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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.

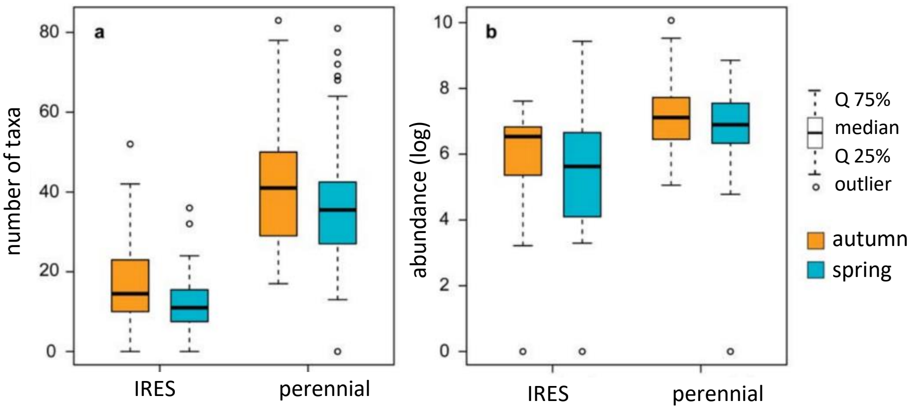


***Figure 1. Differences in number of taxa** (a) **and abundance** (b) **between perennial and IRES sites in spring** (blue) **and autumn** (orange) **seasons, based on the SALAMANDER database analysed in the BIODROUGHT project**. Adapted from Pařil 2015.*

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).

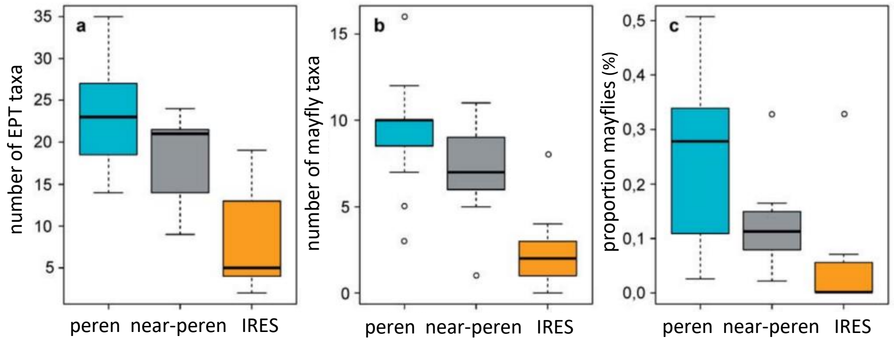
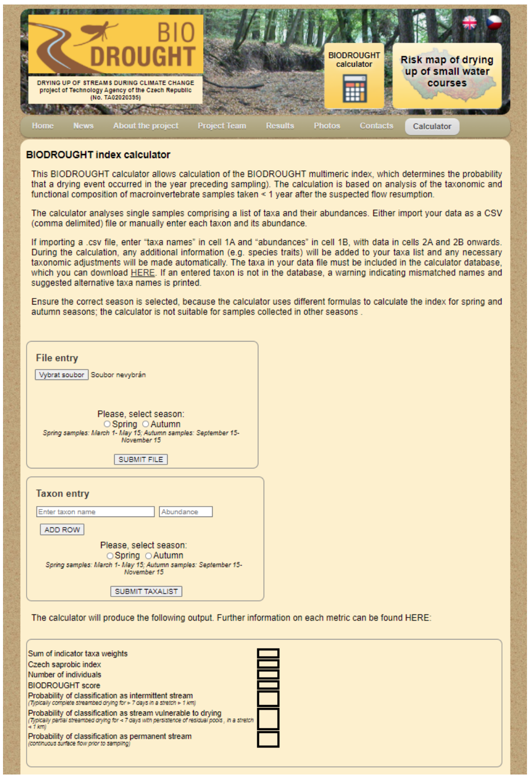


***Figure 2. Gradual decrease in the number of EPT taxa** (a), **number of mayfly taxa** (b) **and proportional representation of Ephemeroptera** (c) **along an intermittence gradient between three defined stream types**. peren = perennial, near–perennial and IRES = intermittent streams. Adapted from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

Soubor nevybrán

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

Taxon entry

Enter taxon name ☐ Abundance ☐

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

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 ☐
(Typically complete streambed drying for > 7 days in a stretch > 1 km)
Probability of classification as stream vulnerable to drying ☐
(Typically partial streambed drying for < 7 days with persistence of residual pools , in a stretch < 1 km)
Probability of classification as permanent stream ☐
(continuous surface flow prior to sampling)

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).

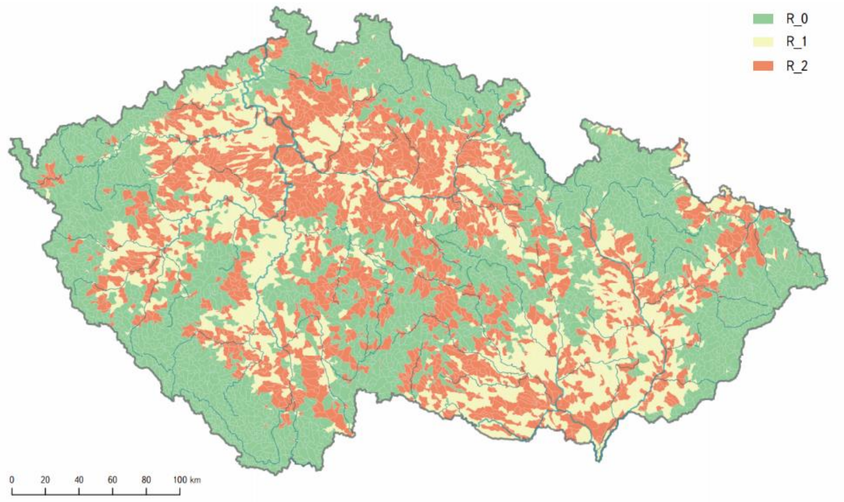


Figure 4. Stream drying–risk classification for all 4th order Czech catchments, developed as part of the BIODROUGHT project. *Stream drying–risk categories: R0 (green) = low, R1 (yellow) = intermediate, R2 (orange) = high. Adapted from Zahrádková et al. 2015.*

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.

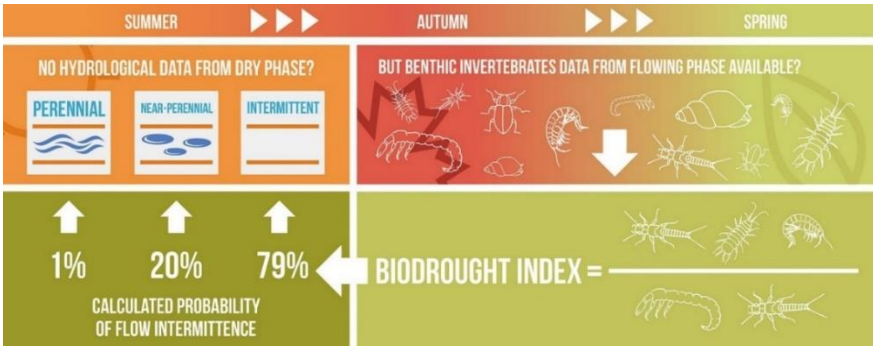


***Figure 5. Schematic illustration showing practical use of the BIODROUGHT index for bioindication of antecedent stream drying in river networks lacking gauging stations** (calculated probability of sample classification to flow categories as an example only). Adapted from Straka et al. 2019.*

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%).

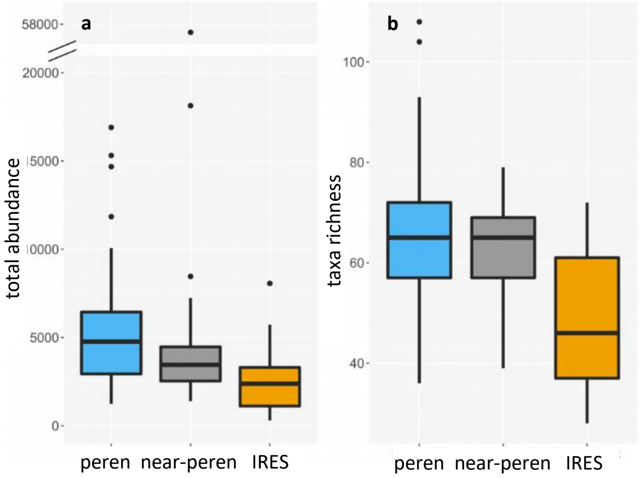


***Figure 6. Differences in macroinvertebrate abundance** (a) **and taxa richness** (b) **over three flow categories** (peren = perennial and IRES = intermittent). Adapted from Straka et al. 2019.*

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 (**<u>Paper 5:</u> 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.

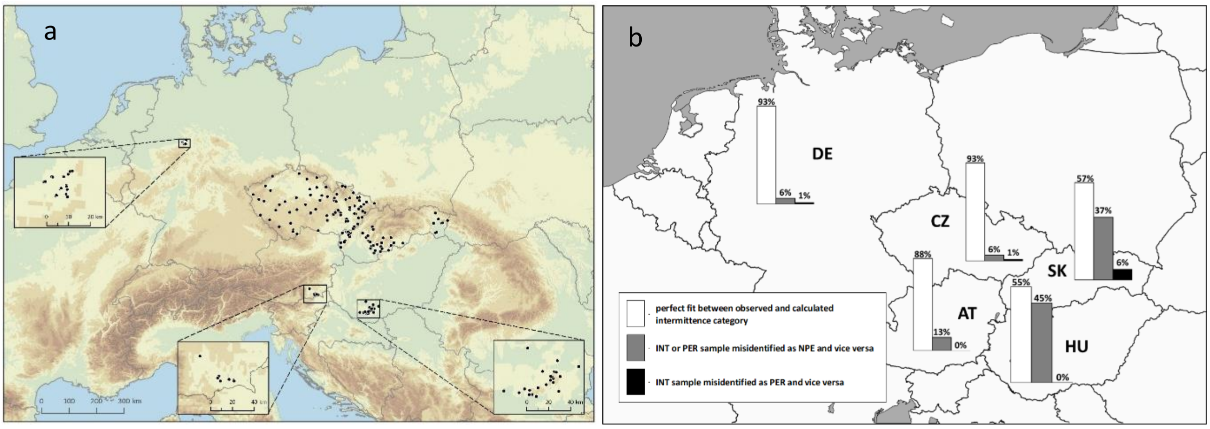


***Figure 6. Maps showing the location of datasets obtained from five Central European countries** (a) **and the percentage of correct sample classifications in each country according to the BIODROUGHT index** (b). Columns indicate success rate (%) of sample classification: white columns = correct classification; 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, Miliša 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.

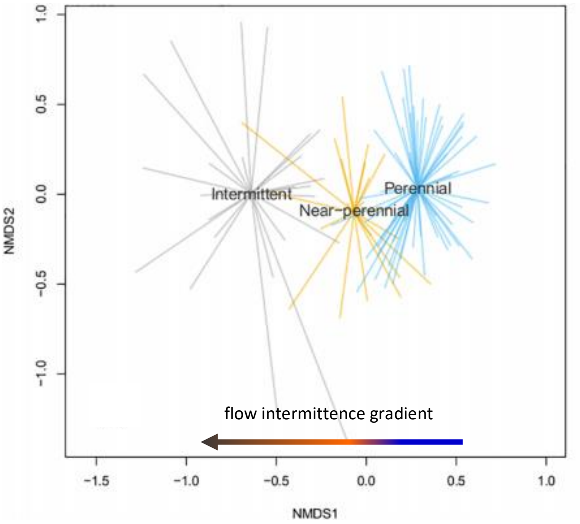


***Figure 7. Increasing MIV community taxonomic variability in three flow categories** (blue = perennial, orange = near–perennial and grey = intermittent) **along a gradient of increasing flow intermittence**. (NMDS ordination plot–based Bray–Curtis distance matrix; adapted from Straka et al. 2021).*

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.

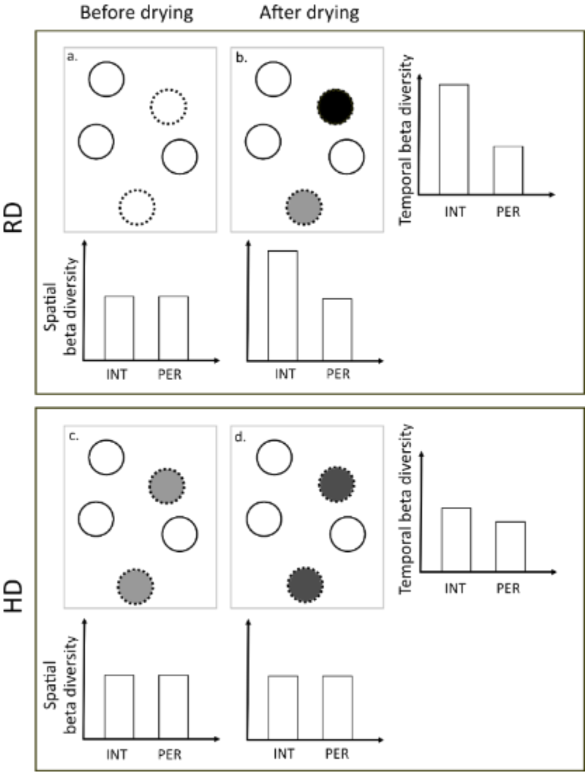


***Figure 8. Expected differences in community structure response to drying in intermittent** (INT) **and perennial** (PER) **rivers over space** (regional beta–diversity) **and time** (before and after dry episodes) **in recently–drying** (RD) **and historically–drying** (HD) **river networks**. Adapted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

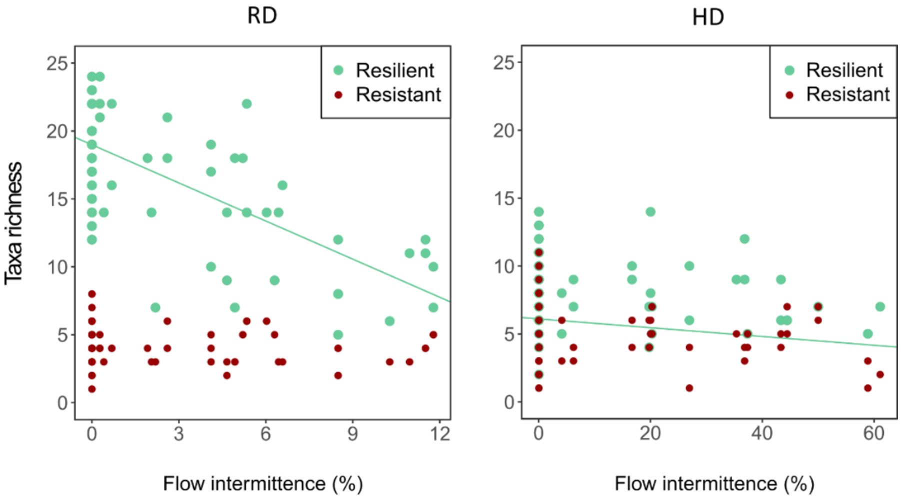


Figure 9. Effect of flow intermittence *(percentage of dry days during a year)* ***on taxa richness of resilient*** *(larger green points)* ***and resistant taxa*** *(small red points)* ***on recently–drying (RD) and historically–drying (HD) networks, showing the linear regression*** *(green line)* ***of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

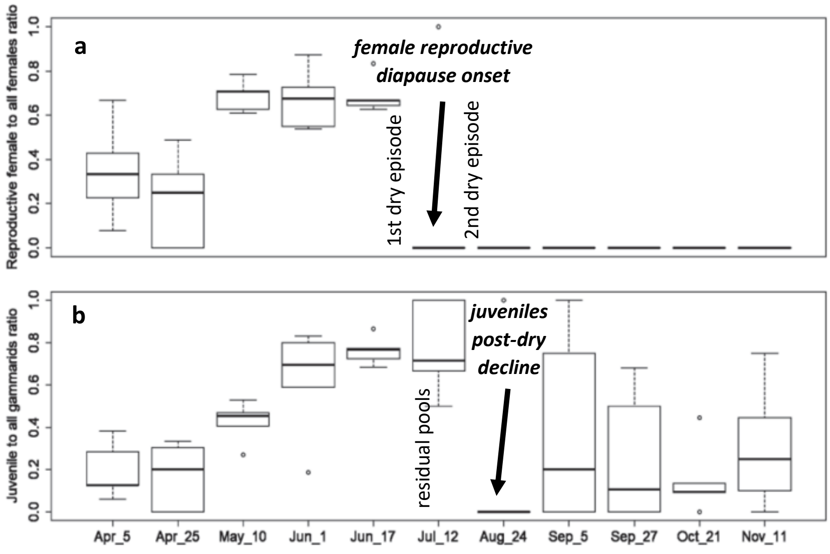


***Figure 10. Proportion of reproductive females to all females** (a) **and juveniles as a proportion of the whole population** (b) **in benthic samples.** A marked decrease in reproductive females is visible immediately after the first dry episode (July 12) and in juveniles after the second dry episode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

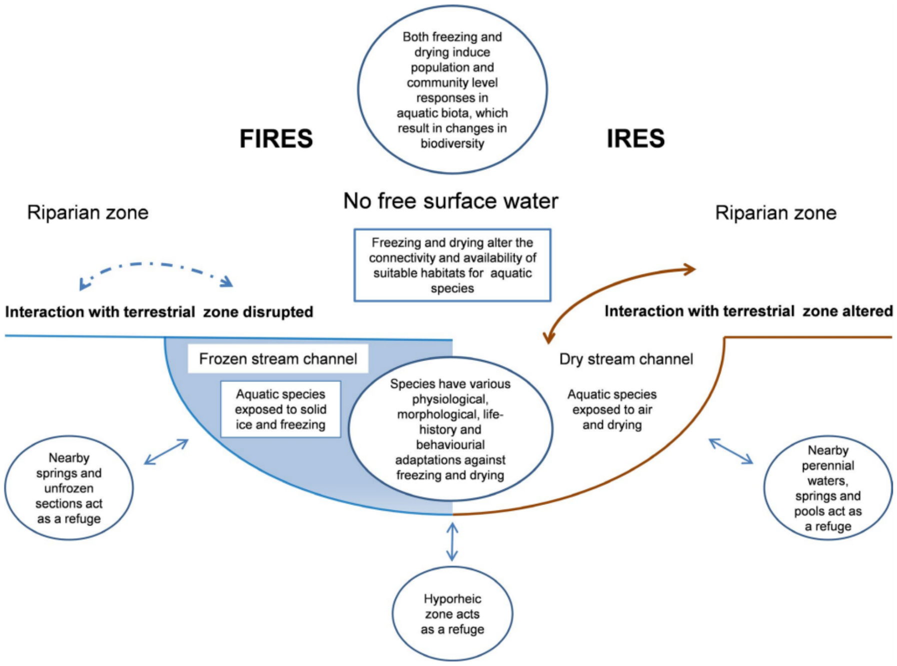


Figure 11. Comparison of habitat connectivity in intermittent rivers and ephemeral streams (IRES) vs. freezing IRES (FIRES)*. Adapted from Tolonen et al. 2019.*

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.

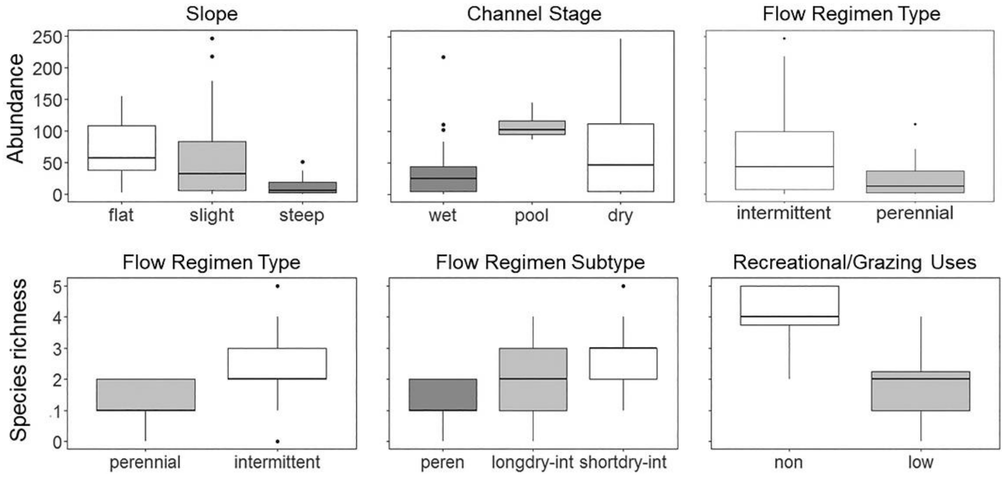


Figure 13. Main factors *(GLMM model)* ***affecting vertebrate species richness and density in Czech rivers****. Adapted from Sanchez Montoya et al. 2022.*

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.

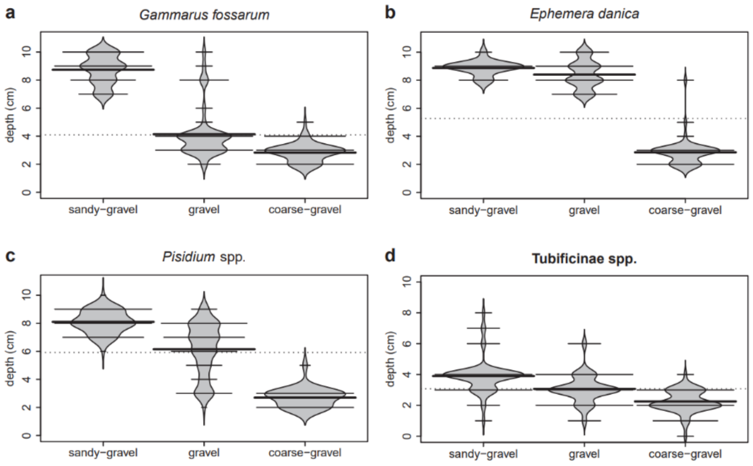


Figure 14. Proportional representation of four model taxa recorded at different sediment depths in experimental mesocosms with three sediment types *(increasing in size from left to right). Adapted from Loskotová et al. 2019.*

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.

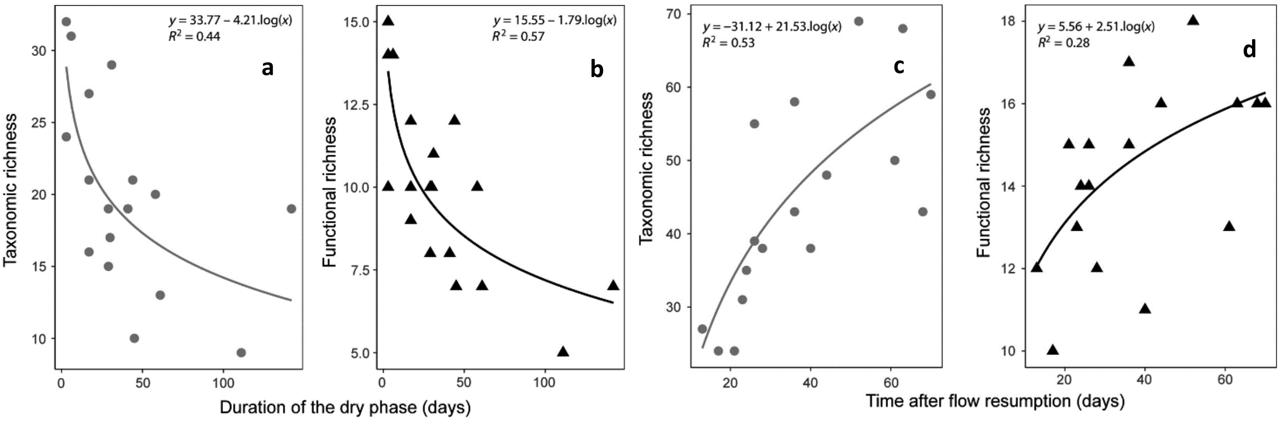


***Figure 15. Changes in MIV community taxonomic and functional richness related to duration of riverbed dry phase** (a, b) **and flow resumption re–watering** (c, d). Adapted from Pařil et al. 2019b.*

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 (**<u>Paper 13:</u> 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.

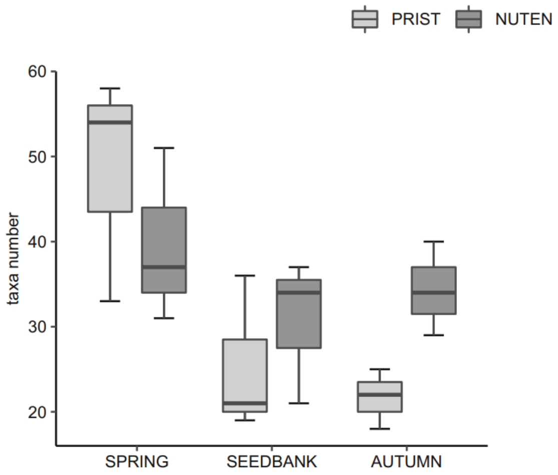


Figure 16. Comparison of seasonal changes in taxa number between nutrient enriched *(NUTEN – dark grey)* ***and pristine*** *(PRIST –light grey****) sites during pre–dry flow*** *(SPRING),* ***summer dry riverbed*** *(SEEDBANK)* ***and post–dry flow phases*** *(AUTUMN). Adapted from Loskotová et al. 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 (**Paper 14: 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.

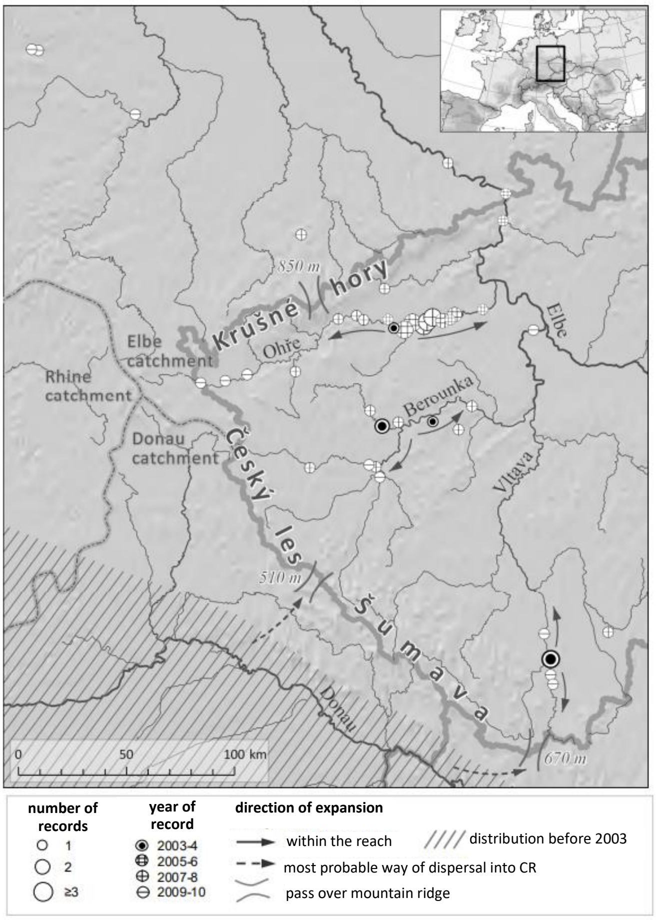


Figure 17. Chronology of Leuctra geniculata expansion into the CR between 2003 and 2010. *The size of circles shows the abundance of recorded larvae, lines across the circles indicate the year of record, dashed arrows indicate possible pathways of dispersal into the CR, and full arrows indicate expected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

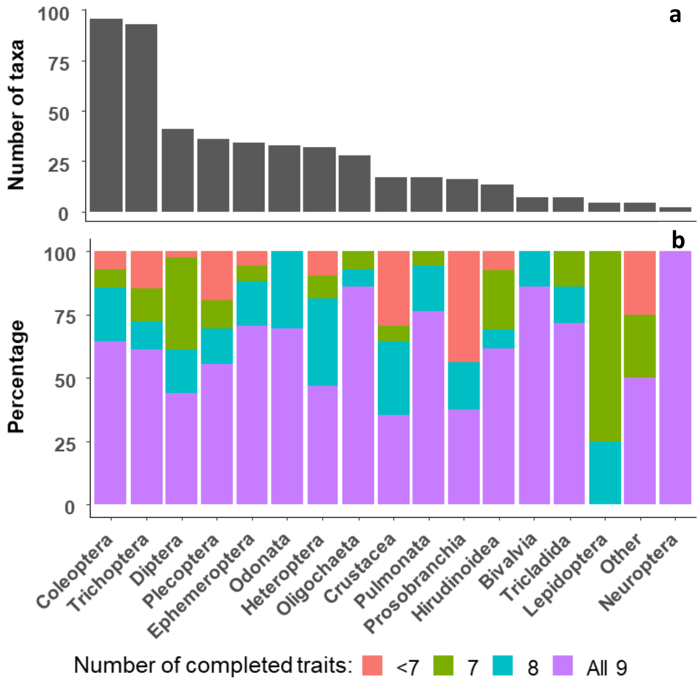


***Figure 18. Total number of taxa** (a) **and completeness (%) of nine traits** (b) **for each MIV group.** The group "Other" includes Hydrozoa, Hymenoptera, Megaloptera and Porifera, each having just one taxon in the database. Adapted from Sarremejane et al. 2021.*

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 (**<u>Paper 19:</u> 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.

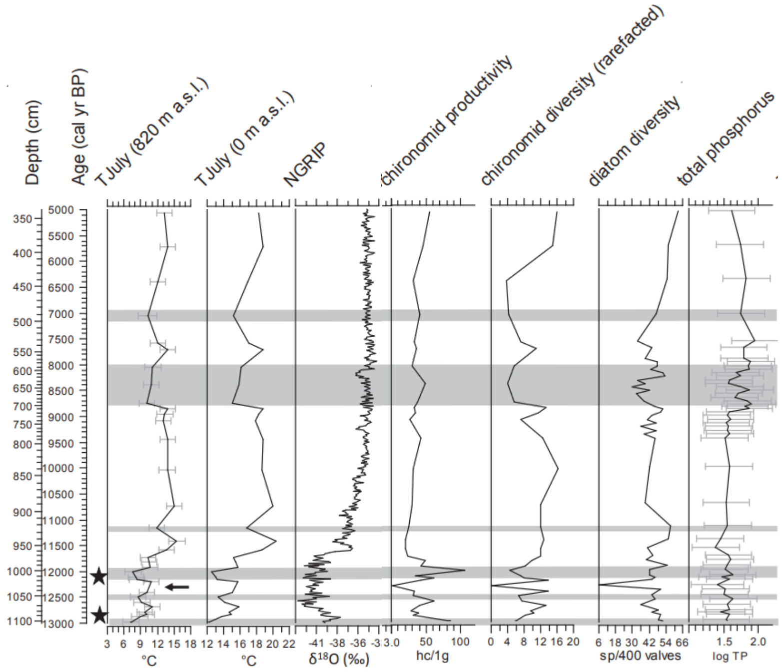


Figure 19. Reconstructed chironomid–based T_{July} *(original values and adjusted to 0 m a.s.l.)* ***from the Hypkaňa profile between 13,000 and 5,000 cal. yr. BP*** *(first two curves). Subsequent curves show changes in NGRIP (North Greenland Ice Core Project), chironomid diversity (number of taxa per 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.

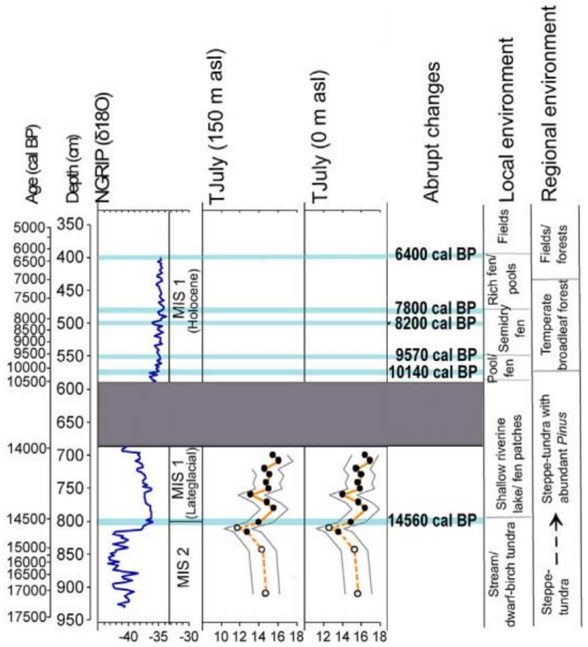


Figure 20. Summary diagram showing results of the Santovka climate chironomid–based paleoreconstruction compared with NGRIP ($d^{18}O$) reconstruction and changes in local and regional environment. Reconstructed chironomid–based T_{July} between ca. 17,000 and 14,000 cal. yr. BP is adjusted to 0 m a.s.l. *(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.

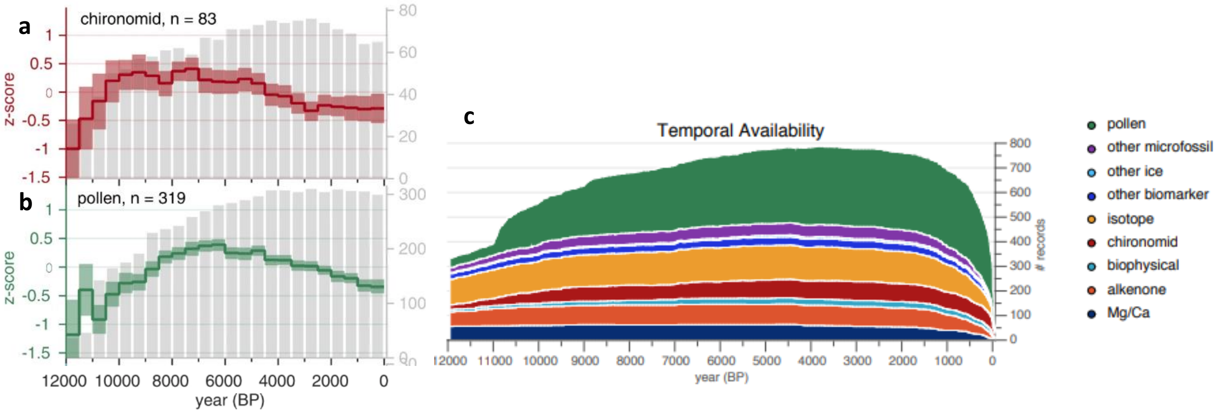


Figure 21. Comparison of major temperature change trends during the Holocene period (last 10,000 cal. yr. BP), reconstructed from (a) chironomid *(n=83)* ***and (b) pollen*** *(n=319)* ***records*** *(z–score = mean of zero and variance of 1 SD over the entire record);* ***Temporal data availability 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).

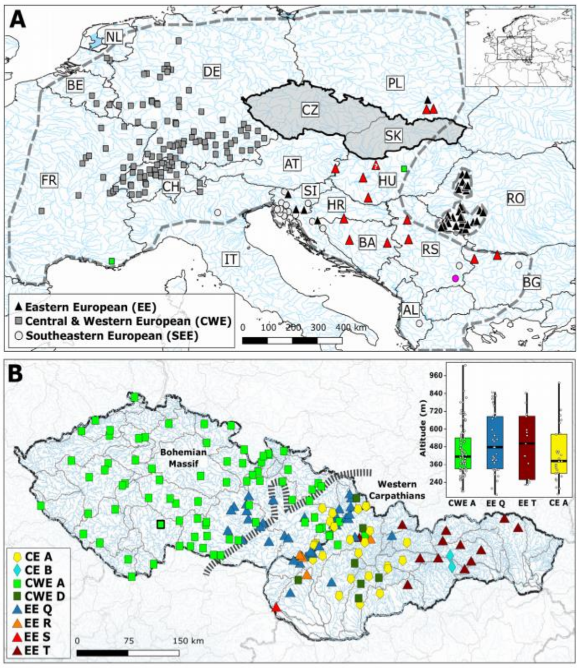


Figure 22. Distribution of eight cryptic Gammarus fossarum lineages across the Czech and Slovak Republics, with the marked biogeographical boundary between the Bohemian Massive and Western Carpathians*. The overlap in lineage altitudinal distribution indicates poor differentiation of ecological preferences 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

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

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