or both. When '*Variable Name*' = 'temperatureComposite,' this time series is the average of winter and summer time series, which were calculated for this database to approximate annual values when no annual values are available, as indicated in the '*QC comments*.' '*Calibration Season*' (when available) specifies the exact month(s) to which the climate variable have been calibrated. For example, many Northern Hemisphere chironomid records are considered to represent summer temperature ('*Seasonality*' = '6 7 8'); however, they are usually calibrated against only July or August temperatures ('*Calibration Season*' = '7' or '8'). For most records, '*Calibration Season*' and '*Seasonality*' are identical.

(7) chronology, including: youngest sample age ('*Min Year*'), oldest sample age ('*Max Year*'), and the median time-series resolution ('*Resolution*'), which is calculated as the median difference between the modeled ages of consecutive proxy samples, extending back 12,000 years. To quantify the frequency of age-control points, '*Ages Per kyr*' is calculated as the average number of radiocarbon (the vast majority of age types), tephra, and U/Th ages per 1000 years back to 12,000 years.

(8) quality control, including the initials of the author(s) ('*QC Certification*') of this data product who was (were) responsible for assuring that an individual record meets the selection criteria, or for justifying the inclusion of records that do not meet the criteria, and for entering additional comments to improve the reusability of the proxy record ('*QC Comments*').

(9) link to the data, including a browser-based interface for LiPD files ('*Link to LiPDverse*') with data-viewing and download capabilities (LiPD and.csv formats).

Number and type of proxy records. In this data descriptor, the term "site" refers to a single location (or limited area) where various analyses were conducted to generate one or more "proxy time series" (Fig. 1). Each proxy time series is interpreted in terms of temperatures for one or more seasons, collectively and generally referred to as "records".

The Temperature 12k database includes proxy time series from 470 terrestrial and 209 marine sites (Suppl. Table 2; Fig. 2). In total, this database comprises 1319 paleotemperature records. Multiple seasons are represented as different records at most sites, especially those based on pollen assemblages. In some cases, multiple records from the same site are based on different proxy types, most commonly planktic foraminifera $\delta^{18}O$, Mg/Ca, and alkenones from the same marine sediment core. The database includes 715 records from lake sediments, 359 records from marine sediments, and 245 from other terrestrial archives (e.g., glacier ice and speleothem). Alkenones and isotopes are the dominant sea-surface temperature proxies, whereas pollen and chironomids are the most common terrestrial temperature proxy types. In addition, the database includes paleotemperature evidence from a wide variety of other proxy types, such as assemblages of vegetation macrofossils from pack-rat middens, dinocysts from marine sediment, the composition of glycerol dialkyl glycerol tetraethers (GDGTs), abundance of chlorophyll from lake sediment, or the isotopic composition of pore ice in permafrost, to name a few.

The most frequent proxy type is pollen. Unlike other compilations of large-scale, pollen-based climate reconstructions, records in this database were screened for resolution and chronological control. In addition, the Temperature 12k database includes links (DOIs) to the primary pollen assemblage and additional data as curated in Neotoma. Most of the pollen-based paleotemperature time series from North America and Europe in this database are from the synthesis of Marsicek *et al.*[16], which used the modern analogue technique to calculate paleotemperatures back to around 11 ka, and screened records using the PalaeoSig[19] package ('randomTF' function with a 95% confidence level). Data from 209 of their 642 sites met our selection criteria. This database expands on the original data archive for ref. [16] (www.ncdc.noaa.gov/paleo-search/study/22992) by also including warmest-month temperatures based on the same pollen data and procedures, along with the mean annual temperatures. The '*Original Data Citation*' for these extended records are therefore indicated as 'this compilation.' In addition, this database includes the native-resolution time series for those datasets, whereas the original data archive features temperatures summarized in 100-year intervals.

The most frequent marine proxy types are $\delta^{18}O$, alkenones ($U^{K'}_{37}$), and Mg/Ca, including data from 135 out of 260 sites that were assimilated from the DADT project. For nearly all of these records, plus those based on TEX_{86}, we generated paleotemperature time series by using the Bayesian calibration methods of Malevich *et al.*[20], Tierney and Tingley[21,22], and Tierney *et al.*[23] respectively, with their published model parameters, as specified in '*Calibration Method*'. The original temperature time series are retained among the ancillary records in the larger collection of temperature-sensitive datasets ('*In Compilation*' = Tverse, see below) and are noted as such in the '*QC Comments*' field. The '*Original Data Citation*' for these datasets refer to the source of the underlying proxy data, some of which include calibrated temperatures from the original studies.

Geographic coverage.

The Temperature 12k database gathers paleotemperature data from every continent and ocean. The geographical distribution of the records, however, is uneven (Fig. 2). Latitudinally, 51% of the sites are located within the zone of 60–30°N, and only 16% are located in the Southern Hemisphere (Fig. 3). The spatial density of sites is comparatively high in North America and Europe and lower across the open ocean and tropical Africa. Data-poor regions reflect a combination of physical impossibility to obtain proxy records (e.g., low sediment accumulation rates in the open ocean and paucity of biogenic materials from extreme environments such as deserts), limited research attention and, in some cases, restricted field and data accessibility (e.g., Siberia).

Record length and resolution.

The temporal distribution of the time series is relatively uniform (Fig. 2b), with different proxy types having similar record lengths. The average record length within the Holocene is 9813 years for this database (n = 816, where only one season (or annual) is counted for each proxy from each site). All sites are represented by records that include at least some data between 8.5 and 3.5 ka. The number of records decreases over the last millennium, especially over the past century, largely because the surface sediment from lakes and oceans are watery and therefore difficult to recover intact, or because the climate interpretation of recently accumulated sediment has been compromised by human activities.

Most of the records (91%) extend back at least 6000 years, thereby encompassing the '6 ka time slice', which is an on-going and long-standing target for paleoclimate modeling[24]. The temporal distribution of time series contrasts with paleotemperature data from the past 2000 years[9], which are dominated by tree-ring records less than 500 years long. This underscores the complementary information afforded by this database when combined with the PAGES 2k Consortium[9]. The median record resolution (Suppl. Table 1, '*Resolution*') of individual time series in the database is 164 years over the Holocene (n = 816, where only one season (or annual) is counted for each proxy from each site) (Fig. 3b). Overall, 15% of these records have 50-year resolution or finer, 39% have 51- to 150-year resolution, and 21% are coarser than 250-year resolution.

Seasonality.

Most of the sites (74%) include at least one record that was interpreted as mean annual temperature, or was calculated as the average of summer and winter values for this data product to represent annual temperature. Most of the sites (64%) have records that are interpreted by the original authors as representing summer temperature, and 39% of the sites include winter paleotemperature estimates. The temporal distribution of records by season through the Holocene (Fig. 2c) shows relatively uniform distribution of seasonal paleotemperature records.

Chronology.

The majority of records in this database are based on sedimentary sequences dated by radiocarbon, and their time series are calibrated to calendar years relative to 1950 CE (BP). Some of the age-depth models are supported by volcanic ash (tephra) whose ages are known, some are augmented by biostratigraphic markers (first-arrival datums for pollen records), and some have ^{210}Pb profiles constraining the age of surface sediments. Speleothems are dated by U/Th methods, and some sequences are annually laminated (varves, ice, wood). For sequences that rely mainly on radiocarbon (n = 613), the average number of ages for records in this database is 1.0 per 1000 years, including the tephra ages. Recalculated age models based on a Bayesian modeling routine[18] are available for many of the time series, including the marine records from the ongoing DADT project (records with '*Calibration Method*' = 'Bay...'), and McKay *et al.*'s Arctic Neoglacial study[25]. The age ensembles are made available as part of the expanded data package for this data product (see below).

Technical Validation

Confirmation that the Temperature 12k dataset accurately represents the temperature at a site or globally would require knowing the actual temperature through time. This can only be determined for the period of instrumental temperature observations. Only a few of the records in this database include a sufficient number of inferred temperatures (samples) over the period of thermometer-based temperature observations for such a determination. In fact, the number of records that include data over the 20th Century is less than any other century of the past 10,000 years. Instead, evidence that the records in the database reflect past temperature fluctuations at each site can typically be found in the original publications associated with each record (Suppl. Table 2, refs. [26–566]). This procedure relies on expert knowledge as represented in peer-reviewed literature to guide the selection of proxy records. Records were not selected or weighted based on how well they correlate with an instrumental target. Expert knowledge can indeed yield a stronger proxy network for paleoclimate reconstructions than screening against instrumental data alone[567].

The validation approach used here focuses on the robustness of the major trends that characterize this diverse dataset. For this, the database was subdivided and summarized statistically to evaluate the extent to which a common signal is represented by various categories of records, or whether the major features of the overall signal are strongly controlled by a particular subset of the records. We also test whether the spatial network of sites in this database is sufficient for representing large areas of the globe; for this, we focus on 30° latitudinal bands, following previous reconstructions of global temperature for the Holocene[5] and the deglacial period[568]. This robustness testing is in addition to basic quality control procedures.

Quality control procedures. The authors of this data product worked in four teams of proxy experts (marine, pollen, chironomid, and other terrestrial archives) to assemble and quality control the data and metadata. They used a web-based data viewer (LiPDverse.com) and other visualization tools to examine displays of the raw data and the metadata. They reviewed the primary literature to assure that the data met the selection criteria for this database. The metadata (Suppl. Table 1, '*QC Certification*') lists the initials of the author(s) of this data descriptor who certified that each proxy record was translated accurately to the database, and that it was interpreted in the literature as related to temperature, and who, in some cases, added notes to help assure appropriate reuse of the data ('*QC Comments*').

In addition to the expert review, each record was analyzed using a series of automated tests to identify those with values that exceed thresholds defined by the physically realistic ranges for a variable. Records were flagged for follow-up if, for example, site coordinates exceeded −90° and 90° latitude, if marine site elevations were positive, if country names did not match those from Natural Earth Data (www.naturalearthdata.com) and ocean basins those from www.marineregions.org, if records contained duplicated ages, or if the temperature exceeded the range of −40 to 50 °C. The automated tests are based on the 'pytest' test framework as described in ref. [569].

Robustness of major trends represented in different subsets of records. Composite time series were generated to characterize the major overall trends in the time series that comprise the dataset and to compare signals contained in various subsets of the database.

Compositing procedures. Our approach follows the data descriptor of the PAGES 2k Consortium[9] temperature database. Briefly, all time series were standardized to z-scores, with a mean of zero and variance of 1 SD over the entire record length (except for the global composite, as specified below). Individual data points were binned by averaging the measurements within 500-year intervals (except for the high-resolution composite described below). We chose 500 years because it is compatible with the minimum 400-year-resolution selection criterion for records in this database, and because this broad bin size substantially mitigates the influence of age uncertainties. The binned records were then gridded spatially using an equal-area grid (4000 grids, each with area = 127,525 km^2, following methods in ref. [12]) to reduce the influence of clustered sites on the composites (except for the proxy-specific composites). The binned time series of various types (as specified below) inside each grid cell were averaged. The gridded data were also averaged into 30° latitudinal (zonal) bands to yield a single composite.

Composite uncertainties. The database contains a wide range of inter-record variance, which necessitates a large sample size to reconstruct regional or global temperature history. The uncertainty in the mean value of the composite at any time is related to both the number of records and their dispersion. To quantify this uncertainty, we used a bootstrap procedure that randomly sampled the proxy data network to generate an ensemble of composites from which the uncertainty was calculated. The procedure samples with replacement[570] whereby the number of randomly selected records equals the total number of records, but individual record could be selected more than once in a given bootstrap sample. The procedure was repeated to generate 1000 datasets from which composites were calculated and the mean and 95% uncertainty intervals were derived. The uncertainty intervals generated by this procedure are widest where a composite is represented by the fewest records and where there is therefore less certainty in the mean value. This approach is different than representing uncertainty according to the dispersion among records, which may not fully reflect the confidence in the mean estimate.

Composite seasonality. Unless otherwise specified, the composites were based on annual records. Where annual records were not available, the summer or winter season was used (all sites where both are available were averaged to generate an annual paleotemperature time series). By combining annual and seasonal records, we assume that the temperature variability represented by the seasonal records correlates with mean annual temperature. PAGES 2k Consortium[9] explored this assumption by correlating seasonal and mean-annual time series using a gridded temperature reanalysis product (HadCRUT4.2). They found that correlations are generally very high ($r > 0.8$) in the tropics, where the mean annual temperature range is small, and low in the extra-tropics, particularly over Northern Hemisphere continental interiors for summer, where the mean annual temperature range is large and dominated by winter synoptic variability. Therefore, summer records located in the tropics may be good surrogates for mean annual temperature, but less so for records located on Northern Hemisphere continents. Even for records that have been calibrated to annual temperature, the extent to which they accurately represent annual temperature depends on multiple factors, including assumptions that underlie the calibration procedures and the part of the seasonal cycle that influences each type of proxy sensor.

Comparison among proxy types. To evaluate the extent to which different proxy types carry a common overall signal, composites were calculated for each of the eight most common proxy types in the database (Fig. 4). No spatial gridding was applied prior to averaging for this comparison so as not to confound the direct comparison among proxy types. The composites all show warming trends during the early Holocene, some reaching peak warmth as early as around 10,000 years ago (e.g., chironomids), and others not until around 6000 years ago (e.g., pollen). By 6000 years ago, all of the composites show a cooling trend.

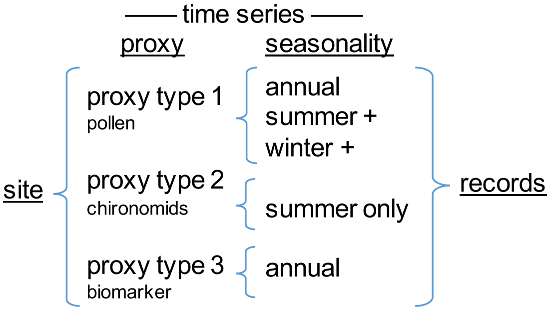

Fig. 1 Nomenclature used in this data descriptor. This example illustrates one study *site* where time series are available for three *proxy types*, each of which is used to infer temperatures for different *seasonality*. This example shows 1 *site* where three *proxy time series* represent five *seasonality time series*, which we collectively and generally call, *records*.

Comparison among seasonal and annual records. Composites were calculated to evaluate the overall differences among records that represent annual, summer and winter temperatures (Fig. 5). For these composites, all of the proxy types were averaged within a grid cell and then across each latitudinal band for annual, summer, and winter time series. Nearly all composites show the general pattern of warming during the early Holocene then are either constant or cooling thereafter.

Comparison between marine and terrestrial records. Composites were calculated to evaluate the overall differences between marine and terrestrial sites (Fig. 6). For these composites, all of the proxy types were averaged within a grid for each of the two settings. The overall tendencies of the aggregated proxy time series from marine and terrestrial archives are similar. Nearly all composites warm during the early Holocene, then are either flat or cool thereafter, with a suggestion that tropical land records cooled more than for tropical sea surfaces.

Comparison between high- versus low-resolution records. Composites were calculated to evaluate the extent to which high- and low-resolution proxy records differ across all sites (Fig. 7). For this comparison, high-resolution records were chosen to have a median resolution finer than 100 years (Fig. 3b). The composites show that the millennial-scale trends of the high- and low-resolution records generally track each other. The high-resolution composites also exhibit greater variability than the low-resolution composites, as expected because the high-resolution composites are based on fewer records and because those records capture higher-frequency variability.

Global mean surface temperature, annual versus winter or summer. A simple global composite of proxy records was calculated as the mean of six, 30° latitude averages, each weighted by the proportion of Earth's area represented by that band (0.067, 0.183, and 0.25 for the high, middle, and low latitude bands, respectively) (Fig. 8). For this composite, records calibrated to temperature (°C) were averaged within grids, then across the latitude bands. To evaluate the effect of combining annual and seasonal records, two composites were calculated: one based on annual records only ($n = 612$) and one based on annual plus either summer or winter values for sites where annual values are not available ($n = 816$). The composites were registered to the temperature scale (left-side x-axis) by aligning the 500 to 1500 CE mean of the composite with the mean of the global temperature reconstruction from the same interval in the PAGES 2k Consortium[571] multi-method median reconstruction. The variance of the Holocene temperature composites (all based on records in units of °C) were not scaled.

Comparison between calibrated and uncalibrated records (Fig. 8). A composite of all relative proxy data ($n = 43$; those not calibrated to temperature in °C and not included in the other global composites in Fig. 8) was calculated for comparison with the calibrated proxy records. The composite of uncalibrated records comprises records from around the globe, but about half (53%) are based on water isotopes in polar ice or speleothems. While the general pattern of the composite based on uncalibrated proxies is similar to that of the calibrated proxies, minor differences are expected, especially considering the limited number of sites and polar bias of the uncalibrated records.

Zonal representativeness. We evaluated the extent to which the spatial network of proxy temperature sites accurately represents the latitudinal surface temperature distribution of the planet (Figs. 9 and 10). Gridded instrumental-based temperatures from two temperature reanalysis data products (ERA20C[572] and HadCRUT[573,574]) were used to evaluate how well the proxy locations represent the mean temperature over each of the 30° latitudinal bands. Instrumental temperature data were binned to decadal resolution to better represent the long time-scales typically integrated by the proxy records. Grid cells corresponding to the locations of proxy records were then averaged and compared with the mean of the entire latitudinal band in which they are located. Temperatures at the proxy site locations are strongly correlated with the latitudinal average.

In addition to the instrumental data, we explored the representativeness of the proxy network using climate models (Fig. 10). Mid-Holocene (6 ka) and preindustrial (0 ka) simulations were analyzed in 12 general circulation models (GCMs) from the Paleoclimate Modelling Intercomparison Project Phase III (PMIP3; experimental

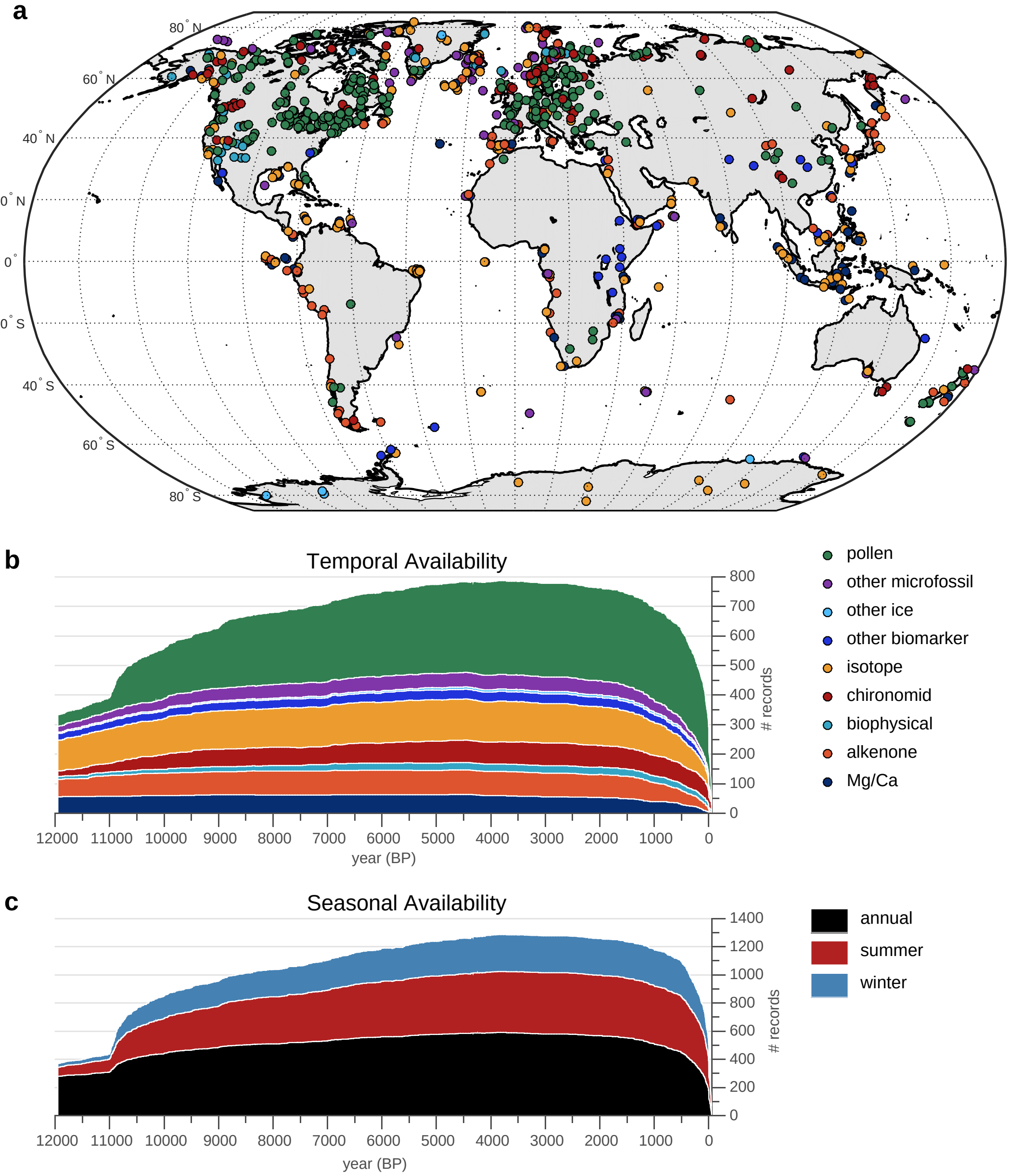

Fig. 2 Spatiotemporal data availability of records in the Temperature 12k database (v. 1.0). (**a**) Geographical distribution of sites (n = 679) by proxy type, coded by color. (**b**) Temporal availability by proxy type, coded by colors as shown in (**a**). Proxy time series (Fig. 1) are represented by only one seasonal (or annual) record for each site, but all proxy types are counted (i.e., some sites include more than one proxy type for the same season; n = 816). Specific proxy types (Suppl. Table 1, 'proxy') are either grouped or treated separately ('*Proxy General*') depending on the number of records available. For example, '*Proxy General*' = 'other microfossils' includes '*Proxy Type*' = dinocysts, foraminifera, diatoms and radiolaria, which together comprise a small number of records and were grouped and separated from the more numerous pollen and chironomid records. '*Proxy General*' = 'other biomarkers' includes TEX_{86}, GDGT, BNA15, LDI, but not alkenones, which are treated separately. '*Proxy General*' = 'other ice' includes boreholes, bubble frequency, gas diffusion, melt-layer frequency, etc., but not isotopes. Refer to Suppl. Table 1 for details. (**c**) Temporal availability of records by seasons (Suppl. Table 1, '*Season General*'). Both annual and seasonal records from the same site are included (n = 1319).

design described in ref. [575]) to assess how well the proxy network represents the temperature of the six latitudinal bands. Compared to the preindustrial period, mid-Holocene simulations are forced by altered astronomical parameters. Ice sheets had already melted to their preindustrial extents. The 12 GCMs are the same as those used in ref. [12] and were analysed using the same procedures. The change in mid-Holocene minus preindustrial temperatures was calculated for both the proxy locations and the latitudinal averages in models. The proxy locations generally explain 94% of the variance in the latitudinal averages of mid-Holocene minus pre-industrial changes across the multiple models. This number increases to 98% for global means computed from area-weighted means of the six latitude bands.

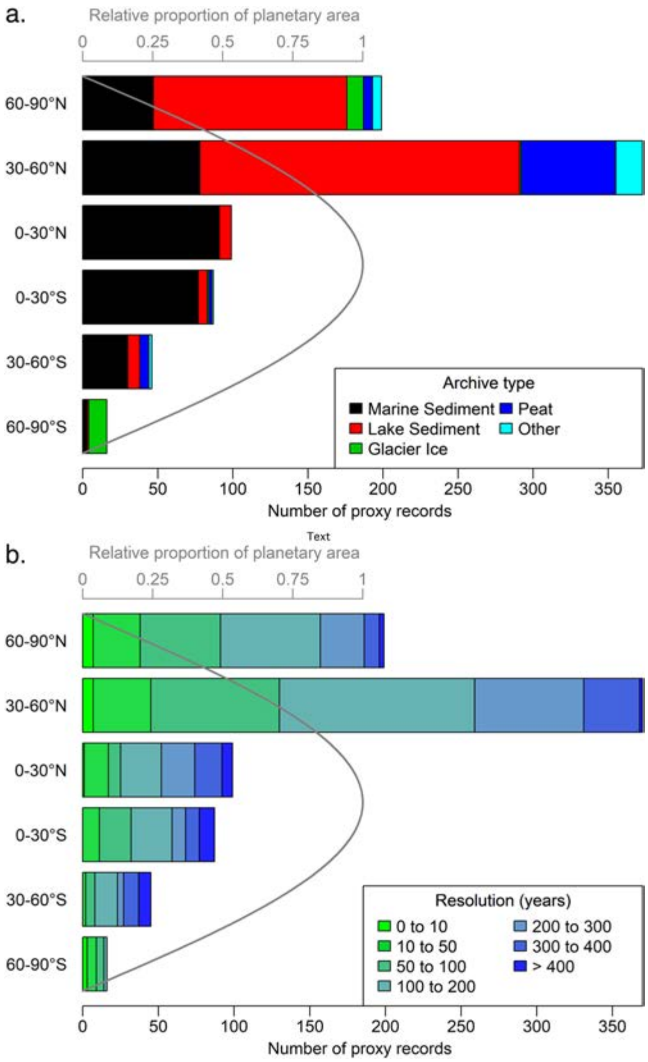

Fig. 3 Latitudinal distribution of records. Frequency of records partitioned in 30° latitude bands according to their (**a**) archive type (Suppl. Table 1, '*Archive Type*'), and (**b**) temporal resolution (Suppl. Table 1, '*Resolution*'). Only one seasonal (or annual) record is counted for each proxy type from a site. Resolution calculated as the median spacing between consecutive proxy samples of each time series, back to 12,000 years.

For these comparisons, the temperature of the grid cells nearest to each proxy-record site were averaged and compared with global and zonal mean temperatures within both the PMIP3 mid-Holocene simulations and the instrumental temperature data over the past 150–100 years. The comparisons show that, within the simulated and reanalysis datasets, the temperatures at the network sites correlate essentially one-to-one with the zonal and global temperatures. The comparison relies on model-smoothed and gridded data, however, and therefore assumes that each proxy location is indicative of the climate of the broader area. Although most proxy archives represent temperature over a relatively large area, we recognize that any proxy records that reflect variations over limited spatial scales (e.g., due to complex local topography) may degrade the network's representation of zonal and global temperature.

Usage Notes

Uncertainties. This database is presently the most comprehensive compilation of a globally distributed, multi-proxy, quality-controlled Holocene paleotemperature time series. It includes records from a variety of terrestrial and marine proxy types, each based on their own principles and procedures, and all of them associated with an extensive literature. Background information about the proxy types and their underlying assumptions is available in textbooks devoted to the topic (e.g., ref. [576]), and specific information about each proxy record is available through the original publications (Suppl. Table 2, refs. [26–566]). In addition to the variety of proxy types, there are a variety of approaches used to characterize uncertainties related to paleotemperature interpretations. There are no standard procedures for either calculating or reporting uncertainties, with some procedures taking analytical uncertainty into account and others focusing on the conversion of proxy measurements into absolute temperature. In most cases, the original studies describe the uncertainties associated with each proxy climate record.

Fig. 4 Major trends according to proxy type. Composites of normalized time series (standard deviation units; includes small portion of uncalibrated, relative proxy records) over the Holocene subdivided by major proxy types (Suppl. Table 1, '*Proxy Type*'). For sites with both annual and seasonal paleotemperature time series, only the annual time series was used ('*Season General*' = 'annual' OR 'summerOnly' OR 'winterOnly'). Shading indicates 95% bootstrap confidence intervals with 1000 replicates. Gray bars indicate the number of records per bin. Specific proxy types are combined or treated separately depending on the number of records available (Suppl. Table 1, '*Proxy General*' and '*Proxy Type*'; see Fig. 2 for explanation).

Among the important uncertainties to consider when using this database are those related to (1) calibration and proxy biases, (2) chronology, (3) spatiotemporal coverage. Depending on the scientific goal, uncertainties related to these and other factors can be substantial. On the other hand, some of these limitations are less important or not applicable, depending on the scientific question they are used to address. For example, calibration uncertainties are often large relative to the small amplitude of Holocene temperature changes, but these uncertainties become less important when investigating the relative magnitude of temperature changes rather than the absolute temperature.

Uncertainties related to calibration and proxy biases. Converting proxy data to paleotemperature estimates at a site-level relies on either (1) statistical procedures using observations of modern systems over the period of instrumentally based observations to infer the quantitative relation between the proxy value and temperature, or (2) transfer functions based on the correlation of biogeochemical properties or taxonomic assemblages over contemporary environmental gradients. These two approaches are referred to as "calibration in time" and "calibration in space," respectively. Uncertainties reported for paleotemperatures based on calibration-in-time procedures are typically relatively small because the method is tuned to reconstruct temperature variability at a single site, although this characterization does not represent all potential uncertainties[577]. In contrast, uncertainties typically reported for calibration-in-space procedures are usually characterized by larger but more globally applicable uncertainties, as the proxy-environment relation is examined over a wide environmental gradient. In this database, author-reported temperature uncertainties are included when they were readily available, which was infrequently and based on a variety of approaches. Some studies characterize uncertainties based on measurement errors, some report apparent calibration uncertainty estimates, while others report rigorously cross-validated uncertainty values.

The wide-ranging approaches that have been used to characterize uncertainties involved in converting proxy values to the paleotemperatures hamper a meaningful and systematic representation of errors. For this reason, some paleoclimate syntheses aimed at large-scale reconstructions apply a single uncertainty estimate to each

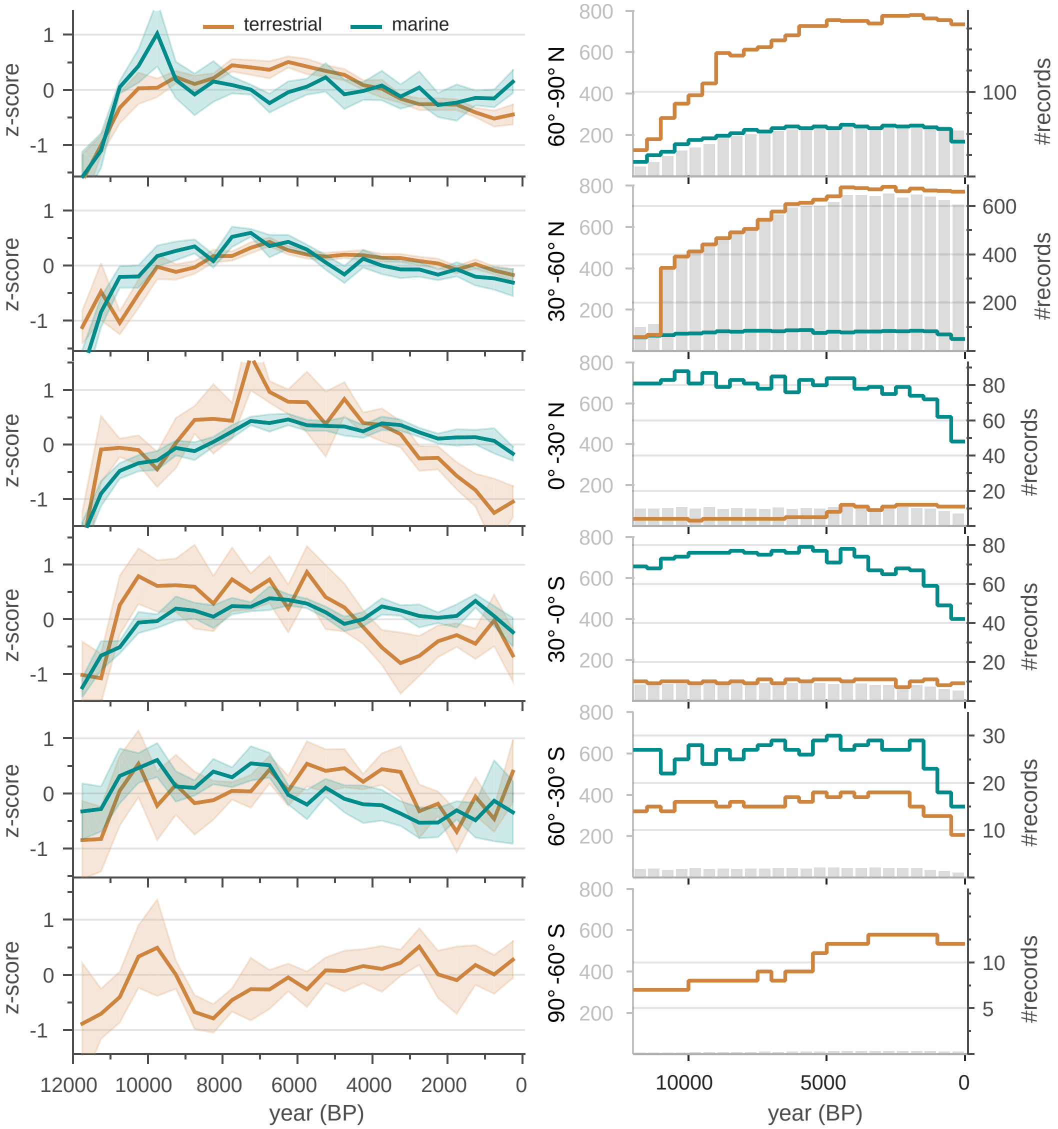

Fig. 5 Comparison among summer, winter and annual records. Composites of normalized time series (standard deviation units; includes small portion of uncalibrated, relative proxy records) over the Holocene subdivided by season, binned at 500 years, averaged on an equal-area grid and then averaged over 30° latitude bands. For sites with both annual and seasonal paleotemperature time series, only the annual time series was used (Suppl. Table 1, '*Season General*' = 'annual' OR 'summerOnly' OR 'winterOnly'). Shading indicates 95% bootstrap confidence intervals with 1000 replicates. The column on the right shows the temporal availability for individual time series comprising the composites for each latitude band. Included are the total number of records per bin (gray bars) plotted on the same y-axis scale (left side, gray) across all latitudes, as well as the number of records by category (colored lines) plotted on a variably zoomed y-axis scale (right side).

proxy type (e.g., ref. [5]). Others apply a single statistical method to calculate uncertainties for published proxy data, often employing expanded calibration datasets and new statistical methods (e.g., ref. [7]). In this database, most of the paleotemperature records based on pollen from North America and Europe are from the large-scale study of ref. [16] and most of the records based on marine sediments (other than those from microfossil assemblages) are calculated for this data product using the Bayesian procedures of refs. [20–23]. For other proxy types, paleotemperature values and their uncertainties are based on multiple generations of analytical and calibration methods.

Biases can arise when proxy types that are most sensitive to summer conditions, common for biological indicators, are scaled to represent mean annual temperatures. Unlike paleotemperatures inferred from microfossil assemblages, other proxy types behave more like temperature sensors in a strict sense, meaning that their temperature signal is biased towards the season when the sensor is most abundant. This recording bias is not always explicitly addressed in the original publications and in these cases we used our expert knowledge to assign the seasonality and water depth ('*Climate Variable Detail*'). However, our knowledge of the ecology of the proxy sensors is still limited and the assumption of a temporally constant recording bias may not always hold true.

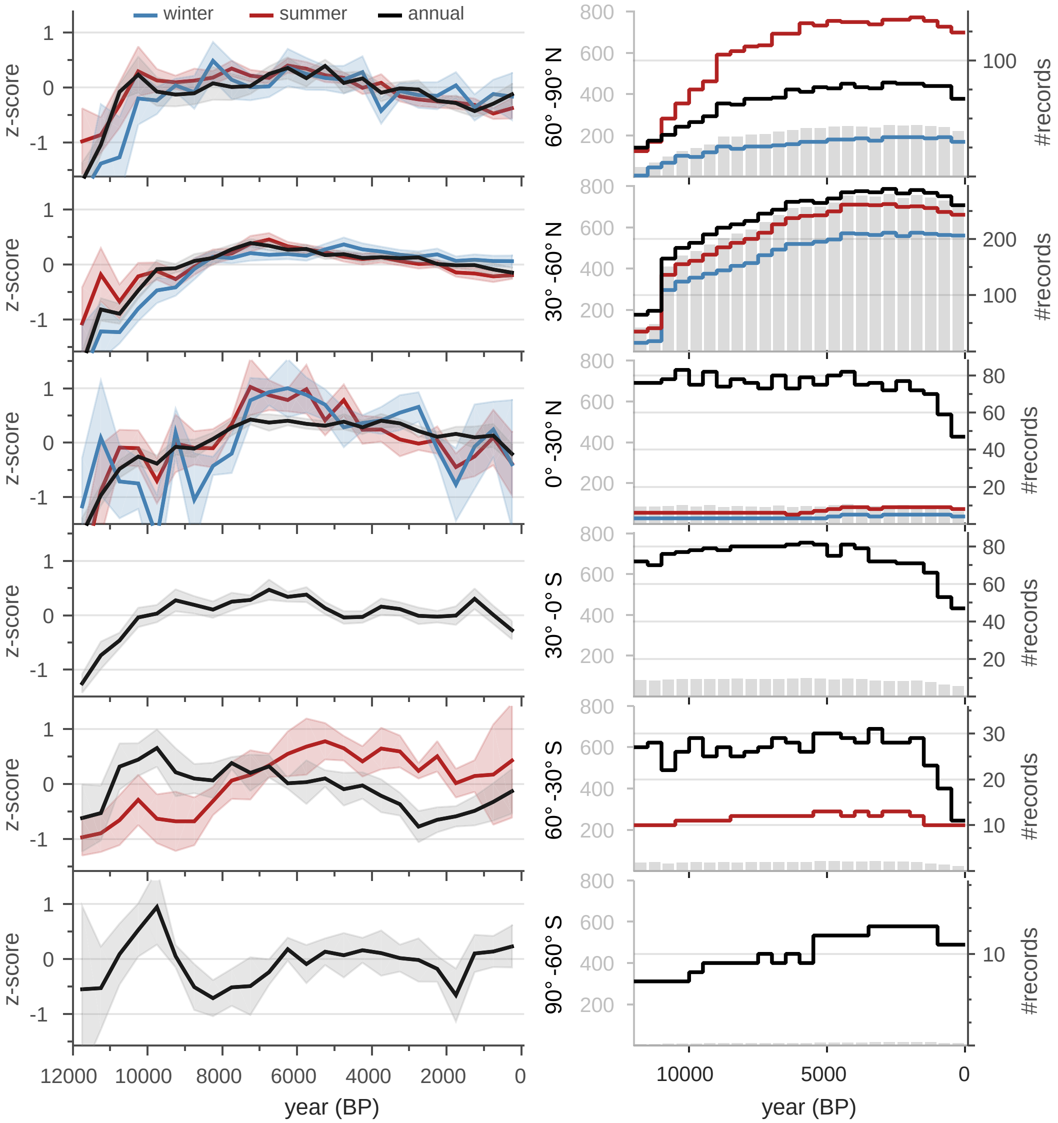

Fig. 6 Comparison between records from terrestrial versus marine sites. Composite time series subdivided terrestrial versus marine archives. Marine sites include some terrestrially based proxy types, such as pollen and some biomarkers; these are represented by '*Climate Variable Detail*' = 'air@surface' rather than 'sea@surface' (Suppl. Table 1). Symbols and procedures same as for Fig. 5.

Uncertainties related to chronology. The 3000-year-maximum spacing between age-control points was chosen as a relatively inclusive screening criterion. The accuracy of the time scales depends mainly on (1) how well the ages represent the true age of the proxy sensor itself, which can be an issue when, for example, a radiocarbon age on bulk sediment is used to represent the age of the proxy sensor (e.g., pollen), and (2) the uniformity of the accumulation rate of the archival medium (dominantly sediments in this database), which governs the accuracy of the interpolated ages of samples between control points[13]. Relative to these uncertainties, the analytical precision of the age determinations is typically minor. The inclusion of the primary chronology data and Bayesian-derived age ensembles for many time series in this database allows users to quantify and incorporate many, but not all, aspects of the age uncertainty into their own analyses.

Uncertainties related to spatiotemporal coverage. The suitability of the database to address different scientific questions depends on the particular spatial and temporal scale. Some regions are covered more densely than others, and the number of records available decreases as the demands for temporal resolution increase. At finer spatial and temporal scales, the number of records with sufficient resolution and geochronological control is limited and typically based on more recent studies. For example, only about 39% of the sites have records with resolution finer than 100 years.

Future directions.

The Temperature 12k database will form the foundation for new studies of Holocene global and regional surface temperature changes, and will help identify future research priorities. This machine-readable database includes multiple parameters for searching and filtering the proxy records, depending

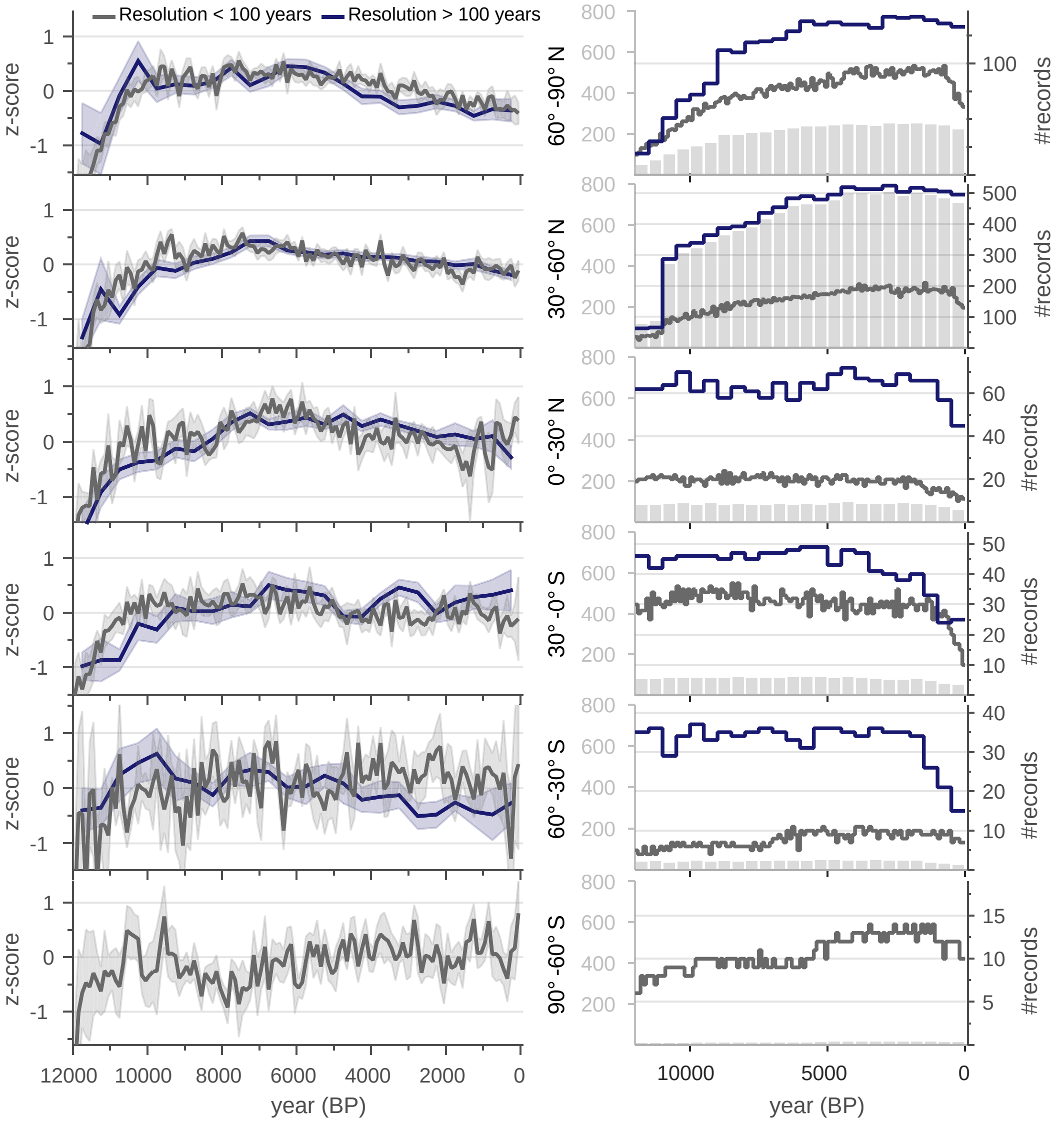

Fig. 7 Comparison between low- and high-resolution records. Composite time series (standard deviation units; left side y-axis) for high-resolution versus low-resolution records binned at 100 and 500 year intervals, respectively. Cut-off between high and low resolution was set as 100 years (median difference between consecutive observations). Symbols and procedures as in Fig. 5.

on the scientific objective. The database can be partitioned to study and compare different proxy types, seasons, and many other attributes. Previous proxy time series from a region can be used to compare with the results of future studies that report Holocene temperatures. Because the Temperature 12k database is relatively comprehensive, it can facilitate broader comparisons, both locally and regionally, than are commonly included in site-level studies. This avoids over-reliance on select records while providing an objective means to recognize aberrant or misinterpreted records through systematic comparison against the full body of other available records.

In addition to the time-series data in this database, an extensive literature describes complementary evidence for Holocene temperature fluctuations based on a variety of data types, including event-based observations (e.g., dated moraines). In the future, such information can be compared with the time-series data in this database to provide a more robust interpretation of past climate changes (e.g., across Alaska[578], Arctic Canada and Greenland[579], and North Atlantic and Fennoscandia[580]).

The simplistic time-series composites included in this data descriptor provide insights into the large-scale patterns of the proxy data, and provide a basis for comparison among different subsets of the diverse database. The area-weighted composite (Fig. 8) also serves as an initial approximation of the global mean surface temperature over the Holocene. This provides a foundation for a more in-depth analysis of temperature history based on this database, including a comparison of statistical methods for reconstructing global mean surface temperature.

The Temperature 12k database represents a concerted effort to generate a comprehensive product, but it is an ongoing effort, with new records continuing to be published. The database includes a large proportion of available published records that meet the selection criteria and that were recovered by the authors of this data product.

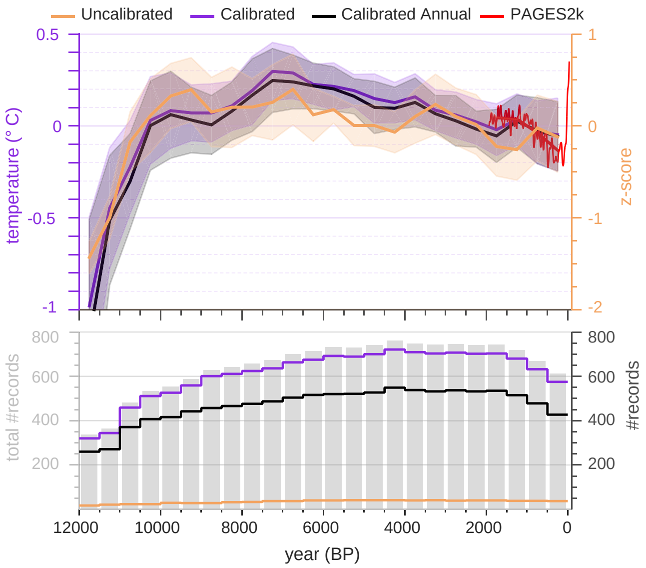

Fig. 8 Comparison between calibrated versus uncalibrated records. Composite time series subdivided by records that are either calibrated to temperature (Suppl. Table 1, '*Units*' = 'degC') or uncalibrated (n = 43; standard deviation units). Two calibrated composites are shown: black = annual records only (n = 612); purple = annual plus either summer or winter records for sites where annual records are not available (n = 816). The calibrated composites were placed on a temperature scale (left x-axis) by aligning the mean of each composite with the mean of the global temperature reconstruction from the PAGES 2k Consortium[571], both over the period 500 and 1500 CE. Red = median of the PAGES 2k multi-method ensemble global mean surface temperature reconstruction binned at 500 years (bold red line) and with 30-year smoothing of annually resolved data (fine red line; data from www.ncdc.noaa.gov/paleo/study/21171). No instrumental data are shown. Symbols and procedures same as for Fig. 5.

Some published records that meet the criteria might have been inadvertently overlooked. Readers who know of missing datasets, especially those from data-poor regions, or who find errors in this version are asked to contact one of the primary authors so that future versions of the database will be more complete and accurate. Rather than issuing errata to this publication, errors and additions will be included in subsequent versions of the database and updated through the online data repository (see below).

Ancillary data. *Additional records within the temperature 12k datasets.* In addition to the paleotemperature records used in the Temperature 12k compilation, the LiPD files also contain ancillary data from the same sites. For some sites, these include the native observations used to derive the paleotemperature values (e.g., Mg/Ca of foraminifera), or other data that are not directly related to climate but represent environmental changes at a study site that might be useful in interpreting the climatic significance of the record (e.g., sedimentary magnetic susceptibility). Some LiPD datasets include proxy data that are sensitive to climate variables other than temperature. These ancillary data are made available along with this data product, but have not been reviewed for accuracy, and some, including most of the pollen-based precipitation records, have not been vetted by peer review. Within the LiPD files, the records that comprise v.1.0.0 of the Temperature 12k database and were used to generate the figures in this data descriptor are discoverable in ref. [7] by filtering the metadata field, '*In Compilation*' = 'Temp12k'.

Temperature-sensitive records in addition to temperature 12k datasets. This database is a quality-controlled and curated subset of data culled from a larger collection of proxy climate data (LiPDverse.org), all structured in LiPD. Many temperature-sensitive proxy records that were gathered as part of this project do not meet the selection criteria for length, resolution, or age control as prescribed for this database. These data are likely useful for addressing scientific objectives that are outside the scope of this compilation. Many of the records have been reviewed by the authors of this data product, as indicated by their initials in '*QC Certification*,' but most have not been reviewed for accuracy. Nonetheless, these data, including about 1110 records from over 560 sites are made available as part of the overall database of temperature-sensitive records. Within the LiPD files (ref. [7], www.ncdc.noaa.gov/paleo/study/27330) these are discoverable by filtering the metadata field, '*In Compilation*' = 'Tverse'.

Ensemble paleotemperature time series and age models. To characterize and quantify uncertainties associated with both chronologies and paleotemperatures, recent paleoclimate studies have featured ensembles of age models and proxy time series, most commonly derived from the posterior distribution of Bayesian statistical methods. Many of the datasets in the Temperature 12k database include age or temperature ensembles, or both. These are included in the expanded version of the Temperature 12k database (Table 2). Most of the age ensembles are associated with previous studies that recalculated age models based on the 'Bacon' modeling routine[18]. These are available for many of the time series, including the marine records from the DADT project (records with

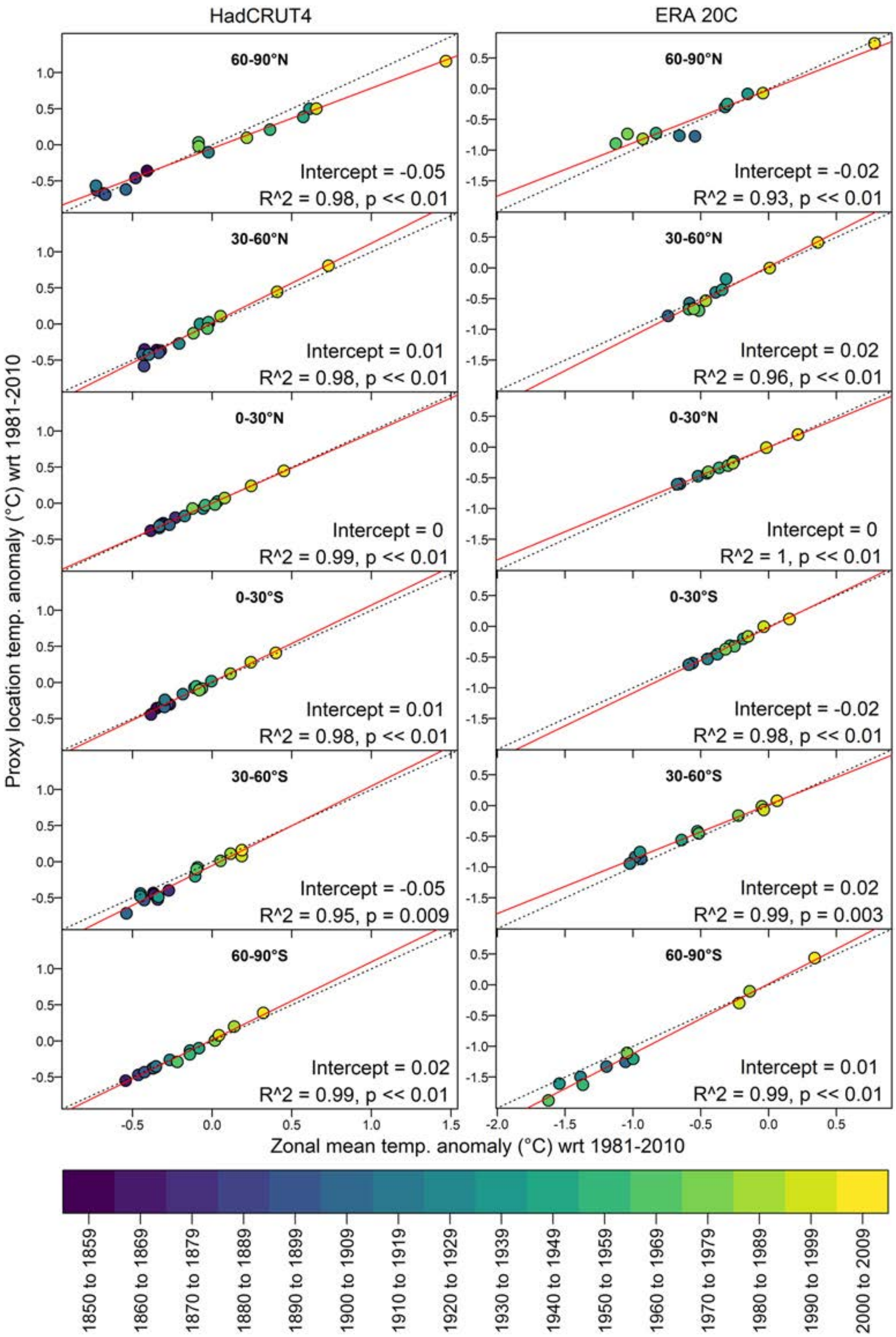

Fig. 9 Zonal representativeness of the proxy network based on instrumental temperature. Scatterplots showing the relation between decadal mean temperature at the proxy locations versus the average of the entire 30° latitudinal zone using gridded instrumental-based temperature reanalysis products: (**a**) HadCRUT4 dataset[573,574], (www.metoffice.gov.uk/hadobs/hadcrut4) and (**b**) ERA20C dataset[572] (www.ecmwf.int/en/forecasts/datasets/reanalysis-datasets/era-20c). In the instrumental dataset, the mean temperature at the proxy locations explain between 93% and 100% of the temperature variance in the latitudinal bands. The spread in data represents the overall temperature trend over the 20th century.

'*Calibration Method*' = 'Bay...'), and McKay *et al*.'s Arctic Neoglacial study[25]. In addition, we generated ensembles of paleotemperature time series for the marine records that were recalibrated for this database. These too are available in the LiPD files in the expanded version of the Temperature 12k database.

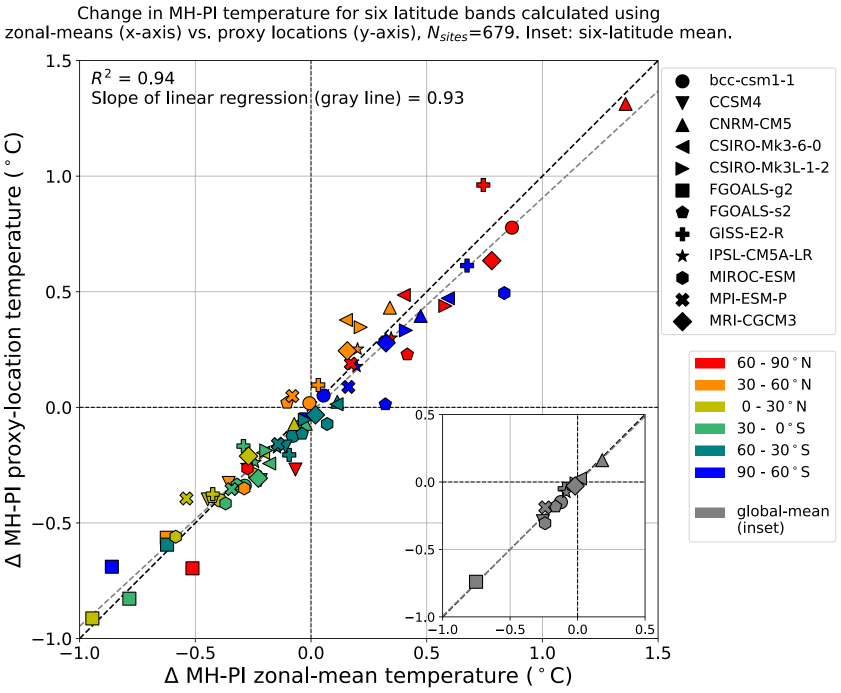

Fig. 10 Zonal representativeness of the proxy network based on modelled temperature. Mid-Holocene minus preindustrial (MH − PI) annual temperature averaged for the proxy locations (y-axis) versus the annual temperature averaged over an entire 30°-wide latitudinal band (x-axis) from 12 PMIP3 climate models (symbols), shown for six latitudinal bands (colors). The proxy network sampled in the models captures the same mid-Holocene annual temperature anomalies as represented by the latitudinal averages. Global-mean values, calculated as the area-weighted mean of the six latitude bands, are shown in the inset. Linear regression of the global-mean values has an R^2 of 0.98 and a slope of 0.99. PMIP3 model output is available at esgf-node.llnl.gov/projects/esgf-llnl.

File name	Contents
LoadData.md	Instructions for loading database (markdown-style text)
Temp12k_directory_LiPD_files	All LiPD files (not zipped)
Temp12k_directory_NOAA_files	All datasets that were deposited at WDS-NOAA Paleoclimatology for the first time as part of this compilation, NOAA template format
Temp12k_v1_0_0_LiPD.zip	Database in LiPD format
Temp12k_v1_0_0.mat	MATLAB-readable database
Temp12k_v1_0_0-ts.pkl	Python-readable database
Temp12k_v1_0_0.Rdata	R-readable database
Temp12k_with_ensembles_v1_0_0_LiPD.zip	Database in LiPD format including available age-model and marine-proxy ensembles
Temp12k_v1_essential_metadata.xlsx	Metadata for Temp12k v.1.0.0 (same as Suppl. Table 1)
Temp12k_v1_record_list.xlsx	Temperature 12k records listed alphabetically
Temp12k_Composite_timeseries.zip	Composite time series shown in Figs. 5–8

Table 2. Contents of files available on the landing page* for Temperature 12k database. *www.ncdc.noaa.gov/paleo/study/27330, DOI: 10.25921/4RY2-G808.

Database format and operability. The site-level proxy and geochronology data and metadata are formatted in the Linked Paleo Data (LiPD) structure. The LiPD framework comprises JSON formatted, standardized files that are machine-readable in multiple programming languages for querying and data extraction[10]. The hierarchical structure allows explicit descriptions at any level and aspect of the database, thereby providing a flexible structure that can accommodate a variety of data and metadata types. The LiPD files comprising this database have also been excerpted and translated into the format of the World Data Service (WDS) NOAA Paleoclimatology where they are archived for long-term reuse.

WDS-NOAA-Paleoclimatology Landing page contents. The entire database is available in LiPD format through WDS-NOAA Paleoclimatology (www.ncdc.noaa.gov/paleo/study/27330; https://doi.org/10.25921/4RY2-G808), with serializations for MATLAB, Python, and R. Any updates to the database will be posted at WDS-NOAA Paleoclimatology. The landing page links to digital versions of the Temperature 12k database, including the metadata in Suppl. Table 1, as well as to the composite time series generated by this study (Table 2).

Versioning scheme. The database versioning scheme for this data product follows the one proposed by McKay and Emile-Geay[10] and used for the PAGES 2k Consortium[9] temperature database. The version number is in the form C1.C2.C3, where C1 is an integer associated with a publication, C2 is a counter updated every time a record is added or removed, and C3 is a counter updated every time a modification is made to the data or metadata in an individual record. The dataset published here is thus v1.0.0 of the Temperature 12k database. Future versions, along with a change log that specifies the modifications associated with each version, will be posted on the WDS-NOAA Paleoclimatology landing page. This versioning applies only to the temperature-sensitive records marked as '*In Compilation*' = Temp12k; changes to ancillary time series are not tracked.

Code availability

Code for working with the LiPD data files, including basic functionality in three programming languages, is available on GitHub (https://github.com/nickmckay/LiPD-utilities). MATLAB code used to map site locations (Fig. 2) and to compute composites (Figs. 4–8) is available at https://github.com/nickmckay/Temperature12k under an MIT license[581].

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Author contributions

The author list is organized in three tiers. The first tier (D.S.K., N.P.M., C.R., M.P.E., B.A.S.D., O.H.E., S.L.J., J.E.T. and C.D.) includes those who did most of the work to produce the database and prepare it for publication, including: coordinating the project, writing the data descriptor, analyzing the data, and creating the figures and tables. The second tier of authors (listed alphabetically: Y.L.A., T.B., O.C., B.M.C., A.E.D., A.d.V., S.E.N., L.J., J.P.M., P.M.S., C.M., A.J.O., K.R., K.M.S., P.S.S., E.K.T., M.S.T., M.T.O. and R.S.V.) includes those who contributed substantially to this data product by assembling, formatting, checking, and providing metadata for a large number of proxy records. The third tier of authors (listed alphabetically: A.A.A., S.B., B.K.B., M.B.R., S.J.B., M.C., M.C.H., L.C.C., J.E.G., J.M.F., A.F.E., W.F., M.C.F., L.C.F., M.L.F., K.G.A., M.G., S.H.A., M.L.H., N.H.O., B.P.I., E.A.I., S.J., D.K.H., K.K.O., P.G.L., I.L.T., L.J.Y., A.F.L., T.P.L., A.W.M., E.K.M., S.B.M., B.G.M., J.I.M., V.M., L.B.N., E.Y.N., P.P.A., E.J.P., M.C.P., R.P.I., M.P.L., D.F.P., A.P.P., A.R.E., S.A.R., S.J.R., N.R.O., S.S., A.E.S., H.S.E., S.S.H., J.M.S.J., B.S., L.S.S., P.T.A., K.J.T., V.V.B., G.V.E., E.R.W., I.R.W., J.M.W., E.Z.H. and S.Z.) includes the many individuals who contributed their data and verified that the records meet the criteria as described in the data descriptor and in some cases, provided comments to facilitate the informed reuse of individual records, or helped improve the contents of this data descriptor. The data certifier for each record is identified by their initials in '*QC Certification*' (Suppl. Table 1). All authors reviewed the manuscript and take responsibility for its content.

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The authors declare no competing interests.

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Epigean gammarids survived millions of years of severe climatic fluctuations in high latitude refugia throughout the Western Carpathians

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ABSTRACT

Isolated glacial refugia have been documented in Central Europe for a number of taxa, but conclusive evidence for epigean aquatic species has remained elusive. Using molecular data (mitochondrial and nuclear markers), we compared the spatial patterns of lineage diversity of the widely distributed *Gammarus fossarum* species complex between two adjacent biogeographically and geomorphologically distinct Central European regions: the Bohemian Massif and the Western Carpathians. We investigated if the observed patterns of spatial diversity are more likely to stem from historical or present-day factors. Phylogenetic and phylogeographic analyses revealed eight phylogenetically diverse lineages: two exhibiting local signatures of recent demographic expansion inhabit both regions, while the other six display a relict distributional pattern and are found only in the Western Carpathians. Molecular dating indicates that these lineages are old and probably diverged throughout the Miocene (7–18 Ma). Furthermore, their distribution does not seem to be constrained by the present boundaries of river catchments or topography. The contrasting spatial patterns of diversity observed between the two regions thus more likely result from historical rather than contemporaneous or recent factors. Our results indicate that despite the high latitude and proximity to the Pleistocene ice sheets, the Western Carpathians functioned as long-term glacial refugia for permanent freshwater fauna, allowing the uninterrupted survival of ancient lineages through millions of years of drastic climatic fluctuations.

1. Introduction

The Pleistocene climatic oscillations have left a strong latitudinal signature on the contemporaneous patterns of European biodiversity. Regions at lower latitudes generally served as refugia and consequently harbour a high diversity that gradually decreases towards the formerly glaciated regions at higher latitudes (Hewitt, 2000). However, recent research has revealed that various groups (especially cold adapted species) have also persisted in or close to previously glaciated areas, in the so-called cryptic or northern refugia (Provan and Bennett, 2008; Tzedakis et al., 2013).

In Central Europe, the presence of cryptic glacial refugia has been suggested in the Western Carpathians and possibly the Bohemian Massif (roughly corresponding to the territories of the present Slovakia and Czechia, respectively; hereafter the BMWC region) among others based on terrestrial mollusc shell fossils (Juřičková et al., 2014a), plant macrofossils (Willis and van Andel, 2004) and pollen deposits (Mitka et al., 2014). These studies indicated that patches of isolated broadleaf forests survived in that region during the last glacial maximum and could have served as refugia for other species as well. Consistent with these results, Western Carpathian glacial refugia have been inferred from molecular data in mammals (Wójcik et al., 2010), species distribution models in trees (Svenning et al., 2008) or a combination of both in terrestrial insects (Homburg et al., 2013; Drees et al., 2016). However, evidence for refugia in aquatic taxa from the BMWC region is inconclusive, although several studies have documented the presence of divergent lineages in the Western Carpathians (Kotlík and Berrebi, 2002; Fijarczyk et al., 2011; Fehér et al., 2013; Zieliński et al., 2013; Theissinger et al., 2013; Copilaş-Ciocianu and Petrusek, 2017). This lack of direct evidence contrasts with the southern half of the Carpathians, which functioned as one of the most important extra-Mediterranean refugia for a wide range of aquatic and terrestrial species, and is considered a major European biodiversity hotspot (Kotlík et al., 2006; Schmitt, 2007; Bálint et al., 2011; Copilaş-Ciocianu and Petrusek, 2015, 2017; Dénes et al., 2015; Wielstra et al., 2015; Hurdu et al., 2016; Mráz and Ronikier, 2016).

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Freshwater amphipods of the genus *Gammarus* Fabricius, 1775 exhibit geographically structured genetic variation with lineages that date as far back as the Miocene, and are geographically distributed in a mosaic-like pattern throughout the Holarctic (Hou et al., 2011; Copilaş-Ciocianu et al., 2014; Copilaş-Ciocianu and Petrusek, 2015, 2017; Katouzian et al., 2016; Mamos et al., 2016). *Gammarus fossarum* Koch, 1836 is one of the most common and diverse gammarid species complexes in Europe (Weiss et al., 2014). Its highest diversity is located in the southwestern Carpathians and the Balkan Peninsula, and consists of tens of microendemic lineages belonging to two major clades (Copilaş-Ciocianu and Petrusek, 2015). In contrast, only a few lineages belonging to another major clade have been detected so far in Central and Western Europe, and these are significantly more widely distributed (Westram et al., 2011; Copilaş-Ciocianu and Petrusek, 2015). Although *G. fossarum* is common in the BMWC region, its diversity there has remained unexplored. Individuals from only two geographically distant localities in the northeastern part of this species complex's range have been analysed genetically: *G. fossarum* sensu stricto (the lineage present at the type locality of the species) was documented from one site in the southern part of the Bohemian Massif (Hou et al., 2011), and a highly divergent lineage was found in the Polish lowlands close to the Western Carpathians (Hou et al., 2007).

The borderline between the Bohemian Massif and the Western Carpathians is an important boundary for the biogeography of freshwater organisms (Illies, 1978) with major shifts occurring in the species composition of permanent aquatic groups such as leeches (Schenková et al., 2009), oligochaetes (Schenková et al., 2010), and gastropods (Horsák and Cernohorsky, 2008; Horsák et al., 2016), as well as insects with aquatic larval stages such as dragonflies (Waldhauser, 2016a,b). Substantial differences in faunal composition are also apparent in terrestrial invertebrates (e.g., Schmitt and Rönn, 2011; Juřičková et al., 2014b). Furthermore, the border between the two regions is considered the most important Central European biogeographical boundary for terrestrial plants (Meusel et al., 1965).

The Bohemian Massif and the Western Carpathians have distinct geomorphological features and geological histories, the former being an old mountain system formed during the late Palaeozoic and is characterized by a moderate relief, while the latter is relatively young, having formed during the Palaeogene, and exhibits a more rugged terrain (Földvary, 1988; Chlupáč et al., 2011; Kováč et al., 2016). Furthermore, the intersection of the boundaries of three major European sea drainage basins (Baltic Sea, Black Sea and North Sea) in the BMWC region may have affected phylogenetic patterns within our focal amphipod group. Collectively, these contrasting characteristics between the two regions might be reflected in the composition of their biotas.

The BMWC region is situated between two regions dominated by two major *G. fossarum* clades with contrasting diversity patterns: a southwestern Carpathian clade to the southeast, comprised of numerous narrowly endemic lineages, and a central-western European clade to the west, with several lineages of considerably broader ranges (Copilaş-Ciocianu and Petrusek, 2015). We presumed that both clades overlap somewhere in the area, which might thus harbour a phylogenetically diverse assemblage of lineages. Along a west-east transect spanning the BMWC boundary, we tested whether the spatial patterns of lineage diversity within the *G. fossarum* complex result from recent or contemporaneous processes (i.e. postglacial colonization and/or specific environmental requirements of the different lineages) or whether ancient historical factors (local survival within glacial refugia) contributed substantially to the present-day situation. In the former case, we would expect a pattern of generally low genetic differentiation within and among lineages, the distribution of which reflects the present-day catchment boundaries or environmental characteristics. In the latter case, we would expect a relict distributional pattern, i.e. deep genetic divergence among endemic lineages that do not differ substantially in their environmental preferences, and whose distribution does not follow the current drainage divides. Along with analysing in detail the lineage composition in the focal BMWC region, we processed additional samples of *G. fossarum* also from other parts of its range (in particular southeast Europe), to at least partially fill gaps in its phylogeography on a large scale.

2. Material and methods

2.1. Sampling

Sampling was focused on a west-east transect that thoroughly covered the territories of Czechia and Slovakia (171 localities). We obtained samples from small streams to medium-sized rivers which have similar ecological conditions throughout the study region. Additional samples complementing the existing datasets were analysed from France, Poland, Hungary, Bosnia and Herzegovina, Serbia and Bulgaria (15 localities) (Fig. 1, Table S1). Specimens were collected by kick sampling between 2010 and 2016 and stored in 96% ethanol. A total of 192 individuals were molecularly analysed, in most cases one per locality. All sequences were submitted to GenBank (accession numbers: KY618212-KY618541; see Table S1).

2.2. Molecular protocols, sequence alignments and data set assembly

We assigned individuals to mitochondrial lineages using the DNA barcoding approach (Hebert et al., 2003). For this purpose, we sequenced two mitochondrial genes, the cytochrome *c* oxidase subunit I (COI) from 145 individuals and the large ribosomal subunit (16S) from 81 individuals for which the COI amplification was unsuccessful (Table S1). The two mitochondrial genes could be used interchangeably for identifying mitochondrial clades because both were sequenced from several individuals per clade and consistently indicated the same pattern. Additionally, we sequenced three nuclear markers for several individuals belonging to each of the distinct mitochondrial lineages. These genes were the large (28S) and small (18S) ribosomal subunits and the elongation factor 1 alpha (EF1α). DNA was extracted using Genomic DNA Kit for tissue (Geneaid Biotech, Taipei) and PCR protocols followed Copilaş-Ciocianu and Petrusek (2015). The primers of Folmer et al. (1994) and Costa et al. (2009) were used to amplify a fragment of at least 600 bp of the COI gene, and for 16S we used the primers 16STf (Macdonald et al., 2005) and 16Sbr (Palumbi et al., 1991) to amplify a 323 bp fragment. For the 28S, 18S and EF1α nuclear markers, we used the primers of Hou et al. (2007, 2011) to amplify ca. 1265, 1837 and 549 bp, respectively.

MUSCLE (Edgar, 2004) implemented in MEGA 6 (Tamura et al., 2013) was used to align the protein-coding genes (COI and EF1α) which were subsequently translated into amino acids in order to examine the presence of stop codons that would indicate potential pseudogenes. Polymorphic sites in the EF1α chromatograms were coded according to the IUPAC ambiguity codes. Ribosomal genes (16S, 18S and 28S) were aligned using MAFFT 7 (Katoh and Standley, 2013) with the Q-INS-i option (Katoh and Toh, 2008).

Data on the *G. fossarum* complex from previous molecular studies (Hou et al., 2007, 2011; Lagrue et al., 2014; Weiss et al., 2014; Wysocka et al., 2014; Copilaş-Ciocianu and Petrusek, 2015; Table S1) were included with the aim of investigating the relationships of the focal lineages in a broader phylogenetic context. Based on the large scale phylogenies of Hou et al. (2011, 2014b), progres-

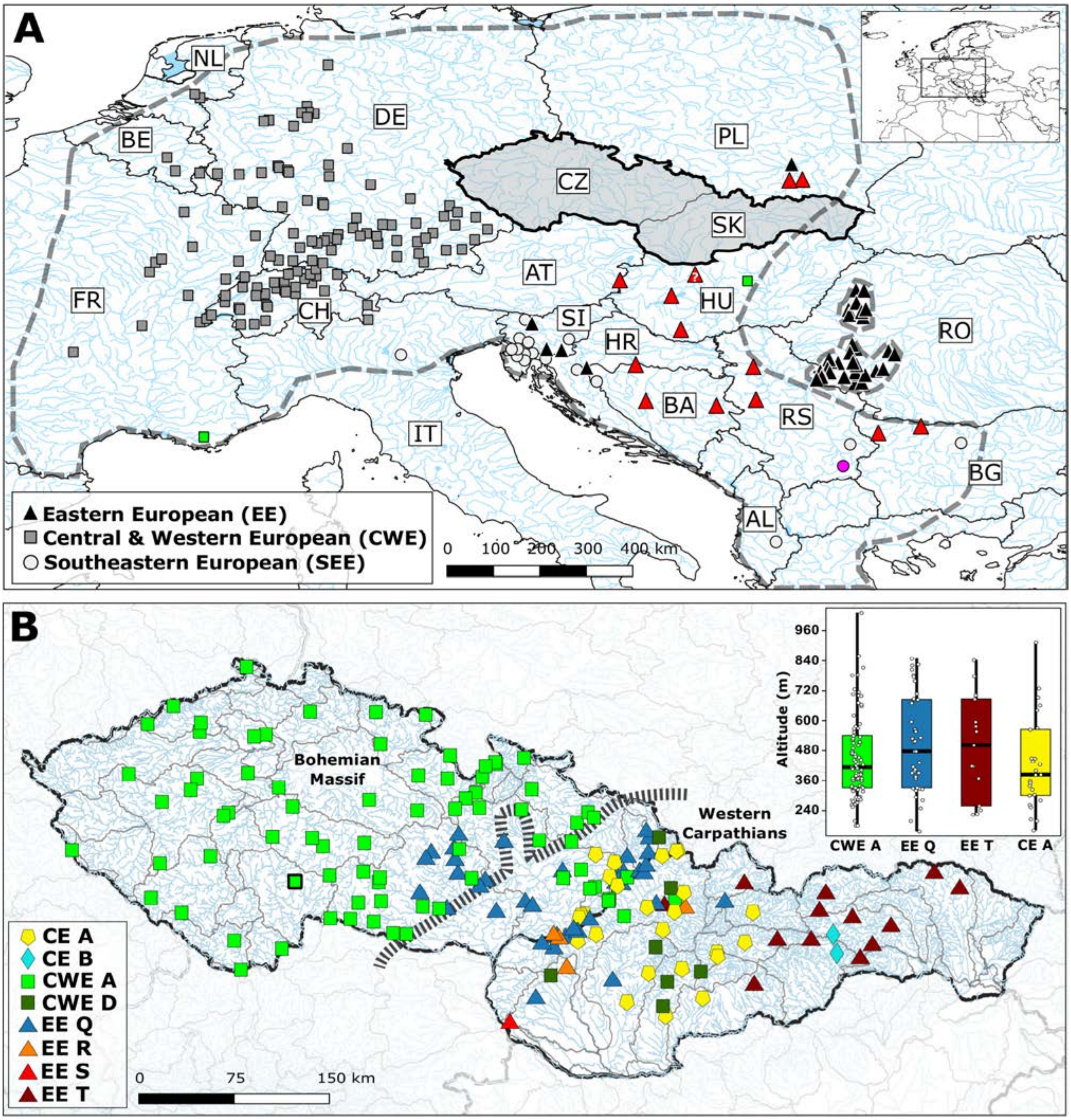

Fig. 1. Spatial patterns in the distribution of mitochondrial lineages of the *Gammarus fossarum* species complex. The nomenclature of the clades follows Fig. 3. (A) Distribution patterns of the three major clades across Europe: Central and Western European clade (CWE), squares; Eastern European clade (EE), triangles; Southeastern European clade (SEE), circles. Coloured symbols indicate new data from the present study, and symbols in grey scale indicate literature data. The known distribution area of the *G. fossarum* species complex is indicated by a dashed line. Countries are indicated by their corresponding two-letter ISO codes: AL, Albania; AT, Austria; BA, Bosnia and Herzegovina; BE, Belgium; BG, Bulgaria; CH, Switzerland; CZ, Czechia; DE, Germany; FR, France; HR, Croatia; HU, Hungary; IT, Italy; NL, Netherlands; PL, Poland; RO, Romania; RS, Serbia; SI, Slovenia; SK, Slovakia. (B) Distributional patterns of mitochondrial lineages in the focal region (Bohemian Massif and Western Carpathians). The colour and shape of the symbols correspond with those in Figs. 2 and 3. A single square with thick outline indicates a record from literature; remaining symbols are results of the present study. The thick grey interrupted line represents a simplified boundary between both geomorphological regions. Thin grey lines delimit the second-order river catchments, and black lines are country borders. Box-plots in the inset depict the altitudinal distribution of the four most widespread lineages; all pair-wise comparisons were non-significant ($p > 0.5$).

sively more distant outgroups were included. Among these there were also the lineages that were used to calibrate the molecular clock: endemic Acanthogammaridae from Lake Baikal (Macdonald et al., 2005), endemic *Gammarus* from Lake Ohrid (Wysocka et al., 2014), and the genera *Rhipidogammarus* and *Sarothrogammarus* (Hou et al., 2011) (see Table S1).

2.3. Lineage diversity analyses

For detecting the number of lineages in the study region, we used a species discovery approach which identifies putative groups without prior information as recommended by Carstens et al. (2013). Therefore, we applied phylogenetic and distance-based methods to each mitochondrial marker in order to identify putative lineages (following Copilaş-Ciocianu and Petrusek, 2015). The datasets of both markers consisted of sequences generated during this study as well as previously published data. The Poisson tree process model, implemented on the web server bPTP (http://species.h-its.org/ptp) (Zhang et al., 2013), was used for the phylogenetic approach to species discovery. Because this method requires a phylogenetic tree as input, we inferred maximum-likelihood (ML) phylogenies for COI and 16S using GARLI 2 (Zwickl, 2006) with five independent search replicates. We used GARLI because it can implement a wide variety of models and consequently, branch lengths (which are a critical parameter for this analysis) might be more accurately estimated in comparison with other programs (Stamatakis, 2006; Zwickl, 2006). Evolutionary models for each gene and codon partition were selected using PartitionFinder 1.1.1 under the corrected Akaike information criterion (AICc) (Lanfear et al., 2012). The SYM + I + Γ, GTR + I + Γ and GTR + Γ models were used for the 1st, 2nd and 3rd codon positions, respectively, in COI, and the GTR + I + Γ model was used for 16S (Table S2). The analysis was run for 300,000 MCMC generations with 20% burn-in. For the distance-based approach, the program Automatic Barcode Gap Discovery (ABGD) was used to identify groups based on the barcode gap (Puillandre et al., 2012). Relative gap width was set to 1, various settings for the maximum intraspecific distance (Pmax) were explored, and genetic distances were calculated using Kimura 2-parameter (K2P) correction (a distance measure frequently used for DNA barcoding approaches; Hebert et al., 2003).

2.4. Phylogenetic analyses and molecular dating

Evolutionary models and the best partitioning schemes for phylogenetic reconstructions were selected with PartitionFinder using AICc. The mitochondrial markers were tested for substitution saturation with the index of Xia et al. (2003) in DAMBE 5.3 (Xia and Xie, 2003). MEGA 6 was used to calculate K2P distances among the focal lineages for each marker.

The phylogenetic relationships among lineages were reconstructed using maximum-likelihood and Bayesian (BI) methods. First, we generated ML trees for each marker to investigate gene tree discordance and then we estimated ML and BI phylogenies for the concatenated mitochondrial markers. For ML we used RAxML 7.2.8 (Stamatakis, 2006) with a thorough ML tree search under the GTRGAMMA model applied for each partition. The protein coding COI and EF1α were further split into codon positions. Statistical support at nodes was assessed with 1000 rapid bootstrap pseudoreplicates. For this analysis we used RAxML instead of GARLI due to its faster computational time in calculating bootstrap support and similar accuracy regarding tree topology (Stamatakis, 2006; Zwickl, 2006). Bayesian analyses were performed in MrBayes 3.2 (Ronquist et al., 2012). Two independent searches of four Metropolis coupled MCMC chains were run for 15 million generations and sampled every 1000 generations with 50% burn-in with the temp parameter set to 0.08. We used the same partitions and evolutionary models as for the GARLI analysis; parameters and rates were unlinked and allowed to vary independently. Sample sizes of parameters were verified with TRACER 1.5 (Drummond and Rambaut, 2007) and the convergence of runs was examined with AWTY (Nylander et al., 2008). Bayesian searches were replicated two times to ensure that the runs converged on the same topology.

Because the above methods detected a potential conflict among the mitochondrial and 28S phylogenies (see Results), we employed two approaches for estimating phylogenetic relationships and divergence times among lineages: species tree estimation using a molecular clock rate, and a supermatrix concatenation using biogeographical calibration points. The purpose of the molecular dating analyses was primarily to examine whether the focal lineages predate the Pleistocene glacial cycles; we did not aim to pinpoint explicit historical events that caused the initial diversification of these lineages. For the first approach we used a fully Bayesian method for estimating a species tree based on the multispecies coalescent model of molecular evolution implemented in *BEAST (Heled and Drummond, 2010) as part of the BEAST 1.8.0 (Drummond et al., 2012) package. This model takes into account the independent evolutionary history of each gene tree embedded in a shared species tree, as opposed to the supermatrix concatenation methods which assumes that gene trees share a single common genealogical history. This approach provides a more accurate tree topology and estimates more realistic divergence times in comparison with simple concatenation methods (McCormack et al., 2011; Ogilvie et al., 2016; Meyer et al., 2016). Because *BEAST requires a priori designation of taxonomic groups, we defined as taxonomic groups the entities identified with the more conservative ABGD method (see above). Since this method cannot work with missing data (i.e. incomplete matrix), we used a restricted data set comprised of 50 terminals assigned to 29 taxonomic groups, which included all lineages identified in our study and representative lineages for the other major clades of the *G. fossarum* species complex. *G. pulex* was used as an outgroup because it is a sister clade to *G. fossarum* (Hou et al., 2011). We originally used the same evolutionary models as described in Table S2, but due to poor mixing we changed the models for the nuclear markers from the complex GTR to the simpler HKY. Since we have no prior knowledge regarding extinction, we used a Yule tree prior to model speciation and random starting topologies were specified for each locus. Based on the cross-validation analyses of Copilaş-Ciocianu and Petrusek (2015), which focused on the same species complex, we used an uncorrelated relaxed molecular clock with a lognormal distribution to estimate divergence times (Drummond et al., 2006). We could not use calibration points in the species tree analysis, as the outgroups needed for calibration were not complete with respect to the number of markers. Thus, based on Copilaş-Ciocianu and Petrusek (2015), we employed a COI molecular rate of 2.3% divergence per million years because it gave very similar estimates to an analysis that was based on biogeographical calibration points (see below). Furthermore, this rate has been successfully used to link present-day patterns of distribution with historical processes in other amphipod taxa as well (Lefébure et al., 2006; Yang et al., 2013; Hou et al., 2014a). We ran the analysis for 200 million generations with a sampling frequency of 2000 generations, and discarded the first 30% of trees as burn-in. Effective sample sizes of parameters were checked with TRACER 1.5.

For the second approach we used supermatrix concatenation in BEAST 1.8.0 and employed biogeographical calibration points to estimate divergence times. The dataset consisted of a total of 80 terminals of which 59 were part of the ingroup. We used the same settings as in the *BEAST analysis and the evolutionary models in Table S2, but ran the MCMC chain for 150 million generations with a sampling frequency of 1000 generations and discarded the first 30% of the trees as burn-in. In order to calibrate the molecular clock, we used the same strategy as in Copilaş-Ciocianu and Petrusek (2015, 2017). Specifically, we employed four calibration points with normally-distributed priors based on biogeographical events. The first and youngest calibration point was constrained at 2 ± 1 Ma and is based on the deepest split within the endemic *Gammarus* species flock in Lake Ohrid (Wysocka et al., 2013, 2014), which coincides with the age of the lake (Lindhorst et al., 2015). The second node was constrained to 16 ± 2 Ma and is based on a Middle Miocene geological subsidence event (Popov et al., 2004) that triggered the diversification of the Eastern European clade of *G. fossarum* (Copilaş-Ciocianu and Petrusek, 2015). The third calibration is based on the radiation of the endemic Acanthogammaridae family in Lake Baikal and was constrained at 28 ± 2 Ma (Sherbakov, 1999; Macdonald et al., 2005; Mats et al., 2011), which is almost as old as the lake itself (Ionov, 2002). The last calibration point assumes that the divergence between the genera *Sarothrogammarus* (Asia) and *Rhipidogammarus* (Europe) is due to the regression of the Paratethys Sea from Central Asia during the Eocene (Popov et al., 2004; Hou et al., 2011) and was constrained at 37 ± 2 Ma. All Bayesian analyses were carried out on XSEDE using the CIPRES Science Gateway (Miller et al., 2010).

2.5. Phylogeographic and demographic analyses

All phylogeographic and demographic analyses were performed only on COI because it was the best represented marker in our dataset, and had the highest amount of variation. Estimates of haplotype and nucleotide diversity for all focal lineages (except one for which we had a single analysed individual) were calculated in DnaSP 5.1 (Librado and Rozas, 2009). Haplotype networks were constructed using the statistical parsimony method (Clement et al., 2000) in POPART 1.7 (Leigh and Bryant, 2015).

To examine the demographic history of lineages we employed three approaches. First, we tested for a recent demographic expansion by applying three neutrality tests, Tajima's *D* (Tajima, 1989) and Fu's F_s (Fu, 1997) implemented in ARLEQUIN 3.5 (Excoffier and Lischer, 2010), and the R_2 test, which is more suitable for small sample sizes (Ramos-Onsins and Rozas, 2002), in DnaSP 5.1. The significance of these tests was evaluated using 1000 simulations.

Second, we performed mismatch distribution analyses (Rogers and Harpending, 1992) and calculated Harpending's raggedness statistic (Hri; Harpending, 1994) in ARLEQUIN 3.5 under a sudden demographic expansion model with 1000 bootstrap replicates. Unimodal frequency distributions of pairwise differences among haplotypes in a population indicate a recent expansion whereas multimodal ones indicate either constant population size or geographical subdivision during its evolutionary history (Marjoram and Donnelly, 1994). The validity of a sudden expansion model was analysed by the sum of squared deviations (SSD) between observed and expected mismatches. Significant SSD and Hri values are considered as evidence for departure from such a model.

Third, to evaluate demographic changes through time, we performed Bayesian Skyline Plot (BSP) analyses (Drummond et al., 2005) in BEAST 1.8. We used a strict molecular clock with the same rate as for the *BEAST analysis (see Section 2.4). The models of molecular evolution were selected for each analysed lineage with PartitionFinder and partitioned in BEAST into codon positions with unlinked substitution rate parameters, rate heterogeneity and base frequencies. We ran the MCMC chain for 50 million generations with a sampling frequency of 1000 generations, and discarded the first 25% as burn-in. Suitable sample sizes of parameters were examined using TRACER 1.5. The BSP analyses were performed two times to ensure convergence on the same results.

2.6. Altitudinal range and geographical distribution patterns of lineages

We also aimed at testing if contemporaneous factors might be involved in shaping the geographical distribution of lineages in the BMWC region. In order to test if topography plays a role, we compared the altitudinal ranges of four of the most widespread lineages using a Kruskal-Wallis one-way ANOVA with Mann-Whitney pairwise comparisons and Bonferroni-corrected alpha values in PAST 2.17 (Hammer et al., 2001). The altitudinal range of a lineage/species can also be interpreted as a proxy for its environmental tolerance (e.g. Gaston and Chown, 1999; Lee et al., 2009). To determine to what extent the catchment boundaries match the geographical distribution patterns of lineages, we used QGIS 2.14.3 (QGIS Development Team, 2016) to plot the distribution of lineages on the boundaries of the second order catchments and also on the major drainages of the Baltic, North and Black seas (data for both types of catchments was downloaded as vector polygon shapefiles from the European Environment Agency http://www.eea.europa.eu/data-and-maps/data/european-river-catchments-1).

3. Results

3.1. Sequence data and lineage diversity

A total of 330 sequences belonging to 192 individuals from 186 localities were obtained in this study (Fig. 1, Tables S1 and S2). None of the protein-coding genes contained stop codons or reading frame shifts after translation into amino acids. Lengths of alignments and character variation are shown in Table S2.

The ABGD method gave concordant results for both COI and 16S by identifying eight lineages in the BMWC region at a maximum intraspecific threshold of 5.9% and 4.3%, respectively (Fig. S1); barcode gaps were observed in both markers (between 5.9 and 12% at COI, and 4.3 and 5% at 16S). As various setting for Pmax indicated the same number of focal lineages, we left the default value of 0.1 in the final analysis. We took a conservative approach and used these entities throughout the paper; these are labelled and colour-coded in Fig. 2. The bPTP approach was consistent with ABGD at COI in six out of eight instances. In the other two cases it further subdivided the lineages EE T and CWE A into four and three sublineages, respectively. With regards to the 16S marker, bPTP was consistent with ABGD in seven cases, in one instance subdividing lineage EE T into two sublineages (Fig. S1). Altogether, these methods indicate that between 8 and 13 lineages are present in the study region. Individuals newly analysed from other countries besides the focal BMWC region were assigned by both ABGD and bPTP to nine COI lineages, and eight to nine lineages for 16S, respectively (Fig. S1). All of these individuals belong to new lineages except for one belonging to Clade E according to Copilaş-Ciocianu and Petrusek (2015).

3.2. Phylogenetic analyses and molecular dating

The substitution saturation test indicated no significant loss in phylogenetic signal ($p < 0.001$). Genetic K2P distances among lineages (detected by the ABGD method) ranged from 18.5 to 30.4% at COI, 10.4 to 20.9% at 16S, 0.2 to 1.3% at 28S, 0.4 to 0.9% at 18S and 0.1 to 2.4% at EF1α (Tables S3–S5). Most of the focal lineages were recovered as monophyletic and distinct at each marker with high support in the ML analyses, except for lineages EE R and EE T, which were not recovered as monophyletic at the EF1α locus, and lineage CWE D at the 18S locus (Figs. S1 and S2). Therefore, in most cases the nuclear phylogenies further support the lineages delimited from the mitochondrial sequence data. The phylogenetic relationships among the lineages were weakly supported at each marker. Separate concatenated phylogenetic analyses on the mitochondrial markers recovered the monophyly of the focal lineages with high statistical support in both BI and ML analyses (Fig. 2A). However, there was some incongruence among the mitochondrial and nuclear 28S phylogenies. The mitochondrial tree recovered lineage EE T as nested within the eastern European clade with relatively strong support, but the phylogenetic analysis based on 28S recovered this lineage as sister to the entire *G. fossarum* species complex (Figs. 2A and S2). No such incongruence was identified between the mitochondrial and the 18S or EF1α gene trees.

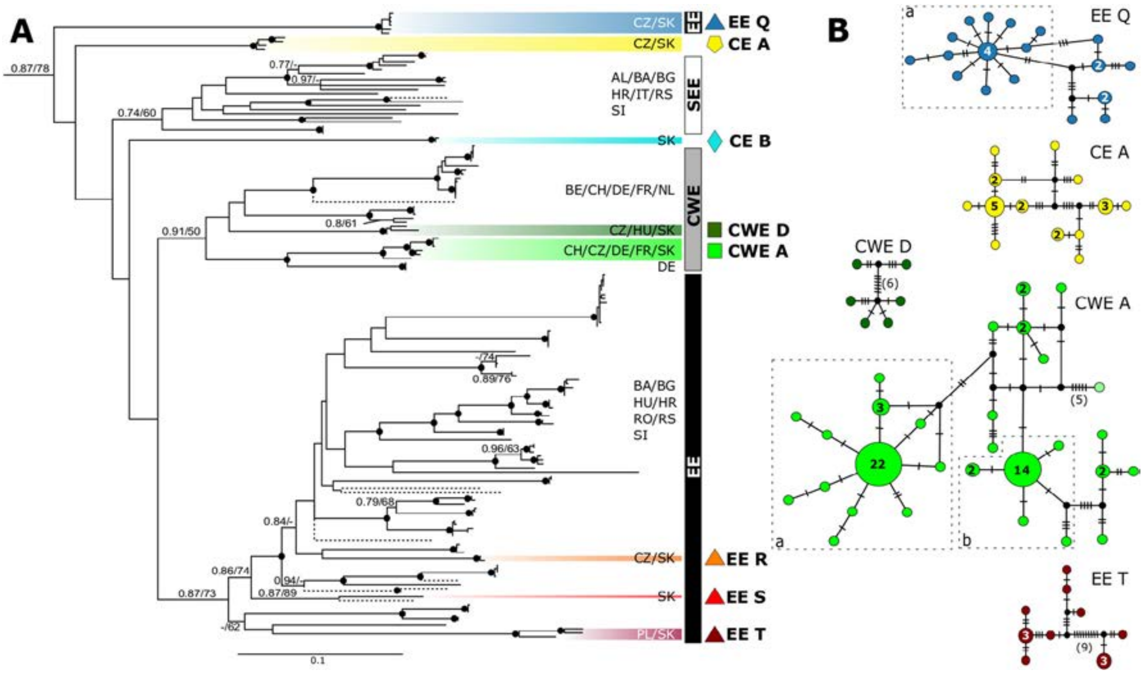

Fig. 2. Phylogenetic relationships of the focal lineages and their corresponding haplotype networks. The nomenclature of the clades follows Fig. 3. (A) Phylogram inferred with ML from mitochondrial sequence data (COI and 16S). Numbers at nodes indicate Bayesian posterior probabilities and ML bootstrap values, respectively (only values above 0.70 and 50% are shown). Black circles indicate strongly supported nodes (Bayesian posterior probabilities and bootstrap values ≥0.95 and 70%, respectively). The focal mitochondrial lineages (as delimited with ABGD) are indicated by coloured symbols and codes that correspond with Figs. 1 and 3. Dotted branches represent additional lineages identified outside the BMWC area (shown by coloured symbols in Fig. 1A). The distribution of focal lineages and major clades is indicated by two-letter country ISO codes (see caption to Fig. 1). Vertical black, grey and white bars indicate the major lineages of the species complex (EE—Eastern European, CWE—Central and Western European and SEE—Southeastern European). (B) COI haplotype networks of the focal lineages. The size of the circles is proportional to the observed frequency of the respective haplotype (sample size is indicated where >1). Hatch marks indicate the number of mutations and black dots indicate unsampled haplotypes.

In order to take into account this incongruence, we performed a multilocus species tree analysis in *BEAST as well as a concatenated supermatrix analysis in BEAST. Both analyses yielded consistent results with previous research (Copilaş-Ciocianu and Petrusek, 2015) by recovering the monophyly of three large clades of the *G. fossarum* species complex: an Eastern European clade (EE, hereafter; labelled as SW Carpathians & SE Europe in Copilaş-Ciocianu and Petrusek (2015)), a Central and Western European clade (CWE, hereafter), and a Southeastern European clade (SEE, hereafter) (Figs. 3 and S3). The distribution of these clades is shown in Fig. 1A. The study of Copilaş-Ciocianu and Petrusek (2015) revealed a clade (named E Europe) which was basal to the entire *G. fossarum* complex. However, at the time, only nuclear data (18S and 28S) obtained from GenBank was available for this lineage (from Hou et al., 2007). In the present study, we can unequivocally confirm that this clade is equivalent with EE T lineage (Table S1; Fig. S2). Although the three main clades are relatively well supported in both analyses, their phylogenetic positions remained weakly supported (Figs. 3 and S3).

The lineages identified from the BMWC region further increase the known diversity of the *G. fossarum* complex by adding six lineages to the already established major clades, as well as two other major clades that have no clear phylogenetic affinity. Although both *BEAST and BEAST recovered largely congruent phylogenetic patterns, we base our lineage nomenclature and discussion on the former analysis due to its higher topological accuracy (McCormack et al., 2011; Ogilvie et al., 2016; Meyer et al., 2016). Based on *BEAST, four of the identified mitochondrial lineages were nested within the EE clade (EE Q, EE R, EE S and EE T) and two into the CWE clade (CWE A and CWE D) (Fig. 3). BEAST recovered the same pattern with the exception that EE Q was basal to the entire species complex, albeit with low support (Fig. S3). The nomenclature of these lineages is extended from Copilaş-Ciocianu and Petrusek (2015) in the case of the EE clade and Müller (2000) in the case of CWE. The remaining two lineages were highly divergent and could not be assigned to any currently known major clade due to low support at internal branches (posterior probability <0.7) in both analyses and were named Central European A and B (CE A and CE B, hereafter) (Fig. 3). Regarding the newly recognized lineages outside the focal area, one was nested within the SEE clade, another one in the CWE clade, while the remaining seven were recovered as belonging to the EE clade (Figs. S1 and S3). However, in the last case, one individual could be sequenced only for 28S and its phylogenetic position was inconclusive (shown with a question mark in Fig. 1A).

There is a striking difference in the distribution of these lineages along the west-east transect across the BMWC region. The Bohemian Massif is inhabited only by lineages CWE A (throughout most of its area) and EE Q (in some easterly located basins), while the Western Carpathians are inhabited by all of them. By far the most widespread lineage is CWE A, which was also encountered in eastern France, Switzerland and southern Germany. For now, it appears that the easternmost edge of its distribution is in the western parts of the Carpathians. This lineage is also known as *G. fossarum* type A and corresponds to *G. fossarum* from its type locality in Regensburg, Germany (Scheepmaker and van Dalfsen, 1989; Müller, 1998, 2000). The other lineages seem to have much more restricted geographical distributions (Fig. 1).

The estimated divergence times are consistent with the previous estimates of Copilaş-Ciocianu and Petrusek (2015) that were based on the same assumptions, and indicate that the main cladogenetic events took place throughout the Miocene (Figs. 3 and S3). The crown age of the *G. fossarum* species complex is estimated at ca. 18.3 Ma in *BEAST (95% HPD: 15.15–21.7 Ma; Fig. 3) and 19.97 Ma in BEAST (95% HPD: 15.73–24.57). According to both

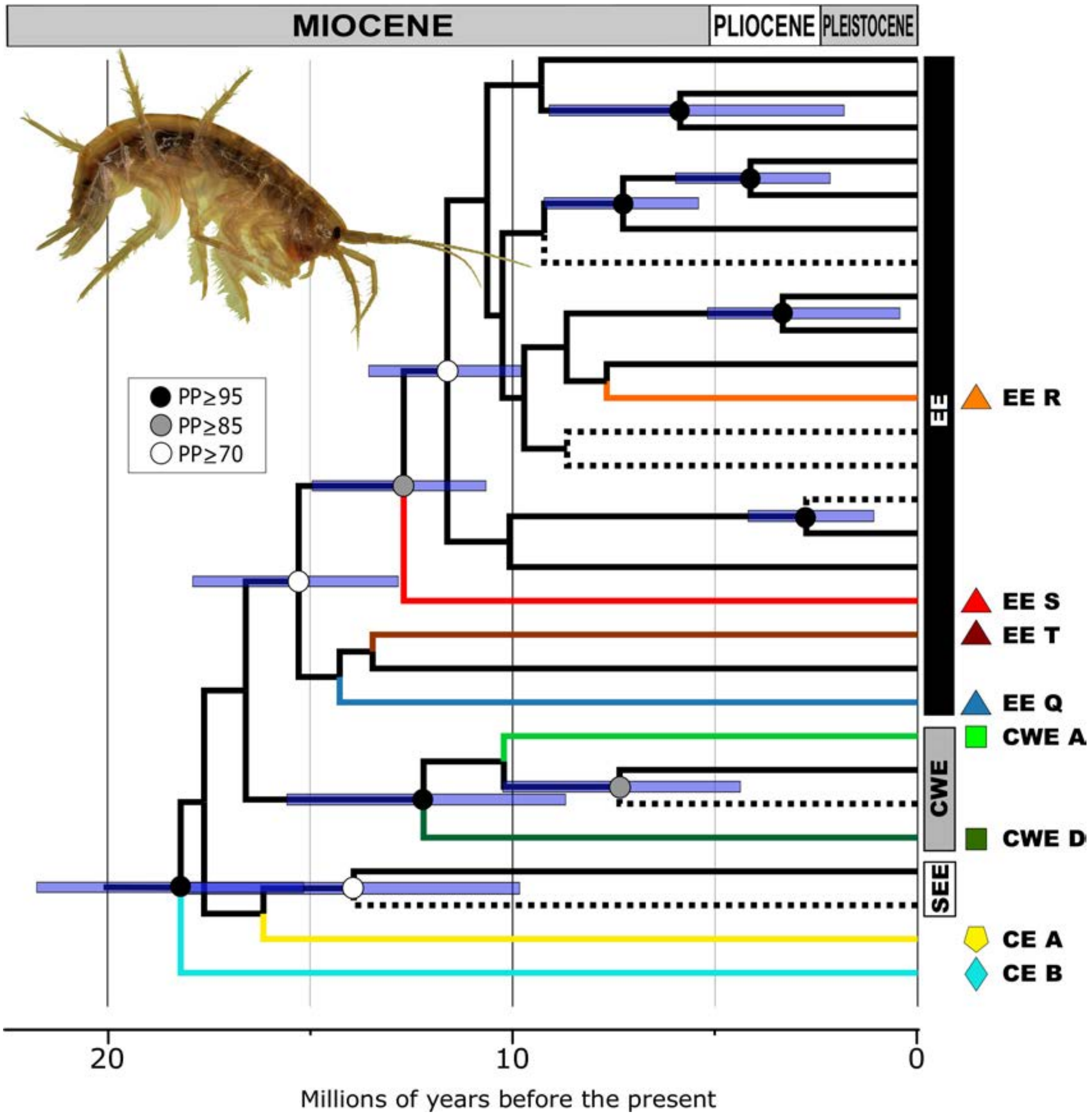

Fig. 3. Dated Bayesian species tree of the *Gammarus fossarum* species complex inferred from all five markers (COI, 16S, 18S, 28S and EF1α). Only posterior probabilities >0.70 are shown at nodes (black ≥ 0.95, grey ≥ 0.85 and white ≥ 0.70). Blue horizontal bars at nodes indicate the 95% HPD intervals of clade ages. The focal mitochondrial lineages are indicated by coloured symbols and codes which correspond with Figs. 1 and 2. Dotted branches represent additional lineages identified outside the focal area. Vertical black, grey and white bars indicate the major lineages of the species complex (EE—Eastern European, CWE—Central and Western European and SEE—Southeastern European). For clarity, the *G. pulex* outgroup has been removed. The inset image depicts a male *G. fossarum* sensu stricto, i.e., from the type locality lineage CWE A (Photograph: Denis Copilaş-Ciocianu).

analyses, all of the focal lineages, except EE R and CWE A, seem to be at least 10 Ma old.

3.3. Phylogeographic and demographic analyses

Haplotype network analyses based on the 602 bp COI fragment revealed that the investigated lineages displayed geographically structured genetic variation (Fig. 2B) with mean intra-lineage genetic distances ranging from 0.1% to 4% (Table S3). Furthermore, the haplotype and nucleotide diversity indices have high values (Table 1). Star-like topologies were observed in two haplogroups (“a” and “b”) of lineage CWE A and one haplogroup (“a”) of lineage EE Q (Fig. 2B).

Demographic analyses were performed on lineages CWE A, EE Q and CE A, for which we had a sufficient sample size (at least 15 individuals per lineage; Table 1). Additionally, lineage EE T was reasonably represented in our samples (13 individuals), but was excluded from these analyses because it was already subdivided into four and two bPTP groups at COI and 16S, respectively (Fig. S1), indicating a significant evolutionary differentiation among its sublineages. Likewise, demographic analyses were performed only on the largest bPTP sublineage of CWE A (Fig. S1); the other two were excluded due to their small sample size. Further mismatch distribution analyses were performed on the haplogroups that displayed a star-like topology in the haplotype networks (lineages CWE A and EE Q). In lineages CWE A and CE A, the neutrality tests were non-significant ($p > 0.1$) while the SSD and Hri had highly significant values ($p < 0.0001$), indicating either a stable population size or a long time of geographical subdivision among populations (Table 1). A similar pattern was observed in lineage EE Q, but the R_2 test indicated population expansion (Table 1). Accordingly, the frequency of pairwise haplotype differences displayed bimodal (CWE A and EE Q) and multimodal (CE A) distributions (Fig. 4B). However, separate analyses on the haplogroups that displayed star-like topologies indicated a recent population expansion. Tajima’s D and R_2 had significant values in haplogroup “a” of lineage CWE A, while only the former test was significant in haplogroup “b” (Table 1). All three tests resulted in significant values in the haplogroup “a” of lineage EE Q (Table 1).

Table 1
Genetic diversity and neutrality tests for the focal lineages of *G. fossarum* in this study (except for EE S for which we had one analysed individual). Significant values are indicated by asterisks and shown in bold.

Lineage	*N*	*S*	*H*	*Hd* (SD)	π (SD)	Tajima's *D*	Fu's F_s	R_2	SSD	Hri
CWE A overall	63	26	24	0.832 (0.037)	0.01442 (0.0008)	−0.850 ns	3.878 ns	0.102 ns	**0.050***	**0.086*****
CWE A group a	31	11	11	0.548 (0.109)	0.00271(0.0007)	**−2.392*****	10.542 ns	**0.121*****	0.116 ns	0.252 ns
CWE A group b	19	8	6	0.468 (0.140)	0.00270 (0.0001)	**−1.813***	8.167 ns	0.138 ns	0.046 ns	0.062 ns
CWE D	6	17	6	1 (0.096)	0.01182 (0.0025)	–	–	–	–	–
EE Q overall	23	22	18	0.968 (0.026)	0.00804 (0.0010)	−0.763 ns	−1.687 ns	**0.125***	**0.014*****	**0.023*****
EE Q group a	15	12	12	0.943 (0.054)	0.00323 (0.0005)	**−1.727***	**−11.683*****	**0.145*****	0.034 ns	0.137 ns
EE R	4	3	2	0.500 (0.265)	0.00266 (0.0014)	–	–	–	–	–
EE T	13	23	9	0.923 (0.057)	0.04107 (0.0046)	–	–	–	–	–
CE A	22	26	13	0.931 (0.036)	0.01400 (0.0013)	0.692 ns	4.652 ns	0.126 ns	**0.026*****	**0.047*****
CE B	2	1	2	1 (0.500)	0.00166 (0.0008)	–	–	–	–	–

N – sample size; S – number of variable sites; H – number of haplotypes; Hd – haplotype diversity; π – nucleotide diversity; SSD – sum of squared deviations; Hri – Harpending's raggedness index; *** $p < 0.0001$, * $p < 0.05$, ns – not significant.

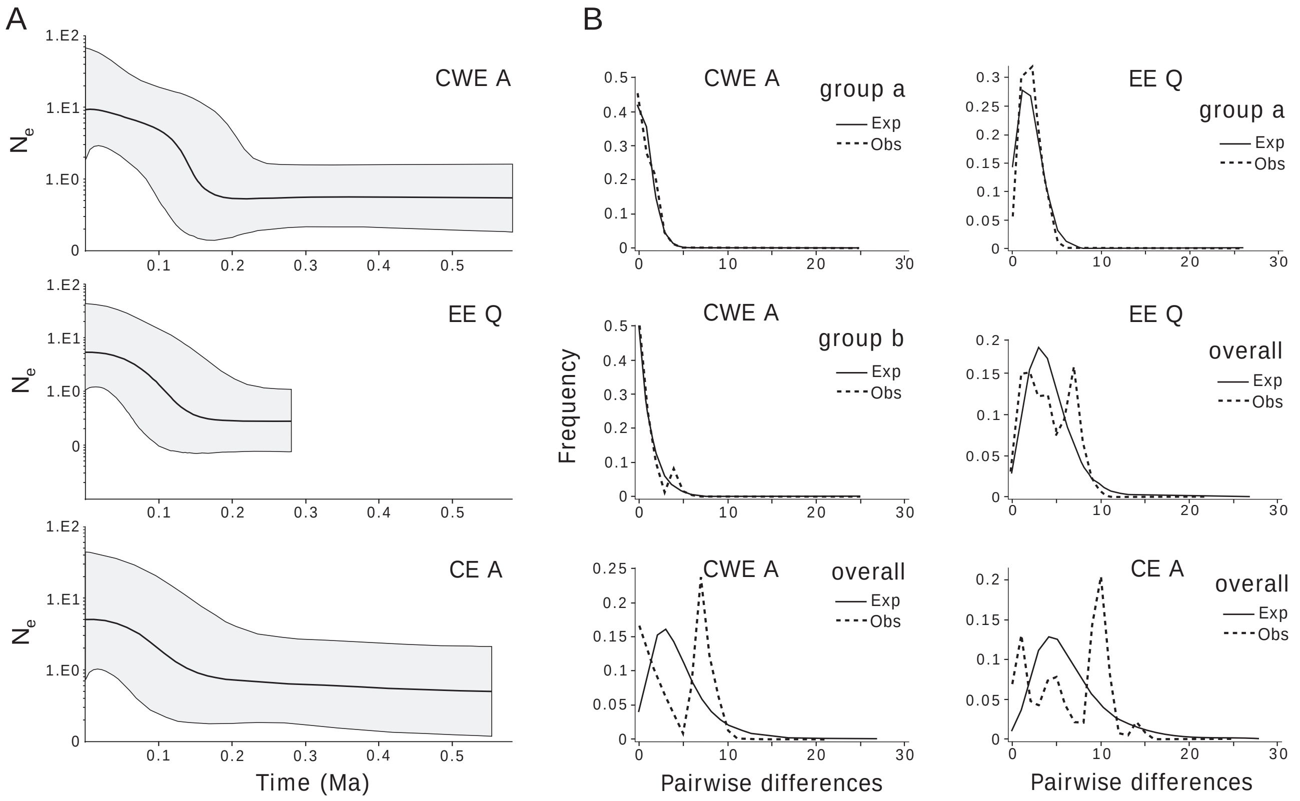

Fig. 4. Bayesian Skyline Plots (BSP) (A) and mismatch distribution analyses (B) of mitochondrial lineages CWE A, EE Q and CE A. Thick black lines in the BSP represent mean effective population size and 95% HPD intervals are shown in grey. Mismatch distribution was also calculated separately for the haplogroups exhibiting a star-like topology in the haplotype networks of lineages CWE A and EE Q (indicated by dashed lines in Fig. 2B).

Consistent with these values, the SSD and Hri were non-significant and the mismatch distributions were unimodal in all three analysed subgroups (Table 1; Fig. 4B). Subgroups "a" and "b" of lineage CWE A have distinct eastern and western geographical distributions, respectively, while a less clear pattern could be observed in subgroup "a" of linage EE Q (Fig. S4). The BSP analyses showed a very similar pattern in all three analysed lineages, indicating a demographic growth that started ca. 150 kyrs ago and plateaued ca. 50 kyrs ago (Fig. 4A).

3.4. Altitudinal range and geographical distribution patterns of lineages

The Kruskal-Wallis test revealed that there were no significant differences in the altitudinal ranges (ca. 150–1000 m) of the four most widespread lineages (CWE A, EE Q, EE T and CE A) ($p > 0.5$ in each pairwise comparison) (Fig. 1B, inset, Table S6), suggesting that they exhibit similar environmental tolerances, and that the environmental conditions or the topography of the region do not play an important role in shaping their geographical distribution. Seven out of eight lineages were represented by more than one locality and the distribution patterns of none of them followed the present boundaries of second order river catchments, i.e. all of them were present in at least two adjacent catchments (Fig. 1B). With respect to the major drainages of the North, Baltic and Black seas (i.e., Elbe, Oder and Danube, respectively), lineage CWE A was present in all three, CWE D, EE Q and EE T were present only in the Baltic and Black Sea drainages, while the remaining four were apparently restricted to the Black sea drainage (Fig. S5).

4. Discussion

The results of this study show that the *G. fossarum* complex in the Bohemian Massif and the Western Carpathians (BMWC) is highly diverse, containing eight lineages that belong to four major clades, and date back to the Miocene. The marked difference in lineage composition and demographic history across the studied biogeographical boundary indicates that at least the Western Carpathians had an important refugial role during the Pleistocene climatic fluctuations.

Our west-east transect across the BMWC region has revealed an important shift in the spatial distribution of lineages along the border of the Bohemian Massif and the Western Carpathians. While only two lineages (CWE A and EE Q) are shared between both regions, the latter is inhabited by six additional lineages. The data available so far suggests that all lineages, except CWE A, have rather narrow geographical distributions. This change in lineage composition between the two regions is concordant with the patterns observed in other aquatic or terrestrial groups of invertebrates (e.g. Schenková et al., 2009, 2010; Horsák and Cernohorsky, 2008; Juřičková et al., 2014b). There is also a discrepancy regarding the demographic history of lineages across the studied biogeographical boundary. Only the ones that are distributed across both the Bohemian Massif and the Western Carpathians (CWE A and EE Q) show signs of local and recent demographic expansion, while the three additional widespread lineages that are restricted to the Western Carpathians (CE A, CWE D, and EE T) do not. The highly localized distributions and divergence of the remaining three lineages from the latter region (CE B, EE R and EE S) would also support a relict survival scenario against that of a recent colonization (Lomolino et al., 2010). Furthermore, the BSP analyses on the widespread lineages CWE A, EE Q and CE A indicate that demographic growth occurred ca. 150 kyr ago, which is consistent with the onset of the last interglacial period (Eemian, 130–115 kyr ago) (Augustin et al., 2004; Dahl-Jensen et al., 2013). Of these, lineages CWE A and EE Q exhibit local groups of haplotypes that bear the signatures of recent expansion. This pattern suggests that during the Last Glacial Maximum these haplogroups survived in geographically distinct refugia from which they expanded during the Holocene. However, the distributions of both the CWE A and EE Q clades are not entirely known, and thus the locations of their refugia might lie outside the BMWC region.

The crown age of the *G. fossarum* species complex estimated in this study to be 18–20 Ma is in good agreement with the 21.4 Ma estimate of Copilaş-Ciocianu and Petrusek (2015). The discordance observed between the mitochondrial and 28S phylogenies regarding the position of the EE T lineage is probably the result of incomplete lineage sorting due to deep coalescence in the 28S marker, however, we also cannot completely rule out an ancient hybridization (Maddison and Knowels, 2006; Degnan and Rosenberg, 2009). Recent simulation research indicates that ancestral gene flow has a more prominent effect on the estimation of divergence times (underestimation) rather than topology in species tree analyses (Leaché et al., 2014). However, both the species tree and the supermatrix concatenation approaches recovered lineage EE T as nested within the major EE clade with strong support, and gave concordant results regarding the main phylogenetic patterns and divergence times.

The lineages found in the Western Carpathians, although less numerous than in the southwestern Carpathians or the Balkan peninsula, seem to be phylogenetically more diverse because they belong to two out of three known major clades of the *G. fossarum* species complex, and two additional lineages (CE A and CE B) cannot be attributed to any currently known major clade. Therefore, the Western Carpathians are one of the most important diversity hotspots for this species complex in Europe. Moreover, most of the focal lineages seem to occupy rather basal positions within their respective major clades and apparently are relatively old, with ages estimated between 7 and 18 Ma. Such a high phylogenetic diversity, old age, basal positions and narrow ranges at relatively high latitudes suggests that this species complex endured millions of years of severe climatic fluctuations and might have had a wider northward geographical distribution during pre-Pleistocene times. It is surprising that such a high and relict diversity may have persisted for so long at high latitudes, in relative proximity to the ice-sheets. To our knowledge, long term survival of old lineages of freshwater organisms at higher latitudes has been so far reported only in subterranean amphipods (Kornobis et al., 2010, 2011; McInerney et al., 2014).

The discovery of several additional lineages outside the BMWC fills important gaps regarding the distribution of the major clades in Europe and indicates that the *G. fossarum* complex still harbours a substantial amount of undetected diversity. The presence of several highly divergent EE lineages in the Pannonian Basin and surrounding regions, including the Western Carpathians, adds further support to the Tisza Land hypothesis proposed in Copilaş-Ciocianu and Petrusek (2015). Tisza Land was a Palaeogene to Early Neogene mountain range that extended throughout the present-day Pannonian Basin, incorporating the southwestern Carpathians (Popov et al., 2004). The hypothesis states that the diversification of the EE clade was triggered by the fragmentation of Tisza Land and its partial submergence under the waters of the Paratethys Sea during the Middle Miocene (ca. 15 Ma) (Kázmer, 1990; Popov et al., 2004).

The current environmental conditions do not seem to explain the distinct patterns of lineage diversity and distribution of *G. fossarum* observed between the Bohemian Massif and the Western Carpathians. There is no difference in altitudinal variation among the widespread lineages, and the sampled habitats (small to medium-sized streams with plant litter) were comparable across the studied region. This likely indicates that topography and current environmental conditions are not major limiting factors to dispersal (Gaston and Chown, 1999; Lee et al., 2009) and that various linages of the complex exhibit similar ecological requirements, as observed in the south-western Carpathians (Copilaş-Ciocianu and Petrusek, 2015). However, even though recent research has suggested that the ecology of cryptic amphipod species can differ, this pattern depends on the scale of the study (Fišer et al., 2015) and historical factors cannot be completely ruled out (Eisenring et al., 2016). The dispersal of these lineages does not seem to be limited by the catchment boundaries either, as most of them can occur in adjacent drainages. Given that these aquatic animals have limited dispersal abilities and cannot actively move across terrestrial drainage divides, it is more likely that this pattern indicates a long-term persistence in the area. Although passive overland dispersal has been previously documented in freshwater amphipods (Swanson, 1984; Bilton et al., 2001), Weiss and Leese (2016) suggest that this type of dispersal occurs rarely and is therefore relevant over long (evolutionary) timescales rather than for on-going dispersal at contemporaneous (ecological) time scales. Another way that these lineages could have crossed the drainage divides is through a stream capture, which occurs when a stream is deviated from its own bed into the bed of a neighbouring stream by headwater erosion (e.g. Waters et al., 2006). As this phenomenon occurs rarely, it is also more relevant for long time scales.

The discrepancy in lineage diversity and distribution between the Bohemian Massif and the Western Carpathians is, thus, better explained by differential survival of lineages across isolated refugia during the Pleistocene glaciations rather than by current environmental factors. The molecular data indicates that at least the

Western Carpathians served as a long term glacial refugium for the *G. fossarum* species complex and that most of the local lineages are actually Tertiary relicts. More specifically, due to their patchy distribution and high divergence, it appears that these lineages survived in isolated refugial areas, which is in agreement with the fossil evidence that indicates the persistence of isolated patches of broadleaf forests in the Western Carpathians and Central Europe in the Pleistocene, including the Last Glacial Maximum (Adams and Faure, 1997; Willis and van Andel, 2004; Magri et al., 2006; Juřičková et al., 2014a; Mitka et al., 2014). Most likely, the heterogeneous topography of the Western Carpathians provided shelter during the adverse climatic conditions of the Pleistocene (Juřičková et al., 2014a) and permanent running waters would have facilitated the survival of aquatic species because such waters are thermally buffered against freezing conditions (Pauls et al., 2006). Furthermore, thermal and mineral springs are widespread across the Western Carpathians in comparison with the Bohemian Massif (Franko and Kolářová, 1985) and might have also played a role in the survival of gammarids not only due to their higher temperature but also thanks to their stability, even during periods of increased aridity that occurred during the glaciation cycles (Franko et al., 2008).

5. Conclusion

The *G. fossarum* lineages inhabiting the Bohemian Massif and the Western Carpathians exhibit contrasting patterns of spatial diversity and demographic histories. While two lineages that show local signatures of recent demographic expansion inhabit both regions, the Western Carpathians harbour six additional lineages that exhibit a relict distributional pattern. Lineages in the studied region are phylogenetically diverse and old, diverging between 7 and 18 Ma, and their distribution does not seem linked to the region's present-day topography or the boundaries of river catchments. Therefore, the distinct patterns of diversity observed between the two areas are best explained by the differential impact of Pleistocene glaciations rather than by contemporaneous factors. Our additional data from outside the focal area also fills some important gaps in elucidating the distribution of the major clades of the complex and indicates that a considerable amount of diversity is still undiscovered. The overall evidence strongly suggests that the Western Carpathians are a major diversity hotspot for the *G. fossarum* complex and functioned as a long-term glacial refugium, enabling ancient lineages to survive millions of years of severe climatic oscillations closer to the Pleistocene ice-sheets than previously thought.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2017.04.027.

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