Limnetica, 45(1): 000-000 (2026). DOI: 10.23818/limn.45.02

eDNA metabarcoding: a non-invasive method to track temporal community dynamics in temporary rivers

Nieves López-Rodríguez[1,2,3,4]*, Dominik Buchner[5,6], Miguel Cañedo-Argüelles[3], Ricard Fontserè[1,7], Florian Leese[5,6], Cesc Múrria[2,8], Núria Bonada[1,2]** and Zeynep Ersoy[1,2]**

[1] FEHM-Lab (Freshwater Ecology, Hydrology and Management), Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Universitat de Barcelona (UB), Diagonal 643, 08028 Barcelona, Catalonia, Spain.
[2] Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona (UB), Diagonal 643, 08028 Barcelona, Catalonia, Spain.
[3] SHE2 group (Surface Hydrology, Ecology and Erosion), FEHM-Lab, Institute of Environmental Assessment and Water Research, IDAEA, CSIC, Barcelona, Catalonia, Spain.
[4] Eurofins-Cavendish, Carretera Bailen-Motril, par. 102 b, Granada, Spain.
[5] Aquatic Ecosystem Research, University of Duisburg-Essen, Universitaetsstrasse 5, 45141 Essen, Germany.
[6] Centre for Water and Environmental Research (ZWU), University of Duisburg-Essen, Universitaetsstrasse 5, 45141 Essen, Germany.
[7] Research group of Ecology, Evolution and Genetics, Biology Department, Vrije Universiteit Brussel (VUB), Pleinlaan 2, 1050 Brussel, Belgium.
[8] ZooSysEvo (Zoological Systematics and Evolution), Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia. Universitat de Barcelona (UB), Diagonal 643, 08028 Barcelona, Catalonia, Spain.

** equal contribution

* Corresponding author: nievesbio95@gmail.com

Received: 25/09/24 Accepted: 25/11/24

ABSTRACT

eDNA metabarcoding: a non-invasive method to track temporal community dynamics in temporary rivers.

Temporary rivers (TRs) are dynamic ecosystems that alternate between hydrological phases (i.e., flowing, disconnected pools, and dry). They are conservation refugia for aquatic species during dry seasons but are often neglected in bioassessment programs. To assess the biological quality of these ecosystems, morphological methods can be invasive, disrupting communities and diminishing their function as refugia. Environmental DNA (eDNA) metabarcoding provides a minimally invasive method, gathering community information from eDNA in water or sediment. We tested the effectiveness of eDNA methods alongside bulk DNA metabarcoding to characterize the macroinvertebrate communities and assess the biological quality of disconnected pools in TRs, comparing them with morphological methods. Additionally, we tested how the community patterns evolve over time using eDNA and how community composition shifts during disconnection. Biological quality was determined through macroinvertebrate indices widely used in Spain (i.e., IBMWP, family richness, and IASPT). eDNA samples were collected biweekly from three TRs in Catalonia, NE Spain. Macroinvertebrates were sampled during the three hydrological phases (connected, disconnecting, and disconnected pools). Macroinvertebrate samples were used to identify organisms using morphology and to sequence bulk DNA. eDNA and bulk DNA samples were analysed via

DNA metabarcoding targeting the mitochondrial COI gene. Although communities determined by sediment eDNA did not detect variations in biotic indices (i.e., IBMWP and family richness), the method was useful to detect the replacement of EPT (Ephemeroptera, Plecoptera, Trichoptera) by OCH (Odonata, Coleoptera, Heteroptera). Additionally, sediment eDNA revealed significant impacts of hydrological changes on meiofauna (Ostracoda, Cladocera, Copepoda), a group often overlooked in stream assessments. These results indicate that sediment eDNA metabarcoding can serve as a valuable tool for the bioassessment of TRs, capturing the transitions between hydrological phases while preserving ecosystem integrity.

KEY WORDS: aquatic macroinvertebrates, meiofauna, intermittent rivers, disconnected pools.

RESUMEN

eDNA metabarcoding: un método no invasivo para detectar cambios en las comunidades de ríos temporales.

Los ríos temporales (TRs) son ecosistemas dinámicos que alternan entre fases hidrológicas (flujo, pozas desconectadas y seco). Los TRs son refugios de conservación para especies acuáticas durante la estación seca, pero no se han considerado en los programas de biomonitoreo. Para su evaluación biológica, los métodos morfológicos pueden ser invasivos, eliminando parte de la comunidad y disminuyendo su función como refugio. El ADN ambiental (eDNA) metabarcoding es una alternativa mínimamente invasiva para recopilar información de la comunidad a partir de agua o sedimento. En este estudio probamos la eficiencia del eDNA metabarcoding junto con ADN masivo para caracterizar las comunidades de macroinvertebrados y evaluar la calidad biológica de los TRs en comparación con los métodos morfológicos. Además, exploramos los patrones de la comunidad bentónica mediante el análisis de cambios a lo largo del proceso de desconexión mediante eDNA metabarcoding. La calidad biológica se determinó a través de tres índices de macroinvertebrados (IBMWP, riqueza e IASPT). Las muestras de eDNA se recolectaron cada 15 días en tres TRs en Cataluña. Los macroinvertebrados se muestrearon en tres momentos (río conectado, durante la desconexión y pozas desconectadas). Las muestras de macroinvertebrados fueron identificadas morfológicamente y procesadas junto con el eDNA mediante análisis de metabarcoding del gen COI. Las muestras de eDNA del sedimento presentaron diferencias significativas en relación a los índices IBMWP y riqueza familiar, pero sí detectaron la sustitución de EPT (Ephemeroptera, Plecoptera, Trichoptera) por OCH (Odonata, Coleoptera, Heteroptera). El eDNA del sedimento también reveló impactos significativos de la temporalidad en la meiofauna (Ostracoda, Cladocera, Copepoda), grupo infravalorado en la evaluación del estado ecológico de los arroyos. Nuestro estudio demuestra el eDNA es una herramienta valiosa para la bioevaluación en TRs, capturando las transiciones entre fases hidrológicas y preservando la integridad de los TRs.

PALABRAS CLAVE: *macroinvertebrados acuáticos, meiofauna, ríos intermitentes, pozas desconectadas.*

INTRODUCTION

Temporary rivers (TRs), also known as intermittent rivers and ephemeral streams, represent over 50% of the global river network (Messager et al., 2021). Their global occurrence is expected to increase with climate change and increasing water demand (Datry et al., 2023), affecting freshwater biodiversity and the benefits that humans obtain from rivers (Fovet et al., 2021; Soria et al., 2017; Stubbington et al., 2017). TRs hydrologically shift between flowing, disconnected pools, and dry phases, leading to significant changes in community composition (Gallart et al., 2017). The changes that occur during the formation of disconnected pools and flow resumption are particularly important for freshwater taxa (Drummond et al., 2015) because disconnected pools provide refugia for many aquatic species during the dry season (e.g., Diptera, Odonata, Coleoptera, and Hemiptera; Pineda-Morante et al., 2022). Moreover, these pools are essential for species' survival and the recolonization of the river network once flow resumes (Bonada et al., 2020; Pineda-Morante et al., 2022). Although there is still little information on the role of TRs in biodiversity organization at the regional level, previous studies suggest that metacommunity assembly is governed by taxa replacement, where habitat heterogeneity promotes colonization by new taxa (Crabot et al., 2021).

TRs have been traditionally excluded from biomonitoring programs such as the European Water Framework Directive (WFD) because of their small size and hydrological dynamism (Stubbing-

ton et al., 2018). However, managers are calling for the development of adapted protocols and tools given the ecological importance of these ecosystems and their prevalence in many river networks around the world (Munné et al., 2021). For example, in Spain, some progress has been made in the assessment of the hydrological and biological quality of TRs (Cid et al., 2017; Soria et al., 2020). The Temporary Rivers Ecological and Hydrological Status (TRESH) software (Gallart et al., 2017) is being officially used to evaluate, quantify, and classify the hydrological regimes of Spanish TRs (MITECO, 2020). Yet, no biological indices exist to assess the biological quality of disconnected pools (Bonada et al., 2024; Ersoy et al., 2024). The use of biological indices based in macroinvertebrates is an accepted approach to detect and quantify the effects of anthropogenic activities on aquatic ecosystems, reflecting environmental quality based on the community composition (Bonada et al., 2005). However, several studies have reported poor performance of biological indices based on macroinvertebrates to assess the biological quality of TRs because they have been developed for perennial rivers (Crabot et al., 2021; Ersoy et al., 2024; Soria et al., 2020), suggesting that they can be only used during the flowing phase (Munné & Prat, 2011; Soria et al., 2017, 2020). During the disconnected pool phase, a decline in diversity can occur due to natural factors (i.e., pool size, presence of predators, natural oxygen depletion) even in pristine sites (Bonada et al., 2020). As a result, it is challenging to distinguish between natural and anthropogenic stress when assessing the biological quality of disconnected pools (Bonada et al., 2024; Cid et al., 2020; Crabot et al., 2021).

In a future scenario of more severe and prolonged droughts (Qiu et al., 2022; Naumann et al., 2018), there is a growing need for alternative sampling methods for biomonitoring TRs, and molecular tools are showing great potential (Leese et al., 2016; Múrria et al., 2024). Environmental DNA (eDNA) metabarcoding is a minimally invasive tool to study communities without the collection of specimens (Hering et al., 2018). eDNA captures the DNA from an environmental sample and can serve to characterize the occurrence and distribution of taxa (Taberlet et al., 2012). eDNA methods hold the potential to provide more precise taxonomic information using faster and cheaper procedures than morphological methods (Pawlowski et al., 2018), and to reduce differences in ecosystem assessments between regions (Blancher et al., 2022). However, while eDNA shows promise for rare and threatened species detection and mapping of population distribution (Harper et al., 2018), it still does not meet the legal requirements of the EU's Habitats Directive (Rasmussen et al., 2021) and the WFD because it cannot quantify species' abundances (Hering et al., 2018). Despite these limitations, eDNA has been used in a variety of organisms and facilitated the inter-calibration of biological indices, from marine bacteria (e.g., the microgAMBI index; Aylagas et al., 2021) to freshwater macroinvertebrates (e.g., the Danish riverine faunistic index, the Swiss IBCH index; Brantschen et al., 2021; Kuntke et al., 2020). Furthermore, previous studies on bulk DNA showed that the IBMWP index could be adapted to permanent rivers in Spain (Fernández et al., 2019; Múrria et al., 2024). Within this context, the study of different eDNA methods can be useful for the adaptation of existing indexes (e.g., IBMWP, family richness, IASPT) or the development of new metrics for ecosystems where morphological methods are not fully developed, such as TRs (Blancher et al., 2022). eDNA is particularly interesting for TRs because it can capture the differences in community composition associated with the flowing, disconnected pool, and dry phases with a minimal impact on the biota (Blackman et al., 2021). Studies show a reasonable overlap in taxa detection between molecular and morphological methods (Keck et al. 2022), and fair agreement in biological indices (e.g., Brantschen et al. 2021, Blackman et al. 2024, Múrria et al. 2024). However, empirical studies evaluating the performance of eDNA methods for assessing the biological quality of TRs are still rare.

The aim of this study was to compare the effectiveness of eDNA and bulk DNA methods to characterize macroinvertebrate communities and assess the biological quality of TRs using morphological and molecular methods. We hypothesized that community composition and biological indices would show good overlap among the

four methods (sediment eDNA, water eDNA, bulk DNA, and morphological method), in particular between different types of samples (sediment eDNA, bulk DNA, and morphological sampling) from the same location, as shown by previous studies conducted during flowing conditions (Brantschen et al., 2021; Fernández et al., 2019; Suren et al., 2024). Additionally, we tested whether communities shifted over time using eDNA methods, focusing on the pool disconnection process. We hypothesized that, during the disconnection process, the communities would be characterized by the disappearance of EPT taxa (Ephemeroptera, Plecoptera, Trichoptera) and the appearance of OCH taxa (Odonata, Coleoptera, Hemiptera), driven by habitat changes from riffles to disconnected pools (Bonada et al., 2020; Pineda-Morante et al., 2022).

MATERIAL AND METHODS

Study area

Three TRs in Catalonia (NE Spain), differing in geology and climate characteristics, were selected: Daró with a siliceous geology and dry-Mediterranean climate, Talamanca with a calcareous geology and dry-Mediterranean climate, and Vallcebre with a calcareous geology and humid-Mediterranean climate (Fig. 1). In each river, two pools were identified, which were connected to riffles at the beginning of the sampling period and became disconnected over time. Each sampling point was visited every two weeks from June to October 2020 to sample eDNA, and macroinvertebrate samples were collected three times (during the connected, disconnecting, and disconnected phases). Also, we installed two modified temperature HOBO® sensors per pool, one in a riffle before the pool and another one inside the pool, to register water presence/absence and determine the disconnection time (Chapin et al., 2014).

Sampling

To minimize the sample collection impact on the macroinvertebrate community, samples were only

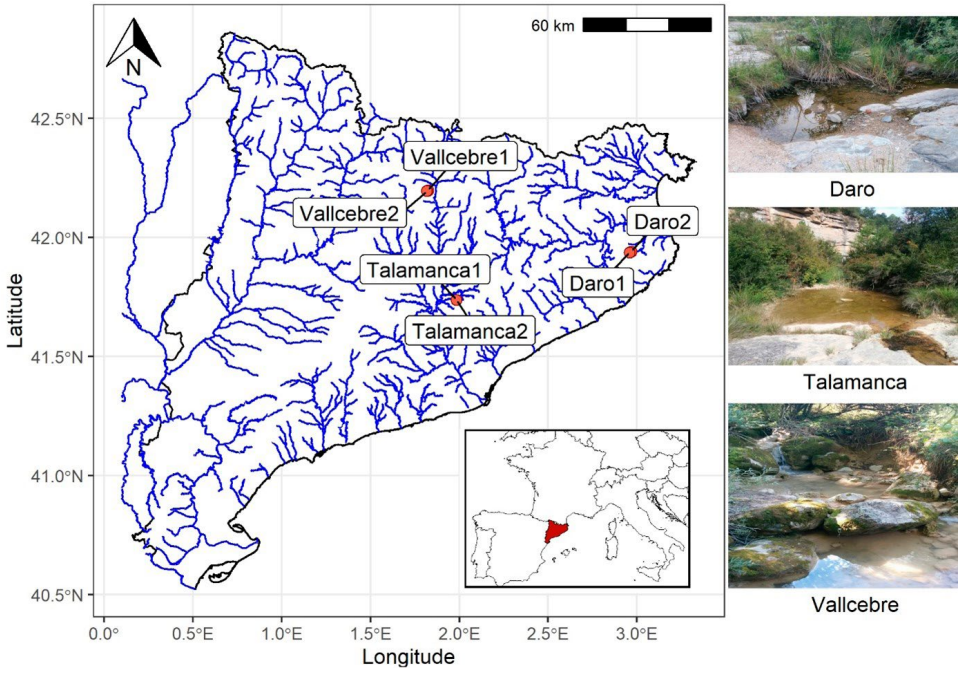

Figure 1. Locations of the disconnected pools sampled across Catalonia, NE Spain. Red dots indicate the locations Vallcebre (1,2), Talamanca (1,2) and Daró (1,2) pools. *Localización de las pozas desconectadas en Cataluña, NE de España. Los puntos rojos indican la localización de las pozas Vallcebre (1,2), Talamanca (1, 2) y Daró (1,2).*

collected three times using a 250 µm mesh size surveys, coinciding with the connected (i.e., flowing), disconnecting (i.e., transitioning from flowing to stagnant), and disconnected pool phases. Samples were collected following standardized protocols for perennial rivers (Jáimez-Cuéllar et al., 2002), but the protocol was adapted following Ersoy et al., (2024) during the disconnected pool phase. Disconnected pool sampling was restricted to half of the pool, leaving the other half unsampled to reduce the impact on the community. The protocol by Jáimez-Cuéllar et al (2002) is notable for its exhaustive and semi-quantitative nature: microhabitats are sampled until no additional taxa are detected. To ensure that this effort was maintained consistently over time, the number of passes through each habitat were recorded to determine the sampling effort per site. Macroinvertebrates were preserved in a 96% not-denatured ethanol solution and transported to the laboratory in a refrigerator. Samples from water and sediment eDNA were collected using bleach-sterilized material and disposable gloves (Bruce et al., 2021, Urycki et al., 2024). Every 15 days between June and October water and sediment samples were collected for eDNA sequencing. One liter of water was collected directly at 10 cm depth of the water column using a plastic bottle, whereas sediments were sampled from the entire surface of the riverbed using sterile syringes (10 mL) and avoiding anoxic zones. Then, eDNA samples were cooled at 4 ºC in the field and preserved at -20ºC until further analyses. To ensure the reliability of the results obtained, a sample of distilled H_2O (dH_2O) was used as negative control and transported during all the sampling procedures in the car and the streams.

Sampling sites located in Vallcebre never got disconnected during the summer of 2020. Thus, the third sampling of macroinvertebrates (i.e., disconnected pool phase) could not be carried out.

Laboratory analyses

For morphological methods we counted the number of individuals and identified them at the family level (except for Oligochaeta and Hydracarina, sub-class and order level, respectively) under a stereomicroscope using standard taxonomic keys (Tachet et al., 2010). We adopted the family level as it is the taxonomic level used in all standardized biological indices in Spain.

Water eDNA and negative control were filtered in the laboratory six months after the sampling period. A 0.22 µm Sterivex-GP Pressure Filter Unit (EMD Millipore, Cat. No: SVGP01050) was used, and the volume filtered varied between 0.2 and 1 L depending on the water turbidity. In parallel, 0.3 g of soil sample were used for the sediment DNA extraction. In addition to water and sediment eDNA samples, once macroinvertebrates were identified, specimens were homogenized and used for molecular analyses as bulk DNA. This bulk DNA sample included a large quantity of DNA extracted from an individual tissue subsample that is a mixture of genetic material from organisms of the entire community. Prior to homogenization, ethanol was removed from bulk samples and the macroinvertebrates were dried using a dehumidification chamber to avoid cross-contamination and homogenized using bleach-sterilized mortar, pestle and liquid nitrogen (N_2). The entire sample was included in the extraction process, as most of the homogenized samples had low weight (less than 0.3 g). All molecular samples (i.e., water eDNA, sediment eDNA, bulk DNA, and sampling negative controls) were extracted without replicates under a UV laminar flow cabin six months after sampling. Contaminations during extraction and amplification were avoided using a UV clean room, bleach, and UV to sterilize all the materials used and the cabin. Individual filtered pipetting tips were also used during all the processes. Extraction and PCR negative controls were held to ensure the reliability of the process. Sediment eDNA and bulk DNA were extracted following commercial instructions of PowerSoil DNA Extraction Kit (PowerSoil, Qiagen), and water eDNA was extracted using PowerWater DNA Extraction Kit (PowerSoil, Qiagen).

The mitochondrial DNA Cytochrome c oxidase I gene (*cox1*, COI) was amplified using 1 µl of each forward and reverse 8-base tagged primers LERAY_XT (Forward-miCOIint-XT: GGWAACWRGWRGRACWITITAYCCYCC; Reverse-jgHCO2198:TAIACYTCIGGRTGICCRAARAAYCA, Wangensteen et al., 2018), 5

µM AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA), 3 µg of bovine serum albumin and 10 ng of purified eDNA/DNA in a total volume of 20 µl per sample. The PCR profile included 10 min at 95°C, 35 cycles of 94°C 1 min, 45°C 1 min and 72°C 1 min, and 5 min at 72°C, following Wangensteen et al., 2018. 8-Base sample tags per sample and 313 bp were used to amplify the extracts, as the long amplicon sequence generated by LERAY_XT primers can improve taxonomic assignment at the species level (Collins et al., 2019) and preliminary studies successfully tested this approach (Fernández et al., 2019; Múrria et al., 2024; Suren et al., 2024). Three 20 µL PCR replicates were analyzed under standard conditions for COI amplifications (Wangensteen et al., 2018) and run in an agarose gel per extraction. All the subsamples from each plate were pooled together, purified, and concentrated using a MinElute PCR purification kit (Qiagen). A Qubit fluorometer was used to quantify DNA concentrations. Three Illumina libraries were built using the Nextflex PCR-free library preparation kit (Perkin-Elmer), which was sequenced in an Illumina MiSeq V3 run using 2 × 250 bp paired-end sequencing (Múrria et al., 2024, Wangensteen et al., 2018).

There was no amplification of water eDNA samples during the PCR process; however, they were also sequenced. Therefore, the results of the water eDNA samples were excluded from the statistical analyses due to their lack of amplification.

Bioinformatics

Sequences were analyzed using the APSCALE_gui (Advanced Pipeline for Simple yet Comprehensive AnaLysEs of DNA metabarcoding data Graphical User Interface) (Macher, 2023). APSCALE_gui is a Graphical User Interface based on Buchner et al. (2022) APSCALE pipeline. VSEARCH (Rognes et al., 2016), Cutadapt (Martin, 2011) and LULU (Frøslev et al., 2017) tools are included on this pipeline. APSCALE is a comprehensive metabarcoding pipeline that automates tasks such as pair-end merging, primer trimming, quality filtering, OTU clustering, denoising and LULU filtering. It includes features such as demultiplexing, dereplication, chimera removal, and re-mapping of Operational Taxonomic Units (OTUs) through a simple single file command line interface. Taxonomy of OTUs was assigned using BOLDigger (Buchner & Leese, 2020). Finally, negative control subtraction and read filter were removed using TaxonTableTools (Macher et al., 2021). Using these tools, different threshold filters were applied to the results to increase reliability. In particular, LULU filtering was used with a 97% similarity threshold (common in LULU) to reduce data noise by identifying and merging rare, similar sequences with more abundant ones, minimizing errors in species identification.

Biotic indices

IBMWP (based on macroinvertebrate richness and the tolerance to organic pollution of each family, Alba-Tercedor et al., 2002), family richness and IASPT (based on the mean tolerance to organic pollution in the macroinvertebrate community, Alba-Tercedor & Sánchez-Ortega, 1988) were used to determine the biological quality (Rico et al., 1992). All biological quality indices were calculated using the "*biomonitoR*" package (Laini et al., 2022) in R version 4.3.1 (R Core Team, 2021).

Statistical analysis

The differences in community composition between methods were assessed through non-metric Multidimensional Scaling (NMDS), using Jaccard dissimilarity matrices based on family presence/absence, and the Analysis of Similarity (ANOSIM), using the "vegan" package (Oksanen et al., 2015). Then, Venn diagrams were plotted using the "ggVennDiagram" package (Gao et al., 2021) to visually assess the overlap of taxa composition across methods. All graphs were produced using the "ggplot2" package (Wickham, 2024). Repeated ANOVA Type III tests for unbalanced and non-independent data were performed to detect differences in biological indices (IBMWP, family richness, IASPT) between methods and time since disconnection using the "car" package (Fox & Weisberg, 2019). The Dunnett's test from the "DescTools" package (Signorell & et al. al., 2017) was used as a non-balanced post-

hoc analysis to test differences between methods, using the morphological method as control.

RESULTS

Metabarcoding analyses generated 7.19 million reads in three libraries. Of these, 6.96 million passed the quality filter. Following bioinformatic processing and cleaning of the raw data with negative controls, 1016 OTUs were obtained. The OTUs sequenced in most of the negative controls showed a low number of reads (between 1 and 15), with only two OTUs presenting a high number of reads (58 and 90). The negative controls of the DNA extraction detected 121 OTUs, most of them with a low number of reads (between 1 and 15) and 4 OTUs with a number of reads ranging between 16 and 51. Lastly, PCR-negative controls yielded 56 OTUs, all of them with a low number of reads (between 1 and 15). These reads were subtracted from all samples to ensure accurate downstream analyses. We detected unequal sequencing depth for the bulk DNA method and the sediment eDNA, with seven times more reads in bulk than in sediment eDNA sequencing (Tables S1 and S2, supplementary information, available at https://www.limnetica.net/en/limnetica). Some OTUs were removed after taxonomic assignment because they were not assigned to freshwater macroinvertebrates (i.e., bacteria, fungi, diatoms), resulting in 489 OTUs. After the filtration process, more than 140 OTUs were assigned to the family Chironomidae, being the most diverse. It was followed by Naididae, Ceratopogoniidae, Cyprididae, Dytiscidae, and Baetidae (Figure S1, supplementary information, available at https://www.limnetica.net/en/limnetica). Caenidae presented the highest percentage of reads, followed by Naididae, Chironomidae, Gomphidae, Simuliidae, Baetidae, and Dytiscideae (Figure S2, supplementary information, available at https://www.limnetica.net/en/limnetica). Furthermore, sediment eDNA detected more families than bulk DNA, and the river with more intermittency (Daró) showed a higher number of taxa than the more permanent one (Vallcebre) (Figure S3, supplementary information, available at https://www.limnetica.net/en/limnetica).

There was a 43% overlap between the morphological and sediment eDNA methods, whereas the morphological and the bulk DNA methods shared 61% of the families (Fig. 2a). Finally, sediment eDNA shared 53% families with the bulk DNA (Fig. 2a). The bulk DNA was the most ef-

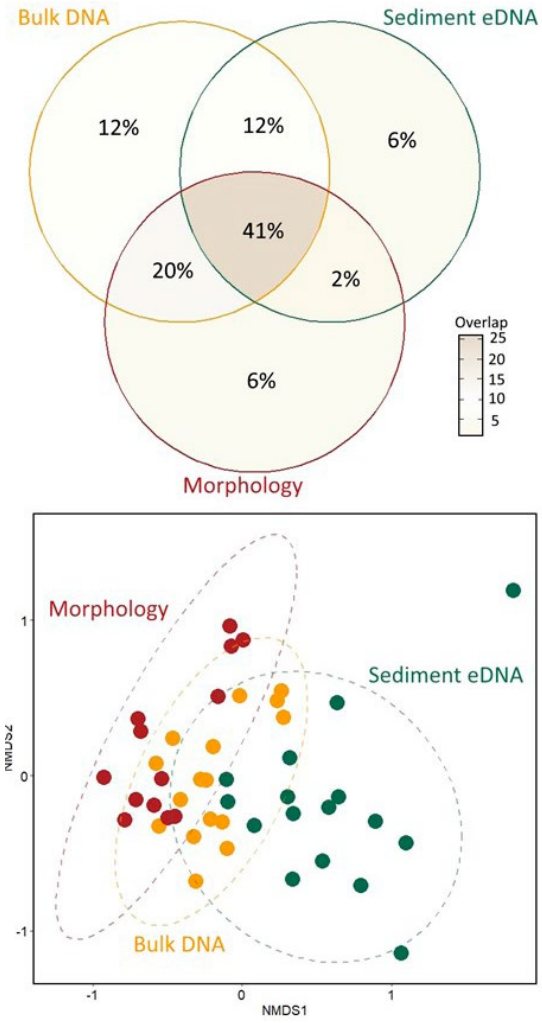

Figure 2. Assessment of effectiveness in capturing community composition among morphological methods (red), bulk DNA (yellow) and sediment eDNA (green) at family level. a) Venn diagram presents 41% of taxa overlap between all methods. b) Non-Metric Multidimensional Scaling (NMDS) based on the Jaccard index show significant differences among methods (ANOSIM R^2 =0.36; *p* value< 0.001). *Evaluación de la eficacia en la captura de la composición de la comunidad entre métodos morfológicos (rojo), ADN masivo (amarillo) y ADNe de sedimento (verde) a nivel de familia. a) El diagrama de Venn presenta un 41% de superposición de taxones entre todos los métodos. b) El Escalamiento Multidimensional No Métrico (NMDS) basado en el índice de Jaccard muestra diferencias significativas entre métodos (ANOSIM R^2 =0,36; valor de* p< *0.001).*

fective method in terms of taxa detection (85% of all detected taxa), followed by the morphological method (69%) and sediment eDNA (61%) (Fig. 2a). According to ANOSIM, there were strong differences between methods in terms of the community composition (p-value=0.001, stress: 0.1348, Fig. 2b).

None of the three calculated indices (IBMWP, family richness, IASPT) responded strongly to the disconnection time (ANOVAs; method: disconnection time, $p>0.1$), but values of family richness and IBMWP differed by sampling method (ANOVAs, method, $p<0.001$; Fig. 3). Particularly, there were differences in family richness and IBMWP between the morphological method and the sediment eDNA (Dunnett's test, $p\leq0.001$; Fig. 3), and no differences between the morphological method and the bulk DNA method (Dunnett's test, $p>0.05$; Fig. 3). Only IASPT showed no differences across the three methods.

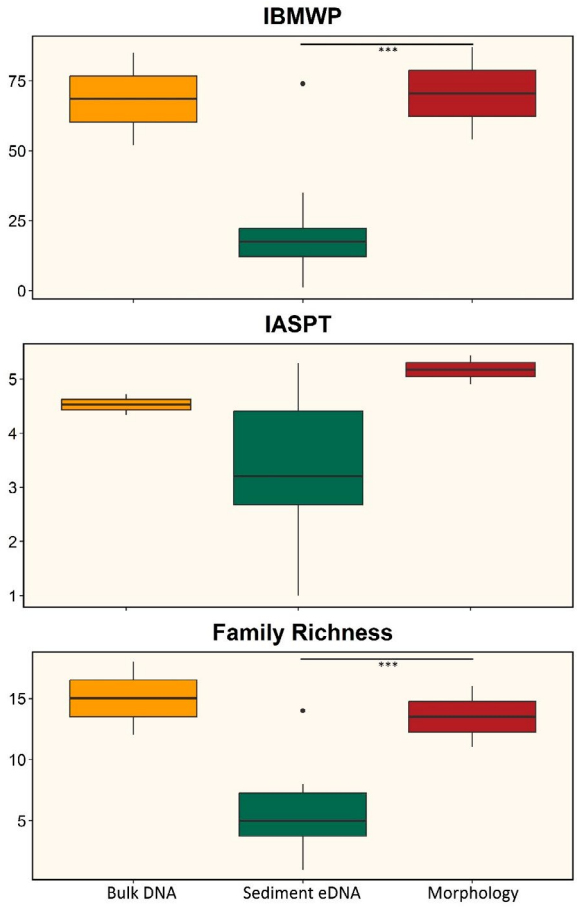

Figure 3. Boxplot for biological indices of macroinvertebrates (IBMWP, family richness, and IASPT) concerning the different methods: morphological methods (red), bulk DNA (yellow), and sediment eDNA (green). The median values (central dark line), 25th and 75th percentile values (box), and the maximum and minimum values are shown. (***) Post-hoc p value<0.001. *Diagrama de cajas para los índices biológicos de macroinvertebrados (IBMWP, riqueza familiar e IASPT) relacionados con los diferentes métodos: métodos morfológicos (rojo), ADN masivo (amarillo) y ADNe de sedimentos (verde). Se muestran los valores de la mediana (línea oscura central), los valores de los percentiles 25 y 75 (cuadro) y los valores máximo y mínimo. (***) Valor* p *post-hoc<0.001.*

Sediment eDNA samples presented strong differences in community composition between the two rivers that had disconnected pools (Daró and Talamanca) and Vallcebre, which never got disconnected (Fig. 4). These differences were not only due to habitat disconnection but also to differences in the sequencing depth across sampling sites. The detected OTUs were grouped into four taxa categories: EPT (Ephemeroptera, Plecoptera, Trichoptera), GOLD (Gasteropoda, OLigochaeta and Diptera), OCC (Ostracoda, Cladocera, Copepoda), and OCH (Odonata, Coleoptera, Hemiptera). EPT taxa were most abundant in July and August, gradually declining as habitat conditions shifted, signaling their sensitivity to flow changes. Conversely, OCH taxa increased in September and October, reflecting their ability to thrive under lentic conditions as habitats evolved. GOLD were abundant across the sampling period, especially in Daró 1 and 2, indicating their stability within these habitats. OCC taxa followed a similar pattern to OCH, with their presence increasing in September and October. On the contrary, in Vallcebre, where the river is permanently connected, OCC remained low and consistently present.

DISCUSSION

The different molecular methods tested yielded different results in terms of community composition. Although the communities identified by the bulk DNA and the morphological methods were highly similar, sediment eDNA failed to detect 26 % of taxa detected by them. This is likely due to the sequencing depth of eDNA samples collected from the sediment, up to seven times lower than in bulk samples. This aligns with findings from other studies, which report the complementarity

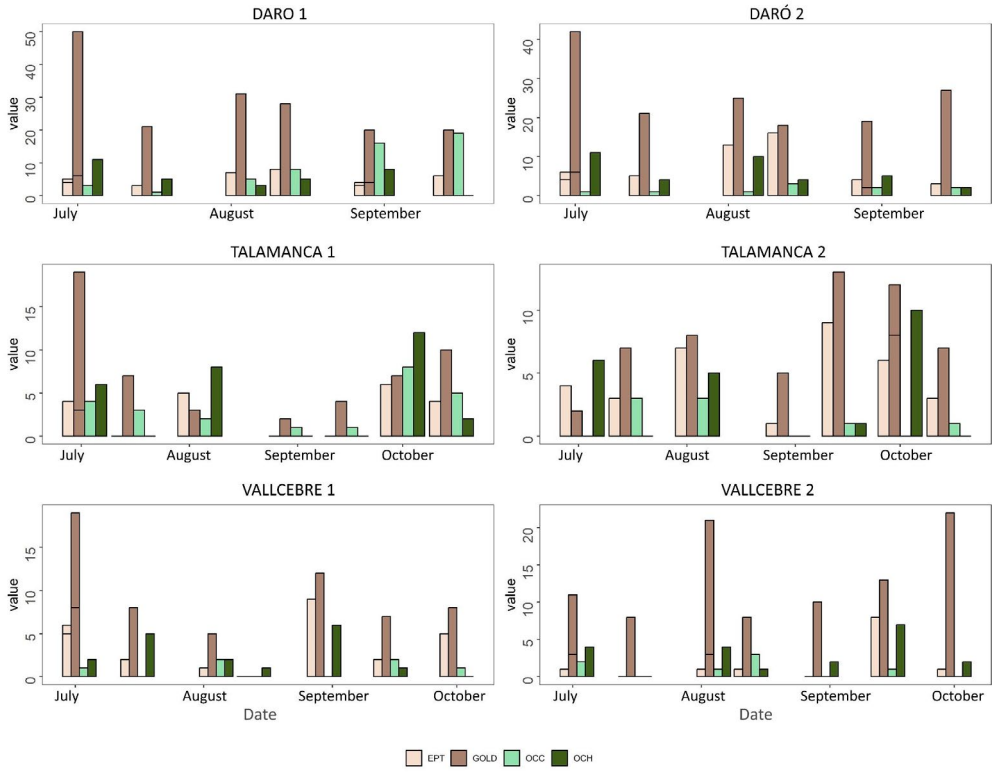

Figure 4. Temporal change in taxonomic composition of sediment eDNA at six pools every 15 days from June to October: Daró 1 and 2 (siliceous-dry Mediterranean climate), Talamanca 1 and 2 (calcareous-dry Mediterranean climate) and Vallcebre (calcareous-humid Mediterranean climate). *Cambio temporal en la composición taxonómica del eDNA de sedimento en seis pozas desconectadas, muestreadas cada 15 días entre junio y octubre: Daró 1 y 2 (clima mediterráneo seco-silíceo), Talamanca 1 y 2 (clima mediterráneo seco-calcáreo) y Vallcebre (clima mediterráneo calcáreo-húmedo)*. EPT (Ephemeroptera, Plecoptera y Trichoptera taxa); GOLD (Gasteropoda, Oligochaeta y Dipera taxa); OCC (Ostracoda, Cladocera y Copepoda taxa); OCH (Odonata, Coleoptera y Heteroptera taxa).

between morphological methods and eDNA approaches (Keck et al., 2022, Múrria et al., 2024). We also found differences in IBMWP and family richness between methods (especially for sediment eDNA). On the contrary, for IASTP, there were no significant discrepancies between methods. This could be explained by the differences in the design of the IASPT and IBMWP indices. As shown by Zamora-Muñoz et al. (1995), the IBMWP index is more dependent on sampling effort than the IASPT, which focuses on the average sensitivity of macroinvertebrate communities to organic pollution. The bulk DNA metabarcoding enables the identification of macroinvertebrate communities with a degree of accuracy comparable to that achieved by morphological methods. However, the intrinsic constraints inherent to bulk DNA methodology (e.g., destructive, time-consuming Mächler et al., 2014) limit its application in TR biomonitoring. Despite capturing a lower number of taxa, eDNA enabled the detection of complementary communities coexisting with macroinvertebrates in TRs during pool disconnection. Consequently, the taxa captured by sediment eDNA provided useful information for the calculation of the IASPT index. It is important to notice that morphological biological indexes (i.e., IBMWP, family richness, and IASPT) are not designed to be used in TRs, and their efficiency in detecting anthropogenic impacts is limited (Ersoy et al., 2024).

Our results on the effect of the disconnection process derived from eDNA methodologies agree with studies that identified macroinvertebrates using morphological methods. As shown by others, the decline of EPT taxa over disconnection time suggests that many of these taxa lack the strategies to cope with drying conditions (Soria

et al., 2020; Bonada et al., 2020). In contrast, the increase of OCH and OCC taxa supports the idea that certain groups are resistant to hydrological fluctuations (Crabot et al., 2021; Soria et al., 2017). Many Ostracoda and Cladocera are commonly found in temporary water bodies (Aguilar-Alberola & Mesquita-Joanes, 2011; Boix et al., 2016). Concordantly, in Vallcebre, where hydrological conditions remained stable, OCC taxa were present in lower abundances. Overall, our results support the use of eDNA for assessing community dynamics in TRs, providing a complementary approach to morphological methods.

Meiofauna (that include a large proportion of OCC taxa; Bonada & Bogan, 2024) is typically under-represented in biomonitoring programs (Stubbington, et al., 2017). However, they constitute an important component of the benthic fauna in TRs (Bonada & Bogan, 2024). Thus, the combination and complementarity of macroinvertebrate and meiofauna in biotic indices (e.g., Boix et al., 2005; Jiménez Palomar, 2012) could provide a significant improvement for biomonitoring disconnected pools. In this regard, eDNA offers a cost-effective approach that should be further explored (Bonada et al., 2024).

Despite the advantages of using eDNA methods in TRs, our results suggest several challenges when applied to the disconnected pools phase. Firstly, natural abiotic and biotic factors may affect the concentration of DNA in TRs, thereby affecting its detection by sequencing technologies, especially for water eDNA. Abiotic factors such as UV-B, pH, salinity, temperature, or the increase of microbes activity and DNases production (Collins et al., 2018; Fernández et al., 2019, URycki et al., 2024), affect TRs during the disconnection process and could result in fast eDNA degradation (Strickler et al., 2015). Moreover, low metabolic activity (e.g., passive movement) and small body size or abundance of some taxa may cause differences in the detection of macroinvertebrate families (Joseph et al., 2022; Strickler et al., 2015). Secondly, the effectiveness of eDNA methods can be highly dependent on the methodology used (Blancher et al., 2022; Dickie et al., 2018). For example, sediment eDNA has lower degradation rates, higher concentrations, and is more resistant to degradation than water eDNA (Bruce et al., 2021). However, sediment eDNA often has higher concentrations of PCR-inhibiting compounds (e.g., humic acids and tanning agents), which are commonly removed using specific DNA extraction methods (Collins et al., 2018). Thirdly, other methodological factors related to sample processing could harm eDNA detection. For example, the absence of water eDNA amplification in our results could be due to transportation, filtration techniques, insufficient preservation, or insufficient volume of water filtered (Bruce et al., 2021, Urycki et al., 2024). While we did not filter the samples on the same day of collection, it is highly recommended to do so to prevent DNA degradation (Goldberg et al., 2016). The longer the time between sampling and filtering, even if samples are frozen, the higher the water eDNA degradation (Yamanaka et al., 2016). We also did not normalize the DNA concentrations of the samples we analyzed, which should be considered in the future to maximize the comparability of the results. Finally, target primers are being designed to optimize the identification of macroinvertebrates and prepared for their use in standardized biomonitoring programs. For example, the use of freshwater primers (fwhF2-EPT-DR2N, 142 bp) may decrease non-target sequences by 99% (Leese et al., 2021), yet it also limits the number of detectable invertebrate taxa due to the lower degeneracy.

In conclusion, sediment eDNA captured fewer taxa compared to morphological and bulk DNA metabarcoding methods, likely due to methodological constraints, particularly the low sequencing depths per sample. Despite this limitation, the information gathered was useful for calculating the IASPT index. Also, the utilization of eDNA from sediments enabled the identification of meiofauna, a biological group that has been largely overlooked in conventional monitoring programs. While eDNA offers significant advantages for monitoring TRs, the presence of abiotic factors (e.g., UV-B, pH, salinity, temperature) and biological factors (microbial activity) during the disconnection phase can impede its detection. Our study highlights the potential of eDNA methods to enhance bioassessment in TRs and deepen our understanding of community dynamics in these ecosystems.

ACKNOWLEDGMENTS

This study was conducted by the FEHM research group (Freshwater Ecology, Hydrology, and Management) and supported by the TRivers-P project (ACA210/18/00022), funded by the Catalan Water Agency; as well as the DRY-Guadalmed project (PID2021-126143OB-C21), funded by the Spanish Ministry of Science, Innovation, and Universities – Agencia Estatal de Investigación and co-financed by ERDF (A Way of Making Europe). With the support of the Industrial Doctorates Plan of the Department of Research and Universities of the Generalitat de Catalunya (DI21-0001), awarded to Nieves López-Rodríguez, Núria Bonada, and the company Eurofins-Cavendish S.L.U. We also thank the two anonymous reviewers for their constructive feedback.

AUTHOR CONTRIBITUTIONS

N.L.R.: Conceptualization, Data curation, Software, Formal analysis, Visualization, Writing: original draft; D.B.: Data curation, Software, Writing: review; M.C.A.: Formal analysis, Visualization, Writing: review; R. F.: Data curation, Methodology, Writing: review; F.L.: Data curation, Software, Supervision, Writing: review; C.M.: Conceptualization, Methodology, Writing: review; N.B.**: Conceptualization, Project administration, Acquisition of funds, Methodology, Supervision, Writing: review; Z.E.**: Conceptualization, Data curation, Formal analysis, Visualization, Supervision, Writing: review.
(**equal contribution)

BIBLIOGRAPHY

Alba-Tercedor, J. & Sánchez - Ortega, A. (1988). Un método rápido y simple para evaluar la calidad biológica de las aguas corrientes basado en el de Hellawell (1978). *Limnetica*, 4(1), 51-66. DOI: 10.23818/limn.04.06

Alba-Tercedor, J., Jáimez-Cuéllar, P., Álvarez, M., Avilés, J., Bonada, N., Casas, J., Mellado, A., Ortega, M., Pardo, I., Prat, N., Rieradevall, M., Robles, S., Sáinz-Cantero, C.E., Sánchez-Ortega, A., Suárez, M.L., Toro, M., Vidal-Albarca, M.R., Vivas, S., Zamora-Muñoz, C. (2002). Caracterización del estado ecológico de ríos mediterráneos ibéricos mediante el índice IBMWP (antes BMWP'). *Limnetica*, 21(2), 175-185. DOI: 10.23818/limn.21.24

Aguilar-Alberola, J.A. & Mesquita-Joanes, F. (2011). Population dynamics and tolerance to desiccation in a crustacean ostracod adapted to life in small ephemeral water bodies. *Limnologica*, 41 (4), 348-355. DOI: 10.1016/j.limno.2011.03.003.

Aylagas, E., Atalah, J., Sánchez-Jerez, P., Pearman, J. K., Casado, N., Asensi, J., Toledo-Guedes, K., & Carvalho, S. (2021). A step towards the validation of bacteria biotic indices using DNA metabarcoding for benthic monitoring. *Molecular Ecology Resources*, 21(6), 1889-1903. DOI: 10.1111/1755-0998.13395.

Blackman, R. C., Altermatt, F., Foulquier, A., Lefébure, T., Gauthier, M., Bouchez, A., Stubbington, R., Weigand, A. M., Leese, F., & Datry, T. (2021). Unlocking our understanding of intermittent rivers and ephemeral streams with genomic tools. *Frontiers in Ecology and the Environment*, 19(10), 574-583. DOI: 10.1002/fee.2404

Blackman, R. C., Carraro, L., Keck, F., & Altermatt, F. (2024). Measuring the state of aquatic environments using eDNA—upscaling spatial resolution of biotic indices. *Philosophical Transactions of The Royal Society B Biological Sciences*, 379(1904). DOI: 10.1098/rstb.2023.0121

Blancher, P., Lefrançois, E., Rimet, F., Vasselon, V., Argillier, C., Arle, J., Beja, P., Boets, P., Boughaba, J., Chauvin, C., Deacon, M., Duncan, W., Ejdung, G., Erba, S., Ferrari, B., Fischer, H., Hänfling, B., Haldin, M., Hering, D., … Bouchez, A. (2022). A strategy for successful integration of DNA-based methods in aquatic monitoring. *Metabarcoding and Metagenomics*, 6, e85652. DOI: 10.3897/mbmg.6.85652

Boix, D., Gascón, S., Sala, J., Martinoy, M., Gifre, J., & Quintana, X. D. (2005). A new index of water quality assessment in Mediterranean wetlands based on crustacean and insect assemblages: the case of Catalunya (NE Iberi-

an peninsula). *Aquatic Conservation: Marine and Freshwater Ecosystems*, 15(6), 635-651.

Boix, D., Kneitel, J., Robson, B. J., Duchet, C., Zúñiga, L., Day, J., Gascón, S., Sala, J., Quintana, X. D., & Blaustein, L. (2016). Invertebrates of Freshwater Temporary Ponds in Mediterranean Climates. *En Springer eBooks* (pp. 141-189). DOI: 10.1007/978-3-319-24978-0_5

Bonada, N., & Bogan, M. T. (2024). Chapter 21-Benthic Animals. En I. D. Jones & J. P. Smol (Eds.), *Wetzel's Limnology* (Fourth Edition) (pp. 621-655). Academic Press. DOI: 10.1016/B978-0-12-822701-5.00021-5

Bonada, N., Cañedo-Argüelles, M., Gallart, F., von Schiller, D., Fortuño, P., Latron, J., Llorens, P., Múrria, C., Soria, M., Vinyoles, D., & Cid, N. (2020). Conservation and Management of Isolated Pools in Temporary Rivers. *Water*, 12(10), Article 10. DOI: 10.3390/w12102870

Bonada, N., Montoya, M. M. S., Cid, N., Acosta, R., Arce, M. I., Cañedo-Argüelles, M., Cunillera-Montcusí, D., Heras, P. de las, Díaz-Redondo, M., Ersoy, Z., Fernández-Calero, J. M., Arnau, N. F., Fortuño, P., Freixinos, Z., Gomà, J., López-Pintor, A., López-Rodríguez, M. J., López-Rodríguez, N., Mellado-Díaz, A., … Prat, N. (2024). Retos y oportunidades para la evaluación del estado ecológico en ríos temporales. *Ecosistemas*, 2655-2655. DOI: 10.7818/ECOS.2655

Bonada, N., Prat, N., Resh, V. H., & Statzner, B. (2005). DEVELOPMENTS IN AQUATIC INSECT BIOMONITORING: A Comparative Analysis of Recent Approaches. *Annual Review Of Entomology*, 51(1), 495-523. DOI: 10.1146/annurev.ento.51.110104.151124

Brantschen, J., Blackman, R. C., Walser, J.-C., & Altermatt, F. (2021). Environmental DNA gives comparable results to morphology-based indices of macroinvertebrates in a large-scale ecological assessment. *PLOS ONE*, 16(9), e0257510. DOI: 10.1371/journal.pone.0257510

Bruce, K., Blackman, R., Bourlat, S. J., Hellström, A. M., Bakker, J., Bista, I., Bohmann, K., Bouchez, A., Brys, R., Clark, K., Elbrecht, V., Fazi, S., Fonseca, V., Hänfling, B., Leese, F., Mächler, E., Mahon, A. R., Meissner, K., Panksep, K., … Deiner, K. (2021). A practical guide to DNA-based methods for biodiversity assessment. *Advanced Books*, 1, e68634. DOI: 10.3897/ab.e68634

Buchner, D., & Leese, F. (2020). BOLDigger – a Python package to identify and organize sequences with the Barcode of Life Data systems. *Metabarcoding and Metagenomics*, 4, e53535. DOI: 10.3897/mbmg.4.53535

Buchner, D., Macher, T.-H., & Leese, F. (2022). APSCALE: Advanced pipeline for simple yet comprehensive analyses of DNA metabarcoding data. *Bioinformatics*, 38(20), 4817-4819. DOI: 10.1093/bioinformatics/btac588

Chapin, T. P., Todd, A. S., & Zeigler, M. P. (2014). Robust, low-cost data loggers for stream temperature, flow intermittency, and relative conductivity monitoring. *Water Resources Research*, 50(8), 6542-6548. DOI: 10.1002/2013WR015158

Cid, N., Bonada, N., Carlson, S. M., Grantham, T. E., Gasith, A., & Resh, V. H. (2017). High Variability Is a Defining Component of Mediterranean-Climate Rivers and Their Biota. *Water*, 9(1), Article 1. DOI: 10.3390/w9010052

Cid, N., Bonada, N., Heino, J., Cañedo-Argüelles, M., Crabot, J., Sarremejane, R., Soininen, J., Stubbington, R., & Datry, T. (2020). A Metacommunity Approach to Improve Biological Assessments in Highly Dynamic Freshwater Ecosystems. *BioScience*, 70(5), 427-438. DOI: 10.1093/biosci/biaa033

Collins, R. A., Bakker, J., Wangensteen, O. S., Soto, A. Z., Corrigan, L., Sims, D. W., Genner, M. J., & Mariani, S. (2019). Non-specific amplification compromises environmental DNA metabarcoding with COI. *Methods in Ecology and Evolution*, 10(11), 1985-2001. DOI: 10.1111/2041-210X.13276

Collins, R. A., Wangensteen, O. S., O'Gorman, E. J., Mariani, S., Sims, D. W., & Genner, M. J. (2018). Persistence of environmental DNA in marine systems. *Communications Biology*, 1(1), 1-11. DOI: 10.1038/s42003-018-0192-6

Crabot, J., Polášek, M., Launay, B., Pařil, P., & Datry, T. (2020). Drying in newly intermittent rivers leads to higher variability of invertebrate communities. *Freshwater Biology*,

66(4), 730-744. DOI: 10.1111/fwb.13673

Crabot, J., Dolédec, S., Forcellini, M., & Datry, T. (2021). The efficiency of invertebrate-based bioassessment for evaluating the ecological status of streams along a gradient of flow intermittence. *Ecological Indicators*, 133, 108440. DOI: 10.1016/j.ecolind.2021.108440

Datry, T., Boulton, A. J., Fritz, K., Stubbington, R., Cid, N., Crabot, J., & Tockner, K. (2023). Non-perennial segments in river networks. *Nature Reviews Earth & Environment*, 4(12), 815-830. DOI: 10.1038/s43017-023-00495-w

Dickie, I. A., Boyer, S., Buckley, H. L., Duncan, R. P., Gardner, P. P., Hogg, I. D., Holdaway, R. J., Lear, G., Makiola, A., Morales, S. E., Powell, J. R., & Weaver, L. (2018). Towards robust and repeatable sampling methods in eDNA-based studies. *Molecular Ecology Resources*, 18(5), 940-952. DOI: 10.1111/1755-0998.12907

Drummond, L. R., McIntosh, A. R., & Larned, S. T. (2015). Invertebrate community dynamics and insect emergence in response to pool drying in a temporary river. *Freshwater Biology*, 60(8), 1596-1612. DOI: 10.1111/fwb.12591

Ersoy, Z., López-Rodríguez, N., Acosta, R., Soria, M., Gomà, J., Gallart, F., Múrria, C., Latron, J., Llorens, P., Fortuño, P., Quevedo-Ortiz, G., Cid, N., Prat, N., Cañedo-Argüelles, M., & Bonada, N. (2024). Evaluating the response of current biotic indices and functional metrics to natural and anthropogenic predictors in disconnected pools of temporary rivers. *The Science Of The Total Environment*, 948, 174825. DOI: 10.1016/j.scitotenv.2024.174825

Fernández, S., Rodríguez-Martínez, S., Martínez, J. L., Garcia-Vazquez, E., & Ardura, A. (2019). How can eDNA contribute in riverine macroinvertebrate assessment? A metabarcoding approach in the Nalón River (Asturias, Northern Spain). *Environmental DNA*, 1(4), 385-401. DOI: 10.1002/edn3.40

Fovet, O., Belemtougri, A., Boithias, L., Braud, I., Charlier, J.-B., Cottet, M., Daudin, K., Dramais, G., Ducharne, A., Folton, N., Grippa, M., Hector, B., Kuppel, S., Le Coz, J., Legal, L., Martin, P., Moatar, F., Molénat, J., Probst, A., … Datry, T. (2021). Intermittent rivers and ephemeral streams: Perspectives for critical zone science and research on socio-ecosystems. *WIREs Water*, 8(4), e1523. DOI: 10.1002/wat2.1523

Fox, J., & Weisberg, S. (2019). An {R} companion to applied regression (3rd ed.). Sage. https://cran.r-project.org/package=car

Frøslev, T. G., Kjøller, R., Bruun, H. H., Ejrnæs, R., Brunbjerg, A. K., Pietroni, C., & Hansen, A. J. (2017). Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. *Nature Communications*, 8(1), 1188. DOI: 10.1038/s41467-017-01312-x

Gallart, F., Cid, N., Latron, J., Llorens, P., Bonada, N., Jeuffroy, J., Jiménez-Argudo, S.-M., Vega, R.-M., Solà, C., Soria, M., Bardina, M., Hernández-Casahuga, A.-J., Fidalgo, A., Estrela, T., Munné, A., & Prat, N. (2017). TREHS: An open-access software tool for investigating and evaluating temporary river regimes as a first step for their ecological status assessment. *Science of The Total Environment*, 607-608, 519-540. DOI: 10.1016/j.scitotenv.2017.06.209

Gao, C.-H., Yu, G., & Cai, P. (2021). ggVennDiagram: An Intuitive, Easy-to-Use, and Highly Customizable R Package to Generate Venn Diagram. Frontiers in Genetics, 12. DOI: 10.3389/fgene.2021.706907

Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., Spear, S. F., McKee, A., Oyler-McCance, S. J., Cornman, R. S., Laramie, M. B., Mahon, A. R., Lance, R. F., Pilliod, D. S., Strickler, K. M., Waits, L. P., Fremier, A. K., Takahara, T., Herder, J. E., & Taberlet, P. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution*, 7(11), 1299-1307. DOI: 10.1111/2041-210X.12595

Harper, L. R., Buxton, A. S., Rees, H. C., Bruce, K., Brys, R., Halfmaerten, D., Read, D. S., Watson, H. V., Sayer, C. D., Jones, E. P., Priestley, V., Mächler, E., Múrria, C., Garcés-Pastor, S., Medupin, C., Burgess, K., Benson, G., Boonham, N., Griffiths, R. A., . . . Hänfling, B. (2018). Prospects and challenges of environmental DNA (eDNA) monitoring in freshwater ponds. *Hydrobiologia*, 826(1), 25-41. DOI:

10.1007/s10750-018-3750-5

Hering, D., Borja, A., Jones, J. I., Pont, D., Boets, P., Bouchez, A., Bruce, K., Drakare, S., Hänfling, B., Kahlert, M., Leese, F., Meissner, K., Mergen, P., Reyjol, Y., Segurado, P., Vogler, A., & Kelly, M. (2018). Implementation options for DNA-based identification into ecological status assessment under the European Water Framework Directive. *Water Research*, 138, 192-205. DOI: 10.1016/j.watres.2018.03.003

Hill, M. J., Heino, J., Thornhill, I., Ryves, D. B., & Wood, P. J. (2017b). Effects of dispersal mode on the environmental and spatial correlates of nestedness and species turnover in pond communities. *Oikos*, 126(11), 1575-1585. DOI: 10.1111/oik.04266

Jáimez-Cuéllar, P., Vivas, S., Bonada, N., Robles, S., Mellado, A., Álvarez, M., Avilés, J., Casas, J., Ortega, M., Pardo, I., Prat, N., Rieradevall, M., Sáinz-Cantero, C.E., Sánchez-Ortega, A., Suárez, M.L., Toro, M., Vidal-Albarca, M.R.,Zamora-Muñoz, C., Alba-Tercedor, J. (2002). Protocolo GUADALMED (PRECE). *Limnetica*, 21(2), 187-204. DOI: 10.23818/limn.21.25

Jiménez Palomar, T. (2012). IBCAEL: Evaluación del estado ecológico de los lagos españoles mediante invertebrados bentónicos. Màster Oficial - Aigua. Anàlisi Interdisciplinària i Gestió Sostenible. https://diposit.ub.edu/dspace/handle/2445/22265

Joseph, C., Faiq, M. E., Li, Z., & Chen, G. (2022). Persistence and degradation dynamics of eDNA affected by environmental factors in aquatic ecosystems. *Hydrobiologia*, 849(19), 4119-4133. DOI: 10.1007/s10750-022-04959-w

Keck, F., Blackman, R. C., Bossart, R., Brantschen, J., Couton, M., Hürlemann, S., Kirschner, D., Locher, N., Zhang, H., & Altermatt, F. (2022b). Meta-analysis shows both congruence and complementarity of DNA and eDNA metabarcoding to traditional methods for biological community assessment. *Molecular Ecology*, 31(6), 1820-1835. DOI: 10.1111/mec.16364

Kuntke, F., de Jonge, N., Hesselsøe, M., & Lund Nielsen, J. (2020). Stream water quality assessment by metabarcoding of invertebrates. *Ecological Indicators*, 111, 105982. DOI: 10.1016/j.ecolind.2019.105982

Laini, A., Guareschi, S., Bolpagni, R., Burgazzi, G., Bruno, D., Gutiérrez-Cánovas, C., Miranda, R., Mondy, C., Várbíró, G., & Cancellario, T. (2022). biomonitoR: An R package for managing ecological data and calculating biomonitoring indices. *PeerJ*, 10, e14183. DOI: 10.7717/peerj.14183

Larned, S. T., Datry, T., Arscott, D. B., & Tockner, K. (2010). Emerging concepts in temporary-river ecology. *Freshwater Biology*, 55(4), 717-738. DOI: 10.1111/j.1365-2427.2009.02322.x

Leese, F., Altermatt, F., Bouchez, A., Ekrem, T., Hering, D., Meissner, K., Mergen, P., Pawlowski, J., Piggott, J., Rimet, F., Steinke, D., Taberlet, P., Weigand, A., Abarenkov, K., Beja, P., Bervoets, L., Björnsdóttir, S., Boets, P., Boggero, A., … Zimmermann, J. (2016). DNAqua-Net: Developing new genetic tools for bioassessment and monitoring of aquatic ecosystems in Europe. *Research Ideas and Outcomes*, 2, e11321. DOI: 10.3897/rio.2.e11321

Leese, F., Sander, M., Buchner, D., Elbrecht, V., Haase, P., & Zizka, V. M. A. (2021). Improved freshwater macroinvertebrate detection from environmental DNA through minimized nontarget amplification. *Environmental DNA*, 3(1), 261-276. DOI: 10.1002/edn3.177

Leigh, C., Bonada, N., Boulton, A. J., Hugueny, B., Larned, S. T., Vorste, R. V., & Datry, T. (2015). Invertebrate assemblage responses and the dual roles of resistance and resilience to drying in intermittent rivers. *Aquatic Sciences*, 78(2), 291-301. DOI: 10.1007/s00027-015-0427-2

Mächler, E., Deiner, K., Steinmann, P., & Altermatt, F. (2014). Utility of environmental DNA for monitoring rare and indicator macroinvertebrate species. *Freshwater Science*, 33(4), 1174-1183. DOI: 10.1086/678128

Macher, T. (2023). *Apscale_GUI [Python]*. https://github.com/TillMacher/apscale_gui (Obra original publicada en 2021)

Macher, T.-H., Beermann, A. J., & Leese, F. (2021). TaxonTableTools: A comprehensive, platform-independent graphical user interface software to explore and visualise DNA metabarcoding data. *Molecular Ecology Resourc-*

es, 21(5), 1705-1714. DOI: 10.1111/1755-0998.13358

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, 17(1), Article 1. DOI: 10.14806/ej.17.1.200

Messager, M. L., Lehner, B., Cockburn, C., Lamouroux, N., Pella, H., Snelder, T., Tockner, K., Trautmann, T., Watt, C., & Datry, T. (2021). Global prevalence of non-perennial rivers and streams. *Nature*, 594(7863), 391-397. DOI: 10.1038/s41586-021-03565-5

MITECO. (2020). *Guía para la evaluación del estado de las aguas superficiales y subterráneas. Ministerio para la Transición Ecológica y el Reto Demográfico*. Madrid, España. .Pp 403. Disponible en: Https://www.miteco.gob.es/es/agua/temas/estado-y-calidad-de-las-aguas/medidatomas-muestras.html.

Munné, A., Bonada, N., Cid, N., Gallart, F., Solà, C., Bardina, M., Rovira, A., Sierra, C., Soria, M., Fortuño, P., Llorens, P., Latron, J., Estrela, T., Fidalgo, A., Serrano, I., Jiménez, S., Vega, R., & Prat, N. (2021). A Proposal to Classify and Assess Ecological Status in Mediterranean Temporary Rivers: Research Insights to Solve Management Needs. *Water*, 13(6), Article 6. DOI: 10.3390/w13060767

Munné, A., & Prat, N. (2011). Effects of Mediterranean climate annual variability on stream biological quality assessment using macroinvertebrate communities. E*cological Indicators*, 11(2), 651-662. DOI: 10.1016/j.ecolind.2010.09.004

Múrria, C., Wangensteen, O. S., Somma, S., Väisänen, L., Fortuño, P., Arnedo, M. A., & Prat, N. (2024). Taxonomic accuracy and complementarity between bulk and eDNA metabarcoding provides an alternative to morphology for biological assessment of freshwater macroinvertebrates. *The Science Of The Total Environment*, 935, 173243. DOI: 10.1016/j.scitotenv.2024.173243

Naumann, G., Alfieri, L., Wyser, K., Mentaschi, L., Betts, R. A., Carrao, H., Spinoni, J., Vogt, J., & Feyen, L. (2018). Global Changes in Drought Conditions Under Different Levels of Warming. *Geophysical Research Letters*, 45(7), 3285-3296. DOI: 10.1002/2017gl076521

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P., O'Hara, B., Simpson, G., Solymos, P., Stevens, H., & Wagner, H. (2015). *Vegan: Community Ecology Package*. R Package Version 2.2-1, 2, 1-2.

Pawlowski, J., Kelly-Quinn, M., Altermatt, F., Apothéloz-Perret-Gentil, L., Beja, P., Boggero, A., Borja, A., Bouchez, A., Cordier, T., Domaizon, I., Feio, M. J., Filipe, A. F., Fornaroli, R., Graf, W., Herder, J., van der Hoorn, B., Iwan Jones, J., Sagova-Mareckova, M., Moritz, C., … Kahlert, M. (2018). The future of biotic indices in the ecogenomic era: Integrating (e)DNA metabarcoding in biological assessment of aquatic ecosystems. *Science of The Total Environment*, 637-638, 1295-1310. DOI: 10.1016/j.scitotenv.2018.05.002

Pineda-Morante, D., Fernández-Calero, J. M., Pölsterl, S., Cunillera-Montcusí, D., Bonada, N., & Cañedo-Argüelles, M. (2022). Local hydrological conditions and spatial connectivity shape invertebrate communities after rewetting in temporary rivers. *Hydrobiologia*, 849(6), 1511-1530. DOI: 10.1007/s10750-022-04799-8

Qiu, J., Shen, Z., & Xie, H. (2022). Drought impacts on hydrology and water quality under climate change. *The Science Of The Total Environment*, 858, 159854. DOI: 10.1016/j.scitotenv.2022.159854

R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. DOI: 10.59350/t79xt-tf203

Rasmussen, J. J., Andersen, L. W., Johnsen, T. J., Thaulow, J., d'Auriac, M. A., Thomsen, S. N., & Hesselsøe, M. (2021). Dead or alive—Old empty shells do not prompt false-positive results in environmental DNA surveys targeting the freshwater pearl mussel (*Margaritifera margaritifera* L.). *Aquatic Conservation: Marine and Freshwater Ecosystems*, 31(9), 2506-2514. DOI: 10.1002/aqc.3677

Rico, E., Rallo, A., Sevillano, M. A., & Arretxe, M. L. (1992). Comparison of several biological indices based on river macroinvertebrate benthic community for assessment of running water quality. *Annales de Limnologie - International Journal of Limnology*, 28(2), Article

2. DOI: 10.1051/limn/1992013

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: A versatile open source tool for metagenomics. *PeerJ*, 4, e2584. DOI: 10.7717/peerj.2584

Signorell, A., & et mult. al. (2017). *DescTools: Tools for descriptive statistics*. R package version 0.99.23DescTools citation info. http://cran.nexr.com/web/packages/DescTools/citation.html

Soria, M., Gutiérrez-Cánovas, C., Bonada, N., Acosta, R., Rodríguez-Lozano, P., Fortuño, P., Burgazzi, G., Vinyoles, D., Gallart, F., Latron, J., Llorens, P., Prat, N., & Cid, N. (2020). Natural disturbances can produce misleading bioassessment results: Identifying metrics to detect anthropogenic impacts in intermittent rivers. *Journal of Applied Ecology*, 57(2), 283-295. DOI: 10.1111/1365-2664.13538

Soria, M., Leigh, C., Datry, T., Bini, L. M., & Bonada, N. (2017). Biodiversity in perennial and intermittent rivers: A meta-analysis. *Oikos*, 126(8), 1078-1089. DOI: 10.1111/oik.04118

Strickler, K. M., Fremier, A. K., & Goldberg, C. S. (2015). Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biological Conservation*, 183, 85-92. DOI: 10.1016/j.biocon.2014.11.038

Stubbington, R., Bogan, M. T., Bonada, N., Boulton, A. J., Datry, T., Leigh, C., & Vander Vorste, R. (2017). Chapter 4.3 - The Biota of Intermittent Rivers and Ephemeral Streams: Aquatic Invertebrates. En T. Datry, N. Bonada, & A. Boulton (Eds.), *Intermittent Rivers and Ephemeral Streams* (pp. 217-243). Academic Press. DOI: 10.1016/B978-0-12-803835-2.00007-3

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

Suren, A. M., Burdon, F. J., & Wilkinson, S. P. (2024). eDNA Is a Useful Environmental Monitoring Tool for Assessing Stream Ecological Health. *Environmental DNA*, 6(4). DOI: 10.1002/edn3.596

Taberlet, P., Coissac, E., Hajibabaei, M., & Rieseberg, L. H. (2012). Environmental DNA. *Molecular Ecology*, 21(8), 1789-1793. DOI: 10.1111/j.1365-294x.2012.05542.x

Tachet, H., Philippe, R., Bournaud, M., & Usseglio-Polatera, P. (2010). *Invertébrés d'Eau Douce*. Systématique, Biologie, Écologie (cnrs editions, Vol. 15).

Urycki, D. R., Kirtane, A. A., Aronoff, R., Avila, C. C., Blackman, R. C., Carraro, L., Evrard, O., Good, S. P., J, D. C. H., López-Rodríguez, N., Mora, D., Schadewell, Y., Schilling, O. S., & Ceperley, N. C. (2024). *A new flow path: eDNA connecting hydrology and biology*. Wiley Interdisciplinary Reviews Water. DOI: 10.1002/wat2.1749

Wangensteen, O. S., Palacín, C., Guardiola, M., & Turon, X. (2018). DNA metabarcoding of littoral hard-bottom communities: High diversity and database gaps revealed by two molecular markers. *PeerJ*, 6, e4705. DOI: 10.7717/peerj.4705

Wickham, H. (2024). *ggplot2: Elegant Graphics for Data Analysis*. Ggplot2. https://ggplot2.tidyverse.org/

Yamanaka, H., Motozawa, H., Tsuji, S., Miyazawa, R. C., Takahara, T., & Minamoto, T. (2016). On-site filtration of water samples for environmental DNA analysis to avoid DNA degradation during transportation. *Ecological Research*, 31(6), 963-967. DOI: 10.1007/s11284-016-1400-9

Zamora-Muñoz, C., Sáinz-Cantero, C. E., Sánchez-Ortega, A., & Alba-Tercedor, J. (1995). Are biological indices BMPW' and ASPT' and their significance regarding water quality seasonally dependent? Factors explaining their variations. *Water Research*, 29(1), 285-290. DOI: 10.1016/0043-1354(94)E0125-P