UNIVERSITAT DE BARCELONA

Macroinvertebrate biodiversity and metacommunity dynamics in temporary rivers: fundamental aspects and insights for conservation planning

José María Fernández Calero

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MACROINVERTEBRATE BIODIVERSITY AND METACOMMUNITY DYNAMICS IN TEMPORARY RIVERS: FUNDAMENTAL ASPECTS AND INSIGHTS FOR CONSERVATION PLANNING

José María Fernández Calero

DOCTORAL THESIS

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Thesis cover and back cover designed by Sofia Faramelli

The Cover

The cover displays detailed illustrations of selected macroinvertebrates, including the adult and nymph forms of *Tyrrhenoleuctra*, an adult Gyrinidae, and a specimen from the Ephemerellidae family

The Back Cover

The back cover displays detailed illustrations of selected macroinvertebrates, including the adult of *Aeshna cyanea*, the nymph form of *Tyrrhenoleuctra*, and *Physella acuta*

Faculty of Biology

Department of Evolutionary Biology, Ecology and

Environmental Sciences

Doctoral program in Ecology, Environmental Sciences and Plant

Physiology

Macroinvertebrate biodiversity and metacommunity dynamics in temporary rivers: fundamental aspects and insights for conservation planning

Biodiversidad de macroinvertebrados y dinámica de metacomunidades en ríos temporales: aspectos fundamentales y perspectivas para la planificación de la conservación

Biodiversitat de macroinvertebrats i dinàmica de metacomunitats en rius temporals: aspectes fonamentals i perspectives per a la planificació de la conservació

Thesis submitted by **José María Fernández Calero** to qualify for the

Doctorate degree by the Universitat de Barcelona

José María Fernández Calero

Barcelona, 2025



Director and Tutor

Dr. Núria Bonada Caparrós

Director

Dr. Miguel Cañedo Argüelles Iglesia

Tanto si crees que puedes, como si crees que no puedes,
estás en lo cierto

Henry Ford

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compañero eterno. Desde que nos conocimos en el instituto, nuestras vidas han estado unidas en muchas ocasiones. Siempre hemos compartido conversaciones de todo tipo (filosóficas, de trabajo, personales…), y espero que el apoyo mutuo que hemos tenido nunca se pierda, estemos más o menos alejados. **Nieves**, entusiasta y tenaz. Hemos compartido muchos años de vivencias personales y profesionales. Gracias por ser parte de mi apoyo durante la tesis, especialmente en la parte genética, que no fue, ni está siendo, tarea fácil. **David Cunillera** y sus STcones (de los…). Aunque tus magníficas métricas de conectividad me han dado más de un dolor de cabeza, jajaja, te doy las gracias por estar siempre dispuesto a resolver dudas estadísticas o de R. Siempre has estado ahí dedicando tu tiempo, te tocara o no, y eso te aseguro que lo valoro muchísimo. **David Pineda**, para mí siempre serás Alberto. Aunque solo compartimos tiempo en lupas durante mi primer año (que era tu año de máster), de esos ratos mirando bichos me llevé una gran amistad. Es imposible olvidar nuestro primer congreso en el SFS de Grand Rapids, una experiencia inolvidable que compartimos con el mítico Alex y que ninguno de los tres olvidará jamás. **Carlota**, ordenada y trabajadora como pocas. Ha sido un placer compartir mesa contigo en estos últimos tiempos. **Martí**, siempre dispuesto a charlar sobre temas de actualidad, una buena compañía en cualquier conversación. **Julie**, gracias por tu apoyo estadístico y por los buenos momentos que pasamos juntos con el grupo. **Álvaro**, gracias por ser un ejemplo claro de cómo se hace y gestiona una tesis. **Diana**, gracias por tu esfuerzo y amor por la limnología, a pesar de las dificultades. **Maria Soria**, simpática y, sin duda, la mejor divulgadora que he conocido. Gracias por tu apoyo científico y personal durante la tesis. Todos ellos son solo algunos de los muchos nombres a los que debo agradecer haber formado parte de este camino. Me harían falta varias páginas más para expresar mi gratitud hacia todas las personas que, de una forma u otra, hicieron de este recorrido algo maravilloso y enriquecedor. Gracias, Guillem Folch, Aida, Manu, Cesc Rivero, Arnau, Jose, Dorotty, Zeynep, Fernanda, Mariella, Anna Ticó, Roger, Alba, Bea, Lidia…

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LIST AND PUBLICATION STATUS OF THE CHAPTERS OF THIS THESIS

List of the status of each chapter of this thesis and supplementary contributions (annexes). The impact factor corresponds to the Journal Citation Reports (JCR):

- **Chapter 1**: [6]David Cunillera-Montcusí, [1,2]José María Fernández-Calero, [16]Sebastian Pölsterl, [1]Roger Argelich, [1,2]Pau Fortuño, [5,1]Núria Cid, [1,2]Núria Bonada & [4]Miguel Cañedo-Argüelles (2023). Navigating through space and time: a methodological approach to quantify spatiotemporal connectivity using stream flow data as a case study. *Methods in Ecology and Evolution*, 14, 1780–1795. https://doi.org/10.1111/2041-210X.14105. Impact Factor (2022): 6,6.

- **Chapter 2**: [1,2]José María Fernández-Calero, [17]Matías Arim, [17]Ana Borthagaray, [6]David Cunillera, [1,2]Núria Bonada & [4]Miguel Cañedo-Argüelles. Current metacommunity paradigms are challenged by Mediterranean temporary rivers (in preparation).

- **Chapter 3**: [1,2]José María Fernández-Calero, [1,2,4]Nieves López-Rodríguez, [4]Julie Crabot, [6]David Cunillera-Montcusí, [5,1]Núria Cid, [3,4]Raúl Acosta, [1,2]Pau Fortuño, [8,9]Maria Soria, [7]Florian Leese, [1,2]Núria Bonada & [4]Miguel Cañedo-Argüelles. DNA-Metabarcoding as a tool to assess metapopulation and metacommunity dynamics in temporary river networks (submitted to *OIKOS*).

- **Chapter 4:** [1,2]José María Fernández-Calero, [12]Daniel von Schiller, [1,2]Guillermo Quevedo-Ortiz, [13]Anna Ticó Pifarré, [5,1]Núria Cid, [4]Miguel Cañedo-Argüelles & [1,2]Núria Bonada. Where do you fly from? Assessing aerial dispersal in temporary rivers using stable isotopes (submitted to *Freshwater Biology*).

- **Chapter 5**: [1,2]José María Fernández-Calero, [6]David Cunillera-Montcusí, [10]Virgilio Hermoso, [1,2]Guillermo Quevedo-Ortiz, [1,2]Pau Fortuño, [3,4]Raúl Acosta, [1]Joan Gomà, [5,1]Núria Cid, [1,2]Dolors Vinyoles, [11]Albert Ruhi, [4]Miguel Cañedo-Argüelles & [1,2]Núria Bonada (2024). Integrating spatiotemporal hydrological connectivity into conservation planning to protect temporary rivers. *Aquatic conservation: Marine and*

Freshwater Ecosystems, 34(3), e4139. https://doi.org/10.1002/aqc.4139. Impact Factor (2023): 2,5.

Supplementary contributions:

- **Annex 1:** [1,2]José María Fernández-Calero, [1,2]Núria Bonada, [4]Miguel Cañedo-Argüelles & [14]José Manuel Tierno de Figueroa (2022). First record of the genus *Tyrrhenoleuctra* Consiglio, (1957) (Plecoptera, Leuctridae) in Northeastern Iberian Peninsula (Catalonia, Spain). *Boletín de la asociación Española de Entomología*. 46 (1-2): 165-168.

- **Annex 2**: [15]Miguel Carles-Tolrá, [4]Miguel Cañedo-Argüelles, [1,2]Núria Bonada & [1,2]José María Fernández Calero (2023). *Dixella graeca* (Pandazis, 1937), especie nueva para Europa continental (Diptera: Dixidae). *Arquivos Entomolóxicos*, 26: 257-260. ISSN: 1989-6581.

- **Annex 3**: [1,2]José María Fernández Calero, [3,4]Raúl Acosta, [1,2]Pau Fortuño, [18]David Pineda-Morante, [5,1]Núria Cid, [4]Miguel Cañedo-Argüelles & [1,2]Núria Bonada (2024). La diversitat d' insectes aquàtics al Parc Natural de Sant Llorenç del Munt i l'Obac. In: Diputació de Barcelona (ed.) *X Trobada d'Estudiosos de Sant Llorenç del Munt i l'Obac*, 10, 371-379. ISBN: 978-84-10278-15-8.

Author affiliations: [1]Freshwater Ecology, Hydrology and Management (FEHM), Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals. Universitat de Barcelona (UB). Barcelona, Spain. [2]Institut de Recerca de la Biodiversitat (IRBio). Universitat de Barcelona (UB). Barcelona, Spain. [3]Institut de Recerca de l'Aigua (IdRA). Universitat de Barcelona (UB). Barcelona, Spain. [4]Freshwater Ecology, Hydrology and Management (FEHM), Institut de Diagnòstic Ambiental i Estudis de l'Aigua (IDAEA), CSIC. Barcelona, Spain. [5]IRTA Marine and Continental Waters Programme, La Ràpita, Catalonia, Spain. [6]Institute of Aquatic Ecology, HUN-REN Centre for Ecological Research. Budapest, Hungary. [7]Aquatic Ecosystem Research, University of Duisburg-Essen. Essen, Germany. [8] Centre d'Estudis dels Rius Mediterranis - Universitat de Vic - Universitat Central de Catalunya, Museu del Ter. Manlleu, Spain. [9]Aquatic Ecology Group, Universitat de Vic - Universitat Central de Catalunya. Vic, Spain. [10]Departamento de Biología Vegetal y Ecología, Universidad de Sevilla. Sevilla, Spain. [11]Department of Environmental Science, Policy, and Management, University of California Berkeley. Berkeley, CA 94720, USA. [12]Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals. Universitat de Barcelona (UB). Barcelona,

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Additionally, José María Fernández Calero contributed to other articles related to the thesis topic during the doctoral program:

- Pau Fortuño, Marina Codina, Jose Luís Herrera, Montserrat Álvarez, Núria Bonada, Jeymmy Milena Walteros-Rodríguez, Nieves López-Rodríguez, José María Fernández-Calero, Carlota Sánchez-Campaña, Raúl Acosta, Narcís Prat, Maria Soria. Reading rivers from libraries: a participatory initiative to assess and improve urbanised rivers using citizen science. *Environmental Science & Policy*. Under review. Impact Factor (2023): 4,9.

- Daniel Escobar-Camacho, Julie Crabot, Rachel Stubbington, Judy England, Romain Sarremejane, Núria Bonada, José María Fernández-Calero, Miguel Cañedo-Argüelles, Carla Ferreira Rezende, Pierre Chanut, Zoltán Csabai, Andrea C. Encalada, Alex Laini, Heikki Mykrä, Nabor Moya, Petr Pařil, Daniela Rosero-López & Thibault Datry (in press). River drying causes local losses and regional gains in aquatic invertebrate metacommunity diversity: a cross-continental comparison. *Global Change Biology*. Impact Factor (2023): 10,8.

- Roger Puig-Gironès, Gemma Bel, Núria Cid, Miguel Cañedo-Argüelles, José María Fernández-Calero, Guillermo Quevedo-Ortiz, Pau Fortuño, Dolors Vinyoles, Joan Real, Eudald Pujol-Buxó & Núria Bonada (2024). Water availability and biological interactions shape amphibian abundance and diversity in Mediterranean temporary rivers. *Science of The Total Environment*, 953, 175917. https://doi.org/10.1016/j.scitotenv.2024.175917. Impact Factor (2023): 8,2

- Bernadett Boóz, Arnold Móra, Márk Ficsór, Petr Pařil, Raúl Acosta, Bea Bartalovics, Thibault Datry, José Maria Fernández-Calero, Maxence Forcellini, Marko Miliša, Heikki Mykrä, Bálint Pernecker, Vendula Polášková, Luka Polović, Henna Snåre & Zoltán Csabai (2024). Neglected dipterans in stream studies. *Journal of Limnology*, 83 (1). https://www.jlimnol.it/jlimnol/article/view/2191. Impact Factor (2023): 0,9.

- Guillermo Quevedo-Ortiz, José María Fernández-Calero, Miguel Cañedo-Argüelles, Daniel von Schiller, Pau Fortuño, Núria Bonada & Joan Gomà (2024). An experimental study to assess resistance and resilience strategies of freshwater diatoms to cope with drying in Mediterranean temporary rivers. *Hydrobiologia* 851, 4293-4306. https://doi.org/10.1007/s10750-024-05585-4. Impact Factor (2023): 2,2.

- Jorge García-Girón, Cesc Múrria, Miquel A. Arnedo, Núria Bonada, Miguel Cañedo-Argüelles, Tomáš Derka, José María Fernández-Calero, Zhengfei Li, José Manuel Tierno de Figueroa, Zhicai Xie & Jani Heino (2024). A time-calibrated 'Tree of Life' of aquatic insects for knitting historical patterns of evolution and measuring extant phylogenetic biodiversity across the world. *Earth-Science Reviews*, Volume 252, 104767. ISSN 0012-8252. https://doi.org/10.1016/j.earscirev.2024.104767. Impact Factor (2023): 10,8.

- Núria Bonada *et al*., (2024). Retos y oportunidades para la evaluación del estado ecológico en ríos temporales. *Ecosistemas*, 2655. https://doi.org/10.7818/ECOS.2655. Impact Factor (2023): 0,8.

- Miriam Colls *et al*., (2024). Impacts of diffuse urban stressors on stream benthic communities and ecosystem functioning: A review. *Limnetica*, 2024, 43(1), 89-108. DOI: 10.23818/limn.43.07. Impact Factor (2023): 1,2.

- David Pineda, José María Fernández-Calero, Sebastian Pölsterl, David Cunillera-Montcusí, Núria Bonada & Miguel Cañedo-Argüelles (2022). Local hydrological conditions and spatial connectivity shape invertebrate communities after rewetting in temporary rivers. *Hydrobiologia*. https://doi.org/10.1007/s10750-022-04799-8. Impact Factor (2022): 2,8.

Furthermore, the findings of this thesis have been personally presented by the author at national and international conferences. A general overview of the **thesis project** was presented as an oral presentation at the XX Meeting of the Iberian Association of Limnology (AIL-2020) and the III Iberoamerican Meeting of Limnology (CIL-2020) (October 2020, Murcia, Spain, online attendance). **Chapter 2** was presented as an oral presentation at the Society for Freshwater Science Meeting (SFS 2021) (May 2021, USA, online attendance), at the 12th Symposium for European Freshwater Sciences (SEFS12) (July 2021, Dublin, Ireland, online attendance), at the Society for Freshwater Science Meeting (JAMS 2022) (May 2022, Grand Rapids, Michigan, USA), at the 2nd Meeting of the Iberian Ecological Society (SIBECOL), at the XXI Conference of the Iberian Association of Limnology (AIL), and at the 21st National Congress of Ecology of the Portuguese Ecological Society (SPECO) (July 2022, Aveiro, Portugal). **Chapter 3** was presented as an oral presentation at the XXII Congress of the Iberian Association of Limnology (AIL 2024) (June 2024, Vigo, Spain). **Chapter 4** was presented as a poster at the 2nd Meeting of the Iberian Ecological Society (SIBECOL), at the XXI conference of the Iberian Association of Limnology (AIL), and at the 21st National Congress of Ecology of the Portuguese Ecological Society (SPECO) (July 2022, Aveiro, Portugal). **Chapter 5** was presented as an oral presentation at the Symposium for European Freshwater Sciences (SEFS 13) (June 2023, Newcastle Upon Tyne, United Kingdom), and as oral presentation at the V Iberoamerican Congress of Limnology and X Argentinean Congress of Limnology (CIL-CAL) (August 2023, Buenos Aires, Argentina).

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ADVISORS' REPORT

Barcelona, 21st March 2025

Dr. Núria Bonada, Full Professor of Ecology and Serra Húnter Fellow in the Department of Evolutionary Biology, Ecology and Environmental Sciences (Section of Ecology) at the University of Barcelona, and Dr. Miguel Cañedo-Argüelles, researcher at the Institute of Environmental Assessment and Water Research (IDAEA-CSIC), as supervisors of the Doctoral Thesis presented by José María Fernández Calero entitled: "Macroinvertebrate biodiversity and metacommunity dynamics in temporary rivers: fundamental aspects and insights for conservation planning".

INFORM,

that the research studies developed by José María Fernández Calero for his Doctoral Thesis have been organized into five chapters, which will correspond to five scientific papers: two already published, two under review and another in preparation. The published papers are:

Chapter 1

CUNILLERA-MONTCUSÍ, D.; FERNÁNDEZ-CALERO, J.M.; PÖLSTERL, S.; ARGELICH, R.; CID, N.; BONADA, N. & CAÑEDO-ARGÜELLES, M. (2023). Navigating through space and time: a methodological approach to quantify spatitemporal connectivity using stream flow data as a stydy case. *Methods in Ecology and Evolution*, 14: 1780-1795.

Impact factor: 6.3

Area: ECOLOGY (Q1)

In this chapter, José María Fernández Calero significantly contributed to sample processing and identification of macroinvertebrates for which the methodological approach was tested.

He was also involved in discussing the data analyses and organizing the paper, as well as reviewing the drafts.

Chapter 5

FERNÁNDEZ-CALERO, J.M.; CUNILLERA-MONTCUSÍ, D.; HERMOSO, V.; QUEVEDO-ORTIZ, G.; FORTUÑO, P.; ACOSTA, R.; GOMÀ, J.; CID, N.; VINYOLES, D.; RUHÍ, A.; CAÑEDO-ARGÜELLES, M. & BONADA, N. (2024). Integrating spatiotemporal hydrological connectivity into conservation planning to protect temporary rivers. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 34: e4139.

Impact factor: 2.5

Area: MARINE & FRESHWATER BIOLOGY (Q1: 24/119), WATER RESOURCES (Q2: 54/128), ENVIRONMENTAL SCIENCES (Q3: 209/358)

And CERTIFY,

that José María Fernández Calero has actively participated on the development of the research and in the writing of each paper. In particular, his contribution included the following tasks: processing all macroinvertebrate samples, setting the objectives of the papers, conceiving and performing the analyses, handling and analyzing the results and writing/reviewing the manuscripts. It should be noted, however, that Chapter 1 was led by the postdoctoral researcher David Cunillera-Montcusí, under whose guidance the work was primarily developed.

Finally, we certify that the co-authors of the papers that conforms this Doctoral Thesis, will not use any of the manuscripts in other Doctoral Thesis.



Núria Bonada



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ABSTRACT

Temporary rivers (TRs) are dynamic ecosystems defined by alternating periods of flow, disconnected pools, and drying, creating a mosaic of habitats that change across space and time. These rivers are found worldwide and comprise more than half of the global river network, with particular prominence in arid and Mediterranean climate regions. Due to their unique dynamics and fragmentation driven by drying, TRs appear to deviate from the general patterns observed in fluvial river networks that determine metacommunity organization. This divergence is attributed to the effects of drying that changes spatiotemporal connectivity. This thesis integrates novel methodologies, empirical studies, and conservation frameworks to advance our understanding of the spatiotemporal dynamics, metacommunity assembly, and biodiversity conservation in Mediterranean TRs. By using macroinvertebrates as model organisms, this study highlights the intricate interplay among hydrological connectivity, environmental variables, and habitat heterogeneity. Together with diverse dispersal strategies, these factors shape biodiversity patterns at both local and regional scales. This thesis aims to deepen our understanding of metacommunity dynamics within a small-scale region, leveraging this knowledge to inform and enhance the conservation and management strategies for these ecosystems.

In **Chapter 1**, a novel methodological framework was developed to quantify the effects of drying on spatial and temporal connectivity together, using presence-absence of water. The resulting connectivity metrics captured hydrological dynamics along time and across stream networks, revealing that spatiotemporally connected sites exhibited higher macroinvertebrate richness and lower community dissimilarity. These metrics demonstrated broad applicability beyond TRs, providing a valuable tool for studying dynamic ecosystems under global change scenarios. **Chapter 2** explores the general diversity patterns of macroinvertebrates and identifies the key drivers of metacommunity assembly at a small regional spatial scale in Mediterranean TRs. Using null models and generalized additive models (GAMs), the study found that alpha diversity was primarily influenced by habitat heterogeneity and spatiotemporal connectivity, particularly for aquatic passive dispersers. In contrast, beta diversity was shaped by environmental gradients and dispersal-related factors. Beta diversity of aerial active dispersers were primarily structured by local environmental

conditions (i.e., species sorting), whereas spatiotemporal connectivity was a key determinant for aquatic passive dispersers (i.e., dispersal limitation). These findings highlight the pivotal role of dispersal strategies in shaping biodiversity patterns in fragmented ecosystems like TRs. In **Chapter 3**, this thesis bridged metapopulation and metacommunity frameworks by investigating the effects of fragmentation on genetic and community variability. Using metabarcoding bulk samples, the study assessed intraspecific genetic diversity within the entire community, and assessed population genetics of two species with opposite dispersal abilities (i.e., *Anax imperator* and *Physella acuta*). In addition, variations in alpha and beta diversity were analyzed by using morphological and molecular data separately. Results showed that spatiotemporal isolation can promote site intraspecific variability in highly permanent and isolated locations. Both morphological and metabarcoding data revealed similar patterns in alpha and beta diversity, suggesting drying and spatiotemporal connectivity influence metacommunity structure when using different identification approaches. This chapter highlighted the value of metabarcoding as a complementary tool to traditional morphological approaches for assessing metapopulation and metacommunities assemblages, and the relevance of drying and connectivity in the biodiversity of TRs. **Chapter 4** employed mesocosm experiments and isotopic enrichment techniques to track the aerial dispersal of macroinvertebrates from disconnected pools during the dry phase. The study found that spatial proximity across disconnected pools significantly shaped community structure, with dispersal decreasing gradually with distance. Aerial dispersal was detected not only for taxa with an aerial stage, but also for obligate aquatic taxa, demonstrating the role of disconnected pools as refuges to sustain biodiversity during the drying phase and to contribute to community recovery during the rewetting. The findings emphasized the critical importance of maintaining disconnected pools in TRs under future drying scenarios to preserve both local and regional biodiversity. Finally, **Chapter 5** addressed the need of integrating the spatiotemporal dynamics of TRs into conservation planning. Using seasonal diatom and macroinvertebrate data, the study combined beta diversity metrics (LCBD and SCBD) and prioritization tools (Marxan) to identify key conservation sites. It demonstrated that spatiotemporal connectivity and habitat heterogeneity were critical drivers of biodiversity patterns and site prioritization. The study provided specific recommendations for promoting the conservation of TRs, including monitoring across hydrological phases and seasons, combining complementary conservation tools, and incorporating spatiotemporal

dynamics into management strategies. In addition, and in relation to biodiversity conservation, this thesis has documented new distribution records for macroinvertebrates inhabiting TRs, including the first record of an ephemeral river specialist stonefly (Leuctridae: *Tyrrhenoleuctra*) for the northeastern Iberian Peninsula, the first record of a dipteran species (Dixidae: *Dixella graeca*) for continental Europe, and numerous new records for the natural park where the research was conducted.

Overall, this thesis highlights the intricate ecological processes shaping biodiversity in Mediterranean TRs and provides innovative methodologies and practical recommendations for their conservation. By integrating spatiotemporal perspectives, metacommunity frameworks, and advanced molecular tools, this work contributes to a more comprehensive understanding of TR ecosystems, which are increasingly threatened by global change.

Keywords: Alpha diversity, beta diversity, conservation, disconnected pools, dispersal strategies, DNA metabarcoding, drying, ephemeral streams, habitat heterogeneity, intermittent rivers, macroinvertebrates, metacommunity, spatial scale, spatiotemporal connectivity.

RESUMEN

Los ríos temporales (RTs) son ecosistemas dinámicos caracterizados por fases alternantes de flujo, pozas desconectadas y períodos de sequía, creando un mosaico de hábitats que varían en el espacio y el tiempo. Estos ríos se encuentran en todo el mundo y constituyen más de la mitad de la red fluvial global, con una especial relevancia en regiones de clima árido y mediterráneo. Debido a sus dinámicas únicas y la fragmentación causada por la sequía, los RTs parecen desviarse de los patrones generales observados en las redes fluviales permanentes que determinan la organización de las metacomunidades. Esta divergencia se atribuye a los efectos de la sequía, que altera la conectividad espacio-temporal. Esta tesis integra metodologías novedosas, estudios empíricos y herramientas de conservación para avanzar en la comprensión de las dinámicas espacio-temporales, el ensamblaje de metacomunidades y la conservación de la biodiversidad en los RTs mediterráneos. Usando

macroinvertebrados como organismos modelo, este estudio destaca la compleja interacción entre la conectividad hidrológica, las variables ambientales y la heterogeneidad del hábitat. Junto con diversas estrategias de dispersión, estos factores moldean los patrones de biodiversidad a escalas locales y regionales. El objetivo de esta tesis es profundizar en la comprensión de las dinámicas de metacomunidades dentro de una región a pequeña escala, aprovechando este conocimiento para informar y mejorar las estrategias de conservación y gestión de estos ecosistemas.

En el **Capítulo 1**, se desarrolla un marco metodológico novedoso para cuantificar los efectos de la sequía sobre la conectividad espacial y temporal, utilizando datos de presencia y ausencia hidrológica. Las métricas de conectividad resultantes capturan las dinámicas hidrológicas a lo largo del tiempo y a través de las redes fluviales, revelando que los sitios con mayor conectividad espacio-temporal presentan mayor riqueza de macroinvertebrados y menor disimilitud comunitaria. Estas métricas demuestran una amplia aplicabilidad más allá de los RTs, ofreciendo una herramienta valiosa para estudiar ecosistemas dinámicos bajo escenarios de cambio global. El **Capítulo 2** explora los patrones generales de diversidad de macroinvertebrados e identifica los principales factores que influyen en el ensamblaje de metacomunidades a pequeña escala regional en los RTs mediterráneos. Utilizando modelos nulos y modelos aditivos generalizados (GAMs), el estudio encuentra que la diversidad alfa está influida principalmente por la heterogeneidad del hábitat y la conectividad espacio-temporal, especialmente en dispersores acuáticos pasivos. Por el contrario, la diversidad beta está determinada por gradientes ambientales y factores relacionados con la dispersión. Los dispersores aéreos activos están estructurados principalmente por condiciones ambientales locales (es decir, *species sorting*), mientras que la conectividad espacio-temporal es un factor clave para los dispersores acuáticos pasivos (es decir, limitación de dispersión). Estos hallazgos subrayan el papel fundamental de las estrategias de dispersión en la configuración de los patrones de biodiversidad en ecosistemas fragmentados como los RTs. En el **Capítulo 3**, la tesis conecta los marcos de metapoblaciones y metacomunidades investigando los efectos de la fragmentación en la variabilidad genética y de comunidades. Usando *bulk sample metabarcoding*, el estudio evalúa la diversidad genética intraespecífica dentro de toda la comunidad y analiza los patrones de estructura poblacional en dos especies con habilidades de dispersión opuestas (*Anax imperator* y *Physella acuta*). Además, se analizan las variaciones

de la diversidad alfa y beta utilizando datos morfológicos y moleculares separadamente. Los resultados muestran que el aislamiento espacio-temporal puede promover la variabilidad intraespecífica en sitios altamente permanentes y aislados. Tanto los datos morfológicos como los de *metabarcoding* revelan patrones similares en diversidad alfa y beta, sugiriendo que la sequía y la conectividad espacio-temporal influyen en la estructura de metacomunidades cuando se usan diferentes enfoques de identificación. El **Capítulo 4** emplea experimentos de mesocosmos y técnicas de enriquecimiento isotópico para rastrear la dispersión aérea de macroinvertebrados desde pozas desconectadas durante la fase seca. El estudio encuentra que la proximidad espacial a las pozas desconectadas influye significativamente en la estructura de las comunidades, con una dispersión que disminuye gradualmente con la distancia. Se detecta dispersión aérea, no solo en taxones acuáticos con fases adultas aéreas, sino también en taxones acuáticos obligados, demostrando el papel de las pozas desconectadas como refugios para mantener la biodiversidad durante la fase seca y contribuir a la recuperación de las comunidades durante la reanudación del caudal. Los hallazgos enfatizaron la importancia crítica de mantener pozas desconectadas en los RTs bajo futuros escenarios de sequía para preservar tanto la biodiversidad local como regional. Finalmente, el **Capítulo 5** aborda los desafíos de integrar las dinámicas espacio-temporales en la planificación de la conservación de los RTs. Usando datos estacionales de diatomeas y macroinvertebrados, el estudio combina métricas de diversidad beta (LCBD y SCBD) y herramientas de priorización (Marxan) para identificar sitios clave para la conservación. Demuestra que la conectividad espacio-temporal y la heterogeneidad del hábitat son factores críticos para los patrones de biodiversidad y la priorización de sitios. Se proporcionan recomendaciones específicas para promover la conservación de los RTs, incluyendo el monitoreo a través de fases y estaciones hidrológicas, la combinación de herramientas complementarias de conservación y la incorporación de dinámicas espacio-temporales en las estrategias de gestión. Además, en relación con la conservación de la biodiversidad, esta tesis documenta nuevos registros de distribución para macroinvertebrados que habitan en RTs, incluyendo el primer registro de un plecóptero especialista en ríos efímeros (Leuctridae: *Tyrrhenoleuctra*) para el noreste de la Península Ibérica, el primer registro de una especie de díptero (Dixidae: *Dixella graeca*) para Europa continental, y numerosos nuevos registros para el parque natural donde se lleva a cabo la investigación.

En conjunto, esta tesis destaca los complejos procesos ecológicos que moldean la biodiversidad en los RTs mediterráneos y proporciona metodologías innovadoras y recomendaciones prácticas para su conservación. Al integrar perspectivas espacio-temporales, el marco de la teoría de metacomunidades y herramientas moleculares avanzadas, este trabajo contribuye a una comprensión más integral de los ecosistemas de RTs, cada vez más amenazados por el cambio global.

Palabras clave: Conectividad espacio-temporal, conservación, diversidad alfa, diversidad beta, escala espacial, estrategias de dispersión, heterogeneidad de hábitat, macroinvertebrados, metabarcodificación de ADN, metacomunidades, pozas desconectadas, ríos efímeros, ríos intermitentes y sequía.

RESUM

Els rius temporals (RTs) són ecosistemes dinàmics caracteritzats per fases de flux, basses desconnectades i lleres seques que s'alternen, creant un mosaic d'hàbitats que canvien en l'espai i en el temps. Aquests rius es troben a tot el món i constitueixen més de la meitat de la xarxa fluvial global, amb una presència particularment destacada a les regions de clima àrid i mediterrani. A causa de les seves dinàmiques úniques i de la fragmentació causada per l'assecatge, les metacomunitats dels RTs semblen diferir dels patrons generals observats en les xarxes fluvials permanents. Aquesta divergència s'atribueix als efectes de l'assecatge, que altera la connectivitat espaial i temporal. Aquesta tesi integra metodologies innovadores, estudis empírics i eines de conservació per avançar en la comprensió de les dinàmiques espacio-temporals, l'assemblatge de metacomunitats i la conservació de la biodiversitat en RTs mediterranis. Utilitzant els macroinvertebrats com a organismes model, aquest estudi posa en relleu la complexa interacció entre la connectivitat hidrològica, les variables ambientals i l'heterogeneïtat dels hàbitats. Juntament amb diverses estratègies de dispersió, aquests factors configuren els patrons de biodiversitat a escales locals i regionals. L'objectiu d'aquesta tesi és aprofundir en la comprensió de les dinàmiques de metacomunitats dins d'una regió a petita escala, aprofitant aquest coneixement per informar i millorar les estratègies de conservació i gestió d'aquests ecosistemes.

Al **Capítol 1**, es desenvolupa un marc metodològic innovador per quantificar conjuntament els efectes de l'assecatge sobre la connectivitat espaial i temporal, utilitzant dades de presència i absència d'aigua. Les mètriques de connectivitat resultants descriuen les dinàmiques hidrològiques al llarg del temps i de les xarxes fluvials, revelant que els llocs connectats espacio-temporalment exhibeixen una major riquesa de macroinvertebrats i una menor dissimilitud de les comunitats. Aquestes mètriques tenen un elevat potencial per ser aplicables més enllà dels RTs, oferint una eina valuosa per estudiar ecosistemes dinàmics en escenaris de canvi global. El **Capítol 2** explora els patrons generals de diversitat dels macroinvertebrats en els RTs estudiats i identifica els principals factors que influeixen en l'assemblatge de metacomunitats en una escala regional petita en RTs mediterranis. Utilitzant models nuls i models additius generalitzats (GAMs), l'estudi troba que la diversitat alfa està principalment influenciada per l'heterogeneïtat dels hàbitats i la connectivitat espacio-temporal, especialment per als dispersors passius aquàtics. En canvi, la diversitat beta està configurada per gradients ambientals i factors relacionats amb la dispersió. Els dispersors aeris actius estan estructurats principalment per condicions ambientals locals (és a dir, *species sorting*), mentre que la connectivitat espai-temporal és un factor clau pels dispersors aquàtics passius (és a dir, limitació de dispersió). Aquests resultats subratllen el paper fonamental de les estratègies de dispersió en la configuració dels patrons de biodiversitat en ecosistemes fragmentats com els RTs. Al **Capítol 3**, la tesi considera les metapoblacions i les metacomunitats en RTs, i investiga els efectes de la fragmentació en la variabilitat genètica i comunitària. Utilitzant *bulk sample metabarcoding*, l'estudi avalua la diversitat genètica intraespecífica de tota la comunitat i analitza els patrons d'estructura genètica de dues espècies amb capacitats de dispersió oposades (*Anax imperator* i *Physella acuta*). A més, els patrons de metacomunitats en diversitat alfa i beta s'analitzen utilitzant dades morfològiques i moleculars separadament. Els resultats mostren que l'aïllament espacio-temporal pot promoure la variabilitat intraespecífica en llocs altament permanents i aïllats. Tant les dades morfològiques com les de *metabarcoding* revelen patrons similars en diversitat alfa i beta, suggerint que l'assecatge i la connectivitat espacio-temporal influeixen en l'estructura de metacomunitats quan s'utilitzen diferents aproximacions d'identificació. El **Capítol 4** empra experiments de mesocosmos i tècniques d'enriquiment isotòpic per rastrejar la dispersió aèria de macroinvertebrats des de basses desconnectades durant la fase seca. L'estudi troba que la proximitat espaial a les basses desconnectades influïa significativament en l'estructura

comunitària, amb una dispersió que disminuïa gradualment amb la distància. Es detecta dispersió aèria, no només en tàxons aquàtics amb adults aeris, sinó també en tàxons estrictament aquàtics, demostrant el paper de les basses desconnectades com a refugis per mantenir la biodiversitat durant la fase seca i contribuir a la recuperació comunitària durant el restabliment del cabal. Els resultats destaquen la importància crítica de mantenir basses desconnectades en RTs sota futurs escenaris d'assecatge per preservar tant la biodiversitat local com la regional. Finalment, el **Capítol 5** aborda els reptes d'integrar dinàmiques espacio-temporals en la planificació de la conservació dels RTs. Utilitzant dades estacionals de diatomees i macroinvertebrats, l'estudi combina mètriques de diversitat beta (LCBD i SCBD) i eines de priorització (Marxan) per identificar llocs clau de conservació. Es demostra que la connectivitat espacio-temporal i l'heterogeneïtat dels hàbitats són factors crítics pels patrons de biodiversitat i la priorització de llocs. Esproporcionen recomanacions específiques per promoure la conservació dels RTs, incloent el monitoratge al llarg de les fases i estacions hidrològiques, la combinació d'eines complementàries de conservació i la incorporació de dinàmiques espacio-temporals en les estratègies de gestió. A més, en relació amb la conservació de la biodiversitat, aquesta tesi documenta nous registres de distribució de macroinvertebrats aquàtics que habiten en RTs, incloent el primer registre d'un plecòpter especialista en rius efímers (Leuctridae: *Tyrrhenoleuctra*) per al nord-est de la Península Ibèrica, el primer registre d'una espècie de dípter (Dixidae: *Dixella graeca*) per a Europa continental, i nombrosos registres nous pel parc natural on es va dur a terme la investigació.

En conjunt, aquesta tesi ressalta els complexos processos ecològics que configuren la biodiversitat en RTs mediterranis i proporciona metodologies innovadores i recomanacions pràctiques per a la seva conservació. Integrant perspectives espacio-temporals, marcs de metacomunitats i eines moleculars avançades, aquest treball contribueix a una comprensió més integral dels ecosistemes de RTs, cada vegada més amenaçats pel canvi global.

Paraules clau: Basses desconnectades, conservació, connectivitat espacio-temporal, diversitat alfa, diversitat beta, escala espacial, estratègies de dispersió, heterogeneïtat d'hàbitat, macroinvertebrats, metabarcoding d'ADN, metacomunitats, rius efímers, rius intermitents i sequera.

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GENERAL INTRODUCTION



The hydrosphere (i.e., all the water on Earth in its various stages or forms) covers three quarters of the Earth's surface. Approximately, 96.5% of the Earth's water volume is contained in the oceans, while only 2.5% constitutes freshwater. Of this freshwater, 68.7% is stored in glaciers or permanent snow. Only 0.26% of the Earth's freshwater is found in lakes, rivers, and reservoirs, which collectively cover approximately 0.8% of the Earth's surface (Araya, 2005; Dudgeon *et al.*, 2006; Carpenter, Stanley & Zanden, 2011). However, despite constituting a small fraction of the Earth's water, freshwater ecosystems harbor a disproportionately high level of species diversity and endemism. Globally, these ecosystems are home to approximately 126,000 species, representing 9.5% of all recognized animal species, underscoring their critical role in maintaining biodiversity. For example, freshwater ecosystems support approximately 45% of all fish species globally and nearly two-thirds of all freshwater vertebrate species (Balian *et al.*, 2008; Carpenter *et al.*, 2011; Dudgeon, 2019), with many of these species increasingly at risk of extinction. In fact, a recent study reveals that about one-quarter of freshwater fauna, including decapod crustaceans, fish, and odonates, is currently threatened with extinction (Sayer *et al.*, 2025).

Freshwater biodiversity is in decline, driven by the inherent vulnerability of freshwater habitats and the pervasive global threats they face (Vörösmarty *et al.*, 2010). Freshwater ecosystems are particularly vulnerable to environmental changes due to their often isolated nature within a terrestrial landscape, while simultaneously serving as both recipients and conduits of processes occurring throughout the basin, which also constrain species dispersal between freshwater patches. This fragmentation restricts organisms' ability to adapt to environmental stressors such as pollution, climate change, and invasive species. Furthermore, freshwater habitats are highly sensitive to fluctuations in water quality and quantity, and such changes can lead to rapid and severe impacts on biodiversity (Dudgeon, 2019). According to the Living Planet Index for vertebrates (Collen *et al.*, 2009) designed by the World Wide Fund for Nature (WWF), 85% of the freshwater species censored in 1970 had disappeared by 2020. This loss far surpasses the declines observed for terrestrial (69%) and marine (56%) species over the same period, underscoring the critical conservation challenges faced by freshwater ecosystems (WWF, *Living Planet Report*, 2024).

Land use changes, pollution, and resource overexploitation are among the most pervasive stressors on freshwater biodiversity, with vertebrate species, such as fish and

amphibians, being the most impacted (Cazzolla Gatti, 2016). In addition, invasive species, whether intentionally or accidentally transported by humans, are causing significant harm to native species and altering the physical, chemical, and ecological conditions of freshwater ecosystems worldwide (Carpenter *et al.*, 2011). At the same time, human water withdrawal accounts for approximately 50% of accessible surface water (Vörösmarty & Sahagian, 2000), with withdrawals for agriculture making up about 70% of total extractions. This will increase due to higher water demands caused by growing human population, shifting dietary patterns and industrial growth (Dudgeon, 2019; WWF, *Living Planet Report*, 2024).

Climate change will exacerbate the above mentioned impacts. Rising global temperatures, shifting precipitation patterns, and increasing the frequency of extreme events, such as floods or droughts, are expected to profoundly impact the flow regimes of inland water systems (Planton *et al.*, 2008; Goodess, 2013; Cazzolla Gatti, 2016; Reid *et al.*, 2019). Notably, climate projections indicate that droughts will become more frequent and intense in the future, further exacerbating the variability of hydrological cycles and posing significant challenges for ecosystem stability and water resource management (Toreti *et al.*, 2024). However, these changes are spatially variable, with certain regions, such as northern Europe and arid areas, being more affected than others (Reid *et al.*, 2019; Walsh *et al.*, 2020). For example, changes in river flow patterns are expected to be more intense in arid regions, representing a significant driver of biodiversity loss (Döll, Fiedler & Zhang, 2009; Döll & Zhang, 2010). This will cause many perennial rivers to cease flowing during certain periods (Datry, Fritz & Leigh, 2016b), increasing the proportion of rivers that dry up at some point during the year (i.e., temporary rivers), which has now risen to over 50% globally (Messager *et al.*, 2021).

TEMPORARY RIVERS: DEFINITION

This thesis will focus on temporary rivers, which are flowing aquatic ecosystems with reaches that stop flowing at some point during their hydrological cycle. These ecosystems are characterized by alternating periods of flow (lotic conditions) and non-flow conditions, which include isolated or disconnected pools (lentic conditions) and dry periods (terrestrial conditions) (Figure 1). There are several names used to refer to these ecosystems, and the

terminology can sometimes be confusing and controversial, depending on the authors (Datry, Bonada & Boulton, 2017a). The most important characteristic for naming these freshwater ecosystems is, first and foremost, the size of the longitudinal freshwater bodies, with "rivers" being larger and deeper than "streams". In addition, the duration of the non-flow conditions (lentic and terrestrial conditions) and its predictability are important factors in classifying these aquatic ecosystems.

In recent years, particularly following the publication of a book by Datry *et al.*, (2017a) that integrates various aspects of these ecosystems, the most used term in a broad sense has become "Intermittent Rivers and Ephemeral Streams," often referred by the well-known acronym "IRES". However, several different terms are used in the literature to refer to IRES. "Intermittent" are the most common term, which could be defined as "natural bodies of water that experience a recurrent dry phase of varying duration" (Williams, 2006), "rivers that flow for 20% - 80% of the year (Matthews, 1988), or "rivers with isolated pools during the dry season that may persist to the end of summer" (Bonada, Rieradevall & Prat, 2007b). "Ephemeral" streams can be defined as those that "flow less than 20% of the time" (Matthews, 1988), or "rivers with dry stream beds in the dry season or even for longer periods" (Bonada *et al.*, 2007b). Another common term is "temporary", which is defined as "waterways that cease to flow at some point in space and time along their course" (Acuña *et al.*, 2014) or "rivers with intermittent discharge" (Davies *et al.*, 1994). Regardless, the literature includes numerous terms, such as episodic, seasonal, non-perennial, and dryland, among others (Datry, Bonada & Boulton, 2017b). In addition, we should not overlook "dry rivers", where surface water is present only for hours or days following heavy rainfall, with the key difference from the temporary rivers being their inability to support aquatic species (Vidal-Abarca *et al.*, 2020). Given the various terminologies and the existing consensus that temporary and seasonal are synonymous with intermittent rivers (Boulton & Lake, 1988; Vidal-Abarca *et al.*, 2020), the term "Temporary Rivers" (hereafter TRs) will be used throughout this thesis in a broad sense to refer to rivers that experience seasonal dry periods, and "Ephemeral Streams" will refer to those with extended dry periods, larger than flowing periods, yet still capable of harboring aquatic communities.

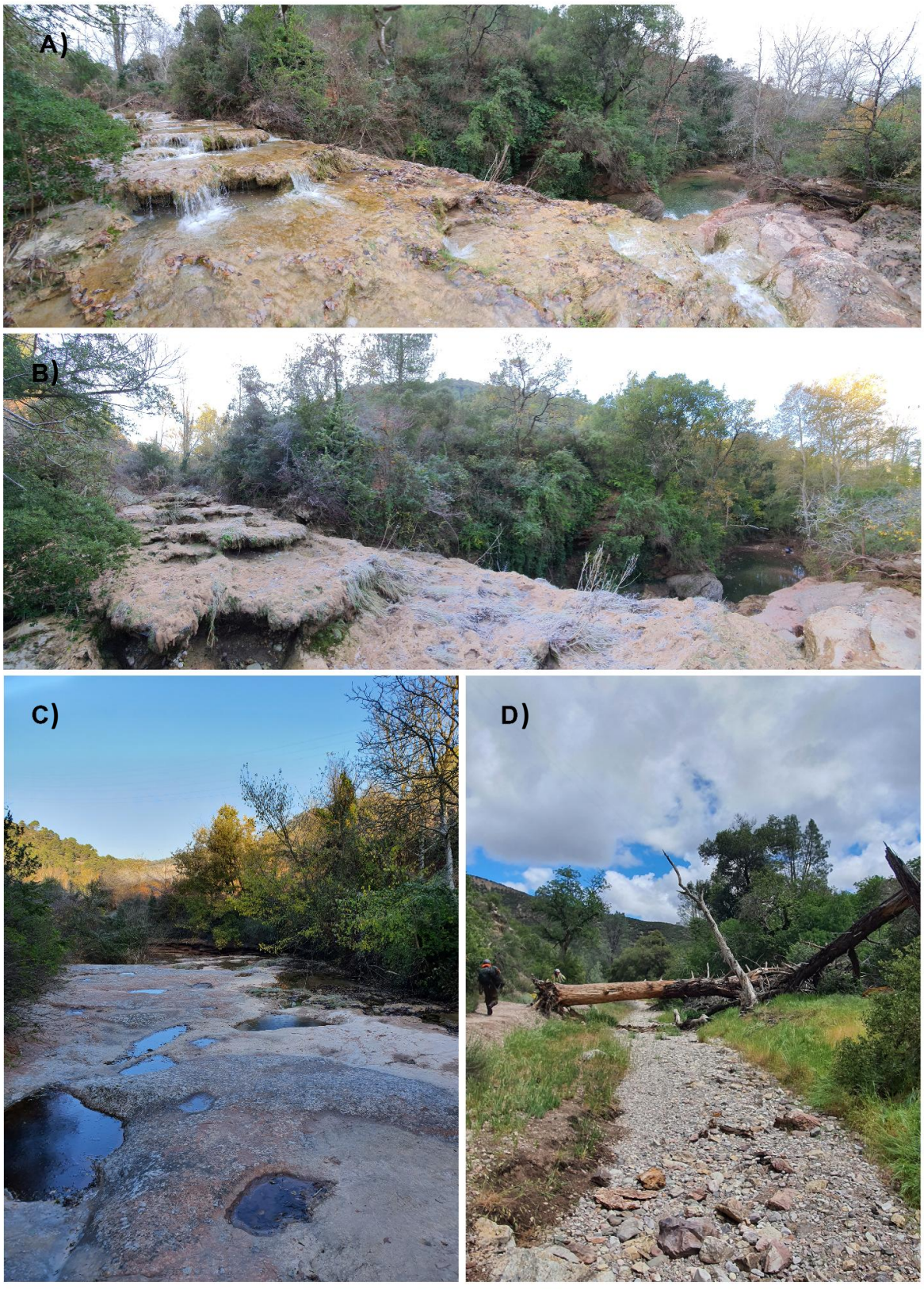


Figure 1. Photographs illustrating the hydrological periods of temporary rivers. **A)** Flowing conditions: the river actively flows through the channel (December 2019). **B)** Non-flowing conditions: the same river reach shown in (A) is completely dry just three weeks earlier (November 2019). **C)** Disconnected pools. **D)** Terrestrial phase. Photos A, B, and C were taken in Sant Llorenç del Munt Natural Park, Spain, and photo D in Pinnacles National Park, California, USA. Photos taken by the author.

HYDROLOGY IN TEMPORARY RIVERS

An important characteristic for describing TRs are their hydrological features. These include their flow regimes, which are governed by the magnitude, frequency, timing, and duration of non-flow periods across different river sections (from entire river networks to microhabitats) and over time (from millenia to hours) (Costigan *et al.*, 2017). At regional scale, the determinants of flow regimes are climate, geology, and human activities, together with catchment conditions (e.g., topography) (Jaeger *et al.*, 2017). In TRs, the most important hydrological factors in characterizing flow intermittence are the frequency and duration of dry periods, which shape both the ecological and geomorphological characteristics of these systems (Larned *et al.*, 2010; Jaeger *et al.*, 2017). For example, a high frequency and prolonged duration of dry periods can alter community composition by reducing the number of species capable of withstanding drying conditions (Datry *et al.*, 2014a). Indeed, the disruption of flow in TRs alters river connectivity in three dimensions: longitudinal, lateral, and vertical (Figure 2). Longitudinal connectivity refers to the unidirectional flow of a river from upstream to downstream, influencing the transport of sediments, nutrients, and drifting organisms. This results, as it also occurs in permanent rivers, in longitudinal gradients in rock sizes (geomorphology), water velocity and the presence of riffles (hydrology), water temperature and oxygen concentration (chemistry), and the distribution of plant and animal communities (ecology) (Allan & Castillo, 2007; Boulton *et al.*, 2017). Considering the longitudinal perspective, drying can occur in headwaters, mid-reaches, or lower reaches of the river network, depending on factors such as climate, groundwater inputs, withdrawal, topography, and precipitation (Bogan, Boersma & Lytle, 2013; Boulton *et al.*, 2017; Dudgeon, 2019). Lateral connectivity refers to the movement of water into the floodplain and riparian zones, which is strongly influenced by topography and infrastructures. This connectivity affects the transport of materials (e.g., sediment and leaves) between the river and adjacent terrestrial habitats. Finally, vertical connectivity refers to the link between the surface water and the hyporheic zone, where some aquatic organisms can survive dry periods due to the water storages in this subsurface area (Bogan, Boersma & Lytle, 2015; Boulton *et al.*, 2017).

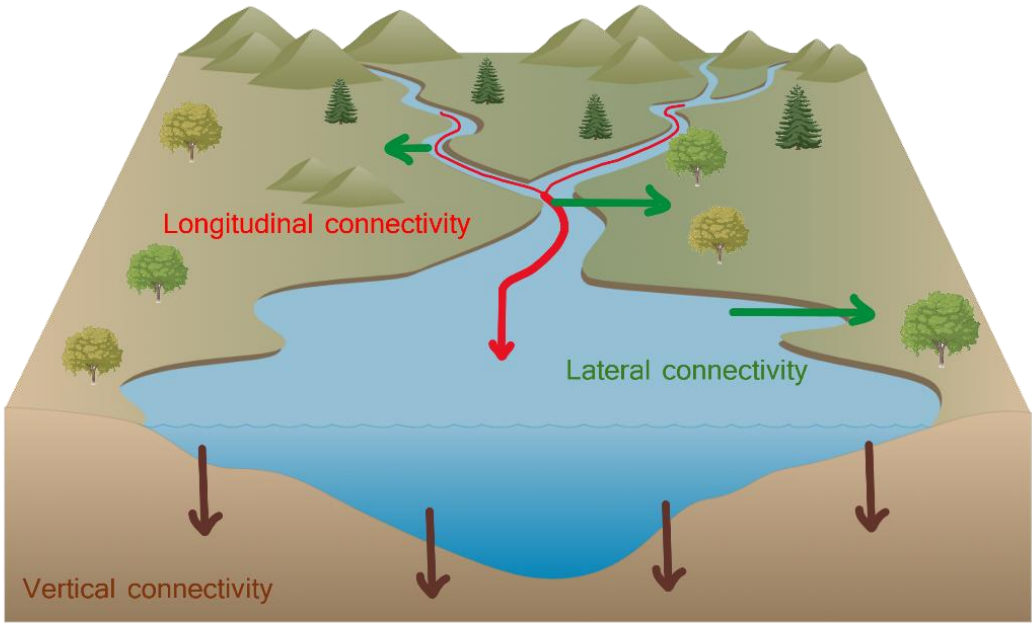


Figure 2. Schematic representation of hydrological connectivity in riverine systems, highlighting the three primary dimensions: longitudinal, lateral, and vertical. Arrows indicate the potential directions of water movement, illustrating the dynamic processes of expansion and contraction within each dimension

Recognizing that the structure and composition of biological communities in TRs are closely linked to temporal hydrological changes, Gallart *et al.*, (2012) defined six aquatic states to capture different hydrological conditions associated with ecological features (mesohabitats) present at the reach scale (Figure 3). The "Hyperrheic state" occurs during floods, when bedload is transported and riffles are the most common macrohabitat. The "Eurheic state" is characterized by abundant riffles, with all mesohabitats available and fully connected. In the "Oligorheic state," pools are the dominant macrohabitat but are still connected by small riffles, supporting both lotic and lentic-like species. These three states represent the flowing phase in TRs. In the "Arheic state," pools become disconnected and the only available macrohabitat, with no visible surface water flow, and only lentic-like species persist. In the "Hyporheic state," there is no surface water, but water remains in the sediment, supporting hyporheic and terrestrial fauna. Finally, the "Edaphic state" occurs when neither water nor moisture is present, and only terrestrial organisms can survive. These three states collectively represent the dry or non-flowing phase in TRs, encompassing conditions such as disconnected pools and dry riverbeds (Gallart *et al.*, 2012) (Figure 3).

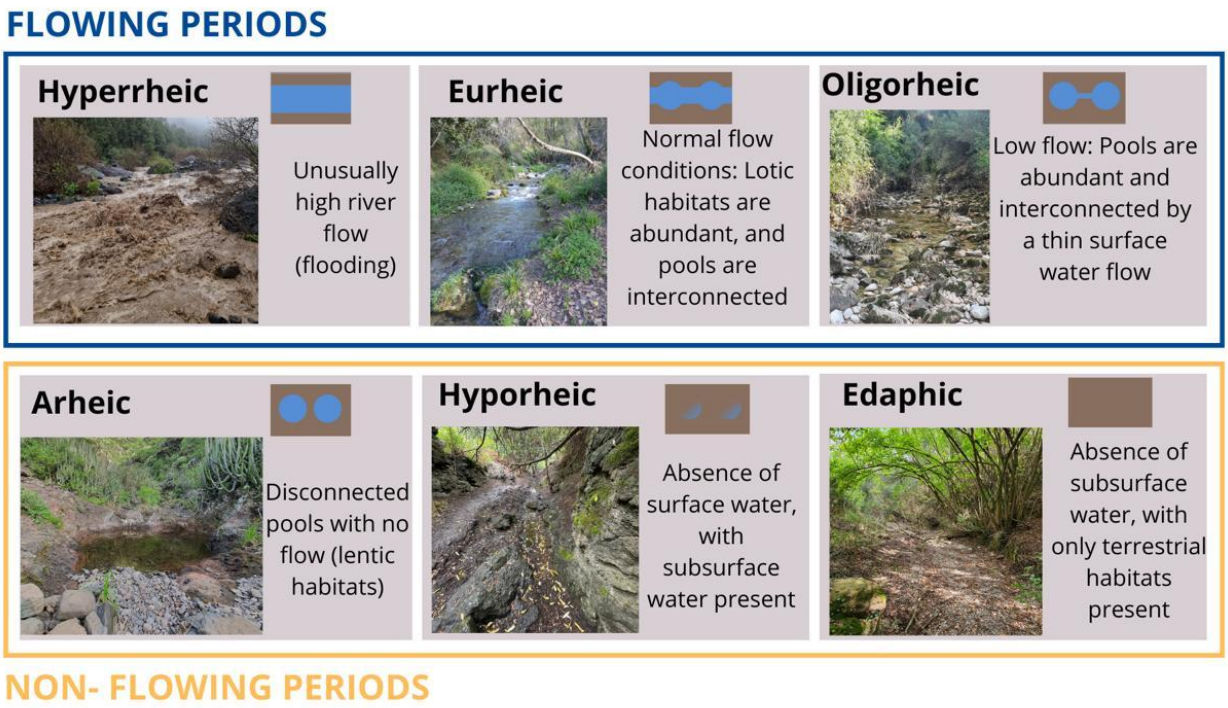


Figure 3. Aquatic states, as classified by Gallart *et al*. (2012), are grouped based on whether they correspond to the flowing or non-flowing phase. Photo of the hyperrheic state courtesy of Tragsa. Photo of the hyporheic state by Raúl Acosta. All other photos were taken by the author.

Thus, TRs alternate between flow and non-flow periods, which directly influence the spatial and temporal dynamics of biological communities and drive transitions from lotic to lentic and eventually to terrestrial species (Datry, Bonada & Heino, 2016a).

MEDITERRANEAN TEMPORARY RIVERS

TRs are among the most prevalent types of freshwater ecosystems globally (Datry, Larned & Tockner, 2014b; Messager *et al.*, 2021). They account for over 50% of the global river network and disproportionately dominate in semiarid and arid regions, such as Australia and the southwestern United States, particularly in Arizona and New Mexico, where they comprise up to 94% of the total river network (Levik *et al*., 2008; Datry *et al.*, 2017b). Although TRs are found in a wide range of climatic regions, including polar, alpine headwaters, temperate, and

tropical climates, they represent prevalent river ecosystems in Mediterranean climate regions.

The Mediterranean climate, characterized by a pronounced seasonality in precipitation and temperature regimes (Figure 4), occurs in five regions around the world: the Mediterranean Basin, coastal California, central Chile, the Cape region of South Africa, and the southwestern and south Australia (Bonada & Resh, 2013). In these regions summers are hot and dry, while winters are cool and wet, resulting in a high and predictable seasonality (Bonada & Resh, 2013). Consequently, TRs in Mediterranean climates are characterized by

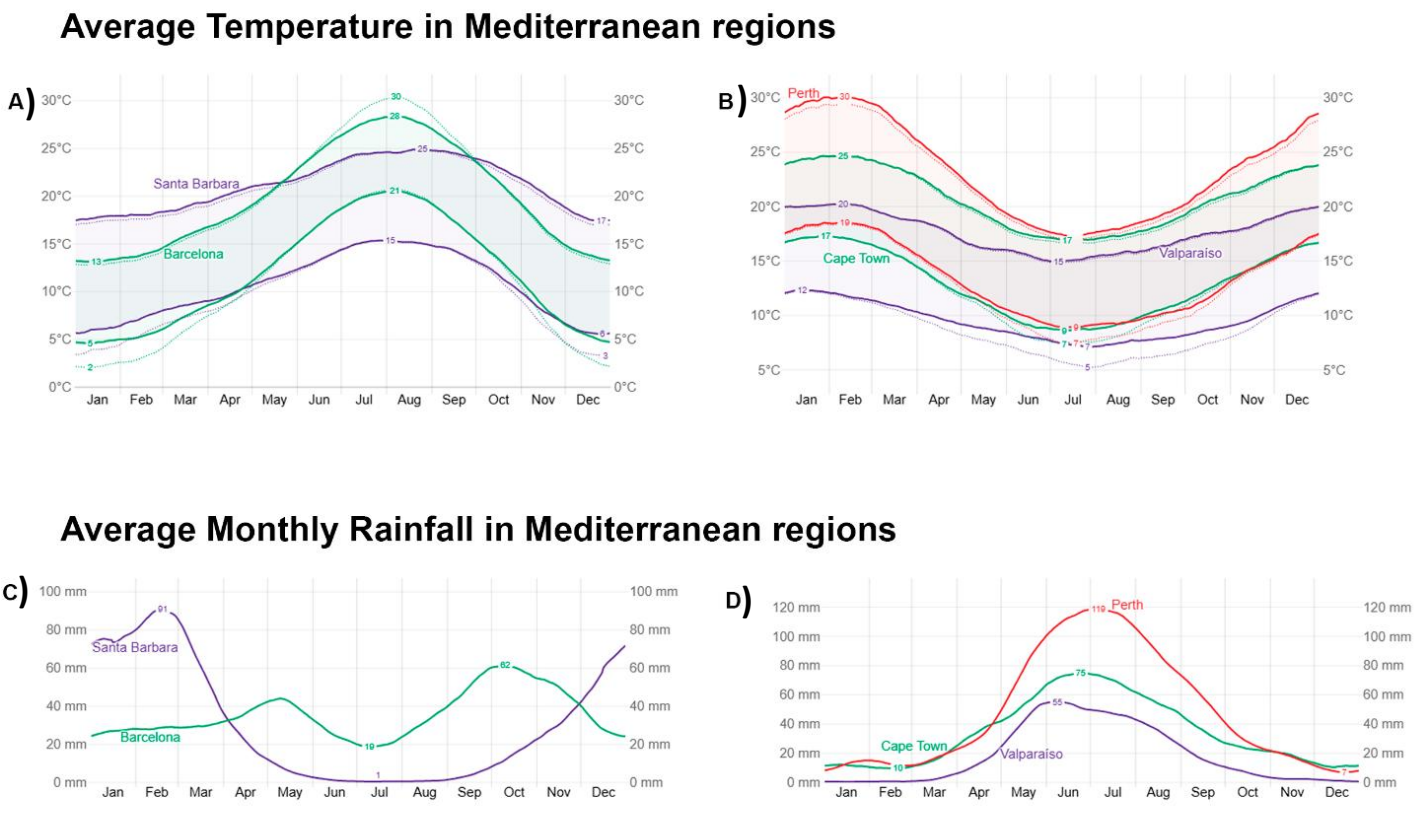


Figure 4. Average temperature and precipitation data for five cities in the five Mediterranean climate regions of the world: Santa Barbara (California, USA), Barcelona (Spain), Perth (Australia), Cape Town (South Africa), and Valparaíso (Chile). **A** and **C** correspond to cities in the Northern Hemisphere, while **B** and **D** correspond to cities in the Southern Hemisphere. **A** and **B)** Average high and low temperatures: Daily average high and low air temperatures measured 2 meters above the ground. The thin dotted lines represent the corresponding perceived temperatures. **C** and **D**) Average rainfall: The accumulated rainfall over a sliding 31-day period, centered on the day in question. These data are based on a statistical analysis of historical hourly weather reports and model reconstructions from January 1, 1980, to December 31, 2016. Graphics are directly obtained from © WeatherSpark.com.

highly seasonal and predictable hydrological patterns (i.e., drying usually occurs in summer) (Tonkin *et al.*, 2017), with distinct flowing and non-flowing periods occurring from mid autumn to early spring, and from mid spring to early autumn, respectively. The seasonality and high predictability of these hydrological patterns shape the ecological features of Mediterranean TRs, leading to well-documented community shifts between flowing and non-flowing phases (Tonkin *et al.*, 2017). These predictable drying periods have resulted in well-established adaptive strategies to cope with drying, such as life-cycle synchronisation, diapause, behavioral adjustments, or tolerance to low oxygen levels (Bonada & Resh, 2013; Sánchez-Montoya *et al.*, 2022). These unique characteristics make Mediterranean TRs ecologically distinct and one of the most important biodiversity hotspots, with high rates of endemicity (Bonada, Dolédec & Statzner, 2007a; Tierno de Figueroa *et al.*, 2013). However, the combined effect of multiple anthropogenic pressures such as water pollution, hydrological alterations, habitat degradation, and climate change, including shifts in precipitation patterns and rising temperatures, has led to a rapid decline in biodiversity, occurring faster than in other regions of the world (Cooper *et al.*, 2013; Cid *et al.*, 2017).

BIODIVERSITY IN TEMPORARY RIVERS

Hydrological variability plays a key role in driving biodiversity in TRs and results in highly dynamic ecosystems of significant conservation value (Datry *et al.*, 2014b; Heino *et al.*, 2015). This diversity is commonly assessed in terms of alpha diversity (the number of species within a given locality), beta diversity (the variation in community composition across different sites), and gamma diversity (the total number of species at a regional scale). TRs often exhibit lower species richness (alpha diversity) compared to perennial rivers, largely due to the dominant effect of flow cessation (Bogan *et al.*, 2013; Soria *et al.*, 2017). However, TRs, particularly those in Mediterranean regions, display greater variation in species composition both between sites (spatial beta diversity) and over time (temporal beta diversity), driven by the predictable seasonal dynamics of their hydrological regimes (Soria *et al.*, 2017, 2024; Tonkin *et al.*, 2017). Beta diversity is commonly partitioned into two components: richness difference and species replacement (Baselga, 2012; Legendre, 2014). Richness difference (nestedness) occurs when the species present at a site are a subset of those found at a more

species-rich site. In contrast, species replacement (turnover) refers to the substitution of species across different sites or environmental conditions (Baselga, 2012) (Figure 5). In TRs as a whole, species replacement often dominates beta diversity because low connectivity during dry periods intensifies environmental filtering, selecting for species uniquely adapted to specific hydrological conditions and creating highly distinct community compositions across sites. Conversely, during periods of high connectivity, increased dispersal facilitates the movement of species from richer to less diverse sites, resulting in nestedness as species from more diverse communities supplement those in less diverse ones, reducing turnover (Datry *et al.*, 2016a). Consequently, the relative contributions of the components of beta diversity fluctuate over time, driven by changes in hydrological connectivity (Ruhí, Datry & Sabo, 2017). For example, TRs experience fluctuating connectivity and environmental conditions between flowing and non-flowing periods, which can limit the survival of lotic-adapted species (e.g., Heptageniidae, Simuliidae) during non-flowing periods. Conversely, species better adapted to lentic conditions (e.g., Dytiscidae, Chaoboridae), are more likely to thrive (Stubbington *et al.*, 2017).

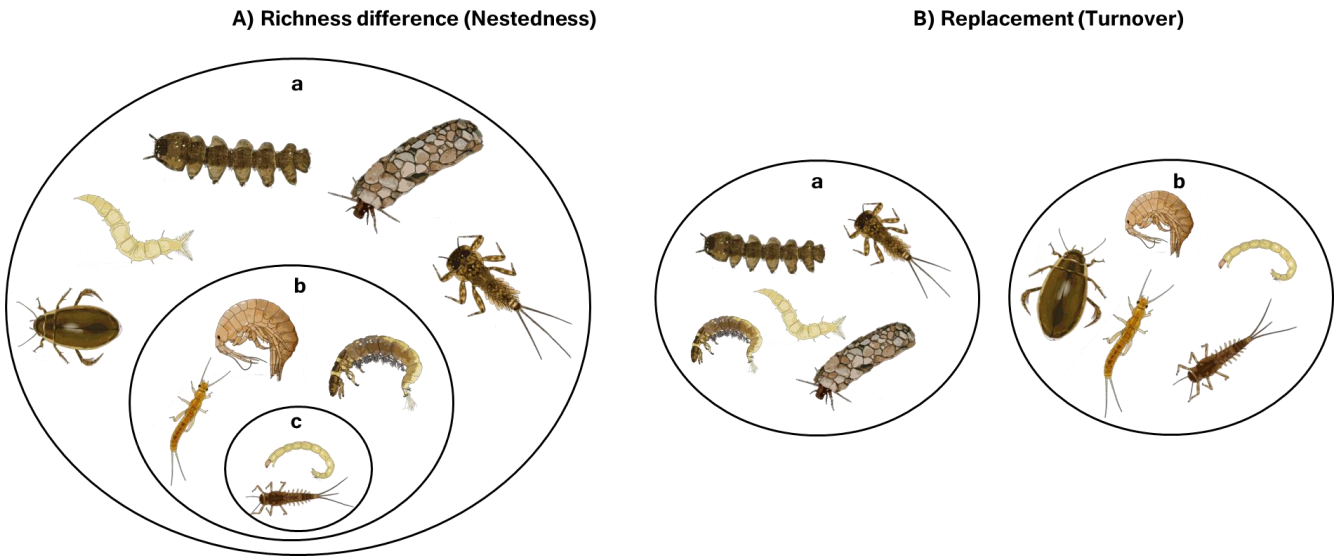


Figure 5. Schematic representation of the two components of beta diversity. **A) Richness difference**: the richer community (a) includes all taxa from the less rich community (c), indicating that differences in community composition are driven by species richness. **B) Replacement**: the two communities do not share any species, reflecting complete species replacement, where differences arise from the substitution of taxa rather than change in richness. Macroinvertebrate drawings by Pau Fortuño.

The dynamic variability of TRs fosters a diverse array of habitats that can sustain a high species diversity. These species exhibit various adaptations to withstand drying, ranging from simple prokaryotes like bacteria and archaea to complex vertebrates, thriving across both aquatic and terrestrial ecosystems. Given that this thesis focuses on the ecology of Mediterranean TRs, this section will provide a comprehensive overview of the biodiversity in TRs, divided into two distinct sections. The first section explores the diversity of non-aquatic macroinvertebrate groups, including Eubacteria, Archaea, Protista, Plants, Fungi, and Animals, all of which play critical roles in the functioning of TR ecosystems. The second section focuses exclusively on aquatic macroinvertebrates, the central subject of this thesis.

Eubacteria, Archaea, Protista, Plants, Fungi, and Animals

Microorganisms (e.g., prokaryotes, protozoa, and certain fungi) are present in nearly all environments and possess diverse metabolic pathways that enable them to thrive in TRs as well as in some of the most extreme habitats on Earth (Stanish *et al.*, 2013). A recent study across various climatic regions shows that bacteria are by far the group with the highest number of operational taxonomic units (OTUs) in TRs worldwide (Foulquier *et al.*, 2024). They can live in both benthic habitats, forming biofilms, and in the water column. However, the diversity of these microorganisms in TRs remains understudied, despite their crucial roles in biogeochemical processes such as carbon, nitrogen, and sulfur cycling, as well as in both aerobic and anaerobic pathways (Brune, Frenzel & Cypionka, 2000; Romaní *et al.*, 2017). Some adaptations to TRs exhibited by these species include the formation of a seed bank of resistant spores or cysts to withstand desiccation (e.g., *Bacillus subtilis*), and the ability of microbial communities in biofilms to retain moisture and nutrients, enhancing survival during dry periods (e.g., Cyanobacteria) (Lennon & Jones, 2011; Battin *et al.*, 2016).

Moreover, as in other ecosystems, primary producers (e.g., cyanobacteria, algae, mosses, and macrophytes) sustain the food web and exhibit specific adaptations to cope with drying in TRs. Many have developed morphological and physiological adaptations to dry periods (Holzinger & Karsten, 2013; Sabater *et al.*, 2017). For example, some diatoms form mucilage structures (e.g., *Cymbella*, *Gomphonema*) that encase cells, protecting them from desiccation, while others migrate from the surface to deeper, moisture-rich sediments

(McKew *et al.*, 2011; Sabater *et al.*, 2017). Macrophytes in TRs serve as vital primary producers, significantly contributing to habitat structure and nutrient cycling. These plants have evolved morphological and physiological adaptations to cope with the hydrological variability characteristic of TRs. For example, some species (e.g., *Ranunculus* spp.) develop extensive root systems capable of accessing deeper, moisture-rich sediments, while others enter a dormant state during dry periods (Sabater *et al.*, 2017). Additionally, many macrophytes feature specialized leaf structures or waxy cuticles that minimize water loss, enhancing their resilience in ephemeral aquatic environments (Holzinger & Karsten, 2013). Similarly, Fungi plays an indispensable role in TRs by decomposing organic matter and facilitating nutrient cycling. Aquatic hyphomycetes are predominant during flowing phases, actively contributing to the breakdown of detritus. To endure dry periods, many Fungi produce resistant spores, enabling them to persist in inhospitable conditions and rapidly recolonize habitats when water flow resumes (Romaní *et al.*, 2017).

Focusing on the animal kingdom, TRs support a wide range of terrestrial invertebrates. During dry conditions, the riverbed provides habitat for diverse invertebrate communities that originally inhabit the shoreline during aquatic periods and then colonize the dry riverbed as conditions change. Many of these invertebrates, such as wolf spiders (Lycosidae), ants (Formicidae), rove beetles (Staphylinidae), and ground beetles (Carabidae), are predators or scavengers that feed on aquatic invertebrates remaining in the dry riverbed (Steward *et al.*, 2017; Sánchez-Montoya *et al.*, 2022). In addition, a wide variety of invertebrates typically found in terrestrial ecosystems are present in TRs, representing several phyla, including Arthropoda, Annelida, Mollusca, and Nematoda. Among them, the most common groups are insects, arachnids (spiders and mites), myriapods (centipedes and millipedes), and springtails (Collembola). These invertebrate communities undergo a succession that aligns with the hydrological cycles, transitioning between aquatic and terrestrial periods (Steward *et al.*, 2017). Some of the most common behavioral adaptations of these organisms (e.g., spider, ants and beetles) include migration to moist refugia or burrowing to avoid desiccation, as well as mechanisms to survive sudden water influxes, such as flotation, seeking higher ground, or burrowing to escape rising water levels (Steward *et al.*, 2017).

Regarding vertebrates, fish are the organisms most closely associated with water in both marine and freshwater ecosystems, as they must remain in water throughout their

entire life cycle. However, even these strictly aquatic organisms have developed adaptations to cope with drying conditions. For example, the climbing perch (*Anabas testudineus*) can move overland and survive in small water bodies for extended periods (Davenport & Matin, 1990; Zworykin, 2012). Growth adaptations are also evident in the three-spined stickleback (*Gasterosteus aculeatus*), a freshwater species widely distributed across Holarctic regions. In the TRs of the Mediterranean Basin, this species halts somatic growth during the summer, in contrast to the different growth patterns observed in the more stable freshwater bodies of northern regions (Clavero, Pou-Rovira & Zamora, 2009). Moreover, most of Europe's endemic fish species are found in the southern peninsulas, which have a Mediterranean climate and served as refugia during glacial periods (Kerezsy *et al.*, 2017). However, fish are not the only vertebrates present in TRs. These habitats, often located in areas where water is a limiting resource, are also utilized by amphibians, reptiles, birds, and mammals for various purposes, including food and water sources, shelter, and nesting sites, during both wet and dry periods (Sánchez-Montoya *et al.*, 2017; Puig-Gironès *et al.*, 2024). Notable adaptations among these groups of vertebrates include reduced metabolic rates to tolerate dehydration in frogs and salamanders (e.g., *Ambystoma tigrinum*), and water storage in tissues in dromedaries (Sánchez-Montoya *et al.*, 2017). Previous groups have not been explored in depth, as the focus of this thesis is on aquatic macroinvertebrates, which are described in the following sections.

Aquatic macroinvertebrates

Aquatic macroinvertebrates, also known as benthic macroinvertebrates, are ubiquitous across freshwater ecosystems and are defined as invertebrates that inhabit freshwater benthic habitats and that are large enough to be seen with the naked eye, typically with a size threshold of 0.5 mm. There are several reasons why aquatic macroinvertebrates were chosen as the focus for this thesis rather than other groups of organisms. Firstly, due to their size, macroinvertebrates are easier to identify and work with than microorganisms. Secondly, macroinvertebrates can be categorized into their main families in the field, without using stereoscopes or microscopes. In contrast, identifying aquatic plants often requires the presence of flower structures, which are available only for short periods of time. Thirdly, compared to terrestrial invertebrates, aquatic macroinvertebrates exhibit lower overall

diversity but are more suitable for studying TR dynamics due to their strong association with the aquatic periods of these ecosystems (Steward *et al.*, 2017; Stubbington *et al.*, 2017). Fourthly, aquatic macroinvertebrates possess diverse dispersal abilities across both spatial and temporal scales (Bogan *et al.*, 2017; Sarremejane *et al.*, 2020a). Finally, their rapid responses to environmental changes and disturbances offer valuable insights into ecosystem dynamics, particularly in the highly variable and transient habitats of TRs. Moreover, macroinvertebrates are sensitive to hydrological variability, with distinct species adapted to lentic or lotic environments (Bonada *et al.*, 2006). These characteristics highlight the suitability of macroinvertebrates as model systems for exploring fundamental ecological and evolutionary processes in TRs, serving as the basis for the specific objectives addressed in this thesis. In this section, the main taxonomic groups observed in TRs during this thesis are described, along with their general ecological roles and adaptations to cope with desiccation in TRs. The notes on dispersal mechanisms for specific groups of organisms are key to understanding how they influence macroinvertebrate community composition in TRs.

Non-Insect Macroinvertebrates: Platyhelminthes, Annelida, Mollusca, Crustacea, and Arachnida

Most species in the phylum Platyhelminthes are parasites, however, Turbellaria are free-living flatworms, with approximately one-fifth of their species found in freshwater environments. Despite significant scientific activity in Europe, numerous regions and habitats remain insufficiently studied, particularly temporary aquatic ecosystems in the Mediterranean Basin (Schockaert *et al.*, 2008). Although uncommon in temporary waters, some Turbellaria species have developed life history strategies to survive the dry periods in pools (Bonada & Bogan, 2024).

The phylum Annelida, characterized by segmented bodies, is primarily represented in freshwater ecosystems by oligochaetes and leeches (Purschke, 2002). The majority of oligochaetes are terrestrial, however, some species and families, such as Naididae and Lumbricidae, have adapted to freshwater environments. They are broadly distributed across various contamination gradients and prefer to inhabit lentic waters, (e.g., pools), with fine sediments (Martin *et al.*, 2008). One of the traits that allow oligochaetes to cope with dry

conditions is their asexual reproduction, which enables rapid colonization and establishment of new populations (Tachet *et al*., 2010).

Mollusca represents the second most diverse phylum in the animal kingdom, surpassed only by Arthropoda. The freshwater species within this phylum primarily include bivalves and snails, belonging to the classes Bivalvia and Gastropoda, respectively, with a total of approximately 5,600 known species (Cummings & Lydeard, 2019). Pulmonate gastropods are the most common mollusks found in temporary waters (Strong *et al.*, 2008). Most snails typically have a spiraled shell and may possess an operculum. A common species in Mediterranean TRs is the Hydrobiidae *Potamopyrgus antipodarum* (Múrria, Bonada & Prat, 2008). Native to New Zealand, this species has become invasive in freshwater ecosystems across Europe and North America. It is highly adaptable and can tolerate dry conditions by burrowing into sediment (Alonso & Castro-Díez, 2015). In contrast, the families Lymnaeidae and Physidae do not have an operculum. They inhabit slow-moving waters and are browsers, feeding on leaves, detritus, and algae. Gastropods inhabiting temporary aquatic habitats encounter significant challenges from desiccation but have evolved remarkable adaptations to conserve water and endure extended dry periods. Pulmonate gastropods, which dominate these environments, are particularly vulnerable due to their thin shells and lack of an operculum. To mitigate desiccation, they burrow into substrates at varying depths, retreat into their shells, and secrete a protective mucous membrane or epiphragm. This response reduces their metabolic activity and enables them to aestivate until favorable conditions return (Alyakrinskaya, 2004). While some species aestivate on the soil surface, others seek refuge beneath decaying vegetation (Alyakrinskaya, 2004; Poznańska *et al.*, 2015; Vilenica *et al.*, 2024). A common characteristic of mollusks is their inability to disperse independently outside of aquatic environments, which greatly limits their dispersal capacity, as they are confined to water for their survival (Kappes & Haase, 2012). However, some species are capable of passive dispersal through other animals (zoochory), which facilitates their movement across water bodies (Van Leeuwen *et al.*, 2013). For example, *Physella acuta* is a widely distributed and highly effective invader of freshwater ecosystems (Vinarski, 2017).

The most primitive group within the phylum Arthropoda is the Chelicerata, with arachnids as the primary representatives. This group includes water mites (Hydrachnidia), with almost all species being freshwater (Thorp & Raupach, 2024). Water mites represent a

highly abundant and diverse group that has traditionally posed challenges due to their complex taxonomy. In lakes and streams, a single square meter can harbor up to 5,000 individuals, encompassing around 75 different species (Proctor *et al.*, 2015). They are free-living and/or ectoparasitic, which aids in their dispersal among different water bodies. Additionally, water mites are broadly distributed across various mesohabitats, with a preference for lentic waters. Some species have also adapted to survive in temporary aquatic habitats by entering diapause during dry periods (Di Sabatino, Gerecke & Martin, 2000).

Among macroinvertebrates, Crustacea are primarily represented in our rivers by gammarids (family Gammaridae) and crayfish. Gammarids are capable of rapidly recolonizing and establishing populations, and they can also burrow into the hyporheic zone during drying conditions (Poff *et al.*, 2006). Regarding crayfish, the red swamp crab (*Procambarus clarkii*), an invasive species from North America, poses a significant threat to the survival of native crab populations in Europe, such as *Austropotamobius pallipes* and *Astacus astacus*, as well as to other organisms, including macrophytes, macroinvertebrates, amphibians, and mollusks (Souty-Grosset *et al.*, 2016). There is much greater diversity of crustaceans in freshwater ecosystems; however, due to their small size, they are not classified as macroinvertebrates (e.g., Copepoda, Ostracoda, and Branchiopoda).

Aquatic insects

Within Arthropoda, and the entire animal kingdom, insects represent the most diverse group in terms of species, with over one million described species in terrestrial and freshwater ecosystems. Estimates suggest there are around 5.5 million insect species, making up 60.4% of freshwater species (Balian *et al.*, 2008; Stork, 2018). In the Mediterranean basin, there are ten taxonomic orders that include aquatic insect species. Five of these orders consist entirely of species that are aquatic during some stage of their life cycle (Ephemeroptera, Plecoptera, Odonata, Megaloptera, and Trichoptera). The remaining orders contain both aquatic and terrestrial species (Hemiptera, Coleoptera, Diptera, Neuroptera, and Lepidoptera) (Boix *et al.*, 2024). This text focuses on the first eight orders mentioned, as Neuroptera and Lepidoptera include only a few aquatic species and are not represented in the TRs of this thesis.

Ephemeroptera

Ephemeroptera, commonly known as mayflies, is a primitive order in which all species have an aquatic nymphal stage and a short-lived aerial adult stage, with adult lifespans ranging from just a few minutes to several days. Mayflies exhibit various body shapes depending on their habitat preferences. For example, members of the family Baetidae have a streamlined body adapted for swimming, while Caenidae (*Caenis*) typically walk on sediments and possess a flattened body with operculate gills that protect their respiratory gills. While mayflies can inhabit a wide range of stream environments, some species are adapted to specific habitats. Rheophilic species, such as *Baetis* (e.g., *Baetis alpinus*) and heptagenids like *Electrogena*, thrive in fast-flowing waters. In contrast, other Baetidae genera, such as *Centroptilum* and *Cloeon*, are adapted to standing waters and can tolerate low oxygen concentrations, often found in TRs (e.g., disconnected pools) (Sartori & Gattolliat, 2024). Additionally, hyporheic habitats can be colonized by Leptophlebiidae genera such as *Thraulus* spp.

Plecoptera

Stoneflies, belonging to the order Plecoptera, have aquatic nymphs and an aerial adult phase. The terrestrial adult form is generally characterized by limited dispersal abilities, typically restricted to short distances. This order is regarded as a reliable indicator of water quality, as they thrive in ecosystems with high dissolved oxygen, cold temperatures, and running waters (Fochetti & Tierno de Figueroa, 2008). Combined with their limited dispersal ability, these factors make stoneflies particularly vulnerable to the impacts of global change (Tierno de Figueroa *et al.*, 2010). This leads to a high level of endemism among stoneflies, with 40.3% of the species found in the Mediterranean Basin being endemic to the region (Tierno de Figueroa *et al.*, 2013). Given the characteristics described above, stoneflies appear to be poorly adapted to TRs. Nevertheless, several species have developed strategies to cope with drying in TRs. For instance, the semivoltine species *Guadalgenus franzi* migrates into the hyporheic zone, where still water persists, to survive dry periods (López-Rodríguez *et al.*, 2009). Another strategy employed by univoltine species, such as *Capnioneura gelesae*, is to grow rapidly during the winter or early spring in their nymphal stages, allowing them to reach

adulthood before the streams dry up (López-Rodríguez *et al.*, 2017). Some specialist species can survive dry periods by entering dormancy, either as nymphs (e.g., *Mesocapnia arizonensis*) or as eggs (e.g., Nemouridae) (Bogan *et al.*, 2015; Stubbington *et al.*, 2017; Bogan,

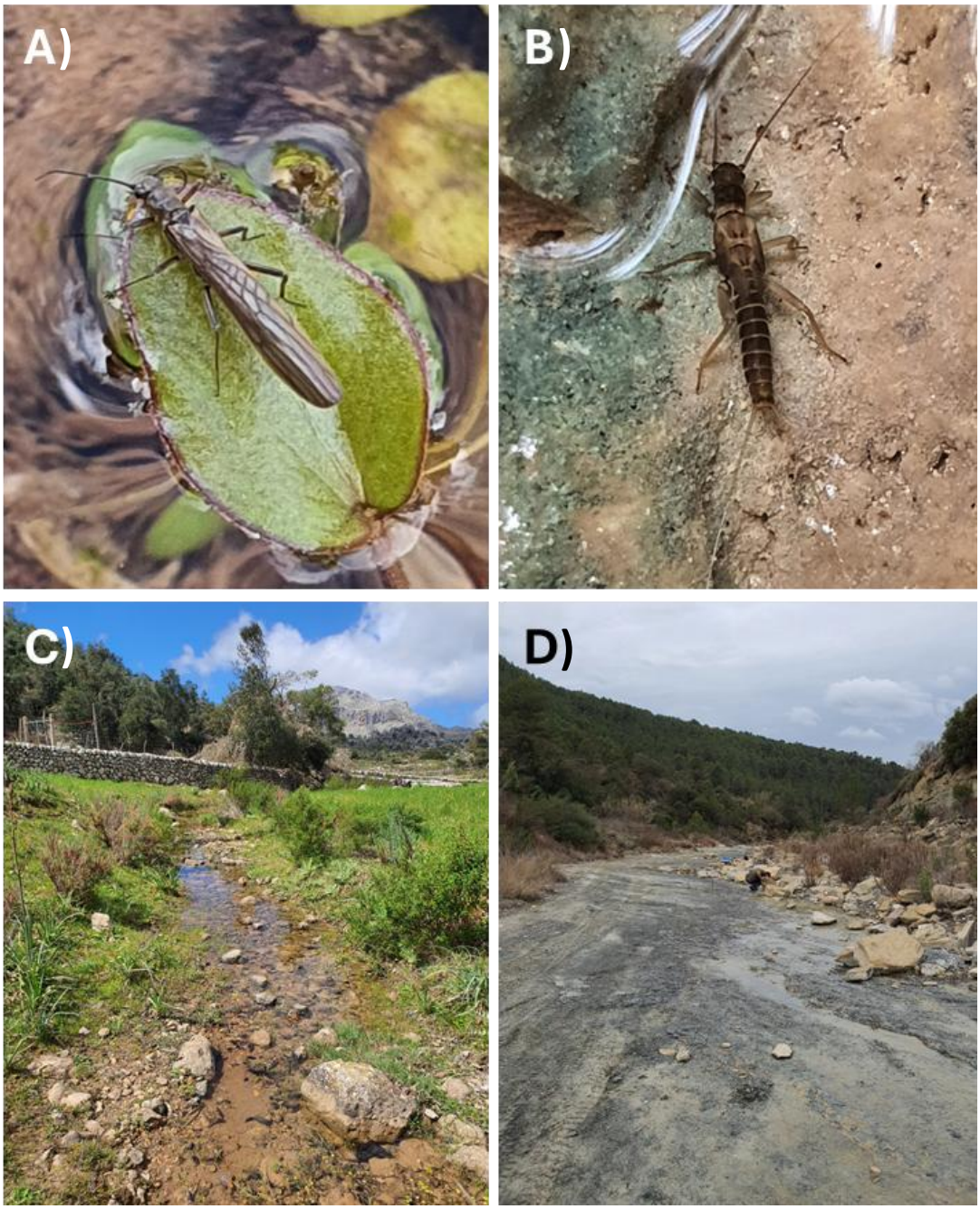


Figure 6. Adult **(A)** and nymph **(B)** stages of *Tyrrhenoleuctra antoninoi*, a stonefly species specialized in ephemeral streams. Photos **(C)** and **(D)** depict typical ephemeral stream habitats for *Tyrrhenoleuctra* spp.: an ephemeral stream in Mallorca, Balearic Islands (Spain) **(C)**, and the Santa Creu stream in Sant Llorenç del Munt i l'Obac Natural Park, Barcelona (Spain) **(D).**

2017). Finally, the genus *Tyrrhenoleuctra* (Figure 6), which is specific to TRs, exhibits asynchrony in nymph development, resulting in overlapping cohorts due to extended flight periods, thereby allowing them to be prepared for the onset of dry periods (Tierno de Figueroa, López-Rodríguez & Fochetti, 2024). In fact, this genus has been cited for the first time in the northeastern Iberian Peninsula (previously reported in northwestern Africa, the southern Iberian Peninsula, the Balearic Islands, Corsica, and Sardinia) during the development of this thesis by its author (Fernández-Calero *et al.*, 2022) **(See Supplementary Contributions: Annex 1).**

Odonata

The Odonata order, which includes damselflies (Zygoptera) and dragonflies (Anisoptera), is another hemimetabolous order (i.e., a gradual metamorphosis, with immature stages resembling adults in form and body proportions) composed entirely of aquatic species, along with Ephemeroptera and Plecoptera. Like these other orders, Odonata have aquatic larval stages and aerial adult forms. They prefer lentic habitats, such as lakes, ponds, and disconnected pools, where they inhabit shorelines with abundant vegetation, with rivers containing strong currents being less favorable. Nevertheless, some species, such as *Coenagrion caerulescens*, *Calopteryx* spp., and members of the family Cordulegastridae require streams with flowing water and vegetation for breeding (Carchini & Hardersen, 2024). Although adult dispersal typically involves local movements, dragonflies are capable of covering long distances due to their large size and strong flying abilities, allowing them to find food, breeding sites, or avoid competition (Minot, Besnard & Husté, 2021). For instance, migration is documented in some odonates. Similar to the monarch butterfly (*Danaus plexippus*), the common green darner (*Anax junius*) is known for its complex life history and migratory movements in North America (May & Matthews, 2008; Hallworth *et al.*, 2018). This remarkable dispersal ability can also be considered a resilience strategy, enabling species to colonizing distant perennial refuges during or after the dry period (Stubbington *et al.*, 2017).

Hemiptera

This order is one of the largest insect orders (the fifth largest overall and the largest among hemimetabolous insects). In the Hemiptera order, all aquatic species belong to the suborder Heteroptera. The infraorder Nepomorpha, known as water bugs, includes species that have developed a fully aquatic life as adults. All Nepomorpha are predators of other invertebrates and small vertebrates (e.g., *Notonecta,* Corixidae), using their adapted raptorial forelegs (e.g., *Naucoris*) for hunting (Cianferoni, 2024). Some of these species, such as *Nepa cinerea* (Nepidae), possess a siphon, a caudal appendage used to capture air from the atmosphere, while others capture air bubbles directly, similar to many beetles. This adaptation allows them to survive during the non-flowing conditions of TRs (e.g., in disconnected pools) under low dissolved oxygen conditions.

On the other hand, the infraorder Gerromorpha, known as semiaquatic bugs, lives on the water's surface, walking or skating. They are the only insects, along with Gyrinidae beetles, that inhabit the upper surface of the water (pleuston). They are predators and scavengers, searching for prey on the surface, where many insects die. Among them, we can find Gerridae (e.g., *Gerris*, *Aquarius*) as well as Veliidae (e.g., *Velia*, *Microvelia*). Water and semiaquatic bugs typically inhabit lentic waters such as lakes, ponds, and slow-flowing streams. However, water bugs are more tolerant to running waters, as they can live in the benthic zone at the bottom of streams. In contrast, semiaquatic bugs require calmer waters that allow them to live on the water surface without being drifted (Cianferoni, 2024).

Megaloptera

Megaloptera is a small holometabolous order, consisting of 354 species, with strictly aquatic larvae (Stork, 2018). The larvae are voracious predators and can be found in various habitats, from large flowing rivers to lakes. However, they prefer soft substrates with detritus and leaves, where they burrow. In spring and summer, the larvae move to shallow shoreline areas, where they pupate, and the adults emerge as aerial insects (Anderson, 2009). For instance, certain megalopterans, similar to some stoneflies, undergo egg or larval diapause stages that allow them to survive prolonged dry periods of up to five years in TRs (Cover, Seo & Resh, 2015; Bonada & Bogan, 2024).

Coleoptera

Coleoptera is the largest group of insects worldwide (around 400,000 named species). The majority of them are terrestrial, with only about 5% being aquatic. Despite this, they represent the second largest group of freshwater arthropods, surpassed only by Diptera (Yee & Kehl, 2015). Along with Nepomorpha, Coleoptera is the only group that has successfully colonized aquatic environments as adults. Water beetles exhibit complex life histories, with both larvae and adults being aquatic in most species. However, Hydraenidae possess terrestrial larvae and egg clutches, which confer an enhanced ability to withstand drying conditions in TRs. Most species live in the water column (pelagic), where they swim and dive, such as adult Dytiscidae, thanks to their hydrodynamic shape and swimming hairs. In contrast, other species are less efficient swimmers and are often found walking (e.g., some Hydraenidae and Hydrophilidae), or as part of the pleuston (e.g., Gyrinidae). In addition, water beetles inhabit a wide range of aquatic habitats, from cold, fast-flowing streams to lakes, estuaries, and ponds. However, they are commonly associated with lentic environments such as lakes, ponds, disconnected pools, or river backwaters (Millán *et al.*, 2024). In fact, Coleoptera, along with Heteroptera and Odonata (OCH), form a broad ecological group of macroinvertebrates that are key indicators of lentic waters (Bonada *et al.*, 2007b), often inhabiting disconnected pools in TRs.

Trichoptera

Trichoptera, commonly known as caddisflies, is a highly diverse order of aquatic insects, with more species than all other strictly aquatic insect orders combined (around 16,200 species). While most larvae are strictly aquatic, adults are aerial and typically fly around the habitats where larvae develop. The most notable characteristic of this group is that certain larvae construct cases using silk produced by modified labial salivary glands. They typically inhabit benthic habitats, residing among stones or leaves, but are also found attached to aquatic plants. Concerning habitat, although preferences vary by species, caddisflies can live in permanent and TRs, ponds, and lakes. Trichoptera, along with Ephemeroptera and Plecoptera (EPT), are most abundant in flowing waters and serve as key indicators of lotic ecosystems (Bonada *et al.*, 2007b). However, some species have developed

adaptations to survive in temporary waters in Mediterranean climates. For example, some Limnephilidae reduce water loss during dry periods through body armoring, while certain Leptoceridae exhibit the ability to fly long distances to colonize new habitats (Poff *et al.*, 2006; Stubbington *et al.*, 2017). Moreover, *Mesophylax aspersus* estivates in caves during its adult life stage, while other species survive the summer as resistant eggs in dry riverbeds (Karaouzas *et al.*, 2024).

Diptera

Diptera, the order of true flies, is one of the most diverse insect orders, and by far the most diverse in aquatic ecosystems with approximately 46,000 aquatic species. They include terrestrial, semiaquatic, and aquatic species. However, aquatic Diptera species are not easily defined, as many larvae live in wet conditions or are considered semiaquatic (e.g., some Calliphoridae and Cecidomyiidae) (Dobson, 2013). In this thesis, following Lencioni, Adler & Courtney, (2024), aquatic Diptera are defined as those that spend most of their life in water as larvae and pupae. Despite being the most diverse group in aquatic ecosystems, Diptera are often the most neglected in scientific studies, partly due to their immense diversity and the extreme difficulty in identifying their larvae, leading to their underrepresentation in aquatic ecology research (Boóz *et al.*, 2024). Diptera are found in nearly all types of aquatic habitats, from lentic waters such as lakes, disconnected pools, ponds, marshes, and artificial pools, to lotic waters like rivers, streams, waterfalls, and madicolous habitats. For example, Chaoboridae are restricted to lentic environments (lakes and long-disconnected pools in streams), while Simuliidae inhabit flowing waters in rivers and streams. Their remarkable plasticity to thrive in suboptimal conditions, combined with their short life cycles, makes them ideal inhabitants of TRs. These traits enable them to rapidly recolonize new habitats. In addition, passive dispersal via drift is a commonly employed strategy, allowing them to colonize downstream sections from perennial upstream reaches efficiently (Stubbington *et al.*, 2017). Other species, such as *Polypedilum vanderplanki*, exhibit remarkable resilience to extreme desiccation by drastically reducing their moisture content and entering a desiccation-tolerant state in which metabolic activity is almost completely suspended (Strachan, Chester & Robson, 2015; Stubbington *et al.*, 2017).

The importance of taxonomic research

While studies on the natural history and taxonomy of aquatic insects have become increasingly rare (Hochkirch *et al*., 2022), they remain fundamental and form the basis of all ecological research. This, together with the great diversity that TRs harbour, motivated me to work at a fine taxonomic resolution and to perform some taxonomic studies that are provided in the **Supplementary Contributions** of this thesis.

METACOMMUNITIES IN TEMPORARY RIVERS

Some of the species described above coexist in the same location at the same time, forming a biological community. The traditional concept of community assumes that it exists on a single scale and is isolated from others (Fauth *et al.*, 1996; Stroud *et al.*, 2015). However, we know that species disperse along the landscape and interact with each other. Thereby, local communities can be expected to be connected to each other and exchange species. This "*set of local communities that exchange colonists of multiple species*" has been termed meta-community (Leibold *et al.*, 2004). Similar to metacommunities, the concept of metapopulations emphasizes that populations interact through migration and dispersal processes (Hanski & Ovaskainen, 2000). These interactions facilitate the exchange of individuals among local populations, enabling the recolonization of vacant habitats, preserving genetic diversity, and buffering against local extinctions. In essence, while metacommunities examine interactions among multiple species across spatially connected communities, metapopulations focus on the population-level dynamics of a single species distributed across a landscape (Hastings & Harrison, 1994).

Overall, dispersal plays a critical role in both local and regional dynamics, influencing population and community assembly within a region (i.e., metapopulation and metacommunity). Some authors argue that biodiversity patterns result from random and stochastic processes (Hubbell, 2001; Rosindell, Hubbell & Etienne, 2011), while others claim that they are mainly structured through deterministic processes (Kraft *et al.*, 2015). However, it appears that these processes are difficult to separate, and ecological communities are likely governed by a combination of both stochastic and deterministic factors (Powell *et al.*,

2015). Regardless of the viewpoint, dispersal is key to understanding how individuals move across the landscape and how connectivity between local communities determines metacommunity structure (Cañedo-Argüelles *et al.*, 2015; Tonkin *et al.*, 2018).

Metacommunity theory aims to explain species composition across space and time through four distinct but non-exclusive paradigms or perspectives (Figure 7) (Leibold *et al.*, 2004; Leibold & Chase, 2017). The first perspective is the “patch-dynamic paradigm”, which assumes that all patches (i.e., discrete areas or sites) are identical and can be potentially colonized by all the species. Extinction in these patches occurs due to both stochastic and deterministic processes. In this paradigm, species are categorized as either strong competitors but poor dispersers, or good dispersers but weaker competitors. As a result, there is a trade-off between species' competitive abilities and their dispersal capabilities, allowing coexistence across patches. The second paradigm, "species-sorting", assumes that patches differ in habitat or abiotic conditions. In this scenario, species are filtered by environmental factors. If species have a wide dispersal range and can access all patches, their distribution is determined primarily by these environmental conditions. Thirdly, the “mass effects” paradigm emphasizes regional spatial dynamics, where patches are well-connected through dispersal. In this framework, species can persist in patches with suboptimal habitat conditions due to high immigration rates from source patches with optimal conditions. The continuous movement of individuals from favorable to less favorable habitats allows species to maintain populations even in areas where they might otherwise be unable to survive. Finally, the “neutral” paradigm assumes that all species have identical competitive and dispersal abilities, meaning species are functionally equivalent. In this paradigm, community composition is shaped by random stochastic processes, including immigration, emigration, extinction, and speciation (Hubbell, 2001; Leibold *et al.*, 2004; Leibold & Chase, 2017).

The dendritic structure of river networks, combined with their unidirectional flow, plays a crucial role in shaping the diversity and composition of the (meta)communities they support, as well as the diverse forces influencing them (e.g., stochasticity, environmental filtering). The connectivity of a community within the network is largely determined by its location, which influences its access to potential colonizers from other sites (Tonkin *et al.*, 2018). Central and lower reaches within the network typically exhibit higher species diversity, driven primarily by dispersal processes (i.e., mass effects). Conversely, communities in

headwater sites are often more isolated, poorer in species and with environmental filtering playing a dominant role in shaping community dynamics through species sorting (Brown & Swan, 2010; Altermatt, Seymour & Martinez, 2013; Altermatt, 2013). The spatial position within river networks is thus relevant to understand community structure but also the flow of matter and energy, shaping the overall meta-ecosystem (Cid *et al.*, 2022).

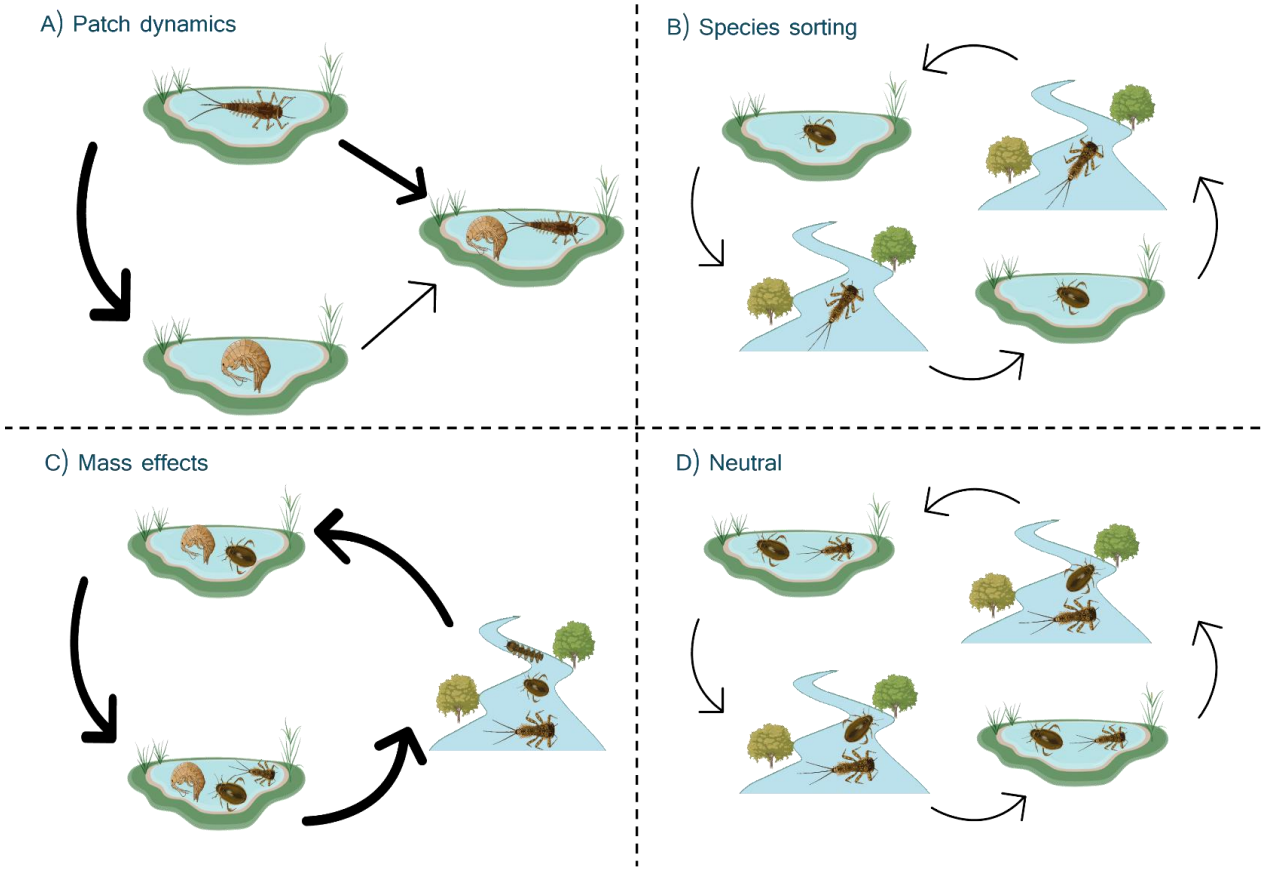


Figure 7. Representation of the four paradigms in metacommunity ecology, adapted for freshwater ecosystems. Solid arrows indicate donor populations or higher dispersal rates. Habitat patches are depicted as pools and flowing streams. **A)** Patch Dynamics Paradigm: all patches are similar, allowing coexistence between strong dispersers (e.g., Baetidae) and strong competitors with lower dispersal capabilities (e.g., Gammaridae). **B)** Species Sorting Paradigm: species distributions are determined by habitat-specific filters, with taxa restricted to particular habitats (e.g., Dytiscidae in pools and Heptageniidae in riffles). **C)** Mass Effects Paradigm: high dispersal rates enable species to persist in suboptimal habitats, resulting in population imbalances (e.g., Heptageniidae having smaller populations in pools compared to Dytiscidae, and vice versa in flowing waters). **D)** Neutral Paradigm: all species are ecologically equivalent, with equal probabilities of colonizing patches, and population dynamics are governed by stochastic processes. Figure adapted from Leibold *et al*. (2004). Macroinvertebrate drawings by Pau Fortuño

Connectivity, dispersal and spatial scale as key metacommunity drivers in temporary rivers

Landscape connectivity is one the main drivers of metacommunity dynamics because it determines species' dispersal (Chisholm, Lindo & Gonzalez, 2011; Tonkin *et al.*, 2018). Traditionally, studies on connectivity have focused on spatial dimensions, often neglecting the temporal component, which is particularly critical in TRs (Fullerton *et al.*, 2010; Uroy *et al.*, 2021). In TRs, connectivity is directly influenced by seasonal variations in hydrological conditions. These periodic hydrological cycles, alternating between aquatic and dry periods, drive transitions from lotic to lentic habitats and, eventually, to terrestrial environments, thereby modifying network connectivity over time (Boulton *et al.*, 2017; **Chapter 1**). Therefore, temporal connectivity plays a pivotal role in shaping metacommunity dynamics in TRs because it determines dispersal. For example, some species that disperse by drift might only be able to colonize certain habitats when the stream network is hydrologically connected (Pineda-Morante *et al.*, 2022). Furthermore, temporal variability fosters species coexistence, as phenological shifts enable species to specialize in different resources or periods of the hydrological cycle (Chase, 2005). Within this context, **Chapter 1** provides a unified theoretical and methodological framework that incorporates both the spatial and temporal aspects of connectivity.

Dispersal mechanisms in temporary rivers

By modifying connectivity, drying can affect dispersal between sites (spatial dispersal or resilience) and within a single site over time (temporal dispersal or resistance), thereby impacting metacommunity dynamics across both spatial and temporal scales (Sarremejane *et al.*, 2020a). Considering the spatial dispersal (which favors resilience to drying), macroinvertebrates can disperse through aquatic or aerial means. Those that disperse by water include strictly aquatic macroinvertebrates, such as snails and flatworms. These movements can occur through drifting in the water column or by moving along the benthos. For instance, although snails can move along the benthos, their upstream dispersal rate is notably slow (Kappes & Haase, 2012). However, snails can also disperse by drifting in the water, allowing them to be among the first colonizers of freshwater habitats (Cañedo-

Argüelles & Rieradevall, 2011). Thus, these organisms reflect a community structure influenced by distances within the river network (Olden, Jackson & Peres-Neto, 2001). Those macroinvertebrates, primarily insects, that can fly are capable of dispersing over long distances and overland between different river branches in the network. For example, some dragonflies can maintain gene flow between populations at very large scales in arid landscapes, thanks to their high air dispersion capability (Razeng *et al.*, 2017). However, it is not only the media (i.e., water vs. air) that is important, the mode of dispersal also plays a key role in determining how macroinvertebrates disperse. Dispersal can be differentiated between active and passive modes. Active dispersers are organisms that intentionally move between habitats or geographic areas using their own means of locomotion (e.g., dragonflies, dytiscids, or backswimmers), while passive dispersers rely on external factors such as drift, wind, or other animals (i.e., zoochory). For instance, small Diptera, including mosquitoes, chironomids, ceratopogonids, and blackflies, are often dispersed by wind (May, 2019). Additionally, macroinvertebrates can disperse through zoochory by attaching to other animals, ranging from invertebrates like mosquitoes (which can carry water mites) to vertebrates such as waterbirds and mammals (Van Leeuwen *et al.*, 2013; Santos *et al.*, 2016; Green *et al.*, 2023). Considering both, the medium and mode of dispersal, the most used trait databases classify macroinvertebrates along a gradient of dispersal into four non-exclusive categories: aerial active, aerial passive, aquatic active, and aquatic passive (Tachet *et al.*, 2010; Sarremejane *et al.*, 2020a). While spatial dispersal plays a significant role in the connectivity of communities, temporal dispersal mechanisms are equally essential for the persistence of aquatic macroinvertebrates during dry periods in TRs.

Many species have evolved temporal dispersal mechanisms to survive periods of desiccation (which favors resistance to drying), including the production of dormant stages that can resist drying and remain viable until water returns. These dormant stages, such as eggs or larvae, allow taxa to endure unfavorable conditions by entering a state of diapause or desiccation resistance (Bonada *et al.*, 2017). This form of temporal dispersal enables macroinvertebrates to survive the absence of surface flow and to reactivate their physiological functions once water resumes. For example, in braided rivers, the hyporheic zone can serve as a refuge for macroinvertebrates, supporting high densities of dormant organisms that recolonize the benthos after flow restoration (Vander Vorste, Malard & Datry,

2016a; Fournier *et al.*, 2023). Additionally, dormant stages can comprise a significant proportion of the macroinvertebrate community, with some studies showing that more than half of the taxa in certain rivers rely on these survival strategies during dry periods (Larned, Datry & Robinson, 2007; Stubbington & Datry, 2013). Conversely, some species exhibit other adaptations such as seeking refuge in the hyporheic zone, from where they can disperse once surface water returns (Bogan *et al.*, 2017). These temporal dispersal mechanisms are essential for maintaining biodiversity and ensuring the recovery of metacommunities in TRs, as they help organisms to persist during drying and recolonize dry areas after flow resumes. However, in the study area of this thesis (the Sant Llorenç del Munt Natural Park, in the Barcelona province), no significant contribution from the hyporheic zone to community recovery after drying is expected. This is likely due to the stream's bedrock substrates and the absence of substantial sediment layers, which resulted in a reduced hyporheic zone and a limited presence of desiccation-resistant species (Chester & Robson, 2011; Folch, 2020).

Understanding dispersal becomes particularly relevant in TRs metacommunities due to the spatial and temporal shifts between lotic and lentic conditions. When rivers stop flowing, species sorting becomes more important as local species composition must cope with the lack of flow and the strong abiotic (e.g., lower oxygen levels) and biotic (e.g., increased competition) filters present in disconnected pools. However, in these new habitats, dispersal plays also a crucial role, as only species with strong dispersal abilities, such as dragonflies or diving beetles, can reach these disconnected pools (Bogan *et al.*, 2017; Bonada *et al.*, 2020). Conversely, during rewetting after the dry phase, species locally colonise from nearby refuges mainly by drift, which can also reach suboptimal environmental conditions leading to mass effects (Datry *et al.*, 2017c; Cid *et al.*, 2020). Therefore, the relative effect of species sorting and dispersal vary with time in TRs (Datry *et al.*, 2017c).

Dispersal is typically assessed in ecological research using biological traits databases (e.g., Tachet *et al*., (2010); Sarremejane *et al*., (2020a)) that assign dispersal categories to each taxon (i.e., potential dispersal), as directly measuring dispersal distances is both challenging and time-consuming (Heino *et al.*, 2017; Sarremejane *et al.*, 2020a). Nonetheless, direct measurements of dispersal movements (i.e., effective dispersal) can be obtained using various methodologies, including physical mark-recapture (Epopa *et al.*, 2017; Ditrich, 2021), isotopic enrichment of larval stages (Macneale, Peckarsky & Likens, 2005; Enss *et al.*, 2024),

and the analysis of genetic structure within populations (Marten, Brändle & Brandl, 2006; Phillipsen *et al.*, 2015). From this foundation arises part of the focus of **Chapters 3 and 4**, which aim to examine the genetic structure of two species with contrasting dispersal abilities and directly evaluate aerial dispersal using isotopic enrichment.

Spatial arrangement, spatial scales, and metacommunity dynamics in drying river networks

In TRs, the spatial positioning of temporary reaches and the unidirectional flow of streams play a crucial role in shaping community composition and governing the spatial drying patterns that define these ecosystems. Within drying river networks, dry reaches may occur in the lower, mid, or headwater sections. Additionally, these drying reaches can exhibit a patchy distribution, or in some cases, the entire river network may become completely dry (Figure 8). Each of these drying patterns creates different network structures that can have different effects on metacommunity assembly (Datry *et al.*, 2016a; Stubbington *et al.*, 2017).

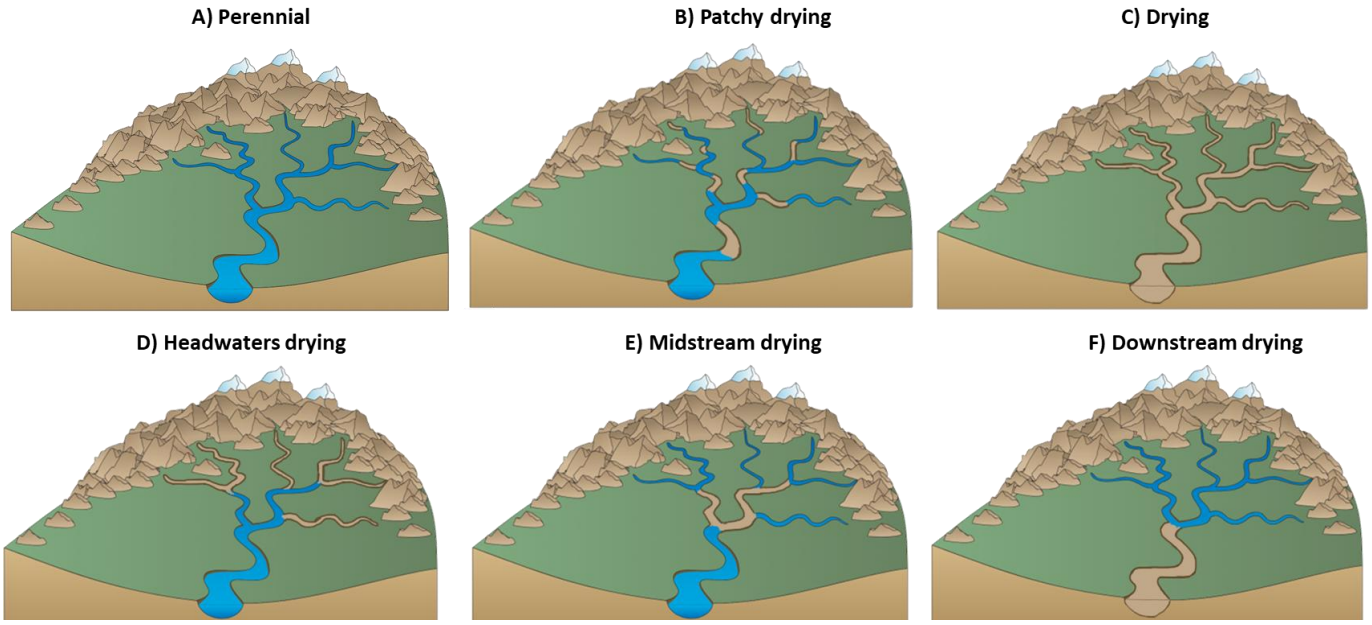


Figure 8. Schematic representation of a dendritic river network, illustrating the spatial distribution of drying reaches. The diagram includes six scenarios: **A)** perennial network, **B)** patchy drying network, **C)** completely drying network, **D)** drying in headwaters, **E)** drying in midstream, and **F)** drying downstream. Blue lines represent permanent reaches, while brown lines indicate dry reaches. The base figure was sourced from https://vecta.io/.

Headwater dry reaches generally exhibit higher spatial beta diversity compared to downstream reaches. However, the species replacement (i.e., turnover component) is lower in headwater reaches, with richness differences serving as the dominant driver of spatial beta diversity (Crabot *et al.*, 2020). This pattern can be attributed to the isolation of temporary headwater sites, where dispersal limitations make recolonization challenging, resulting in lower turnover compared to more connected parts of the network. The observed patterns also depend on the dispersal capacities of the organisms. For example, aerial dispersers tend to exhibit greater community turnover owing to their ability to reach isolated sites more effectively than aquatic dispersers, which typically show higher richness differences (Crabot *et al.*, 2020). Consequently, the position of temporary reaches within the river network and the dispersal traits of organisms collectively determine community composition, highlighting the importance of spatial drying patterns in structuring biodiversity in TRs (Datry *et al.*, 2016a). The spatial positioning of temporary reaches interacts with broader spatial scales, further influencing metacommunity dynamics and the relative roles of environmental and spatial factors.

Besides the temporal changes in metacommunity drivers in TRs, the spatial scale under consideration also plays a significant role in metacommunity assembly (Heino *et al.*, 2015; Viana & Chase, 2019). The spatial scale ranges from microsites (i.e., areas that can support only a single individual) to regions (i.e., a collection of local communities) (Leibold *et al.*, 2004). However, there is no consensus among researchers on how to define these scales (Heino *et al.*, 2015). In streams, a local community can refer to individual riffles or pools within a reach (Grönroos & Heino, 2012), a section that includes both riffles and pools (Roque *et al.*, 2010), or even an entire stream (Lamouroux, Poff & Angermeier, 2002). Similarly, a metacommunity can encompass a single stream, an entire river basin, or larger geographical regions (Grönroos & Heino, 2012; Landeiro *et al.*, 2012; Heino *et al.*, 2015). Following Heino *et al*. (2015), for a given species or group of species, increased spatial distance results in greater dispersal limitation (Soininen, 2012). Conversely, at smaller spatial scales, high rates of dispersal can be expected between local communities, leading to mass effects (i.e., species can occupy sub-optimal patches through colonization from optimal patches). This can diminish the influence of local environmental conditions when the spatial extent is very small. In contrast, the species sorting paradigm requires sufficient dispersal to detect variations in

community assembly based on environmental conditions (Leibold *et al.*, 2004; Heino *et al.*, 2015). Overall, it is expected that environmental conditions have a limited influence on community structure at both small and large spatial scales but play a more significant role at intermediate spatial scales (i.e., species sorting). In contrast, spatial factors are expected to be more influential at the extremes: mass effects dominate at small spatial extents, while dispersal limitation becomes more important at larger spatial scales (Figure 9) (Heino *et al.*, 2015). However, in TRs the fragmentation caused by drying limits dispersal (Cañedo-Argüelles *et al.*, 2015; Tonkin *et al.*, 2018) and could prevent mass effects, thereby leading to species sorting and/or dispersal limitation even at small spatial scales like the one assessed in this thesis (Figure 9). This is the main research question addressed in **Chapter 2**, in which traditional metacommunity paradigms developed for perennial river networks are challenged.

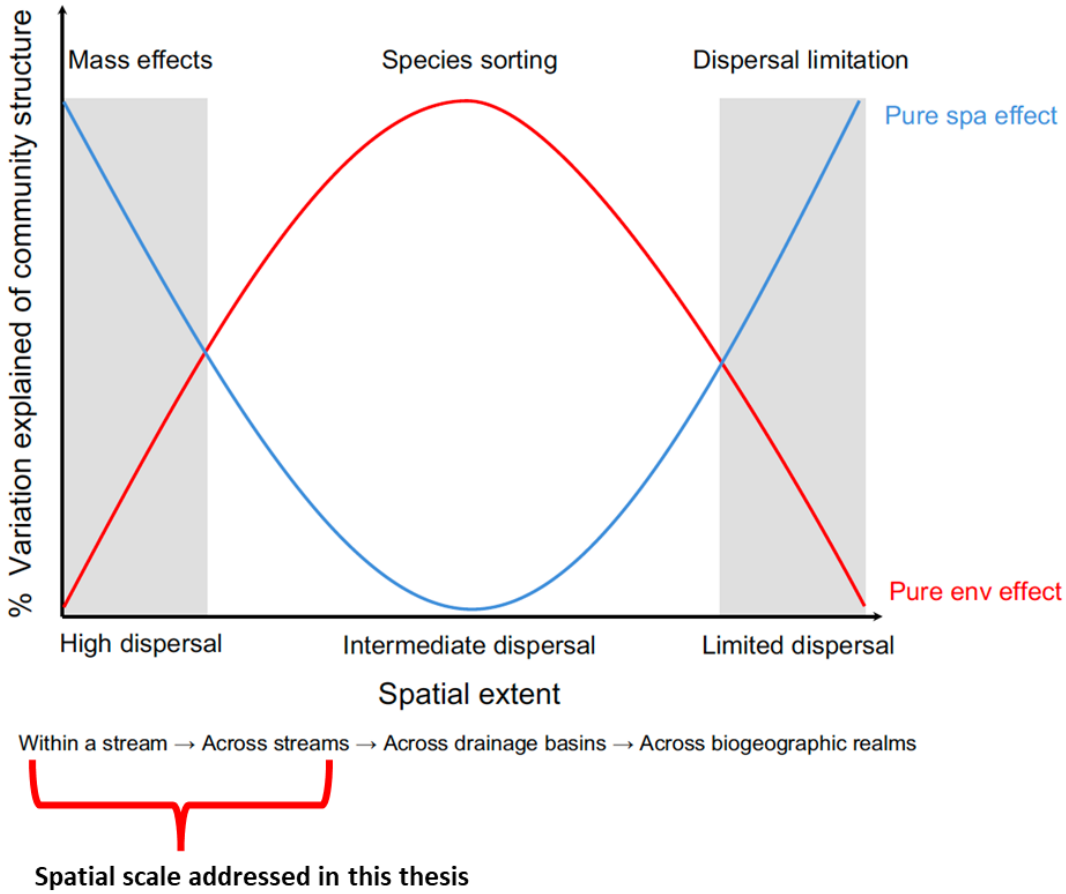


Figure 9. Conceptual model illustrating the relative contributions of environmental filtering and spatial processes in structuring metacommunities. The model emphasizes how the relative importance of these processes shifts across spatial scales. Source: Heino *et al.* (2015).

NOVEL MOLECULAR APPROACHES IN COMMUNITY ECOLOGY

The study of metacommunities in TRs is currently facing new challenges but is also benefiting from emerging technologies that are enhancing our understanding of ecological patterns. Among these innovations, molecular techniques have been particularly instrumental in reshaping ecological research, offering new applications and insights. This section will trace the evolution of these technologies, demonstrating how they are being used, while also acknowledging the limitations that still persist.

Molecular technologies advanced significantly in the late 20th century, particularly with the advent of PCR-based DNA amplification (Mullis & Faloona, 1987; Saiki *et al.*, 1988) and the development of universal primers (Taberlet *et al.*, 1991). These breakthroughs addressed key challenges in species identification, culminating in the establishment of standardized DNA barcoding by Hebert *et al.*, (2003). This method uses short DNA fragments, such as the mitochondrial cytochrome c oxidase I (COI) gene for animals, to enhance identification accuracy. Traditional morphological approaches often fall short due to challenges like phenotypic plasticity, cryptic species, and difficulties in identifying developmental stages or sexes. DNA barcoding provides a faster and more reliable alternative for biodiversity studies (Hebert *et al.*, 2003; Ficetola & Taberlet, 2023).

Over the past two decades, DNA barcoding has transformed ecological research by enabling species identification, comprehensive taxonomic inventories, population genetics studies, and conservation planning (Cristescu, 2014). However, barcoding is limited to individual specimens in good condition, which poses constraints for large-scale biodiversity assessments (Taberlet *et al.*, 2012). To address these limitations, DNA metabarcoding was developed, leveraging high-throughput sequencing technologies to assess entire communities. Metabarcoding facilitates the taxonomic identification of multiple species from a single sample, using either bulk sample analysis or environmental DNA (eDNA) techniques (Hajibabaei *et al.*, 2011; Ruppert, Kline & Rahman, 2019).

Bulk metabarcoding involves analyzing pooled specimens, often focusing on specific taxonomic groups, and aligns closely with traditional morphological methods (Laini *et al.*, 2020). In contrast, eDNA metabarcoding retrieves degraded DNA from environmental

samples such as water, soil, or sediments, enabling non-invasive assessments of community composition. While eDNA is advantageous for large-scale ecosystem monitoring, it can be less effective at detecting certain bioindicator species due to issues like DNA degradation or low species abundance (Macher *et al.*, 2018; Ruppert *et al.*, 2019). These two approaches are complementary, with their choice depending on the study's objectives. Bulk metabarcoding is particularly suited for detailed analyses of community composition, while eDNA excels at assessing broader ecological stressors and ecosystem health (Múrria *et al.*, 2024).

Application of molecular tools in temporary rivers

All these molecular methodologies have been widely applied in inland aquatic ecosystems, including TRs. Metabarcoding enables the study of community dynamics in TRs and emerges as a complementary method for assessing metacommunity structures in these highly fragmented ecosystems (Blackman *et al.*, 2021). Given that hydrological fragmentation can result in isolated populations, spatiotemporal dynamism can alter the genetic structure of aquatic organisms, affecting both microevolutionary and macroevolutionary ecological processes (Bonada *et al.*, 2017).

Connecting dispersal to genetic structure, it can be inferred that the genetic composition of a species reflects its dispersal abilities (Chester *et al.*, 2014). Generally, it is assumed that increased dispersal facilitates greater gene exchange, which in turn enhances the genetic similarity among populations (Heino *et al.*, 2017; May, 2019). For example, an insightful study by Phillipsen *et al.*, (2015) illustrates these patterns in a drying river network in Arizona. They examined three insect species to investigate the relationship between genetic structure and dispersal ability. The researchers found that a perennial water inhabitant exhibited population differentiation attributed to genetic drift. In contrast, a generalist beetle, known for its robust aerial capabilities, displayed random mating without any discernible spatial pattern. Conversely, the stonefly specialist adapted to TRs (*Mesocapnia arizonensis*) demonstrated an isolation-by-distance pattern, indicative of its limited flying range. However, Phillipsen *et al*. (2015) did not employ a metabarcoding approach; instead, they utilized microsatellite markers, as the study of metapopulations using metabarcoding is still in its infancy. Nevertheless, metabarcoding techniques can facilitate the

investigation of intraspecific genetic variation based on the analyzed barcoding markers. This specific variation is referred to as Exact Sequence Variants (ESVs), which are unique DNA sequences distinguished by their precise nucleotide composition (Elbrecht *et al.*, 2018; Porter & Hajibabaei, 2020; Zizka, Weiss & Leese, 2020). From this foundation, **Chapter 3** aims to assess the effects of fragmentation on intraspecific variability and genetic structure, with a focus on how these patterns are influenced by dispersal abilities. Additionally, it compares the effectiveness of morphological and metabarcoding approaches in capturing metacommunity dynamics in TRs.

CONSERVATION OF TEMPORARY RIVERS FROM A METACOMMUNITY PERSPECTIVE

TRs represent a significant portion of global river networks (Messager *et al.*, 2021) and are increasingly recognized for their ecological importance. However, their conservation presents unique challenges due to their intermittent nature and heightened vulnerability to both natural and anthropogenic disturbances. While TRs face many of the same threats as perennial rivers—such as habitat degradation, pollution, and invasive species—they are particularly sensitive to alterations in hydrological regimes caused by water withdrawals (Figure 10), intensive agriculture, and land-use changes (Chiu *et al.*, 2017). Climate change further exacerbates these pressures by extending dry periods and reducing flow permanence, with significant implications for biodiversity and ecosystem functioning (Chiu *et al.*, 2017). Hydrological alterations can transform perennial rivers into TRs or, conversely, convert natural TRs into perennial systems, often due to human activities such as irrigation or wastewater effluent discharge (Lawrence *et al.*, 2014). These shifts can significantly disrupt ecological processes, resulting in habitat loss, changes in species composition, and diminished resilience of aquatic ecosystems. Understanding these unique dynamics is essential for developing conservation strategies that address the distinctive characteristics and vulnerabilities of TRs. However, despite their ecological importance and the pressing challenges they face, TRs are often overlooked in current water management and policy frameworks, further hindering their effective conservation and sustainable management (Fritz, Cid & Autrey, 2017).

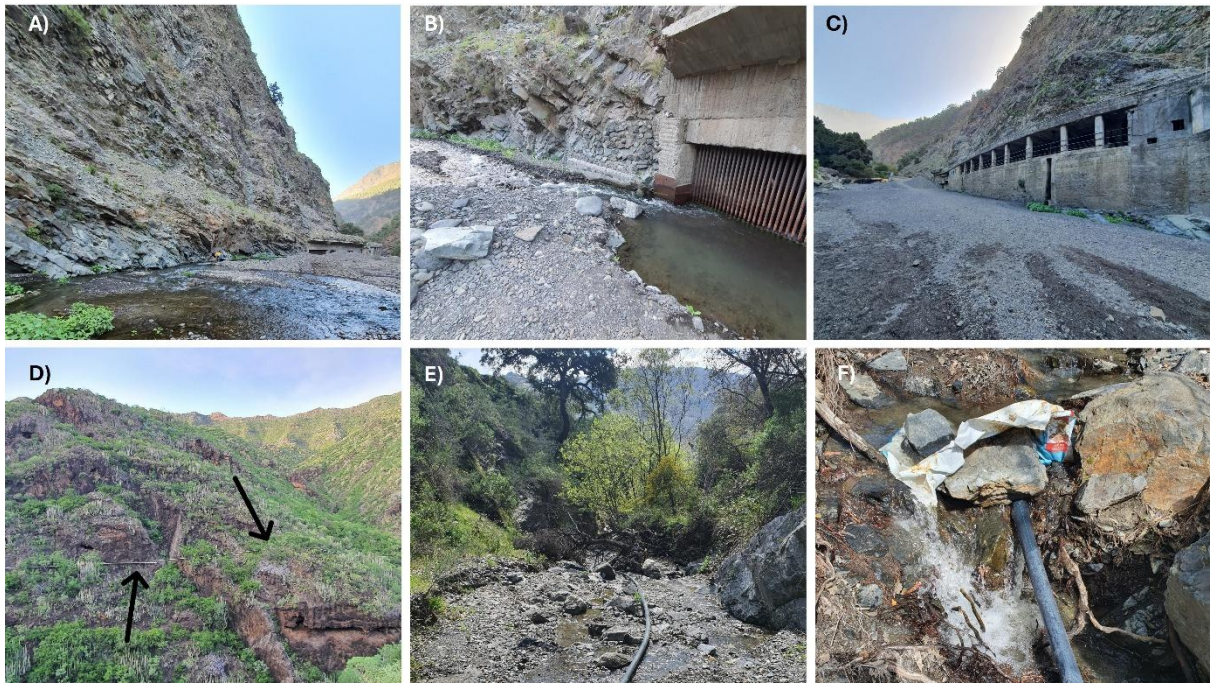


Figure 10. Superficial water withdrawal in temporary rivers. Photos **A**, **B**, and **C** show the same river at three stages: immediately before water extraction, when water is flowing **(A)**; during the extraction process **(B)**; and after extraction, when the riverbed is dry **(C)**. These images were captured at the boundary of *La Caldera de Taburiente* National Park, *Barranco de las Angustias*, La Palma (Canary Islands, Spain). Photo **D** shows water extraction pipes (indicated by arrows) crossing a ravine in *Barranco de Igueste*, Anaga Rural Park, Tenerife (Canary Islands, Spain). Photos **E** and **F** illustrate water extraction infrastructure in a temporary river within the Genal Basin (Málaga, Spain). Photos by the author.

Overall, TRs have been largely neglected by water managers and policy-makers (Acuña *et al.*, 2014; Fritz *et al.*, 2017; Marshall *et al.*, 2018; Datry *et al.*, 2023). For example, in the European Union, the Water Framework Directive (WFD) (2000/60/CE) aims at achieving the good ecological status (defined according to physicochemical, biological and hydrogeomorphological quality elements) of European water bodies. However, this does not apply to many TRs because they are too small to be considered a water body (Kristensen & Globevnik, 2014). Even when they are included into monitoring programs the assessment of their ecological status proves challenging. While metrics for evaluating the physicochemical and biological quality of perennial rivers have been developed and widely applied, their reliability in TRs is low, mainly due to the fluctuating hydrological regimes and the absence of perennial flow (Soria *et al.*, 2020; Bonada *et al.*, 2024). Moreover, the conventional methods

also struggle to differentiate between natural (e.g., caused by drying) and anthropogenic disturbances (e.g., chemical pollution), leading to potentially inaccurate evaluations (Soria *et al.*, 2020; Bonada *et al.*, 2024). The unique nature of TRs demands methods that account for the dynamic alternation between flow and dry periods, which strongly influence their biological composition (Munné & Prat, 2011).

Current biological metrics, often calibrated for perennial conditions, fail to properly assess TRs, particularly disconnected pools, which naturally support lower species richness (Bonada *et al.*, 2020, 2024). For example, stoneflies—key indicators in indices like the IBMWP due to their pollution sensitivity—are typically restricted to flowing waters (Alba Tercedor *et al.*, 2002). Their absence in disconnected pools does not necessarily reflect poor water quality. Additionally, the predominance of generalist species with broad environmental tolerances complicates the use of traditional tools in these ecosystems (Prat *et al.*, 2014). To address these challenges, alternative approaches such as functional diversity metrics, including functional redundancy, have been proposed. These metrics provide valuable insights into ecosystem resilience and stability, offering a more accurate reflection of TR ecological functioning compared to traditional taxonomic methods (Guillemot *et al.*, 2011; Soria *et al.*, 2024).

In Spain, conservation efforts, such as the establishment of river protected areas (*reservas naturales fluviales*), have predominantly been guided by geomorphological and landscape criteria rather than focusing specifically on freshwater biodiversity or ecological processes. These protected areas are often concentrated in mountainous regions, which are highly valued for their aesthetic and geomorphological significance (Urquiaga & Martín, 2017; Cañedo-Argüelles *et al.*, 2019). This focus often excludes the middle and lower river reaches, typically more degraded or less studied ecologically. As a result, the existing network of protected areas is fragmented, failing to function as effective ecological corridors essential for maintaining population connectivity across aquatic ecosystems. This fragmentation is a major driver of species extinction (Hermoso *et al.*, 2011). At the European level, the Natura 2000 network, established under the Birds Directive (Directive 2009/147/EC) and the Habitats Directive (Council Directive 92/43/EEC), serves as a cornerstone for habitat and species protection. However, the network has faced criticism for its limited inclusion of river ecosystems and its shortcomings in safeguarding freshwater biodiversity (Hermoso *et al.*,

2019). While Natura 2000 represents a vital tool for European conservation, it has yet to adequately address the unique ecological requirements of freshwater and TRs ecosystems, leaving significant gaps in the protection of these critical habitats.

TRs, which constitute a significant portion of Spain's river network, are notably underrepresented in conservation frameworks despite their ecological significance. Although TRs often support lower biodiversity compared to perennial rivers, their conservation value is undeniable (Acuña *et al.*, 2014; Soria *et al.*, 2017). These systems harbor unique species adapted to endure or recover from dry periods (Bogan *et al.*, 2017), exhibit distinctive ecological dynamics (Datry *et al.*, 2017b), and provide essential ecosystem services (Datry *et al.*, 2018). However, TRs also suffer from a lack of social recognition and awareness, as their temporary nature often leads to their ecological roles being undervalued. Local communities are frequently unaware of the ecosystem services they provide, further hindering their effective conservation and management (Vidal-Abarca *et al.*, 2023; Rodríguez-Lozano, 2024).

Given these limitations, incorporating advanced ecological frameworks such as the metacommunity approach offers a pathway to overcome these challenges and improve conservation and management efforts in TRs (Cid *et al.*, 2020). Traditional assessments often overlook the dynamic and interconnected nature of TRs, focusing solely on local conditions and alpha diversity. However, a metacommunity approach provides a broader perspective, acknowledging the importance of regional processes such as dispersal, connectivity, and the spatial configuration of habitats (Cid *et al.*, 2020). This perspective is particularly relevant in TRs, where the interplay between environmental filters, mass effects, and dispersal limitation shapes community structure across spatial and temporal scales. For instance, sites with low species richness do not necessarily indicate poor ecological quality but may reflect dispersal limitations due to isolation or the transient nature of available habitats (Heino, 2013b; Cid *et al.*, 2020). Recognizing these dynamics is crucial for effective conservation planning, as it highlights the need to maintain connectivity between habitat patches and to identify key refugia—such as disconnected pools—that facilitate recolonization and sustain metacommunity dynamics during dry periods (Bonada *et al.*, 2020; **Chapter 4**). Protecting these refugia not only preserves biodiversity but also enhances ecosystem resilience by ensuring that source populations remain viable, enabling recolonization after flow resumes (Chase *et al.*, 2020; Pineda-Morante *et al.*, 2022).

From a conservation perspective, adopting a metacommunity approach involves prioritizing sites that maximize regional biodiversity and ecological functioning. This includes accounting for beta diversity which is often driven by spatial heterogeneity and hydrological variability in TRs (Soria *et al.*, 2024). Sites with high beta diversity may act as reservoirs of unique species or serve as stepping stones, connecting populations across the network and mitigating the effects of fragmentation (Ruhí *et al.*, 2017; Cid *et al.*, 2020; Rodríguez-Lozano *et al.*, 2023). In TRs, where hydrological regimes are highly variable, conservation strategies must also address temporal dynamics (Datry *et al.*, 2014b). Seasonal drying and rewetting create opportunities for species sorting, while prolonged fragmentation during dry periods can exacerbate dispersal limitations (Datry *et al.*, 2016a). By integrating long-term monitoring and metacommunity dynamics into biomonitoring efforts, conservation planning can better anticipate how changing hydrological patterns, intensified by climate change, might impact biodiversity. For example, restoring or maintaining regional connectivity can help mitigate the effects of habitat loss and fragmentation, ensuring that both local and regional biodiversity are preserved (Chase *et al.*, 2020; Cid *et al.*, 2020).

Incorporating these considerations into conservation strategies aligns with the broader meta-systems perspective, which highlights the interconnectedness of landscapes and the cascading effects of changes at one scale on broader ecological networks (Chase *et al.*, 2020; Cid *et al.*, 2022). This framework emphasizes the interaction between local and regional mechanisms in shaping ecosystems (Heino, 2013b). Its application is particularly valuable for TR ecosystems, where the dendritic structure and spatiotemporal patterns of connectivity and fragmentation pose unique management challenges (Tonkin *et al.*, 2018). By integrating meta-systems theory, river managers can predict population responses to anthropogenic stressors at the regional scale and identify key source populations and dispersal routes. Such an approach is crucial for maintaining ecosystem resilience and ensuring the long-term effectiveness of conservation efforts (Schiesari *et al.*, 2019; Cid *et al.*, 2022).

In this context, **Chapter 5** focuses on addressing these challenges by identifying priority conservation sites within TR networks. By integrating two complementary approaches—local contributions to beta diversity and systematic conservation planning—this

chapter highlights critical conservation areas and offers actionable, TR-specific guidelines to support biodiversity conservation and enhance ecosystem resilience.

OBJECTIVES



The general objective of this thesis is **to examine how drying affects the spatiotemporal connectivity of TRs, its effects on (meta)community dynamics and its implications for conservation.**

To address this overarching objective, this thesis is organized into five chapters, each developed as an independent study. All research was conducted within the same study area, the Natural Park of Sant Llorenç del Munt i l'Obac, located in the province of Barcelona (northeast Spain). This area is characterized by a seasonal Mediterranean climate and is dominated by TRs shaped by the region's unique karst geology. Seven streams were studied, exhibiting a wide range of hydrological conditions. These diverse conditions provide an ideal setting to explore how drying affects spatiotemporal connectivity of TRs (**Chapter 1**) and how this drives metacommunity assembly at a small spatial scale (**Chapter 2**) and using different identification approaches (**Chapter 3**), depending on the dispersal abilities of the species (**Chapter 4**). Taking into account the complex spatiotemporal dynamics of TRs, I argue that specific conservation and management strategies under the metacommunity framework are urgently needed to ensure their preservation (**Chapter 5**), as these ecosystems provide refuges for unique adapted species (**Supplementary Contributions**) and are highly vulnerable to global change.

Data for **Chapters 1, 2, and 5** were collected across these seven streams throughout different seasons, providing a comprehensive view of seasonal variations. **Chapter 3** is focused specifically on spring and summer data, when habitat fragmentation is most pronounced. Lastly, **Chapter 4** involved a field experiment conducted in one of the streams during the dry season, enabling a focused analysis of dispersal dynamics within a controlled fieldwork setting. The specific objectives of the thesis, corresponding to each of the five chapters, are as follows:

- To empirically quantify drying and spatiotemporal connectivity across the TRs to be used as predictors of community structure at local and regional scales and considering different dispersal modes (**Chapter 1**).
- To provide a comprehensive overview of diversity patterns and identify the drivers of metacommunity assembly at a small spatial scale, considering the differences in the dispersal abilities of the taxa (**Chapter 2**).

- To analyze the effects of hydrological fragmentation on metapopulation and metacommunity dynamics in TRs (**Chapter 3**).
- To directly assess the aerial dispersal abilities of aquatic macroinvertebrates in TRs through a field mesocosm experiment using an isotopic tracer during the disconnected pools phase (**Chapter 4**).
- To identify priority sites and key drivers for the conservation of aquatic biodiversity in TRs by evaluating two distinct conservation prioritization approaches using diatom and macroinvertebrate data (**Chapter 5**)

La historia se repite, ese es uno de los errores de la historia

Charles Darwin

CHAPTER 1

Navigating through space and time: a methodological approach to quantify spatiotemporal connectivity using stream flow data as a case study

David Cunillera-Montcusí, José María Fernández-Calero, Sebastian Pölsterl, Roger Argelich, Pau Fortuño, Núria Cid, Núria Bonada & Miguel Cañedo-Argüelles



ABSTRACT

The growing interest in combining spatial and temporal patterns in nature has been fostered by the current availability of high frequency measurements. However, we still lack a methodological framework to process and interpret spatiotemporal datasets into meaningful values, adaptable to different time-windows and/or responding to different spatial structures. Here we developed and tested a framework to evaluate spatiotemporal connectivity using two new measures: the Spatiotemporal connectivity (*STcon*) and the Spatiotemporal connectivity matrix (*STconmat*). To obtain these measures, we consider a set of spatially connected sites within a temporally dynamic network. These measures are calculated from a spatiotemporal matrix where spatial and temporal connections across sites are captured. These connections respond to a determined network structure and assign different values to these connections and generate different scenarios from which we obtain the spatiotemporal connectivity. We developed these measures by using a dataset of stream flow state spanning a 513-day period obtained from data loggers installed in seven temporary streams. These measures allowed us to characterize connectivity among stream reaches and relate spatiotemporal patterns with macroinvertebrate community structure and composition. Spatiotemporal connectivity differed within and among streams, with *STcon* and *STconmat* capturing different hydrological patterns. Macroinvertebrate richness and diversity were higher in more spatiotemporally connected sites. Community dissimilarity was related to *STconmat* showing that more spatiotemporally connected sites had similar communities for active and passive dispersers. Interestingly, both groups were related with spatiotemporal connectivity patterns for some of the analysed scenarios, highlighting the relevance of spatiotemporal connectivity in dynamic systems. As we exemplified, the proposed framework can help to disentangle and quantify spatiotemporal dynamics or be applied in the conservation of dynamic systems such as temporary streams. However, the current framework is not limited to the temporal and spatial features of temporary streams. It can be extended to other ecosystems by including different time windows, and/or consider different network structures to assess spatiotemporal patterns. Such spatiotemporal measures are especially relevant in a context of global change, with the spatiotemporal dynamics of ecosystems being heavily disrupted by human activities.

Keywords: Intermittent rivers, ephemeral streams, spatial connectivity, temporal connectivity, network structure, spatiotemporal graphs

INTRODUCTION

The interaction between populations, communities and ecosystems within a landscape has a prominent role in ecology (Gounand *et al*., 2020; Hanski, 1999; McArthur & Wilson, 1967). To better understand this interaction the so-called meta-system framework has been developed (Cid *et al*., 2022; Gounand *et al*., 2018; Schiesari *et al*., 2019). This framework asserts that landscape connectivity drives exchanges of matter, energy, and organisms among localities within a landscape, affecting ecosystem structure, functioning and dynamics (Battin *et al*., 2009; Dale, 2017; L. G. Larsen *et al*., 2012). For example, several studies have highlighted the relevance of connectivity in driving species' diversity and abundance (Borthagaray *et al*., 2020; Economo & Keitt, 2010), community turnover (Epele *et al*., 2021; Horváth *et al*., 2016), population synchrony (S. Larsen *et al*., 2021; Sarremejane *et al*., 2021), mutualistic interactions (Ferreira *et al*., 2020; Monteiro *et al*., 2022), species' extinctions (Horváth *et al*., 2019), vulnerability to species invasions (Drake *et al*., 2017) and ecosystem services (Mitchell *et al*., 2013). Therefore, connectivity is emerging as one of the main drivers of community assembly and ecosystem functioning as it varies the strength of dispersal (Leibold & Chase, 2018; Thompson *et al*., 2020; Vellend, 2016) and affects biodiversity, which is linked to ecosystem properties such as resistance or productivity (Thompson *et al*., 2017; Tonkin, Heino, *et al*., 2018). Nevertheless, most of these examples are focused on spatial connectivity, while the temporal facet of connectivity remains relatively understudied (Uroy *et al*., 2021).

Metapopulations, metacommunities and meta-ecosystems have been generally assessed using a snapshot approach that assumes temporal stability (Holyoak *et al*., 2020; Uroy *et al*., 2021). In the specific case of metacommunities, most studies have considered spatial or temporal connectivity individually while communities experience both temporal and spatial variation in connectivity affecting populations (Kurihara, 2007), communities (Cañedo-Argüelles *et al*., 2020; Dong *et al*., 2017), and ecological processes (Ding *et al*., 2013; Sadchatheeswaran *et al*., 2021). Whereas spatial connectivity refers to how communities are connected within a landscape (Moritz *et al*., 2013), temporal connectivity refers to how communities are connected in time, e.g., across multiple years (Holyoak *et al*., 2020). Within this context, considering only one facet of connectivity can lead to misleading conclusions about the importance of landscape connectivity for the exchange of species between local

communities (Castillo-Escrivà *et al*., 2020; Huang *et al*., 2020; Rasmussen *et al*., 2013). To date, few studies have assessed how connectivity changes simultaneously in space and time, defined here as spatiotemporal connectivity, and how these can affect natural systems (Fullerton *et al*., 2010; Uroy *et al*., 2021).

Nowadays, new technologies such as satellite high frequency sensors (Burel & Baudry, 2005; Ducklow *et al*., 2009; Pekel *et al*., 2016), high time frequency measuring devices such as GPS (Bischof *et al*., 2019; Fattebert *et al*., 2015; Foley & Sillero-Zubiri, 2020) or data loggers (Crabot *et al*., 2020; Jensen *et al*., 2019; Vander Vorste *et al*., 2016b) offer good opportunities to characterise spatiotemporal connectivity because they capture high spatial and temporal resolution data that can be later combined into spatiotemporal measures (Martensen *et al*., 2017; Uroy *et al*., 2021). Some studies have used long-term datasets to assess the temporal variation of spatial processes through consecutive snap-shot analyses (Cañedo-Argüelles *et al*., 2020; Leppänen *et al*., 2019; Rouissi *et al*., 2014), but in these studies the interaction between different time steps is not considered. In contrast, other studies (Huang *et al*., 2020; Martensen *et al*., 2017; Thompson *et al*., 2017) simultaneously consider the interaction between spatial and temporal dimensions by including the effect of connectivity through more than one time-step (e.g., the connectivity of a given timepoint has an effect on the next timepoints and this effect propagates in time). However, few examples exist where spatial and temporal variation are simultaneously considered to estimate spatiotemporal connectivity (Uroy *et al*., 2021). The lack of a clear framework from which to calculate spatiotemporal connectivity values or assess different time-windows (i.e., number of time steps considered to impact connectivity) is limiting the development of such approximations. Even though technologies to capture spatiotemporal connectivity changes are nowadays available, we still need a methodological framework for obtaining connectivity measures that integrate spatial and temporal patterns (Uroy *et al*., 2021).

Temporary river networks are suitable systems to analyse and develop a framework to assess spatiotemporal connectivity due to their high hydrological variability in space and time (Datry *et al*., 2014b). Spatially, fluvial systems are driven by an up- to down-stream unidirectional flow following a dendritic structure which affects the distribution and presence of species (Altermatt, 2013; Altermatt *et al*., 2020; Borthagaray *et al*., 2020; Schmera *et al*., 2018). Temporally, they are subjected to a strong seasonal and/or interannual variation, with

recurrent drying and flowing periods determining habitat availability (Bonada *et al*., 2020; Datry *et al*., 2016a; Valente-Neto *et al*., 2020). Finally, species inhabiting these systems have two different strategies to withstand drying and thus coping with spatiotemporal variability: resisting *in situ* as dormant stage (temporal dispersal) or recolonizing from nearby refuges after flow resumption (spatial dispersal; He *et al*., 2020; Heino, 2013a; Sarremejane *et al*., 2020a). These different ways of interacting with the landscape can be related with different network structures or dispersal abilities, thereby informing about changes in network connectivity (Cañedo-Argüelles *et al*., 2020; Pineda-Morante *et al*., 2022; Tonkin, Altermatt, *et al*., 2018). Consequently, in temporary streams, spatiotemporal connectivity among communities is promoted by unidirectional or by multidirectional dispersal (Borthagaray *et al*., 2015; Seymour *et al*., 2015; Tonkin, Altermatt, *et al*., 2018). A unidirectional or directed network would be related with species that are aquatic obligates and that follow the stream dendritic structure to move from one site to another, hereafter passive dispersers. On the other hand, a multidirectional or undirected network would be related with species that can fly in all directions, with no need of following the stream course, hereafter aerial dispersers. So far, flow data obtained using high-frequency temperature data loggers has been used to characterise the local hydrological conditions of temporary streams (e.g., drying duration, number of consecutive dry days, days with disconnected pools, days after flow return) and its effects on aquatic communities (Arias-Real *et al*., 2021; B-Béres *et al*., 2019; Beesley & Prince, 2010; Crabot *et al*., 2020). However, this information has not yet been used to develop spatiotemporal connectivity measures.

In this study, we developed a methodological framework to assess spatiotemporal connectivity focusing on Mediterranean streams as a case study. Using high frequency data on water presence/absence from a set of temporary streams, we propose two measures of connectivity (Figure 1): Spatiotemporal connectivity (*STcon*) and Spatiotemporal connectivity matrix (*STconmat*). *STcon* measures average site connectivity for each individual site and thus can be used as a predictor of community structural and functional metrics such as local species richness or Shannon-Wiener diversity. *STconmat* represents these connectivity values by pairs of sites so as a spatiotemporal connectivity matrix and allows to assess changes in community composition (e.g., Jaccard dissimilarity, Bray-Curtis dissimilarity). Here, we used

these two measures to explain changes in the structure of macroinvertebrate communities at the local and regional scales to illustrate their relevance for capturing ecological processes.

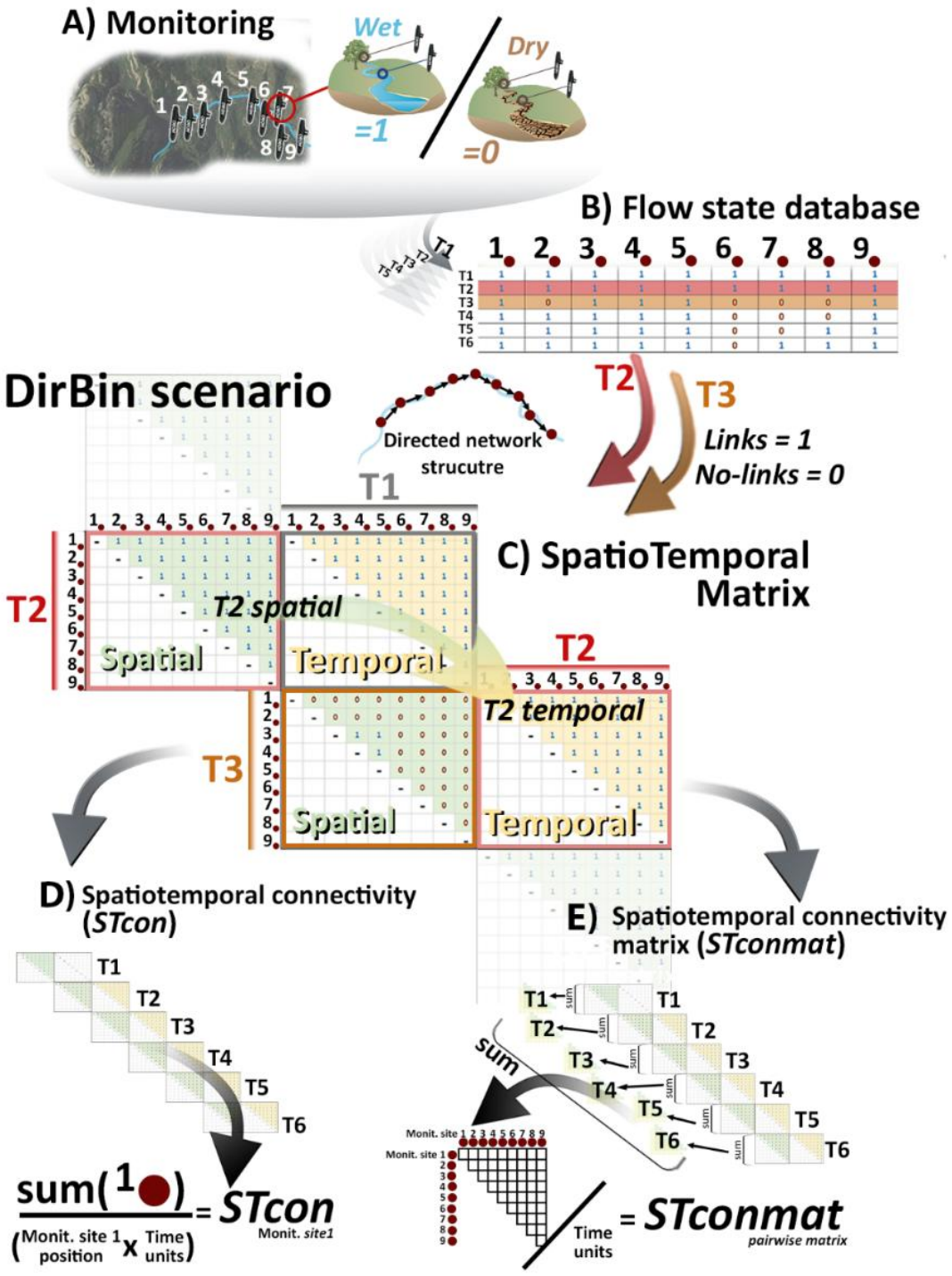


Figure 1. Description continued on the next page.

Figure 1: Conceptual graphical representation of how the *STcon* and *STconmat* are calculated using the example of Vall d'Horta stream (VH). A) The data loggers are deployed in the field and monitor the presence (i.e., wet) or absence (i.e., dry) of water. B) These values are then presented in a flow state database where rows represent each time unit (i.e., 6 days, from T1 to T6) and columns every monitored site (from 1 to 9). Each cell of the flow state database is filled with a value of "1" if the monitored site was "wet" that day (e.g., all sites in day 1, T2, were defined as 1) or with a value of "0" if the monitored site was "dry" that day (e.g., in day 2, T3, monitored site 2, 6,7 and 8 were defined as 0). C) Using the flow state database, the spatiotemporal matrix is built based on a defined scenario, in this case we used a directed network structure and a binary quantification of links and no-links (DirBin scenario). For each row of the flow state matrix (e.g., T2) a pairwise matrix is constructed where all the possible spatial links, based on which monitored sites are "wet" and which are "dry", are assessed. This defines the Spatial connections for a specific time (green part of the matrix). Temporal connections are filled based on the same connections of that time (T2) but introduced in the spatiotemporal matrix in the following time step (T3). D) *STcon* is calculated for each site individually (e.g., Site 1) and is the average of how often a site has been connected to all its potential neighbours (e.g., Site 1 has 8 potential neighbours) during the studied time (6 units of time). D) *STconmat* corresponds to the sum of all the spatiotemporal matrices into one unique matrix later divided by the studied time (6 units of time).

METHODS

Study sites and sampling

The dataset used for developing and testing the current framework comes from a study carried out in Sant Llorenç del Munt i l'Obac Natural Park, a protected area in the Vallès Occidental region (Catalonia, NE Spain). The area has a Mediterranean climate and the underlying geology is dominated by karst limestone. These characteristics make surface flows heavily linked to rainfall events and thus streams in the region have marked flowing/drying periods, especially during the summer. We selected seven temporary streams that were sampled in November 2019 as part of a yearly seasonal monitoring where streams were monitored from July 2018 until November 2019. All streams had similar instream characteristics and aquatic macroinvertebrates were sampled following a multihabitat design and identified at the lowest possible taxonomic level, mostly species or genus. Information on sampling location, procedure, characteristics, and specific taxonomic identification can be found in Chapters 2 and 5, as well as in Pineda-Morante *et al*. (2022).

Flow state monitoring and database building

A total of 69 temperature data loggers (HOBO Pendant® Temperature/Light logger, hereafter loggers) were used to assess flow state in the seven selected streams (Figure 1A), with between 10-15 loggers per stream (Figure S1 and Pineda-Morante *et al*., 2022). These data loggers were installed in July 2018 and kept in the streams until December 2019. At each study site, a logger was submersed into the water to record water temperature (°C) and, for each stream, a logger was placed outside the water (e.g., hanging from a tree) to record air temperature (°C). The loggers recorded temperature at hourly intervals for 513 days. We calculated the daily mean temperature and compared the diurnal temperature variation of the installed loggers to assess if each site had enough water to cover the data logger, hereafter wet, or no surface water, hereafter dry comparing air and instream temperatures, following Gungle (2006). Those estimations were validated using field observations and time-lapse images taken for some streams. Finally, we used a logistic regression model including stream characteristics to correct the missing values generated due to loggers malfunctioning or loss due to climatic events (e.g., temperatures below 0°C, floods). Specific details about the model and the process of predicting missing data can be found in Pineda-Morante *et al*. (2022) supplementary materials. The final dataset resulted in a table with the daily wet/dry values (rows) for each logger (columns) for each one of the monitored streams. In this table a wet day with presence of surface water, flowing or as disconnected pools, is represented by "1" and a dry day by "0". This dataset constitutes the basic information from which we later calculated all the spatiotemporal indices. In this work we used the entire 513 days period to calculate all the spatiotemporal indices. Using the same flow state database obtained with the data loggers, we also calculated several local hydrological metrics commonly used to characterise surface flow state in temporary streams (Arias-Real *et al*., 2021; Crabot *et al*., 2020; Pineda-Morante *et al*., 2022), such as the total number of dry days (TotDur), the number of changes from wet to dry days (TotNum), and the average number of dry days for each drying event (TotLeng).

Building spatiotemporal matrices based on logger data

Spatiotemporal connectivity was obtained after building a spatiotemporal matrix, hereafter ST matrix, for each stream. Each row and column of the ST matrix corresponded to a monitored site with a data logger deployed at a specific time (Figure 1B). Therefore, the matrix was as big as many sites and times were monitored. Then, the ST matrix was filled with information regarding the two levels of connectivity that we assessed: spatial and temporal.

Spatial connection

Spatial connections were built based on a defined stream network structure (Figure 1C). A directed structure follows an upstream-downstream direction (Figure S2) and an undirected structure implies that all nodes can be reached from everywhere (Figure S2). This structure was related to the dispersal modes of the organisms that we wanted to study. We assumed that passive dispersers would disperse mostly downstream by drift so they would mainly respond to a directed network. Contrarily, active dispersers that disperse by flight would mostly respond to an undirected network. In the two networks used to create the ST matrix, each node represented a monitored site and each link an effective connection between sites. If the node's flow state was considered as wet, the connection with other nodes was possible. Following the paths defined by the network, the number of reachable nodes from each monitored site was assessed (Figure 1C Spatial). In the case that a monitored site was dry for that day, the connection with this dry site or throughout it was not possible.

Temporal connections

Temporal connections were built in the same way as spatial links (Figure 1C Temporal). All the possible connections within the network for a specific time were assessed based on the same network structure of the spatial ones. However, the temporal links were entered in the ST matrix on the following time step. In other words, we considered that the connection between the nodes on a given day would influence their connectivity on the following day (Figure 1C T2 Temporal connections).

Link and no-link values

For each node, all the spatial and temporal connections with its neighbours were filled with the values corresponding to what we defined as the *link* or *no-link* value (Figure 1C). The *link* value is the value that we give when dispersal can occur between two nodes. In this study this occurs when the flow state is 1 between a pair of nodes and the path to reach them. Contrarily, the *no-link* value is the value given when dispersal is not possible or in our case, there is at least one 0 in the path between a given pair of nodes. *Link* and *No-link* values can be modified depending on how connected and unconnected sites are defined. These values can be modified in the function *spat_temp_index,* an R function created to calculate the spatiotemporal connectivity metrics (Online Supplementary Material), to define different scenarios. Therefore, the current framework allows for calibrating spatiotemporal connections between sites and can include any ecologically meaningful network structure or link interpretation (Cañedo-Argüelles *et al*., 2015; Phillipsen & Lytle, 2013).

Binary and Weighted scenarios

In this study we designed four different scenarios from which we calculated *STcon* and *STconmat* to exemplify different possibilities of the current framework. Each scenario combined a different network structure, directed or undirected, and a different way of quantifying the *links* and *no-links (*binary and weighted). For the two **binary scenarios,** the directed binary (hereafter DirBin) and the undirected binary (hereafter UndBin) the *link* value was defined as 1 and the *no-link* value as 0. Therefore, *STcon* and *STconmat* were quantifying the number of times that each site was connected to its neighbours. For these scenarios, high values meant that a site was highly connected to its potential neighbours during the considered time.

For the other two **weighted scenarios,** the directed weighted (DirWei) and the undirected weighted (UndWei), we added an additional variable: the Euclidean distance between sites. Here, the *link* value was 0.1 and the *no-link* value was 1, and these values were multiplied by the distance between the two nodes. Thus, for the weighted scenarios we assumed that the presence of water between two sites was facilitating dispersal. Consequently, when connection was possible, the Euclidean distance between two sites was

reduced by 90% and when connection was not possible the Euclidean distance between these two sites was preserved. Therefore, *STcon* and *STconmat* calculated for the DirWei and UndWei scenarios were assessing dispersal resistance. For these scenarios, high and low resistance values indicate that the connectivity between two sites is low and high, respectively.

Spatiotemporal connectivity measures

Once the ST matrix was filled with the corresponding *link* and *no-link* values, *STcon* and *STconmat* were calculated. Depending on the desired output, connectivity can be either calculated for each site individually resulting on the *STcon* measure, or as a pairwise matrix that incorporates the relation of each site with all the other pairs within the network, resulting in the *STconmat*.

The *STcon* corresponds to the average of all the spatial and temporal links for each individual node (Figure 1D). To calculate it, we summed all the times that a node had been connected to its neighbours both spatially and temporally and later we divided that value by the amount of time considered and the total potential neighbours that the individual node can have: $STcon = \sum_{Tx}(S_i+T_i)/ Nneigh_i*TTU$ .Where $\sum_{Tx}$ is the sum of values through all considered times, *Si* and *Ti* are the spatial and temporal connections of a specific node *i* with its neighbours, *Nneigh* corresponds to all the potential neighbours that node i has, and TTU is the total time units considered. Therefore, *STcon* represents an average value of how often an individual node has been connected to all their potential neighbours (Figure 1C). In the case of a directed network structure, the value is calculated considering each site's outflow path, which identifies the relevance of each site as a source of colonists for all their neighbours. As *STcon* is averaged by the total potential neighbours of each site, *STcon* can be compared within sites of the same system and among systems. For the *STconmat* we summed the ST matrix in a unique pairwise matrix including all stream sites (Figure 1E). We then divided this pairwise matrix by the amount of time considered being $STconmat=[(S(i_1-n_1)…n)+(T(i_1-n_1)…n)]/TTU$, where S and T are the spatial and temporal links for each pair of nodes, and TTU the total time units considered. Therefore, *STconmat* is averaging daily spatial and temporal connections for each pair of nodes. Both *STcon* and *STconmat* capture the

spatiotemporal connectivity of each site during the studied period and their values are directly related to the values assigned to *link* and *no-link*.

Testing the spatiotemporal connectivity measures

For each one of the four scenarios, DirBin, DirWei, UndBin and UndWei, we calculated *STcon* and *STconmat* for the entire monitored period of 513 days. We conducted a principal components analysis, PCA, including the *STcon* values for each scenario and the three local hydrological variables (TotNum, TotDur and TotLeng) to analyse how they were correlated based on the Euclidean distances between them, using *prcomp* function from stats package (R-Core Team, 2019). We used the *adonis* function from vegan (Oksanen *et al*., 2010) to test if upstream-downstream position or stream ID were presenting similar values in this multivariate space using a permutational analysis of variance (PERMANOVA) and expecting that the high hydrological variability in the sampled streams would result in significant differences among them. In addition, we used Spearman correlations for the same set of variables to test their correlation and quantify the strength of their relationship.

As *STcon* and *STconmat* differ in their structure, the approach to characterise each stream was also different. First, we related *STcon* with the relative position of each monitored site within each stream network in an up- to downstream direction. Second, we analysed the differences in *STconmat* for each stream in a multivariate space through a non-metric multidimensional scaling (NMDS). As *STconmat* is a pairwise matrix, the values are equivalent to spatiotemporal distances between sites and can be represented in a bidimensional space.

Finally, to test *STcon* and *STconmat* against macroinvertebrate community's metrics we used the period of 480 days between the start of the monitoring (26/07/2018) until the biological sampling campaign (18-21/11/2019) to build the spatiotemporal connectivity metrics. We considered this period for the four different scenarios (DirBin, DirWei, UndBin and UndWei). As the two network structures were related with different dispersal abilities (i.e., directed for aquatic passive and undirected for aerial active), we split the community matrix in these two dispersal groups based on available biological trait data (Pineda-Morante *et al*., 2022; Sarremejane *et al*., 2020a; Tachet *et al*., 2010). We did not account for aquatic active and aerial passive dispersers because of their low abundance representing both around

12% of sample's total abundance. For both aquatic passive and aerial active dispersers we calculated taxonomic richness, Shannon-Wiener diversity, relative dispersal traits abundance and compared them against *STcon*. We also calculated two beta diversity proxies: Jaccard, based on presence-absence, and Bray-Curtis, based on abundance data and compared them against *STconmat*. These metrics were calculated using the vegan package (Oksanen *et al*., 2010). Finally, linear mixed models were used to analyse the relationship between *STcon* and the calculated community metrics (taxonomic richness, Shannon-Wiener diversity, relative dispersal traits abundance) using the package lme4 (Wood & Scheipl, 2013) and considering stream identity as a random factor to control for variability between the different streams. We analysed all pairwise distance matrices with Mantel tests relating *STconmat* with the two community dissimilarity metrics (Bray-Curtis and Jaccard indices) and using the vegan package (Oksanen *et al*., 2010).

RESULTS

Spatiotemporal connectivity and network structure

The selected streams showed strong differences in their spatiotemporal patterns in relation to network structure. In the binary scenarios DirBin and UndBin, where direct connectivity is quantified, both *STcon* (Figure 2A) and *STconmat* (Figure 2B) captured wet/dry patterns for each monitored site. For example, sites that dry more often and for longer durations had lower values of *STcon* for the DirBin and UndBin scenarios (Figure 2, site highlighted in red). When the network structure was directed (DirBin), the sites that were dry more often were impacting other sites located above them by reducing their *STcon* and *STconmat* values as these sites cannot connect to their downstream neighbours when an intermediate site is dry (Figure 2 DirBin, sites above the one highlighted in red). On the contrary, this effect was not observed for the undirected network structure (Figure 2; UndBin). In weighted scenarios, (Figure 2 DirWei and UndWei), where *STcon* and *STconmat* quantified dispersal resistance, the highest values were found at upstream sites when there was a directed structure, DirWei, whereas the drier sites had the greatest values of *STcon* and *STconmat* in the undirected network UndWei (Figure 2 site highlighted in red). In weighted scenarios, *STcon* and *STconmat*

captured the interaction between distance and drying so when a site contained water the distance between two sites decreased concomitantly with *STcon* and *STconmat* values. Contrarily, when a site was dry, the distance corresponded to the Euclidean distance between the sites leading to an increase of *STcon* and *STconmat* values. The complete dataset with

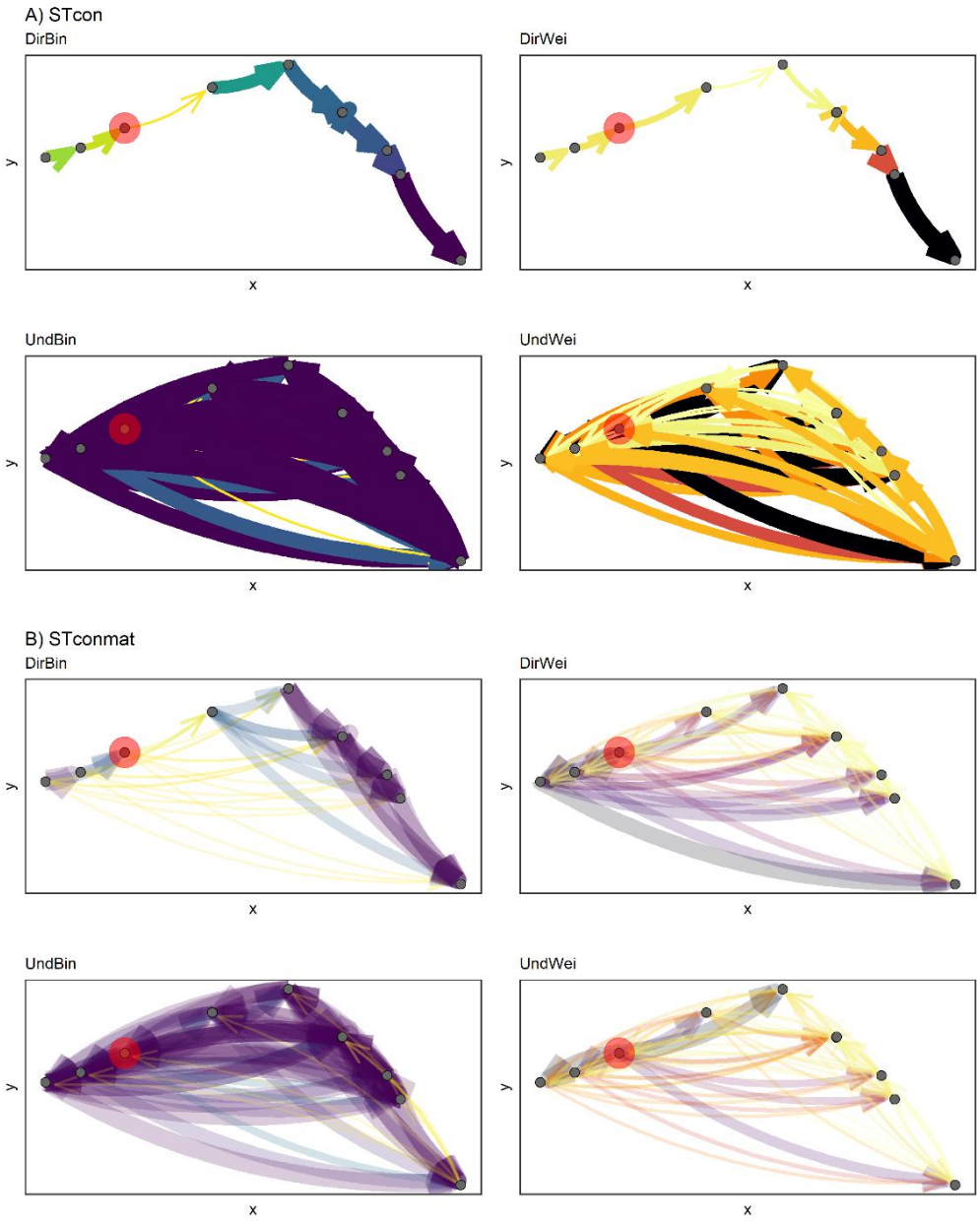


Figure 2. A) *STcon* and **B)** *STconmat* values for the Vall d'Horta stream (VH). Colour viridis gradient and size correspond to *STcon* and *STconmat* values. For DirBin and UndBin scenarios purple/blue and bigger sized arrows indicate higher connectivity while yellow and smaller arrows indicate isolation. For DirWei and UndWei grey/red and bigger sized arrows indicate higher dispersal resistance while orange/yellow and smaller arrows indicate less resistance. We highlighted in red the monitored site 3 just to ease its location in the network. All the data is calculated based on the whole monitored time window (513 days). See the other studied streams in the Supplementary Material Figure S3.

STcon and *STconmat* values for the four scenarios and for all the monitored streams can be seen in Figure S3 and Table S1.

Spatiotemporal connectivity and local flow state

When comparing the spatiotemporal indices with previously existing indices based on flow state data (i.e., TotDur, TotNum and TotLeng) we observed that there were negative correlations between the *STcon* and the TotDur (DirBin= -0.69 and UndBin= -0.65) and TotNum (DirBin = -0.51 and UndBin = -0.53) for both directed and undirected networks (Figure 3). Thus, higher values of *STcon* (i.e., high spatiotemporal connectivity), were associated with lower values of TotDur (i.e., total duration of drying events) and TotNum (i.e., frequency of drying events). Spearman correlation analysis highlighted these negative correlations (Figure S4). Finally, for the PERMANOVA analysis we found significant differences across sites position

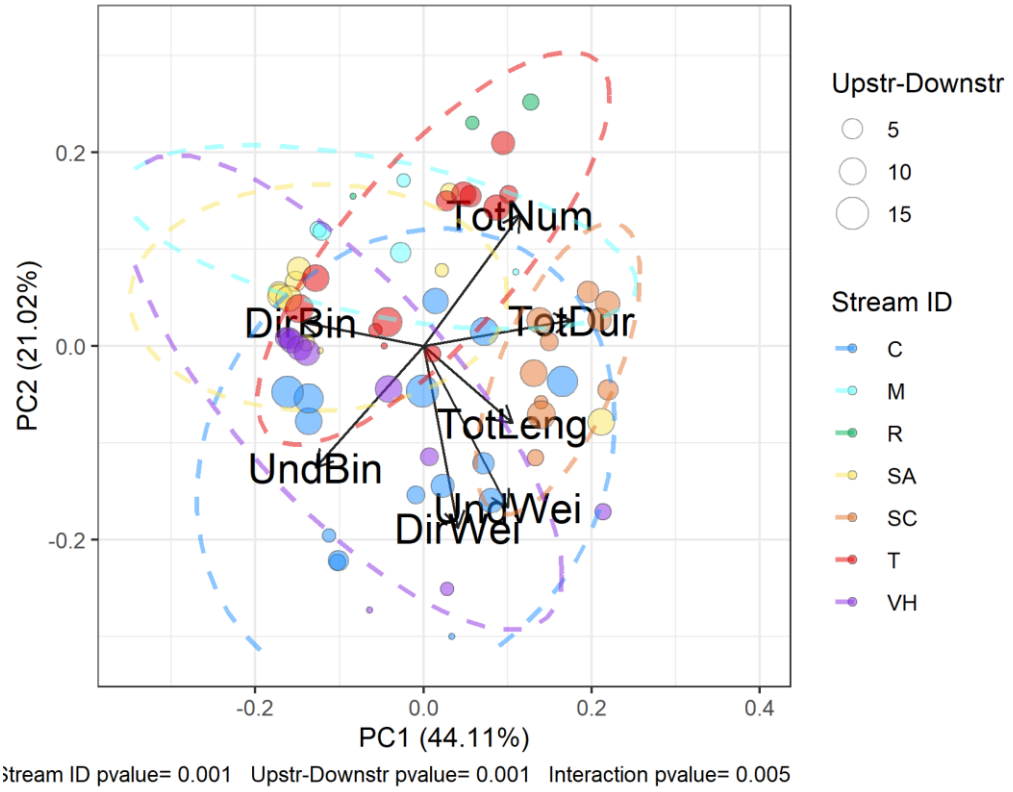


Figure 3. PCA plot for *STcon* indices for each one of the four scenarios: Directed binary (DirBin), Directed Weighted (DirWei), Undirected Binary (UndBin), Undirected Weighted (UndWei) and the hydrological variables: the total duration of drying events (TotDur), the frequency of drying events (TotNum), and the average length of each drying event (TotLeng). All the data is calculated based on the whole monitored time window (513 days).

in the PCA between streams and between site position within streams (i.e., upstream-downstream), and a significant interaction of both factors as expected (Figure 3).

Stream spatiotemporal characterization

STcon differed between streams and between upstream and downstream sections. For the binary non-weighted scenarios (Figure 4A; DirBin and UndBin), there was a general increase in *STcon* towards downstream sites meaning that downstream sites presented higher spatiotemporal connectivity (Figure 4A; DirBin), except for the M and SA streams (where no clear tendency was found). On the other hand, the indices followed an inverse trend in the weighted scenarios as dispersal resistance increased towards upstream reaches, Figure 4A; DirWei and UndWei), especially in the directed scenario (Figure 4A; DirWei). *STcon* captured drier sites impact on connectivity for directed networks (Figure 4A; DirBin and DirWei), which disappeared for undirected scenarios (Figure 4A, UndBin and UndWei). The NMDS performed using the *STconmat* values (Figure 4B) illustrated the differences in spatiotemporal connectivity between sites within each of the streams. Widely dispersed sites (Figure 4B, VH stream) experienced a higher temporal variation in connectivity than clustered sites (Figure 4B, SC and C streams). *STcon* values for each individual stream and scenario are available in Figure S5.

Testing spatiotemporal connectivity against macroinvertebrates communities

The linear mixed models showed several significant relationships between *STcon* calculated for each of the four scenarios and the three site-specific biodiversity metrics (taxonomic richness, Shannon-Wiener diversity, and dispersal traits abundance) for the two dispersal groups (Figure 5A, B, C). *STcon* was negatively related with the richness of passive dispersers in the DirWei scenario, whereas *STcon* was positively related with the taxonomic richness of both passive and active dispersers in the UndBin scenario. Shannon-Wiener diversity of passive and active dispersers as well as the dispersal traits abundance of passive dispersers were positively related with *STcon* in the UndBin scenario. We also identified significant relationships between *STconmat* and community dissimilarity (Figure 5D, E). Passive and

active dispersers responded significantly to both directed and undirected weighted scenarios, with Bray-Curtis dissimilarity positively related with *STconmat* in the DirWei and UndWei scenarios. Jaccard dissimilarity for passive dispersers was positively related with *STconmat* in the DirBin and UndBin scenarios. Contrastingly, Jaccard dissimilarity for active dispersers was negatively related with *STconmat* in the DirBin scenario. Finally, Jaccard dissimilarity for active

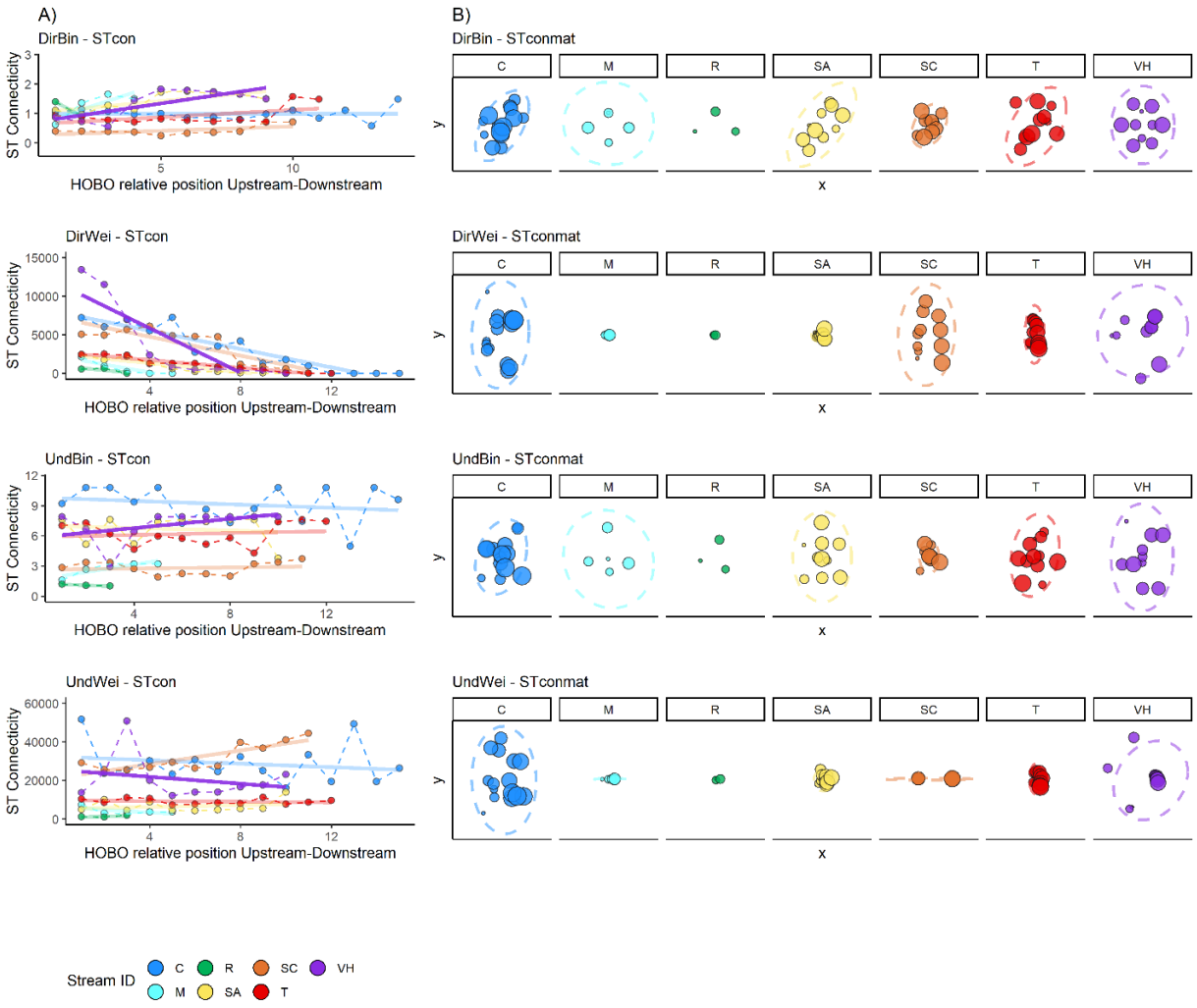


Figure 4. A) Monitored streams *STcon* values (y-axis) ordered along their relative position from upstream to downstream (x-axis). **B)** NMDS with *STconmat* values of each monitored stream. More compact clouds of points mean a greater similarity between sites in terms of spatiotemporal connectivity (i.e., all sites dry similarly). More dispersed clouds indicate a greater variability within the stream in terms of drying. Circle size indicates upstream (smaller) or downstream (bigger) site position. Each plot row corresponds to one of the four scenarios: Directed binary (DirBin), Directed Weighted (DirWei), Undirected Binary (UndBin), Undirected Weighted (UndWei). All the data is calculated based on the whole monitored time window (513 days). Individual stream *STcon* plots can be found in Supplementary Figure S5.

dispersers was positively related with *STconmat* in the undirected weighted scenario (UndWei). All community metrics and model effect sizes can be found in Figure S6 and Table S2.

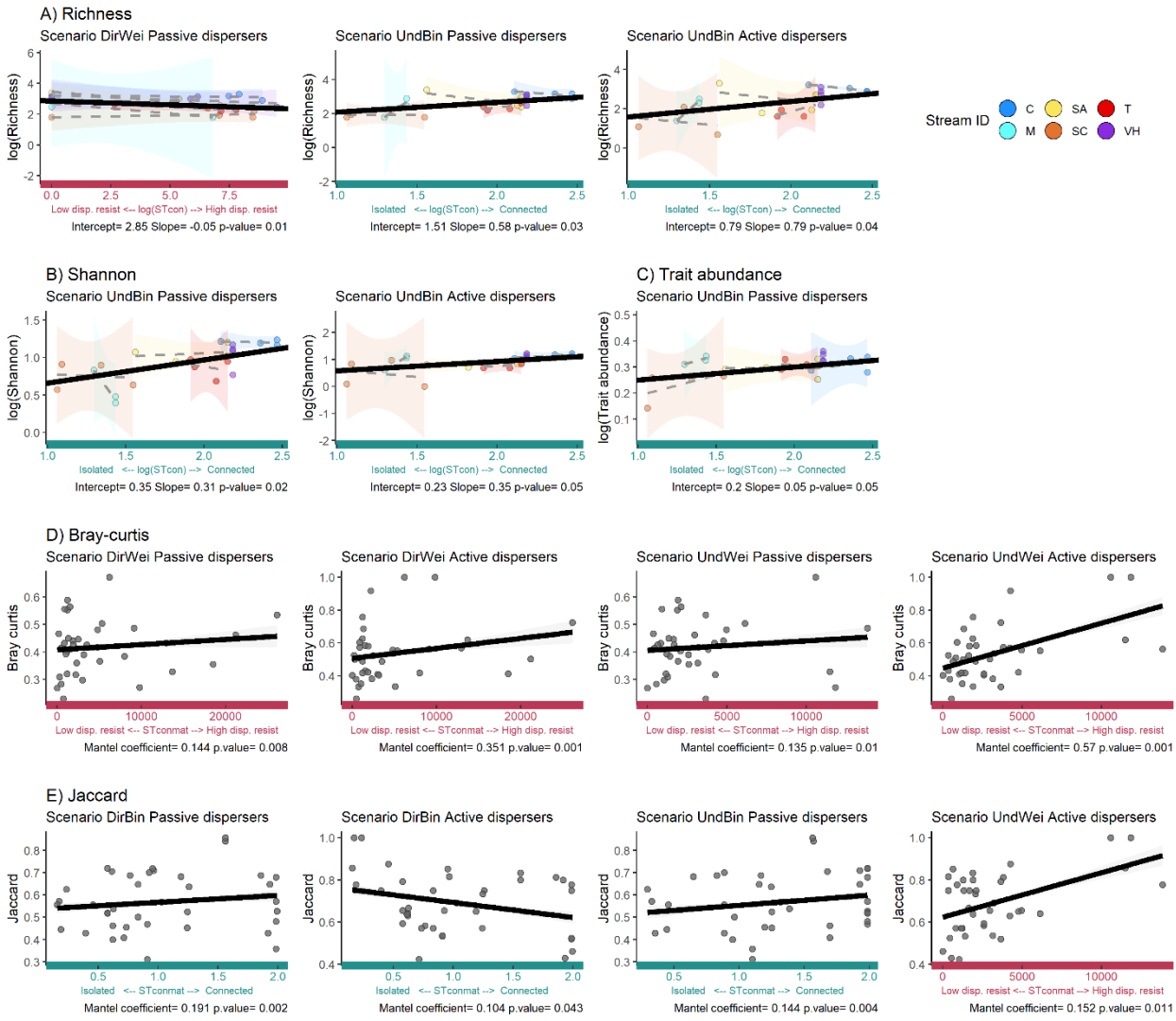


Figure 5. Significant linear mixed models between *STcon* **(A, B, C)** and *STconamt* **(D,E)** and biological metrics calculated for passive aquatic and active aerial dispersers. **A)** Richness, **B)** Shannon, **C)** Trait abundance, **D)** Pairwise metrics (i.e., Jaccard and Bray-Curtis indices). Note that *STcon* values for DirBin and UndBin scenarios (green x axes) quantify how much a site has been connected, whereas values for UndWei and DirWei scenarios (red x axes) quantify the dispersal resistance of each site. All the data is calculated based on the time window between the beginning and the sampling date (480 days). See all other results for all the metrics and scenarios in Supplementary Material Figure S6.

DISCUSSION

We present a new methodological framework from which spatiotemporal connectivity can be calculated combining both spatial and temporal dimensions in one unique value. Our spatiotemporal connectivity measures use data obtained at a continuous time interval (e.g., minutes, days, months, years) and consider a particular spatial structure (i.e., dendritic network or undirected network in our case). We developed this approach using a high frequency database of flow state where daily wet/dry values of seven streams were recorded for 513 days (Pineda-Morante *et al*., 2022). Past studies used daily monitoring to assess the spatial variation (i.e., wet or dry reaches) and/or to characterise temporal variation (e.g., seasonal changes) of river networks following a snapshot approach (Crabot *et al*., 2020; Pineda-Morante *et al*., 2022). Our framework allows merging spatial and temporal dynamics within the same connectivity value, while being adaptable to different temporal windows, monitoring studies, and systems (e.g., months or years with datasets like Mouton *et al*., 2022; Perera *et al*., 2020; Ridl *et al*., 2018). In addition, the framework can incorporate non-binary connections based on e.g., distances, resistance (e.g., non-transitable areas), barriers (e.g., dams, forests) or dispersal pathways (e.g., wind or flow). Therefore, the proposed framework can be extended to any system presenting a spatial structure that has been monitored through time and with enough detail to build a network structure that captures fluxes of individuals, species, energy, or matter.

The spatiotemporal connectivity measures developed here captured distinct network connectivity patterns. For example, they showed differences among headwaters and downstream reaches and accounted for how dry reaches acted as dispersal barriers. From a management point of view, such information could prove useful in defining stream typologies according to their spatiotemporal trends and associate different management practices to them (Bonada *et al*., 2020; Datry *et al*., 2014b; Gallart *et al*., 2017). For example, when assessing temporary stream biological quality, one could assign relevance to the terrestrial and/or the aquatic biota according to stream spatiotemporal connectivity patterns (Steward *et al*., 2022). Furthermore, these spatiotemporal measures can be useful to understand how network connectivity determines the fluxes of energy, matter, and organisms within the river (Cid *et al*., 2022; Gounand *et al*., 2018; Martensen *et al*., 2017; Uroy *et al*., 2021). Although

we used high time frequency data loggers to obtain flow state data, the current framework could be applied to data coming from gauging stations (Monk *et al*., 2008; Stocks *et al*., 2021; Walker *et al*., 2016) or from hydrological models (Costigan *et al*., 2017; Döll & Schmied, 2012; Yu *et al*., 2020), which could be used to define the values assigned to the "links" and "no links" to assess connectivity. In this sense, instead of binary values of connectivity we could incorporate flow, wind velocity or even permeability values between sites as far as this data would be available at the same temporal resolution. In terms of drying patterns, we found that *STcon* values showed negative correlations with TotNum, TotDur, and TotLeng in binary scenarios. These correlations were already expected because both *STcon* and hydrological metrics are quantifying intermittence similarly. For example, high values of *STcon* for the scenario DirBin were negatively correlated with the duration of dry events (TotDur), meaning that highly spatiotemporally connected sites are also the ones which generally have shorter dry events. Nevertheless, these results also suggest that by only using the *STcon* we would be already capturing drying patterns and the usage of local hydrological metrics such as TotNum, TotDur and TotLeng would not be necessary. Overall, *STcon* and *STconmat* present a great potential to characterize spatiotemporal patterns in streams, representing a powerful tool to guide conservation and restoration actions given the importance of spatiotemporal connectivity for population/community dynamics and ecosystem functioning (Chase *et al*., 2020; Cid *et al*., 2020).

Macroinvertebrate communities significantly responded to *STcon*, with different responses for active aerial and passive dispersers, thereby suggesting that our indices captured their dispersal dynamics. Species richness and Shannon diversity increased with greater spatiotemporal connectivity in the UndBin scenario, which seems to quantify the impacts of drying on aquatic communities (Crabot *et al*., 2020; Pineda-Morante *et al*., 2022). Furthermore, passive dispersers responded negatively to the DirWei scenario, with the communities experiencing higher resistance to dispersal showing lower richness. Thus, drier and more distant sites had fewer taxa, as seen in other studies (Brooks *et al*., 2018; Elliott, 2002; Fonseca, 1999; Kappes & Haase, 2012; O'Hop & Wallace, 1983). The differences between active and passive dispersers regarding dispersal resistance highlighted the relevance of overland pathways for active dispersers (Bogan & Boersma, 2012; Cañedo-Argüelles *et al*., 2015; Phillipsen & Lytle, 2013). Passive dispersers abundance responded

positively to *STcon* in the UndBin scenario, indicating that perennial sites would tend to accumulate higher abundances of this dispersal group (Hershkovitz & Gasith, 2013). Interestingly, the fact that passive dispersers appear related with undirected scenarios could be linked to the small distances between sites, which could be overcome during wet conditions (Kappes & Haase, 2012; Razeng *et al*., 2016) and/or through dispersal vectors such as wind (Cáceres & Soluk, 2002; Epele *et al*., 2021; Vanschoenwinkel, Gielen, *et al*., 2008a) or animals such as birds and terrestrial mammals (Figuerola & Green, 2002; Frisch *et al*., 2007; Vanschoenwinkel, Waterkeyn, *et al*., 2008b).

Regarding *STconmat,* both weighted scenarios (DirWei and UndWei) had positive relationships with dissimilarity indices suggesting that dryer and more distant sites harboured more unique communities. Such pattern was probably linked to a combination of dispersal limitation and harsher environmental conditions imposed by drying events leading to higher dispersal resistance and the development of distinct communities (Bonada *et al*., 2020; Steward *et al*., 2022; Valente-Neto *et al*., 2020). On the contrary, the binary scenarios (DriBin and UndBin) resulted in contrasting patterns depending on the dispersal group for presence-absence data. In this scenario, passive and active dispersers were positively and negatively (respectively) related to spatiotemporal connectivity. This suggests that more connected sites tend to have passive dispersers communities with different species composition while active dispersers communities tend to be more similar. Thus, when sites are hydrologically connected for longer periods of time the communities of passive dispersers tend to diverge in their composition. Since many passive dispersers are lost with drying and it takes time for them to recolonise rewetted streams (Bogan & Boersma, 2012; Kappes & Haase, 2012), more perennial sites tend to diverge in their composition as greater species number promotes niche partitioning between habitat patches. On the other hand, in the case of active dispersers, as the spatiotemporal connection between sites increases the communities become more homogeneous due to mass effects (Gansfort *et al*., 2021; Heino *et al*., 2015; Sarremejane *et al*., 2017).

Overall, although the study sites were close to each other (mean distance between sites within a stream was around 1km and all the streams were located within an approximate range of 100 km^2), the macroinvertebrate communities showed significant responses to spatiotemporal connectivity. This highlights the importance of quantifying spatiotemporal

connectivity in highly dynamic systems such as temporary streams (Cid *et al*., 2020; Datry *et al*., 2016a). Finally, *STconmat* proved useful to incorporate pairwise metrics (e.g., beta-diversity calculated with Jaccard or Bray-Curtis indices) within spatiotemporal analysis of temporary systems (Khattar *et al*., 2021; Legendre & De Cáceres, 2013), which is key to better capture assembly processes or improve management of spatiotemporally variable ecosystems (Bo *et al*., 2020; Boyé *et al*., 2019; Lamy *et al*., 2015; Ruhí *et al*., 2017; Sobek *et al*., 2009).

In dendritic networks such as fluvial systems, connectivity has been acknowledged for a long time as a key driver of diversity and functioning at population, community and/or ecosystem levels (Fullerton *et al*., 2010; Vannote *et al*., 1980). Indeed, several indices have been developed to assess the degree of fragmentation generated by dams (Baldan *et al*., 2022; Cote *et al*., 2009) or connectivity indices accounting for longitudinal and lateral connectivity (Rivers-Moore *et al*., 2016). However, the inclusion of both spatial and temporal connectivity facets has been poorly explored so far (Fullerton *et al*., 2010; Uroy *et al*., 2021). We believe that the proposed framework represents a first step towards a better inclusion of spatiotemporal connectivity in general ecology, extending beyond our study case and being potentially applied to any ecosystem. For example, our measures could be useful to incorporate network connectivity into biodiversity conservation using systematic planning approaches (Hermoso *et al*., 2012). Also, *STcon* and *STconmat* can serve to assess the effectiveness of restoration measures as the community response to habitat restoration will depend on spatiotemporal connectivity to some extent (Cid *et al*., 2022). At the same time, it complements other methods like remote sensing (Bishop-Taylor *et al*., 2017) by specifically quantifying spatiotemporal dynamics and incorporating multiple time-windows.

Overall, the proposed framework has a strong potential to be used at several temporal scales (e.g., days, months, years) and across different levels of biological organisation (e.g., populations, communities, ecosystems). Besides flow state, other metrics that vary spatiotemporally and define connectivity such as land use could be used (Firmiano *et al*., 2021). Furthermore, the current function could be extended to include other network structures by defining the number of neighbours at which dispersal is considered effective, either setting distance thresholds or dispersal decay with distance (Borthagaray *et al*., 2015; Muneepeerakul *et al*., 2008; Radinger & Wolter, 2014). In addition, another aspect that could

be further developed within this framework is related to antecedent spatiotemporal connectivity (i.e., the temporal scale considered by *STcon* and *STconmat*). In the approach presented here, the ST matrix considers the spatial structure from the previous day, thereby considering two time steps (i.e., what happened on day 1 has an impact on day 2 but not on day 3; Uroy *et al*., 2021). Both the defined network structure and the time span are case-specific and could be adjusted to the characteristics of the studied system and the focal organism (e.g., organism life-span, a pollution event). So far, the main limitation of this framework is still the lack of databases that include high spatial and temporal resolutions at relevant scales. Furthermore, the adaptation of the framework to different ecosystems and network structures should be made with caution. For example, the parameters used to calculate *STcon* and *STconmat* need to be defined for each system, because the results can be considerably different depending on the designed scenario. Nonetheless, the adaptability of our framework suggests that it can still be expanded to incorporate more facets of the interaction between spatial and temporal processes. In this regard, we provide the R code (see Online Supplementary Material) to facilitate and promote future studies.

The quantification of spatiotemporal connectivity is key for any ecosystems subjected to high seasonal and/or interannual variability, or affected by natural (e.g., wildfires, hurricanes, floods, droughts) or human-driven disturbances (e.g., invasive species, point-source pollution). Moreover, environmental conditions are becoming globally harsher and more variable, with an expected increase in the frequency, duration, and variability of natural and human-driven disturbances (IPCC, 2022). Consequently, we need to further explore and develop frameworks that can capture such variability in space and time (Uroy *et al*., 2021). Capturing and assessing the spatiotemporal dynamics at a meta-system scale is a key step towards guiding future conservation and management actions (Chase *et al*., 2020; Cid *et al*., 2020; Schiesari *et al*., 2019). We encourage the inclusion of spatiotemporal connectivity in future studies in the field of meta-system ecology, restoration ecology and biodiversity conservation to test and challenge our method so we can gain insights into spatiotemporal processes.

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AUTHOR CONTRIBUTIONS

David Cunillera-Montcusí conceptualized the framework and leaded the paper in the analysis, writing, interpretation and edition. Miguel Cañedo-Argüelles and Núria Bonada conceptualized the study, collected the data, interpreted the results, wrote, and edited the manuscript. Núria Cid collected the data, interpreted the results, wrote, and edited the manuscript. José María Fernández-Calero, Sebastian Pölster, Roger Argelich and Pau Fortuño collected the data and edited the manuscript.

Tot és posible fins que es demostri el contrari

David Cunillera-Montcusí

CHAPTER 2

Current metacommunity paradigms are challenged by Mediterranean temporary rivers

José María Fernández-Calero, Matías Arim, Ana Borthagaray, David Cunillera-Montcusí, Núria Bonada & Miguel Cañedo-Argüelles



ABSTRACT

According to available metacommunity studies in stream networks, at small spatial scales, species should be able to occupy non-preferential habitats through immigration from suitable habitats because dispersal should not be limited (i.e., mass effects). However, there is some evidence that in temporary rivers (TRs) stream network fragmentation caused by drying events could lead to dispersal limitation even at small spatial scales, preventing mass effects. Thus, we hypothesize network connectivity should have a significant effect on species replacement between nearby local communities in TRs, preventing mass effects and leading to dispersal limitation for certain taxa. To test this hypothesis, we assessed metacommunity assembly of aquatic macroinvertebrates with diverse dispersal strategies in Mediterranean TRs at a small spatial scale. We investigated alpha and beta diversity patterns in relation to local and regional predictors using null models and generalized additive models (GAMs), including habitat heterogeneity, physicochemical conditions, fish abundance, drying duration, and spatiotemporal connectivity. Our findings revealed significant seasonal variability in macroinvertebrate richness and composition, with relatively high gamma diversity considering the small spatial scale of the study area. Alpha diversity of aquatic passive dispersers was primarily driven by habitat heterogeneity and spatiotemporal connectivity. In contrast, beta diversity, considering all taxa, was shaped by environmental gradients and dispersal-related factors. Beta diversity of aerial active dispersers was predominantly influenced by local environmental variables, such as habitat heterogeneity and physicochemical gradients, whereas aquatic passive dispersers were strongly shaped by spatiotemporal connectivity. Thus, we found evidence of species sorting for aerial dispersers and dispersal limitation for aquatic dispersers. This suggests that current metacommunity paradigms designed for perennial stream networks should be revised for TRs. This is relevant if we take into account that more than 50% of the global stream network is temporary, and this percentage will likely increase in a future scenario of climate change and increased water demand.

Keywords: Connectivity, dispersal, habitat heterogeneity, macroinvertebrates, metacommunity, spatial scale

INTRODUCTION

Metacommunity theory has become a fundamental framework in ecology, offering insights into how local and regional processes shape biodiversity patterns across spatially structured habitats (Leibold *et al.*, 2004; Chase, 2005). By linking communities through dispersal, this framework integrates species interactions, environmental gradients, and spatial connectivity, addressing ecological dynamics at multiple scales (Leibold *et al.*, 2004). Four main paradigms have been proposed to explain metacommunity dynamics (Leibold *et al.*, 2004): species sorting, driven by environmental filtering and niche differentiation; neutral theory, focusing on stochastic processes like random dispersal and extinction; mass effects, when dispersal can override local environmental conditions; and patch dynamics, which examines colonization-extinction dynamics across discrete habitat patches. Although these paradigms are not intended to represent the entire spectrum of metacommunity dynamics (Brown *et al.*, 2017), they are useful to assess the different drivers that determine metacommunity assembly.

Scale plays a pivotal role in the study of metacommunities, particularly in river networks, where different paradigms prevail depending on the spatial extent of the analysis (Brown *et al.*, 2011; Tonkin *et al.*, 2018; Pouget, Pottier & Jabot, 2021). According to Heino *et al.* (2015), the influence of spatial and environmental factors on metacommunity structure varies across spatial scales. At larger spatial extents, dispersal limitation becomes more pronounced, leading to greater differentiation between communities as species face challenges in moving across distant habitats (Múrria *et al.*, 2010). In contrast, at smaller spatial scales, high dispersal rates between closely connected communities are expected (i.e., mass effects). This enables species to occupy sub-optimal habitats through the emigration of individuals from optimal habitats, thereby reducing the influence of local environmental conditions on community assembly. According to this, it is reasonable to assume that community turnover (i.e., beta-diversity) would be low at small spatial scales, with strong dispersers dominating the communities. Finally, species sorting typically occurs at intermediate spatial scales, at which species can track environmental gradients but dispersal is limited to some extent (Leibold *et al.*, 2004; Heino *et al.*, 2015).

Temporary rivers (TRs) can potentially challenge this paradigm because they have a strong hydrological variability that shapes network connectivity (i.e., connectivity between local communities) and results in high a spatiotemporal dynamism, even at small spatial scales (Larned *et al.*, 2010; Bonada & Resh, 2013; Datry, Larned & Tockner, 2014; Datry *et al.*, 2017c). These ecosystems, characterized by alternating phases of flow, disconnected pools, and dry riverbeds create a mosaic of habitats that shift in space and time (Datry, Bonada & Heino, 2016a; Bonada *et al.*, 2020; Cunillera-Montcusí *et al.*, 2023). This variability imposes both strong environmental filters on local communities and dispersal limitation due to the fragmentation of the stream network by drying. For example, Chase (2007) and Soininen (2012) highlighted how environmental constraints in drying habitats can amplify the effects of niche-based filtering, while simultaneously restricting dispersal. Also, Cañedo-Argüelles *et al.* (2015) showed that network fragmentation by drying affected dispersal dynamics of aquatic macroinvertebrates, which dispersed overland using perennial pools as stepping stones instead of using the stream channel as a dispersal corridor (as commonly reported for perennial streams).

Macroinvertebrates are widely recognized as an excellent model for studying metacommunity dynamics in TRs. These organisms exhibit diverse biological and ecological traits, including varying dispersal strategies that enable them to persist in the highly dynamic and often harsh conditions of TRs (Stubbington *et al.*, 2017). Many species have adaptations to cope with drying, such as desiccation-resistant eggs, diapause stages, or the ability to burrow into substrates to avoid desiccation (i.e., resistance strategies) (Bogan *et al.*, 2017). Their dispersal strategies can be categorized as aerial active dispersal (e.g., flight in winged adult stages), aerial passive dispersal (e.g., wind-mediated transport of eggs or lightweight individuals), aquatic active dispersal (e.g., movement within the water column or across the benthos), and aquatic passive dispersal (e.g., drift with water flow) (Tachet *et al*., 2010; Sarremejane *et al.*, 2020a). Fragmentation of the river network due to drying significantly impacts these groups in distinct ways. Aerial active dispersers are the least constrained, as is their ability to fly overland across the river network and colonize isolated patches or rewetting habitats, often acting as the primary recolonizers once flow resumes (Cañedo-Argüelles *et al.*, 2015; Pineda-Morante *et al.*, 2022). Aerial passive dispersers, while also capable of moving over fragmented networks, are more reliant on external vectors like wind, making their

colonization success more stochastic and dependent on environmental conditions (Bilton, Freeland & Okamura, 2001). In contrast, aquatic active dispersers, which move within the water column or across the benthos, are severely restricted by the loss of continuous aquatic habitats. Fragmentation isolates these taxa in disconnected pools, limiting their ability to disperse and recolonize new habitats, which can result in reduced genetic exchange and localized extinctions (Bohonak & Jenkins, 2003). Similarly, aquatic passive dispersers, such as taxa relying on drift, are directly dependent on flowing water for their dispersal (Kappes & Haase, 2012). Prolonged drying and fragmentation can limit their dispersal and their access to perennial refugia, often leading to reduced species richness and homogenization of communities in isolated sections of the river. Overall, drying-induced fragmentation exacerbates dispersal limitations for aquatic taxa, while aerial dispersers demonstrate greater resilience, albeit with varying degrees of dependence on habitat availability and external dispersal vectors (Datry *et al.*, 2016a). Thus, aquatic macroinvertebrates are ideal model organisms to test our hypothesis (i.e., fragmentation by drying should prevent mass effects) because they capture a wide dispersal gradient, with different groups of taxa showing different dispersal limitations.

The main aim of this study is to assess if spatiotemporal connectivity can significantly affect metacommunity assembly of aquatic macroinvertebrates in Mediterranean TRs at a small spatial scale. We hypothesize that fragmentation by drying should prevent mass effects, leading to dispersal limitation of aquatic dispersers and species sorting (reinforced by harsh environmental conditions) in aerial dispersers (Figure 1). The specific objectives of the study are to: (1) provide a descriptive overview of the spatiotemporal variation in alpha and beta diversity at a small spatial scale; and (2) investigate the dominant drivers of metacommunity assembly at a small spatial scale and how they differ based on the dispersal modes of the taxa.

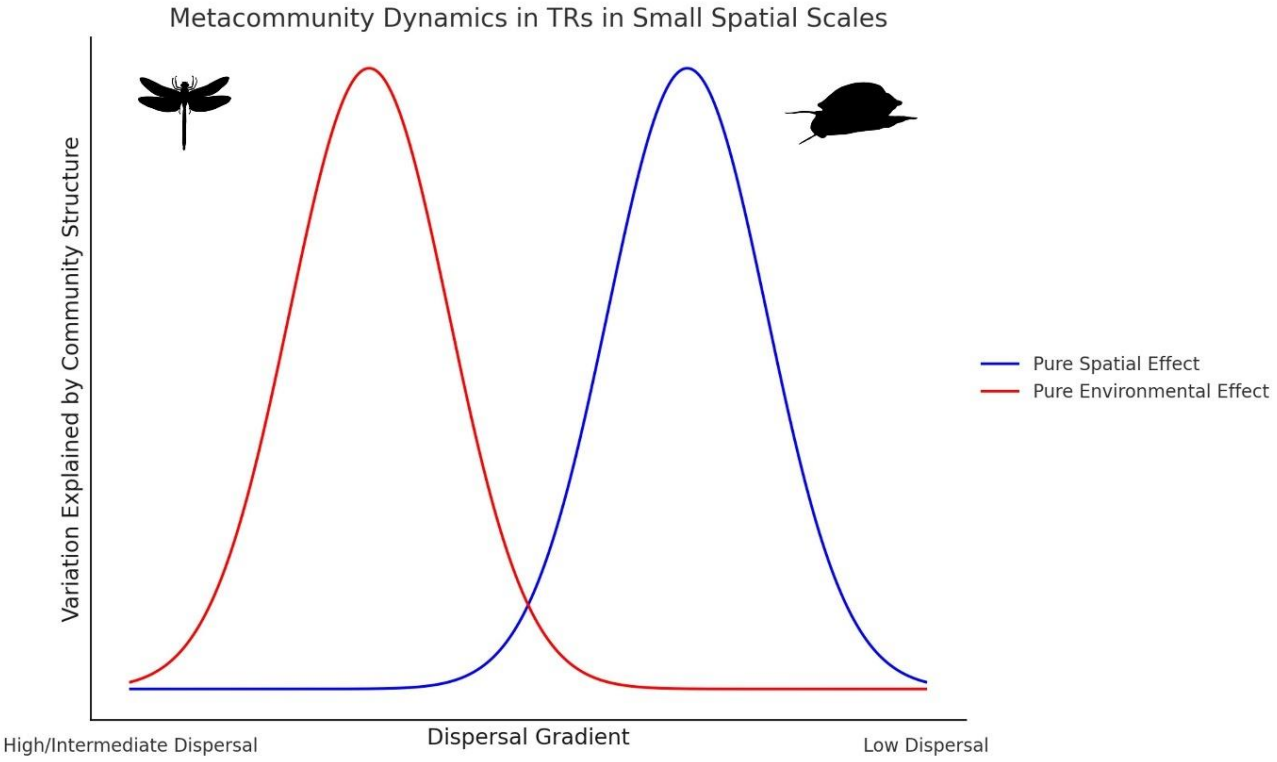


Figure 1. The diagram illustrates the variation in community structure explained along a dispersal gradient in temporary rivers. Dispersal limitation is the dominant process under low dispersal conditions, whereas species sorting governs community dynamics under intermediate to high dispersal conditions. The contributions of pure spatial effects (blue curve) and pure environmental effects (red curve) are highlighted as key drivers of metacommunity dynamics across the gradient. Conceptual model adapted from Heino *et al*. (2015).

METHODS

Study area

The study was conducted in the Sant Llorenç del Munt i l'Obac Natural Park, a 137 km² protected area located in the northeastern Iberian Peninsula (Spain) (Figure 2). This region experiences a Mediterranean climate, characterized by hot and dry summers and cool and wet winters, with intense and irregular rainfall, primarily occurring in autumn. The area's geology is predominantly karstic, which results in highly dynamic hydrological patterns. Streams typically exhibit a significant seasonal flow variability, transitioning from flowing

water to disconnected pools, and sometimes to completely dry riverbeds, especially in summer. The TRs selected for this study included Vall d'Horta, Sanana, Santa Creu, Rellinars, Talamanca, Castelló, and Mura. These rivers encompass a broad gradient of hydrological conditions, ranging from permanent (e.g., Vall d' Horta) to ephemeral (e.g., Rellinars) reaches. These TRs span two major river basins, the Besòs and the Llobregat, both of which discharge into the Mediterranean Sea. Sampling was conducted throughout 2019 during the four seasons (winter, spring, summer and autumn: January, May, July, and November, respectively) to capture the intra-annual variability in flow permanence across different hydrological phases.

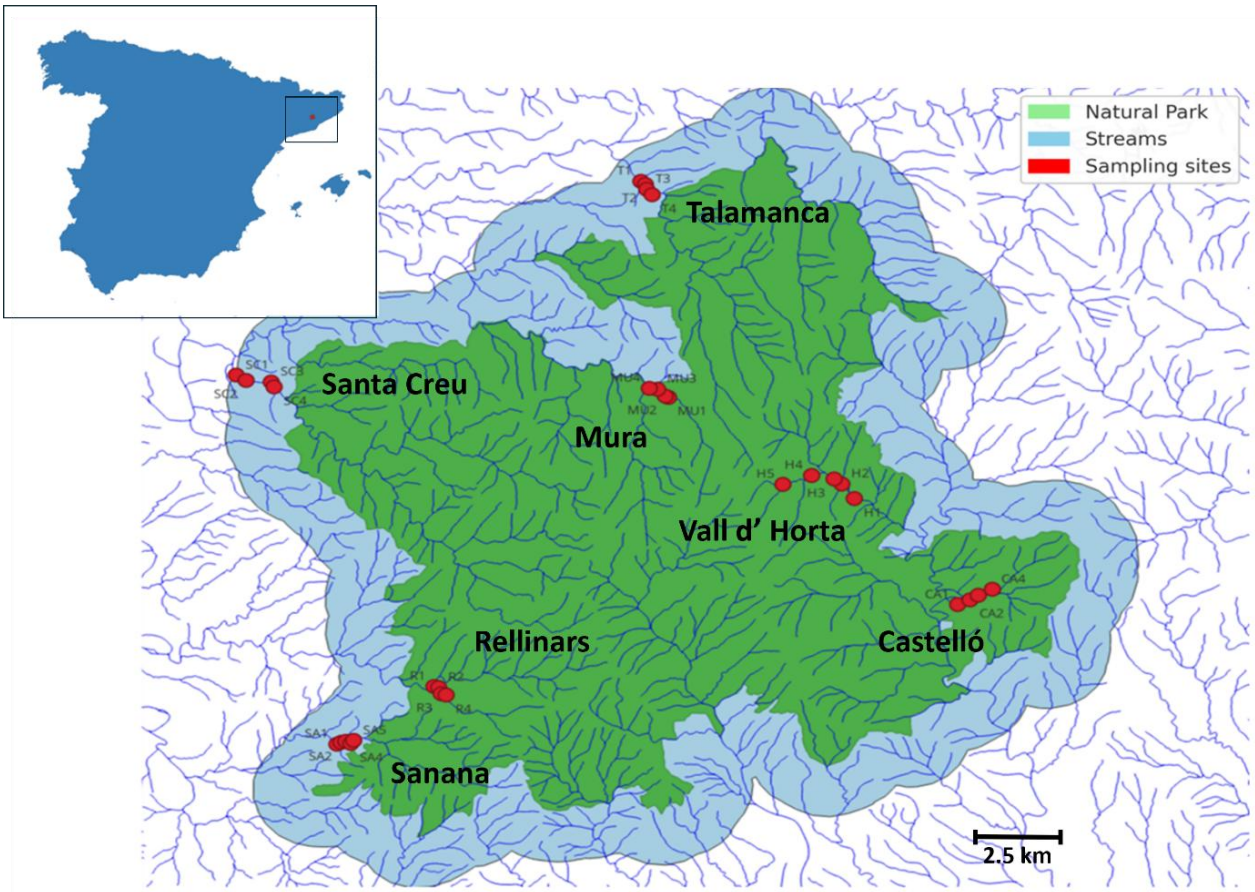


Figure 2. Maps of Sant Llorenç del Munt i l'Obac. The inset map of Spain shows the location of the park, highlighted within a rectangle. The central map displays the sampling points across seven streams. The green area represents the natural park, while the blue-shaded buffer zone encompasses all sampling points.

Biological and environmental data collection

Macroinvertebrate samples were collected from 30 sites across seven streams. The sampling sites were located within a relatively small area, with points from the same streams separated by only a few hundred meters. Dry sites were excluded from sampling, resulting in 30 samples in winter, 25 in spring, 21 in summer, and 25 in autumn. A 250 µm hand net was used to sample all available habitats along a 100-meter reach at each site. Samples were preserved in 96% ethanol and stored in a freezer until sorting and taxonomic identification were done in the laboratory. Macroinvertebrates were identified and counted under a binocular stereoscope to the lowest possible taxonomic level, typically to genus, except for Oligochaeta and Chironomidae, which were identified to subfamily or tribe level. To classify macroinvertebrates into one of four dispersal modes (aerial active, aerial passive, aquatic active, or aquatic passive), the fuzzy coding approach from Tachet *et al*. (2010) and Sarremejane *et al*. (2020a) was applied. Taxa were assigned to the category with the highest coding value (0–3). In cases where a taxon had equal coding values in multiple categories, it was placed in the strongest dispersal class according to the following hierarchy: aerial active > aerial passive > aquatic active > aquatic passive. This hierarchy reflects the higher colonization potential of aerial and active strategies compared to aquatic and passive ones. For taxa not included in Tachet *et al*. (2010) or Sarremejane *et al.* (2020a), assignments were made at the nearest taxonomic level (e.g., coding all Psychodidae genera at the family level). Subsequently, only aerial active and aquatic passive groups were used, as the other two groups lacked sufficient taxa to ensure robust statistical analyses.

Fish were sampled to serve as a biological predictor for macroinvertebrate communities, employing electrofishing techniques in accordance with the official protocol established by the Spanish government (MAGRAMA, 2015). Tricaine methanesulfonate (MS-222) was used as an anesthetic to minimize stress, and after identification and counting, the fish were returned to the stream. Four fish species were identified in the field by experts across the studied streams, although their distribution varied substantially. No fish was detected in the Vall d'Horta, Rellinars, and Santa Creu streams. The native species—*Barbus haasi*, *B. meridionalis*, and *Squalius laietanus*—coexisted with the invasive exotic cyprinid *Phoxinus septimaniae* × *P. dragarum* in certain streams. *B. meridionalis* was exclusively

recorded in the Castelló stream within the Besòs river basin, whereas *B. haasi* was confined to streams in the Llobregat river basin, specifically Mura, Sanana, and Talamanca. *Squalius laietanus* and *P. septimaniae × dragarum* were found co-occurring in the Mura stream, also within the Llobregat river basin. Then, fish abundances were used as a predictor variable to explain macroinvertebrate diversity.

Physicochemical parameters were measured with a YSI Professional Plus Multiparameter Probe, which recorded dissolved oxygen (DO in mg/L), oxygen saturation percentage (DO %), water temperature (°C), pH, conductivity (EC in µS/cm), specific conductivity (SPC in µS/cm), and total dissolved solids (TDS in mg/L). Habitat complexity was assessed using the Fluvial Habitat Index (IHF) (Pardo *et al.*, 2002), which evaluates various factors such as substrate type and distribution in riffles and pools (B1), riffle frequency (B2), substrate diversity (B3), flow velocity and depth regime (B4), channel shading (B5), habitat heterogeneity (e.g., leaf litter, branches, wood, or roots) (B6), and aquatic vegetation cover (B7).

To assess multicollinearity among the physicochemical and IHF variables, a Spearman's correlation matrix was computed using the "cor" function from R software. Highly correlated variables (with a correlation coefficient greater than 0.75) were removed from the dataset using the "findCorrelation" function from the "caret" package (Kuhn, 2008). To analyze the structure of the physicochemical and habitat heterogeneity datasets and identify the primary sources of variation, Principal Component Analysis (PCA) was performed separately for each dataset using the "dudi.pca" function from the "ade4" package (Dray & Dufour, 2007). Data were standardized to ensure comparability across variables. The PCA results were visualized using a biplot generated with the "fviz_pca_biplot" function from the "factoextra" package, which facilitated the exploration of relationships between physicochemical variables, the IHF, and seasonal patterns. To enhance interpretation, 90% confidence ellipses were added to the biplot for each seasonal group. Principal component scores for the first axis were extracted from the PCA results for subsequent analysis.

For hydrological analysis, 69 temperature data loggers (HOBO Pendant®) were deployed at various sites to monitor both riffle and pool habitats, with one logger per stream recording air temperature. Temperature data were recorded hourly over a 513-day period

(July 2018–December 2019). This setup allowed us to determine the wet or dry conditions of each site throughout the study period (Gungle, 2006). Additional details are provided in Pineda-Morante *et al.* (2022) and Cunillera-Montcusí *et al.* (2023).

The hydrological information was used to calculate several local and regional hydrological metrics: the total number of dry days (hereafter TotDur) (Crabot *et al.*, 2020; Arias-Real *et al.*, 2021; Pineda-Morante *et al.*, 2022) and four spatiotemporal connectivity variables (Cunillera-Montcusí *et al*., 2023). To calculate spatiotemporal hydrological connectivity variables (hereafter *STcon*) for each sampling site, a presence/absence of water dataset was used. Two scenarios proposed by Cunillera-Montcusí *et al.* (2023) were considered: a binary scenario (connection between sites as 1 and no connection as 0) and a weighted scenario (connection between sites weighted by the Euclidean distance between them). Two types of network structures were evaluated for each scenario: directed (with upstream to downstream directionality) and undirected (where all sites are connected to each other). Four *STcon* values were derived: directed binary (DirBin), undirected binary (UndBin), directed weighted (DirWei), and undirected weighted (UndWei). In the binary scenarios, higher *STcon* values indicate that a site was highly connected to its neighbors during the monitored period (i.e., high connectivity), while in the weighted scenarios, higher values suggest that a site was more isolated relative to its neighbors, indicating high dispersal resistance. Given the small distances between sampling sites and the relatively straight nature of the streams, Euclidean distances were used to weight both the binary and undirected scenarios. For further details on *STcon*, refer to Cunillera-Montcusí *et al*. (2023).

Statistical analysis

To examine beta diversity components across seasons, the dataset was partitioned into seasonal subsets, and beta diversity metrics were calculated for each subset. The "beta.div.comp" function from the "adespatial" package was used (Dray *et al.*, 2022), with the Jaccard index calculated on abundance data. This analysis yielded information on two components of beta diversity: species replacement and richness difference. All graphical illustrations were created using the "ggplot2" package (Wickham *et al.*, 2024).

Null models were built to estimate expected diversity values under random assembly scenarios and compare them to observed values (Gotelli & Graves, 1996). This approach yielded Z-scores, which indicate whether diversity metrics deviated from random expectations, providing insights into community assembly processes. Sampling sites were replicated across four seasons, with abundance data aggregated by site to generate a single value per site.

The null model approach involved randomizing species compositions while maintaining observed species abundance distributions and community sizes. First, observed alpha diversity (species richness) and beta diversity (Jaccard dissimilarity index) were calculated. Then, null communities were generated by sampling species randomly from the regional species pool, constrained by the total observed abundances within each community. Z-scores for alpha (Zα) and beta (Zβ) diversity were computed by comparing observed values to the mean and standard deviation of the null distributions. The Z-scores were calculated using the formula:

$$Z\,\alpha/\beta = (\text{Observed } \alpha/\beta \text{ Diversity} - \text{Mean } (\alpha/\beta \text{ Diversity}_{null})) / \text{SD } (\alpha/\beta \text{ Diversity}_{null})$$

The analyses were repeated for all taxa, as well as for aerial active and aquatic passive dispersers.

To ensure robust null distributions for both alpha and beta diversity, 2000 replicate null models were generated. This extensive number of replicates enhanced the statistical reliability of the null expectations, offering a precise basis for comparing observed diversity metrics. Z-scores for alpha and beta diversity that deviate significantly from zero—whether higher or lower—indicate that meta-community assembly is influenced by deterministic processes, such as environmental filtering or competitive interactions. In contrast, Z-scores close to zero suggest a random community assembly, with species distributions reflecting stochastic events rather than ecological processes.

Following the calculation of Z-scores for alpha and beta diversity, Generalized Additive Models (GAMs) were employed to investigate the potential environmental drivers shaping these diversity patterns while accounting for potential non-linear relationships. The predictor variables examined included: the first principal component of the PCA using physicochemical

variables (PC1 FQ) and habitat heterogeneity (PC1 IHF); the total number of dry days (TotDur), four spatiotemporal connectivity metrics (DirBin, DirWei, UndBin, and UndWei) and the abundance of *Barbus* species (i.e., *B. haasi* and *B. meridionalis*) as well as non-*Barbus* species (i.e., *Phoxinus* spp. and *S. laietanus*). Fish species were grouped into *Barbus* and non-*Barbus* categories based on ecological and phylogenetic considerations. *Barbus* species are benthic feeders with similar trophic niches and a strong reliance on substrate habitats. In contrast, non-*Barbus* species are more pelagic, with broader habitat tolerances and differing ecological roles.

Each predictor was included in the models as both linear and quadratic terms. The function “gam” from the “mgcv” package was used to fit smooth terms with cubic splines, providing flexibility in capturing complex relationships between the predictors and diversity measures (Wood, 2017). For the model selection, the best-fitting models for each environmental variable were selected using the “bestglm” function, based on the Akaike Information Criterion (AIC). The models included both linear and quadratic terms to ensure that the best-fitting and most parsimonious model was selected for each predictor variable.

To explore the influence of predictor variables on alpha and beta diversity Z-scores, partial residual plots were generated. These plots illustrate the isolated effects of each predictor while accounting for the influence of other covariates in the model. The X-axis in these plots represents the predictor variable, while the Y-axis displays the adjusted response (partial residuals) for alpha and beta diversity.

RESULTS

A total of 178 distinct taxa were identified across the seven streams and four seasons. The distribution of taxa across seasons revealed notable differences in richness: 134 in winter, 132 in spring, 128 in summer, and 98 in autumn. In terms of relative abundance, Diptera was the most dominant order across all four seasons, comprising 51% of the total abundance in winter and 77% in spring. Ephemeroptera was the second most abundant order, with relative abundance ranging from 14% in spring to 27% in summer (Figure 3). The majority of taxa were

classified as aerial active dispersers (115), followed by aquatic passive (33), aquatic active (21), and the least represented group, aerial passive dispersers (7).

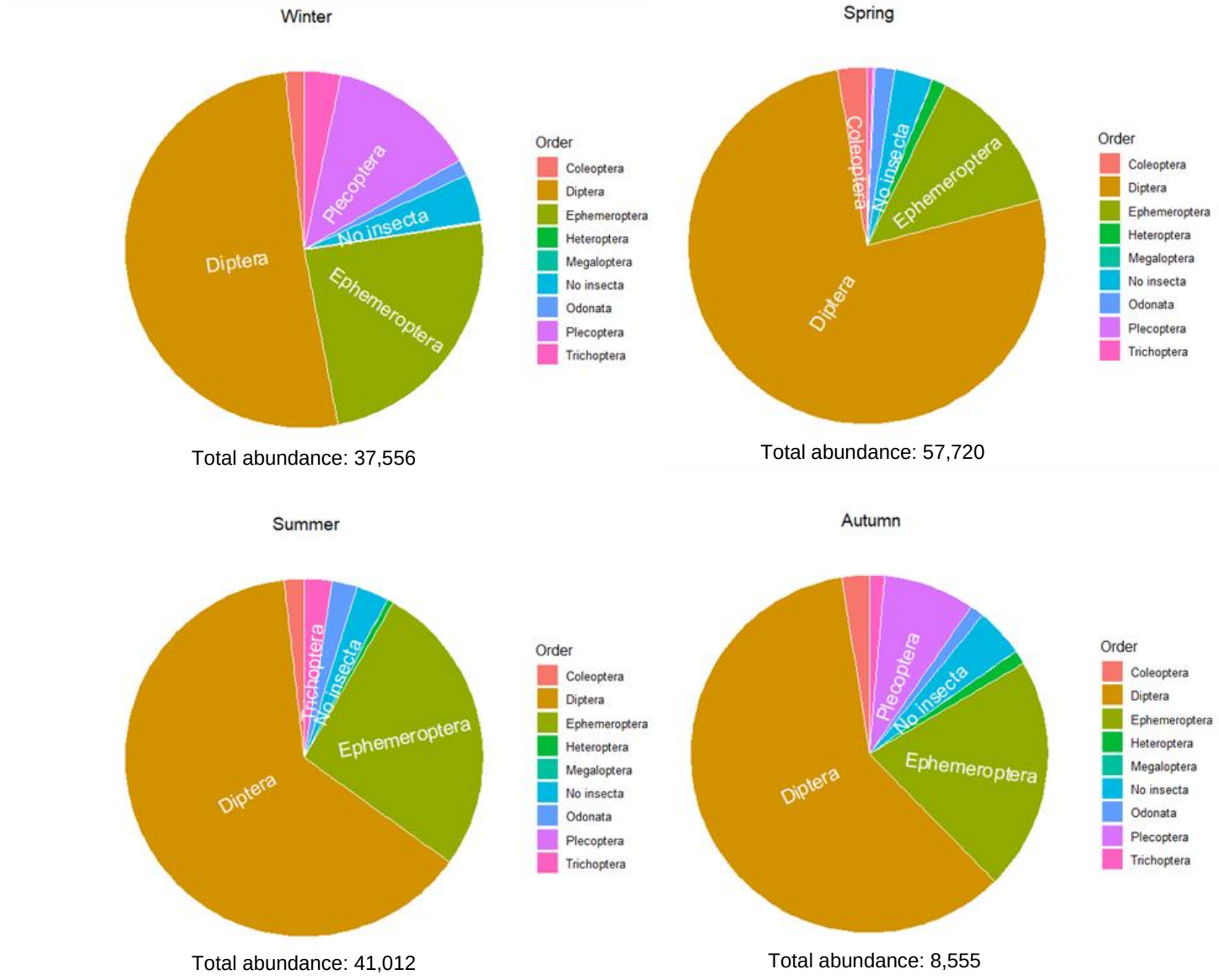


Figure 3. Pie charts illustrating the relative abundance of different macroinvertebrate orders across the four seasons. Each segment represents a specific order, with the size proportional to its abundance relative to the other orders. The labels within the chart indicate the four most abundant orders. The "No Insecta" group represents non-insect macroinvertebrates, such as Mollusca, Annelida, and Crustacea. Total abundance for each season is represented below the corresponding pie charts.

The mean taxa richness per site was 32.7 (± 12.5). The highest richness values were recorded in the Vall d'Horta stream (site H1), with 61 taxa observed during the summer, likely attributable to the low intermittence in three of its five studied reaches, which provide more stable habitat conditions. In contrast, more ephemeral streams, such as Rellinars and Santa Creu, experienced frequent drying events across several reaches, leading to lower richness values (Figure 4).

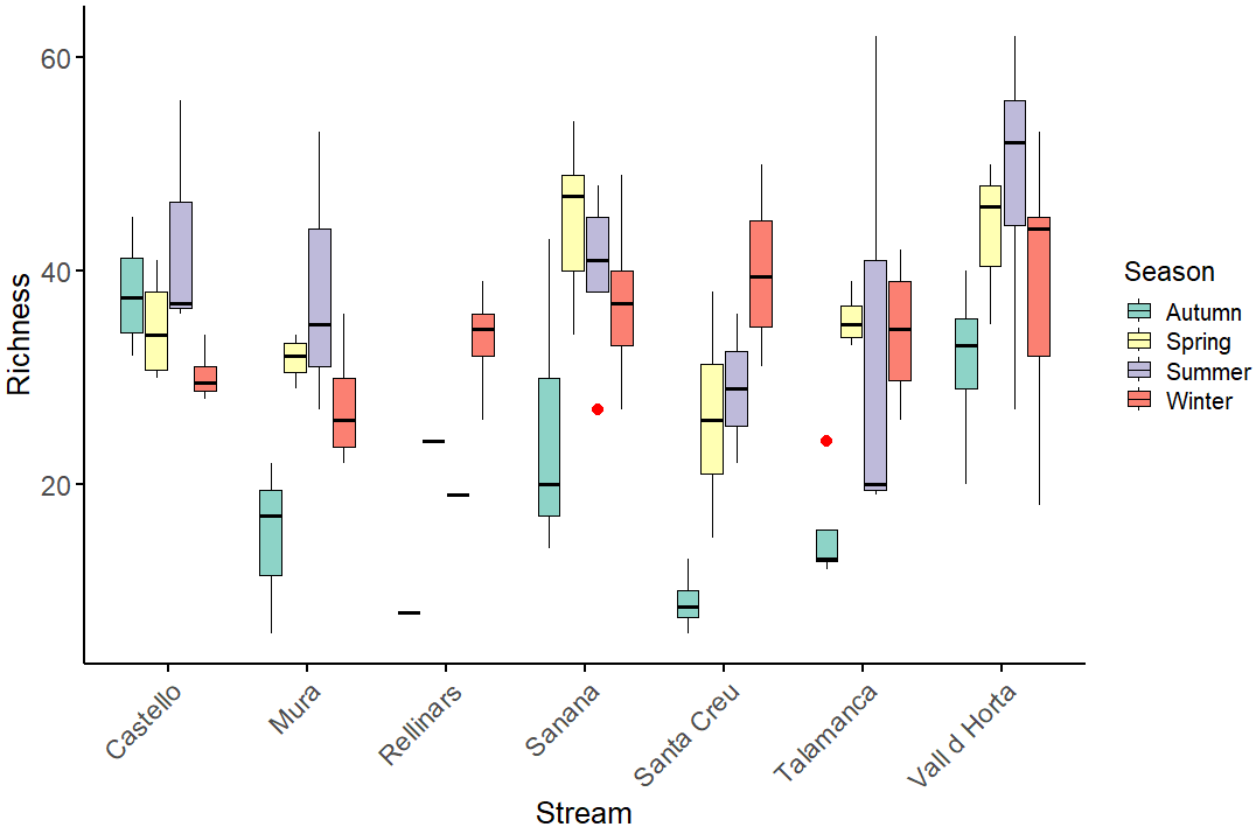


Figure 4. Boxplot showing species richness across different streams and seasons. Each box represents the distribution of richness values within a stream, grouped by season, with outliers highlighted in red.

Regarding beta diversity, richness difference (i.e., nestedness) exceeded replacement (i.e., turnover) in all seasons except winter, when the contributions of both components were more balanced (Figure 5; see the Supplementary Material for detailed insights into the beta diversity components of specific sites).

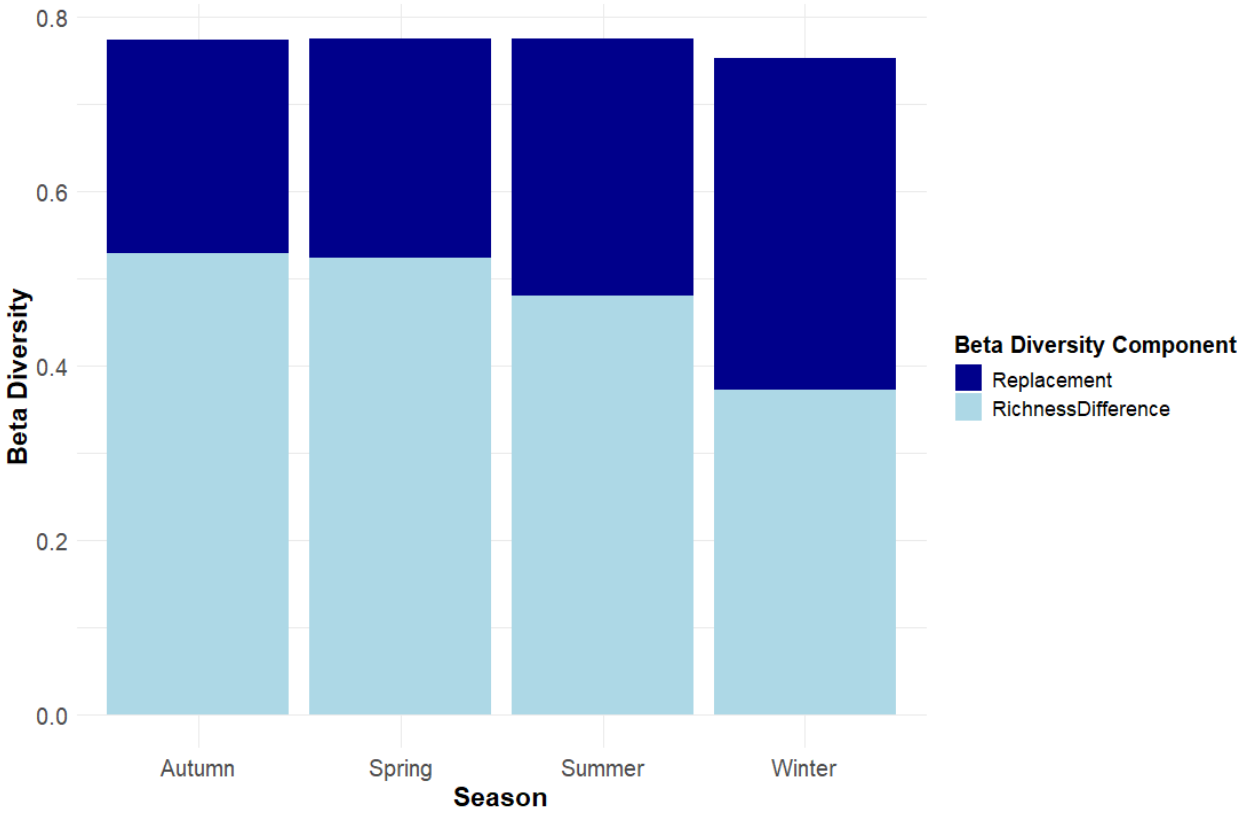


Figure 5. Stacked bar plot showing the contributions of beta diversity components—replacement (turnover) and richness difference (nestedness)—across seasons.

The first two axes of the Principal Component Analysis (PCA) explained 39.1% and 30% of the variance for the physicochemical variables, respectively. Similarly, for the IHF variables, the first and second axes accounted for 37.5% and 20.5% of the variance (Figure 6).

The null models showed that alpha diversity values were generally lower than those expected under random assembly (i.e., lower than zero). Conversely, beta diversity values exceeded null expectations (Figure 7). The Z-scores of aquatic passive dispersers were closer to random expectations than those of aerial active dispersers.

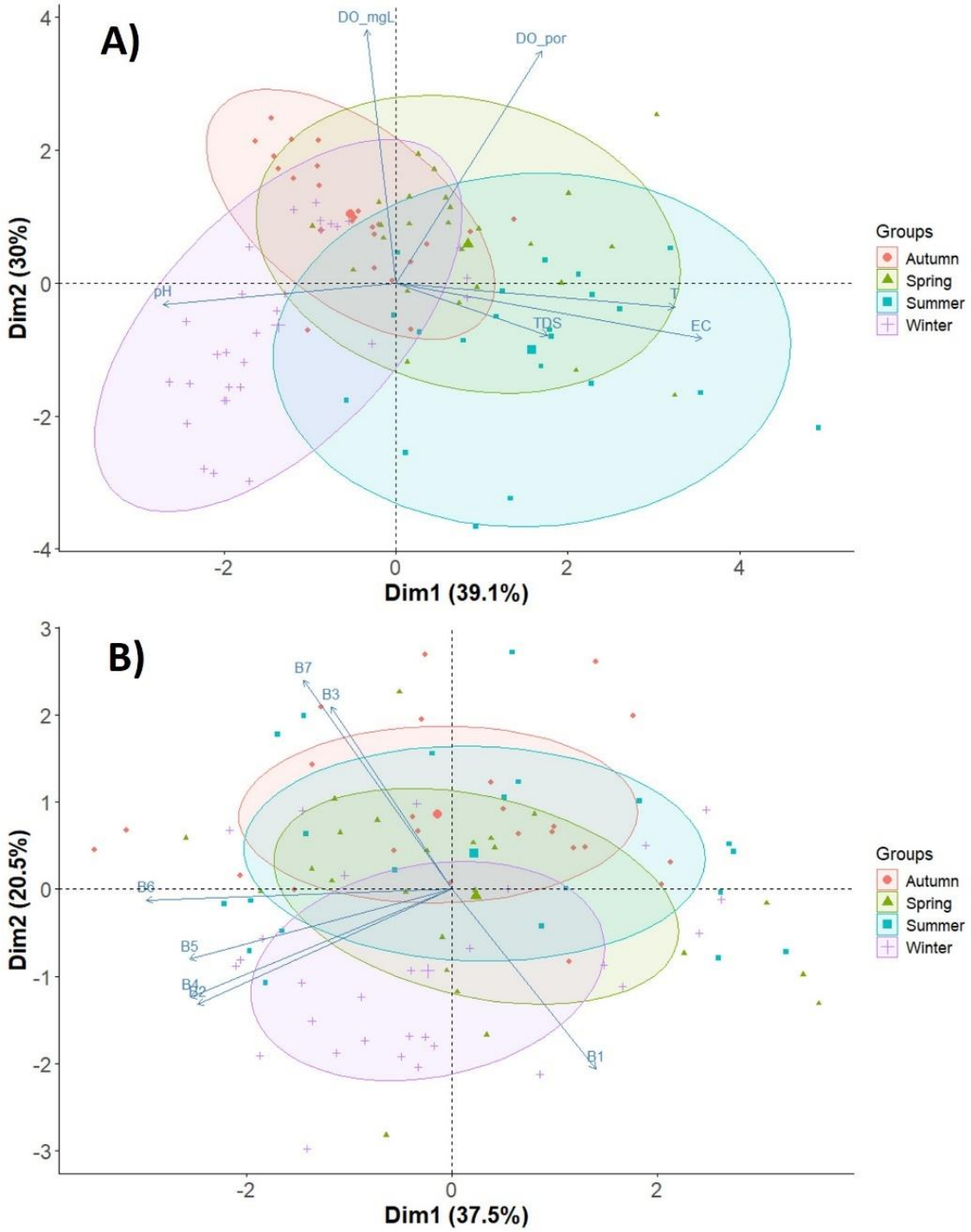


Figure 6. Principal Component Analysis (PCA) biplot of physicochemical (A) and habitat heterogeneity variables (B). Arrows represent variable loadings, while points indicate individual samples colored by season. In (A), physicochemical parameters include dissolved oxygen (DO_mgL), oxygen saturation percentage (DO_por), water temperature (T), pH, conductivity (EC), and total dissolved solids (TDS). In (B), habitat heterogeneity variables include substrate type and distribution in riffles and pools (B1), riffle frequency (B2), substrate diversity (B3), flow velocity and depth regime (B4), channel shading (B5), habitat heterogeneity (e.g., leaf litter, branches, wood, or roots) (B6), and aquatic vegetation cover (B7). The 90% confidence ellipses illustrate how observations cluster according to seasonal differences.

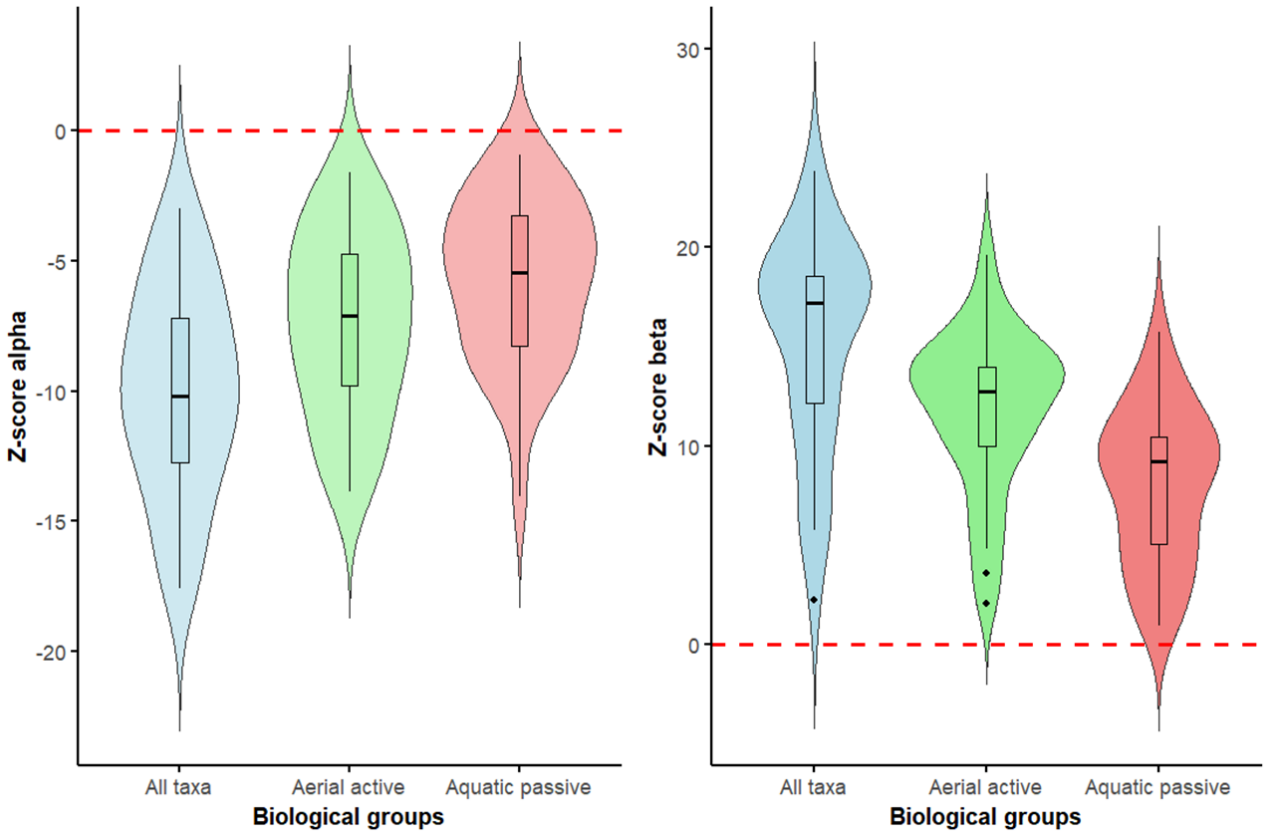


Figure 7. Violin plots showing the distribution of Z-scores for alpha and beta diversity for All taxa, aerial active dispersers, and aquatic passive dispersers. The dashed red line indicates a Z-score of 0, representing the threshold between observed values falling below and exceeding expected null values.

According to the GAM models, alpha diversity was not strongly influenced by the tested predictors for all taxa and aerial active dispersers. However, for aquatic passive dispersers, the model shows a significantly better fit (R^2 = 0.52), with a strong relationship between alpha diversity and habitat heterogeneity, spatiotemporal connectivity, and the abundance of non-*Barbus* fish species (Table 1). Higher alpha diversity values in aquatic passive dispersers were associated with negative PCA IHF scores, which correspond to greater habitat heterogeneity in riffles, flow velocities, and in-channel structural complexity (Figure 8). Furthermore, both spatiotemporal connectivity and the abundance of non-*Barbus* species were found to negatively influence alpha diversity in aquatic passive dispersers.

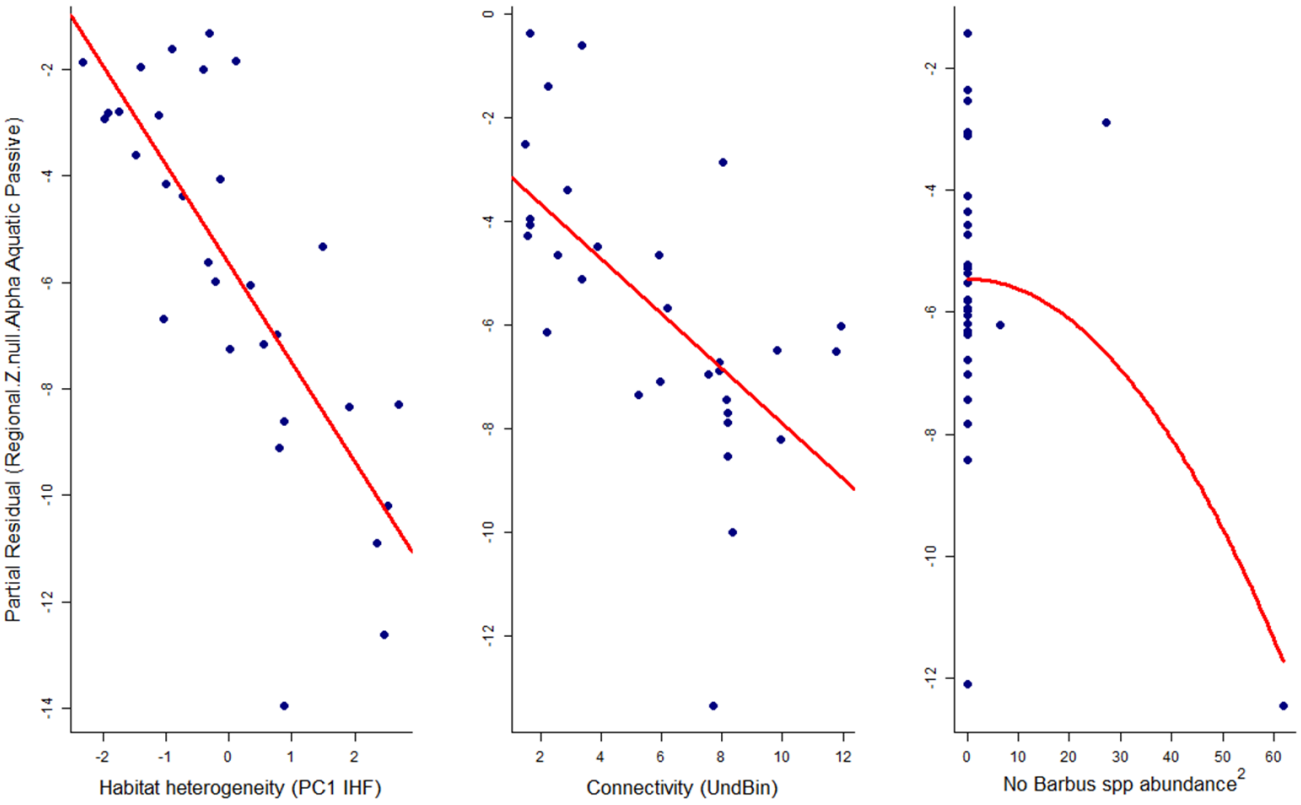


Figure 8. Partial residual plots illustrating the relationship between alpha diversity (Z-scores) of aquatic passive dispersers and significative key predictors. The predictors include (A) habitat heterogeneity (PC1 IHF), (B) spatiotemporal connectivity (UndBin), and (C) the abundance of non-*Barbus* fish species squared. The squared term is included to capture potential non-linear effects. The red line represents the fitted relationship from the GAM model.

Regarding beta diversity, the GAM models were significant for all biological groups and exhibited higher R^2 values than the alpha diversity models (Table 1). Physicochemical conditions and drying duration were selected as significant predictors of beta diversity when considering all taxa (Figure 9). For aerial active dispersers, local predictors such as habitat heterogeneity and physicochemical conditions were the most significant. In contrast, for aquatic passive dispersers, while habitat heterogeneity (IHF) remained significant, spatiotemporal connectivity was most important (Figure 10).

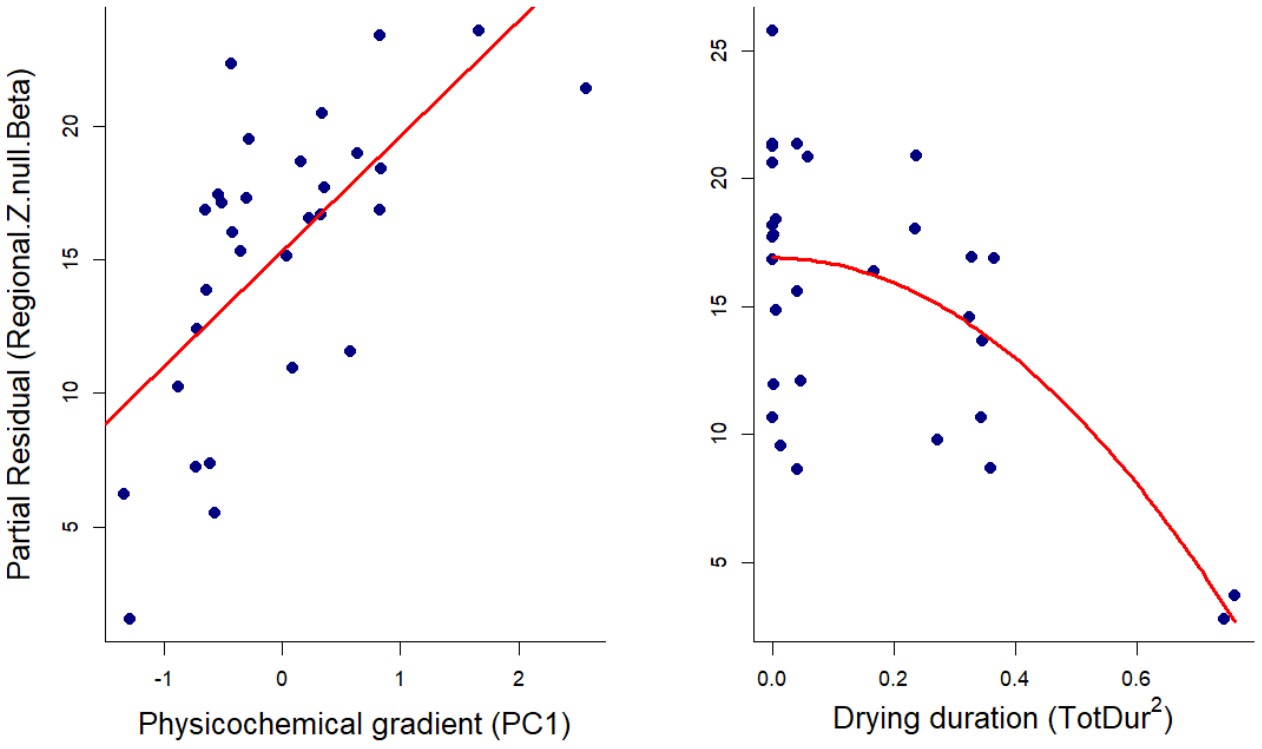


Figure 9. Partial residual plots showing the relationship between all taxa beta diversity (Z-scores) and the two significant key predictors. (A) Physicochemical gradient (PC1 FQ) and (B) drying duration squared (TotDur²). The squared term is included to capture potential non-linear effects. The red line represents the fitted relationships from the GAM models.

Table 1. Summary of GAM model results for alpha and beta diversity for all taxa, aerial active and aquatic passive dispersal groups. The squared term is included to capture potential non-linear effects.

Biological categories	Diversity	Predictors	Model R^2	Model p value	Estimate	Std. Error	p value
All taxa	Alpha	$(PC1\ IHF)^2$	0.07	0.15	0.5	0.33	0.147
	Beta	PC1 FQ	0.42	0.002 **	4.3	1.18	0.001 **
		ab_*Barbus*			0.15	0.07	0.05
		$(TotDur)^2$			-24.32	6.39	< 0.001***
Aerial active	Alpha	TotDur	0.25	0.05	5.38	2.85	0.07
		ab_*Barbus*			-0.26	0.13	0.05
		$(ab\_Barbus)^2$			0.004	0.002	0.14
	Beta	PC1 FQ	0.41	0.002 **	2.7	0.88	0.004 **
		PC1 IHF			-1.06	0.44	0.02*
		$(PC1\ IHF)^2$			-0.9	0.34	0.01*
Aquatic passive	Alpha	PC1 IHF	0.55	< 0.001***	-1.85	0.35	< 0.001 ***
		UndBin			-0.53	0.15	0.002**
		$(ab\_no\_Barbus)^2$			-0.001	0.0006	0.01*
	Beta	PC1 IHF	0.5	< 0.001 ***	1.45	0.45	0.003 **
		UndBin			3.1	0.76	< 0.001***
		$(UndBin)^2$			-0.18	0.05	0.03**

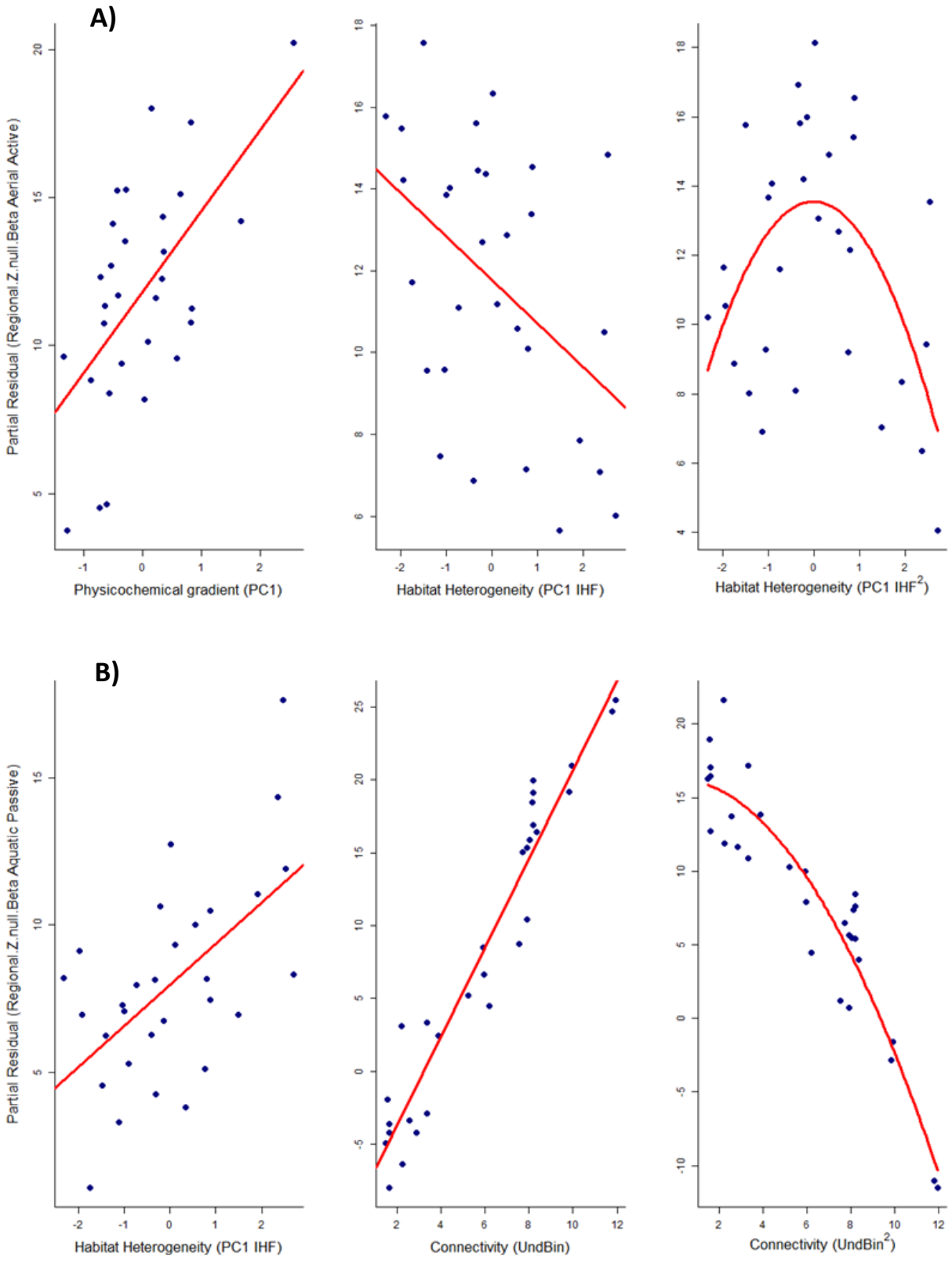


Figure 10. Partial residual plots illustrating the relationships between beta diversity (Z- scores) and significant predictors for (A) aerial active and (B) aquatic passive dispersers. The squared term is included to capture potential non-linear effects. The red line represents the fitted relationships from the GAM models.

DISCUSSION

Overall, this study revealed significant differences in species richness across seasons and streams, with relatively high gamma diversity when accounting for the small spatial scale of the study area. Although the total number of taxa identified was lower compared to larger-scale studies, this is expected given the limited spatial extent. Despite the small geographic area, the study documented substantial macroinvertebrate diversity, emphasizing the ability of TRs to support diverse communities due to their spatiotemporal variability. Diptera, dominated by Chironomidae, exhibited remarkable ecological plasticity, thriving under fluctuating hydrological conditions. Beta diversity analysis revealed that richness differences exceeded species replacement, likely reflecting the restricted regional species pool and subset community structure at this spatial scale, contrasting with previous studies in TRs conducted at larger spatial scales (Ruhí, Datry & Sabo, 2017; Rogosch & Olden, 2019).

Alpha diversity values lower than expected under the null model suggest that community assembly was shaped by deterministic processes such as environmental filtering, where specific conditions select for certain species, thereby reducing local diversity (Chase, 2007). In contrast, beta diversity values exceeded null expectations, showing that the differences between communities were greater than expected by chance. This could be due to spatial isolation associated with network fragmentation. This has been reported for aquatic macroinvertebrate communities in high mountain streams, where spatial isolation caused by mountain barriers resulted in high beta diversity (Finn *et al.*, 2011). Beta diversity could also be promoted by environmental heterogeneity. TRs are very dynamic systems in space and time, with strong environmental fluctuations that can favor the coexistence of species and the differentiation of communities (Bogan & Lytle, 2007). These patterns remained consistent across different dispersal categories, suggesting that, regardless of dispersal mode, these metacommunities are not structured by random processes. However, for both alpha and beta diversity, aquatic passive dispersers showed values closer to random expectations compared to aerial active dispersers, possibly due to the limited species pool available for this dispersal group within our spatial study area.

The alpha diversity of aquatic passive dispersers was decreased along the habitat heterogeneity gradient. Low values of this gradient are associated with factors such as a high

frequency of riffles (B2), distinct flow velocity and depth regimes (B4), a greater percentage of shade in the channel (B5), and the presence of structural habitat heterogeneity elements (e.g., leaf litter, branches, wood, or roots) (B6). This aligns with previous studies showing that flowing water and habitat heterogeneity promote macroinvertebrate alpha diversity (Palmer, Menninger & Bernhardt, 2010). Complex habitats provide a variety of microhabitats, each capable of supporting species with distinct ecological requirements. Such heterogeneity reduces interspecific competition by facilitating niche partitioning, thereby promoting species coexistence (Tews *et al.*, 2004). Additionally, structural complexity—such as leaf litter, wood, and vegetation—offers critical refugia from predators (e.g., fish) and buffers against harsh environmental conditions (Flecker & Allan, 1984). Since fish are typically found in connected and permanent sites in TRs (Bonada *et al.*, 2020), the fact that higher connectivity was associated with lower alpha diversity for aquatic passive species could be related with an increase in fish predation, which can significantly reduce aquatic macroinvertebrate richness (Oscoz et al., 2006). Another possible explanation is that connectivity favours the arrival of different species, thereby increasing density-dependent processes (e.g., predation, competition) and reducing the number of species (Salau *et al.*, 2012; LeCraw, Kratina & Srivastava, 2014). Furthermore, reduced connectivity may promote higher diversity by limiting dispersal, as aquatic organisms are constrained to remain in isolated habitats. In such conditions, local species may establish and coexist more effectively due to reduced competition from distant populations, despite the potential risk of dispersal limitation.

Beta diversity increased along the gradient of physicochemical conditions, characterized by elevated temperatures and higher conductivity, typically associated with summer conditions. This pattern may be linked to the presence of disconnected pools, which create heterogeneous environments that support distinct communities (Bonada *et al.*, 2020; Soria *et al.*, 2024). However, under prolonged drying conditions, as highlighted by Vander Vorste *et al.* (2021), environmental filtering tends to reduce community differentiation, leading to nestedness patterns and a decline in regional diversity. In permanent flowing waters or stable disconnected pools, broader ecological niches and more predictable conditions allow for higher species turnover and elevated beta diversity (Bonada *et al.*, 2020). In contrast, sites experiencing extended drying predominantly support generalist species capable of rapid recolonization (e.g., chironomids) (Sundermann *et al.*, 2007; Płóciennik *et al.*,

2018), resulting in greater community homogenization. Thus, while stable disconnected pools can promote diverse and distinct assemblages (Larned *et al.*, 2010), persistent drying conditions drive homogenization through stringent environmental filtering (Datry *et al.*, 2014b).

According to our results, our initial hypothesis can be accepted: fragmentation by drying in TRs prevents mass effects in aquatic macroinvertebrates at small spatial scales, leading to species sorting and dispersal limitation depending on the dispersal ability of the taxa. Network connectivity had a negligible effect on metacommunity assembly of aerial active dispersers. This could be expected given the small spatial scale of our study area and the fine spatial resolution used (with sampling sites being separated by less than 1 kilometer in some cases). Aerial active disperses include species that are able to fly over several kilometers (May & Matthews, 2008) and they can overcome dispersal barriers caused by stream drying by using perennial pools as stepping stones (Bogan & Boersma, 2012; Saura, Bodin & Fortin, 2014; Cañedo-Argüelles *et al.*, 2015). However, the combination of harsh environmental conditions imposed by drying events (Leigh & Datry, 2017; Bonada *et al.*, 2020) and certain dispersal limitations that could arise from the scarcity of perennial pools in the study area and prevent mass effects, resulted in local environmental and habitat conditions significantly affecting the metacommunity dynamics of aerial active dispersers. In the case of aquatic dispersers, hydrological connectivity emerged as a significant driver of metacommunity assembly, in spite of the small spatial scale and fine spatial grain of our study. This aligns with previous studies that showed how fragmentation by drying imposed a strong dispersal barrier for aquatic macroinvertebrates (Cañedo-Argüelles *et al.*, 2020; Pineda-Morante *et al.*, 2022; Cunillera-Montcusí *et al.*, 2023).

Considering our results we argue that metacommunity paradigms emerging from studies in perennial river and stream networks should be revisited for TRs. This is important because TRs represent the majority of the global river network (Messager *et al.*, 2021) and will become even more prevalent due to increased water demand and climate change (Döll *et al.*, 2024). Also, assessing the effect of spatiotemporal connectivity on metacommunity assembly has important implication for biodiversity conservation, e.g., species might be absent from suitable habitats due to dispersal limitations (Heino, 2013a; Tonkin *et al.*, 2018). Finally, incorporating a meta-systems perspective—extending beyond traditional

metacommunity frameworks—could offer deeper insights into landscape-scale dynamics and ecosystem functioning (Cid *et al.*, 2022). Such an integrative approach is crucial for developing sustainable management strategies in TRs.

AUTHORS' CONTRIBUTIONS

José María Fernández-Calero conceived the research ideas and led the development of the paper, including contextualization, data analysis, interpretation, writing, and editing. He also collected field data and sorted and identified macroinvertebrates. Miguel Cañedo-Argüelles contributed to data collection, research conceptualization, results interpretation, manuscript writing and editing, and secured funding. Núria Bonada supported the research conceptualization, results interpretation, manuscript writing and editing, and secured funding. Matías Arim and Ana Borthagaray led the statistical analyses and contributed to results interpretation. David Cunillera-Montcusí calculated the spatiotemporal connectivity metrics and supported results interpretation.

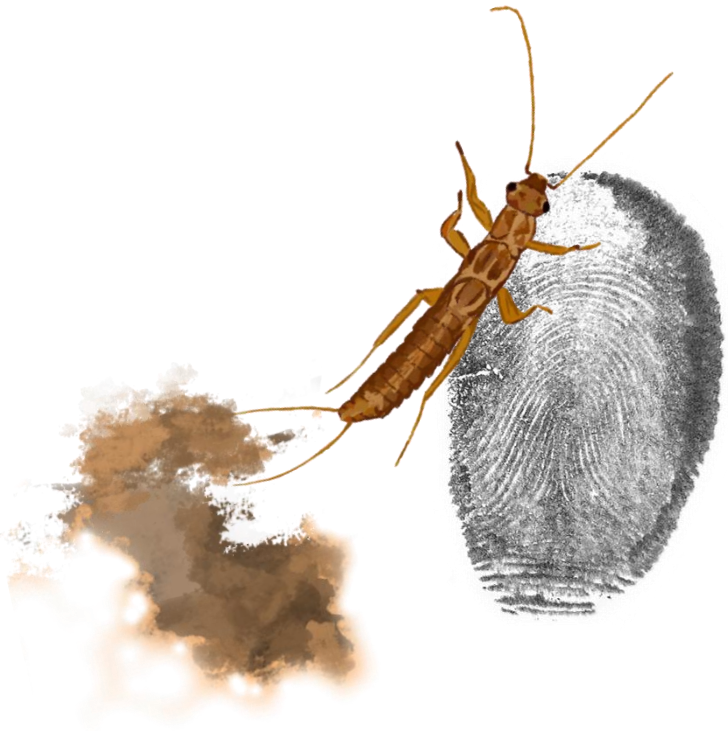
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Zig Ziglar

CHAPTER 3

DNA-Metabarcoding as a tool to assess metapopulation and metacommunity dynamics in temporary river networks

José María Fernández-Calero, Nieves López-Rodríguez, Julie Crabot, David Cunillera-Montcusí, Núria Cid, Raúl Acosta, Pau Fortuño, Maria Soria, Florian Leese, Núria Bonada & Miguel Cañedo-Argüelles



ABSTRACT

Fragmentation by drying disrupts river connectivity, influencing the exchange of genes between local populations (metapopulations) and the exchange of species between local communities (metacommunities). Thus, combining studies at the (meta)population and (meta)community level is key to understanding how river network fragmentation might shape regional biodiversity. Metabarcoding offers a powerful and cost-effective tool to do this. In this study, we first assessed how fragmentation affects the intraspecific variability within the entire community and within two selected aquatic macroinvertebrate species (i.e., the gastropod *Physella acuta* and the dragonfly *Anax imperator*) by using Exact Sequence Variants (ESVs) of the mitochondrial cytochrome c oxidase subunit I gene (COI) obtained via bulk sample metabarcoding. Then, we analyzed metacommunity variability of aquatic macroinvertebrates using metabarcoding bulk samples and comparing them with morphological identifications. We sampled seven Mediterranean temporary rivers in a small protected area near Barcelona (Spain). Then, we identified macroinvertebrates using traditional methods and subsequently employed DNA metabarcoding of bulk samples to obtain ESVs and identify taxa through clustering. Our results suggested a slight increase in site intraspecific variability per site at perennial and highly isolated locations by drying, potentially indicating that spatial and temporal isolation promotes genetic variation. Also, we found significant genetic differentiation of populations between locations for strictly aquatic species (*P. acuta*), whereas strong fliers (*A. imperator*) presented no discernable spatial pattern of intraspecific variability. Both morphological and metabarcoding data revealed similar patterns in species richness and beta diversity, suggesting that drying and spatiotemporal connectivity influence metacommunity structure independently from taxa identification approach. However, morphological data exhibited a stronger relationship with these factors. Overall, our results highlight the value of metabarcoding as a tool for jointly assessing metapopulations and metacommunities, providing valuable information for biodiversity conservation at the regional scale.

Keywords: Bulk sample, drying, intraspecific variability, isolation, macroinvertebrates, spatiotemporal connectivity.

INTRODUCTION

Habitat fragmentation, a major driver of ecological and evolutionary changes, is also a leading cause of species loss and ecosystem degradation (Crooks *et al.,* 2017, Brauer & Beheregaray 2020, Gauthier *et al.,* 2021, Kuipers *et al.,* 2021). Fragmentation disrupts natural habitat connectivity, hindering organism dispersal across their overall range and the specific pathways they use to move (Pardini *et al.,* 2018, Horváth *et al.,* 2019). This limits gene flow between populations, exacerbating their vulnerability to environmental changes and diseases, thereby increasing the risk of local extinction (Dixo *et al.,* 2009, Harrisson *et al.,* 2012, Wright *et al.,* 2015). Overall, fragmentation influences the exchange of genes between local populations (i.e., metapopulations) and the exchange of species between local communities (i.e., metacommunities), affecting biodiversity at the network scale (Vellend 2010, Erős & Campbell Grant 2015).

In the context of river networks, fragmentation can shape biological communities by disrupting the natural flow of water and sediments, affecting ecological processes and reducing connectivity between upstream and downstream habitats (Erős & Campbell Grant 2015, Sofi *et al.,* 2023). River fragmentation arises when the dendritic structure of the river network is altered either artificially (e.g., dams or barriers) or naturally (e.g., drying reaches) with the concomitant impact on overall connectivity (Fuller *et al.,* 2015, Rodeles *et al.,* 2021, Baldan *et al.,* 2023). Furthermore, natural disturbances such as wildfires or floods can also significantly change river structure and favor the connection or isolation of existing habitats (Richards *et al.,* 2002, Verkaik *et al.,* 2013).

In a world where river networks are increasingly fragmented by dams and by drying, studying consequences on both metapopulation and metacommunity level and deriving suitable biological conservation strategies for maximum benefit is key, especially for temporary rivers (TRs) (Heino *et al.,* 2015, Datry *et al.,* 2017b, Fernández-Calero *et al.,* 2024). TRs are highly dynamic ecosystems with strong spatial and temporal variability, alternating between flowing, disconnected pool and dry phases (Gallart *et al.,* 2012, Bonada *et al.,* 2024). These contrasting phases are also reflected in their biological communities, strongly influenced by changes in local environmental conditions and dispersal (Sousa-Santos *et al.,* 2014, Tonkin *et al.,* 2018, Cunillera-Montcusí *et al.,* 2023). Consequently, TRs often harbor

diverse and specialized species with a wide range of dispersal modes and capacities to cope with these fluctuations (Sarremejane *et al.,* 2017, Cañedo-Argüelles *et al.,* 2020, Pineda-Morante *et al.,* 2022, Fernández-Calero *et al.,* 2022). For instance, winged insects such as dragonflies can exhibit significant long-distance dispersal, colonizing TRs across the landscape (Sarremejane *et al.,* 2020a), whereas flightless invertebrates such as some crustaceans or snails rely on passive dispersal leading to more localized movements (Kappes & Haase 2012, Weiss & Leese 2016). Together with habitat fragmentation, these differences in dispersal abilities can lead to genetic differentiation among populations. For example, Phillipsen *et al.,* (2015) found that flightless species *Abedus herberti* exhibited population differentiation attributed to genetic drift, while *Mesocapnia arizonensis*, a specialist adapted to TRs, showed an isolation-by-distance pattern indicative of its limited flying range. At the same time, the relative importance of environmental and spatial factors for metacommunity assembly largely depends on the dispersal abilities of the species (Cañedo-Argüelles *et al.,* 2015).

Traditional methods based on morphological taxonomy, while informative, may be incomplete for capturing diversity trends linked to drying in TRs due to the usually incomplete biodiversity estimates due to taxonomic limitations (e.g., Finn *et al.,* 2006, Blackman *et al.,* 2021). In contrast, the development of molecular techniques (barcoding, eDNA metabarcoding, microsatellites) offer greater efficiency and scalability for studying TRs biodiversity, potentially connecting metapopulation and metacommunity dynamics (Deiner *et al.,* 2016, Laini *et al.,* 2020, 2023, Blackman *et al.,* 2021). Among these, metabarcoding has emerged as a powerful tool for studying community composition and dynamics due to its ability to detect a wide range of taxa at lower taxonomic levels (Bertrand *et al.,* 2006, Vilmi *et al.,* 2016, Elbrecht *et al.,* 2017a), all while being time and cost-effective (Pawlowski *et al.,* 2018). In the context of metacommunity dynamics, metabarcoding enables the analysis of entire communities within river systems, allowing for the detection of shifts in biodiversity due to environmental changes, pollution, or habitat fragmentation (Ruppert *et al.,* 2019). For instance, metabarcoding can reveal changes in the community structure of macroinvertebrates (Laini *et al.,* 2020) by detecting a broader range of species compared to traditional taxonomy including the introduction of invasive species (Blackman *et al.,* 2022). For metapopulations, the use of high-resolution markers such as microsatellites or single-nucleotide polymorphisms (SNPs) has proven effective in examining genetic intraspecific

variability structures in species with varying dispersal abilities in landscapes (Phillipsen *et al.,* 2015, Weiss & Leese 2016, Weiss *et al.,* 2022). However, also with metabarcoding approaches it is possible to study intraspecific genetic variation based on the barcoding marker that is analyzed, i.e., so called Exact Sequence Variants (ESVs) (Elbrecht *et al.,* 2018, Zizka *et al.,* 2020). Furthermore, metabarcoding can also be used to regularly monitor the presence and abundance of specific taxa within and across river fragments, or detect local extinctions and recolonizations (Zizka *et al.,* 2020). This is particularly valuable for taxa that are difficult to detect using traditional methods, such as cryptic species, populations with early stages of development, species that are difficult to identify morphologically, or those that are hard to capture (Elbrecht *et al.,* 2017a, Kuntke *et al.,* 2020). Despite the growing use of molecular techniques, few studies have combined traditional and molecular techniques so far in the study of metacommunity dynamics, which can provide complementary information at both metacommunity and metapopulation levels (Phillipsen *et al.,* 2015, Elbrecht *et al.,* 2018, Laini *et al.,* 2020, 2023).

In this study, we used TRs located in a small protected area in northeastern Spain as a model system to assess the effects of fragmentation on aquatic metapopulation and metacommunity structure. First, we assessed the intraspecific variability at both the entire community level and for two macroinvertebrate species considering drying and spatiotemporal connectivity gradients as explanatory variables. The two macroinvertebrate species had contrasted dispersal abilities: *Anax imperator* Leach, 1815 (i.e., a large dragonfly with a strong aerial active dispersal capacity; (Minot *et al.,* 2021) and *Physella acuta* (Draparnaud, 1805) (i.e., a strictly aquatic pulmonate snail with an aquatic passive dispersal mode; Tachet *et al.,* 2010). Second, we compared the effectiveness of morphological and metabarcoding approaches in capturing metacommunity dynamics in TRs at a small spatial scale. We posed two hypotheses regarding intraspecific variability at the community level (H1 & H2), intraspecific variability across space between the two invertebrate species (H3), and considering overall diversity and composition comparison (H4 & H5). These hypotheses are outlined below:

(H1) Drying and spatiotemporal connectivity gradients are expected to have minimal influence on intraspecific variability at the site level, as all sites are likely to experience low

dispersal limitation due to the limited spatial scale of our study (but we acknowledge its potential; see Heino *et al.,* (2015)).

(H2) Genetic richness based on ESVs differs between aquatic passive and aerial active species, with passive dispersers exhibiting higher ESV richness values than aerial active species due to their more limited dispersal capabilities. In addition, we expect greater discrepancies between ESV richness and DNA-metabarcoding-based richness in aquatic passive dispersers than in aerial active dispersers.

(H3) Populations of the aquatic snail *P. acuta* show a higher genetic differentiation between populations and are therefore more spatially structured than populations of the dragonfly *A. imperator* (May & Matthews 2008, Kappes & Haase 2012, Minot *et al.,* 2021).

(H4) At community level, alpha diversity estimates increase with reduced drying and greater spatiotemporal connectivity gradients, for both morphological and metabarcoding identifications techniques (Datry *et al.,* 2014b, Schriever *et al.,* 2015, Anton-Pardo *et al.,* 2019).

(H5) Dissimilarity (i.e., beta diversity) estimates between communities inferred using morphological identification and metabarcoding are affected by hydrological connectivity, while we do not expect a significant effect of the distance decay relationship (Cañedo-Argüelles *et al.,* 2015, Sarremejane *et al.,* 2020b, Laini *et al.,* 2020).

However, we anticipate metabarcoding to provide more robust and comprehensive results compared to morphological assessments for H4 and H5. The accuracy of the models is demonstrated specifically by higher R^2 values, which we expect to indicate better explanatory power and greater stability of metabarcoding technique.

METHODS

Study area

The study focused on seven headwater TRs from the Llobregat and Besòs river basins, located in the *Sant Llorenç del Munt i l'Obac* Natural Park (Catalonia, NE Spain) and distributed with an area of 93.3 km^2 (Bonada *et al.,* 2007b, Fernández-Calero *et al.,* 2024) (Figure 1). We sampled four sites in Talamanca, Santa Creu, Mura, Rellinars, and Castelló rivers each, and five sites in Sanana and Vall d'Horta rivers each (Figure 1). The Mediterranean climate and karstic geology of the area results in high seasonal and inter-annual variations in precipitation and flow (Bonada & Resh 2013, Cid *et al.,* 2017). During the sampling period (May-July 2019),

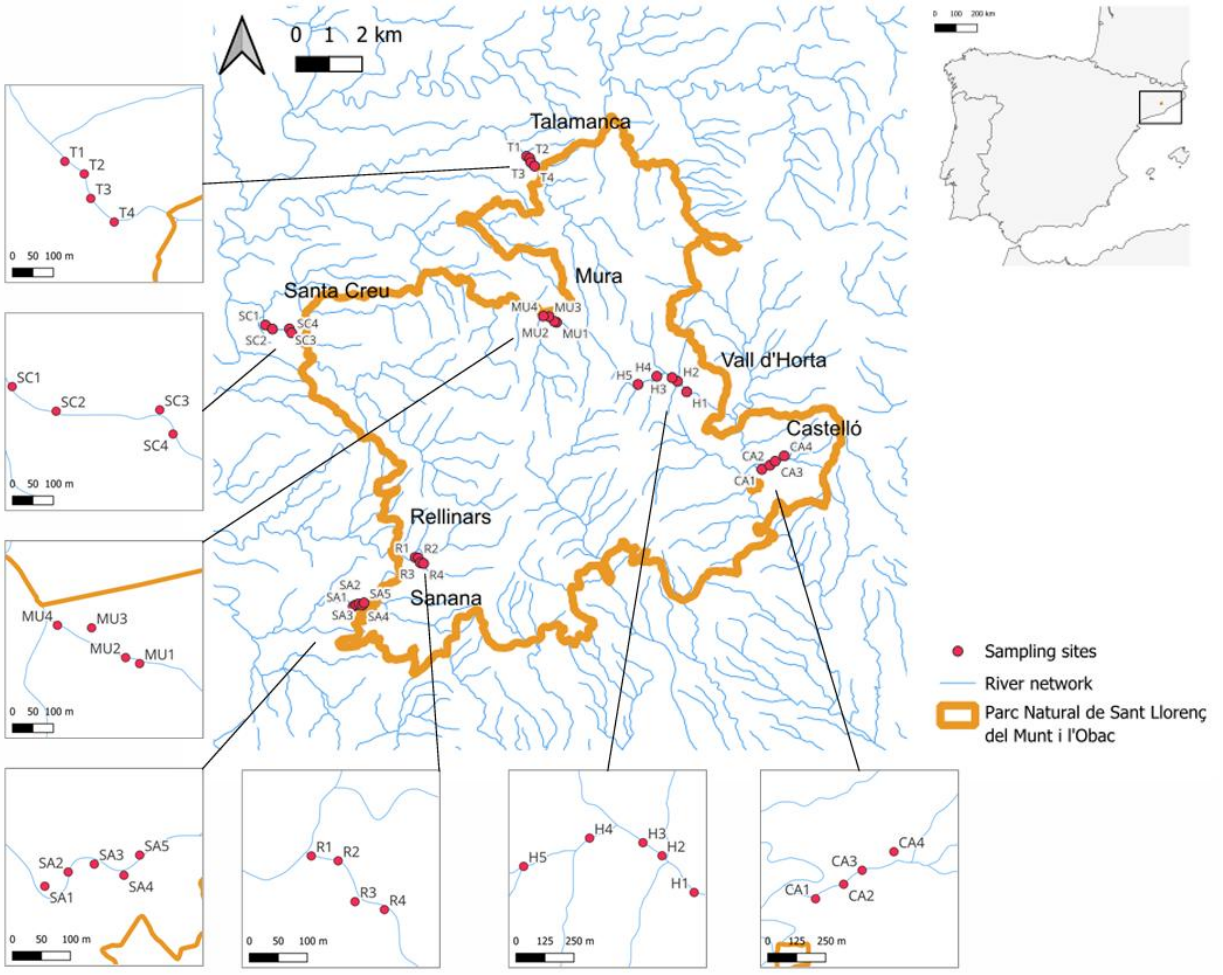


Figure 1. Study area of the 7 headwater temporary rivers sampled in *Sant Llorenç del Munt i l'Obac* Natural Park (Catalonia, Spain). Inset (top right) shows the park's location within the Iberian Peninsula. Detailed views depict each sampled river, with red dots marking sampling points.

the study sites typically transition through different aquatic phases: flow, disconnected pools and dry riverbeds (Bonada *et al.,* 2007b).

Morphological and metabarcoding macroinvertebrate data

We sampled macroinvertebrates from a total of 25 sites in spring (May 2019) and 21 sites in summer (July 2019). Dry sites were excluded from sampling, with 5 dry sites in spring and 9 in summer. We used a 250 µm hand net and sampled all available habitats along a 100 meters reach. We preserved samples with 96% ethanol and then stored it in the freezer until their sorting and taxonomic identification in the lab. We used a binocular stereoscope to identify macroinvertebrates at the lowest possible taxonomic resolution, usually genus.

For eDNA metabarcoding analysis, we opted to use bulk sample DNA from macroinvertebrates rather than directly from water or sediment. This approach can be particularly useful when focusing on specific groups of organisms, as it minimizes the background noise associated with eDNA from other organisms (Hajibabaei *et al.,* 2019, Gleason *et al.,* 2021). To complement the analysis of metabarcoding-based richness, we employed ESVs for a more accurate assessment of the true sequence diversity within our samples, which are comparable (but not always identical) to haplotypes in population genetics (Callahan *et al.,* 2017). We used a few specimens of each taxon found in the macroinvertebrate samples for DNA metabarcoding, including tissue fragments for large individuals (i.e., Odonata, Heteroptera) or whole individuals for the remaining and smaller taxa (e.g., Chironomidae, Baetidae). We then dried the samples to remove ethanol, extracted DNA using PowerSoil DNA extraction kits (PowerSoil, Qiagen) and amplified it using LERAY_XT primers (Leray *et al.,* 2013, Wangensteen *et al.,* 2018) with 8-base sample tags (Forward-miCOIint-XT:GGWAACWRGWRGRACWITITAYCCYCC; Reverse-jgHCO2198:TAIACYTCIGGRTGICCRAARAAYCA). The comparatively long amplicon sequence generated by LERAY_XT primers can improve taxonomic assignment at species level (Collins *et al.,* 2019). After PCR, we purified amplicons and pooled them using Minelute PCR purification (Wangensteen *et al.,* 2018) and ligated Illumina adapters. For sequencing, we used the Illumina MiSeq platform (and the 600 cycle kit, i.e., 2x300 by paired-end sequencing) and analyzed the data using the APSCALE_gui pipeline (Buchner *et al.,* 2022) which utilizes

VSEARCH (Rognes *et al.,* 2016), Cutadapt (Martin 2011) and LULU (Frøslev *et al.,* 2017). To assign taxonomy to the obtained OTUs and ESVs, we utilized the BOLDigger software v4 (Buchner & Leese 2020) by comparing our OTUs to BOLD (Ratnasingham & Hebert 2007) within this software. For statistical analysis of the metabarcoding data we used the software TaxonTableTools (Macher *et al.,* 2021). First, we subtracted reads found per OTU/ESV in the negative controls from all other samples individually (per column). For taxonomic classification of OTUs and ESVs, we adopted a conservative approach, considering only those with at least 85% similarity to reference sequences in BOLD database. OTUs with a similarity of ≥ 90% were assigned to family level, ≥ 95% to genus level and ≥ 98% to species level (Elbrecht *et al.,* 2017a). Finally, to obtain the most detailed picture of species richness within our samples, we defined and analyzed Exact Sequence Variants (ESVs), as they represent unique sequence variants, differing by even a single nucleotide. We used these ESVs for taxonomic assignments up to the species level to estimate detected species richness. In addition, some previous studies have demonstrated that the use of metabarcoding through ESVs can facilitate the detection of true haplotypes, showing applications for population genetics (Serrana & Watanabe 2023). Therefore, we used ESVs from our two selected species (*P. acuta* and *A. imperator*) to assess their intraspecific variability.

Dispersal trait data

We used fuzzy coding from Tachet *et al.* (2010) and Sarremejane *et al*. (2020a) to classify taxa into four dispersal categories: aerial active, aerial passive, aquatic active, and aquatic passive. We assigned each taxon to the dispersal category with the highest coding value (i.e., from 0 = no affinity to 3 = maximum affinity). If a given taxon could be equally assigned to more than one category, it was assigned to the strong dispersal category (i.e., aerial active > aerial passive > aquatic active > aquatic passive). The reason for this assignment is that an organism that is capable of dispersing in both, “aerial/aquatic” and “active/passive” modes, has a greater potential to colonize new sites through active and aerial dispersal than passive and aquatic dispersal. Taxa that were not included in Tachet *et al.* (2010) and Sarremejane *et al*. (2020a) were assigned to the closest taxonomic level (e.g., all genera of Psychodidae were coded to the family level). For Chironomidae, while the database from Serra *et al*. (2016) offered valuable genus-level information, our morphological identifications reached only the

subfamily or tribe level. To address this limitation, we calculated the mode of each trait of all the taxa included within each category (subfamily or tribe) and then assigned it to our respective subfamilies or tribes. In cases of ties, assignments were made based on expert judgment. We generated two dispersal datasets, one for the taxa list obtained with traditional methods based on morphology, and a second one for the taxa list obtained with metabarcoding. For the metabarcoding analysis, we focused solely on taxa identified at the genus or species level to ensure accurate association of ecological traits with the identified organisms. No trait information for aquatic mites (Hydrachnidia) was available, therefore we removed this taxon from our trait analysis.

Local drying and spatiotemporal connectivity

We assessed the impact of drying on river network connectivity for each sampling site using the framework proposed in Cunillera-Montcusí *et al.* (2023), which resulted in two local drying metrics (TotNum and TotDur) and two regional spatiotemporal connectivity metrics (DirBin and DirWei). To do so, we first monitored the studied rivers using temperature data loggers that were used to obtain daily information on the aquatic states (i.e., wet or dry) for each sampling site (Pineda-Morante *et al.,* 2022, Cunillera-Montcusí *et al.,* 2023). Monitoring of the rivers began in July 2018 and continued for an entire hydrological cycle, encompassing both spring (May 2019, 298 days) and summer (July 2019, 348 days) sampling campaigns. We used the daily recordings of wet/dry state for each site to calculate local drying metrics and the spatiotemporal connectivity between sites. As local drying metrics, we calculated the number of dry periods (TotNum), and the number of dry days (TotDur) for each site. As spatiotemporal connectivity metrics, we calculated the *STcon* and *STconmat* indices (Cunillera-Montcusí *et al.,* 2023). *STcon* summarizes how often each site has been connected to all its potential neighbors and *STconmat* refers to the same connectivity but between each pair of sites. We considered the entire monitored period until each sampling date for calculating all these hydrological metrics.

Following Cunillera-Montcusí *et al.* (2023), we defined directed connectivity scenarios where sites were connected following the watercourse direction from upstream to downstream. Furthermore, we considered two different ways of weighting connections

between sites: 1) Binary (i.e., connections are considered only as 1 or 0, 1 for connected and 0 for not connected); and 2) Weighted (i.e., connections are weighted by the network distance between sites). Binary scenarios quantify the number of times that each site has been connected to its neighbors with higher values meaning higher spatiotemporal connectivity. On the other hand, for the weighted scenarios we assumed that the presence of water between two sites was facilitating dispersal. Consequently, when connection was possible the distance between two sites was reduced by 90% and when connection was not possible the distance between these two sites was preserved. Therefore, higher values for weighted scenarios implied a greater resistance to dispersal between pairs of reaches (i.e., drying frequently and being far from each other). We combined connectivity weights to obtain two connectivity scenarios from which to calculate *STcon* and *STconmat*: Directed Binary (hereafter DirBin) and Directed Weighted (hereafter DirWei). The *STcon* and *STconmat* values for each scenario were considered in the following analyses as independent variables providing different information about drying impacts on fluvial connectivity.

Statistical analysis

We tested the correlation between hydrological variables (TotNum and TotDur) using the function "findCorrelation" from "caret" R package (Kuhn 2008). We used a Spearman correlation cut-off of 0.75. We then discarded the variable with the higher mean absolute correlation (TotNum) to avoid collinearity.

Site intraspecific variability and genetic structure of metapopulations

We first tested the influence of drying and spatiotemporal connectivity on intraspecific variability at the site level (H1). To do that, we created a metric by dividing the ESV richness of a site by the DNA-metabarcoding-based richness, which we refer to as the mean site intraspecific variability (hereafter "site intraspecific variability"). We used this metric as a response variable in generalized linear mixed models (see next section). To examine whether the dispersal mode could influence genetic richness (DNA-metabarcoding-based and ESVs richness) (H2) we compared different dispersal group (i.e., aerial active, aerial passive, aquatic active and aquatic passive) by visually comparing DNA-metabarcoding-based richness and

ESV richness values in order to compare species and haplotypes and between the whole community dispersal groups. To assess the genetic structure of the 2 studied species across the study area (H3), we performed a Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson 2001) on *P. acuta* (an aquatic passive disperser) and *A. imperator* (an aerial active disperser), using the function "adonis2" of the "vegan" package (Oksanen *et al.,* 2022) to test whether their ESV richness differed between rivers. These species were chosen to represent the two dispersal groups because they had several ESV and sufficient frequency across sites to compute this analysis. As PERMANOVA can show misleading significant results if ESV richness has high within-rivers variation (dispersion) but low across-rivers differences, PERMANOVA was validated through a permutation test of multivariate homogeneity of groups dispersions (PERMDISP) (Anderson *et al.,* 2006) using the "betadisp" function of the "vegan" package (Oksanen *et al.,* 2022). Variation in ESV richness was calculated using the Jaccard dissimilarity for presence-absence data, and significance was assessed using 999 permutations.

Metacommunities

We calculated morphological richness and metabarcoding-based species richness as our response variables using the function "diversity" from the "vegan" package (Oksanen *et al.,* 2022). To assess if macroinvertebrate richness increases along gradients of drying and network connectivity (H4) we used generalized linear mixed models (GLMMs) to assess changes in richness (following a Poisson error distribution) and in site intraspecific variability (using a Gamma distribution) (see previous section) along gradients of drying duration (TotDur) and spatiotemporal connectivity (DirBin and DirWei). First, we included in all models sites nested in rivers as our random factor structure. Then, we selected the best structure of fixed factors, with all possible interactions among the response variables, and we built the best model using the Akaike Information Criterion corrected for a small sample size (AICc) (Burnham & Anderson 2002). Afterwards, we selected between the models with lower AICc values (ΔAICc < 2). Two coefficients of determination were reported to measure the accuracy of the models: the marginal (R^2m) and conditional (R^2c), which respectively describe the proportion of variance explained by fixed effects, and by fixed and random effects combined (i.e., the total model) (Nakagawa & Schielzeth 2013). The coefficients of determination were

calculated using the trigamma function as recommended by Nakagawa *et al.* (2017). Effects were identified by a significant (p value < 0.05) or potential (0.05 < p value < 0.25). The "glmer" function in the "lme4" package (Bates *et al.,* 2015) was used to perform the GLMM analyses.

We used Multiple Regression on distance Matrices (MRM; (Manly 1986, Smouse *et al.,* 1986)) to assess how beta diversity (i.e., Jaccard dissimilarity) varied along gradients of geographic distance, drying duration (TotDur), and spatiotemporal connectivity (*STconmat*, including both the binary and weighted connections) (H5). Geographic distances were calculated using the "dist" function in R, based on the geographic coordinates (longitude and latitude) of the sampling sites. MRM is an extension of partial Mantel tests that allows modeling non-linear or nonparametric relationship between the response distance matrix, here the beta diversity, and explanatory distance matrices such as geographic or environmental distances (Legendre *et al.,* 1994, Lichstein 2007). We then ran the MRM analysis with the "MRM" function of the "ecodist" package (Goslee & Urban 2007). Models were computed separately for each season (spring and summer) and each method (morphological and metabarcoding data), resulting in 4 models in total. RStudio 4.3.0 was used for all the analyses (R Core Team 2024).

RESULTS

We identified a total of 148 taxa morphologically. Taxa richness varied across samples, ranging from 15 to 62, with an average of 37 per sample. We obtained a total of 2,838,849 reads for the 46 metabarcoding samples after filtering. We excluded 6 samples that had no reads. The rest of the samples showed an uneven distribution of reads, ranging from 540 to 367,918 with an average of 69,240.22 reads. We included three negative controls to test for possible cross-contamination in the laboratory. Negative-control read numbers ranged from 10 to 451 with a mean of 186.3 reads per sample. The majority of these negative control reads was assigned to Actinopterygii and *Paracentrotus lividus* (species being studied in the same laboratory by other research groups). We identified 918 ESVs according to metabarcoding analyses. After removing bacteria, fungi and meiofauna, we kept 564 ESVs belonging to aquatic macroinvertebrate taxa. ESV richness per site ranged between 5 and 118, with an average of 45 per site. Following the taxonomic assignment of the ESVs into taxonomic

categories, we obtained 209 DNA-metabarcoding-based taxa, ranging from 3 to 55 taxa per site, with an average of 26.

Site intraspecific variability and spatial genetic structure of metapopulations

Site intraspecific variability did not vary strongly along drying and spatiotemporal connectivity gradients (Table 1). However, trends were found for drying (TotDur) and dispersal resistance (DirWei), with greater site intraspecific variability in more permanent and spatiotemporally isolated sites (Figure 2).

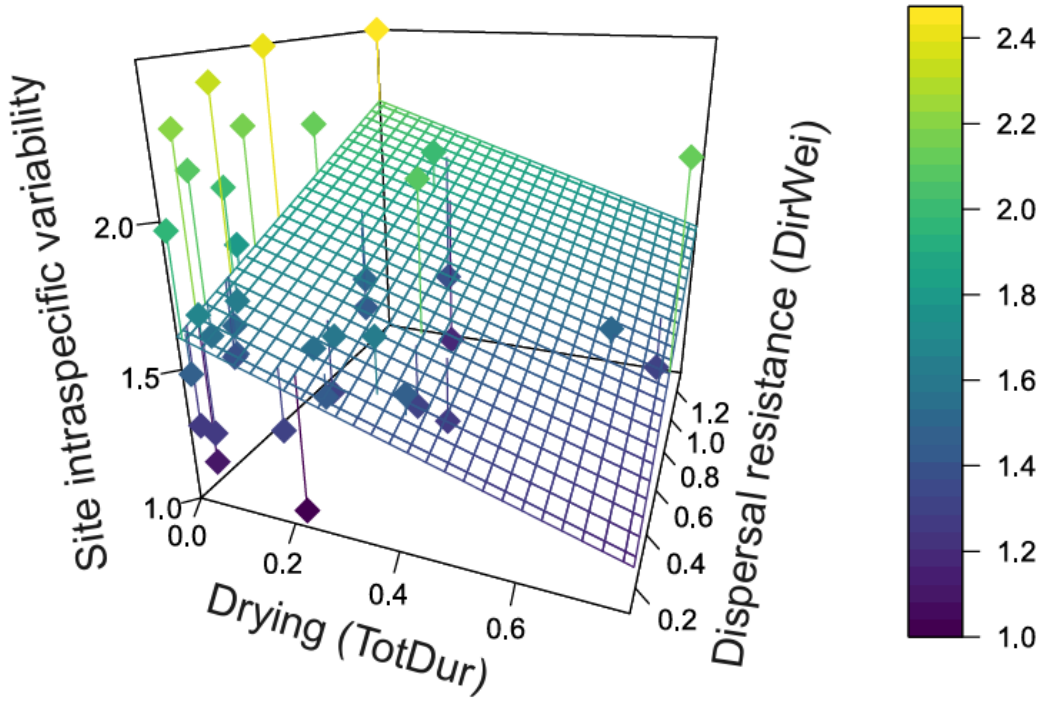


Figure 2. Relationship between site intraspecific variability and selected hydrological variables for the best-fitted GLMMs: frequency of dry days (TotDur) and dispersal resistance (DirWei). The color gradient represents site intraspecific variability, with yellow tones indicating higher values and cooler tones indicating lower values. One outlier "MU3 spring sample" was removed to enhance the visualization of the main data trends. The exclusion was based on its extreme deviation from the central data distribution. The original plot (including MU3 spring) can be found in Supporting Information.

A total of 338 ESV (60 %) and 163 DNA-metabarcoding-based taxa (78 %) could be assigned to species or genus and, therefore, associated with dispersal traits. Aerial active was the most common dispersal group in our rivers (66% and 75% of the DNA-metabarcoding-based taxa and ESV, respectively), followed by aquatic passive (16% and 12%). The difference between DNA-metabarcoding-based richness and ESV richness was higher for aerial active than for aquatic passive dispersers (Figure 3).

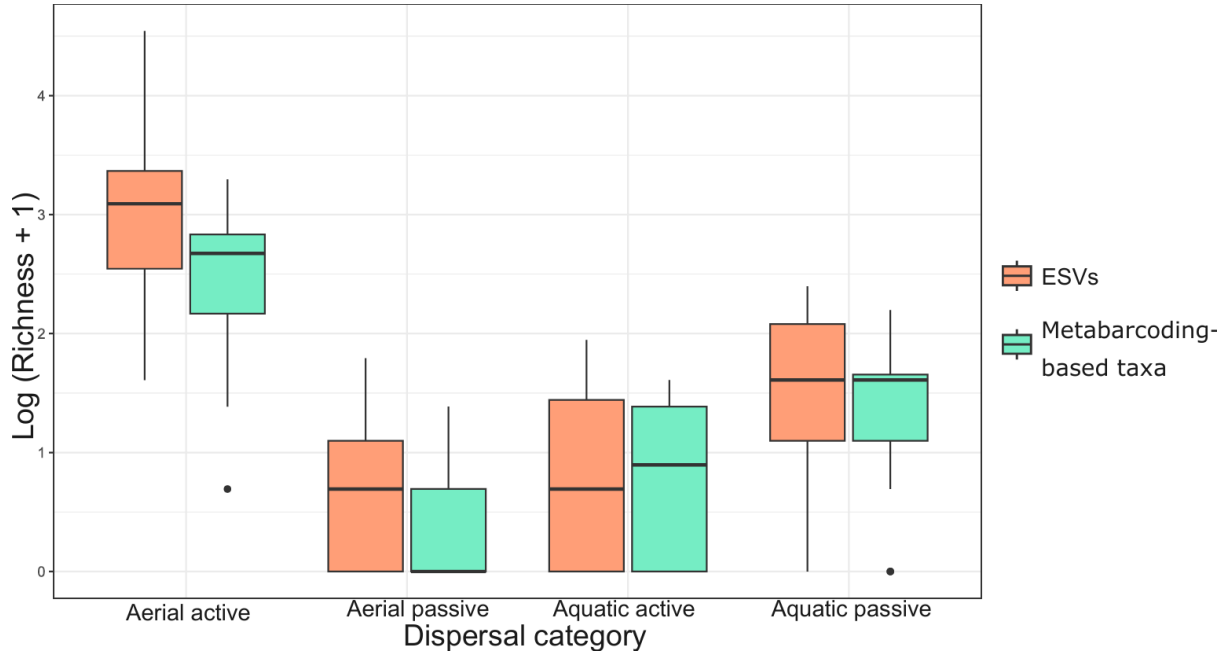


Figure 3. Boxplot of log-transformed richness for dispersal groups using DNA-metabarcoding-based and ESV approaches.

The distribution of ESVs significantly differed between rivers for *P. acuta* (PERMANOVA: R^2 = 0.74, F = 5.77, p = 0.001; PERMDISP test: F = 3.19, p = 0.018) with the different ESVs mostly restricted to one or two rivers (Figure 4a). Contrarily, ESVs of *A. imperator* did not show a strong spatial differentiation (PERMANOVA: R^2 = 0.42, F = 0.89, p = 0.581; PERMDISP: F = 5.55, p = 0.176) (Figure 4b).

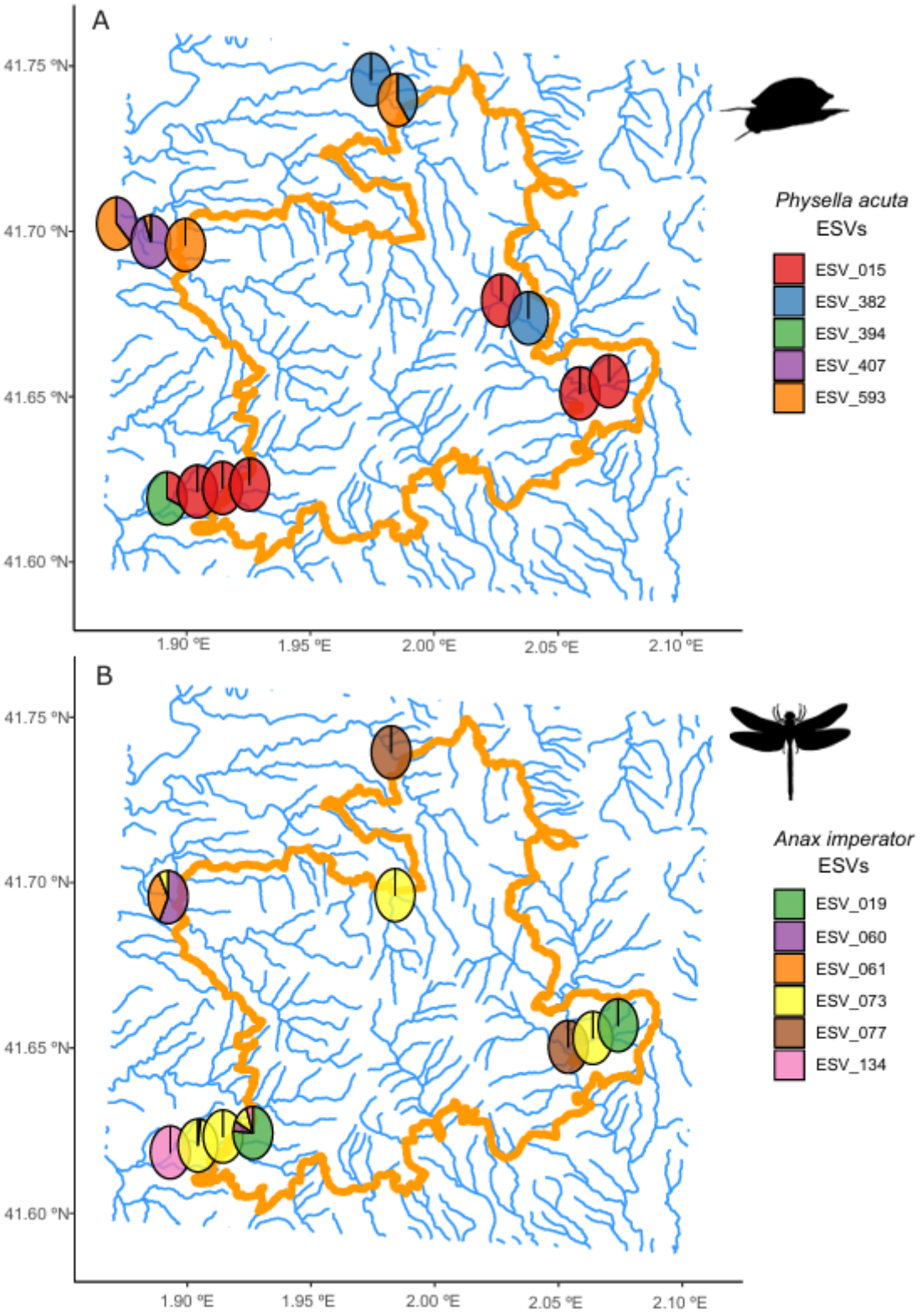


Figure 4. Maps showing distribution of the different haplotypes (i.e., ESVs) across sites for populations of *Physella acuta* **(A)** and *Anax imperator* **(B)**.

Metacommunities

When analyzing the match between morphological and metabarcoding identification at the genus level we observed a 45% overlap, with 24% and 31 % exclusively identified through morphology and metabarcoding, respectively (Figure 5). Genera only identified through morphology were predominantly non-Chironomidae Diptera, followed by Coleoptera, and Trichoptera. Conversely, the genera identified through metabarcoding that had not been morphologically identified were primarily non-biting midges (i.e., Chironomidae), oligochaetes, and water mites (Trombidiformes) (Figure 5. A taxa list for both methods is provided in the Supporting Information). Chironomidae, oligochaetes, and aquatic mites were assigned to species (26%, 52%, 38%) and genus (20%, 26%, 24%), respectively. Only two of the genera exclusively identified through morphology (*Cyphon* and *Satchelliella*) were absent from the BOLD System.

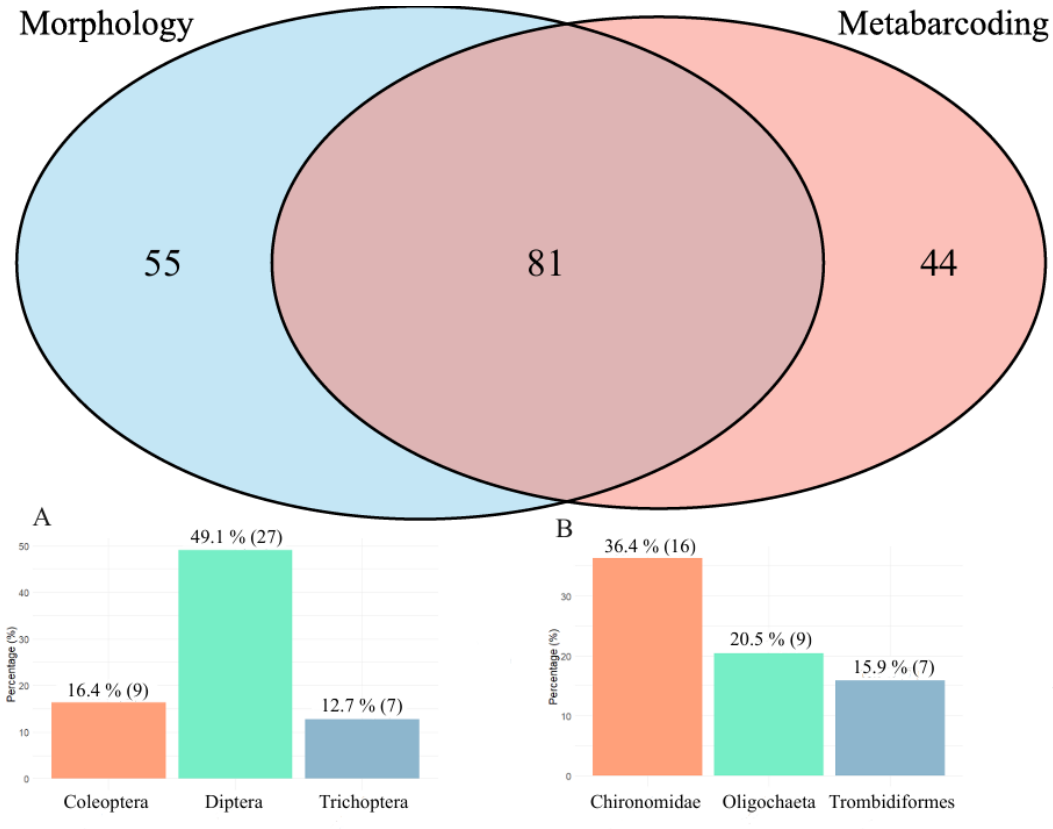


Figure 5. Venn diagram illustrating the overlap between morphological and metabarcoding identification at the genus level. Barplots displaying the three primary groups exclusively identified in **A)** morphology and **B)** metabarcoding. Diptera identified only by morphology correspond to non-chironomid Diptera.

We found that TotDur was the main driver of taxa richness. TotDur consistently emerged as a significant predictor across all models, regardless of whether the approach was morphological or metabarcoding (Table 1). The interaction between TotDur and DirBin provided the best-fitted model to explain morphological richness, and the interaction between TotDur and DirWei to explain DNA-metabarcoding-based richness (Table 1). In both cases, richness was higher at perennial and spatiotemporally connected sites (Figure 6) (the relationship between morphologic and DNA-metabarcoding-based richness and the three predictor variables can be found in the Supporting Information). Models that assessed changes in richness along drying (TotDur) and spatiotemporal connectivity gradients (DirBin) were stronger for morphological richness than for DNA-metabarcoding-based richness (Table 1).

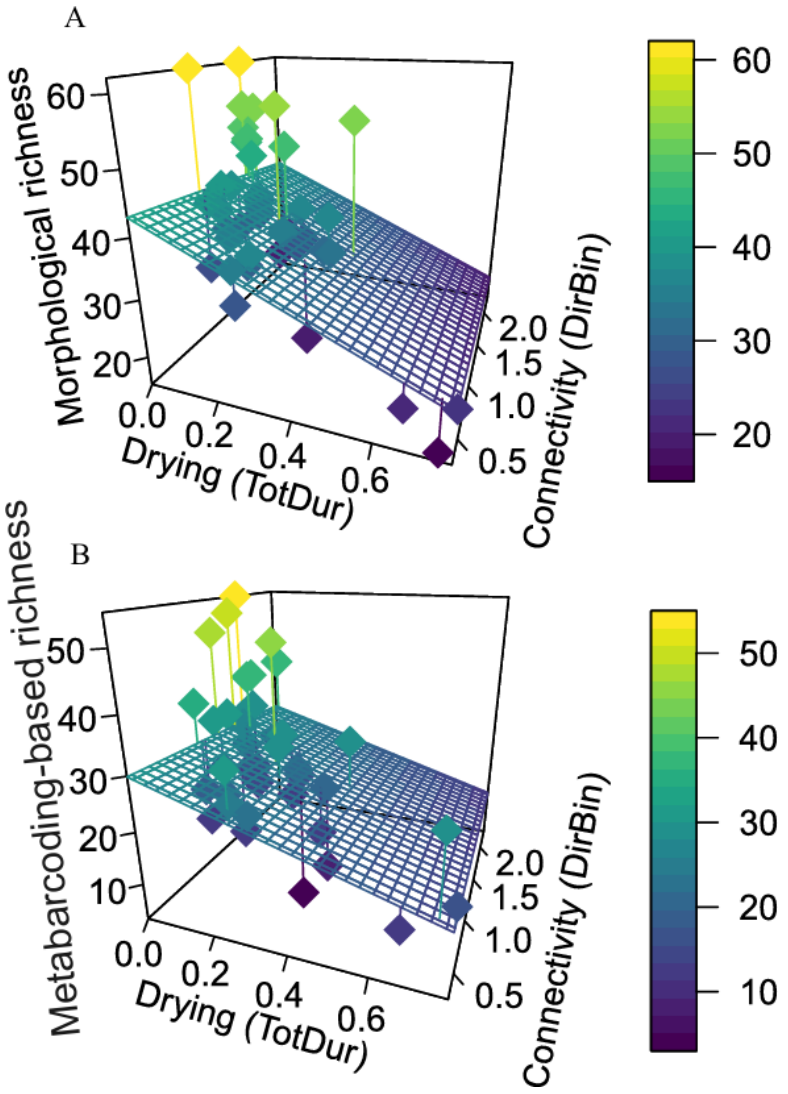


Figure 6. Relationship between morphologic and DNA-metabarcoding-based richness and predictor variables. **A)** Relationship between morphological taxa richness, frequency of days in which the stream site was dry (TotDur), and spatiotemporal connectivity (DirBin); **B)** relationship between DNA-metabarcoding-based richness, frequency of days in which the stream site was dry (TotDur), and spatiotemporal connectivity (DirBin). The color gradient represents richness, with yellow tones indicating higher richness and cooler tones indicating lower richness.

Table 1. GLMM results on site intraspecific variability, morphological and DNA-metabarcoding-based richness with predictor variables. We show the best fitted models according to ΔAICc < 2. R2m and R2c correspond to the marginal and conditional coefficients of determination respectively.

	Models	Predictors	Estimate	Std. Error	p value	R^2m	R^2c	AICc
Site Intraspecific variability	TotDur	TotDur	-0.421	0.295	0.153	0.088	0.49	48.54
	TotDur+DirWei	TotDur	-0.498	0.282	0.077.	0.13	0.49	49.85
		DirWei	0.225	0.179	0.209			
	DirWei	DirWei	0.148	0.201	0.462	0.02	0.46	49.97
Morphology	DirBin*DirWei	DirBin	-0.3	0.2	0.127	0.344	0.7	309.55
		DirWei	-1.109	0.396	0.005**			
		DirBin:DirWei	1.078	0.312	0.0005***			
	TotDur	TotDur	-0.68	0.29	0.019*	0.219	0.706	309.95
	TotDur*DirBin	TotDur	-1.538	0.516	0.002**	0.36	0.732	311.13
		DirBin	-0.12	0.146	0.41			
		TotDur:DirBin	1.035	0.494	0.036*			
Metabarcoding	TotDur	TotDur	-0.763	0.098	0.04*	0.14	0.79	351.13
	TotDur*DirWei	TotDur	-2.424	0.918	0.008**	0.284	0.798	351.81
		DirWei	-0.025	0.274	0.925			
		TotDur:DirWei	2.482	1.331	0.062.			
	TotDur+DirWei	TotDur	-0.841	0.362	0.02*	0.168	0.791	352.33
		DirWei	0.289	0.233	0.21			

Drivers of beta diversity based on morphology and on metabarcoding varied between seasons. Overall, morphological beta diversity showed a stronger response to drying and spatiotemporal connectivity distances than metabarcoding dissimilarity (Table 2). The MRMs that included only spring data explained the highest percentage of beta diversity for each methodology (56% and 22% for morphology and metabarcoding, respectively). Significant distance decay relationships were found for geographic distance across different seasons and approaches (Table 2). The results indicated that pairs of sites with higher distances in spatiotemporal connectivity (DirBin) exhibited lower beta diversity, whereas higher dissimilarity in terms of drying duration (TotDur) and dispersal resistance (DirWei) were associated with higher beta diversity (Table 2). On one hand, these results indicated that more spatiotemporally connected pairs of sites (i.e., high DirBin *STconmat* values) had more similar communities. On the other hand, more dry and isolated sites (i.e., high TotDur and DirWei *STconmat* values) presented markedly different communities with respect to their permanent and connected counterparts (Table 2).

Table 2. MRM results on Jaccard dissimilarity of morphological and metabarcoding. Relationships were assessed separately across network structures and seasons.

		Global model		Geographic distances		Connectivity (DirBin)		Dispersal resistance (DirWei)		Drying (TotDur)	
		R^2	p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value
Morphology	Spring	0.56	0.01**	0.44	0.01**	-0.10	0.01**	0.06	0.02*	0.15	0.02*
	Summer	0.17	0.03*	0.25	0.01**	-0.04	0.170	-0.01	0.600	-0.01	0.810
Metabarcoding	Spring	0.22	0.01**	0.22	0.01**	0.01	0.200	0.00	0.720	-0.12	0.02*
	Summer	0.12	0.03*	0.14	0.01**	0.01	0.410	0.00	0.530	12.58	0.03*

DISCUSSION

Intraspecific variability and genetic richness in relation to river network drying

As hypothesized (H1), site intraspecific variability did not respond strongly to local hydrological conditions or spatiotemporal connectivity. This is likely due to the small spatial scale of our study and the similar environmental conditions (i.e., geological and physico-chemical conditions) of our rivers (Bonada *et al.,* 2007b, Fernández-Calero *et al.,* 2024), which should favor gene exchange between local populations. In addition, it could result from the interaction of two opposing forces: drying acting as an environmental filter that eliminates haplotypes, and drying promoting spatial and temporal heterogeneity, which could facilitate the coexistence of haplotypes (Bogan & Lytle 2007). However, although we found no statistically significant trends, we observed that intraspecific variability increased in sites with higher dispersal resistance (DirWei) (i.e., more isolated and perennial reaches). This result suggests that the isolation of populations over space and time can result in enhanced intraspecific diversity at local scale (Alp *et al.,* 2012, Bonada *et al.,* 2017, Golden *et al.,* 2021). As previous studies have demonstrated, isolation reduces gene flow between populations (e.g., isolation by distance) (Watanabe *et al.,* 2010, Alp *et al.,* 2012). Therefore, it can be suggested that genetic drift and local adaptation play a more prominent role in this context. This is most likely related with the fact that permanent disconnected pools can serve as refugia to a great diversity of taxa and haplotypes during the dry season (Bonada *et al.,* 2020, Pineda-Morante *et al.,* 2022). This phenomenon is consistent with previous studies in arid landscapes (Meffe & Vrijenhoek 1988, Bonada *et al.,* 2017) or aquatic habitats such as ponds

(Michels *et al.,* 2003) in which genetic structures are not associated with river networks and are strongly dependent on dispersal abilities (Boix *et al.,* 2016, Tonkin *et al.,* 2018).

As predicted (H2), our results showed higher ESV richness and DNA-metabarcoding-based richness for aerial active dispersers than for the rest of the taxa. This aligns with the expectation of aerial dispersal overland being the main dispersal pathway to sustain source-sink dynamics in fragmented stream networks (Cañedo-Argüelles *et al.,* 2015, Razeng *et al.,* 2016). However, contrary to our initial expectations (H2), our findings revealed a higher degree of differentiation between genetic richness for aerial active taxa than for aquatic passive dispersers. As previously stated, because of the limited aquatic passive species in our study (fewer than ten), our sampling design may not have fully detected a stronger differentiation of haplotypes in aquatic passive organisms, as might be expected in other permanent rivers networks and climates where snails and mussels are highly predominant and diverse (Haag 2012, Perez Rocha *et al.,* 2023).

Genetic structure of macroinvertebrate populations with different dispersal modes

Despite our small spatial scale, we found stronger intraspecific differences among the populations of *P. acuta* than in *A. imperator* (H3). The difference between both species aligns with previous findings comparing species with different dispersal abilities. Dragonflies usually fly long distances to find mating sites and good food resources (Popova *et al.,* 2017). Moreover, *Anax* species can fly hundreds of kilometers in migratory movements, as reported for *A. junius* (May & Matthews 2008, Minot *et al.,* 2021), covering a large home range (Hallworth *et al.,* 2018). Instead, *P. acuta,* is the most widespread freshwater snail species and ranks among the most effective invaders of freshwater ecosystems (Vinarski 2017). Several studies conducted at both large and small spatial scales did not find an effect of isolation by distance on the population structures of this snail (Walters *et al.,* 2022, Dumidae *et al.,* 2024). These studies focused on ponds and lentic water bodies, which are usually permanent. However, Bousset *et al.* (2004) and Jarne *et al.* (2021) found differences in genetic population structures at similar spatial scales to ours in temporary systems, ponds, and rivers. According to our results, habitat fragmentation due to drying appears to be the main driver

of genetic structure in *P. acuta* populations, regardless of the spatial scale considered. In addition, this snail can be carried between freshwater patches by larger animals, such as waterbirds, or attached to mammals (Van Leeuwen *et al.,* 2013). Although zoochory has been extensively documented for *P. acuta* in lentic habitats (i.e., wetlands, temporary ponds) (Vanschoenwinkel *et al.,* 2008b, Van Leeuwen *et al.,* 2013), studies focusing on its occurrence in pristine headwater rivers remains scarce. In contrast with previous studies, our study focused on a region characterized by small water bodies (i.e., temporary rivers) where the presence of waterbirds is limited compared to large wetlands. This, combined with the mountainous landscape and the dendritic structure of river networks, limits the dispersal of individuals between sub-basins, thus fostering barriers to gene flow and leading to population structure similar to what has been found for species with limited dispersal abilities in mountainous or headwater environments (Finn *et al.,* 2006, 2011).

Morphological and molecular methods to describe metacommunity dynamics

The fact that some taxa (mostly Diptera and Coleoptera) were only identified through morphological analyses was probably due to their small size and sclerification, respectively. A significant proportion of the Diptera taxa present in our samples have been identified from a single or a few individuals, with body sizes that were notably small. Despite an attempt being made to address the imbalance in biomass compared to other larger taxa (e.g., Odonata or Heteroptera), this may result in an under-representation of DNA extraction from bulk samples, which could lead to the lack of detection of small taxa (Elbrecht *et al.,* 2017b). In addition, some aquatic beetles (e.g., Dytiscidae, Elmidae) tend to be underrepresented in DNA extraction due to sclerification. This has been reported in several works (Carew *et al.,* 2018, Marquina *et al.,* 2019). There the authors suggest that a longer incubation in the DNA extraction buffer is required to release sufficient DNA for metabarcoding. It seems that the incompleteness of barcode reference libraries is not the reason for our divergence, since only two of the genera identified were not coded in BOLD. It should be noted, however, that we did not compare data at species level because most of the taxa were morphologically identified at the genus level. We encourage future studies at the species level to address the substantial underrepresentation of ecological studies and barcoded taxa in the Iberian

Peninsula, especially Diptera with only 23% of the species barcoded (Múrria *et al.,* 2020, Boóz *et al.,* 2024). On the other hand, as expected, some taxa that are difficult to identify morphologically (e.g., Chironomidae, Oligochaeta, Hydracarina) were identified using metabarcoding. However, fewer than half of the sequences of these taxa were assigned to the species level, which highlights the importance of sequencing these understudied groups. The difficulty in the morphological identification of these taxa is often due to their small size, morphological similarity between species and the presence of morphologically undetectable cryptic species (Gustafsson *et al.,* 2009). The ability of metabarcoding to identify these and other challenging taxa underscores its value as a tool for ecological research and environmental monitoring. By providing accurate species-level identification, barcoding and metabarcoding improve our understanding of biodiversity in aquatic ecosystems (Carew *et al.,* 2013, Vivien *et al.,* 2017, Bush *et al.,* 2019).

In line with H4, we found that both morphological and DNA-metabarcoding-based richness decreases with drying (TotDur) and increases with spatiotemporal connectivity (DirBin), thereby suggesting that more permanent and connected sites contain a higher number of taxa. This is consistent with previous studies conducted at regional (Schriever *et al.,* 2015, Pineda-Morante *et al.,* 2022) and global (Datry *et al.,* 2014b) scales. Our study shows that drying and connectivity act as strong environmental filters at small spatial scales (Pineda-Morante *et al.,* 2022) and can be effectively detected at both morphological and metabarcoding levels. Although we did not expect this to affect community dissimilarity (H5) we found a significant distance decay relationship for both morphological and metabarcoding beta diversity. According to Heino *et al.* (2015) and He *et al.* (2020), high dispersal rates between communities are expected at small spatial scales, allowing species to persist in unsuitable environments. Our current results challenge these statements for drying river networks suggesting that fragmentation caused by drying is limiting dispersal (Cañedo-Argüelles *et al.,* 2015) even at small spatial scales, with important implications for conservation planning (Fernández-Calero *et al.,* 2024). Furthermore, we found that both local hydrological conditions and regional hydrological connectivity were strong drivers of metacommunity organization, with a higher effect during spring than summer. This is probably related to the fact that during spring there is a wide hydrological gradient, with most of the rivers connected and flowing (i.e., flowing, connected pools, isolated pools), whereas

during summer disconnected pools and dry sites dominate the stream network. This gradient of hydrological conditions during spring could promote the differentiation between communities according to environmental and spatial processes. For example, disconnected pools have communities that are very different from flowing reaches due to changes in habitat condition and water chemistry (Bonada *et al.,* 2020). Also, strictly aquatic dispersers should be able to reach sites that are hydrologically connected, but their dispersal would be limited by dry reaches (Cunillera-Montcusí *et al.,* 2023). This highlights the importance of the seasonal variation in hydrological conditions for shaping aquatic macroinvertebrate communities in TRS, as reported by previous studies (Sarremejane *et al.,* 2017, Pineda-Morante *et al.,* 2022, Cunillera-Montcusí *et al.,* 2023).

Metabarcoding enables the detection of greater diversity than morphological identification (Elbrecht *et al.,* 2017a), although similar patterns in taxonomic compositions are frequently observed (Remmel *et al.,* 2024). This was consistent with our findings as the patterns detected by the two methods were the same for both alpha and beta diversity. However, our models were more robust using morphological than metabarcoding data. This may be due to the fact that some species, (e.g., Oligochaeta and Chironomidae), are ubiquitous across the river network, leading to less community dissimilarity between sites.

Conclusions and management implications

Overall, in this study we provide valuable insights into the potential use of metabarcoding to capture drivers that shape aquatic biodiversity in fragmented river networks. Although eDNA metabarcoding techniques have been extensively explored in recent years, their application has primarily focused on biomonitoring programs focused on water quality assessments (Hering *et al.,* 2018, Pérez-Burillo *et al.,* 2020, Múrria *et al.,* 2024) and conservation (Miqueleiz *et al.,* 2022, Corral-Lou & Doadrio 2023). Notwithstanding, the use of metabarcoding for studying other facets of biodiversity such as intraspecific genetic diversity remains limited due to the technique's constraints in detecting haplotypes (Elbrecht *et al.,* 2018). For example, tag switching, primer bias, sequencing errors and the inability to differentiate it from mutations are the main limitations of metabarcoding for obtaining haplotypic information (Shokralla *et al.,* 2014, Esling *et al.,* 2015, Elbrecht & Leese 2015, Tremblay *et al.,* 2015). Although our

investigation clearly advances current knowledge, some important limitations must be acknowledged. Firstly, we have to consider that in larger spatial scales the reported patterns would likely change. For instance, a robust pattern of intraspecific variability along drying gradients could be expected at larger spatial scales, with isolated sites probably showing a greater intraspecific variability. Secondly, our data have a high proportion of aerial active dispersers. Thus, it would be interesting to contrast our results in larger study areas, as we mentioned above, and in other climatic regions where the proportion of other dispersal modes may be greater. Finally, of sample homogenization prior to library preparation could lead to improvements in the uneven sequencing depth between samples. This homogenization, combined with the use of a second nuclear pair of primers, might be an opportunity to test deeply intraspecific patterns in our study, as we could see in Weigand *et al.* (2017).

Further research applying metabarcoding has the potential to elucidate evolutionary processes such as speciation in landscapes fragmented by drying, hitherto addressed with other methodologies (e.g., barcoding) (Finn *et al.,* 2011, Múrria *et al.,* 2013). At the same time, we show that metacommunity dynamics can successfully be captured by metabarcoding approaches, although the strength of some relationships might be modified by the overrepresentation of certain taxa (e.g., Chironomidae). We highlight that species richness and site intraspecific variability were equally determined by drying. However, while richness (morphological and DNA-metabarcoding-based) was favored by connectivity, site intraspecific variability was promoted by isolation. Indeed, fragmentation due to drying appears to be a determining factor for cosmopolitan freshwater snails like *P. acuta*, while showing no effect on strong dispersers like *A. imperator*. In fact, metabarcoding provides flawless taxonomic identification and presents the same patterns as morphologic identification for both alpha and beta diversity at small spatial scales despite the gaps found between these two approaches. In addition, metabarcoding allows to detect species neglected by morphology that will be useful for future research in the study area and the general knowledge about species that inhabit TRs. Based on our results, metabarcoding bulk samples can be a good option compared to traditional morphological methods to inform conservation and management decisions and elucidate general community ecology patterns across different spatial scales. This approach is advantageous due to its efficiency in terms of

time and cost (Elbrecht *et al.,* 2017a). Nevertheless, as indicated by Macher *et al.* (2018), alternative approaches to metabarcoding bulk samples, such as eDNA, should be considered for other purposes, such as the assessment of the impact of stressors at larger scales.

AUTHORS' CONTRIBUTIONS

José María Fernández-Calero conceived the ideas and leaded the paper in the contextualization, analysis, writing, interpretation and edition. He also collected the data, sorted, identified macroinvertebrates and performed laboratory work for metabarcoding. Miguel Cañedo-Argüelles collected the data, conceptualized the paper, interpreted the results, wrote and edited the manuscript and obtained funding. Núria Bonada conceptualized the paper, interpreted the results, wrote and edited the manuscript and obtained funding. Nieves López-Rodríguez performed laboratory work for metabarcoding and edited the manuscript. Julie Crabot analyzed data, interpreted the results, wrote and edited the manuscript. David Cunillera-Montcusí calculated spatiotemporal connectivity metrics, interpreted the results, wrote and edited the manuscript. Maria Soria interpreted the results, wrote and edited the manuscript. Núria Cid collected the data, interpreted the results, wrote and edited the manuscript and obtained funding. Florian Leese interpreted the results and edited the manuscript. Raúl Acosta collected the data, identified macroinvertebrates and edited the manuscript. Pau Fortuño collected the data and identified macroinvertebrates.

Cada día sabemos más y entendemos menos

Albert Einstein

CHAPTER 4

Where do you fly from? Assessing aerial dispersal in temporary rivers using stable isotopes

José María Fernández-Calero, Daniel von Schiller, Guillermo Quevedo-Ortiz, Anna Ticó Pifarré, Núria Cid, Miguel Cañedo-Argüelles & Núria Bonada



ABSTRACT

Dispersal is a crucial ecological process that facilitates gene flow and species exchange within ecosystems. In temporary rivers (TRs), dispersal is a key driver of community assembly because it determines the ability of the organisms to adapt to seasonal drying and maintain stable populations. In particular, disconnected pools (DPs) in TRs function as critical ecological refuges during dry periods, preserving local and regional biodiversity, and acting as sources for aerial macroinvertebrate dispersal. Aquatic macroinvertebrate traits have been used as a proxy to estimate dispersal. However, studies measuring the actual dispersal of aquatic macroinvertebrates in TRs are very limited. In this study, we investigated macroinvertebrate dispersal in TRs with DPs remaining during the non-flowing phase by applying isotopic enrichment in the DPs and using mesocosms to assess dispersal. Our aim was to assess the ability of aquatic macroinvertebrates to aerially disperse in TRs with DPs. The experiment was conducted in a headwater Mediterranean TR stream in northeast Spain, characterized by flow intermittence during summer. We used eight uncolonized mesocosms installed along the dry stream channel, with four placed upstream and four downstream from five closely spaced DPs. Labeled ammonium chloride ($^{15}NH_4Cl$) was employed as isotopic tracer to mark DPs and track macroinvertebrate dispersal. We first assessed the effectiveness of ^{15}N isotopic tracers in tracking aquatic macroinvertebrate aerial dispersal. Then, we examined dispersal distance upstream and downstream of the DPs, predicting that community similarity between mesocosms and DPs would decrease with increasing geographic distance, highlighting the key role of spatial proximity in shaping community structure. Isotopic enrichment increased over time in DPs, with a proportion of enriched individuals varying with life cycle stages (e.g., larvae or adults). Enriched *Cloeon* sp. and Chironomini larvae were the most abundant taxa captured in both DPs and mesocosms. Enriched aquatic obligate taxa (e.g., Mollusca, water mites) were also present in the mesocosms. There was a gradual decrease in enriched isotopic samples both upstream and downstream from DPs. Community similarity analysis revealed significant differences between DPs and mesocosms, whereas mesocosms placed upstream and downstream clustered together. Additionally, weak positive correlations were observed between environmental (i.e., physicochemical variables) and geographic distances and community composition. Our findings contribute to the understanding of aquatic macroinvertebrate dispersal mechanisms through the use of isotopic enrichment techniques,

pioneering their use in TRs and successfully detecting isotopic marking in subsequent generations. We emphasize the importance of maintaining DPs in TR networks to host colonizers that will facilitate community recovery during and after drying, either through active aerial dispersal of aquatic insect adults or passive aerial dispersal through zoochory. Considering future drying scenarios, the conservation (and potential promotion) of DPs as refuges should be prioritized to avoid local and regional biodiversity loss in TRs.

Keywords: Disconnected pools, isotopic enrichment, macroinvertebrates, mesocosms, resilience.

INTRODUCTION

Dispersal refers to the movement of organisms between different habitat patches (Bilton *et al*., 2001). It is a key ecological process that ensures gene flow between local populations (i.e., metapopulations), species exchange between local communities (i.e., metacommunities), and material and energy flows in ecosystems (i.e., metaecosystems) (Bohonak & Jenkins, 2003; Gounand *et al*., 2018; Mouquet & Loreau, 2003; Olivieri *et al*., 1995; Raffard *et al*., 2022). Dispersal is driven by a combination of intrinsic factors that depend on the organism's dispersal abilities, such as flight capability, and extrinsic factors such as wind, geographical barriers, or habitat fragmentation (Epele *et al*., 2021; Gauthier *et al*., 2021). Each organism has different dispersal modes that can vary depending on their life cycle and development stages. In freshwater ecosystems, some aquatic macroinvertebrates (such as Mollusca and Crustacea) remain strictly aquatic throughout their life cycle, while others (e.g., Ephemeroptera and Diptera) have an adult stage that is entirely aerial. Additionally, some species transition between aquatic and aerial environments at different life stages (e.g., Heteroptera or Coleoptera) (Cianferoni, 2024; Lencioni *et al*., 2024; Millán *et al*., 2024; Sartori & Gattolliat, 2024).

Temporary rivers (TRs) are fluvial ecosystems that stop flowing or dry completely at some point in time and space. They experience a dynamic transition between flowing water (lotic conditions), disconnected pools (lentic conditions) and terrestrial habitats (dry riverbeds) (Datry *et al*., 2016a; Bonada *et al*., 2024). They are the most common river type on Earth, and are becoming more frequent in many regions due to climate change and increased water demand (Messager *et al*., 2021). Macroinvertebrate communities in TRs experience recurrent shifts of colonization and extinction and, therefore, understanding dispersal processes in TRs is key for biodiversity conservation (Bruno *et al*., 2022; Fernández-Calero *et al*., 2024) and to ensure the resilience of these ecosystems to global change. In this context, disconnected pools (DPs) serve as ecologically transitional habitats and play an essential role as refuges during dry periods, sustaining aquatic life and helping maintain local and regional biodiversity. Factors such as pool permanence, size, predation pressure, physicochemical conditions, and time since disconnection from river flow significantly influence their ecological function (Anusa *et al*., 2012; McIntosh *et al*., 2017). Despite their importance, DPs

have received limited attention, highlighting the need for comprehensive research to guide effective conservation and management strategies (Bonada *et al*., 2020; Robson *et al*., 2013).

Dispersal in TRs is particularly relevant before and after the non-flowing phase. Before the non-flowing phase, macroinvertebrates that are unable to produce resistance strategies to survive locally need to find aquatic refuges to complete their life cycles (Bogan *et al*., 2017). After the non-flowing phase, these refuges are supposed to largely contribute to the community recovery (i.e., resilience) during flow reconnection (Fournier *et al*., 2023; Pineda-Morante *et al*., 2022). The dispersal mechanisms to cope with drying or to recover from it are diverse. Some taxa produce desiccation-resistance dormant stages or remain in diapause, reactivating their physiological activities after the non-flowing phase. These resistance strategies are also referred to as dispersal through time (Bonada *et al*., 2017), and can be very relevant in TRs. For example, in braided rivers the hyporheos can host high abundances of macroinvertebrates that will recolonize the benthos once surface water returns (Fournier *et al*., 2023; Vander Vorste *et al*., 2016a). Also, desiccation-resistance dormant stages can represent more than half of the macroinvertebrate taxa present in some rivers (Larned *et al*., 2007; Stubbington & Datry, 2013). In contrast, many taxa employ mechanisms like seeking refuge in DPs or in upstream or downstream perennial reaches from where they will disperse after the non-flowing phase (Bogan *et al*., 2017). These resilient strategies are also referred to as dispersal in space (Bonada *et al*., 2017) and rely on the ability of organisms to disperse, the landscape configuration, and the presence of refuges (Bogan *et al*., 2017; Hershkovitz & Gasith, 2013; Stubbington & Datry, 2013). Drift, a common dispersal process in perennial rivers, is considered a resilience pathway in TRs, facilitating the movement of organisms through the aquatic habitat (Bogan *et al*., 2017). Alternatively, dispersal through the aerial adult stage becomes a critical and widespread mode of macroinvertebrate dispersal in TRs allowing organisms to fly overland to look for refuges scattered throughout the landscape (Bogan *et al*., 2017; Cañedo-Argüelles *et al*., 2015; Tonkin *et al*., 2018). For example, some Odonata species are capable of flying up to hundreds of kilometers (Hallworth *et al*., 2018; May & Matthews, 2008; Minot *et al*., 2021), allowing them to disperse widely across different river catchments.

In spite of the ecological relevance of dispersal and the wide range of dispersal strategies that exist in aquatic macroinvertebrates (Bohonak & Jenkins, 2003), direct

measurements of dispersal are still rare, and dispersal capacities are most often inferred using traits from the literature (Sarremejane *et al*., 2020a). For example, only 9.3% of European EPT species (Ephemeroptera, Plecoptera, and Trichoptera) empirical data on dispersal distances are available (Peredo Arce *et al*., 2021). Physical mark-recapture methods have been useful for these purposes. For instance, color dye powders were employed to mark *Anopheles* mosquitoes and track their dispersal in relation with the incidence of malaria in Burkina Faso (Epopa *et al*., 2017). Other types of labels have been used to permanently mark specific individuals; for example, water beetles (Dytiscidae) and semiaquatic bugs (Hemiptera: Gerromorpha) have been successfully labelled by attaching numbered waterproof paper tags to their elytra using an adhesive (Davy-Bowker, 2002; Ditrich, 2021). All these methods are individually-based and impractical when the purpose is to get a general idea of the dispersal of the whole population or community, regardless of their size.

Stable isotopes (e.g., ^{15}N) have been widely employed in freshwater systems to study food webs, trophic interactions and diet composition (Boecklen *et al*., 2011; Coat *et al*., 2009; Quinby *et al*., 2020; Tank *et al*., 2000). However, they can also be used to track dispersal while avoiding the drawbacks of other techniques, such as their invasive nature, which can alter the behavioral patterns of the animals being studied (Bilton *et al*., 2001). For example, Briers *et al*. (2004) and Macneale *et al*. (2005) used ^{15}N enrichment to label large quantities of stream macroinvertebrates, directly testing adult dispersal between headwater streams at the population level in two stonefly species (*Leuctra inermis* and *L. ferruginea*). These and other studies focused on grazers species because they feed directly on algae, which initially assimilate ^{15}N and then transfer it through the trophic chain (Kumar & Bera, 2020). Nevertheless, Enss *et al*. (2024) have recently demonstrated that labeling with stable isotopes in streams is feasible across multiple taxa and various feeding types (e.g., filter feeders and predators). Despite recent advances, the use of stable isotopes for assessing dispersal in aquatic macroinvertebrates is still under development. Labeling techniques in rivers have been tested primarily under lotic conditions, often focusing on a single species (Briers *et al*., 2004; Macneale *et al*., 2005) rather than across multiple taxa (Enss *et al*., 2024). Moreover, our understanding of individual dispersal distances traveled by macroinvertebrates remains limited, and no studies have so far addressed the labeling of dispersal processes through oviposition and colonization in new habitats for subsequent generations.

In this study, we investigate the ability of aquatic macroinvertebrates to aerially disperse in TRs with DPs. We hypothesized DPs as refuges that host macroinvertebrates during the non-flowing phase and that potentially contribute to community recovery after the non-flowing phase (Bonada *et al*., 2020). We performed a field experiment using mesocosms to exclude other potential temporal and spatial dispersal pathways in TRs, such as the use of the hyporheic zone, desiccation-resistant dormant stages, drift, and swimming. Our first objective (O1) was to test the efficiency of an isotopic tracer (^{15}N) in tracking the dispersal of macroinvertebrates across different life stages and over time. For this objective, we hypothesized (H1) that the concentration of the isotopic labeling of macroinvertebrates would increase along time in enriched DPs, taking more time to reach the maximum for taxa with an aerial adult life stage (e.g., Ephemeroptera and Diptera) than for species with an aquatic and aerial (semiaquatic) adult life stage (e.g., Hemiptera and Coleoptera). This expectation is based on the trophic position of aerial adults, which are predominantly grazers during their larval stages, whereas semiaquatic adults tend to be predatory (Enss *et al*., 2024). We also expected a lower proportion of isotopically enriched aerial adults emerging from DPs compared to their corresponding larvae (H2), as nitrogen is acquired during the larval stage and the isotopic signal becomes diluted as their body changes from one stage to another. Consequently, enriched larvae in a mesocosm should have a lower isotopic signal but still would indicate that a flying female moved from the enriched DPs, laid eggs, and larvae emerged. Therefore, we did not anticipate aquatic obligate colonization (e.g., Mollusca and water mites). We also hypothesized (H3) that the proportion of enriched individuals would decrease with increasing distance from our enriched DPs, as distance influences the diversity of DPs through colonization and extinction dynamics (Bonada *et al*., 2020). Our second objective (O2) was to assess the differences between DPs and mesocosms in terms of community structure to assess how these DPs could contribute as sources of colonizers. For this second objective, we hypothesized (H4) that the communities of DPs and mesocosms would become more similar with time and that their similarity would be inversely related to the distance between them. By using uncolonized mesocosms we limited other kinds of community recovery pathways (either through resistance and resilience strategies). Moreover, we expected (H5) that the distance between natural DPs and mesocosms would be more important than the differences in their environmental conditions for structuring aquatic macroinvertebrate communities. This was expected due to the similarity of the

physico-chemical conditions between the mesocosms, the short duration of the experiment, and the small scale of the study area.

METHODS

Study area and experimental set-up

The experiment was carried out in late summer in Vall d'Horta, a TR located in a natural area (Sant Llorenç del Munt i l'Obac Natural Park) in northeast Spain. This area has a typical seasonal Mediterranean climate, with hot and dry summers (for further information about the study area see Fernández-Calero *et al*. (2024) and Pineda-Morante *et al*. (2022)). The stream exhibits a characteristic pool-riffle morphology with a high proportion of bedrock that allows the formation of DPs that might persist in summer, therefore acting as refuges (Figure S2 and S3 Supporting Information 1). The stream is divided into a temporary upstream section with DPs, where the experiment was conducted during the non-flowing phase, and a perennial downstream section. Five successive and very close DPs, which retained water year-round, were identified within a 25-m stretch. These pools were separated by only a few centimeters, with a maximum distance of two to three meters between consecutive pools. The DPs varied in depths from 8 cm to 146 cm, in width from 70 cm to 410 cm, and in length from 110 cm to 530 cm (Figure S2 Supporting Information 1). At the same stream reach, eight uncolonized 150-liter mesocosms (119x79x42 cm) were installed along the dry riverbed (Figure S1 Supporting Information 1). Four mesocosms were located at 50 m (M1), 120 m (M2), 228 m (M3), and 398 m (M4) upstream of the uppermost DPs, and four additional mesocosms were placed at 50 m (M5), 94 m (M6), 504 m (M7), and 564 m (M8) downstream of the lowermost DPs. Mesocosms M7 and M8 were the closest to the perennial reach (96 and 36 m away from it, respectively) (Figure 1A). The perennial stream reach was located at 600 meters away downstream of the DPs (Figure 1A). Canopy cover in the mesocosms ranged from 29% to 93%, with an average of 54%. To avoid introducing macroinvertebrate propagules (eggs or small larvae) with water, the mesocosms were filled with commercial bottled mineral water, which had a composition similar to that of the study area (see Quevedo-Ortiz *et al*. (2024) for further details). In addition, 4 sterile stones, sterilized in

bleach for 24 hours, were placed within each mesocosm, providing a suitable habitat and potential refuge for macroinvertebrates, as well as to facilitate algal colonization (Quevedo-Ortiz *et al*., 2024). A flight interception trap was set up next to each mesocosm to catch insects flying upstream or downstream. Each trap consisted of a 50x50 cm intercepting sheet and a collecting container that was 50 cm long, 8 cm high, and 5.5 cm thick (for a better view of the trap design, please refer to Figure S3 Supporting Information 1). The traps were positioned 1 meter above ground level, on the opposite side of the expected dispersal direction of macroinvertebrates emerging from the DPs. For upstream mesocosms (M1, M2, M3, M4), traps were placed upstream, while for downstream mesocosms (M5, M6, M7, M8), traps were positioned downstream (Figure 1A). This placement ensured that dispersing macroinvertebrates would first encounter the mesocosm water for colonization or egg deposition, without being intercepted by the traps prior to reaching the water. Two additional interception traps were placed along the lateral sides of DPs to capture insects dispersing laterally into the adjacent forest. Two emergence traps were installed on DPs and one in the downstream perennial section four days before the start of the experiment (Figure S3 Supporting Information 1). We used “Amphibious Emergence Traps (BT2008)” emergence traps manufactured by MegaView Science Co., Ltd. Each trap measured 110x110x110 cm. In addition, hand net sampling was conducted during each scheduled sampling time throughout the experiment to collect flying individuals in the surrounding areas of both the DPs and mesocosms. The data from individuals collected through hand net sampling were not included in the analysis of aquatic communities (e.g., DPs and mesocosms). Instead, they were used exclusively to assess the spatial distribution of these individuals in relation to isotopic enrichment.

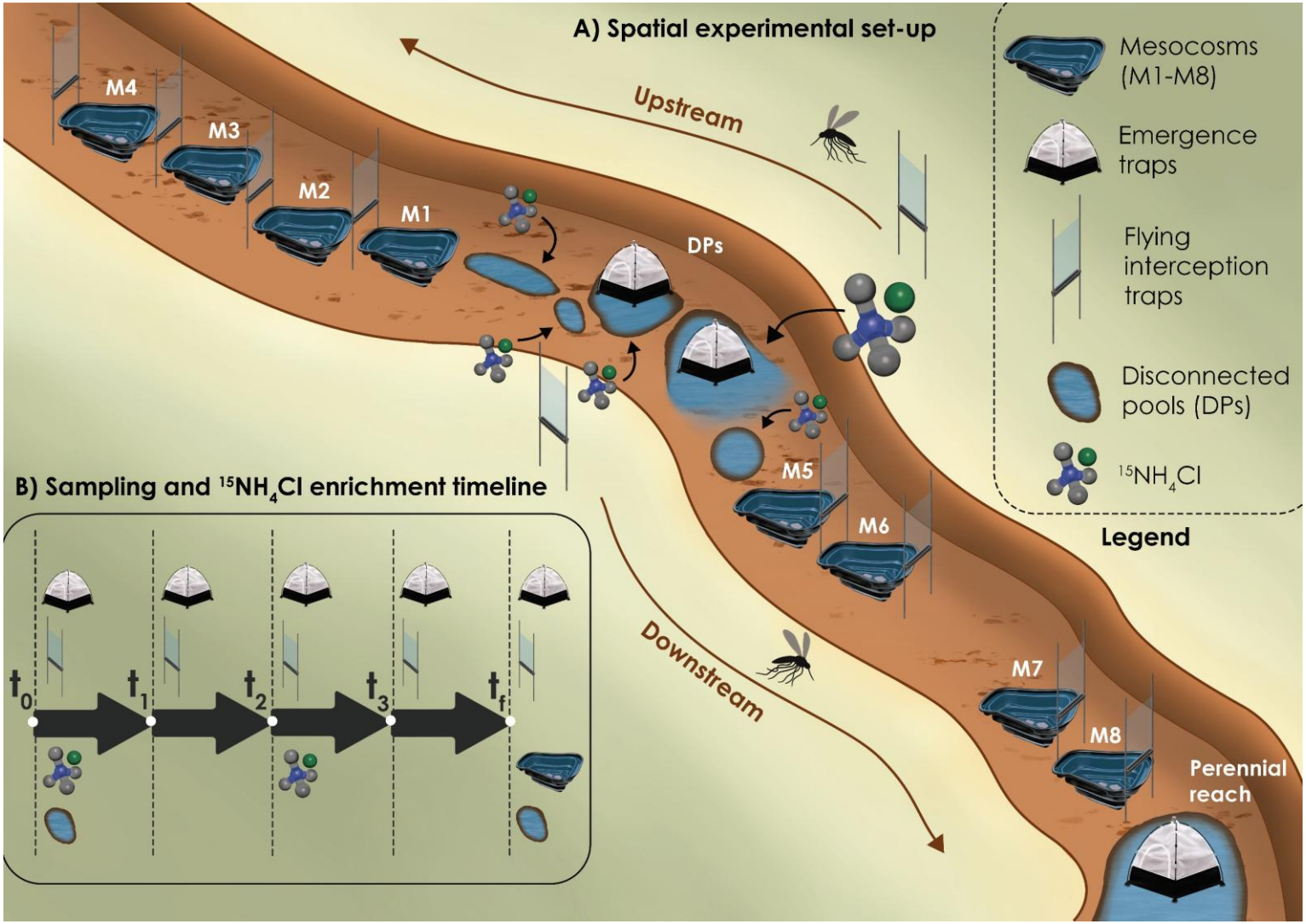


Figure 1. A) Experimental set-up: Enriched disconnected natural pools (DPs) with mesocosms (M) located upstream and downstream. Non-enriched perennial reach located downstream of the mesocosms. Emergence traps and flight interception traps are shown in their spatial arrangement along the river section. Additionally, ^{15}N-enriched DPs are indicated. **B)** Sampling and enrichment timeline: Above the timeline, the sampling periods for the emergence and flight interception traps are indicated. Below the timeline, the sampling periods for the disconnected natural pools and mesocosms are shown. The timing of the ^{15}N addition is also indicated, occurring at t_0 and t_2. The figure shows that the emergence and interception traps were sampled throughout the experiment (from t_0 to t_f), while the DPs were sampled only at t_0 and t_f, and mesocosms were sampled only at t_f. Drawing by Sofia Faramelli.

Timing, sampling and environmental data

The experiment started on the 6th of September 2021 (t_0), when the mesocosms were filled and the isotopic tracer ($^{15}NH_4Cl$) was added to the DPs (see next section). Pre-addition macroinvertebrate field samples (i.e., control samples) were collected from the DPs (t_0) using a 250 µm mesh size hand net by kicking. Additionally, pre-addition samples were obtained from emergence traps, which were set during the 4 days leading up to t_0. Samples from the emergence and flight interception traps were collected weekly, on the 13th (t_1), 22nd (t_2), and 29th (t_3) of September (Figure 1B). The experiment was completed on the 4th of October 2021 (t_f), when field samples were collected from the emergence and flight interception traps. Additionally, macroinvertebrates were sampled from the mesocosms (single sampling at t_f) and from DPs (sampled twice, at t_0 and t_f) (Figure 1B). In the DPs, sampling was standardized by proportionally covering all available microhabitats while maintaining a consistent sampling effort across sites. In mesocosms, the entire water content was drained through the hand net to ensure the comprehensive collection of macroinvertebrates. Macroinvertebrates were preserved in 96% ethanol and stored under refrigeration until identification and isotopic analysis were completed. All macroinvertebrates were identified and counted to the lowest possible taxonomic resolution (species, genus, or family level) using a binocular stereoscope. General macroinvertebrate and insect identification keys were used (e.g., Tachet *et al*., 2010; Merrit *et al*., 2019), along with specific keys for certain groups (e.g., Jaagumagi, 1982; Harker, 1999; Vieira-Lanero, 2000; Askew, 2004; Millán *et al*., 2014; Conesa-García, 2022).

Abiotic variables were measured once a week in each DP and mesocosm, simultaneously with the macroinvertebrate sample collection. Oxygen saturation (%DO), water temperature (T; ºC), pH and specific conductivity (SPC; µS/cm) were measured using a multiparameter probe (YSI Professional plus Multiparameter Probe). Data loggers (HOBO Pendant® Temperature/Light logger) were also used to continuously measure water temperature at each sampling site (Figure S4 Supporting Information 1). Furthermore, canopy cover was measured in each DP and mesocosm using a spherical densiometer. The dry weight of organic matter accumulated in the mesocosms during the experiment was determined by drying the material collected during the final sampling (primarily leaves) in an oven at 60°C for 48 hours.

Experimental ^{15}N enrichment and isotopic analysis

^{15}N was used as an isotopic tracer to enrich the DPs. Ten grams of ammonium chloride ($^{15}NH_4Cl$) was added at t_0 and five grams at t_2 (Figure 1B) to DPs in order to label the autotrophic and detrital resources used as food by the macroinvertebrates living there (Macneale *et al*., 2005). To ensure uniform distribution, the $^{15}NH_4Cl$ was dissolved in water using a watering can and evenly dispersed throughout the DPs. Pre-addition field samples from kick samples and emergence traps were used to adjust the ^{15}N threshold and determine whether post-addition samples were labeled or not.

For the isotopic analysis, a total of 275 isotopic samples (hereafter, Iso-samples) of macroinvertebrates were selected from DPs, mesocosms, flight interception, and emergence traps. Of these, 43 were pre-addition Iso-samples that served as controls, and 232 were post-addition Iso-samples. Each Iso sample corresponded to a single taxon per sampling date (t_0, t_1, …t_f) and per site (DPs or mesocosms). Taxa that lacked active aerial dispersal abilities, such as snails and meiofauna (e.g., Copepoda and Ostracoda), were pooled together if collected from the same site and date. For some of these taxa, like Copepoda and Ostracoda, the pooling was necessary because they are very small and their biomass did not meet the minimum threshold required for isotopic analysis. For small-sized taxa (e.g., Chironomidae (Diptera), *Cloeon* sp. (Ephemeroptera: Baetidae)), multiple individuals were chosen from each Iso sample. For medium size taxa (e.g., *Stictonectes*, *Agabus* (Coleoptera: Dytiscidae), *Hydraena* (Coleoptera: Hydraenidae)), the selection varied from one to several individuals chosen, depending on the sample's abundance. Finally, for large size taxa (e.g., *Aeshna cyanea* (Odonata: Aeshnidae), *Chalcolestes viridis* (Odonata: Lestidae)), a piece of tissue (usually abdomen) was used for isotopic analysis. A total of 61 taxa were included in the 275 Iso-samples. However, this analysis does not encompass the entire community. In mesocosms, the isotopic analysis represented an average of 83% of the taxa present. For other sampling methods, the isotopic analysis accounted for 76% of the taxa from emergence traps and 63% from FI traps (Table S2 in Supporting Information 2). Taxa with very low abundances and small sizes were excluded from the analysis, as their biomass was insufficient to meet the minimum required for isotopic analysis. Nevertheless, the isotopic results provide a robust representation of the community, encompassing the majority of the taxa present. We then

removed the alcohol by evaporation and dried the Iso-samples at 60ºC for six hours before lyophilizing them at -55ºC and 0.05 mBar for two days.

The freeze-dried Iso-samples were ground and weighed to the nearest 0.3 mg, encapsulated in tins, and stored until analysis. Encapsulated Iso-samples of macroinvertebrates were analyzed for N (as a percentage of the total dry mass) and the ^{15}N isotopic signal by the EA-IRMS technique (Elemental Analysis—Isotope Ratio Mass Spectrometry) using a FlashIRMS elemental analyzer interfaced with a Delta V Advantage isotope ratio mass spectrometer (IRMS) via a Conflo IV interface (ThermoFisher Scientific, Germany). Measurement precision was 0.3 ‰ for $\delta^{15}N$ of non-enriched Iso-samples (Table with isotopic enrichment values for each Iso sample and taxa is provided in the Table S1 in Supporting Information 2).

Biological categories

Macroinvertebrates were categorized into four non-overlapping groups based on their life cycle, developmental stages, and consequently, their dispersal capacity: *semiaquatic adults, aerial adults, aquatic larvae,* and *aquatic obligates*. *Semiaquatic adults* were defined as macroinvertebrates whose adult life stage is entirely or primarily developed in water (including its surface), such as adult stages of beetles (e.g., Dytiscidae, Gyrinidae) or backswimmers (Notonectidae, Corixidae). *Aerial adults* were defined as macroinvertebrates whose adult phase primarily occurs outside of water, inhabiting terrestrial or aerial environments, with examples including adult stages of non-biting midges (i.e., Chironomidae), mosquitoes (i.e., Culicidae), or mayflies (e.g., Baetidae). The *aquatic larvae* (hereafter larvae) group included all macroinvertebrates that are strictly aquatic in the larval stage but have adult stages that are wholly or partially aerial, encompassing all larval stages from the two previous categories. Finally, the *aquatic obligates* were included in the group of macroinvertebrates that obligatorily complete their entire life cycle in water and are incapable of aerial dispersal by themselves, such as snails (e.g., Physidae), meiofauna (e.g., Copepoda and Ostracoda), and water mites (i.e., Hydrachnidiae).

Statistical analysis

The temporal variation in $\delta^{15}N$ ($\log(\delta^{15}N)$) was analyzed to track the incorporation of ^{15}N in macroinvertebrate tissues exposed to enriched DPs. This analysis was conducted for the two biological groups sampled in emergence traps: semiaquatic adults and aerial adults. For each group, linear, exponential, quadratic, and cubic polynomial models were fitted to evaluate the relationship between time and $\delta^{15}N$ values. The goodness of fit was assessed using the coefficient of determination (R^2) and the Akaike Information Criterion (AIC) for each model. Pre-addition Iso-samples were used to establish the δ ^{15}N threshold for isotopic labeling assessment. A more conservative approach, based on the method outlined by Enss *et al*. (2024), was adopted by using the maximum nitrogen value instead of the mean, as used in their study. The computation involved adding twice the standard deviation to the maximum ^{15}N value, as shown below:

$$\delta^{15}N\ threshold = Max\ \delta^{15}N + 2 * SD(\delta^{15}N)$$

Values above the threshold were considered “enriched” and values below the threshold were considered “non-enriched”.

The relative proportion of enriched and non-enriched Iso-samples along the distance gradient was described using 179 Iso-samples from mesocosms, flight interception traps, and kick Iso-samples from DPs after isotopic enrichment. To illustrate the relative proportions across different sites, a stacked bar plot was generated using the ggplot function in the ggplot2 package (Wickham, 2016) in R, where the mesocosms were ordered according to the upstream and downstream directions of the stream in relation to DPs.

To analyze the macroinvertebrate community composition, the abundance data were first transformed using a log-transformation (log(x+1)). A Non-Metric Multidimensional Scaling (NMDS) analysis was then performed using the vegan package in R. The analysis was based on the Bray-Curtis distance, considering a two-dimensional solution and a maximum of 50 iterations using the metaMDS function. This approach allowed us to visualize the differences in community structure across the stream position (source, upstream and downstream) and habitat samples (DPs and mesocosms). To statistically test the effects of stream position and habitat on community composition, a Permutational Multivariate

Analysis of Variance (PERMANOVA) (Anderson, 2001, 2017) was performed using the adonis2 function from the vegan package (Oksanen *et al*., 2022). Two separate analyses were conducted: one to evaluate the effect of stream position and another to assess the effect of habitat. Both analyses were based on Bray-Curtis dissimilarities, with 999 permutations to determine the significance of the factors. Cladocera, Copepoda, and Ostracoda were not included in this analysis.

To analyze the environmental and pairwise distances drivers of community composition, mean values of abiotic variables (pH, %DO, T, and SPC) were calculated across sampling periods and standardized using the scale function. Subsequently, Euclidean distances were calculated using the vegdist function from the vegan package (Oksanen *et al*., 2022) in R. Pairwise stream distances (in meters) were standardized using the scale function. To analyze the relationship between community dissimilarity (beta diversity based on Bray-Curtis distances), geographic distance, and environmental factors, a Mantel test was performed with abundance data, using the mantel function from the vegan package (Oksanen *et al*., 2022) in R.

RESULTS

During the experiment, 38,538 individuals were collected representing a total of 103 taxa. Of these, 33,675 individuals were collected through kick sampling, with 13,633 from DPs and 20,042 from mesocosms. A total of 76 taxa were identified from DPs and 29 taxa from mesocosms. *Cloeon* sp. and Chironomini were the most abundant taxa in DPs (relative abundance 0.37 and 0.14 respectively) and mesocosms (0.56 and 0.27 respectively). Four other taxa were present in all samples from both DPs and mesocosms (*Culex* sp., Hydrachnidiae, *Hydraena* sp. and Pentaneurini). A total of 10 taxa were consistently present in the DPs at both sampling times, but were found only in some of the mesocosms (*Aedes* sp., *Ancylus fluviatilis*, *Chaoborus* sp., *Graptodytes* sp., *Gyrinus* sp., Macropelopinii, *Notonecta maculata*, *Oulimnius* sp., *Oxycera* sp., and *Stictonectes* sp.). Taxonomic richness per sample ranged from 47 to 36 in DPs and from 18 to 9 in mesocosms (Table S1 in Supporting Information 3).

A total of 4,863 individuals were identified from emergence and flight interception traps representing 36 taxa belonging to 19 families. In flight interception, although *Cloeon dipterum* (relative abundance 0.24) was the second most abundant taxon, beetles as a group were the most abundant, with *Hydraena* sp., *Anacaena* sp., (Coleoptera: Hydrophilidae) and



Figure 2. Examples of adult aquatic macroinvertebrates collected in the emergence traps. **A, B** and **C)** Adult Chironomidae with water mites (Hydrachnidiae) attached. **D)** Adult female of *Caenis luctuosa* with egg clutches.

Dryops sp. (Coleoptera: Dryopidae) together accounting for a combined relative abundance of 0.51 (0.30, 0.12, and 0.09, respectively). No aquatic macroinvertebrates were found in the two lateral flight interception traps (Table S3 in Supporting Information 3). The most abundant taxa in the emergence traps were Chironomini, followed by *Cloeon simile* and *C. dipterum* (relative abundance of 0.46, 0.12 and 0.09, respectively) (Table S2 in Supporting Information 3). A total of 409 water mite larvae were found attached to adult Chironomidae and 7 to adult Culicidae in the emergence traps (Figure 2). Taxa richness in emergence traps varied from 7 to 18 across different time samples, whereas in flight interception traps it ranged from 0 to 5. The kick samples from DPs and mesocosms captured the highest number of taxa by far, followed by the emergence and flight interception traps (Figure 3) (Table S4 in Supporting Information 3). Additionally, five adult flying individuals were sampled using a hand net: One individuals of *Aeshna cyanea* and *Crocothemis erythraea* (Odonata: Libellulidae), and three of *Chalcolestes viridis*.

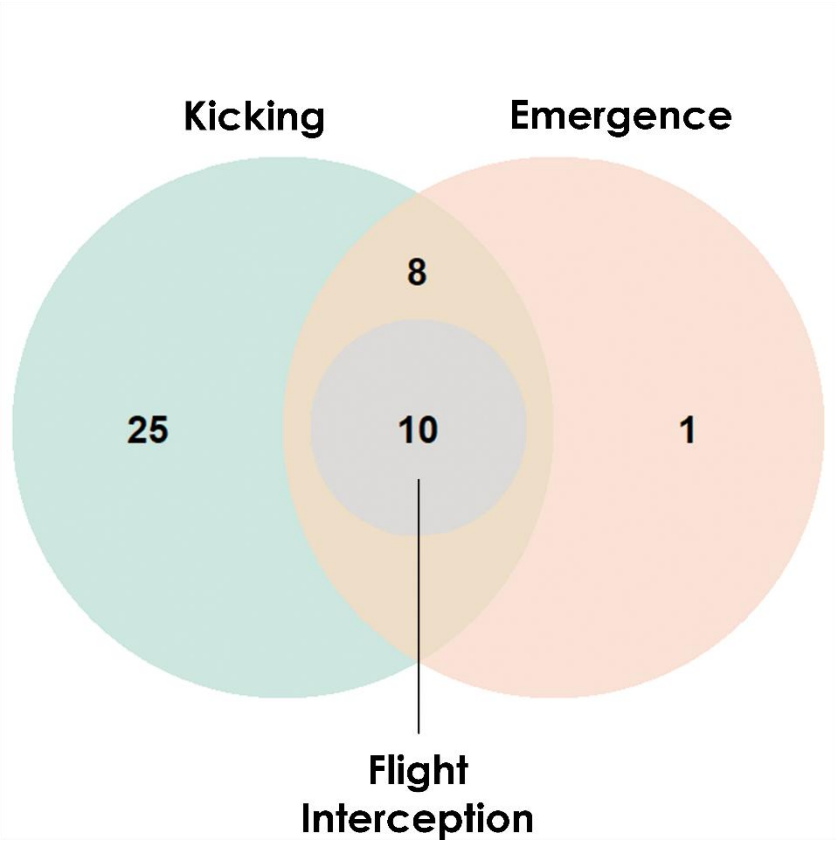


Figure 3. Venn diagram showing the overlap of macroinvertebrate families among sampling devices.

For aerial adults, the cubic polynomial model provided the best fit (R^2 = 0.85, AIC = 164, $p < 0.001$), while for semiaquatic adults, the linear model was the most suitable (R^2 = 0.49, AIC = 64, p = 0.001). See Table S3 in Supporting Information 2 for additional model results. ^{15}N levels increased earlier in semiaquatic adults (e.g., Coleoptera); however, from the second week onwards, aerial adults (e.g., Ephemeroptera and Diptera) showed higher ^{15}N content (Figure 4). See Figures S1 and S2 in Supporting Information 2 for graphical representations of the models.

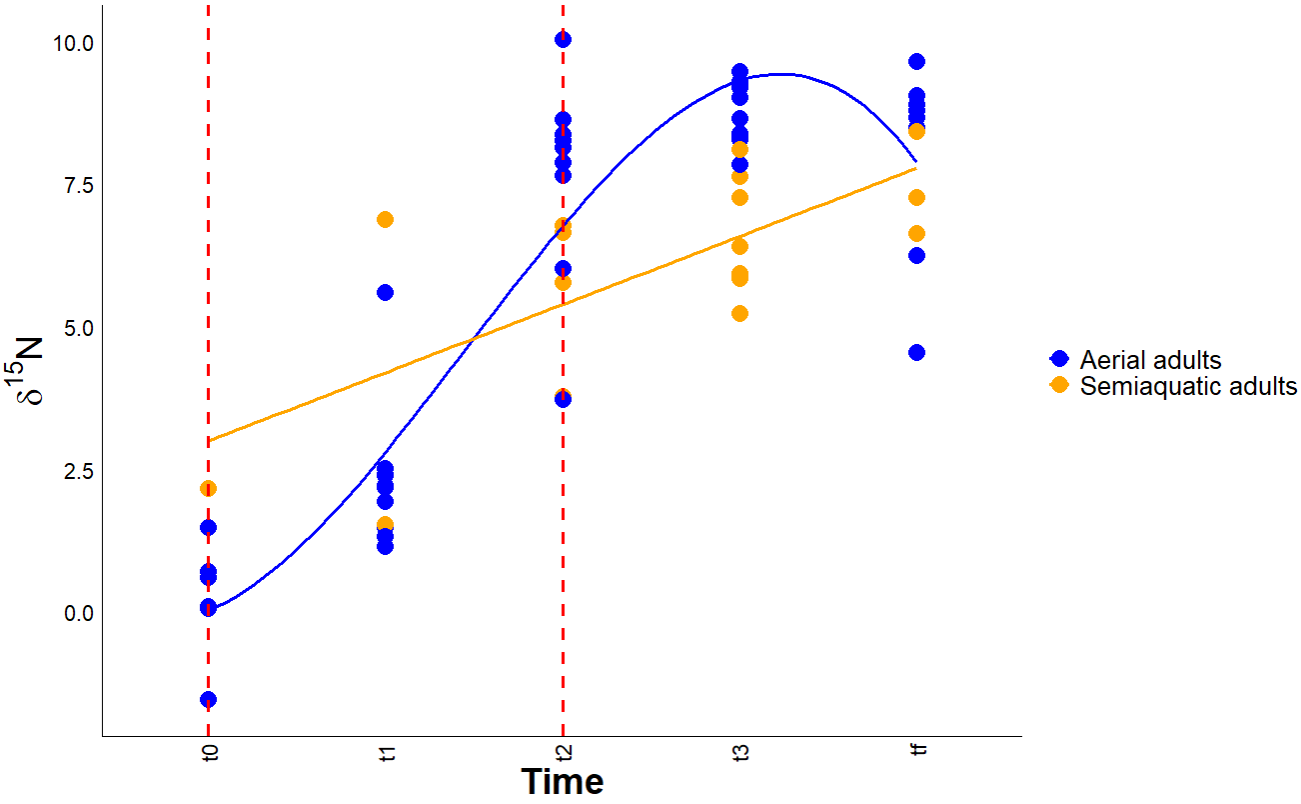


Figure 4. Temporal variation in log-transformed $\delta^{15}N$ values for aerial and semiaquatic adult biological groups of Iso-samples from emergence traps. Points represent individual Iso sample measurements of log ($\delta^{15}N$) over time. A cubic polynomial fit is applied to the aerial adult's group, while a linear model fit is applied to the semiaquatic adults group. Vertical dashed red lines indicate the ^{15}N addition events.

Enriched individuals were found in Iso-samples obtained with all sampling methodologies (i.e., kicking, emergence traps, flight interception, and hand net), and across different phenological stages and generations, including immature stages (nymphs or larvae),

adults, and immature stages of the next generation. Among the 232 Iso-samples collected after the ^{15}N addition, a total of 123 (53 %) were enriched. The proportion of enriched Iso-samples was higher in DPs (81% ± 0.08) than in the mesocosms (36 % ± 0.1) (Figure 5) (Figure S3 in Supporting Information 2). In the mesocosms, aerial adults (captured by flight interception traps) had the lowest marking proportion (9%) followed by semiaquatic adults (39%). Enriched larvae of some taxa were also detected in the mesocosms (54%), presumably the next generation of those who lived in the enriched DPs, such as Chironomidae, Culicidae, and *Cloeon* sp., as well as in aquatic obligates taxa (56%), including snails (*Potamopyrgus antipodarum* (Gastropoda: Hydrobiidae)), water mites, and meiofauna (Copepoda and Ostracoda) (Figure 5).

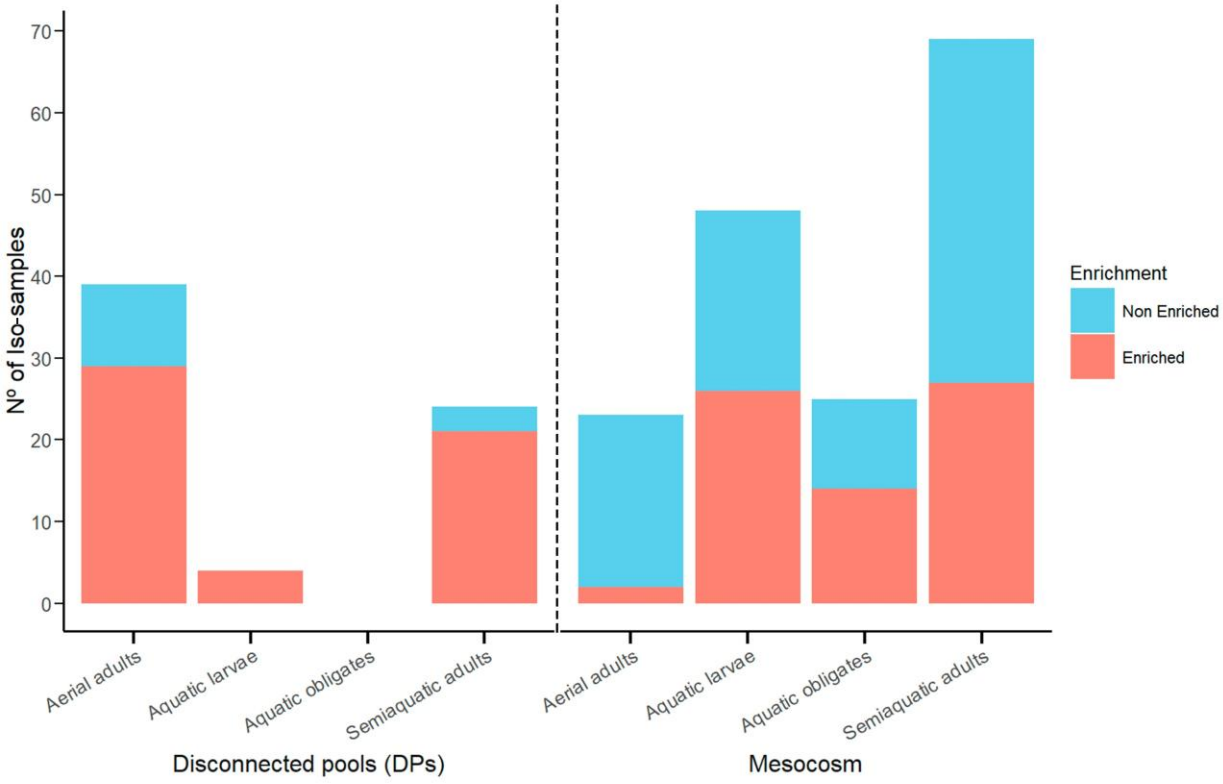


Figure 5. Number of enriched and non-enriched isotopic samples (Iso-samples) in mesocosms and disconnected pools by biological categories.

Of the 179 kick and flight interception Iso-samples collected after the ^{15}N addition, all the Iso-samples from DPs were enriched. The mesocosms closest to the DPs (M1 and M5) had the highest proportion of enriched Iso-samples (71% and 78% respectively). Furthermore, the

relative proportion of enriched Iso-samples followed a stepwise pattern showing a similar decrease downstream and upstream of the natural DPs (source) (Figure 6). Nevertheless, enriched Iso-samples of Ephemeroptera and Coleoptera were found even in the most distant mesocosms. In M4, located 398 meters upstream, *Caenis* sp. (Ephemeroptera: Caenidae) and *Graptodytes* sp. were enriched, while at the opposite end of the study stream, situated 564 meters downstream from the enriched natural pools, only *Hydraena* sp. was enriched.

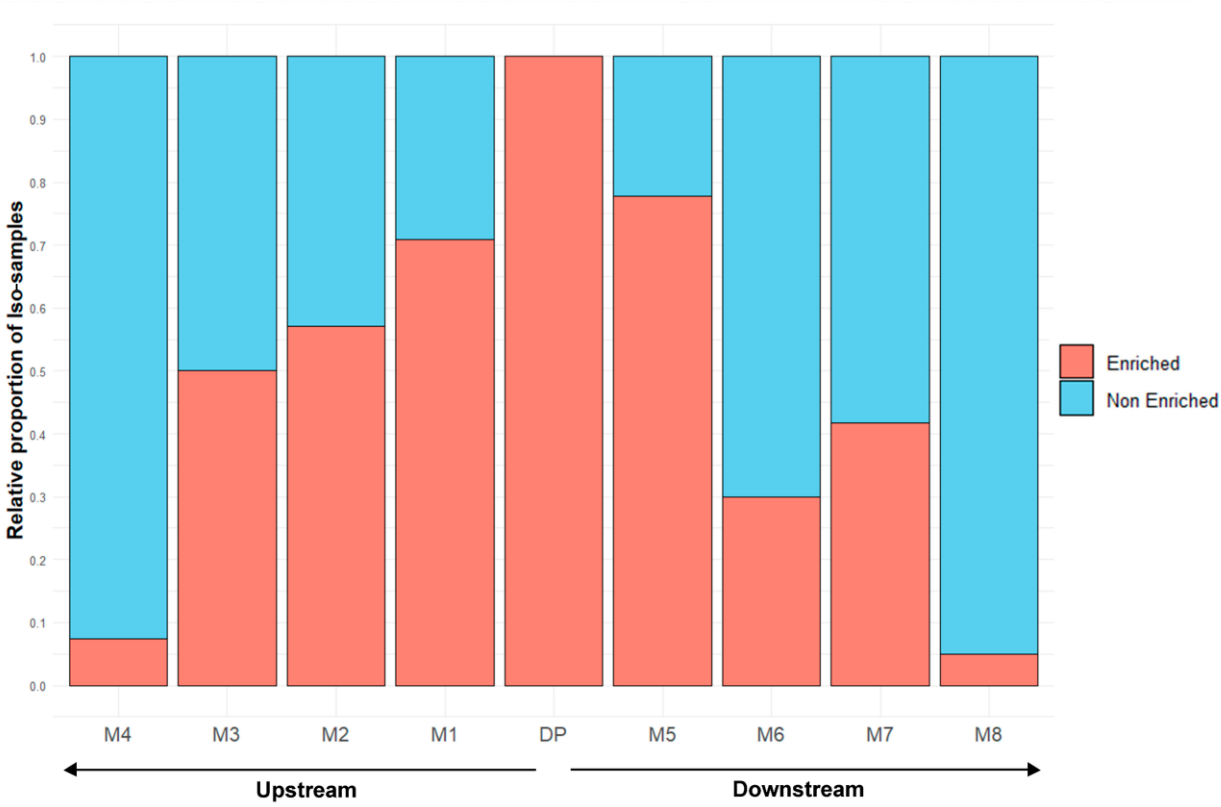


Figure 6. Relative proportions of enriched and non-enriched isotopic samples (Iso-samples). Upstream direction is from DP to M4, and downstream direction is from DP to M8.

A similar stepwise pattern was observed for Coleoptera (Figure 7a). A more random distribution was noted for Diptera and aquatic obligates (snails, water mites, Copepoda, and Ostracoda) in the upstream and downstream areas (Figure 7b and 7c), whereas Ephemeroptera displayed a preference for upstream colonization (Figure 7d).

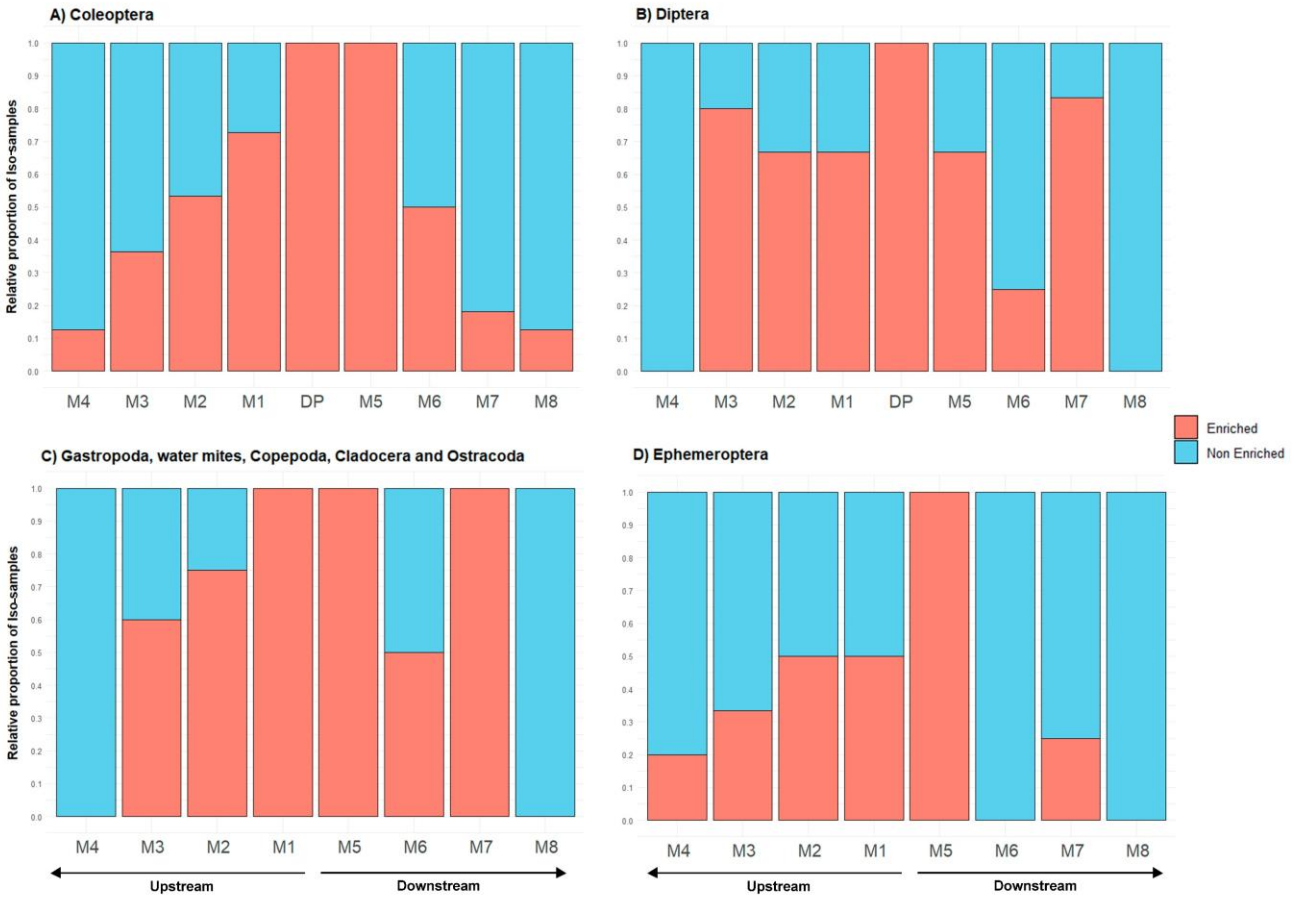


Figure 7. Relative proportion of enriched and non-enriched isotopic samples (Iso-samples). Upstream direction is from DP to M4, and downstream direction is from DP to M8. **A)** Coleoptera (semiaquatic adults), **B)** Diptera, **C)** Gastropoda, water mites, Copepoda and Ostracoda (aquatic obligate species) and **D)** Ephemeroptera, (aerial adults).

The NMDS analysis (stress = 0.06) showed no differentiation in community composition within natural pools (DPs and perennial reach) between t_0 and t_f communities. PERMANOVA analysis showed significant differences in community composition between habitats (mesocosms and natural pools) (R^2 = 0.45, F = 8.47, p = 0.003) and stream position (upstream, downstream, and source) (R^2 = 0.56, F = 5.91, p = 0.001) (Figure 8).

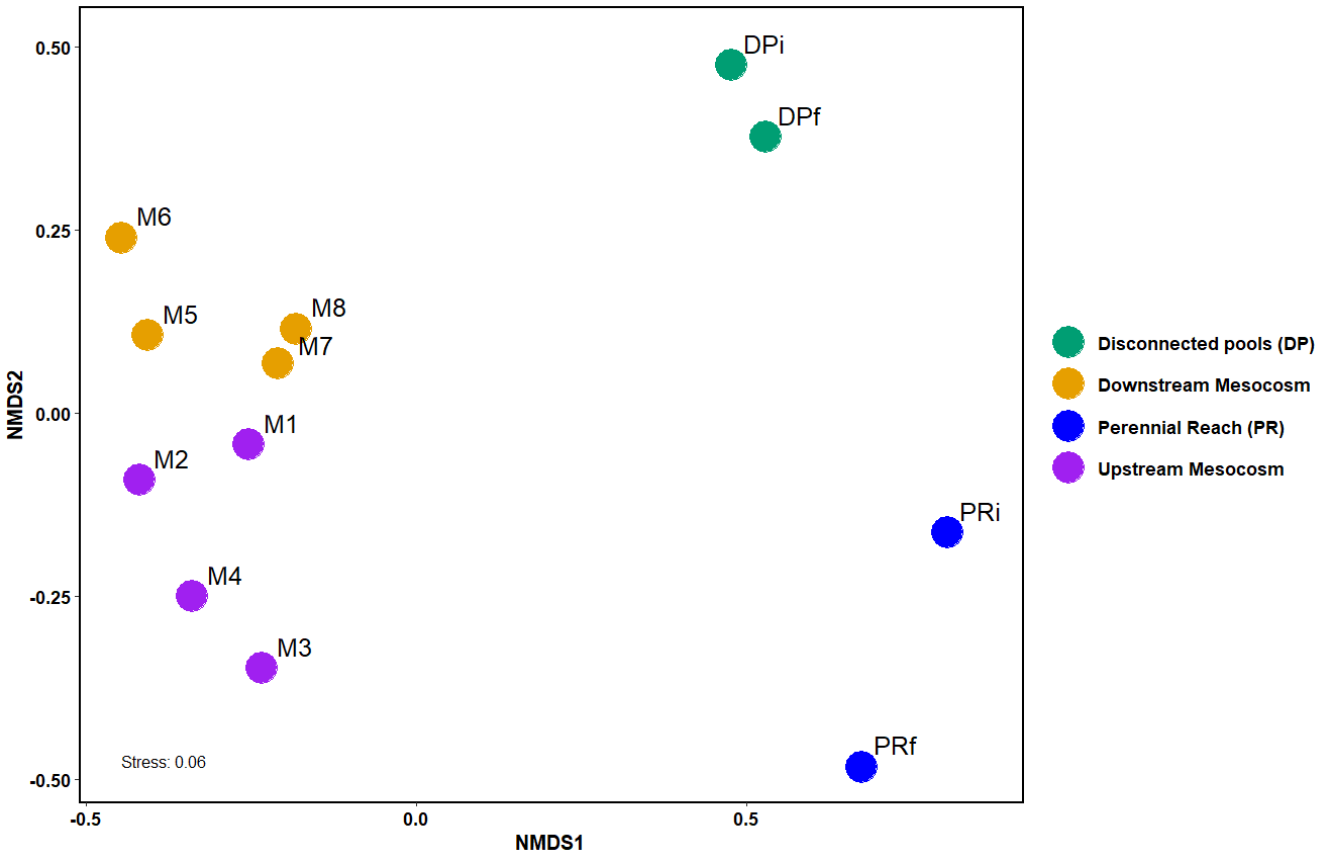


Figure 8. NMDS showing samples separated by their position along the stream (source, upstream, or downstream) and habitat type (natural pool or mesocosm). DPi: natural disconnected pools at the beginning of the experiment; DPf: natural disconnected pools at the end of the experiment; PRi: natural perennial reach at the beginning of the experiment; PRf: natural perennial reach at the end of the experiment. Mesocosms are labeled with their respective codes (M1, M2, M3, etc.).

The Mantel test indicated a positive but not statistically significant relationship of the Bray-Curtis community dissimilarity with environmental distance (Mantel statistic r = 0.37; p value = 0.07) and geographic distance (Mantel statistic r = 0.24; p value = 0.06) with (Figure 9).

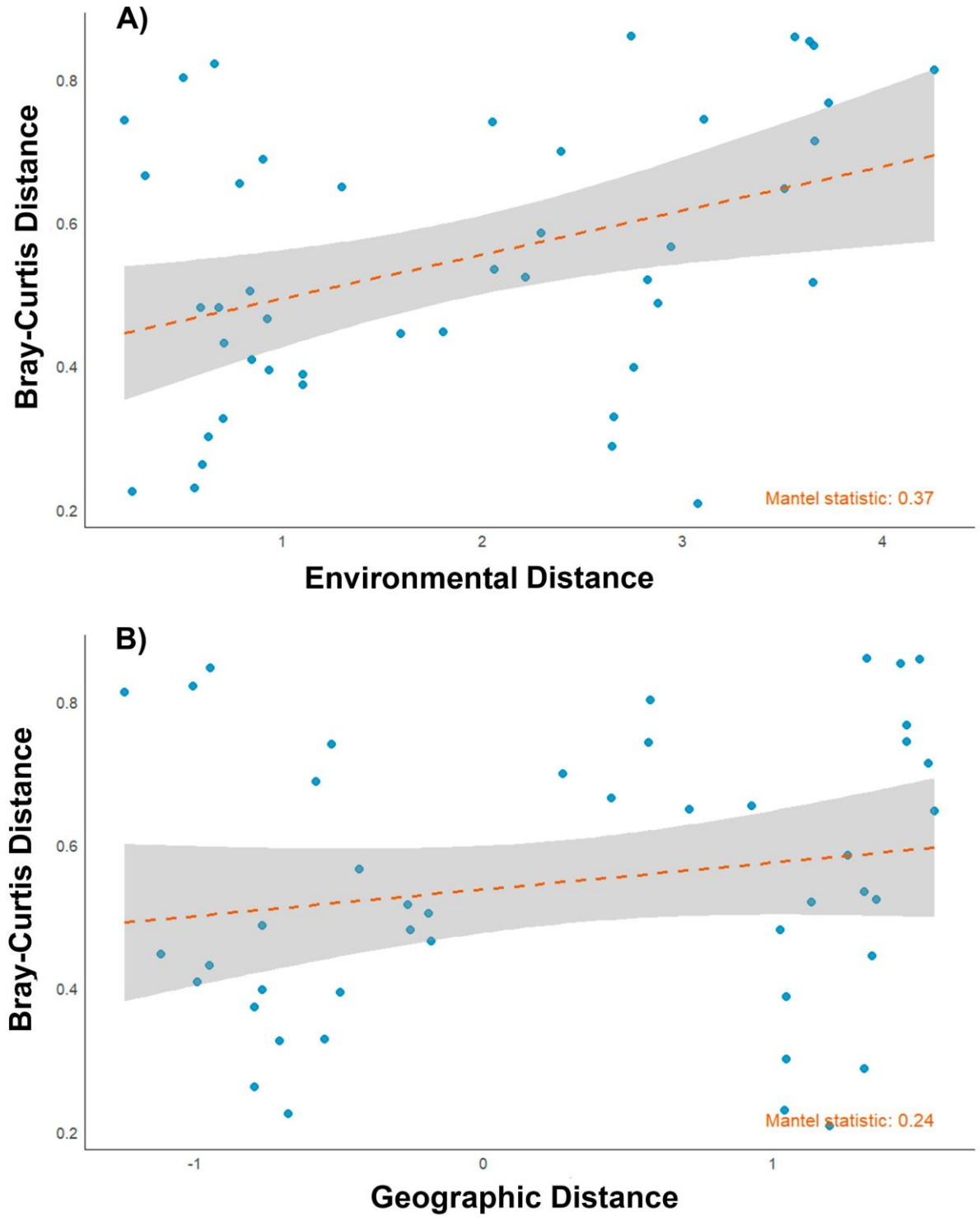


Figure 9. Correlation of community dissimilarity (Bray-Curtis distance) with environmental **(A)** and geographic **(B)** distances. No significant correlations were found.

DISCUSSION

Overall, our study provides strong evidence for the role of DPs as a crucial sources of aerial dispersers in TRs. Specifically, our findings support H1, demonstrating that isotopic enrichment effectively tracked macroinvertebrate dispersal, as enriched organisms were captured using the various sampling methods employed (i.e., kick sampling, emergence, and flight interception traps). More than half (53%) of the collected Iso-samples were enriched, this percentage being significantly higher than those reported for stonefly populations in headwater streams (5% and 8%) by Briers *et al*. (2004) and Macneale *et al*. (2005), but lower than the 83% observed by Enss *et al*. (2024) for their 12 taxa. As we hypothesized in H1, aerial adults (e.g., Ephemeroptera, Diptera) reached their peak ^{15}N levels later than semiaquatic adults (e.g., Heteroptera, Coleoptera). This pattern is consistent with the trophic levels occupied by the representative species within these biological categories. Given that we only collected adult stages in emergence traps, larval stages of grazers—primarily Ephemeroptera and Diptera—first become enriched by feeding on ^{15}N -labeled biofilms (Enss *et al*., 2024). Subsequently, the predatory stages of semiaquatic adults, such as Heteroptera and Coleoptera (e.g., *Hydrometra stagnorum* (Hemiptera: Hydrometridae), *Agabus* sp., *Graptodytes* sp.), feed on these enriched larvae, thus becoming enriched earlier than aerial adults. Finally, the enriched larvae of aerial adult species emerge as adults (e.g., *Cloeon* spp, Chironomidae) with higher levels of ^{15}N enrichment compared to their semiaquatic adult counterparts. In addition, the elapsed time from ^{15}N addition to sample collection was consistent with the phenology of these species (*C. dipterum*, *C. simile*, *Caenis luctuosa*, and Chironomidae), which exhibit multivoltine life cycles (i.e., two or more generations per year) with two short summer generations and one longer in winter (Cayrou & Céréghino, 2003, 2005; Płóciennik *et al*., 2018), a pattern that is even more pronounced in semiarid regions like our study area (Perán *et al*., 1999).

Our results support H2, as the greater enrichment of semiaquatic adults compared to aerial adults can be attributed to the immigration of aerial adults from nearby non-enriched small pools or rain-formed puddles during the experiment, as well as to the dilution of the isotopic signal in aerial adults. For instance, generalist species like mosquitoes (Culicidae) can rapidly colonize mesocosms (Bogan & Boersma, 2012) because their multivoltine life cycles

and their ability to breath aerially allow them to survive in puddles or small pools (Bonada *et al*., 2020; Foster & Walker, 2019). However, species more adapted to lentic environments (e.g., Heteroptera, Coleoptera), with larger body sizes, need greater habitat complexity, larger water bodies, and more time to develop a mature community (Bonada *et al*., 2020). Thus, the semiaquatic adults colonizing the mesocosms primarily originated from the enriched DPs, the only mature DPs in the surrounding area. Surprisingly, larvae and aquatic obligate organisms in the mesocosms were also enriched to a great extent. The presence of enriched larvae indicates the transfer of the isotopic tracer to the next generation, allowing us to track dispersal across generations. Due to the rapid life cycles in summer, which can be completed in a few weeks (Cayrou & Céréghino, 2003, 2005), and the ability of some species, like *C. dipterum*, to reproduce through ovoviviparity (Brittain, 1982), the duration of this experiment was likely sufficient to detect the isotopic tracer in the larvae of the subsequent generation. While labeling methodologies have been tested for single species or communities, they have typically been applied within the same individuals or generations (Briers *et al*., 2004; Enss *et al*., 2024; Macneale *et al*., 2005). Our findings highlight the potential of isotopic tracking for studying dispersal through oviposition and the recolonization of new habitats during and after the non-flowing phase.

Despite the short duration of the experiment and the inability of aquatic obligate species to fly, water mites, Mollusca, Copepoda, and Ostracoda unexpectedly colonized the mesocosms from the enriched DPs. We found that water mites reached mesocosm attached to adults of chironomids and mosquitoes. Water mites have an ectoparasitic larval stage on nematocerous flies, which provides them with nutrition and aids in dispersal (Santos *et al*., 2016; Smith *et al*., 2010). Ectoparasitic water mite larvae feed on host fluids, while the adult and some nymph stages are typically predators of invertebrates (Dhooria, 2016; Smith *et al*., 2010). Therefore, the enrichment of water mites could occur through the fluids of enriched adult flies or by preying on other enriched invertebrates in the mesocosms. On the other hand, enriched snails, which could not be further identified due to their short development time and small size in the mesocosms, along with Copepoda and Ostracoda, likely reached the mesocosms through passive transport via zoochory. For instance, waterbirds are excellent dispersal vectors for a wide range of organisms in freshwater ecosystems, while mammals can disperse propagules attached to their bodies (epizoochory) (Green *et al*., 2023; Van

Leeuwen *et al*., 2013). In our study area, Quevedo-Ortiz *et al*. (2024) reported, using camera traps near the mesocosms, the presence of several wild mammals around the study area during the experiment, including wild boar (*Sus scrofa*), squirrel (*Sciurus vulgaris*), beech marten (*Martes foina*), and field mouse (*Apodemus sylvaticus*), as well as roe deer (*Capreolus capreolus*) and weasel (*Mustela nivalis*). In addition, Quevedo-Ortiz *et al*. (2024) empirically demonstrated the dispersal of algae (diatoms) by domestic dogs in the area. These findings support the idea that wild and domestic fauna could also act as passive dispersers of small and aquatic obligates macroinvertebrates (e.g., Copepoda, Ostracoda, Mollusca, small insects) (Allen, 2007; Incagnone *et al*., 2015), potentially playing a crucial role in their dispersal and recolonization of habitats during the non-flowing phase in fragmented freshwater systems such as TRs.

Regarding the dispersal distance, H3 was confirmed, as enriched macroinvertebrates were found throughout the entire study area, covering almost one kilometer. This suggests that isolated DPs can serve not only as refuges but also as sources of propagules capable of recolonizing surrounding waters during and following dry periods. As expected in H3, the further the distance from the enriched DPs, the lower the proportion of macroinvertebrates that colonized them. This aligns with the findings of Briers *et al*. (2004) and Macneale *et al*. (2004, 2005), who observed this pattern in headwater stonefly populations in perennial rivers. In our study, dispersal from DPs appeared to occur both downstream and upstream, with no clear directional pattern. Furthermore, we observed distinct patterns when analyzing different taxonomic orders. Coleoptera exhibited a stepwise dispersal pattern from DPs, mirroring the general trend. It is likely that the overall community dispersal patterns are heavily influenced by Coleoptera, as this order accounted for the largest proportion of taxa richness in the community Iso-samples (~40%). The stepwise enrichment (i.e., lower enrichment as we moved away from the enriched DPs) showed by beetles may be attributed to the directed aerial active dispersal between the DPs (where they fed and became enriched), and the mesocosms where they seek suitable habitats (e.g., food, lower competition pressure). On the other hand, Ephemeroptera showed a weak tendency for upstream dispersal. Some aquatic insects are well known to compensate for drifting downstream by flying upstream in streams (May, 2019; Müller, 1982), a behavior that has been the main colonization pathway of some mayflies in perennial headwaters (Galatowitsch

& Batzer, 2011). For example, Flecker & Allan (1988) demonstrated that female *Baetis* sp. (Ephemeroptera: Baetidae) exhibit a clear upstream flight pattern. Flies (e.g., Chironomidae, Culicidae, Ceratopogonidae) and aquatic obligates taxa (Mollusca, water mites, Copepoda and Ostracoda) typically rely on passive dispersal strategies, which depend on weather conditions such as temperature, humidity, and wind direction (Vebrová *et al*., 2018) or passive transport via zoochory (as stated above) (Green *et al*., 2023). This can explain the more stochastic enrichment patterns (e.g., upstream and downstream of the enriched DPs) shown by these taxa in our study. Moreover, few studies have analyzed isotopic enrichment across multiple aquatic macroinvertebrate taxa. For example, Enss *et al*. (2024) conducted community-level enrichment with 12 macroinvertebrate taxa, but their measurements focused primarily on downstream enrichment, detecting marked invertebrates up to 2 km downstream from the addition point. In contrast, our study was limited in downstream distance, as the furthest mesocosm was located 564 m downstream, where we found enriched beetles (*Hydraena* spp.). The low proportion of enriched Iso-samples in downstream mesocosm (M7 and M8) can be explained by their proximity to the non-enriched perennial reach located further downstream that could serve as an alternative colonizing source. Upstream of the experimental site, the stream is highly ephemeral and hydrologically isolated, lacking permanent pools due to steep mountainous terrain that prevents stable water formation. The nearest perennial streams are located downstream, making overland dispersal unlikely except from adjacent valleys, where only temporary rain-induced puddles may provide limited additional habitat.

Regarding the community composition, as stated in H4, we observed distinct clustering between mesocosms and DPs. Additionally, mesocosm communities showed clear differentiation based on their upstream or downstream position. This aligns with the stepwise dispersal pattern of enrichment discussed earlier. The fact that the mesocosms had a different community composition when compared with DPs is most likely related with the fact that they do not replicate the habitat complexity of DPs and the duration of the experiment was not long enough to allow the establishment of a mature community (Bogan & Boersma, 2012; Cáceres & Soluk, 2002).

Contrary to our hypothesis H5, neither environmental factors nor spatial distance from DPs appeared to play a significant role in structuring the mesocosm communities. This

may be explained by the relatively short length of the study area (less than 1 km), which likely prevented dispersal limitations (Heino *et al*., 2015). Nonetheless, despite the physico-chemical conditions of the water were uniform across the mesocosms at the start of the experiment, a weak relationship with community composition emerged over time. This could be due to changes in the biogeochemical conditions within the mesocosms, driven by both abiotic and biotic factors. Biotic factors may have influenced the chemical conditions throughout the experiment due to the colonization of microorganisms such as green algae and diatoms, in addition to the presence of macroinvertebrates (Bonada *et al*., 2020; Quevedo-Ortiz *et al*., 2024). Furthermore, abiotic conditions may vary between mesocosms due to differences in factors such as organism abundance (i.e., biotic factors) and canopy cover. These variations can affect the accumulation of organic detritus (e.g., leaves from adjacent trees), light availability, and, consequently, the levels of dissolved oxygen and temperature (Fellman *et al*., 2011). In fact, the canopy cover of our mesocosms varied widely, ranging from 31% to 93%, and, similarly, the dry weight of organic matter showed considerable fluctuation. These variations may be a primary factor influencing community structure, as they can alter physico-chemical conditions and provide refuges for macroinvertebrates.

Our results highlight the importance of aerial dispersal processes and local environmental factors in shaping community composition during the recolonization process in TRs throughout the non-flowing phase. The recovery of communities during and after dry disturbances is influenced by both local and regional factors. Local factors include the intensity of drying periods (e.g., number of zero-flow days) and habitat conditions (e.g., presence of a hyporheic humid zone in alluvial debris), while regional factors involve site isolation and species pool dispersal abilities (Datry *et al*., 2017c; Stubbington *et al*., 2017). Regional climate also plays a key role in shaping recovery after flow resumption (Bogan *et al*., 2017). For instance, in desert climate systems with prolonged dry periods, as well as in mountain streams experiencing intermittency, hyporheic refuges facilitate faster community recolonization. However, species richness recovers more slowly through drift or aerial colonization compared to alluvial river systems in temperate climates (Bogan *et al*., 2015, 2017; Bruno *et al*., 2020). In our study area, Folch (2020) conducted a sediment rewetting experiment to quantify the contributions of the hyporheic zone and seedbank to community

recovery after drying. The experiment found no contribution from the hyporheic zone, likely due to the stream characteristics (i.e., dominated by rocky substrates ranging from exposed bedrock to thin sediment layers) resulting in a reduced or negligible hyporheic zone and a low abundance of drying-resistant species. Similar patterns were observed by Chester & Robson, (2011) in Mediterranean-climate rivers in southern Australia, where bedrock dominance and a lack of hyporheic zones were associated with lower macroinvertebrate richness. However, studies in other stream of the northeastern Iberian Peninsula have demonstrated that both the seedbank and hyporheic zone can contribute to community recovery after rewetting, although the taxa present are often subsets of those found in benthic communities (Viza *et al*., 2023). Given that our study focuses on aerial dispersion, these findings support our hypothesis that the geological characteristics of our stream—rather than just the regional climate—favor aerial dispersal as the primary mechanism for recolonization from nearby permanent refuges in TRs.

Fournier *et al*. (2023) showed how the relative importance of different recolonization pathways—including aerial dispersal, downstream drift, upstream locomotion, and vertical substrate migration, as outlined by Merritt & Cummins (1996)—varied along a gradient of isolation from permanent water, finding that isolation significantly influences the relative contribution of these pathways to post-drying community composition. For instance, these authors suggest that resistance strategies increase in relative importance as the distance from permanent water increases, while resilience strategies (e.g., drift or aerial colonization) become more significant in closer proximity to rewetted habitats in downstream direction. These findings align with our results, which indicate that distance is a key driver for the taxa colonizing the mesocosms. However, we observed these patterns in both downstream and upstream directions from the DPs source, in contrast to the predominantly downstream dispersal reported in rewetting experiments (Fournier *et al*., 2023). This difference likely arises because, unlike rewetting studies where flow resumption drives colonization, our system remained patchily dry, presumable forcing individuals to disperse in multiple directions looking for available habitats (Cañedo-Argüelles, *et al*., 2015).

While our research has made significant contributions to current knowledge, certain limitations should be noted. Firstly, our experiment was conducted in only one stream with a reduced length. An experiment involving multiple TRs—some with permanent upstream

sections and others with permanent downstream sections—would likely reveal more robust patterns. Additionally, extending the length of the stream could facilitate the assessment of macroinvertebrate dispersal over greater distances. Secondly, the study period was relatively short. We conducted the experiment in late summer when the stream had dried up, resulting in a typical DPs community. The study could not be extended further due to anticipated rainfall in early autumn and the risk of mesocosms being washed away. Extending the experiment across an entire hydrological year would provide valuable insights into how aerial dispersal patterns change with varying environmental conditions and how these dynamics shape metacommunity structures over time. Thirdly, there was a lack of species-level identification for all taxa involved in the experiment. A greater taxonomic resolution, e.g., as identifying Mollusca or water mites at the species level, could enhance our understanding of their life cycles and natural histories, providing more precise information on when and where enrichment occurs. For example, in future studies, a deeper understanding of the species' biological cycles could help clarify whether water mites were enriched during their larval or adult stages, and provide more precise information on when enrichment occurs at each stage. In addition, it is important to note that our study was too short to fully capture the life-cycle duration of K-strategists such as some Coleoptera and Odonata, leading to an over-representation of R-strategists like Chironomidae. Consequently, we encourage further investigations to incorporate longer study periods and more temporal replicates, so the relationship between life-cycle duration, enrichment patterns and dispersal can be further assessed. Direct individual-level measurements of dispersal patterns are still underutilized, while many studies rely on indirect dispersal methods, such as database-based assessments of aerial dispersal in TRs.

Our findings should be considered when planning management and conservation actions in TRs. Recognizing the role of aerial dispersal from DPs and their function as refuges during the non-flowing phase is critical for protecting freshwater biodiversity and identifying key conservation priorities in TRs (Bonada *et al*., 2020; Bruno *et al*., 2022; Fernández-Calero *et al*., 2024). Furthermore, our study underscores the importance of resilience and ecological functioning during the non-flowing phase within TR metacommunities, where species must navigate both temporal and spatial environmental variability (Cunillera-Montcusí *et al*., 2023). Given the impacts of climate and land-use changes, TRs are expected to become more

prevalent, particularly in vulnerable regions such as those with Mediterranean climates (Döll & Schmied, 2012; Pumo *et al*., 2016). As a result, the importance of DPs as refuges will increase, helping to preserve regional biodiversity in drying stream networks. These DPs serve as vital sources of propagules, supporting viable populations of macroinvertebrates throughout the dry season and facilitating their recolonization when flow resumes in the river network (Bonada *et al*., 2020; Hill & Milner, 2018). This function is especially crucial for obligate aquatic fauna, such as fish, that rely on DPs to survive non-flowing phases. For example, in our study region, species like *Barbus haasi* have been shown to depend on these refuges to persist during dry periods (Aparicio & De Sostoa, 1999). Based on our results, DPs could serve as nature-based solutions in drying stream systems by strategically maintaining or enhancing their presence along river networks to function as refuges, thereby supporting regional biodiversity conservation. However, caution is warranted, as some studies highlight potential negative impacts of water retention structures on freshwater ecosystems, including degraded water quality and increased spread of invasive species (Morden *et al*., 2022). Thus, incorporating DPs into management and conservation actions requires careful context-specific evaluation within drying river networks to effectively preserve freshwater biodiversity.

AUTHORS' CONTRIBUTIONS

Conceptualisation: José María Fernández-Calero, Daniel von Schiller, Núria Cid, Miguel Cañedo-Argüelles and Núria Bonada. Developing methods: José María Fernández-Calero, Daniel von Schiller and Núria Bonada. Conducting the research: José María Fernández-Calero, Daniel von Schiller, Guilermo Quevedo-Ortiz, Anna Ticó Pifarré, Miguel Cañedo-Argüelles and Núria Bonada. Data analysis: José María Fernández-Calero. Data interpretation: José María Fernández-Calero, Daniel von Schiller, Núria Cid, Miguel Cañedo-Argüelles and Núria Bonada. Preparation of figures and tables: José María Fernández-Calero. Writing: José María Fernández-Calero, Daniel von Schiller, Anna Ticó Pifarré, Núria Cid, Miguel Cañedo-Argüelles and Núria Bonada.

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CHAPTER 5

Integrating spatiotemporal hydrological connectivity into conservation planning to protect temporary rivers

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ABSTRACT

Temporary rivers (TRs) have been largely overlooked in conservation assessments. Because TRs are such dynamic ecosystems, spatial and temporal aspects of their hydrology and ecology need to be taken into account when designing conservation plans. The aim of this paper is to propose a set of recommendations that could be useful for managers to do this, using seasonal diatom and macroinvertebrate data from north-eastern Spain as a case study. Beta diversity was partitioned into local and species contributions to beta diversity (LCBD and SCBD). Additionally, priority conservation sites covering the spatial distribution of all species were identified using Marxan and the selection frequency (MSF) of the sites served as a measure of the relative irreplaceability of each site. Using both approaches (beta diversity and Marxan), the effects of changing spatiotemporal connectivity and habitat heterogeneity on the selection and prioritization of sites to be conserved were assessed. It was found that LCBD and MSF ranged widely both in space and time. However, LCBD and MSF were weakly related. Marxan adequately represented all taxa by selecting a few sites, while LCBD selected communities with higher differentiation but not necessarily those with rare species. In addition, SCBDs assigned low values to rare taxa, thus care must be taken when using this index for conservation planning. Spatiotemporal connectivity and local habitat heterogeneity played a critical role at the regional and local scales, driving site prioritization. Overall, we recommend: 1) monitoring multiple hydrological phases to encompass the different community types and capturing total diversity; 2) using Marxan and LCBD in combination, to benefit from their complementary insights; and 3) integrating spatiotemporal isolation and habitat heterogeneity into conservation plans, since they are the main drivers of community variation over space and time in TRs.

Keywords: Conservation planning, Marxan, aquatic macroinvertebrates, diatoms, temporary rivers, habitat connectivity, habitat heterogeneity, local contribution to beta diversity, species contribution to beta diversity

INTRODUCTION

Preserving freshwater biodiversity is one of the most pressing global challenges of our time (IPBES, 2019). Pressures on freshwater biodiversity and freshwater habitats have exponentially increased during the last decades, mainly due to habitat destruction, fragmentation, pollution, invasive species, and overexploitation (Harrison *et al*., 2018; Albert *et al*., 2021). Consequently, freshwater species are disappearing at alarming rates (over 83% have become extinct since 1970), faster than their marine and terrestrial counterparts (Harrison *et al*., 2018). This is expected to worsen in the future, as a result of climate change and increasing human water demands (Dudgeon, 2019). Within this context, there is an urgent need to improve current conservation efforts to address the particular management needs of freshwater ecosystems, mostly overlooked under current conservation priorities (Hermoso *et al*., 2016).

Temporary rivers (hereafter TRs) are generally described as watercourses that stop flowing in both space and time (Acuña *et al*., 2014; Datry, Bonada & Boulton, 2017b; Datry *et al*., 2018), and are also known as non-perennial, intermittent, ephemeral, or episodic. TRs are one of the most common freshwater habitats in the planet (Messager *et al*, 2021), and they are expected to become even more widespread in the future due to water scarcity (Kumar *et al*., 2014; Mekonnen & Hoekstra, 2016; Vliet *et al*., 2021). TRs are characterised by shifting wet and dry phases (Gallart *et al*., 2012), which change spatiotemporal hydrological connectivity (Cunillera-Montcusí *et al*., 2023), constraint species dispersal, and affect metacommunity dynamics (Cañedo-Argüelles *et al*., 2020). This dynamism can have challenging implications for the conservation and biomonitoring of TRs (Datry *et al*., 2023) because available habitats and species to be potentially protected and/or used as bioindicators change dramatically in space and through time. Additionally, TRs are among the most damaged and vulnerable freshwater habitats in the world (Datry *et al*., 2014a; Skoulikidis *et al*., 2017; Fovet *et al*., 2021). Despite some efforts have been done in some parts of the world (e.g., partial recognition in the EU Water Framework Directive and Habitats Directive; Fritz, Cid & Autrey, 2017), they still remain neglected by policy-makers (Leigh *et al*., 2015; Datry *et al*., 2023), and conservation plans have largely ignored them (Bruno *et al*., 2022). The few existing studies on the conservation of TRs argue that priority sites for

conservation can vary over time in these ecosystems (Ruhí, Datry & Sabo, 2017; Rodríguez-Lozano *et al*., 2023). For example, when present, disconnected pools might play a vital role as biodiversity refugia during the dry season (e.g., fish or amphibians) (Hermoso, Ward & Kennard, 2013; Bonada *et al*., 2020), or can be used as stepping-stones habitats, favouring connections across the disconnected river network (Datry *et al*., 2018; Sánchez-Montoya *et al*., 2022). However, the integration of spatiotemporal changes of TRs into conservation plans remains neglected.

There are different conservation approaches that could be used to select and prioritise sites to be preserved. One of them is to partition beta diversity to obtain the relative contribution of sites or species as in Legendre & De Cáceres (2013). This framework allows assessing the relative contribution of each sampling unit to beta diversity, i.e., local contribution to beta diversity (LCBD), and the relative contribution of each species to beta diversity (SCBD). Considering a set of sites (i.e., regional scale), LCBD is an indicator of the biological uniqueness of each site, i.e., a very different community composition compared with the regional mean. Thus, large values of LCBD could be considered as indicators of priority sites for conservation, as they reflect unusual species combinations or species-poor sites with restoration needs (high LCBD and low alpha diversity) (Legendre & De Cáceres, 2013; Legendre, 2014). The LCBD value identifies relatively unique communities in terms of their community differentiation, although not necessarily the communities with the rarest species. Therefore, setting conservation priorities based only on LCBD could lead to underrepresenting these rare species, which are often the most threatened ones. Importantly, LCBD values can change over time, reflecting metacommunity dynamics processes (i.e., habitats that may operate as sources, or sinks, to whole river networks) (Ruhí, Datry & Sabo, 2017). In turn, SCBD indicates species with high variation in abundance between different sites in a region. Usually, species with high SCBD values have a large, or moderate, occupancy across sites and high abundance, resulting in the most important species contributing to the uniqueness (LCBD) of the different sites (Heino & Grönroos, 2017). These beta partitioning approaches have increasingly been used for to identify conservation sites based on different organisms, such as diatoms and macroinvertebrates (Heino & Grönroos, 2017; Vilmi, Karjalainen & Heino, 2017; Rodríguez-Lozano *et al*., 2023), fish (Xia *et al*., 2022), terrestrial arthropods (Sánchez-Montoya *et al*., 2020), and terrestrial plants (Kirk *et al*., 2021).

An alternative approach for selecting and prioritising sites to be conserved are systematic planning (Margules & Pressey, 2000), which have been used in rivers (Linke, Turak & Nel, 2011) and, only recently, in TRs (Bruno *et al*., 2022). Among these tools, one of the most widely used is Marxan (Ball, Possingham & Watts, 2009), which relies on the principle of complementarity to find the best combination of sites to be protected to meet a set of conservation targets at the minimum cost. Unlike LCBD-based approaches, Marxan may be used to identify priority sites based on a specific predefined target, rather than just considering sites very far from the median community composition in a regional pool. Thus, Marxan-based conservation planning may be particularly beneficial when community composition (and thus centroids) vary strongly over space and time and the main conservation target is total regional gamma diversity.

In this study, we aim to propose a set of recommendations that can be useful for managers when designing conservation plans in TRs. To do so, the above-mentioned conservation prioritisation approaches were used to identify priority conservation sites in TRs, and to assess the effects of changing spatiotemporal connectivity on the selection and prioritisation of selected sites to be managed. Since connectivity plays an important role in structuring aquatic communities in TRs (Cid *et al*., 2022), the focus was on two groups of organisms with contrasting dispersal abilities: diatoms and macroinvertebrates. Diatoms are widely dispersed (Kristiansen, 1996; Bogan *et al*., 2017) by water flow, wind, or animal vectors such as mammals and birds (Romero *et al*., 2003; Liu *et al*., 2013; Leone *et al*., 2014; Manning *et al*., 2021). Macroinvertebrates have two main dispersal modes (Bilton, Freeland & Okamura, 2001): i) active dispersers (e.g., adult dragonflies) can reach very long distances and they usually fly oriented upstream (Bogan & Boersma, 2012; French & McCauley, 2019); whereas ii) passive dispersers (e.g., larvae of non-biting midges) might drift downstream (Brown *et al*., 2011; Sarremejane *et al*., 2020a) or restrict their movements to the benthic zone (Kappes & Haase, 2012).

Firstly, we hypothesised that priority conservation sites and species change across seasons in response to changes in hydrological conditions, because drying determines biodiversity and habitat availability (Leigh & Datry, 2017; Ruhí, Datry & Sabo, 2017). This would suggest that conservation actions should be adaptive and priority sites to be conserved should vary in space and time. Secondly, we hypothesised that both conservation approaches

mentioned above are complementary. Whereas LCBD can identify sites with unique species composition, systematic planning tools are based on the complementarity principle and, therefore, these sites might not necessarily be selected. In addition, SCBD is not designed to select rare species but those that occur in a moderate number of sites in high abundances or have narrow niche breadth (Pozzobom *et al*., 2020; Xia *et al*., 2022), whereas rare species could be used as a selection criteria in systematic planning tools. Thirdly, we hypothesised that environmental drivers behind selected sites could inform us whether the conservation of local habitat or spatiotemporal connectivity should be preserved (or enhanced) to ensure current conservation status. For example, when local habitat variables are relevant, measures to enhance habitat diversity or to improve water quality should be implemented, whereas if drying is important, measures should consider solutions that reduce its effects and increase connectivity (Datry, Bonada & Heino, 2016a).

METHODS

Study area

The study was carried out in the Sant Llorenç del Munt i l'Obac Natural Park, a 140 km^2 protected area in northeast Spain with a typical Mediterranean climate (i.e., dry summers and heavy rains during autumn). The geology of the area is mainly karstic with permeable substrates (Anglés *et al*., 2017), but with abundant exposed bedrock in some areas. The flora of the natural park is typically Mediterranean, dominated by evergreen oaks (Q*uercus ilex*) and several species of pines (*Pinus halepensis*, *P. nigra,* and *P. sylvestris*). Some of the main species of shrubs are the common box (*Buxus sempervirens*) or heath species (*Erica* spp.). Two species of native barbels (*Barbus haasi* or *B. meridionalis*, depending on river basin) inhabit the study area, as well as the native *Squalius laietanus,* and the invasive exotic cyprinid (*Phoxinus septimaniae* x *P. dragarum)* (Corral-Lou *et al*., 2019). Thirty sites were sampled distributed among seven TRs: Vall d'Horta (H), Sanana (SA), Santa Creu (SC), Rellinars (R), Talamanca (T), Castelló (CA) and Mura (MU). These TRs covered a wide hydrological gradient, with Flow Permanence values (i.e., percentage of days with wet reaches in each site, FP) ranging from 33.14% to 99.80% for the 513 monitored days (from July 2018 to December

2019) (see Supporting Information S2 for all FP values) (Pineda-Morante *et al*., 2022). The samples were collected once per season during 2019: winter (January), spring (May), summer (July), and autumn (November).

Diatom and macroinvertebrate data

Diatoms were sampled by brushing the surface of three submerged stones with a toothbrush. The samples were preserved in the freezer using 70% ethanol. Then, organic matter was removed with hydrogen peroxide at 100 ºC, rinsed with distilled water and prepared permanent slides with Naphrax ®. Afterwards, diatoms were identified to species level with a DIC Polyvar Zeiss microscope with a built-in camera, counting a minimum of 500 diatom valves per sample. Macroinvertebrates were collected along 100 metres reaches using a 250 µm mesh size hand net by kicking all the available habitats. Before sampling, each studied reach was carefully inspected to identify all the available habitats based on expert judgement (including different items, such as substrate type, flow conditions, and aquatic vegetation). The samples were preserved in 96% ethanol and stored them in the freezer until their sorting and identification. Likewise, all macroinvertebrates were identified and counted using a binocular stereoscope to the lowest possible taxonomic resolution (usually genus). Once all diatoms and macroinvertebrates were identified and counted, a logarithmic transformation was applied (log x + 1) to abundances values of each taxon.

Environmental and hydrological data

The abiotic variables were measured once per reach in each season. Dissolved oxygen (DO mg/L), oxygen saturation (DO %), water temperature (T °C), pH, conductivity (EC µS/cm), specific conductivity (SPC µS/cm) and total dissolved solids (TDS mg/L) were measured using a multiparameter probe (YSI Professional plus Multiparameter Probe). A characterisation of the habitat heterogeneity was also conducted by calculating the "Fluvial Habitat Index" (IHF) (Pardo *et al*., 2002), which accounts for the embeddedness in riffles and sedimentation in pools (B1), the frequency of riffles (B2), the substrate composition (B3), the flow velocity and depth regimes (B4), the percentage of shade in the channel (B5), the presence of habitat heterogeneity elements (e.g., leaf litter, branches and woods, or roots) (B6) and the aquatic

vegetation coverage (B7). Presence/absence of water was registered from July 2018 to December 2019 using temperature data loggers deployed at each sampling site (Pineda-Morante *et al*., 2022). This information was used to calculate different hydrological metrics: the total number of dry days or duration of drying events (hereafter TotDur), the frequency of drying events or number of drying events (hereafter TotNum) and the average length of each drying event (hereafter TotLeng) (Crabot *et al*., 2020; Arias-Real *et al*., 2021; Pineda-Morante *et al*., 2022). In addition, the flow permanence was calculated as the percentage of the days that reaches were wet along two different time windows: 50 days before each sampling campaign (FP50), and 100 days before each sampling campaign (FP100). Two different time windows were considered, since organisms with different life cycles will be affected differently depending on when and how long sites were dried.

A presence/absence of water dataset was utilized to calculate spatiotemporal hydrological connectivity variables (hereafter *STcon*) for each sampling site, and considering the two scenarios proposed in Cunillera-Montcusí *et al*. (2023): a binary scenario (i.e., connection between sites as 1 and no connection as 0) and a weighted scenario (i.e., connection between sites weighted by the Euclidean distance between them). In addition, two types of network structures were considered for each of these two scenarios: directed (i.e., with an upstream to downstream directionality) and undirected (i.e., where all sites are connected between each other). Four *STcon* values were finally obtained: directed and binary, undirected binary, directed weighted and undirected weighted (DirBin, UndBin, DirWei, and UndWei, respectively). For binary scenarios, higher *STcon* values imply that a site has been highly connected with all its potential neighbours during the monitored time (i.e., high connectivity), whereas weighted scenarios with higher values imply that a site has been highly disconnected during the monitored time and it is located in an isolated position from its neighbours (i.e., high dispersal resistance). As distances between sampling sites for each stream were small, and streams were relatively straight, Euclidean distances could be used to weight both Binary and Undirected scenarios. See more details about *STcon* in Cunillera-Montcusí *et al*. (2023).

Beta diversity partitioning: LCBD and SCBD

Community uniqueness results from partitioning beta diversity indices between sites (LCBD) and species (SCBD). LCBD calculates the uniqueness of the community across several sites and indicates how far each individual site is with respect to the mean centroid sample. The sum of the LCBDs across all sites equals 1, being the highest values of LCBD corresponding to communities with different species composition (Legendre, 2014). Then, sites with significantly different LCBD values were identified (p-value < 0.05 after Holm correction for multiple comparisons; Holm, 1979; Borcard, Gillet & Legendre, 2011). LCBD values for each season were plotted to explore sites values temporal variation. Moreover, SCBD calculates the species contribution to beta diversity, which represents the relative contribution of each taxa to beta diversity. For this, a "rose plot" was plotted to visually explore the different contributions of each taxa to beta diversity and the relationship with occupancy and abundance. The "beta.div" function (Legendre & De Cáceres, 2013) in the R package "adespatial" (Dray *et al*., 2022) was used to calculate LCBD and SCBD, and the "ggplot" function in the "ggplot2" (Wickham, 2016) to plot the results of the analyses.

Systematic planning tools: MSF

To identify a minimum set of sites that collectively achieve a coverage target, the systematic planning tool Marxan was used (Ball, Possingham & Watts, 2009). Marxan relies on a simulated annealing optimization algorithm to identify a minimum set of sites that meet user-defined targets at a minimum cost. A representation target of 20% of the sites where each species was found was set (i.e., occupancy of each specie*0.2), keeping a minimum target of 1 for those taxa that were present in less than 5 sites. Given the high rate of taxa turnover in our dataset, with most taxa restricted to a few sites, high representation targets could only be achieved for all taxa at the same time by selecting almost all sites simultaneously. To address this problem, a bootstrap analysis was conducted (Bruno *et al*., 2022), by attempting to achieve the targets for a given proportion of all taxa each time. For this, 1000 random samples were selected containing 50% of the taxa from the total pool and identified the minimum set of locations to cover the representation targets explained above. This decision was based on the work of Bruno *et al*. (2022), who tested three different thresholds (50%,

75%, 90%) and found that the results were consistent across the different bootstrap thresholds used. Given the lack of estimates of conservation costs across the study area, a constant cost across all locations were used (e.g., Hermoso *et al*., 2021) and no connectivity penalty measure was included, similar to Bruno *et al*. (2022). With this configuration, Marxan was ran 100 times, using a million iterations each, and kept the solution with the lowest objective function score across those runs as our best solution, for each of the bootstrap selection of taxa. All best solutions were summarised by calculating the selection frequency of each location across the 1000 solutions, calling this variable Marxan Selection Frequency (MSF). In this way, the irreplaceability of each site at representing the taxa was measured, regardless of the particular combination of taxa selected in each of the 1000 bootstraps. As previously described above for LCBD, MSF values for each season were plotted to explore changes in space and time.

Finally, in order to compare results obtained by LCBD and MSF approaches, taxon richness per site was calculated using the "vegan" package (Oksanen *et al*., 2022), and compared values among seasons using a Kruskal-Wallis test. In case of differences between seasons, a Mann-Whitney-Wilcoxon test was conducted.

Environmental and hydrological models

Finally, all predictor variables were normalised (i.e., water chemistry, hydrology, habitat, spatiotemporal connectivity) using the "scale" function from R Base Package and tested their correlation between them using the function "findCorrelation" from "caret" R package (Kuhn, 2008). The Spearman correlation cut-off was set at 0.7 and then calculated the mean absolute correlation of each variable with the rest of the variables in the dataset, eliminating those with the highest mean absolute correlation. For the diatom data, a total of 16 variables were considered to test with LCBD and MSF. Previously, 7 variables were deleted (TotDur, TotNum, FP100, B6, EC, SPC and DO %) that had a high Spearman correlation. For the macroinvertebrate dataset, 6 of the 23 variables were eliminated due to their high Spearman correlation (TotDur, TotNum, FP100, EC, SPC and DO %), resulting in a total of 17 variables to test with LCBD and MSF.

In order to analyse the complementarity of LCBD and MSF, we compared data obtained by the two approaches and with richness using beta regression models for LCBD, and linear mixed-effect models (LMM) for MSF. All models included streams and sites as random effects, and season as fixed effect. For LMM, the "lme" function in the package "nlme" was used (Pinheiro *et al*., 2022), whereas for beta regression models, the "glmmTMB" function in the "glmmTMB" (Brooks *et al*., 2017) was employed. Finally, in order to determine the environmental drivers behind selected sites by LCBD and MSF, similar models were run between each approach and the predictor variables. Two different coefficients of determination were reported, denoted R^2. Marginal R^2 (R^2m) describes the proportion of variance of fixed factors and conditional R^2 (R^2c), that takes into account fixed and random effects (i.e., the total model) (Nakagawa & Schielzeth, 2013).

Rstudio 4.3.0 was used for all the analyses and Muff *et al*. (2022) was followed to report the results in the language of evidence.

RESULTS

A total of 55,654 diatoms belonging to 222 species and 144,846 macroinvertebrates belonging to 178 different taxa were collected (diatom species and macroinvertebrate taxa can be found in Appendix 1 and 2). Diatoms richness ranged between 14 and 65, with an average of 34. The Kruskal-Wallis test showed no significant difference in richness among seasons ($p = 0.141$). Macroinvertebrates richness ranged between 6 and 62, with an average of 34. The Kruskal-Wallis test showed strong differences in richness among seasons ($p < 0.001$). The strongest differences were found between autumn and the rest of the seasons (see Mann-Whitney-Wilcoxon test for further information in Supporting Information S1).

According to the Holm-corrected LCBD values, four sites strongly contributing to beta diversity which belonged to the same stream and two different seasons for diatoms were found (H1W, $p = 0.009$; H2W, $p = 0.009$; H3A, $p = 0.037$; H5A, $p = 0.037$). Four sites were also found for macroinvertebrates, belonging to three different streams and three different seasons (H5W, $p = 0.039$; MU2A, $p = 0.01$; SC2SP, $p = 0.01$; SC3A, $p = 0.03$). A high spatiotemporal variability in LCBD for both diatoms and macroinvertebrates was found (Figure 1). For example, some ephemeral sites (e.g., R1 and R2) were completely dry for most

of the year and LCBD ranged from 0 to 0.011, whereas some perennial reaches (e.g., H1) maintained very similar LCBD values throughout the seasons for macroinvertebrates (Figure 1 C-D). MSF also showed a very strong seasonal variation for both diatoms and macroinvertebrates. For example, for diatoms, only two sites (T3 and CA1) were selected by MSF during summer (T3 and CA1) (Figure 1B).

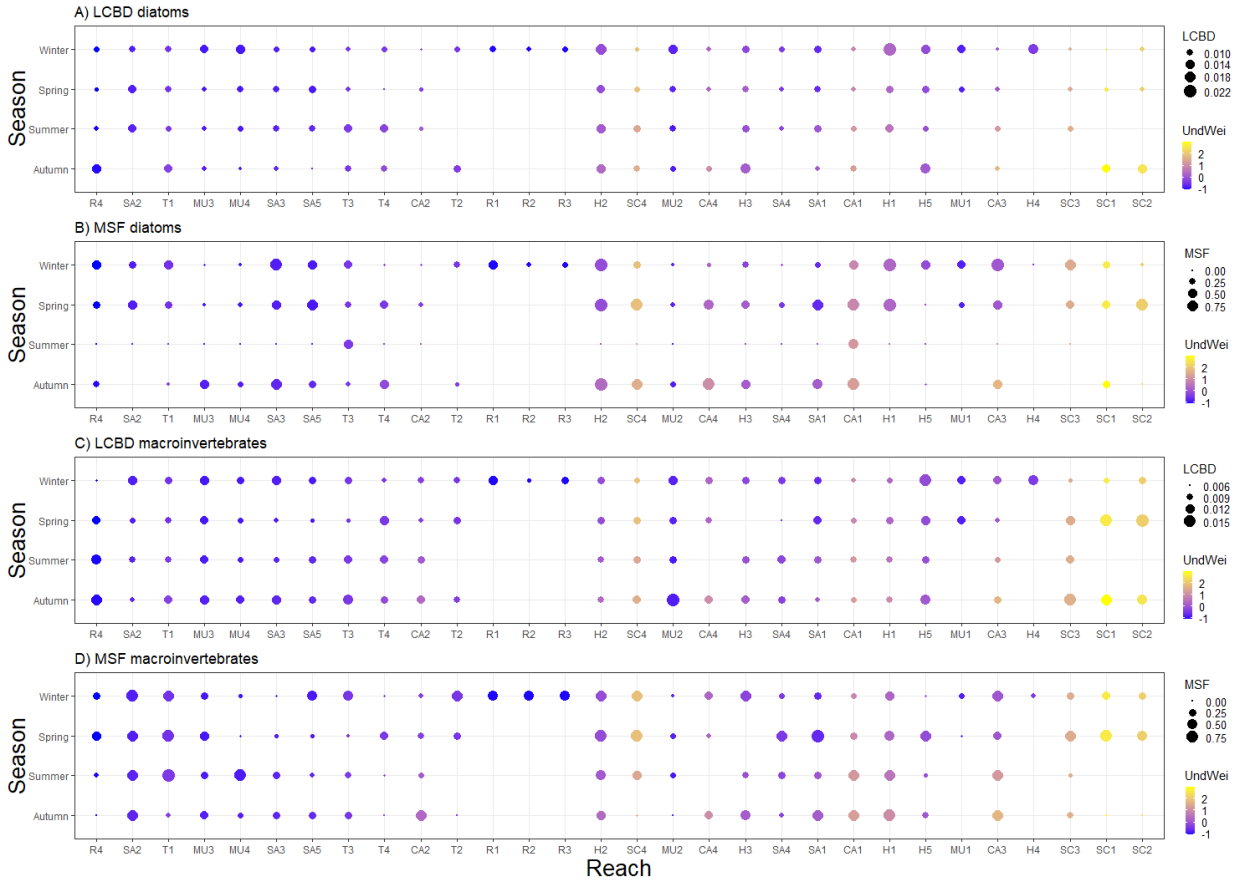


Figure 1. Bubble plots showing Local Contribution to Beta Diversity (LCBD) values and Marxan's Selection Frequency (MSF) for the four seasons and across sites. **(A-B)** Diatoms and **(C-D)** macroinvertebrates values. Bubble plot size represents LCBD and MSF values. The order of the reaches is in increasing order of dispersal resistance (UndWei) values.

No strong relationship was found between LCBD and MSF (diatoms: $p = 0.641$; $R^2m = 0.002$; $R^2c = 0.125$; macroinvertebrates: $p = 0.264$; $R^2m = 0.012$; $R^2c = 0.211$). LCBD showed a negative relationship with species richness, which was weak for diatoms ($p = 0.026$; $R^2m = 0.077$; $R^2c = 0.445$) and very strong for macroinvertebrates ($p < 0.0001$; $R^2m = 0.355$; $R^2c =$

0.411). Contrarily to LCBD, MSF showed a strong positive relationship with richness for both, diatoms ($p < 0.0001$; $R^2m = 0.298$; $R^2c = 0.304$) and macroinvertebrates; ($p < 0.0001$; $R^2m = 0.443$; $R^2c = 0.46$) (Figure 2).

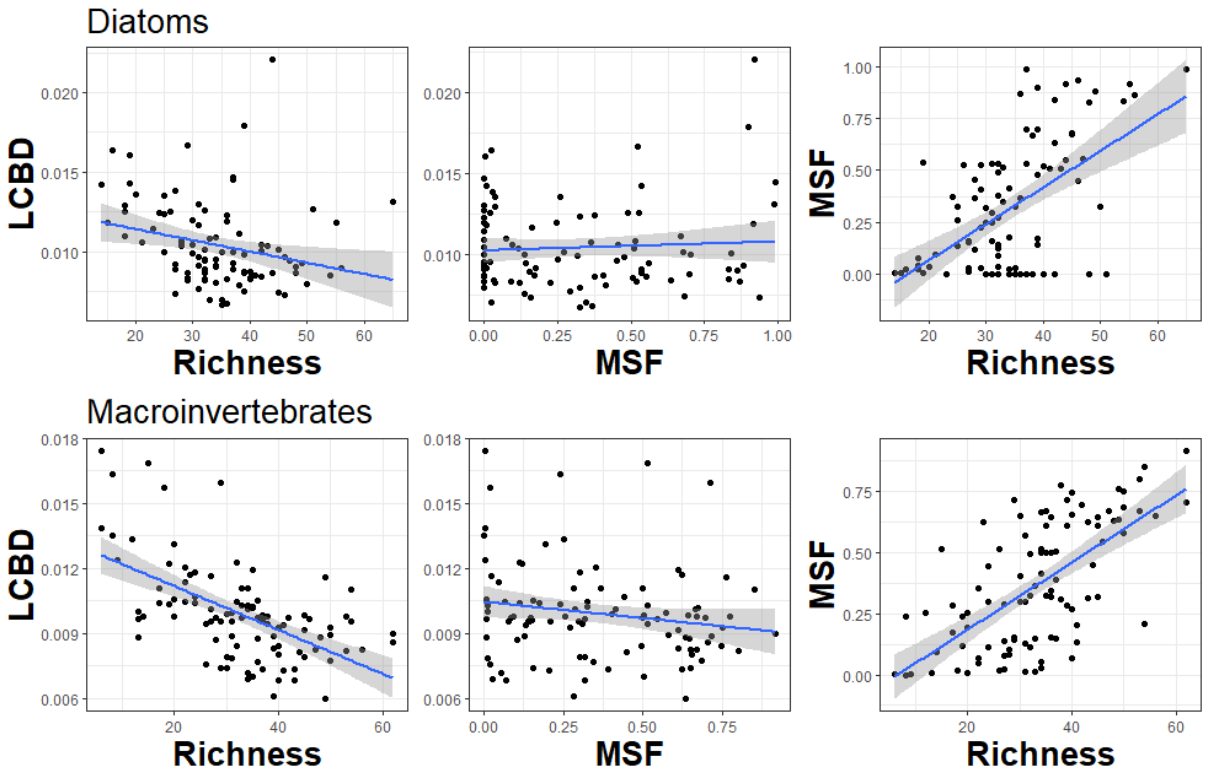


Figure 2. Relationship between Local Contribution to Beta Diversity (LCBD), Marxan's Selection Frequency (MSF) and taxa richness for diatoms and macroinvertebrates.

SCBD values ranged from 0 to 0.024 for diatoms, and from 0.0001 to 0.035 for macroinvertebrates. SCBD showed an unimodal relationship with species occupancy when presence/absence data was used (Figure 3 B-D), whereas it increased exponentially with occupancy when using abundances (Figure 3 A-C).

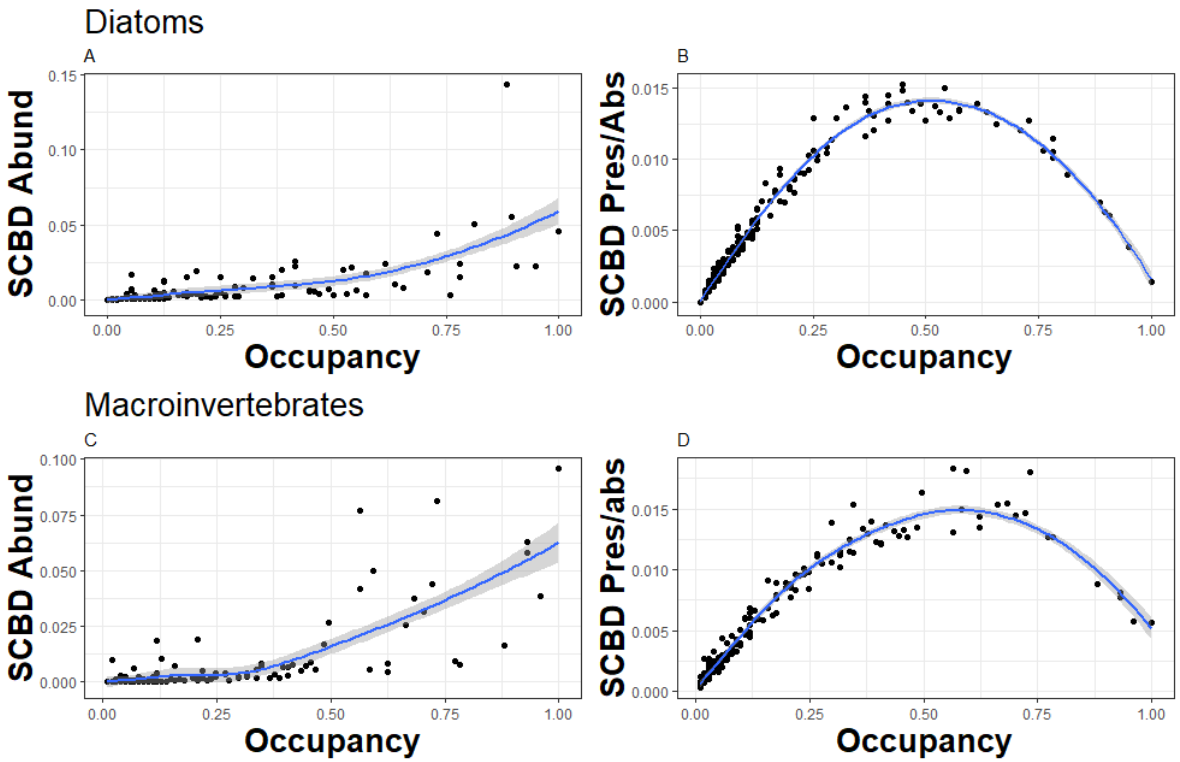


Figure 3. Relationship between occupancy and abundance or presence/absence data for diatoms **(A-B)** and macroinvertebrates **(C-D)**.

A great seasonal variability was found in the contribution of each taxa to beta diversity (Figure 4). For diatoms, several species showed high SCBD values in three seasons (e.g., *Brachysira neoexilis, B. neglectissima, Encyonopsis subminuta, Gomphonema lateripunctatum, G. tergestinum, Amphora pediculus* and *Achnanthidium pyrenaicum)* whereas other species showed high SCBD values for only one season (e.g., few *Fragilaria* spp. in winter, *Cocconeis placentula* in spring or *Navicula antonii* in autumn). In the case of macroinvertebrates there were seasonal variations in SCBD. For example, *Bidessus* exclusively contributed to beta diversity in spring, whereas *Habroleptoides* greatly contributed in winter. However, some taxa (e.g., *Dasyhelea* or *Cloeon*) greatly contributed to beta diversity across seasons (Figure 4). SCBD did not seem to be related to the rarity of the taxa, since some taxa that were exclusively found in ephemeral streams (e.g., *Tyrrhenoleuctra* and *Mesophylax*), showed very low SCBD values (0.003 and 0.001, respectively). In the case of diatoms, several species showed high SCBD values in three seasons (e.g., *Brachysira neoexilis, B. neglectissima,*

Encyonopsis subminuta, Gomphonema lateripunctatum, G. tergestinum, Amphora pediculus and Achnanthidium pyrenaicum) whereas other species showed high SCBD values for only one season (e.g., few *Fragilaria* spp. In winter, *Cocconeis placentula* in spring or *Navicula antonii* in autumn).

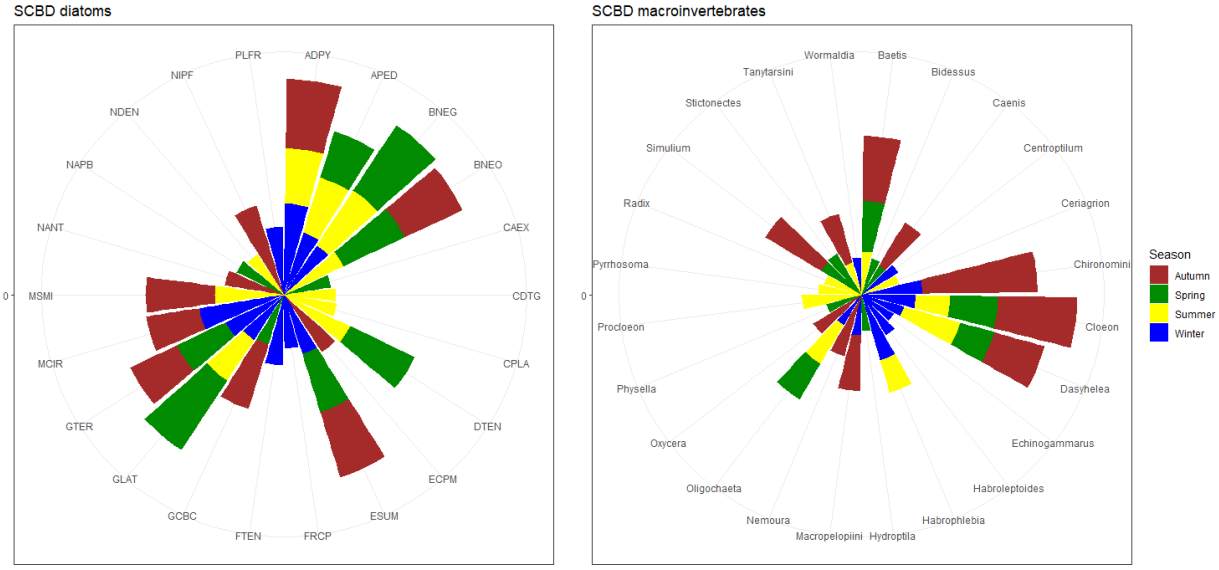


Figure 4. Rose plot of the most contributing taxa to beta diversity (SCBD) across seasons for diatoms and macroinvertebrates. Diatom species codes can be found in Appendix 1.

According to beta regression models using diatoms, LCBD did not show any relationship with the predictor variables, whereas MSF showed a strong positive relationship with two variables: dissolved oxygen (DO mg/L) ($F = 7.61$; $p = 0.007$) and temperature ($F = 7.81$; $p = 0.007$); and a weak relationship with two spatiotemporal connectivity metrics: connectivity (UndBin) ($F = 3.021$; $p = 0.087$) and dispersal resistance (UndWei) ($F = 3.559$; $p = 0.064$) (Figure 5) (for more information about the rest of the variables see Supporting Information S1). For macroinvertebrates, there was a strong relationship between LCBD and three habitat heterogeneity variables: embeddedness in riffles and sedimentation in pools (B1) ($Z = 2.98$; $p = 0.003$), frequency of riffles (B2) ($Z = -2.62$; $p = 0.008$), the substrate composition (B3) ($Z = 3.08$; $p = 0.002$). Other two variables related to spatiotemporal connectivity showed a moderate relationship with LCBD: directed weighted scenario (DirWei: $Z = 2.27$; $p = 0.023$) and undirected binary scenario (UndBin: $Z = -2.52$; $p = 0.012$). Finally, two

habitat heterogeneity variables showed a weak relationship with LCBD: flow velocity and depth regimes (B4) (Z = -1.86; p = 0.062) and aquatic vegetation cover (B7) (Z = -1.65; p = 0.098). For MSF using macroinvertebrates models only showed a strong relationship with aquatic vegetation cover (B7) (F = 10.37; p = 0.02) (for more information about the rest of the variables see Supporting Information S1).

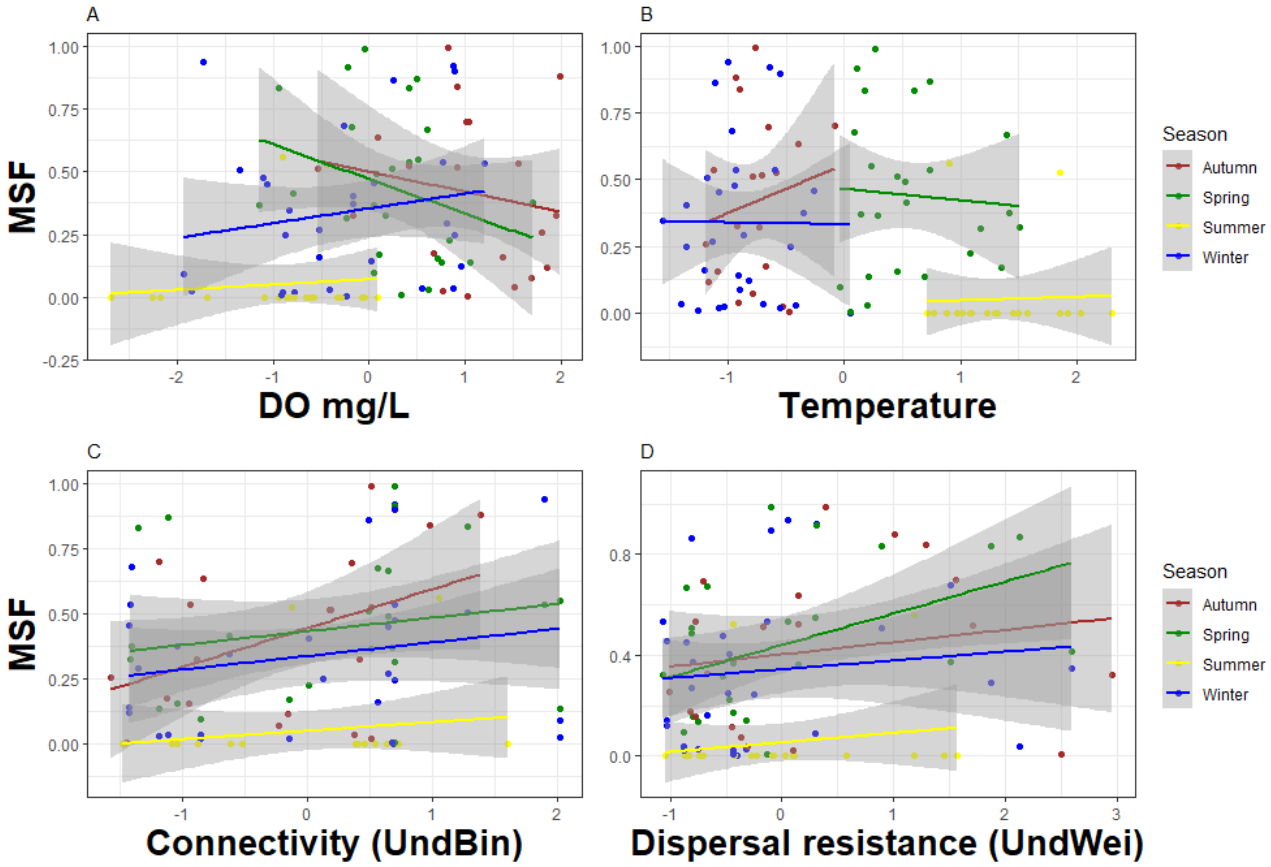


Figure 5. Relationships between predictor variables and Marxan's selection frequency (MSF) for diatoms with p-values < 0.1 in each season. DO mg/L refers to dissolved oxygen, temperature to the temperature on water, UndBin to the connectivity between each pair of reaches without any given direction, and UndWei to the dispersal resistance (or spatiotemporal isolation) between reaches not following a downstream direction.

The relationship of LCBD with the variables selected by the beta regression models experienced seasonal variations (Figure 6). For example, DirWei (i.e., the dispersal resistance, or spatiotemporal isolation, between reaches following a downstream direction) was positively related with LCBD in all seasons, whereas UndBin (i.e., the connectivity between each pair of reaches without any given direction) was negatively correlated with LCBD in all seasons except for winter (Figure 6E).

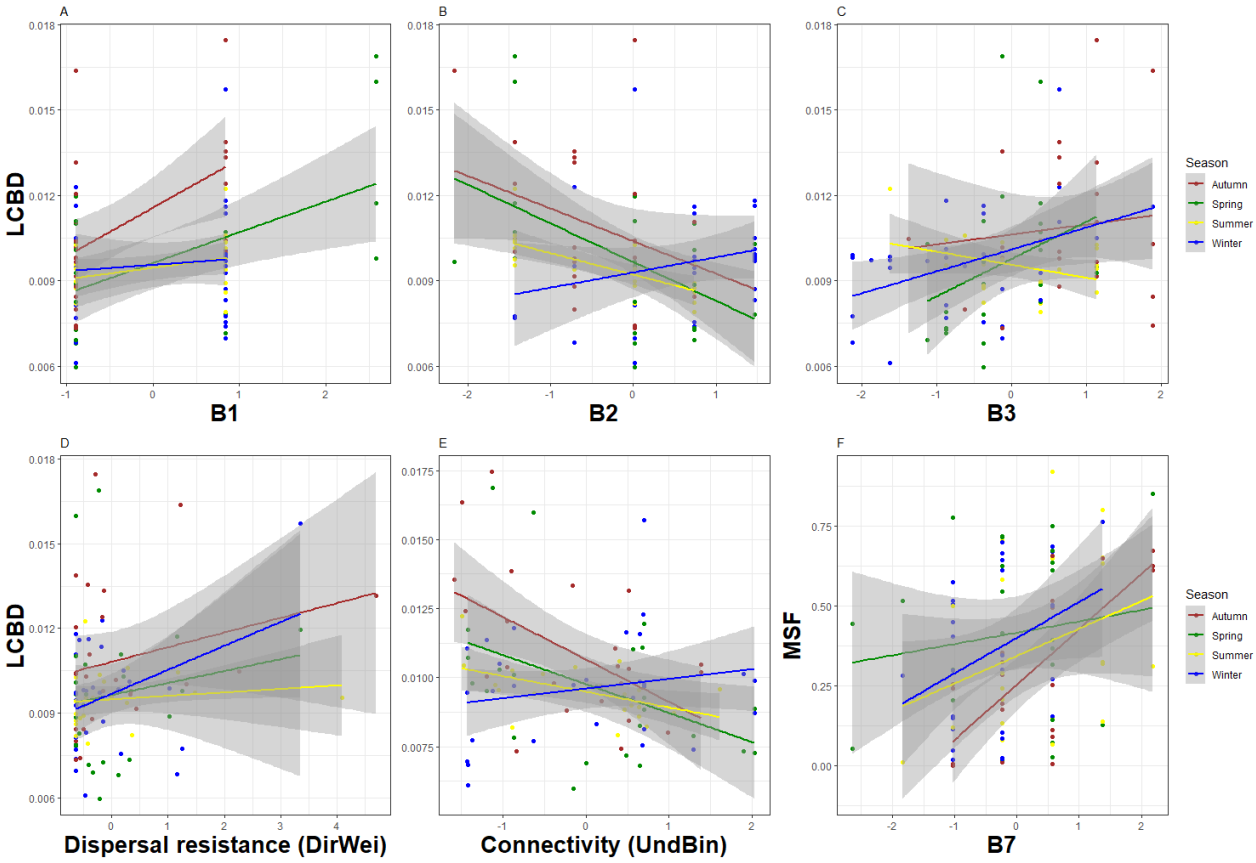


Figure 6. Relationships between predictor variables and Local Contribution to Beta Diversity (LCBD) or Marxan's Selection Frequency (MSF) values for macroinvertebrates with p-values < 0.05 in each season. "B1" refers to embeddedness in riffles and sedimentation in pools, "B2" to the frequency of riffles, "B3" to the substrate composition, "DirWei" to the dispersal resistance (or spatiotemporal isolation) between reaches following a downstream direction, "UndBin" to the connectivity between each pair of reaches without any given direction, and "B7" to the aquatic vegetation cover.

DISCUSSION

In spite of covering a small study area with similar climatic, geological, and habitat characteristics, selected sites for conservation using LCBD and MSF varied widely in space and time, which agrees with our first hypothesis. Given our results, we advise conservation plans to properly capture the spatiotemporal hydrological variability of TRs and be able to accommodate the changing conditions. As we have hypothesised, TRs are highly dynamic systems that create a patchy landscape in which flowing reaches can co-occur with disconnected pools and dry riverbeds within a single network. This favours the co-existence of different communities (e.g., aquatic and terrestrial) due to multiple factors, such as habitat heterogeneity or the existence of spatially isolated sites that serve as a refugia for weak competitors (Larned *et al*., 2010; Bonada *et al*., 2020). Moreover, this landscape drastically changes over time. For example, in late summer, a dry fragmented stream network can be rapidly transformed into a fully connected network after heavy precipitation events (Pineda-Morante *et al*., 2022). Taking this into account, planning conservation on the basis of snap-shot aquatic biodiversity surveys would lead to strongly biased and misguided decisions that could hamper conservation efforts.

MSF and LCBD showed a strong spatiotemporal variability, but they were weakly related. In agreement with our second hypothesis, MSF usually selects a combination of sites with high local richness and very rare taxa (Epele *et al*., 2021). In our study, under some circumstances, MSF was able to adequately represent all taxa by selecting a few sites (e.g., only two sites for diatoms in summer). On the contrary, LCBD selected the communities with a higher relative weight at the level of community differentiation but not necessarily the communities with rare or endemic species. Thus, this approach does not seem adequate to design conservation strategies in rivers when these rare or endemic species occur and have a high conservation value, such as in TRs (Bogan, 2017; Stubbington *et al*., 2017; Fernández-Calero *et al*., 2022). In these cases, MSF with its principle of complementarity and its flexibility in the application of specific targets, would be a more reliable tool. However, LCBD selected communities with higher differentiation and low richness that were not necessarily selected by MSF (e.g., site R4 in autumn or H5 in winter (Figure 1)). This highlights the conservation

value of TRs and specially of those with low richness as a result of low flow permanence (Datry *et al*., 2014a).

According to previous studies (Siqueira *et al*., 2009; Heino & Grönroos, 2017) taxa with high abundances and occupancy across sites, which are often the least threatened taxa, present a greater contribution to beta diversity. Concordantly, we found a very strong relationship between species occupancy and abundance with SCBD for diatoms and macroinvertebrates. This suggests that SCBD alone is not a suitable metric to assess the conservation value of a given taxa, since it is strongly driven by locally abundant and regionally common species, rather than rare or endemic species with high conservation value (Rodríguez-Lozano *et al*., 2023). For example, the genus *Tyrrhenoleuctra*, a specialist taxon of ephemeral rivers, whose nymphal presence only occurs in winter and early spring, did not strongly contribute to SCBD in our study. However, this is a rare and vulnerable taxa with restricted geographical distribution (Fochetti *et al*., 2009; Fernández-Calero *et al*., 2022) that should be considered in conservation plans and probably requires specific conservation actions.

Despite the use of SCBD is not recommended for conservation, we found different diatom and macroinvertebrate taxa strongly contributing to beta diversity depending on the season. In the case of diatoms, species characteristic of oligotrophic calcareous rivers contributed the most to beta diversity (e.g., *Brachysira neoexilis, Gomphonema lateripunctatum, G. tergestinum* and *Encyonema subminuta*), as well as generalist species (e.g., *Achnanthidium pyrenaicum* and *Amphora pediculus*). This aligns with previous studies showing that commonly occurring diatom taxa greatly contribute to beta diversity (Vilmi, Karjalainen & Heino, 2017; Szabó *et al*., 2019). For macroinvertebrates, the differential seasonal contribution of taxa might be explained by changes in habitat variability and flow permanence (Bonada, Rieradevall & Prat, 2007b), which influences habitat preference and phenology (Porst *et al*., 2012; Woods, Kaz & Giam, 2021). For example, in agreement with previous studies (Bonada, Rieradevall & Prat, 2007b; Bogan *et al*., 2017), Ephemeroptera, Plecoptera, and Trichoptera (EPT) were more abundant in winter and autumn, during flowing conditions and mild temperatures, whereas Odonata, Coleoptera, and Hemiptera (OCH) dominated in spring and summer, when many stream reaches might have disconnected pools and temperatures are high. Thus, riffle-adapted taxa, such as *Simulium* and *Habroleptoides*

(Horne, Bennison & Davis, 1992), showed high SCBD values in winter and autumn, whereas in spring and summer lentic-adapted taxa, such as *Bidessus, Stictonectes, Gerris,* or *Sympetrum* (Letsch, Gottsberger & Ware, 2016; Buffagni, 2021), showed the highest SCBD values. However, there were many generalist taxa that contributed significantly to beta diversity throughout most of the year (e.g., *Cloeon* and *Dasyhelea*).

Concerning our third hypothesis, local conditions largely determined the conservation importance of our study sites. Physicochemical and habitat variables were the most important drivers of LCBD and MSF for diatoms and macroinvertebrates, respectively. We found that dissolved oxygen and temperature drove diatom MSF, in line with previous works showing that local physicochemical conditions shaped diatom communities (Soininen & Teittinen, 2019). Diatoms also responded to environmental heterogeneity, but this might be directly related to the species-area relationship (SARs) (i.e., larger samples, more richness) (Soininen & Teittinen, 2019). For macroinvertebrates, embeddedness in riffles and sedimentation in pools (B1), along with substrate composition (B3), and aquatic vegetation cover (B7), determined LCBD across seasons, suggesting that, in general, increasing habitat heterogeneity might promote community turnover through a niche partitioning. For example, rock characteristics can determine caddisfly oviposition patterns and influence population dynamics (Reich *et al*., 2011). Overall, our findings illustrate the importance of preserving local habitat heterogeneity to support diverse macroinvertebrate communities.

Our results agree with that more spatiotemporally isolated sites in TRs showed a stronger contribution to LCBD (Ruhí, Datry & Sabo, 2017; Crabot *et al*., 2020). However, there were important temporal variations in the relationship between LCBD and hydrological connectivity. When the network was fully connected in winter (after the rewetting), LCBD values linearly increased with spatiotemporal connectivity, most likely because there are fewer dispersal restrictions and each species occupies its preferential niche (Warfe *et al*., 2012; Fletcher *et al*., 2016; Leibold & Chase, 2017; Thompson, Rayfield & Gonzalez, 2017). In the remaining seasons, the communities with highest LCBD values were located in the most spatiotemporally isolated sites, suggesting that isolation promotes beta diversity (e.g., by allowing rare species with low competitive advantage to persist), which has already been shown for high mountain streams (Finn *et al*., 2011). Hydrological connectivity also played a role in the selection of sites by MSF, with spatially isolated sites being often selected to

represent rare taxa. Overall, our results suggest that conservation plans should adequately capture the natural hydrological variability of drying river networks, because the networks can also have temporary reaches with high conservation value (Hermoso, Ward & Kennard, 2013; Hamdhani, Eppehimer & Bogan, 2020; Vander Vorste *et al*., 2020; Naia *et al*., 2021).

TRs are one of the most widespread and vulnerable river systems on the planet (Chiu *et al*., 2017; Messager *et al*., 2021; Datry *et al*. 2023), but host unique freshwater biodiversity adapted to these ecosystems (e.g., *Mesophylax*, *Tyrrhenoleuctra*) (Magoulick & Kobza, 2003; Bonada *et al*., 2020; Vander Vorste *et al*., 2020; Yu *et al*., 2022). In addition, TRs also support semi aquatic and terrestrial taxa (i.e., invertebrates and vertebrates) that use dry channels and riparian habitats as new habitats and migration corridors (Sánchez-Montoya *et al*., 2017). These singular river ecosystems should have specific conservation strategies to ensure their biodiversity and functionality. However, specific protection plans including TRs are still lacking (Datry *et al*. 2023). For example, in Europe, even though the Annex I of the Habitats Directive (HD; Council Directive 92/43/EEC) describes habitat types that refer to TRs (i.e., Intermittently flowing Mediterranean rivers of the Paspalo-Agrostidion, and Riparian formations on intermittent Mediterranean watercourses with *Rhododendron ponticum*, *Salix*, and other species), this has resulted in limited conservation and policy actions related to the protection of these ecosystems (Fritz, Cid & Autrey, 2017; Magand *et al*., 2020; Datry *et al*., 2023). In the United States, many temporary waterbodies are protected under a range of policy instruments at the local, State, and Federal levels. However, protections for TRs at the Federal level have been increasingly threatened over the past decades, owing to Supreme Court rulings (Sulliván & Gardner, 2023). A recent Supreme Court decision (Sackett v. US Environmental Protection Agency) has ruled that for a “wetland” (including streams and rivers) to receive protection under the Clean Water Act of 1972, it must have a continuous surface connection with a larger body of water (i.e., an ocean, a lake, or a larger perennial river). This decision threatens protection to TRs, which strongly dominate the subarid and arid regions of the U.S. Southwest (Levick *et al*., 2008). Moreover, as many rivers across the U.S. Southwest continues to lose flow due to climate change and overallocation of freshwater resources (Ruhí, Olden & Sabo, 2016), often shifting from perennial to intermittent flow regimes, using physical but not biological connectivity as a condition for stream protection may enable further degradation in the near future.

In addition, many rivers around the world are expected to become temporary and unpredictable during the next decades due to climate change (Döll & Schmied, 2012). Thus, conservation plans that incorporate the spatiotemporal variability of temporary rivers, will become increasingly needed to effectively protect freshwater biodiversity. Therefore, based on our results, we suggest a set of recommendations to guide future conservation plans for TRs that help to ensure their biodiversity and functionality. Firstly, we strongly recommend regular assessments across seasons or, at least, during the dry season (including disconnected pools), the rewetting period, and the fully connected period to have a full characterization of biodiversity. Secondly, we recommend the use of beta diversity components such as LCBD and conservation planning methods (MSF) as complementary approaches to determine and prioritize sites to be conserved. LCBD is able to detect unique species composition even in sites with low richness as a result of low flow permanence (Datry *et al*., 2014a), whereas MSF could be used as a tool to design specific criteria, e.g., to include rare or singular species. Thirdly, focusing on a local perspective, we encourage the preservation of local habitat heterogeneity such as the substrate composition or instream aquatic vegetation. Fourthly, in a regional perspective, we encourage the conservation of spatiotemporal isolated communities with low species richness because these sites usually host unique communities with high LCBD values. Fifthly, we argue that natural spatiotemporal connectivity should be enforced and be case specific (Cid *et al*., 2022). For example, in historically isolated populations and communities such as the ones in our study, anthropogenic water discharges that could increase hydrological connectivity but decrease biodiversity of the TRs should be avoided.

Although our study provides valuable insights about the spatiotemporal dynamics of biodiversity patterns in TRs and how it can influence conservation decisions, it also has limitations that should be consider if our approach is applied to other areas. Firstly, our study area covers a small spatial scale with very homogeneous environmental conditions, which could potentially result in homogeneous community dynamics. However, we showed that even under similar environmental conditions community dynamics differ because of differences in spatiotemporal hydrological dynamics. Secondly, the temporal duration of the study was only one year, and the patterns observed may not reflect long-term trends. Although the year of the study was a normal hydrological year in the area, in a drier year,

communities can behave completely different (Cid *et al*., 2017). This highlights the need to incorporate data coming from multiple years in conservation planning studies, specially in areas with high interannual variability, such the Mediterranean region (Cid *et al*., 2017; Bonada *et al*., 2020). Thirdly, our study only considers diatoms and macroinvertebrates, which may not represent the full complexity of the ecosystem. Data on other aquatic (e.g., fish, amphibians) or even terrestrial organisms would be recommended if the approach needs to be applied to other areas. Future research should address these limitations when applying our approach to other TRs (and even perennial) in order to increase the performance of conservation strategies.

Finally, conservation plans need to be flexible and adapted to each hydrological scenario. For example, in the wet season the connectivity of the river network and microhabitat heterogeneity (riffles and pools) should be maintained at the reach scale. However, during the dry season, maintaining disconnected pools is key to sustaining refuges for aquatic biodiversity. All these recommendations stand as broad guidelines that should be specifically adapted to the particular characteristics of each TR but keeping in mind the relevance of these ecosystems at the global scale (Messager *et al*., 2021), especially considering future global change scenarios.

AUTHOR CONTRIBUTIONS

José María Fernández-Calero leaded the paper in the contextualization, analysis, writing, interpretation and edition. He also collected the data, sorted and identified macroinvertebrates. Núria Bonada conceptualised the paper, interpreted the results, wrote and edited the manuscript and obtained funding. Miguel Cañedo-Argüelles collected the data, conceptualised the paper, interpreted the results, wrote and edited the manuscript and obtained funding. David Cunillera-Montcusí calculated spatiotemporal connectivity metrics, interpreted the results, wrote and edited the manuscript. Núria Cid collected the data, interpreted the results, edited the manuscript and obtained funding. Albert Ruhí conceptualised the paper, interpreted the results and edited the manuscript. Dolors Vinyoles collected the data and edited the manuscript. Guillermo Quevedo-Ortiz identified diatom and edited the manuscript. Joan Gomà interpreted diatom results and edited the manuscript.

Virgilio Hermoso interpreted the results, wrote and edited the manuscript. Pau Fortuño and Raúl Acosta collected the data, identified macroinvertebrates and edited the manuscript.

GENERAL DISCUSSION



This thesis offers a comprehensive overview of how flow intermittence in TRs influences metacommunity dynamics at a small spatial scale. To achieve this, a range of methodologies were employed, including traditional morphological approaches, field-based mesocosm experiments, and advanced molecular techniques. Additionally, it provides new insights into the aquatic macroinvertebrate diversity of TRs together with recommendations for conservation planning.

First, I present a novel methodological framework for assessing spatiotemporal connectivity in TRs, accounting for the varying dispersal capabilities of macroinvertebrates across different hydrological phases (**Chapter 1**). Fragmentation and changes in spatiotemporal connectivity are shown to directly influence metacommunity dynamics in drying river networks, with their effects depending on the dispersal strategies of the taxa (**Chapter 2**). Furthermore, fragmentation caused by desiccation promotes the spatial structuring of metapopulations and metacommunities along spatiotemporal connectivity gradients (**Chapter 3**). During the dry phase, resistance and resilience strategies become critical, with disconnected pools emerging as stepping stones and refuges through a mesocosm field experiment (**Chapter 4**). Building on these findings, conservation guidelines for TRs are proposed, addressing their spatiotemporal variability (**Chapter 5**) and the unique species they support (**Supplementary Contributions: Annexes 1, 2, and 3**).

GENERAL PATTERNS IN MACROINVERTEBRATE DIVERSITY

We found differences in total species richness across seasons and streams, along with a relatively high gamma diversity (total taxa) for the sampled study area. The seasonal variability observed in macroinvertebrate species richness and community composition likely reflects the complex interplay between abiotic (i.e., flow conditions, flow permanence, or temperature, among others) and biotic factors (i.e., species-specific life cycles and ecological interactions). The phenological adaptations of species inhabiting Mediterranean TRs are highly synchronised to predictable seasonal environmental changes, ensuring survival and maximizing fitness in these dynamic ecosystems (Lytle & Poff, 2004; Bogan & Lytle, 2007; Bonada & Resh, 2013).

The values reported for species richness for our study (178 taxa) can be considered relatively low compared to other studies conducted in TRs, which report approximately 230 taxa (e.g., Cañedo-Argüelles *et al.*, 2015; Bruno *et al.*, 2022) (**Chapter 2**). However, it is important to highlight that the spatial scale of most existing studies is significantly larger than the study area analyzed in this thesis. For instance, Crabot *et al.* (2020) reported a total of 232 taxa with a mean richness of 26 (± 9) taxa per site in a study spanning a gradient of intermittence in southeastern France. Their study encompassed several catchments, each covering hundreds of square kilometers, sampled across multiple seasons. The extensive dataset of nearly 1,000 samples exceeds by far the 101 samples collected for this thesis. This disparity in sample size and spatial scale may contribute to differences in richness estimates. In contrast, this thesis focuses on a finer taxonomic resolution, particularly for groups such as Diptera, where genus-level identification was systematically performed, except in a few cases (e.g., Chironomidae). This level of detail is typically rare, as most ecological studies identify Diptera only to the family level (Boóz *et al.*, 2024). Moreover, the findings highlight significant spatial and temporal variability in macroinvertebrate communities, both across different streams and seasons, as illustrated in **Chapter 2**. Despite the relatively small spatial scale of the study area (137 km^2), with sampling reaches located only a few hundred meters apart within each stream and a few kilometers between rivers, the observed macroinvertebrate diversity is remarkably high. This high diversity emphasizes the ecological importance of TRs, where the high spatiotemporal variability of the environmental conditions promotes the co-existence of species (Bogan & Lytle, 2007).

The pronounced dominance of Diptera highlights their capacity to thrive under the fluctuating flow conditions of TRs (**Chapter 2**). Many dipteran larvae exhibit rapid growth rates, high fecundity, and the ability to exploit a broad range of microhabitats, which confer significant advantages in ephemeral or unstable aquatic environments. Beyond their extensive distribution and remarkable diversity, Diptera often rank among the most abundant groups in freshwater ecosystems (Sundermann *et al.*, 2007; Boóz *et al.*, 2024). Within Diptera, the family Chironomidae is considered one of the most widespread and ecologically versatile macroinvertebrate families worldwide, inhabiting diverse ecosystems and habitats (Ferrington, 2008). In fact, Chironomidae represented the most abundant group in our study area, being found in all localities across all seasons. Similarly, the family Simuliidae is also

highly abundant in riffles when flow conditions are well maintained (Currie & Adler, 2008). Overall, Diptera display a broad range of tolerance, allowing some species to occupy pristine environments while others withstand various forms of environmental stress and degradation, including heavily polluted habitats (Courtney *et al.*, 2009). This ecological plasticity helps to explain why dipterans consistently prevail under diverse flow regimes in TRs.

The beta diversity partitioning results from the **Chapter 2**, which show that richness difference exceeded species replacement, contrast with previous research in TRs that typically identifies species replacement as the dominant driver of beta diversity (Ruhí *et al.*, 2017; Rogosch & Olden, 2019). This discrepancy may be attributed to the small spatial scale of our study, which likely restricts regional (gamma) diversity and leads to taxa-poor communities being subsets of taxa-rich communities.

HYDROLOGICAL CONNECTIVITY AS A MAJOR DRIVING FORCE SHAPING MACROINVERTEBRATE METACOMMUNITIES IN TEMPORARY RIVERS

A key finding of this work is the identification of distinct diversity patterns and highly diverse communities within a remarkably small spatial scale. Sampling points were located between a few hundred meters within the same stream (e.g., 300 - 600 meters) and up to approximately 15 kilometers apart across different streams. This limited spatial extent ensured a consistent climatic context and comparable environmental conditions, such as geology and precipitation, across all sampling sites.

Within the conceptual framework of metacommunities in stream networks, this spatial scale should result in a minimal influence of environmental factors on metacommunity structure, with spatial effects dominating due to mass effects (Heino *et al.*, 2015). Contrary to this expectation, this thesis reveals that metacommunity assembly can be driven by environmental filtering and dispersal limitation, primarily influenced by fragmentation from drying and variations in spatiotemporal connectivity, in TRs at small spatial scales (**Chapters 1, 2, 3, 4, and 5**). These findings highlight the critical role of hydrology as a structuring force for aquatic macroinvertebrate metacommunities in TRs (**Chapter 1**). In addition, **Chapter 2**

explores the factors influencing the diversity patterns observed in the studied streams, emphasizing the role of local habitat heterogeneity and physicochemical conditions, which are closely tied to the hydrological constraints. In the studied TRs, macroinvertebrate assemblages were shaped by environmental filtering driven by hydrological changes, particularly during the dry season. For example, community homogenization (i.e., decreased beta diversity) occurred during prolonged drying periods (**Chapter 2**), forcing species to adopt resilience strategies, such as aerial dispersal to permanent pools in the surrounding area (**Chapter 4**). In addition to its local influence, hydrology shaped metacommunities due to its effect on network connectivity. For example, perennial and spatiotemporally isolated sites promote intraspecific variability at the community level and genetic differentiation in the population structure of strictly aquatic species, driven by hydrological fragmentation (**Chapter 3**), while also enhancing the uniqueness of biological communities (**Chapter 5**).

In **Chapter 1**, we develop a methodological framework for assessing spatiotemporal connectivity metrics (i.e., integrating both spatial and temporal dimensions into a single metric) as predictors of community dynamics based on hydrological data. This thesis makes a substantial contribution to the field, as connectivity has traditionally been spatially assessed, with temporal aspects often overlooked or assessed independently (Fullerton *et al.*, 2010; Uroy *et al.*, 2021). By jointly examining both spatial and temporal variations, this framework provides a more holistic view of connectivity, which is essential for understanding the complexity of dynamic ecosystems such as TRs (Datry *et al.*, 2014b). Moreover, the flexibility of these metrics allows adaptation to a variety of ecosystems, broadening their applicability across diverse freshwater habitats. For instance, they could be applied to lakes or pond systems by incorporating non-directional connectivity scenarios.

In TRs, connectivity is fundamentally shaped by hydrological dynamics, which dictate how organisms interact with their environment across both temporal and spatial dimensions. These dynamics, particularly the dispersal capacities of organisms, heavily influence how they respond to fragmentation and seasonal drying, ultimately driving the structure and composition of metacommunities. Therefore, strong aerial dispersers, such as dragonflies, can traverse large distances to colonize new habitats, resulting in weak spatial patterns of genetic differentiation (Minot *et al.*, 2021; **Chapter 3**). In contrast, strictly aquatic organisms, such as snails, are unable to disperse overland and depend on connectivity within the aquatic

network (Kappes & Haase, 2012; **Chapter 2**), resulting in more structured patterns of genetic differentiation (**Chapter 3**). In the aerial dispersal experiment conducted as part of this thesis (**Chapter 4**), it is demonstrated that disconnected pools serve as vital refuges for macroinvertebrates. These disconnected pools facilitate recolonization of nearby water bodies and the entire river network during rewetting phases, highlighting their ecological importance in sustaining biodiversity and supporting metacommunity dynamics in TRs (Bonada *et al.*, 2020; Pineda-Morante *et al.*, 2022; **Chapter 4**). In addition, a key contribution of this thesis is the innovative application of stable isotopes to monitor spatial dispersal in TRs. This technique not only enables the tracking of dispersal events but also allows for tracing dispersal across subsequent generations, offering insights previously unattainable in such studies. This advancement provides a valuable tool for studying connectivity and dispersal patterns in aquatic ecosystems through direct and measurable insights into dispersal (Heino *et al.*, 2017; **Chapter 4**). The findings revealed that the distance to disconnected pools significantly influenced the composition of macroinvertebrates colonizing new habitats, thereby shaping potential alpha diversity. Interestingly, despite the dispersal constraints of strictly aquatic organisms, such as water mites and snails, these taxa demonstrated the ability to colonize new water bodies within the short experimental timeframe. This observation underscores the adaptability and rapid dispersal capacity of even highly specialized aquatic taxa, illustrating the resilience of aquatic communities inhabiting TRs. However, no resistance strategies were identified in the study area. As part of the same thesis project, rewetting sediment experiments were conducted to investigate the presence of resistance propagules in the hyporheos of the streams study area (Folch, 2020). The absence of such strategies may be attributed to the shallow depth of the hyporheic zone and the prevalence of bedrock in many stream reaches (Chester & Robson, 2011). These findings suggest that macroinvertebrate strategies in these TRs are predominantly resilience-based rather than resistance-based (**Chapter 4**). Building on this, the diverse spatial dispersal strategies (i.e., resilience) of macroinvertebrates are the predominant mechanisms for colonizing spatiotemporally isolated sites within drying river networks, highlighting resilience as the primary strategy shaping community composition in Mediterranean TRs, rather than resistance (**Chapters 3 and 4**). However, it was also demonstrated in some Mediterranean TRs of the northeastern Iberian Peninsula that resistance strategies are present, contributing to community composition after flow resumption (Viza *et al.*, 2023).

Moreover, the dynamics of spatiotemporal connectivity, driven by drying, act as strong environmental filters that shape not only local diversity (i.e., alpha diversity) but also community turnover between sites (spatial beta diversity) and across seasons (temporal variation in spatial beta diversity) (Heino *et al*., 2024; **Chapters 1, 2, 3, and 5**). The observed pattern of reduced alpha diversity with extended drying periods is consistent across diverse regions and climates (Soria *et al.*, 2017). Drying exerts a significant disturbance on habitat conditions, such as by eliminating riffle habitats and reducing dissolved oxygen levels in disconnected pools (Datry *et al.*, 2016a; Bonada *et al.*, 2020). Only species with resistant or dormant life stages can survive these conditions locally, highlighting the selective pressures imposed by drying events (Stubbington & Datry, 2013). This trend extends beyond macroinvertebrates to encompass microbial communities (Schreckinger *et al.*, 2021), diatoms (Falasco *et al.*, 2021; Tornés *et al.*, 2021), and fish (Benejam *et al.*, 2010; Kerezsy *et al.*, 2017), suggesting a pervasive global pattern among freshwater organisms.

Thereby the interaction between dispersal and hydrological connectivity is critical for maintaining biodiversity within these highly variable systems (Datry *et al.*, 2014b; Leigh & Datry, 2017; Heino *et al.*, 2017; **Chapters 3 and 4**). For instance, longitudinal connectivity, which is crucial for downstream dispersal processes such as drift, is disrupted during dry periods (Bogan *et al.*, 2013). This disconnection spatially confines certain species to isolated patches, limiting their ability to move through the river network (Cañedo-Argüelles *et al.*, 2015). Macroinvertebrates with limited spatial dispersal capabilities must develop strategies to withstand adverse environmental conditions *in situ*, exhibiting resistance to temporal variability. This dual challenge—navigating the constraints of spatial isolation while adapting to temporal fluctuations—highlights the intricate ecological dynamics characteristic of TR ecosystems.

The degree of spatiotemporal connectivity within TRs further modulates this balance, directly influencing alpha and beta diversity patterns. Our findings indicate that macroinvertebrate alpha diversity generally increases with connectivity and permanence in TRs (**Chapters 1 and 3**), aligning with global trends documented in TRs studies (Datry *et al.*, 2014b; Schriever *et al.*, 2015; Leigh & Datry, 2017). High connectivity facilitates dispersal, which can enhance alpha diversity at local sites by enabling the arrival and establishment of a broader range of species. However, this increased connectivity may simultaneously reduce

beta diversity by homogenizing communities across sites. In contrast, low connectivity promotes greater beta diversity by allowing isolated populations to diverge independently, minimizing mass effects and promoting species sorting (Leibold *et al.*, 2004; Heino *et al.*, 2015; **Chapters 1, 3, and 5**). Notably, these patterns were consistent across both morphological and genetic data examined in our studied streams (**Chapter 3**).

This thesis shows that beta diversity increases with drying and isolation, with more connected and perennial sites showing lower beta diversity values (**Chapters 1, 3, and 5**). Sites experiencing higher spatiotemporal isolation, tend to host more unique communities, likely due to the limitations imposed on dispersal and niche filtering (**Chapters 1, 3, and 5**). However, spatiotemporal connectivity showed contrasting effects on beta diversity depending on the dispersal strategy: beta diversity showed a negative relationship with connectivity for aquatic passive dispersers, while no significant relationship was observed for aerial active dispersers. This suggests that aerial active dispersers tend to be dominated by a few taxa with strong dispersal abilities when the network is hydrologically connected, although they are still constrained by environmental conditions, implying the absence of mass effects (**Chapter 2**). In the case of aquatic passive dispersers, hydrological connectivity led to a decrease in alpha diversity, likely due to biotic interactions (**Chapter 2**). This, together with the low regional species pool for this group in our study area and their limited dispersal (Kappes & Haase, 2012) could help to explain the increase in their beta diversity with increasing hydrological connectivity. Aligning with this, as discussed in **Chapter 5**, sites characterized by low alpha diversity frequently display elevated Local Contribution to Beta Diversity (LCBD) values.

When beta diversity was decomposed into its main components (i.e., species replacement and richness difference) we found that species replacement (turnover) is higher during winter than in the rest of the seasons (**Chapter 2**). This pattern suggests higher niche partitioning during winter, when more habitats are available (i.e., riffles and pools), most species are present at the larval stage and the network is hydrologically connected facilitating that each species occupies its preferential habitat. The predominance of richness difference across most seasons is likely a consequence of the small spatial scale of this study, where local species gain or loss drives richness differences more prominently than species replacement. Conversely, during other hydrological phases, beta diversity appears to be more influenced

by richness differences, driven by species gain or loss associated with fluctuating habitat availability. Additionally, specific hydrological dynamics, such as the frequency and duration of drying events, can lead to local extinctions, thereby filtering the species pool.

Overall, isolated and permanent sites (i.e., spatiotemporally isolated permanent pools), exhibited the highest intraspecific variability (**Chapter 3**). This suggests that evolutionary forces, including genetic drift and local adaptations, play a significant role in shaping biodiversity in these isolated environments. While perennial rivers in Mediterranean climates also exhibit community shifts, driven by abiotic factors such as temperature, humidity, and precipitation linked to phenological changes (Gasith & Resh, 1999; Bonada & Resh, 2013), TRs exhibit even more pronounced spatial and temporal variability. This increased variability—manifested through distinct communities across seasons and hydrological phases—contributes to higher beta diversity compared to the relatively stable conditions found in perennial systems (Leigh *et al.*, 2019; Crabot *et al.*, 2020). Together, these findings underscore the interplay between hydrological connectivity, evolutionary processes, and the spatial variability of communities, highlighting the distinct biodiversity patterns in TR ecosystems.

IMPLICATIONS FOR MANAGEMENT AND CONSERVATION OF TEMPORARY RIVERS

Despite being the most widespread river ecosystem globally (Messager *et al.*, 2021), TRs often lack adequate legal protection, as existing environmental directives frequently disregard or fail to accommodate their dynamic characteristics (Acuña *et al.*, 2014; Datry *et al.*, 2023). The biological communities used as indicators for ecological status assessments in TRs exhibit pronounced temporal variability, contrasting with the relative stability typical of perennial aquatic ecosystems. Consequently, the timing of assessments in TRs can yield inconsistent outcomes, increasing the risk of misinterpretation. Such variability often reflects the natural dynamics of TRs rather than pollution or anthropogenic stressors. For example, in regions with Mediterranean climates, the observed spatiotemporal variability in these communities is primarily driven by the natural hydrological and ecological processes intrinsic to these systems (Gallart *et al.*, 2012). This thesis highlights the necessity of accounting for the

spatiotemporal dynamics and variations of biological communities in TRs to develop more effective and adaptive conservation or restoration strategies (**Chapter 5**). In particular, the role of connectivity in shaping communities shifts with the seasons: in winter, increased connectivity facilitates niche partitioning, enhancing community diversity and species replacement, while in the dry season, fragmentation fosters the development of unique communities with elevated conservation value (Soria *et al.*, 2024). However, persistent threats, such as habitat degradation—including the loss of aquatic vegetation—and water abstraction, underscore the urgent need for conservation efforts that are not only locally tailored but also effectively address these challenges across multiple temporal scales.

A clear example of habitats that exhibit varying conservation value depending on the season are disconnected pools. During summer, these pools serve as critical refuges for populations that are highly isolated and vulnerable due to their limited dispersal abilities and strictly aquatic life cycles (e.g., fish species) (Bonada *et al.*, 2020). In this context, maintaining disconnected pools emerges as a critical conservation measure, as these habitats not only support local biodiversity but also function as regional reservoirs of species, playing a crucial role in recolonizing newly available habitats and acting as stepping stones (Saura, Bodin & Fortin, 2014; Heino *et al.*, 2017; Soria *et al.*, 2024; **Chapter 4**). However, in winter, the significance of these disconnected pools may diminish, as their role in supporting biodiversity may be less critical during periods of increased connectivity in the river network.

As climate change intensifies the frequency and severity of extreme droughts across many regions, freshwater ecosystems are experiencing unprecedented pressures. This is expected to lead to an increase in the prevalence of TRs, with a shift toward more ephemeral streams and a decline in permanent refugia, posing significant challenges to the persistence of freshwater biodiversity (Döll & Schmied, 2012; Döll *et al.*, 2024). Therefore, the implementation of conservation and management actions should focus on maintaining natural permanent refugia and promoting nature-base solutions that are environmentally sound. In this sense, the installation of artificial pools has been proposed as a promising solution to safeguard critical habitats for vulnerable populations during dry phases, thereby bolstering the resilience of the broader ecosystem (Global Water Partnership Central and Eastern Europe, 2015). However, this thesis emphasizes prioritizing the conservation of natural disconnected permanent pools (**Chapter 4**) over the installation of artificial pools, as

the latter may sometimes prove counterproductive. In fact, the implementation of artificial pools requires careful consideration, as potential unintended consequences may compromise their effectiveness. For example, altering natural hydrological regimes can lead to diverse outcomes depending on the region, such as eutrophication in artificially retained waters (Habets *et al.*, 2018). This can deplete oxygen levels or promote algal blooms, threatening the survival of native species. Moreover, these structures might enable the proliferation of invasive species across the river network, further destabilizing ecological integrity (Morden *et al.*, 2022). Therefore, the use of such structures should be approached with caution and guided by a comprehensive assessment of the specific context in which they are implemented. This evaluation should carefully consider the potential advantages and disadvantages at the ecosystem level, ensuring that the benefits outweigh the risks and contribute positively—not only to maintaining and enhancing local species richness but also within a regional framework that supports overall biodiversity at a broader scale (**Chapter 4**). Building on this, the methods and results of this thesis provide valuable tools for identifying optimal locations to prioritize the conservation of natural disconnected pools, with a focus on dispersal patterns and metacommunity dynamics (Heino *et al.*, 2017; Cid *et al.*, 2020; **Chapters 4 and 5**).

Overall, adopting a metacommunity and metasystem perspective is crucial when establishing conservation measures, as it emphasizes that the system should not be managed in the traditional, locality-by-locality and niche approaches, but rather within a broader regional context (Cid *et al.*, 2020, 2022). Identifying both local and regional drivers of community dynamics is crucial for designing effective conservation and management plans to protect species and their habitats. The decision to prioritize connectivity restoration should be guided by whether diversity patterns are driven primarily by spatial factors (e.g., dispersal limitations) or local environmental variables. If spatial processes are the dominant drivers, restoring connectivity is key. Conversely, if local environmental factors primarily determine diversity, conservation efforts should focus on restoring or maintaining local habitat quality. This distinction is fundamental within the metacommunity framework and has profound implications for conservation and restoration strategies. For instance, the endangered *Iberochondrostoma almacai* in the Iberian Peninsula highlights the importance of addressing connectivity issues in fragmented habitats (Sousa *et al.*, 2010; Cid *et al.*, 2022). Conversely, in

cases where historically isolated populations are threatened by changes in natural hydrological regimes—such as the invasion of non-native species facilitated by altered flow patterns—efforts should aim to preserve or restore the natural isolation and local habitat characteristics that sustain these unique communities (Larned *et al.*, 2010; Ruhi *et al.*, 2019).

Another important consideration is the choice of tools or approaches used to select priority conservation areas, as different methods can yield varying results. Therefore, it is essential to clearly define the primary conservation objectives based on the specific system being studied. In this thesis, two alternative approaches are proposed: beta diversity partitioning and systematic planning, using the software Marxan. While beta diversity is useful for identifying unique localities based on community composition, it does not effectively distinguish specific species that may be rare or highly specialized, thus not addressing one of the key needs of conservation strategies. On the other hand, systematic conservation planning, as outlined in **Chapter 5**, provides greater flexibility by enabling the prioritization of different variables based on the specific characteristics of the system being managed and the resources available. This approach can also incorporate spatiotemporal connectivity values and consider the costs—whether human or economic—associated with implementing particular conservation or management strategies. Consequently, systematic planning is better suited to the practical realities of conservation action in a regional context. Moreover, systematic conservation planning facilitates the identification of priority conservation areas by focusing on species uniquely adapted to specific habitats. In dynamic ecosystems such as TRs, where habitat conditions fluctuate significantly, tools like Marxan can play a pivotal role. By identifying critical areas that support species dependent on temporary or ephemeral flow regimes, these approaches enhance the effectiveness and efficiency of biodiversity protection strategies. However, their success depends on comprehensive and detailed knowledge of the species inhabiting each site and their ecological adaptations to intermittence.

ADVANCING KNOWLEDGE OF AQUATIC MACRO-INVERTEBRATE BIODIVERSITY IN TEMPORARY RIVERS: A KEY CHALLENGE FOR CONSERVATION

Building on the importance of understanding species uniquely adapted to the dynamic conditions of TRs, this thesis investigates the biodiversity of aquatic macroinvertebrates, addressing key knowledge gaps through detailed taxonomic records and descriptions of the species' ecological characteristics. The findings enhance our understanding of aquatic insect communities in TRs by providing new species records from the local study area, the Natural Park of Sant Llorenç del Munt i l'Obac (NE Spain). Some of these records document species previously unknown to the park, while others expand our knowledge to broader regions, including the Iberian Peninsula and mainland Europe. This foundational data is crucial for informing conservation planning and management strategies specifically tailored to these unique ecosystems.

Throughout the course of this thesis, 26 new records of aquatic insect species or genera were documented in the TRs of the study area, excluding Diptera larvae. As detailed in **Supplementary Contributions: Annex 3,** most of these species are widely distributed across the Iberian Peninsula but had not previously been reported within the Natural Park of Sant Llorenç del Munt i l'Obac. Notably, two of these records represent a significant expansion in the known distribution range for two taxa: *Tyrrhenoleuctra* sp. (Plecoptera, Leuctridae) and *Dixella graeca* (Diptera, Dixidae). The record of the genus *Tyrrhenoleuctra* represents the first occurrence of this genus in the northeastern Iberian Peninsula (**See Supplementary Contributions: Annex 1**). This stonefly is a specialist of ephemeral streams and is endemic to western Mediterranean waterways, with its distribution primarily concentrated in the southern regions of the Iberian Peninsula, as well as in North Africa, the Balearic Islands, Corsica, and Sardinia (Fochetti *et al.*, 2009; Figueroa & Fochetti, 2014). In contrast, *Dixella graeca* marks the first record of this species in mainland Europe (**See Supplementary Contributions: Annex 2**), previously cited only in Great Britain and Sardinia.

Despite numerous studies of rivers conducted in this natural area and throughout this region of Spain over the past few decades (Rieradevall, Bonada & Prat, 1999; Bonada *et al.*,

2007b; Rodríguez-Lozano *et al.*, 2015) by various research groups and public agencies (e.g., FEHM-lab, Catalan Water Agency), this thesis reveals a significant gap in fundamental research regarding the biology and distribution of aquatic macroinvertebrate species. This may be attributed to the tendency of ecological studies to underestimate the importance of fine taxonomic identification of aquatic macroinvertebrates, or the relevant information may remain unpublished as gray literature. The immature stages (nymphs and larvae) that inhabit water lack sufficient morphological characteristics for accurate identification, particularly among certain groups like Diptera, where even family-level identification can be challenging (Dobson, 2013; Boóz *et al.*, 2024). Additionally, biomonitoring programs designed to assess water quality typically require identification at the family level. This limitation hinders a deeper understanding of species composition at the study sites, as such detailed identification is often beyond the scope of these assessments. Moreover, most studies are conducted during spring and summer, often overlooking other seasons, such as winter, when species with different life-history adaptations may emerge. For example, *Tyrrhenoleuctra* sp. was identified during the winter sampling campaign, as its nymphs inhabit streams primarily during the wet phase of ephemeral streams, which occurs mainly in winter and early spring.

Building upon this, it is essential to note that, historically, taxonomic and natural history research has predominantly focused on perennial rivers, with TRs remaining relatively understudied until recent decades (Leigh *et al.*, 2015). Although initial studies on TRs ecology were descriptive and focused on natural history (e.g., Allee & Torvik, 1927), more recent research has increasingly centered on assessment and management (Prat *et al.*, 2014; Datry *et al.*, 2014b; Acuña *et al.*, 2014), metacommunity ecology and biodiversity comparisons with perennial rivers (Cañedo-Argüelles *et al.*, 2015; Datry *et al.*, 2016a; Soria *et al.*, 2017), hydrological dynamics (Bunn & Arthington, 2002; Costigan *et al.*, 2017; Gallart *et al.*, 2017), as well as theoretical and conceptual frameworks (Larned *et al.*, 2010; Datry *et al.*, 2014b). Despite this progress, our understanding of the natural histories of species associated with TRs remains limited and is frequently overlooked. In the current context of a freshwater biodiversity crisis (Harrison *et al.*, 2018) and with climate change accelerating the intermittence of many river systems (Döll & Schmied, 2012), studies that deepen our knowledge of fluvial biodiversity at the species level are essential. Comprehensive sampling was encouraged across all seasonal and phenological stages, as well as examining multiple life

stages of macroinvertebrates (i.e., larval, pupal, and adult stages) to capture a complete picture of biodiversity in TRs. Studies such as ours (**Supplementary Contributions: Annexes 1, 2, and 3**) provide a foundational knowledge base that is essential for developing targeted conservation and management strategies to protect aquatic biodiversity.

CHALLENGES, RESEARCH GAPS, AND FUTURE DIRECTIONS IN TEMPORARY RIVERS

Emerging research gaps from this thesis

Approaches to quantifying hydrological metrics

Throughout this thesis, several methodological limitations were encountered. One of the primary challenges was the quantification of stream hydrology, which was necessary for the implementation of hydrological and connectivity metrics. Using temperature data loggers, a high-resolution spatiotemporal dataset was collected, enabling precise assessments of stream drying patterns (e.g., number of dry days per site) and spatiotemporal connectivity metrics. This approach provided valuable insights but also presented methodological challenges and limitations. Data loggers, being small devices designed to record environmental data at specific points, are susceptible to displacement by external factors such as animal interactions and flooding. When dislodged to streambanks or other non-aquatic areas, the data loggers may incorrectly register a dry condition for a site that is actually submerged, leading to potential inaccuracies. Additionally, data loggers are limited to detecting only the presence or absence of water, providing limited insight into the aquatic conditions of streams. For instance, they cannot inherently distinguish between disconnected pools and hydrologically connected water bodies, unless additional loggers are installed (e.g., one in the riffle of the entrance of the pool and another in the deepest part of the pool). During the sampling campaign for this thesis, 64% of data loggers experienced data loss, primarily due to malfunctions on colder days and displacement during flooding events. Additionally, vandalism emerged as a significant limitation of this methodology. To mitigate these data gaps, we employed a logistic regression model incorporating variables such as slope, geology, temperature, and precipitation. Reconstructed data were validated with field

observations and time-lapse photography in selected streams, ensuring improved accuracy in capturing stream hydrology dynamics (Pineda-Morante *et al.*, 2022; **Chapter 1**).

Drawing on this experience, this thesis advocates for the adoption of alternative methods in future research to evaluate hydrological conditions in Mediterranean streams prone to recurrent flooding. Emerging technologies, such as high-frequency satellite sensors, offer promising applications (Pekel *et al.*, 2016). Moreover, the utilization of image acquisition from unmanned aerial vehicles (UAVs), commonly known as drones, has gained traction in recent years, providing high-resolution datasets for small areas while offering numerous advantages over traditional survey methods (Scalici *et al.*, 2021). These advantages include hyperspatial resolution and the capacity to acquire spatially explicit and continuous data more quickly, easily, flexibly, and frequently than conventional approaches (Woodget & Austrums, 2017). However, achieving high spatial resolution in small-scale stream environments remains challenging. For instance, in reaches where vegetation is dense and close to the water, the use of satellite imagery and UAVs becomes impractical. While personal visual monitoring or interviews may serve as straightforward approaches to assess temporal variations in hydrological conditions, they tend to be highly time-consuming and logistically impractical (Noto *et al.*, 2024).

In this context, the use of time-lapse cameras offers a viable alternative for monitoring the hydrological status over time, as recently demonstrated by Noto *et al.* (2024). Additionally, the cost of a temperature data logger (HOBO Pendant® Temperature/Light Logger) is comparable to that of a photo-trap camera. In addition, emerging tools, such as smartphone applications like DischargeApp (https://discharge.ch/) and similar technologies, offer valuable capabilities for monitoring water presence or absence in intermittent rivers. Their ability to measure flow characteristics and water levels without invasive equipment makes them particularly suitable for studying dynamic systems like TRs, where water availability can change rapidly over space and time. Focusing on TRs, the DRYRivERS app (https://www.dryver.eu/citizen-science/introduction) is a citizen science tool specifically designed to collect and map information on drying events in rivers and streams. This mobile application allows users to record hydrological conditions (e.g., flowing water, disconnected pools, or completely dry channels) offline while in the field, accompanied by geolocation data and photographs. By utilizing smartphones or other portable devices to collect and analyze

hydrological data, these tools enable efficient and cost-effective assessments, even in remote or challenging environments.

Resistance adaptations in temporary rivers

A major limitation in the study of TRs is the lack of biological information on resistance adaptations that species employ to withstand drying. In our study area, resilience appears to be the dominant strategy for coping with drying, as demonstrated in **Chapter 4** and in the rewetting seedbank experiment conducted by Folch (2020). However, our limited understanding of species' life histories and adaptations hinders a comprehensive explanation for the presence and dynamics of seedbanks. Hypotheses such as the absence of a well-developed hyporheic zone, the predominance of exposed riverbed reaches, or the prevalence of other strategies to cope with drying in such predictable climates (e.g., life cycle synchronization), offer partial insights but remain inconclusive. Long-term studies, which are currently scarce, are critical for addressing these gaps. Existing research has primarily focused on population structure and life stage synchronization (Bogan *et al.*, 2017), leaving substantial gaps in our understanding of interannual variability. Comparing wet and dry hydrological years could provide valuable insights, as substrate moisture is a key determinant of seedbank viability (Larned *et al.*, 2007; Stubbington & Datry, 2013). Furthermore, identifying the spatial distribution of seed refuges within the river network can provide valuable insights into the dynamics of (meta)communities and inform conservation strategies aimed at supporting species recovery in TRs. For instance, if these refuges are primarily located in headwaters, does recolonization occur primarily via drift during rewetting, resulting in limited seedbank presence in downstream reaches? While some experimental studies have begun to explore these topics (Fournier *et al.*, 2023), many fundamental questions remain unanswered, underscoring significant gaps in our understanding of the role and dynamics of seedbank refuges in TRs. This knowledge gap is particularly urgent in the context of climate change, which is projected to increase the frequency and intensity of droughts (Döll *et al.*, 2024), potentially rendering current resistance strategies less effective. Additionally, the relative contribution of resistance strategies likely varies across climatic zones and TR types globally (Stubbington & Datry, 2013; Leigh *et al.*, 2016), underscoring the importance of conducting broader, comparative research to inform adaptive conservation strategies.

Challenges and innovations in quantifying spatial dispersal

As highlighted throughout this thesis, dispersal is a fundamental process for understanding (meta)community dynamics (Heino *et al.*, 2017), particularly within TRs (Cañedo-Argüelles *et al.*, 2015; Tonkin *et al.*, 2018). This process operates across diverse spatial and temporal scales. At shorter temporal and smaller spatial scales, dispersal involves individuals/species moving from a population/community to colonize new habitats (Bilton, Freeland & Okamura, 2001; Heino *et al.*, 2017), as explored in **Chapter 4**. Over longer temporal scales, such as evolutionary timeframes, or larger spatial scales dispersal can result in population/species isolation, ultimately driving speciation processes (Bonada *et al.*, 2017). To deepen our understanding of short-term dispersal, integrating laboratory and field experiments is strongly recommended to establish robust datasets and minimize reliance on generalized values at the family or genus level present in traits databases (e.g., Tachet *et al.*, 2010; Sarremejane *et al.*, 2020a). Stable isotope analysis represents a particularly effective and non-invasive method for such investigations (Macneale *et al.*, 2005; Enss *et al.*, 2024; **Chapter 4**). In this thesis, the dispersal field experiment was constrained to a single stream with one macroinvertebrate propagule source (i.e., a set of disconnected pools). Expanding these studies across multiple streams and broader spatial scales would enhance their applicability and scope. Future experiments could incorporate stable isotopes of various elements (e.g., nitrogen, phosphorus, carbon), assigning each isotope to a distinct source, such as a specific stream or disconnected pool. Investigating these dynamics within diverse spatial configurations of TRs, such as those with permanent downstream and temporary upstream sections or vice versa, could yield significant insights. Furthermore, understanding how dispersal mechanisms function in TRs across varying climates and geographic regions would provide a more comprehensive perspective, as these dynamics are likely to differ in response to environmental and climatic contexts.

Molecular tools can be particularly effective for investigating dispersal processes at short and long-term temporal scales if appropriate markers are used. In this thesis, metabarcoding of bulk samples was used to obtain a community-level overview. However, species-specific studies employing high-resolution markers, such as microsatellites, are crucial for examining intraspecific variability and speciation processes within fragmented

ecosystems (Phillipsen & Lytle, 2013; Phillipsen *et al.*, 2015). For robust and reliable results, it is important to include a sufficiently representative sample size for each species under study—ideally at least 10 individuals per species—while ensuring consistent sampling effort across sites. This methodological rigor enhances the reliability of data and facilitates the detection of evolutionary patterns shaped by ecosystem fragmentation.

Metapopulations and evolutionary challenges in temporary rivers

TRs impose unique selective pressures across biological organization levels, from populations to ecosystems. The inherent hydrological variability and fragmentation in TRs generate complex connectivity patterns that significantly influence dispersal processes, shaping gene flow, genetic drift, and local adaptation (Bonada *et al.*, 2017; Heino *et al.*, 2017). However, the impact of drying events on the genetic structure of (meta)populations in macroinvertebrates remains poorly understood. Processes such as recolonization and population persistence via refugia (e.g., disconnected pools or hyporheic zones) and their contributions to regional genetic diversity have been largely overlooked. While dispersal strategies are recognized as critical for connectivity (Cañedo-Argüelles *et al.*, 2015), there is limited understanding of how hydrological isolation during dry phases affects genetic drift, local adaptation, or population bottlenecks, particularly in passive aquatic dispersers, which are expected to exhibit stronger genetic structuring compared to aerial dispersers (Bonada *et al.*, 2017).

Empirical studies (e.g., Phillipsen & Lytle, 2013; Phillipsen *et al.*, 2015) have offered initial insights into metapopulations in TRs. However, further research is needed to explore, for example, how intraspecific variability within populations varies according to spatial drying positions in the river network. Such studies could provide valuable insights into patterns of speciation over evolutionary timescales. A significant research gap lies in the scarcity of long-term studies linking hydrological cycles with metapopulation dynamics and evolutionary trajectories. Capturing interannual variability and the cumulative impacts of repeated drying and rewetting cycles is vital for understanding these processes.

Additionally, anthropogenic pressures —such as altered flow regimes, water extraction, and climate change— exacerbate selection pressures and disrupt natural

connectivity, potentially leading to unforeseen genetic and ecological consequences. Bridging these knowledge gaps requires the integration of molecular tools, ecological modeling, and field studies to unravel the interplay between hydrological variability, metapopulation processes, and evolutionary dynamics in TRs. Such knowledge is essential for predicting species' adaptive capacities and for developing conservation strategies to safeguard biodiversity in these increasingly vulnerable ecosystems.

Broader challenges and future perspectives in the management of temporary rivers

There are several other challenges related to TRs that go beyond the main aim of this thesis:

- **Bioassessment of disconnected pools in temporary rivers.** The Water Framework Directive (WFD) overlooks TRs, posing challenges in assessing their ecological status, particularly for disconnected pools. Disconnected pools, critical refuges for aquatic species, are characterized by fluctuating water chemistry, variable species compositions, and sporadic connectivity, making them difficult to assess using existing frameworks. While tools like TREHS (Temporal Rivers Ecological and Hydrological Status) have improved hydrological evaluations (Gallart *et al.*, 2017), the lack of standardized biological indices for these habitats remains a significant gap. Traditional methods fail to capture the ecological complexities of disconnected pools, which often host species with high contamination tolerance and low richness (Bonada *et al.*, 2020, 2024; Ersoy *et al.*, 2024). However, further research is needed to validate these methods and address their application in routine monitoring. Developing tailored methodologies for TRs is increasingly critical under climate change, as drying events become more frequent. Current initiatives, such as the DRY-Guadalmed project, aim to enhance monitoring and conservation efforts for these vulnerable ecosystems.

- **Limited knowledge and bioassessment strategies for dry phases in temporary rivers.** Dry phases in TRs serve critical ecological functions, acting as corridors and providing essential resources such as water, food, shelter, and breeding areas. However, their assessment presents unique challenges, as traditional approaches designed for

aquatic ecosystems often fail to address the ecological role of dry channels. These habitats are dominated by terrestrial invertebrates, which are abundant and sensitive to anthropogenic impacts, making them promising bioindicators (Sánchez-Montoya, 2024). Despite their potential, little is known about their tolerance to various environmental stressors, and further research is needed to validate their application (Steward *et al.*, 2018). Additionally, riparian vegetation and plants colonizing dry riverbeds show promise as indicators of sediment composition and geomorphological changes, but their use requires standardization and further investigation (Westwood *et al.*, 2021; Bonada *et al.*, 2024). Addressing these gaps is essential for effective monitoring and conservation of dry phases in TRs, especially as climate change exacerbates their prevalence.

- **Neglected riparian zones: the interplay between aquatic and terrestrial ecosystems.** In addition, another often overlooked aspect of TRs is the riparian zones, located at the interface between aquatic and terrestrial environments. These zones play a crucial role in biodiversity conservation and the provision of ecosystem services in both perennial and TRs. Despite the global prevalence of TRs, these ecosystems and their riparian zones are frequently neglected in management and biomonitoring policies, increasing their vulnerability. Therefore, it is urgent to integrate the monitoring and assessment of riparian zones into environmental management policies for both aquatic and adjacent terrestrial areas (Bruno & Velasco, 2024).

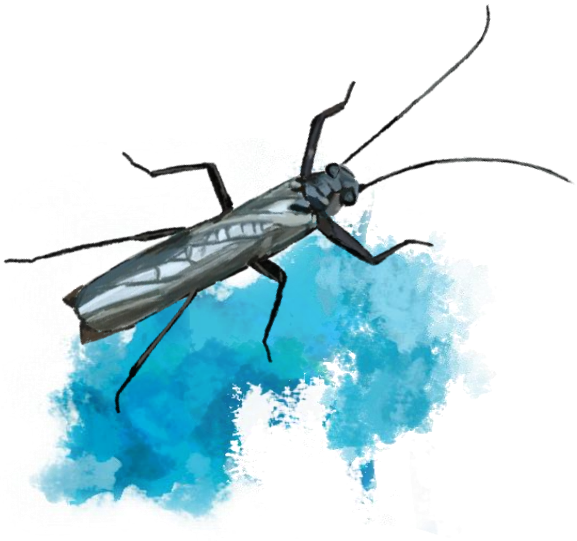
- **Differentiating between natural and hydrologically impacted TRs.** Distinguishing between natural and hydrologically impacted TRs is challenging, as some TRs are naturally temporary, while others are impacted by human activities (e.g., water extraction) or have become permanent due to wastewater or irrigation effluents (Chiu *et al.*, 2017). Accurate differentiation requires knowledge of the stream's natural flow regime (Gallart *et al.*, 2017), which can be obtained from precipitation-runoff models, historical records, or rivers in similar conditions. Developing alternative metrics for hydrological alteration is crucial, as traditional metrics based on flow regimes are difficult to apply to TRs. Tools like TREHS offer valuable approaches for estimating and comparing hydrological alterations using available data and biological communities (Bonada *et al.*, 2024).

- **Differentiating between natural and anthropogenic disturbances**. TRs support species that are well-adapted to their dynamic conditions, yet their communities are often dominated by a few generalist species capable of tolerating fluctuating environments (Bogan *et al.*, 2017). This creates challenges in distinguishing between natural disturbances, such as temporal variations, and anthropogenic impacts, particularly when both occur simultaneously (Bonada & Resh, 2013; Soria *et al.*, 2020). The high spatial and temporal variability of TRs further complicates the identification of reference communities. While certain taxa, such as Hydrophilidae, can indicate a reference TR without the need for hydrological data (Cid *et al.*, 2016), the considerable variability of these communities raises concerns about the viability of fixed reference conditions. A more adaptable approach, integrating long-term interannual data and novel biological quality indices, may be essential for accurately assessing TRs (Bonada *et al.*, 2024).

- **Incorporating functional metrics into the assessment, management, and conservation of TRs**. Integrating taxonomic and functional metrics is essential to fully capture the spectrum of biodiversity patterns and ecosystem functioning in TRs. Taxonomic metrics offer valuable insights into species composition and diversity, while functional metrics shed light on the ecological roles species play, providing a deeper understanding of community responses to environmental variability. Measuring these metrics across spatial and temporal scales is vital to unravel the complex dynamics of TRs (Soria *et al.*, 2024). For example, functional redundancy analyses can serve as early warning systems for ecosystem changes, enabling timely interventions to prevent critical tipping points and the loss of macroinvertebrate-driven ecosystem functions (Rosenfeld, 2002; Boersma *et al.*, 2014; Soria *et al.*, 2024). Additionally, Soria *et al.* (2020) highlighted how the presence or absence of filter-feeding organisms can act as indicators of anthropogenic impacts in stream ecosystems regardless of flow intermittence. Thus, the development of standardized methodologies that integrate both taxonomic and functional approaches while accounting for the unique traits of TRs is imperative. Such an approach will enhance ecological assessments and bolster conservation strategies for these dynamic ecosystems.

- **Evaluation of the ecological status in TRs combining physicochemical, biological, and hydrogeomorphological indicators.** Besides the challenges related to the performance of biological indices in TRs, evaluating the ecological status of TRs is also challenging due to the natural variability of physicochemical parameters, which can resemble the impacts of human-induced disturbances. For example, nutrient concentrations like phosphorus and nitrogen in disconnected pools may signal eutrophication, but these levels can also naturally increase due to evaporation or the mineralization of organic matter (Fellman *et al.*, 2011). Similarly, dissolved oxygen levels can fluctuate significantly, potentially reaching anoxic conditions, which can disrupt nutrient cycles and affect water quality (Gómez *et al.*, 2017). These complexities make it difficult to rely on standard physicochemical indicators for assessing the ecological health of TRs, particularly when anthropogenic influences, such as urban or industrial effluents, exacerbate these conditions. Furthermore, the physical and hydrogeomorphological features of TRs, including sediment composition and flow dynamics, are essential for maintaining habitat quality. However, the effectiveness of current hydrogeomorphological indices for assessing TRs remains uncertain. Emerging methods that address Mediterranean river conditions are promising, but further research is necessary to refine and validate these tools before their implementation in regular bioassessments (Bonada *et al.*, 2024).

CONCLUSIONS



In this section, the conclusions of the five chapters are presented, along with the overarching conclusions of the thesis.

The specific conclusions of each chapter are as follows:

- **Chapter 1** introduced spatiotemporal connectivity metrics that provided a novel framework for assessing connectivity in dynamic ecosystems, with a particular focus on TRs. *STcon* measures the average connectivity of each site and can served as a predictor for local community structure, such as alpha diversity (e.g., species richness). In contrast, *STconmat* quantifies the connectivity values between pairs of sites, facilitating the assessment of changes in community composition (e.g., beta diversity metrics such as Jaccard or Bray-Curtis dissimilarity). The results underscored the critical role of spatiotemporal connectivity in shaping biodiversity, with more connected sites exhibiting higher species richness and diversity, depending on dispersal modes. This framework provided valuable insights into the role of spatiotemporal dynamics in conservation, offering a robust tool for understanding the impacts of global change on ecological connectivity across various ecosystems.

- **Chapter 2** demonstrated the critical role of spatiotemporal connectivity in shaping metacommunity assembly in Mediterranean TRs, particularly at small spatial scales. Contrary to traditional expectations of mass effects dominating at such scales, the results revealed that network fragmentation caused by drying events could lead to significant dispersal limitation for certain taxa, notably aquatic passive dispersers. Alpha diversity was primarily driven by habitat heterogeneity and spatiotemporal connectivity, highlighting the importance of localized environmental conditions and connectivity in maintaining biodiversity. Conversely, beta diversity patterns were shaped by environmental gradients and dispersal dynamics, with aerial active dispersers exhibiting species sorting due to their capacity to overcome dispersal barriers, while aquatic passive dispersers were constrained by limited connectivity. These findings highlight the need to revisit and adapt current metacommunity paradigms, which were largely developed for perennial stream networks, to account for the unique dynamics of TRs.

- **Chapter 3** demonstrated the impact of river fragmentation, driven by drying, on intraspecific variability and on the metacommunity structure of aquatic macroinvertebrates using metabarcoding and traditional morphological approaches. The results showed that spatiotemporal isolation increased intraspecific genetic variability, particularly in isolated perennial sites. Strictly aquatic species exhibited more pronounced genetic differentiation compared to species with higher dispersal abilities. Additionally, both metabarcoding and traditional morphological methods revealed similar patterns in species richness and beta diversity. However, morphological data showed a stronger correlation with metrics of drying and spatiotemporal variability. These findings highlighted the potential value of metabarcoding for assessing genetic diversity and community composition and for linking metapopulation and metacommunity levels.

- **Chapter 4** presented a field experiment on macroinvertebrate dispersal in TRs, concluding that dispersal was a crucial process for maintaining biodiversity in these dynamic ecosystems, particularly in disconnected pools that acted as biodiversity refuges during dry periods. Isotopic enrichment techniques were successfully employed to demonstrate that aquatic macroinvertebrates could disperse aerially (actively but also passively) between disconnected pools and mesocosms, with significant differences in community composition observed depending on the distance from the source. These findings highlighted the importance of preserving disconnected pools within TR networks to support species recovery and maintain biodiversity, particularly under future drying conditions. Consequently, conservation strategies should have prioritized the protection and restoration of disconnected pools to safeguard both local and regional biodiversity in TRs.

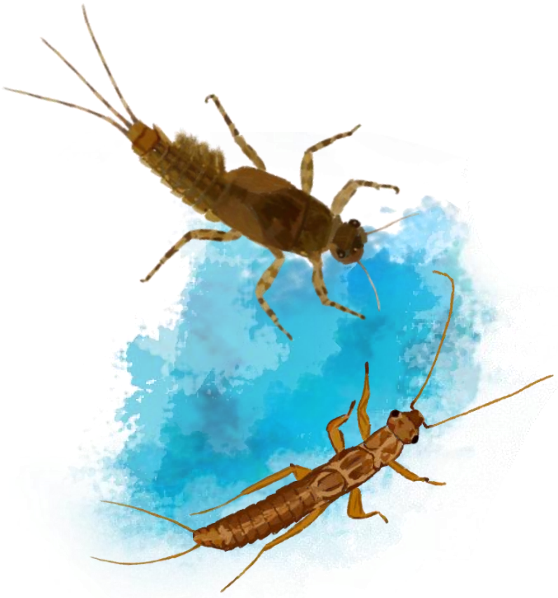
- **Chapter 5** underscored the importance of incorporating spatiotemporal connectivity and habitat heterogeneity into conservation planning for TRs, as these factors were critical drivers of community variation across space and time. The results suggested that using a combination of indices, such as LCBD and Marxan Selection Frequency, was valuable for identifying and prioritizing conservation sites, offering complementary insights into biodiversity and species rarity. The findings emphasized the need to monitor multiple hydrological phases to capture the full diversity of

communities, and stressed the importance of considering both connectivity and habitat heterogeneity at regional and local scales when designing conservation strategies for TRs.

The general conclusions of this thesis are as follows:

- The recordings of new species show that the aquatic fauna of TRs still remains poorly explored, and more studies at fine taxonomic resolution are needed.
- Hydrological connectivity in TRs plays a crucial role in the dynamics of metacommunities, even at very small spatial scales.
- The dispersal modes of organisms are fundamental in shaping the structure of populations and communities.
- Molecular tools, such as metabarcoding, hold significant potential for metapopulation and metacommunity studies. However, further development is required to fully address evolutionary questions and the dynamics of population and community isolation.
- The conservation and management of TRs should account for their spatiotemporal variability and adopt a meta-ecosystem perspective that integrates both local and regional scales.
- Disconnected pools appear as key habitats for biodiversity conservation in TRs.

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SUPPLEMENTARY CONTRIBUTIONS: ANNEXES 1, 2, AND 3

- **Annex 1:** First record of the genus *Tyrrhenoleuctra* Consiglio, 1957 (Plecoptera, Leuctridae) in Northeastern Iberian Peninsula (Catalonia, Spain)
- **Annex 2:** *Dixella graeca* (Pandazis, 1937), new species for mainland Europe (Diptera: Dixidae)
- **Annex 3:** The diversity of aquatic insects in the Sant Llorenç del Munt i l'Obac Natural Park

ANNEX 1

Primera cita del género *Tyrrhenoleuctra* Consiglio, 1957 (Plecoptera, Leuctridae) en el noreste de la península ibérica (Cataluña, España)

First record of the genus *Tyrrhenoleuctra* Consiglio, 1957 (Plecoptera, Leuctridae) in Northeastern Iberian Peninsula (Catalonia, Spain)

Los plecópteros son insectos que habitan preferentemente aguas de montaña de corrientes rápidas y bien oxigenadas (DEWALT *et al*., 2015). Esto, junto a su escasa capacidad de dispersión, los hace un grupo muy vulnerable a los efectos del cambio climático, principalmente en el sur de Europa, donde se espera que muchos ríos permanentes se conviertan en temporales (DÖLL & SCHMIED, 2012; LARNED *et al*., 2010; TIERNO DE FIGUEROA *et al*., 2010). No obstante, también hay especies de plecópteros de diversos géneros y familias adaptadas a vivir en ríos temporales y que presentan distintas estrategias de resistencia a los periodos de sequía (e.g. HYNES, 1976; WILLIAMS, 2006; LÓPEZ-RODRÍGUEZ *et al*., 2009a, 2009b). Un ejemplo de ello son las especies del género *Tyrrhenoleuctra* Consiglio, 1957, que se encuentran distribuidas en ríos temporales de la parte occidental de la cuenca mediterránea, llegando incluso a hallarse en arroyos a nivel del mar (FOCHETTI *et al*., 2009). Este género cuenta en la actualidad con cinco especies conocidas: *Tyrrhenoleuctra zavattarii* (Consiglio, 1956), endémica de las islas de Córcega y Cerdeña, *T. antoninoi* Fochetti & Tierno de Figueroa, 2009, endémica de las Islas Baleares, *T. tangerina* (Navás, 1922), que se encuentra en Marruecos, Argelia y España (sur de la provincia de Cádiz), y dos especies, *T. minuta* (Klapálek, 1901) y *T. lusohispanica* Tierno de Figueroa & Fochetti, 2014, ambas presentes en el sur de la península ibérica y, en el caso de esta última, también en el oeste peninsular hasta el norte de Portugal (FOCHETTI *et al*., 2009; TIERNO DE FIGUEROA & FOCHETTI, 2014, FERREIRA *et al*., 2020). La gran variabilidad inter e intraespecífica observada en los caracteres morfológicos tradicionalmente utilizados en la identificación de las especies de este género hacen indispensable el uso de herramientas moleculares para su correcta asignación específica (FOCHETTI *et al*., 2009).

El objetivo de este trabajo es dar a conocer la primera cita para el género *Tyrrhenoleuctra* en el noreste de la península ibérica. El material estudiado se recolectó en el Parque Natural de Sant Llorenç del Munt i l'Obac, en la provincia de Barcelona, el día 15/01/2019. Este parque natural tiene un clima mediterráneo estacional con lluvias irregulares que se concentran en invierno y con una época estival seca. La geología de la zona es de tipo kárstica. Se monitorizaron varios arroyos con sensores de temperatura (HOBO *Pendant Temperature/Light logger*) durante 513 días consecutivos para detectar la presencia o ausencia de agua (desde 26/07/2018 a 20/12/2019), y se tomaron muestras de macroinvertebrados en varios tramos (se pueden encontrar más detalles sobre la metodología de recolección y análisis de datos en PINEDA-MORANTE *et al*., 2022). Los ejemplares de *Tyrrhenoleuctra* se recolectaron en dos puntos diferentes. En la riera de Rellinars (R1) (41º 37' 57.1"N 1º 55' 56.9"E, 400 m.s.n.m.) caracterizada por una escasa frecuencia de rápidos y cantos medianamente fijados por arena, con grandes claros arbóreos y vegetación acuática compuesta por briófitos, algas incrustantes y caráceas. También se recogieron en cuatro localidades de la riera de Santa Creu, separadas apenas 1 km entre sí: SC1 (41º 41'53"N 1º 52' 58.8"E, 220 m.s.n.m.), SC2 (41º 41' 48.9"N 1º 53' 07.7"E, 224 m.s.n.m.), SC3 (41º 41' 48"N

1º 53' 29.7"E, 235 m.s.n.m.) y SC4 (41º 41' 44.2"N 1º 53' 32.3"E, 238 m.s.n.m.). Toda esta riera presenta escasa vegetación riparia y zonas de pozas y rápidos con distintos tamaños de cantos y arena.

Cabe destacar que, en el momento del muestreo, muchas zonas estaban congeladas debido a las bajas temperaturas (Figura 1A), aunque los parámetros físico-químicos no presentaban valores fuera de lo habitual (Tabla I).

Se recolectaron un total de 78 ninfas de *Tyrrhenoleuctra* (Figs. 1C, 1D): 19 en R1, 9 en SC1, 45 en SC2, 1 en SC3 y 4 en SC4.

Este hallazgo representa la primera cita de *Tyrrhenoleuctra* en el noreste peninsular y para Cataluña, siendo éste el único territorio conocido que alberga los tres géneros de Leuctridae [*Leuctra* Stephens (1836), *Pachyleuctra* Despax (1929) y *Tyrrhenoleuctra*] presentes en el Paleártico occidental (ZWICK, 2004).

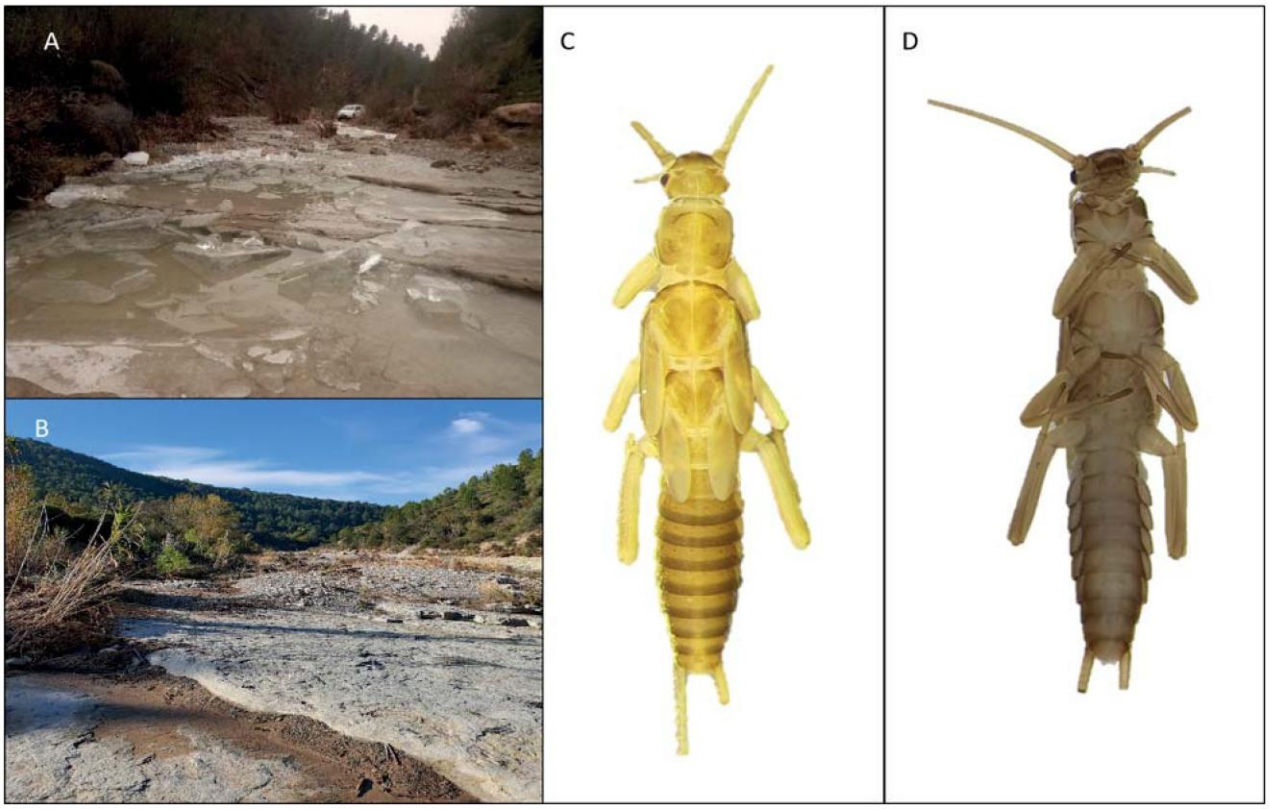


Fig. 1. A) Riera de Santa Creu en invierno durante el muestreo. B) Riera de Santa Creu en otoño. C) Ninfa de *Tyrrhenoleuctra* en visión dorsal. D) Ninfa de *Tyrrhenoleuctra* en visión ventral donde pueden apreciarse la separación entre terguitos y esternitos abdominales.
Fig. 1. A) Riera de Santa Creu in Winter during the sampling. B) Riera de Santa Creu in autumn. C) Nymph of *Tyrrhenoleuctra* in dorsal view. D) Nymph of *Tyrrhenoleuctra* in ventral view where the separation between abdominal tergites and sternites can be appreciated.

Tabla I. Parámetros físico-químicos de las localidades de muestreo el 15 de enero de 2019.
Table I. Physicochemical parameters of the sampling sites on January 15th 2019.

Riera	Código	pH	Oxígeno (mg/L)	Oxígeno (%)	Temperatura ºC	Conductividad específica(µS/cm)
Santa Creu	SC1	8,73	7,05	58	0,2	803,9
Santa Creu	SC2	8,38	11,18	95,4	1,4	804,5
Santa Creu	SC3	8,49	8,42	75,1	4,8	886
Santa Creu	SC4	7,99	11	97,63	5,6	911
Rellinars	R1	7,36	9,19	86,44	10,3	696

Los datos hidrológicos de las rieras muestran un alto porcentaje de intermitencia (porcentaje de días en los que el cauce no lleva agua respecto al total de días estudiados), con valores entre 44,25 % y 72,32% (Tabla II). Esto, junto con la baja altitud sobre el nivel del mar (entre 220 y 400 m.s.n.m.), coincide con la tipología de ríos en los que habitan normalmente las poblaciones de este género (e.g. FOCHETTI *et al*., 2009).

Tabla II. Características hidrológicas de las localidades de muestreo estudiadas a lo largo del periodo de estudio. El caudal fue medido el 15 de enero de 2019.
Table II. Hydrological characteristics of the studied sites throughout the study period. The flow was measured on January 15th 2019.

Código	Días totales de estudio	Intermitencia de flujo (%)	Caudal (l/s)
SC1	513	44,24951267	0,16
SC2	513	66,08187135	1,32
SC3	513	65,10721248	0,73275
SC4	513	46,71864847	0,65075
R1	513	72,31968811	2,57

Resulta llamativo el hecho de que este género no haya sido previamente detectado en Cataluña, dada la cantidad de estudios realizados en sus medios fluviales. Una posible explicación podría ser una colonización reciente, si bien parece poco probable dada la escasa capacidad de dispersión de estos organismos y la distancia a zonas más próximas con presencia confirmada de este taxón. Parece más probable que, a pesar de ser un área muy estudiada, este género no se hubiera detectado con anterioridad por otros motivos como: 1) haber pasado desapercibidos porque en muchos estudios de ecología fluvial y calidad de las aguas los ejemplares son identificados a nivel de familia [posiblemente incluyendo este género erróneamente entre los Capniidae, dada la nítida separación entre terguitos y esternitos más allá del cuarto segmento abdominal en las ninfas (Fig. 1D), un carácter habitualmente empleado en las claves a nivel de familia para distinguir Capniidae de Leuctridae a nivel europeo, aunque recomendamos ver ZWICK (2004) para una identificación más precisa], o 2) a que los ambientes fluviales temporales han recibido a nivel global menor atención, comparativamente con los medios permanentes, en cuanto a los estudios taxonómicos de ciertos grupos animales como el de los plecópteros.

Futuros estudios morfológicos, etológicos y principalmente moleculares, utilizando adultos y ninfas, nos permitirán la caracterización específica de estas poblaciones de *Tyrrhenoleuctra* de Cataluña. No obstante, es importante, dado el nivel taxonómico que habitualmente se emplea en numerosos estudios ecológicos de ríos, destacar la presencia en el área de este género, de características biológicas y autoecológicas peculiares (e.g. GRAF *et al*., 2008; LÓPEZ-RODRÍGUEZ *et al*., 2009a) y que supone una notable ampliación de la distribución previamente conocida en la península ibérica.

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ANNEX 2

NOTA / NOTE

Dixella graeca (Pandazis, 1937), especie nueva para Europa continental (Diptera: Dixidae)

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Resumen: El díxido *Dixella graeca* (Pandazis) (Diptera) se cita de Europa continental por primera vez, a partir de material capturado en España. Los ejemplares fueron cogidos mediante una trampa de emergencia situada en una poza desconectada de un río temporal en la provincia de Barcelona.
Palabras clave: Diptera, Dixidae, *Dixella graeca*, faunística, España, Europa continental.

Abstract: *Dixella graeca* (Pandazis, 1937), new species for mainland Europe (Diptera: Dixidae). The dixid *Dixella graeca* (Pandazis) (Diptera) is recorded for the first time from mainland Europe, from material collected in Spain. The specimens were caught in an emergency trap placed in a disconnected pool of a temporary river in the province of Barcelona.
Key words: Diptera, Dixidae, *Dixella graeca*, faunistics, Spain, mainland Europe.

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Los Dixidae son una familia de dípteros nematóceros de tamaño pequeño (2-6 mm) y con un número reducido de especies (menos de 200 a nivel mundial y 32 en Europa), de las cuales en la Península Ibérica se conocen 8 en España, 3 en Andorra y 2 en Portugal, todas ellas pertenecientes a dos géneros: *Dixa* Meigen 1818, con 12 especies, y *Dixella* Dyar & Shannon 1924, con 20 (Wagner & Báez, 2002; Wagner, 2004; Marshall, 2012).

Las larvas, todas acuáticas y filtradoras, son fáciles de reconocer, entre otros caracteres, por su forma de U cuando descansan y, normalmente, se las puede encontrar en la superficie, cerca de la orilla y de los tallos de las plantas que sobresalen del agua. Las larvas de *Dixa* prefieren aguas rápidas, mientras que las de *Dixella* las prefieren estancadas, aunque ambos géneros se pueden encontrar en aguas lentas (Oosterbroek, 2006; Marshall, 2012).

Por su parte, los adultos son delicados, de patas largas, pareciendo tipuloideos muy pequeños, aunque se distinguen fácilmente por tener la vena R_{2+3} bifurcada y fuertemente arqueada. No se alimentan y se les puede encontrar cerca del agua donde se desarrollan las larvas, formando a menudo nubes de apareamiento (Oosterbroek, 2006; Marshall, 2012).



Fig. 1.- Vista general de la trampa de emergencia y su ubicación.

En esta nota se presenta una novedad faunística obtenida durante un estudio entomológico llevado a cabo por los tres últimos autores en el Parque Natural de Sant Llorenç del Munt i l'Obac, provincia de Barcelona. El estudio se llevó a cabo del 6 de septiembre al 5 de octubre de 2021. Durante este tiempo, se instaló una trampa de emergencia sobre la superficie del agua en una poza desconectada en el Torrent de la Vall d'Horta (41.6785662, 2.0273655, 513 m s.n.m.) (Fig. 1). La trampa, de 1,1x1,1 m, flotaba en la superficie y presentaba una tela periférica en la base de unos 10 cm que se sumergía para impedir que entraran organismos externos vía aérea. Los individuos que emergían quedaban atrapados en un bote con alcohol etílico al 70% colocado en el extremo superior de la trampa. La recolecta de muestras se realizó semanalmente durante el periodo de estudio. Entre todo el material entomológico obtenido, destacamos aquí una pequeña muestra de 18 ejemplares del género *Dixella* que, siguiendo la clave de Disney (1999), se han identificado como *Dixella graeca* (Pandazis, 1937) (Fig. 2).

Material estudiado:
1♀ (6-13/9/2021), 1♀ (13-22/9/2021), 1♂ y 2♀♀ (22-29/9/2021), 4♂♂ y 9♀♀ (29/9-5/10/2021).

El material, identificado por el primer autor, se halla conservado en alcohol 70° en la colección particular del mismo.

Las larvas de esta especie están citadas en mayo, agosto y octubre. El hábitat larval incluye *Phragmites* spp. y vegetación a orillas de estanques o cuerpos de agua pequeños. En general, las hembras ponen entre 30 y 40 huevos por puesta, pero se han llegado a contabilizar hasta 83. El periodo de vuelo de los adultos es en agosto y septiembre (Disney, 1999), coincidiendo con nuestra época de muestreo.



Fig. 2.- Habitus de *Dixella graeca* (Pandazis). Longitud del cuerpo = 1,9 mm.

En cuanto a su distribución, hasta ahora esta especie se conocía únicamente de Gran Bretaña y la isla de Cerdeña (Italia) (Wagner, 2004 y com. pers.). Por lo tanto, estas capturas representan la primera cita de esta especie para Europa continental.

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ANNEX 3

La diversitat d'insectes aquàtics al Parc Natural de Sant Llorenç del Munt i l'Obac

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Resum

Els rius i rieres temporals són el principal ecosistema fluvial del parc. L'estudi d'aquest ecosistema s'ha incrementat en els últims anys, però encara queden aspectes bàsics de la seva biodiversitat per descobrir.

En aquest treball se citen 25 nous tàxons d'insectes aquàtics descoberts pel projecte MECODISPER en set rieres mostrejades dins del parc. Són tàxons de sis ordres, majoritàriament d'àmplia distribució a la península ibèrica, però que no havien estat citats prèviament al parc. Cal destacar el gènere *Tyrrhenoleuctra*, troballa que representa la primera cita per tot el nord-est de la península. S'han trobat nous tàxons durant les quatre estacions de l'any i a totes les rieres estudiades. Aquest treball posa de manifest el valor de conservació de les rieres del parc i la necessitat de continuar estudiant-ne la biodiversitat, encara força desconeguda.

Paraules clau
Rius temporals, insectes aquàtics, biodiversitat, faunística

Resumen

La diversidad de insectos acuáticos en el Parque Natural de Sant Llorenç del Munt i l'Obac

Los ríos y arroyos temporales son el principal ecosistema fluvial del parque. El estudio de este ecosistema se ha incrementado en los últimos años, pero todavía quedan aspectos básicos de su biodiversidad por descubrir.

En este trabajo se citan 25 nuevos taxones de insectos acuáticos descubiertos por el proyecto MECODISPER en siete arroyos muestreados dentro del parque. Son taxones de seis órdenes, mayoritariamente de amplia distribución en la península ibérica, pero que no habían sido citados previamente en el parque. Hay que destacar el género *Tyrrhenoleuctra*, hallazgo que representa la primera cita para todo el nordeste de la península. Se han encontrado nuevos taxones durante las cuatro estaciones del año y en todos los arroyos estudiados. Este trabajo pone de manifiesto el valor de conservación de los arroyos del parque y la necesidad de continuar estudiando su biodiversidad, aún bastante desconocida.

Palabras clave
Ríos temporales, insectos acuáticos, biodiversidad, faunística

Abstract

The diversity of aquatic insects in the Sant Llorenç del Munt i l'Obac Natural Park

The temporary rivers and stream are the main fluvial ecosystem in the park. The study of this ecosystem has increased in recent years, but there are still basic aspects of its biodiversity to be discovered.

This work cites 25 new taxa of aquatic insects discovered by the MECODISPER project in seven streams sampled within the park. They are taxa of six orders, mostly widely distributed in the Iberian Peninsula, but which had not previously been mentioned in the park. The *Tyrrhenoleuctra* genus should be highlighted, a find that represents the first mention for the entire north-east of the peninsula. New taxa have been found during the four seasons of the year and in all the streams studied. This work highlights the conservation value of the park's streams and the need to continue studying their biodiversity, which is still largely unknown.

Keywords
Temporary rivers, aquatic insects, biodiversity, fauna

Introducció

Els rius i rieres temporals constitueixen el 60 % de tots els rius i rieres a escala global (Messager *et al.*, 2021), i s'estima que ocupen més del 50 % de la longitud fluvial de la península ibèrica. En les últimes dècades, els rius i rieres temporals han augmentat a causa del canvi climàtic i les pressions antròpiques sobre l'ús de l'aigua (Larned *et al.*, 2010). S'espera que aquesta tendència es mantingui en el futur i converteixi molts rius permanents en temporals, principalment a les regions del planeta que es troben sota escenaris de canvi climàtic més extrems (Döll i Schmied, 2012; Datry *et al.*, 2014). Això comportarà una disminució de la connectivitat hidrològica i una pèrdua d'hàbitats per als organismes aquàtics que habiten en aquests sistemes.

Típicament, els rius i rieres temporals naturals presenten comunitats de macroinvertebrats aquàtics (constituïdes majoritàriament per insectes) amb valors de riquesa i diversitat alfa menors que els rius permanents (Soria *et al.*, 2017). No obstant això, la seva gran variabilitat ambiental i el seu aïllament hidrològic promouen la diversificació d'espècies i d'estratègies ecològiques, que resulta en un augment de la diversitat beta (Schriever i Lytle, 2016). És a dir, aquests ecosistemes alberguen un nombre baix d'espècies de manera local, però promouen la diversitat regional perquè afavoreixen el reemplaçament d'espècies en el temps i l'espai.

A més, és d'esperar que aquests organismes presentin estratègies de resistència per sobreviure durant el període de sequera (com la diapausa o la quiescència d'ous i larves d'insectes; dispersió temporal), o de resiliència per permetre'ls desplaçar-se a zones més favorables per tornar posteriorment, quan les condicions siguin favorables (dispersió espacial). Per això, moltes espècies de macroinvertebrats aquàtics depenen directament de romanents d'aigua o gorgs que es mantenen al llit del riu, o en rius més pròxims, i que actuen com a refugi (Arthington *et al.*, 2010). Malgrat l'interès dels macroinvertebrats aquàtics dels rius i rieres temporals, el seu coneixement taxonòmic és encara molt limitat, sobretot dels grups més complexos.

Les rieres del Parc Natural de Sant Llorenç del Munt i l'Obac presenten un ampli gradient de temporalitat, des de rieres permanents fins a rieres molt efímeres, i, per tant, són un marc ideal per a l'estudi de les comunitats de macroinvertebrats aquàtics i la seva resposta a la temporalitat (Bonada *et al.*, 2007; Pineda-Morante *et al.*, 2022). El projecte MECODISPER, finançat pel Ministeri de Ciència i Innovació, ha estat estudiant les respostes d'aquests organismes a la temporalitat en diverses rieres del parc. L'objectiu d'aquest treball és donar a conèixer les noves cites d'insectes aquàtics trobades en les mostres del projecte MECODISPER i que no havien estat prèviament citades al parc. En aquest treball no s'inclouen els tàxons de l'ordre dels dípters a causa de la seva complexitat taxonòmica, que requeriria un estudi més detallat.

Material i mètodes

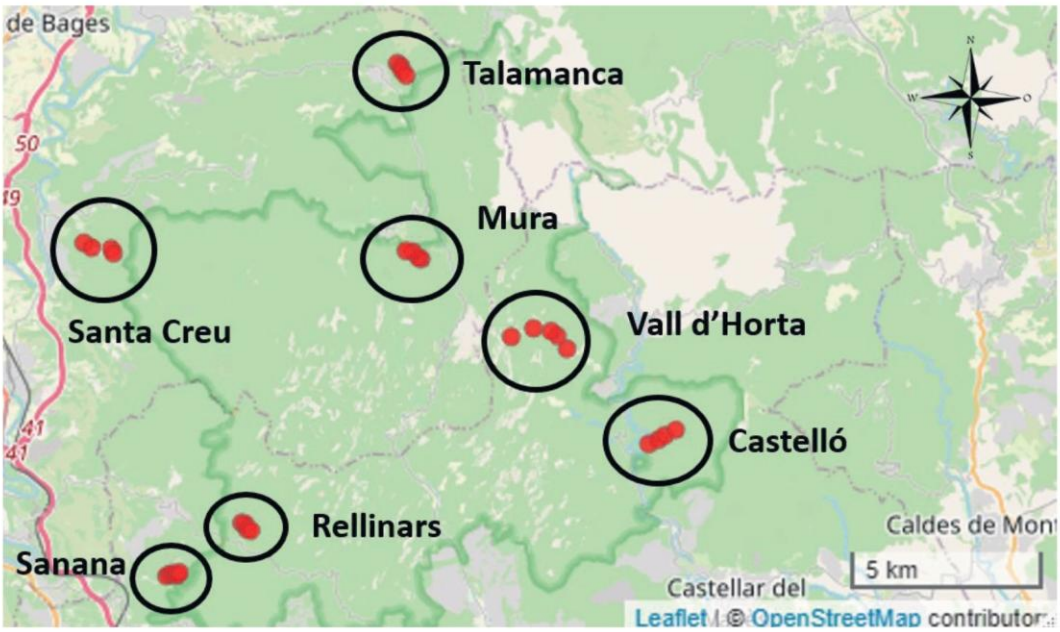
Àrea d'estudi

L'estudi es va dur a terme en set rieres del Parc Natural de Sant Llorenç del Munt i l'Obac i els seus voltants, a la província de Barcelona (Figura 1). Aquesta zona presenta un clima mediterrani estacional, amb pluges irregulars a la tardor i la primavera,

fortes pluges a l'hivern i estius secs. La geologia del parc és predominantment càrstica, la qual cosa afavoreix la infiltració de l'aigua superficial. Tot i així, també es troben trams amb roca mare que permeten mantenir aigua en forma de basses desconnectades a l'estiu.

Les rieres estudiades van ser Mura (o Nespres), Castelló, Talamanca, Vall d'Horta, Santa Creu, Rellinars i Sanana. Aquestes rieres cobreixen un ampli gradient hidrològic. La Vall d'Horta és la més permanent i Rellinars, la més efímera. A cada riera s'han estudiat entre quatre i cinc punts de mostreig separats uns 200 o 500 metres.

Figura 1. Mapa del Parc Natural de Sant Llorenç del Munt i l'Obac amb les diferents rieres mostrejades i la seva localització. Imatge obtinguda d'*OpenStreetMap contributor*.



Recollida de mostres

Els mostrejos es van dur a terme de manera estacional (hivern, primavera, estiu i tardor) durant l'any 2019 en trenta punts de mostreig repartits per les set rieres. Es van prendre mostres de macroinvertebrats aquàtics en trams d'uns 100 metres amb una xarxa *kick* de 250 micròmetres de llum de malla i es van mostrejar tots els hàbitats disponibles.

També es va fer un experiment de colonització al final de l'època seca (setembre del 2021), en què es van instal·lar vuit basses artificials al llit sec del torrent de la Font del Llor, conca de la riera de la Vall d'Horta. Aquestes basses es van disposar aigües amunt i avall de les basses desconnectades naturals que serveixen de refugi per als organismes aquàtics durant la fase seca. També es van instal·lar tres trampes d'emergència d'adults d'insectes aquàtics a les basses naturals (Figura 2). Es van mostrejar ambdós tipus de basses des del 6 de setembre fins al 5 d'octubre del 2021, recollint mostres setmanals de les trampes d'emergència, mostrejant a l'inici i al final les basses desconnectades i recollint mostres de les basses artificials al final de l'experiment.

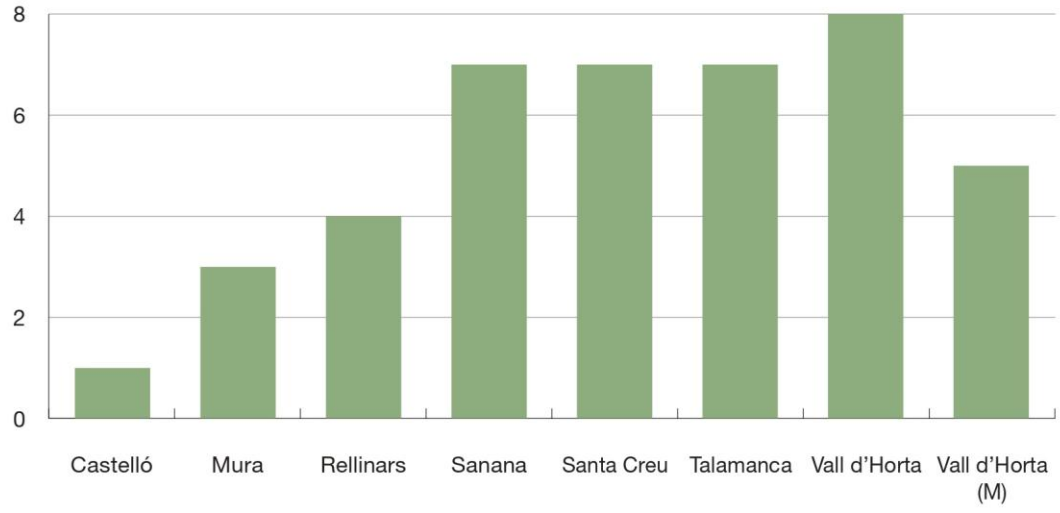
Figura 2. Trampa d'emergència instal·lada en una bassa desconnectada natural



Resultats

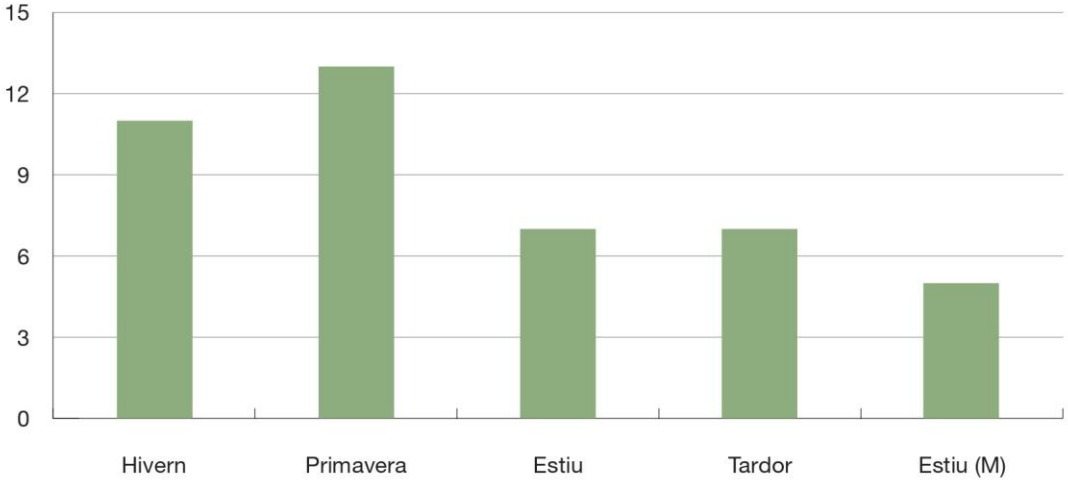
Es varen trobar 25 nous tàxons en totes les mostres recol·lectades, 19 dels quals es varen poder identificar a nivell d'espècie i 6, de gènere. Aquestes cites es distribueixen en 59 mostres al llarg de l'espai (diferents rieres) i del temps (diferents estacions), de les 101 mostres recol·lectades en total. A més, aquests tàxons es varen trobar en els diferents rieres mostrejades: Vall d'Horta (8), Sanana (7), Santa Creu (7), Talamanca (7), experiment Vall d'Horta (5), Rellinars (4), Mura (3) i Castelló (1) (Figura 3a). Al llarg de les estacions, i si considerem per separat l'experiment de Vall d'Horta (realitzat a finals d'estiu), el nombre de tàxons citats en cada estació seria el següent: hivern (11), primavera (13), estiu (7), tardor (7), experiment Vall d'horta (finals d'estiu) (5) (Figura 3b). L'ordre més representat és el dels coleòpters (Figura 4).

Figura 3a. Nombre de cites noves en cadascuna de les set rieres



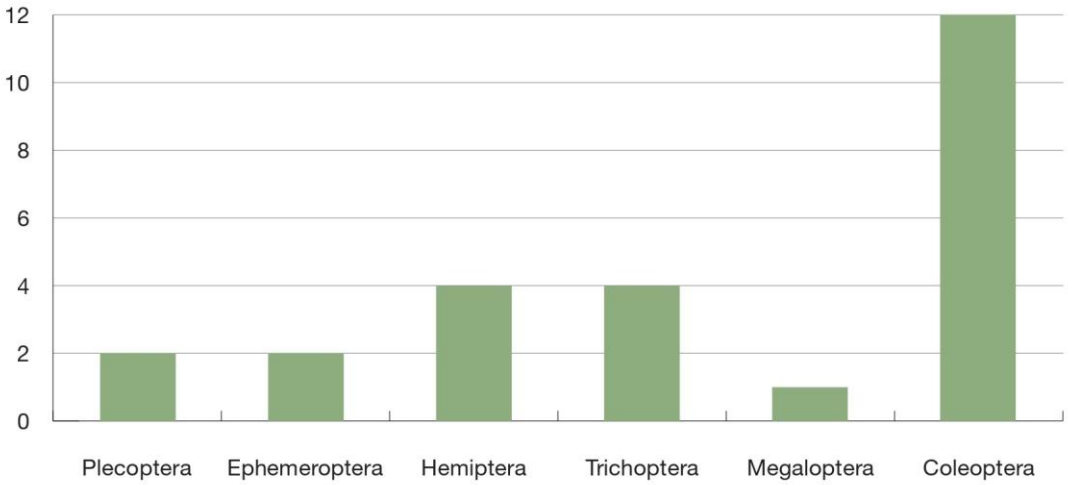
Vall d'Horta (M) fa referència a les mostres de l'experiment.

Figura 3b. Nombre de cites noves a cada estació i a l'experiment de colonització



Estiu (M) fa referència a les mostres de l'experiment.

Figura 4. Nombre de cites noves per ordre



- Ordre Plecoptera

  - ***Siphonoperla torrentium* (Pictet, 1842) (família Chloroperlidae)**
    7 nimfes (Vall d'Horta, 14/01/2019)
  - ***Tyrrhenoleuctra* Consiglio, 1957 (família Leuctridae)**
    19 nimfes (Rellinars, 15/01/2019; Santa Creu, 15/01/2019) (Figura 5)
    (Prèviament publicat a Fernández-Calero *et al.*, 2022.)

Figura 5. Nimfa del gènere *Tyrrhenoleuctra* a la riera de Santa Creu



- Ordre Ephemeroptera
  - ***Caenis luctuosa* (Burmeister, 1839) (família Caenidae)**
    184 adults (27♂, 157♀) (experiment Vall d'Horta, 04/10/2021)
  - ***Electrogena lateralis* (Curtis, 1834) (família Heptageniidae)**
    43 nimfes (Vall d'Horta, 14/01/2019), 1 (Vall d'Horta, 27/05/2019), 1 (Vall d'Horta, 21/11/2019), 39 (Sanana, 08/01/2019), 1 (Sanana, 18/11/2019), 1 (Mura, 07/01/2019) i 1 ♂ adult (experiment Vall d'Horta, 29/09/2021)

- Ordre Hemiptera
  - ***Naucoris maculatus* (Fabricius, 1798) (família Naucoridae)**
    18 adults (Sanana, 10/07/2019), 7 (Sanana, 23/05/2019), 1 (Sanana, 18/11/2019) i 1 (Talamanca, 09/07/2019)
  - ***Velia caprai* (Tamanini, 1947) (família Veliidae)**
    1 adult (experiment Vall d'Horta, 06/09/2021)
  - ***Parasigara perdubia* (Rey, 1894) (família Corixidae)**
    33 adults (experiment Vall d'Horta, 04/10/2021)
  - ***Plea minutissima* (Leach, 1818) (família Pleidae)**
    1 adult (Sanana, 08/01/2019) i 1 (Santa Creu, 27/05/2019)

- Ordre Trichoptera
  - ***Stactobia* (McLachlan, 1880) (família Hydroptilidae)**
    2 larves (Rellinars, 15/01/2019), 1 (Sanana, 18/11/2019), 7 (Sanana, 10/07/2019), 30 (Talamanca, 09/01/2019), 6 (Talamanca, 25/05/2019) i 21 (Talamanca, 09/07/2019)
  - ***Adicella reducta* (McLachlan, 1865) (família Leptoceridae)**
    1 larva (Vall d'Horta, 14/01/2019)

- ***Halesus* (Stephens, 1837) (família Limnephilidae)**
  1 larva (Vall d'Horta, 27/05/2019)
- ***Polycentropus kingi* (McLachlan, 1881) (família Polycentropodidae)**
  1 larva (Vall d'Horta, 21/11/2019)

- Ordre Megaloptera

  - ***Sialis lutaria* (Linnaeus, 1758) (família Sialidae)**
    1 larva (Vall d'Horta, 21/11/2019), 9 (Vall d'Horta, 27/05/2019), 7 (Vall d'Horta, 14/01/2019), 6 (Sanana, 08/01/2019), 14 (Sanana, 23/05/2019), 1 (Sanana, 18/11/2019), 1 (Talamanca, 09/07/2019) i 1 (Talamanca, 09/01/2019)

- Ordre Coleoptera

  - ***Agabus biguttatus* (Olivier, 1795) (família Dytiscidae)**
    1 adult (Talamanca, 22/05/2019)
  - ***Agabus bipustulatus* (Linnaeus, 1767) (família Dytiscidae)**
    1 adult (experiment Vall d'Horta, 04/10/2021)
  - ***Agabus nebulosus* (Forster, 1771) (família Dytiscidae)**
    3 adults (Vall d'Horta, 27/05/2019)
  - ***Dytiscus marginalis* (Linnaeus, 1758) (família Dytiscidae)**
    2 adults (Castelló, 20/11/2019)
  - ***Hydroglyphus geminus* (Fabricius, 1792) (família Dytiscidae)**
    1 adult (Santa Creu, 15/01/2019) i 1 (Talamanca, 09/07/2019)
  - ***Ilybius meridionalis* (Aubé, 1837) (família Dytiscidae)**
    1 adult (Mura, 09/07/2019)
  - ***Yola bicarinata* (Latreille, 1804) (família Dytiscidae)**
    1 adult (Rellinars, 15/01/2019), 1 (Santa Creu, 11/07/2019), 1 (Sanana, 10/07/2019) i 11 (Talamanca, 09/07/2019)
  - ***Helophorus* (Fabricius, 1775) (família Helophoridae)**
    1 adult (Santa Creu, 27/05/2019)
  - ***Ochthebius* (Leach, 1815) (família Hydraenidae)**
    1 adult (Santa Creu, 15/01/2019) i 1 (Talamanca, 22/05/2019)
  - ***Berosus (Berosus) signaticollis* (Charpentier, 1825) (família Hydrophilidae)**
    4 adults i 3 larves (Santa Creu, 15/01/2019), 2 adults (Santa Creu, 27/05/2019) i 1 adult i 2 larves (Santa Creu, 11/07/2019)
  - ***Paracymus* (C. G. Thomson, 1867) (família Hydrophilidae)**
    1 larva (Rellinars, 27/05/2019), 1 (Vall d'Horta, 21/11/2019) i 1 (Mura, 19/11/2019)
  - ***Noterus clavicornis* (De Geer, 1774) (família Noteridae)**
    1 adult (Sanana, 23/05/2019)

Discussió i conclusions

El nostre estudi posa de manifest que la diversitat i la distribució d'insectes aquàtics als rius i rieres temporals encara es troba lluny de ser coneguda. En aquest treball es presenten 25 noves cites pel Parc Natural de Sant Llorenç del Munt i l'Obac, trobades en diverses rieres que difereixen hidrològicament.

El tàxon més rellevant que s'ha trobat és el gènere de plecòpter *Tyrrhenoleuctra*. Es tracta de la primera cita en tot el nord-est de la península ibèrica. Això pot ser degut, segons Fernández-Calero *et al.* (2022), als pocs estudis realitzats en rius i rieres temporals (que són l'hàbitat propi d'aquest insecte) i al fet que, d'acord amb la seva fenologia, les nimfes es troben presents a les rieres durant l'hivern, coincidint amb l'època en la qual usualment es duen a terme menys campanyes de mostreig.

Tots els altres tàxons citats tenen una àmplia distribució a la península ibèrica, o fins i tot a Europa. Els coleòpters són l'ordre animal més divers (Zhang, 2011), i també és l'ordre que aporta més cites noves al parc. El fet que no hagin estat citats anteriorment al parc segurament està lligat a la falta d'estudis d'insectes aquàtics adults. Generalment, els estudis d'insectes en ecosistemes fluvials se centren en els estadis aquàtics de nimfes o larves, les quals en moltes ocasions no tenen desenvolupades les característiques morfològiques suficients per ser identificades a nivell d'espècie. En el nostre cas, l'estudi d'insectes adults recollits en les trampes d'emergència ens ha ajudat a reconèixer l'espècie d'alguns tàxons. A més, tot i que s'hagin dut a terme molts estudis de seguiment de la fauna fluvial del parc (Rieradevall *et al.*, 1999; Bonada *et al.*, 2007; CARIMED, 2023), en el millor dels casos, els macroinvertebrats s'han identificat a nivell de gènere, atès que els primers treballs tenien finalitats de monitoratge de la qualitat de les aigües, que només requereixen identificacions a nivell de la família.

En els nostres resultats es pot apreciar que hi ha diferències entre les rieres estudiades pel què fa al nombre de noves cites. Cal destacar que les rieres amb menys cites (Castelló i Mura) són les que porten estudiant-se des de fa més temps per diferents projectes del grup de recerca (entre els quals destaca el projecte CARIMED, 2023). Això posa de manifest la importància de dur a terme mostrejos periòdics dels rius i rieres per conèixer-ne la biodiversitat. També destaca la variació de cites trobades al llarg de l'any: l'estiu i la tardor són les estacions en les quals s'han trobat menys cites. A l'estiu, presumiblement perquè és l'estació més estudiada, i a la tardor, perquè, a causa de la sequera estival, la riquesa taxonòmica és més baixa, ja que les comunitats es troben en procés de recolonització (Pineda-Morante *et al.*, 2022).

Amb aquest treball s'amplia significativament el coneixement de la biodiversitat fluvial del parc. A més, es posa de manifest la necessitat d'impulsar estudis sobre la distribució i la història natural d'insectes aquàtics en ecosistemes aparentment més oblidats, com són els rius i rieres temporals. En el context actual de crisi de biodiversitat d'aigua dolça (Harrison *et al.*, 2018), i amb el canvi climàtic augmentant la temporalitat de cada cop més cursos fluvials (Döll i Schmied, 2012), es fa urgent la implementació d'estudis que aportin coneixement sobre la biodiversitat fluvial a nivell d'espècie. En aquest sentit, estudis com el nostre poden servir per establir la línia base de coneixement a partir de la qual dissenyar plans i estratègies de conservació específiques per a la biodiversitat aquàtica en espais protegits.

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SUPPORTING INFORMATION
(OR SUPPLEMENTARY MATERIAL)

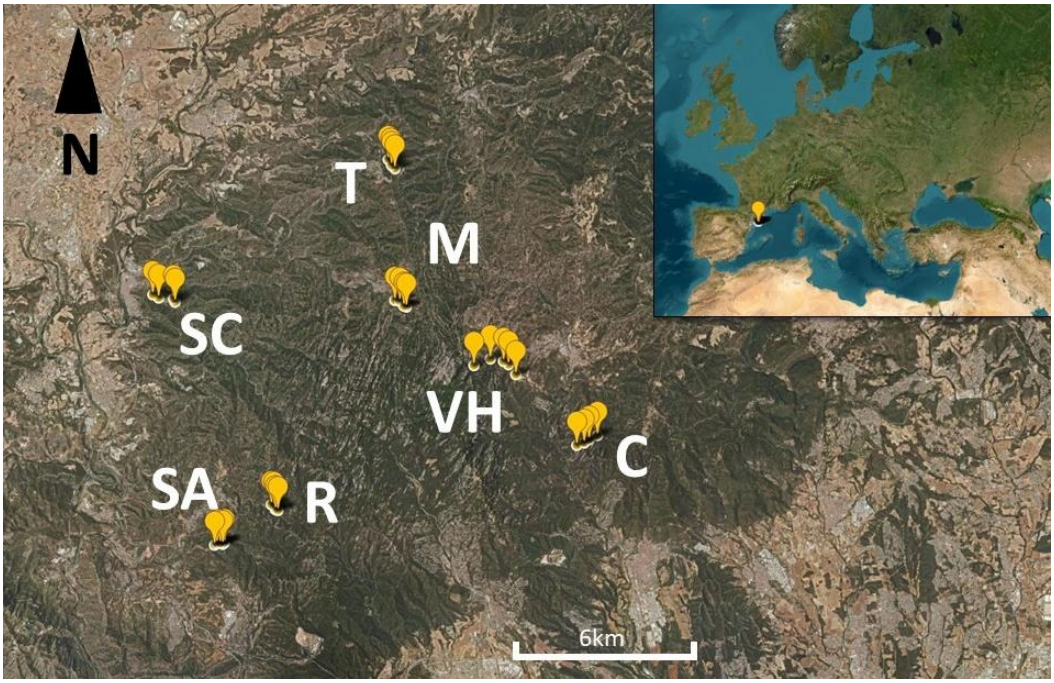
- **Chapter 1**
- **Chapter 2**
- **Chapter 3**
- **Chapter 4**
- **Chapter 5**
- **Tables of biological and environmental data**

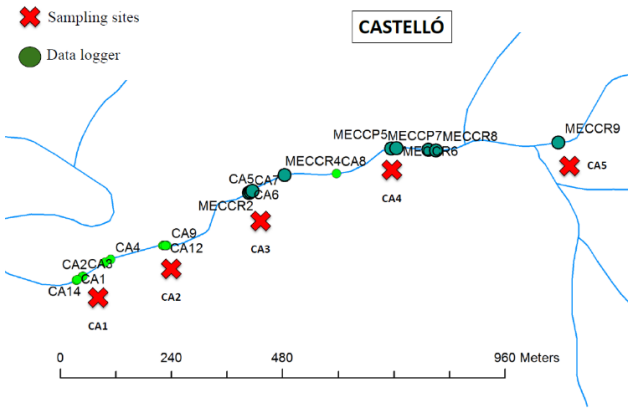
CHAPTER 1:

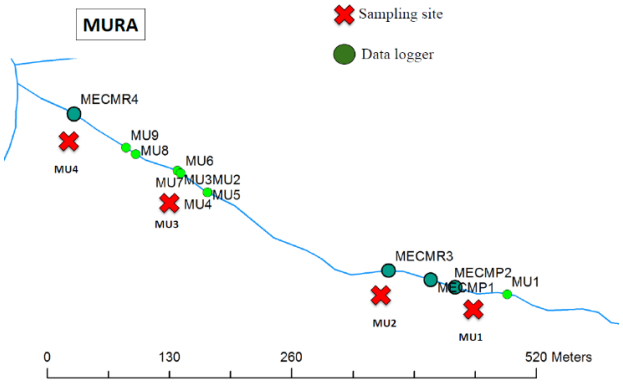
Navigating through space and time: a methodological approach to quantify spatiotemporal connectivity using stream flow data as a case study

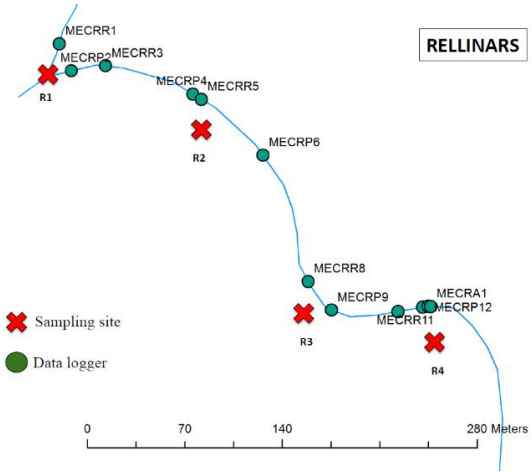
Please note that Table S1 and Table S2 for this chapter are not included in this section. To access the complete Supplementary Information, please visit the publication's webpage at the following link: https://besjournals.onlinelibrary.wiley.com/doi/full/10.1111/2041-210X.14105

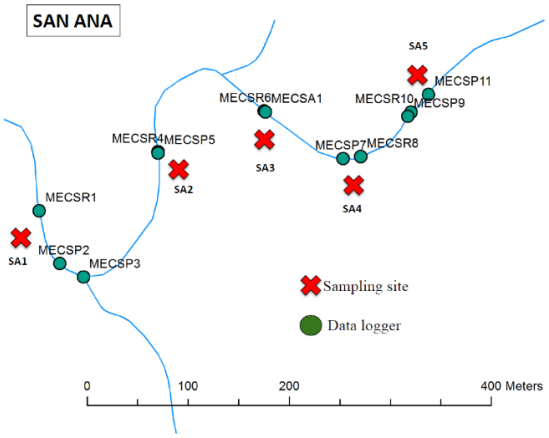
Figure S1: Maps of the main study area located in Sant Llorenç del Munt i l'Obac natural park and of each one of the monitored streams with the sampling locations and data loggers deployed to monitor flow states. Castelló (C), Mura (M), Rellinars (R), San Ana (SA), Santa Creu (SC), Talamanca (T) and Vall d'horta (VH). Note that the number of data loggers deployed in each stream varied due to differences in stream morphology and the number of reaches that were normally dry in that stream.

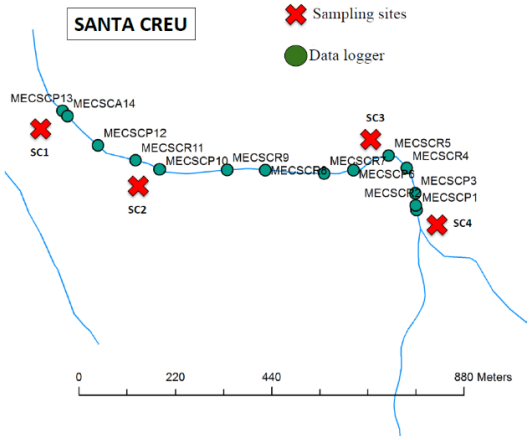


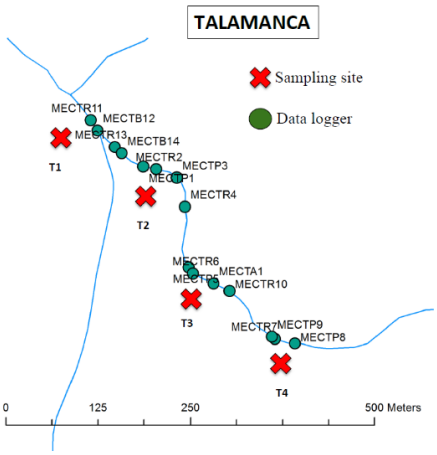

Sampling sites
Data logger
CASTELLÓ
MECCP5MECCP7MECCR8
MECCR6
MECCR9
MECCR4CA8
CA5CA7
CA6
MECCR2
CA9
CA4
CA12
CA2CA3
CA1
CA14
CA1
CA2
CA3
CA4
CA5
0
240
480
960 Meters


MURA
Sampling site
Data logger
MECMR4
MU9
MU8
MU6
MU4
MU7
MU3MU2
MU5
MU4
MU3
MECMR3
MECMP2
MECMP1
MU1
MU2
MU1
0
130
260
520 Meters


RELLINARS
MECRR1
MECRP2MECRR3
MECRP4MECRR5
MECRP6
MECRR8
MECRP9
MECRA1
MECRP12
MECRR11
R1
R2
R3
R4
Sampling site
Data logger
0
70
140
280 Meters


SAN ANA
SA5
MECSP11
MECSR6MECSA1
MECSR10MECSP9
MECSR4MECSP5
MECSP7MECSR8
MECSR1
MECSP2
MECSP3
SA1
SA2
SA3
SA4
Sampling site
Data logger
0
100
200
400 Meters


SANTA CREU
Sampling sites
Data logger
MECSCP13MECSCA14
MECSCP12
MECSCR11
MECSCP10
MECSCR9
MECSCR8
MECSCR7
MECSCR6
MECSCR5
MECSCR4
MECSCP3
MECSCP2
MECSCP1
SC1
SC2
SC3
SC4
0
220
440
880 Meters


TALAMANCA
Sampling site
Data logger
MECTR11
MECTB12
MECTR13
MECTB14
MECTR2
MECTP3
MECTP1
MECTR4
MECTR6
MECTA1
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T2
T3
T4
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250
500 Meters

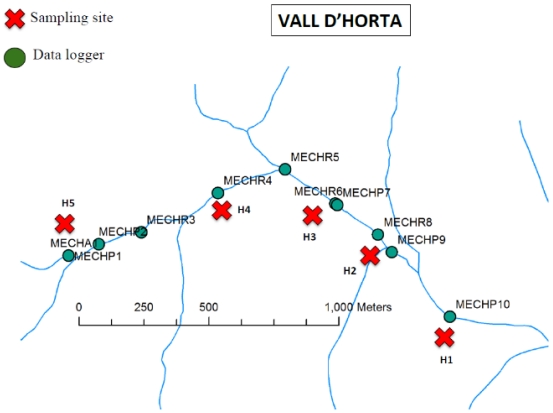
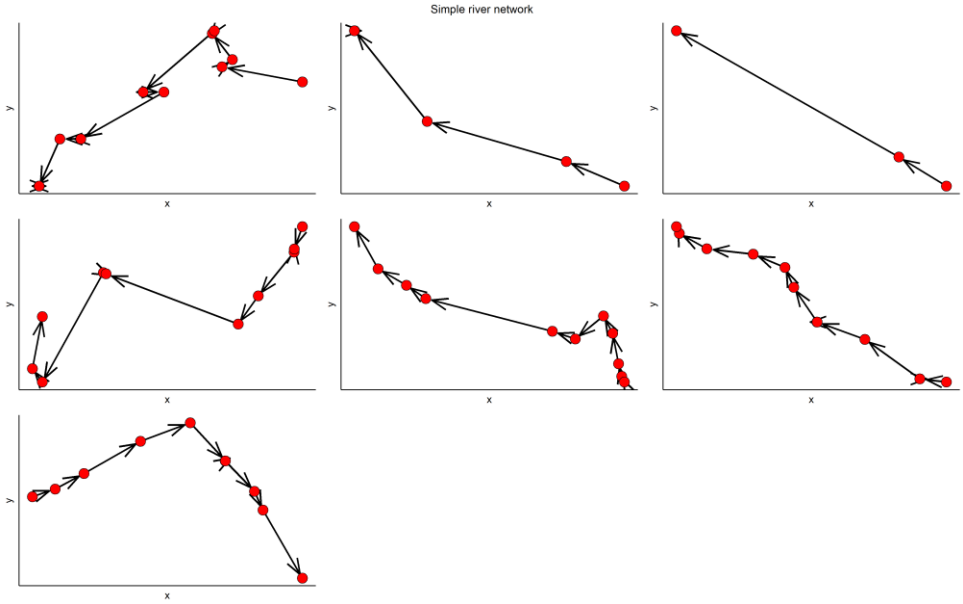

Sampling site
Data logger
VALL D'HORTA
MECHR5
MECHR4
MECHR6MECHP7
MECHR3
MECHR8
MECHP9
MECHA
MECHP1
H1
H2
H3
H4
H5
0
250
500
1,000 Meters
MECHP10

Figure S2: Network representation of all monitored streams C, M, R, SA, SC, T and VH respectively. Two network structure were considered: **A)** directed, which was used for the DirBin and DirWei scenarios and **B)** undirected, which was used for the UndBin and UndWei scenarios.

A) Directed scenarios network strucutre



B) Undirected scenarios network strucutre

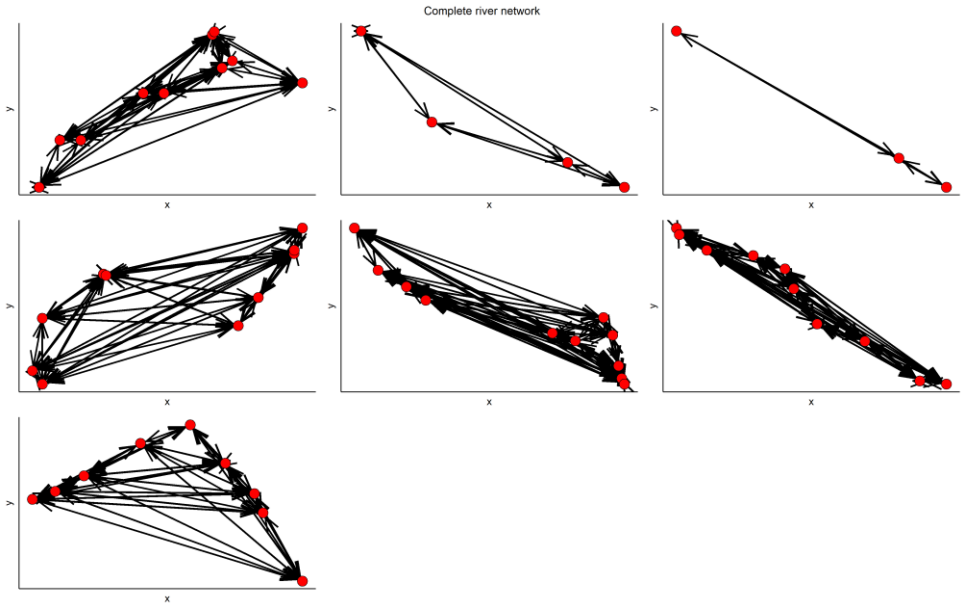
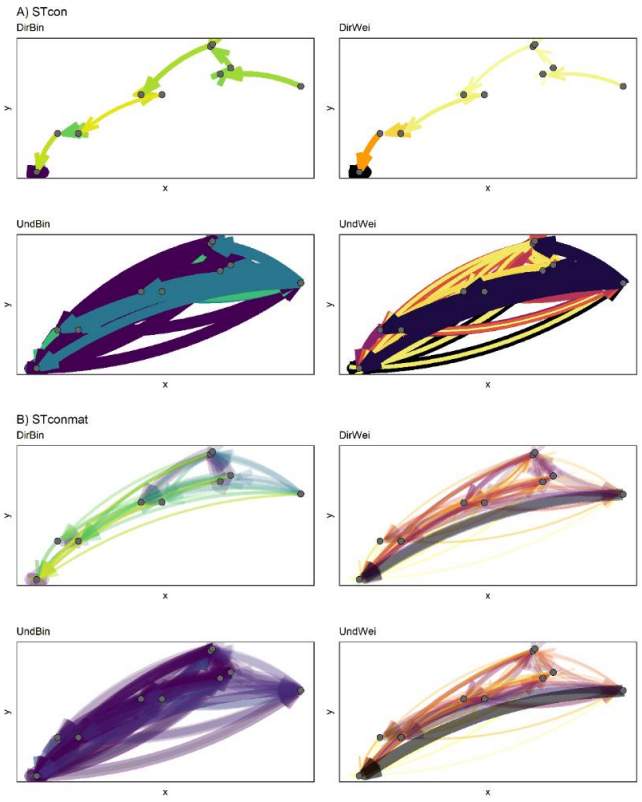


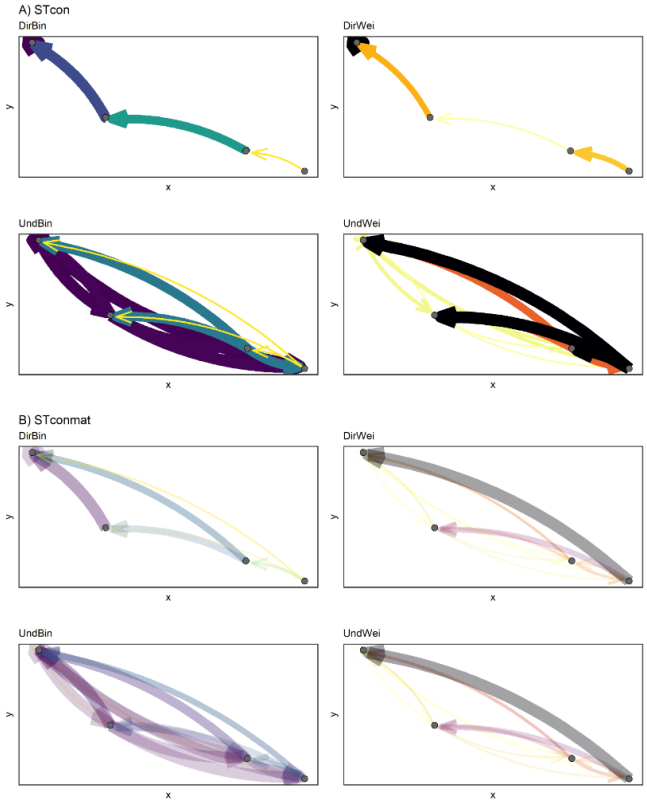
Figure S3: *STcon* and *STconmat* graphical representation for the seven monitored streams: C, M, R, SA, SC, T and VH respectively. The four calculated scenarios are represented in each part: DirBin; DirWei; UndBin; UndWei. The viridis colour gradient and arrow size illustrates the corresponding values of *STcon* and *STconmat*. For DirBin and UndBin scenarios a gradient from purple/blue to green/yellow indicates high to low spatiotemporal connectivity respectively. For DirWei and UndWei a gradient from grey/purple to red/yellow indicates greater or lower

spatiotemporal resistance to dispersal. Binary and Weighted scenarios quantify differently connectivity as they focus on only number of connections or how big is the distance to be connected.

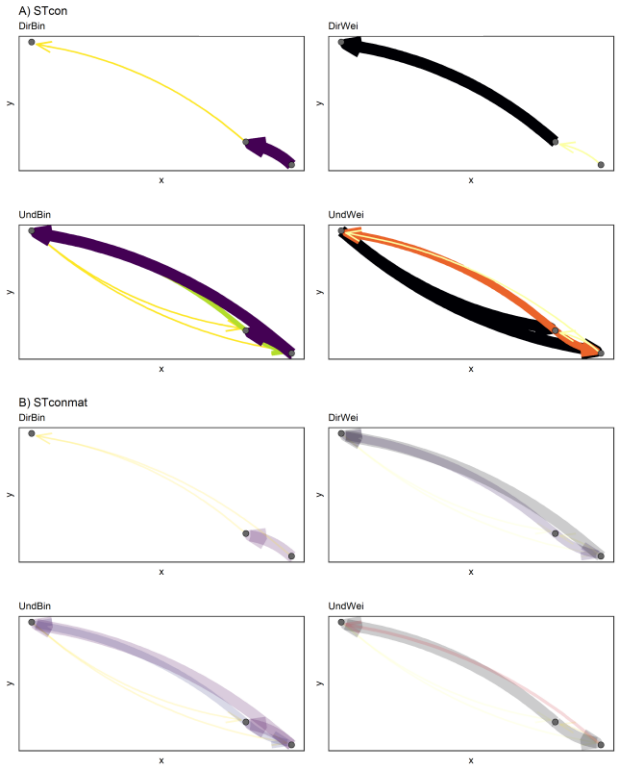
Castelló stream



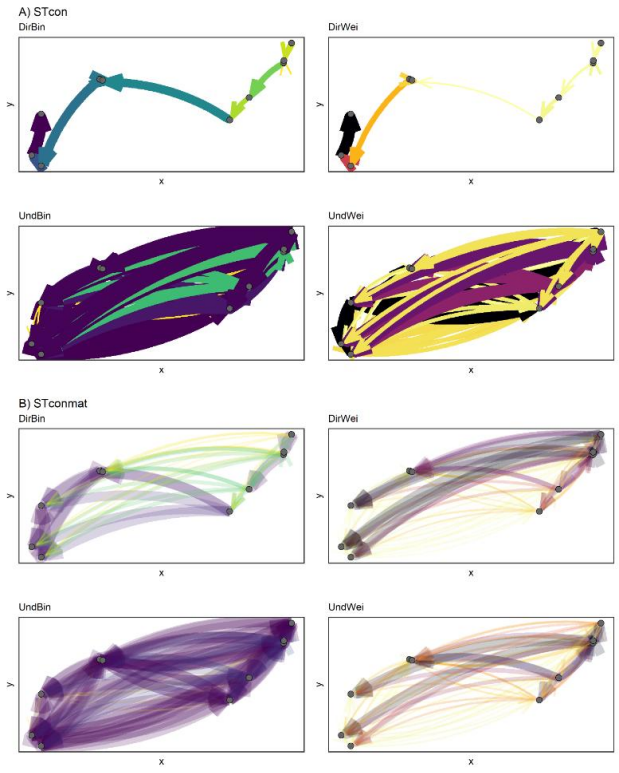
Mura stream



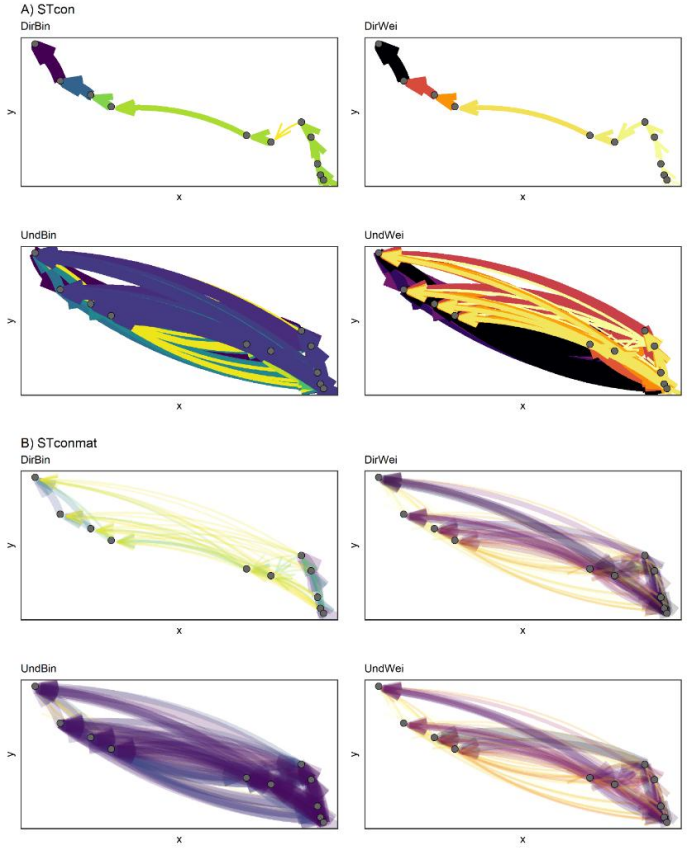
Rellinars stream



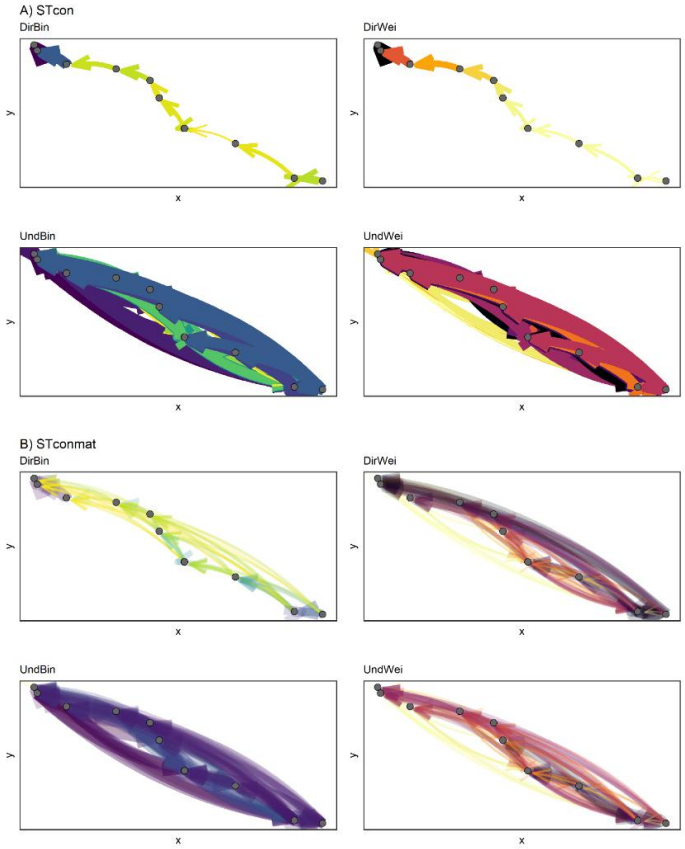
Sanana stream



Santa Creu stream



Talamanca stream



Vall d'Horta stream

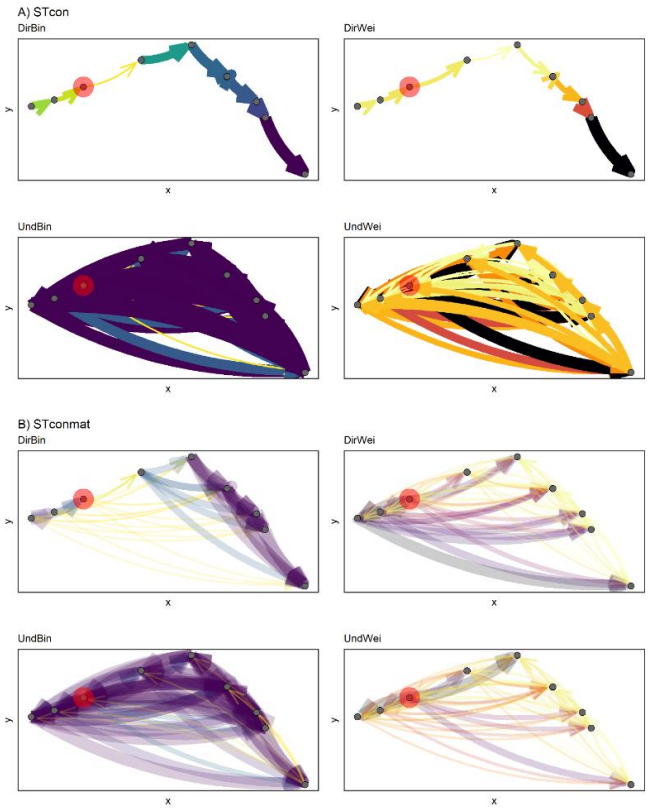


Figure S4: Spearman correlation matrix including *STcon* values calculated for the four scenarios DirBin, DirWei, UndBin, and UndWei. Spearman rho values for each pair are represented in the upper triangle section of the plot. Pairs with a significant correlation were indicated with a black asterisk next to Spearmen correlation rho.

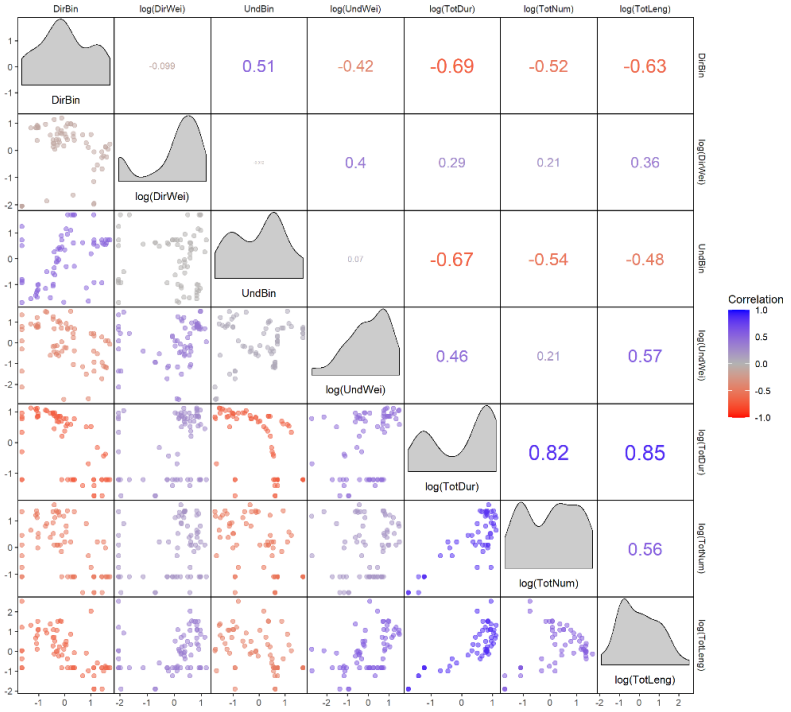
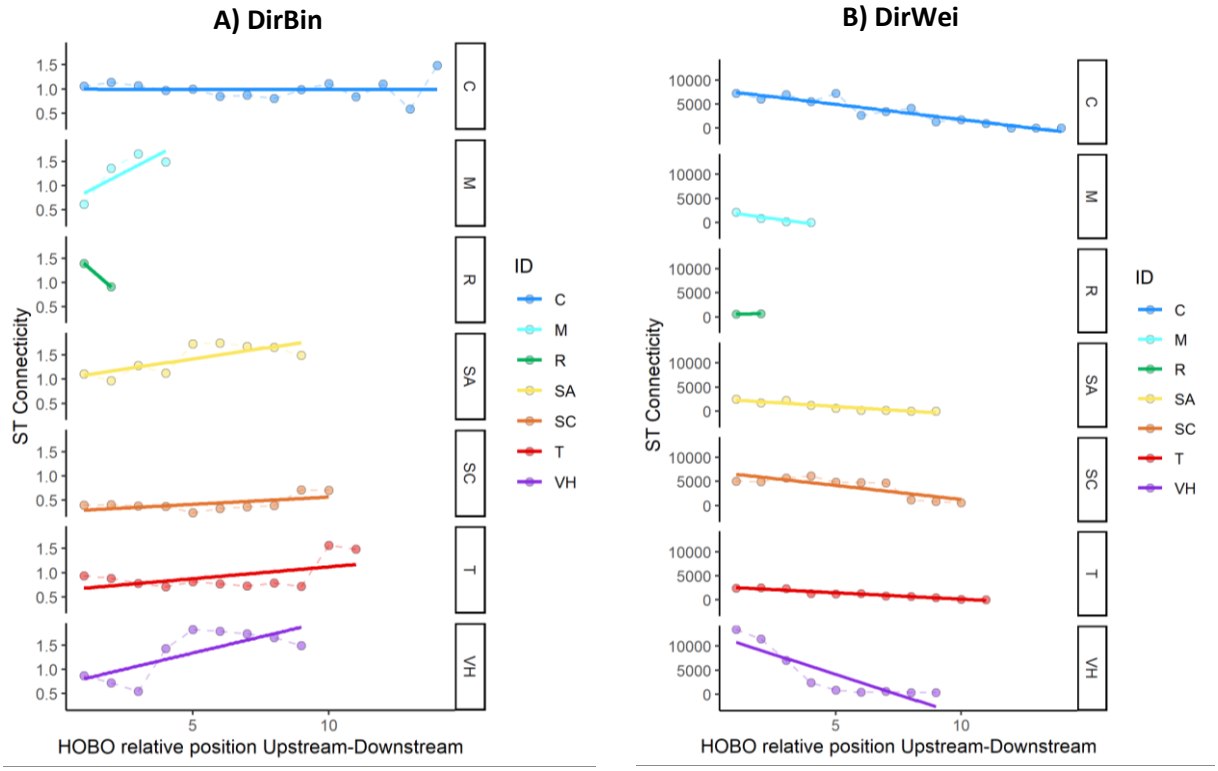


Figure S5: *STcon* values for each stream and for each one of the four scenarios. **A)** DirBin, **B)** DirWei, **C)** UndBin, **D)** UndWei. Note that these plots represent the same values as Figure 4 but here they are shown for each individual stream.



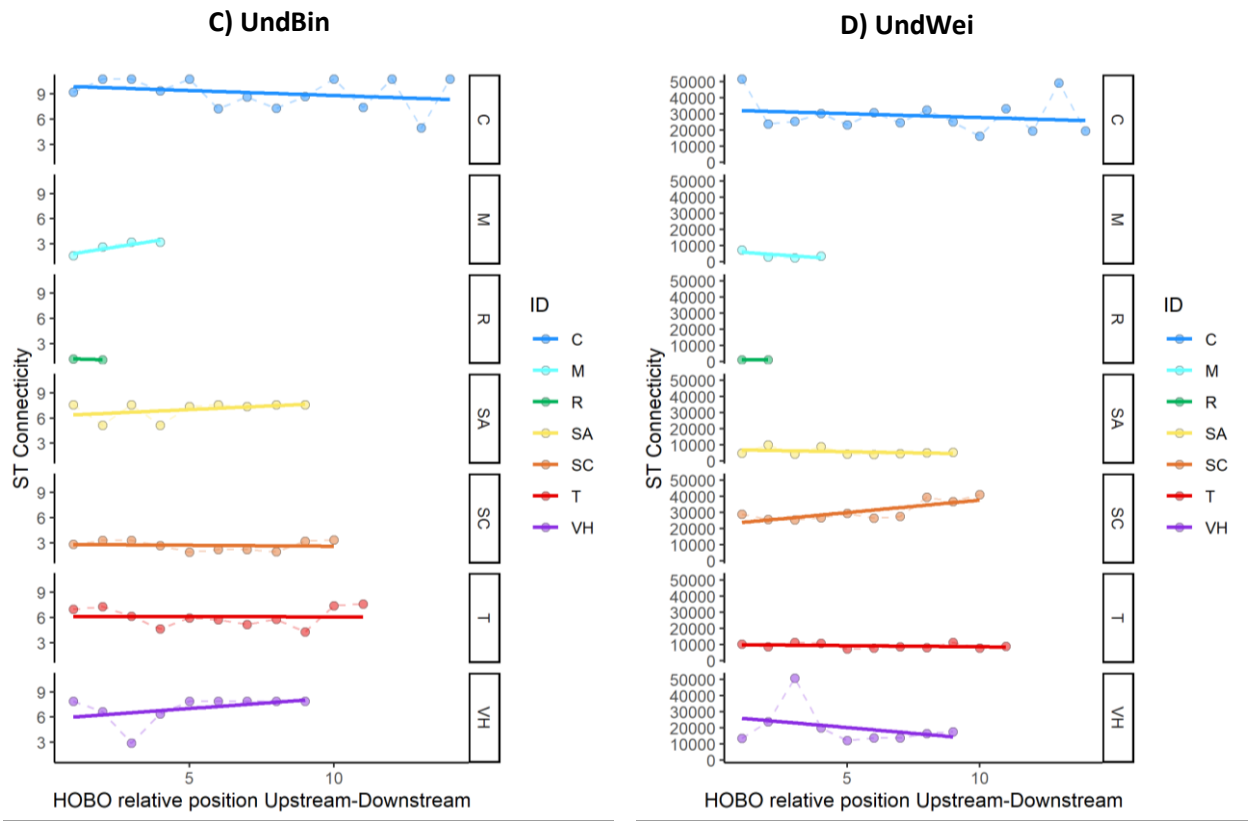
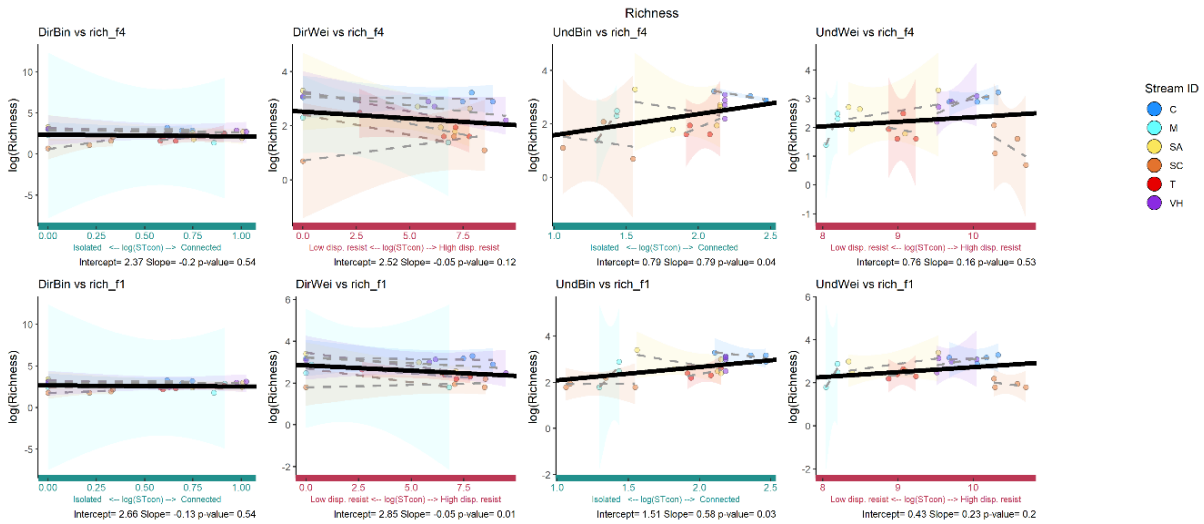
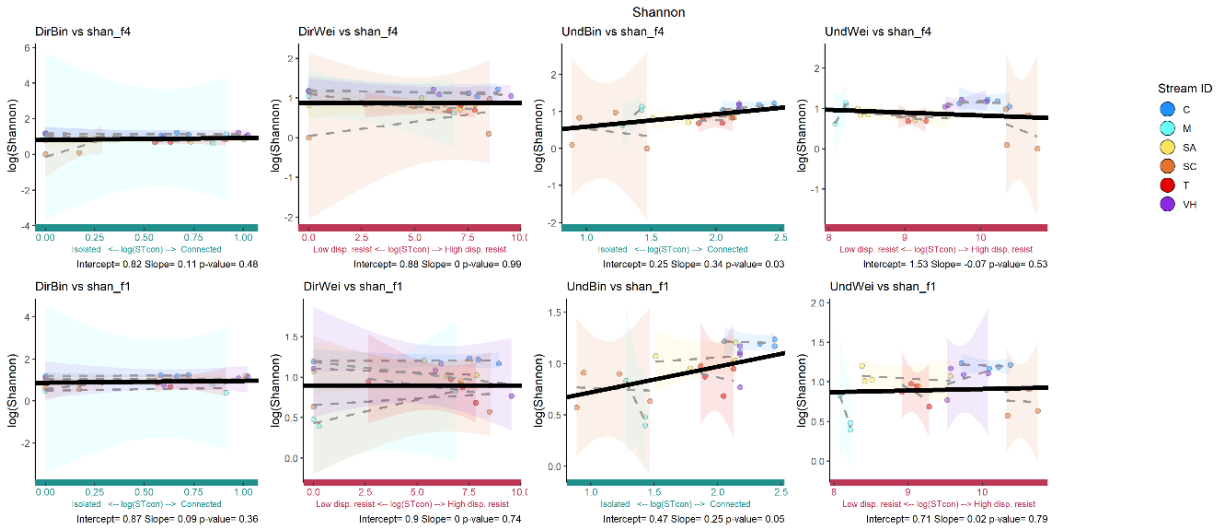


Figure S6: All community metrics results for the six monitored streams from which enough biological data was obtained (stream was not considered here R). Each box corresponds to an individual community metric: **A)** Taxonomic Richness, **B)** Shannon Wiener-diversity, **C)** Trait abundance, **D)** Jaccard dissimilarity, and **E)** Bray Curtis dissimilarity. In each box, the first row of plots correspond to active dispersers (group of trait f4) and the second row of plots to passive dispersers (group of trait f1). Note the X axes of plots must be read carefully for each scenario: for DirBin and UndBin higher *STcon* values mean higher spatiotemporal connectivity whereas for DirWei and UndWei higher *STcon* values mean greater spatiotemporal resistance to dispersal.

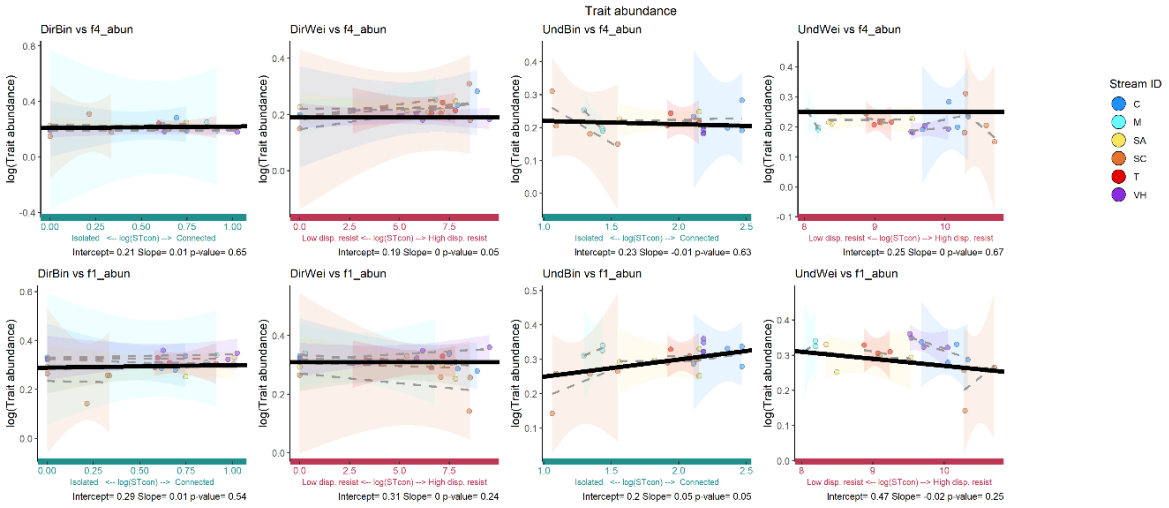
A) Taxonomic richness



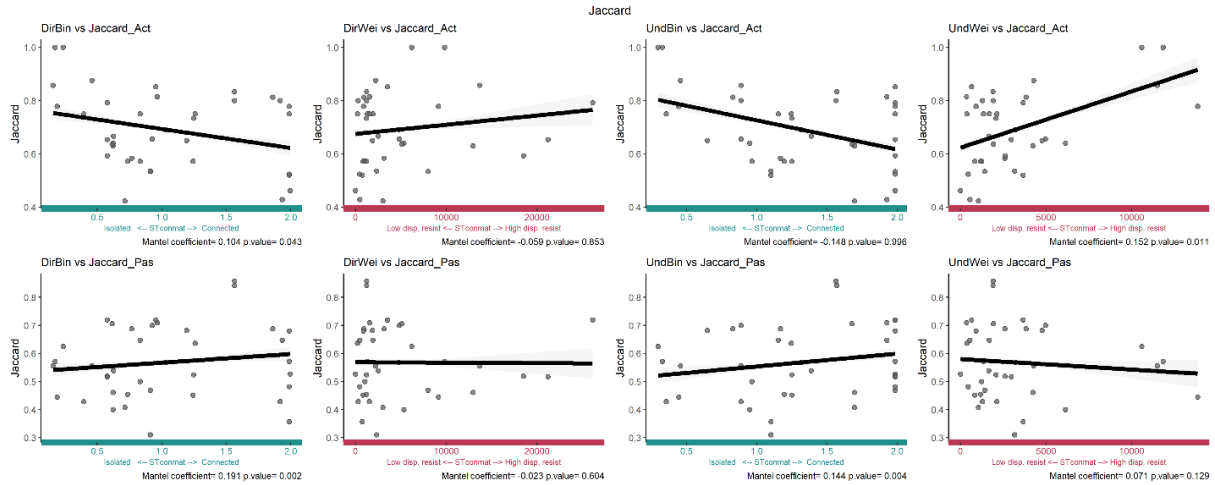
B) Shannon-Wiener diversity



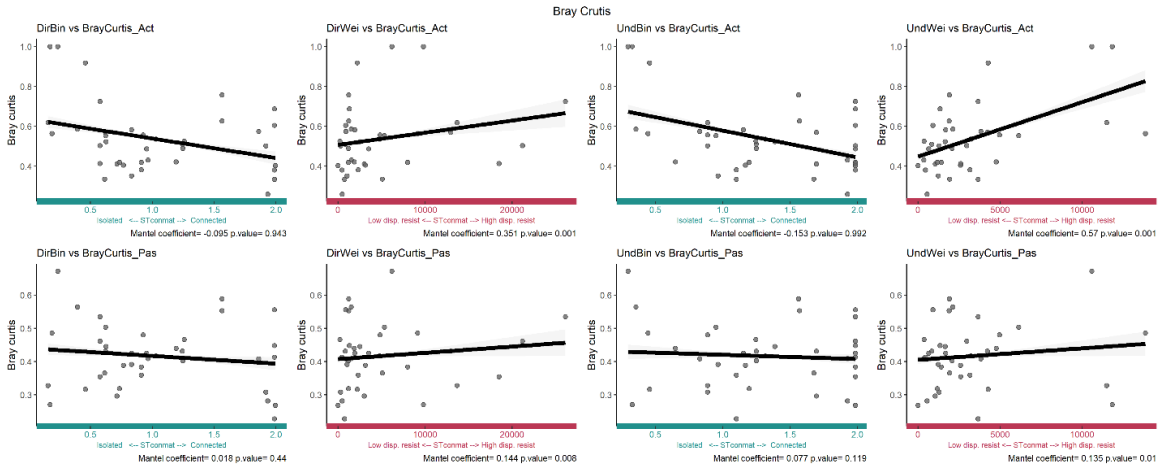
C) Traits abundance



D) Jaccard similarity index



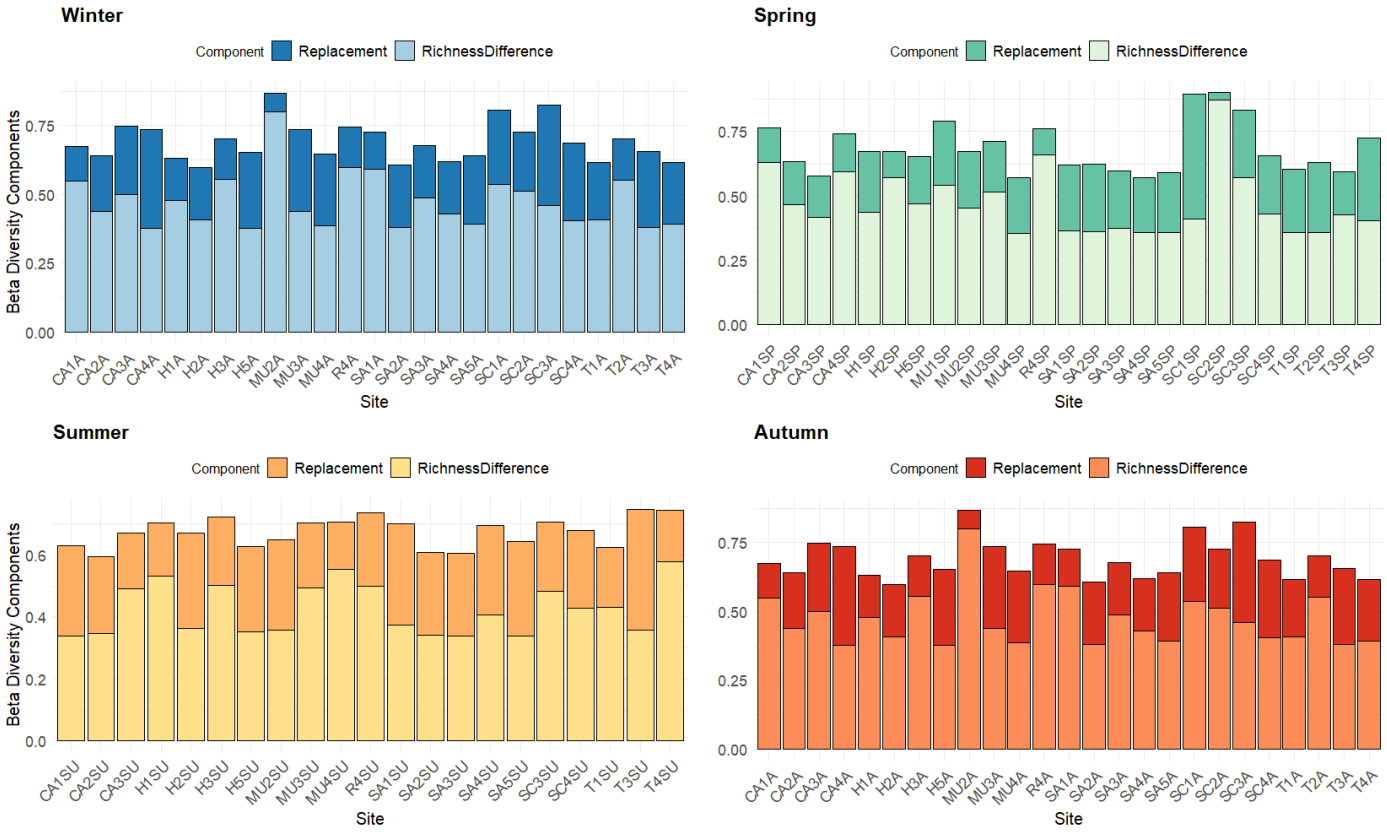
E) Bray-Curtis index



CHAPTER 2:

Current metacommunity paradigms are challenged by mediterranean temporary rivers

Figure S1. Stacked bar plots illustrating the contributions of beta diversity components—replacement (turnover) and richness difference (nestedness)—across sites during the four seasons.



CHAPTER 3:

Metabarcoding as a tool to assess metapopulation and metacommunity dynamics in temporary river networks

Figure S1: Original relationship between site intraspecific variability and selected hydrological variables for the best-fitted GLMMs: frequency of dry days (TotDur) and dispersal resistance (DirWei). The color gradient represents site intraspecific variability, with yellow tones indicating higher values and cooler tones indicating lower values.

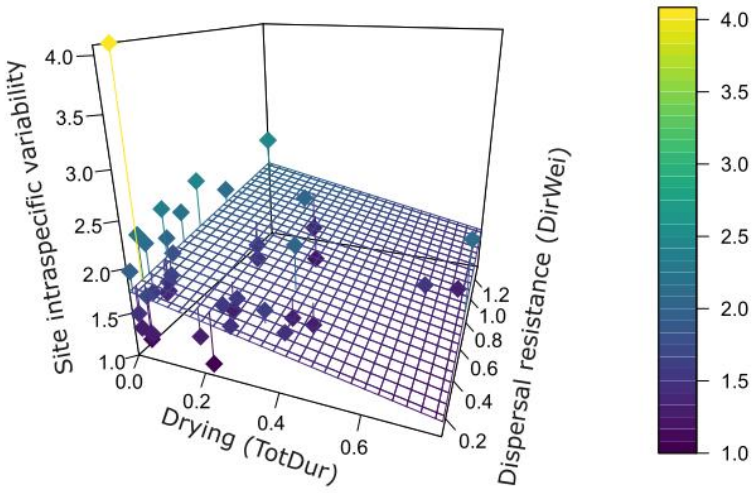


Figure S2: Original boxplot of richness for dispersal groups using DNA-metabarcoding-based and ESV approaches.

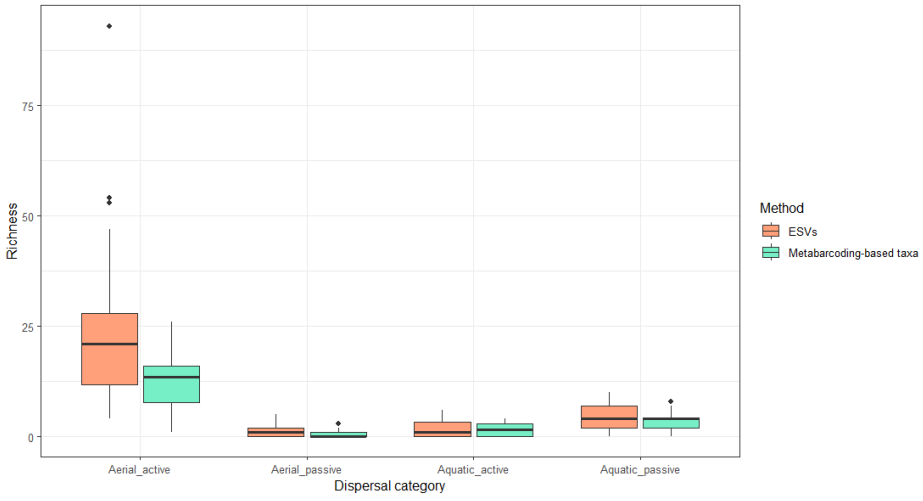


Figure S3: Relationship between morphologic and DNA-metabarcoding-based richness and predictor variables: drying (TotDur), spatiotemporal connectivity (DirBin) and dispersal resistance (DirWei).

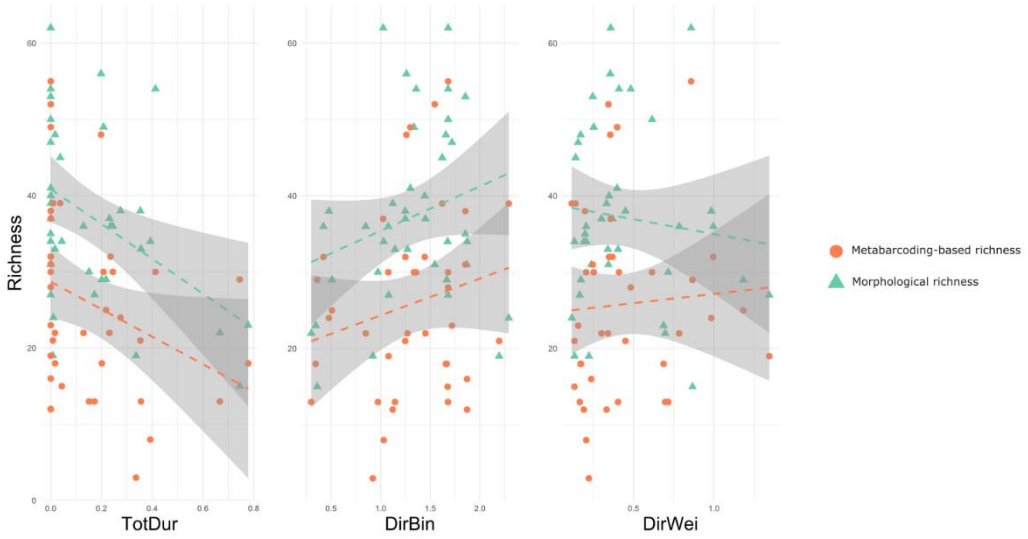


Table S1: List of Taxa Identified by Morphology.

Order	Family	Genus
Diptera	Culicidae	Aedes
Odonata	Aeshnidae	Aeshna
Coleoptera	Dytiscidae	Agabus
Coleoptera	Hydrophilidae	Anacaena
Odonata	Aeshnidae	Anax
Basommatophora	Ancylidae	Ancylus
Basommatophora	Planorbidae	Anisus
Diptera	Culicidae	Anopheles
Hemiptera	Gerridae	Aquarius
Hemiptera	Corixidae	
Diptera	Athericidae	Atherix
Diptera	Ceratopogonidae	Atrichopogon
Ephemeroptera	Baetidae	Baetis
Trichoptera	Beraeidae	Beraea
Coleoptera	Hydrophilidae	Berosus
Diptera	Ceratopogonidae	Bezzia
Coleoptera	Dytiscidae	Bidessus
Odonata	Aeshnidae	Boyeria
Diptera	Ceratopogonidae	Brachypogon
Ephemeroptera	Caenidae	Caenis
Odonata	Calopterygidae	Calopteryx
Ephemeroptera	Baetidae	Centroptilum
Odonata	Coenagrionidae	Ceriagrion
Odonata	Lestidae	Chalcolestes
Diptera	Chaoboridae	Chaoborus
Diptera	Empididae	Chelifera
Diptera	Chironomidae	
Diptera	Rhagionidae	Chrysopilus
Diptera	Tabanidae	Chrysops
Diptera	Empididae	Clinocera
Ephemeroptera	Baetidae	Cloeon
Odonata	Coenagrionidae	Coenagrion
Odonata	Coenagrionidae	
Odonata	Cordulegastridae	Cordulegaster
Odonata	Libellulidae	Crocothemis
Diptera	Culicidae	Culex
Diptera	Ceratopogonidae	Culicoides
Coleoptera	Scirtidae	Cyphon
Diptera	Ceratopogonidae	Dasyhelea
Coleoptera	Dytiscidae	Deronectes
Diptera	Limoniidae	Dicranota
Diptera	Dixidae	Dixa
Diptera	Dixidae	Dixella
Coleoptera	Dryopidae	Dryops
Seriata	Dugesiidae	Dugesia
Coleoptera	Dytiscidae	Dytiscus
Amphipoda	Gammaridae	Echinogammarus
Coleoptera	Elmidae	Elmis
Coleoptera	Scirtidae	Elodes
Hirudinoidea	Erpobdellidae	Erpobdella
Diptera	Ceratopogonidae	Forcypomyia

Order	Family	Genus
Basommatophora	Lymnaeidae	Galba
Hemiptera	Gerridae	Gerris
Odonata	Gomphidae	Gomphus
Coleoptera	Dytiscidae	Graptodytes
Coleoptera	Gyrinidae	Gyrinus
Ephemeroptera	Leptophlebiidae	Habrophlebia
Coleoptera	Haliplidae	Haliplus
Diptera	Limoniidae	Helius
Rhynchobdellida	Glossiphoniidae	Helobdella
Coleoptera	Hydrophilidae	Helochares
Coleoptera	Helophoridae	Helophorus
Diptera	Empididae	Hemerodromia
Diptera	Limoniidae	Hexatoma
Trombidiformes		
Coleoptera	Hydraenidae	Hydraena
Coleoptera	Dytiscidae	Hydroglyphus
Hemiptera	Hydrometridae	Hydrometra
Coleoptera	Hydrophilidae	Hydrophilus
Coleoptera	Dytiscidae	Hydroporus
Trichoptera	Hydropsychidae	Hydropsyche
Trichoptera	Hydroptilidae	Hydroptila
Coleoptera	Dytiscidae	Ilybius
Odonata	Coenagrionidae	Ischnura
Coleoptera	Hydrophilidae	Laccobius
Coleoptera	Dytiscidae	Laccophilus
Plecoptera	Leuctridae	Leuctra
Coleoptera	Hydraenidae	Limnebius
Trichoptera	Limnephilidae	Limnephilus
Diptera	Muscidae	Limnophora
Diptera	Limoniidae	
Diptera	Muscidae	Lispe
Basommatophora	Lymnaeidae	Lymnaea
Diptera	Chironomidae	
Hemiptera	Corixidae	Micronecta
Hemiptera	Veliidae	Microvelia
Diptera	Limoniidae	Molophilus
Trichoptera	Leptoceridae	Mystacides
Hemiptera	Naucoridae	Naucoris
Plecoptera	Nemouridae	Nemoura
Hemiptera	Nepidae	Nepa
Diptera	Ceratopogonidae	Nilobezzia
Coleoptera	Noteridae	Noterus
Hemiptera	Notonectidae	Notonecta
Coleoptera	Hydraenidae	Ochthebius
Odonata	Gomphidae	Onychogomphus
Odonata	Libellulidae	Orthetrum
Diptera	Chironomidae	
Coleoptera	Elmidae	Oulimnius
Diptera	Stratiomyidae	Oxycera
Trichoptera	Hydroptilidae	Oxyethira
Hemiptera	Corixidae	Parasigara
Coleoptera	Haliplidae	Peltodytes
Diptera	Chironomidae	
Diptera	Psychodidae	Pericoma
Diptera	Psychodidae	Peripsychoda
Trichoptera	Philopotamidae	Philopotamus
Basommatophora	Physidae	Physella
Diptera	Limoniidae	Pilaria
Odonata	Platycnemididae	Platycnemis
Hemiptera	Pleidae	Plea
Trichoptera	Polycentropodidae	Plectrocnemia
Trichoptera	Polycentropodidae	Polycentropus
Littorinimorpha	Tateidae	Potamopyrgus
Diptera	Tipulidae	Prionocera
Decapoda	Cambaridae	Procambarus
Ephemeroptera	Baetidae	Procloeon
Diptera	Chironomidae	Prodiamesa
Diptera	Psychodidae	Psychoda
Diptera	Ptychopteridae	Ptychoptera
Odonata	Coenagrionidae	Pyrrhosoma
Basommatophora	Lymnaeidae	Radix
Coleoptera	Elmidae	Riolus
Diptera	Psychodidae	Satchelliella
Ephemeroptera	Ephemerellidae	Serratella
Trichoptera	Leptoceridae	Setodes
Megaloptera	Sialidae	Sialis
Hemiptera	Corixidae	Sigara
Diptera	Simuliidae	Simulium
Sphaeriida	Sphaeriidae	Sphaerium
Trichoptera	Hydroptilidae	Stactobia
Coleoptera	Dytiscidae	Stictonectes
Diptera	Stratiomyidae	Stratiomys
Odonata	Libellulidae	Sympetrum
Diptera	Tabanidae	Tabanus
Diptera	Dolichopodidae	Tachytrechus
Diptera	Chironomidae	
Ephemeroptera	Leptophlebiidae	Thraulus
Trichoptera	Psychomyiidae	Tinodes
Diptera	Tipulidae	Tipula
Diptera	Psychodidae	Tonnoiriella
Hemiptera	Veliidae	Velia
Hemiptera	Veliidae	
Trichoptera	Philopotamidae	Wormaldia
Coleoptera	Dytiscidae	Yola

Table S2: List of Taxa Identified by Metabarcoding.

Order	Family	Genus
Ephemeroptera	Caenidae	Caenis
Ephemeroptera	Leptophlebiidae	
Odonata	Lestidae	Chalcolestes
Odonata	Libellulidae	Sympetrum
Odonata	Aeshnidae	Aeshna
Coleoptera	Gyrinidae	Gyrinus
Ephemeroptera	Baetidae	Cloeon
Odonata	Lestidae	Sympecma
Diptera	Stratiomyidae	Stratiomys
Hemiptera	Notonectidae	Notonecta
Coleoptera	Hydrophilidae	Hydrophilus
Hemiptera	Nepidae	Nepa
	Physidae	Physella
Odonata	Platycnemididae	Platycnemis
Diptera	Culicidae	Culiseta
Odonata	Calopterygidae	Calopteryx
Odonata	Aeshnidae	Anax
Odonata	Cordulegastridae	Cordulegaster
Hemiptera	Gerridae	Aquarius
Diptera	Stratiomyidae	
Odonata	Gomphidae	Gomphus
Ephemeroptera	Baetidae	
Trichoptera	Hydropsychidae	Hydropsyche
Diptera	Tabanidae	Tabanus
Diptera	Stratiomyidae	Oxycera
Odonata	Coenagrionidae	Ceriagrion
Coleoptera	Elmidae	Oulimnius
Odonata	Libellulidae	Orthetrum
Diptera		
Hemiptera	Nepidae	
Trombidiformes	Lebertiidae	Lebertia
Diptera	Culicidae	Culex
Odonata	Coenagrionidae	Ischnura
Coleoptera	Hydrophilidae	Helochares
Odonata	Coenagrionidae	Pyrrhosoma
Odonata	Gomphidae	Onychogomphus
Diptera	Chironomidae	Chironomus
Trombidiformes	Limnesiidae	Limnesia
Hemiptera	Potamocoridae	Naucoris
Tubificida	Naididae	Potamothrix
Trombidiformes	Arrenuridae	Arrenurus
Hemiptera	Gerridae	Gerris
Hemiptera	Corixidae	Parasigara
Odonata	Lestidae	
Coleoptera	Dytiscidae	Stictonectes
Trombidiformes	Hygrobatidae	Hygrobates
Trichoptera	Psychomyiidae	Tinodes
Trichoptera	Hydroptilidae	Hydroptila
Odonata		
Coleoptera	Hydrophilidae	Laccobius
Trombidiformes	Sperchontidae	Sperchon

Order	Family	Genus
Coleoptera	Dytiscidae	Graptodytes
Hemiptera		
Diptera	Tipulidae	Tipula
Ephemeroptera	Ephemerellidae	Serratella
Plecoptera	Nemouridae	Nemoura
Diptera	Simuliidae	Simulium
Diptera	Chironomidae	
Odonata	Aeshnidae	Boyeria
Coleoptera	Dryopidae	
Diptera	Ceratopogonidae	Dasyhelea
Odonata	Aeshnidae	
Trichoptera	Limnephilidae	Halesus
Hygrophila	Lymnaeidae	Radix
Odonata	Calopterygidae	
Diptera	Chironomidae	Microtendipes
Coleoptera	Dytiscidae	Laccophilus
Ephemeroptera	Leptophlebiidae	Habrophlebia
Odonata	Libellulidae	
Trichoptera	Polycentropodidae	
Diptera	Psychodidae	Peripsychoda
Odonata	Cordulegastridae	
Hemiptera	Corixidae	
Coleoptera	Hydraenidae	Hydraena
Diptera	Culicidae	
Diptera	Chironomidae	Ablabesmyia
Trombidiformes		
Lumbriculida	Lumbriculidae	Lumbriculus
Littorinimorpha	Tateidae	Potamopyrgus
Tubificida	Naididae	Tubifex
Coleoptera	Dytiscidae	Dytiscus
Trichoptera		
Hemiptera	Hydrometridae	Hydrometra
Coleoptera	Dytiscidae	Bidessus
Diptera	Ceratopogonidae	
Coleoptera	Hydrophilidae	Anacaena
Coleoptera	Dryopidae	Dryops
Trichoptera	Limnephilidae	Limnephilus
Coleoptera	Hydrophilidae	
Diptera	Chironomidae	Limnophyes
Odonata	Gomphidae	
Plecoptera	Leuctridae	Leuctra
Coleoptera	Dytiscidae	Deronectes
Diptera	Chironomidae	Cricotopus
Coleoptera	Dytiscidae	
Ephemeroptera	Baetidae	Baetis
Amphipoda	Gammaridae	Echinogammarus
Coleoptera	Hydrophilidae	Berosus
Ephemeroptera	Baetidae	Centroptilum
Diptera	Psychodidae	
Diptera	Muscidae	Limnophora

Order	Family	Genus
Trombidiformes	Hydrodromidae	Hydrodroma
Coleoptera	Scirtidae	
Sphaeriida	Sphaeriidae	Pisidium
Coleoptera	Dytiscidae	Agabus
Odonata	Coenagrionidae	Coenagrion
Diptera	Dolichopodidae	
Diptera	Tabanidae	Chrysops
Diptera	Stratiomyidae	Vanoyia
Diptera	Limoniidae	Erioptera
Diptera	Empididae	
Tubificida	Naididae	Pristina
Coleoptera		
Coleoptera	Hydraenidae	Limnebius
Lumbriculida	Lumbriculidae	
Trombidiformes	Pionidae	Forelia
Odonata	Libellulidae	Libellula
Hemiptera	Veliidae	Velia
Diptera	Chironomidae	Procladius
Tubificida	Naididae	
Coleoptera	Haliplidae	Haliplus
Hemiptera	Gerridae	
Diptera	Chironomidae	Psectrocladius
Tubificida	Naididae	Nais
Rhynchobdellida	Glossiphoniidae	Helobdella
Diptera	Chironomidae	Tanytarsus
Trombidiformes	Eupodidae	
Ephemeroptera	Baetidae	Procloeon
Diptera	Psychodidae	Pericoma
Coleoptera	Elmidae	Riolus
Diptera	Athericidae	
Hemiptera	Corixidae	Corixa
Tubificida	Naididae	Chaetogaster
Hemiptera	Corixidae	Micronecta
Ephemeroptera	Heptageniidae	Electrogena
Trichoptera	Leptoceridae	Mystacides
Diptera	Chironomidae	Rheopelopia
Diptera	Chironomidae	Polypedilum
Diptera	Chironomidae	Parametriocnemus
Diptera	Muscidae	
Diptera	Limoniidae	Molophilus
Coleoptera	Dytiscidae	Hydroporus
Coleoptera	Haliplidae	Peltodytes
Diptera	Tabanidae	
Diptera	Psychodidae	Pneumia
Trichoptera	Polycentropodidae	Polycentropus
Tubificida	Naididae	Limnodrilus
Diptera	Chironomidae	Corynoneura
Diptera	Chironomidae	Psectrotanypus
Odonata	Libellulidae	Crocothemis
Diptera	Chironomidae	Rheotanytarsus
Tubificida	Naididae	Bothrioneurum
Odonata	Coenagrionidae	Erythromma
Diptera	Chironomidae	Prodiamesa
Hemiptera	Veliidae	Microvelia
Crassiclitellata	Lumbricidae	Eiseniella
Diptera	Chironomidae	Macropelopia
Arhynchobdellida		
Diptera	Chironomidae	Paracricotopus
Plecoptera	Perlodidae	Isoperla

CHAPTER 4:

Where do you fly from? Assessing aerial dispersal in temporary rivers using stable isotopes

Supporting Information 1

Figure S1. Mesocosms: **A)** Mesocosm equipped with a flight interception trap. **B)** Mesocosm showing four sterile stones placed for algal colonization, along with a temperature data logger (HOBO Pendant® Temperature/Light logger) attached to one of the stones.



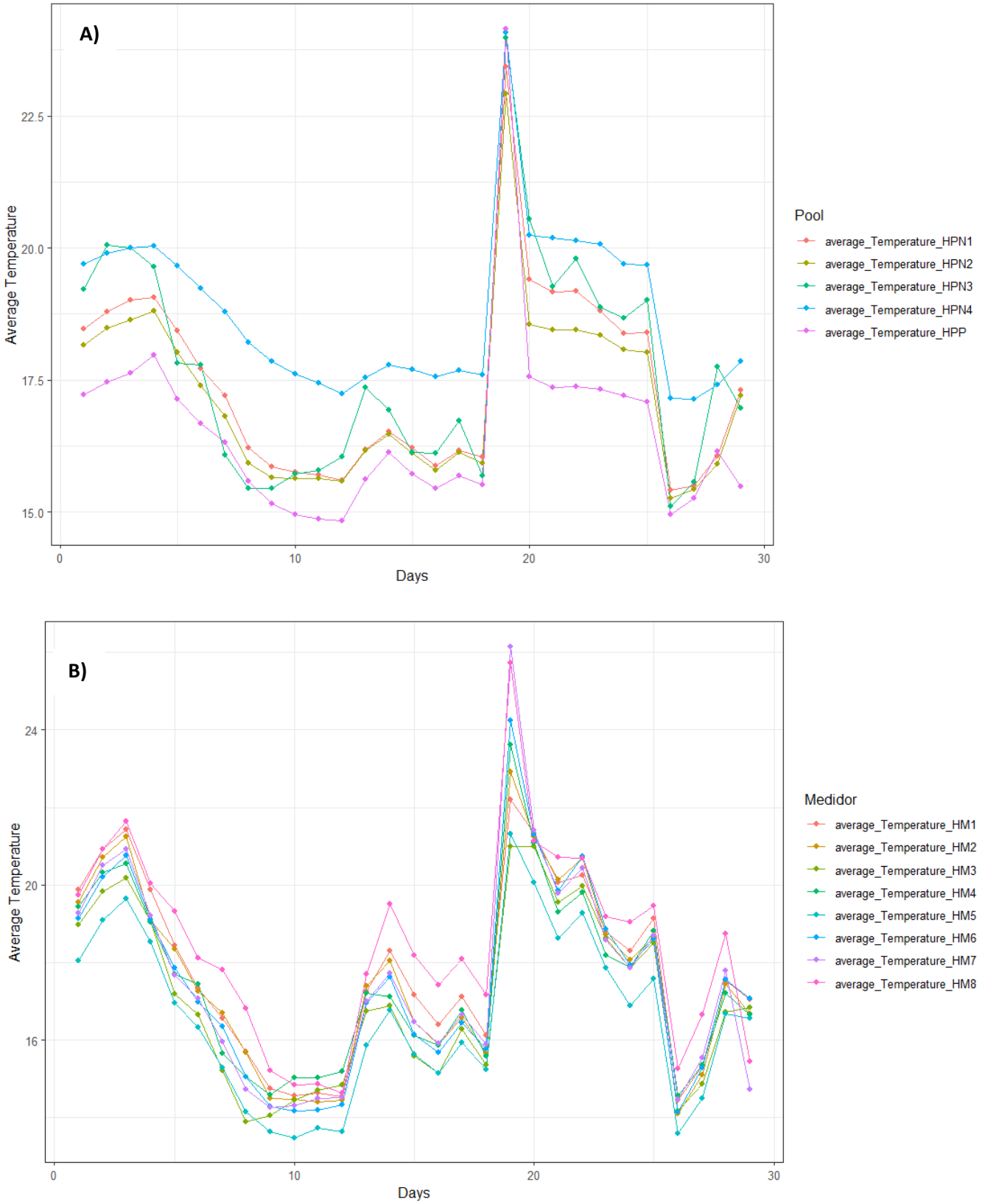
Figure S2. Enriched disconnected natural pools.



Figure S3. A) Disconnected natural pool with emergence trap. **B)** Flight interception trap.



Figure S4. Average temperature in degrees Celsius per day during the experiment measured with temperature data logger (HOBO Pendant® Temperature/Light logger). **A)** Average temperature of the five enriched disconnected natural pools. **B)** Average temperature of the eight mesocosms.



Supporting Information 2

Table S1. Table presenting raw data on isotopic enrichment samples (Iso-samples) across sampling points (sites) and time periods, organized by taxa and biological categories. Each row of the table represents an Iso sample.

Site	Time	Trap	Group	Taxa	Biological category	$\delta^{15}N$
DP	t0	Kick	Acari	Hydracarina	Aquatic obligates	2.769738
M1	tf	Mesocosm	Acari	Hydracarina	Aquatic obligates	950.9862
M2	tf	Mesocosm	Acari	Hydracarina	Aquatic obligates	2531.974
M3	tf	Mesocosm	Acari	Hydracarina	Aquatic obligates	28.34222
M4	tf	Mesocosm	Acari	Hydracarina	Aquatic obligates	13.93892
M5	tf	Mesocosm	Acari	Hydracarina	Aquatic obligates	3370.793
M6	tf	Mesocosm	Acari	Hydracarina	Aquatic obligates	11.76109
M7	tf	Mesocosm	Acari	Hydracarina	Aquatic obligates	35.07983
M8	tf	Mesocosm	Acari	Hydracarina	Aquatic obligates	4.899767
M8	tf	Mesocosm	Cladocera	Cladocera	Aquatic obligates	8.075002
DP	t0	Kick	Coleoptera	*Haliplus*	Semiaquatic adults	-5.133766
DP	t0	Kick	Coleoptera	*Agabus brunneus*	Semiaquatic adults	8.935834
DP	t0	Kick	Coleoptera	*Agabus didymus*	Semiaquatic adults	8.935834
DP	t0	Kick	Coleoptera	*Agabus bipustulatus*	Semiaquatic adults	8.935834
DP	t0	Kick	Coleoptera	*Deronectes*	Semiaquatic adults	0.565414
DP	t0	Kick	Coleoptera	*Stictonectes*	Semiaquatic adults	0.555395
DP	t0	Kick	Coleoptera	*Graptodytes*	Semiaquatic adults	1.338932
DP	t0	Kick	Coleoptera	*Hydraena*	Semiaquatic adults	-3.073725
DP	t0	Kick	Coleoptera	*Dryops*	Semiaquatic adults	1.112487
DP	t0	Kick	Coleoptera	*Anacaena*	Semiaquatic adults	-2.005629
DP	t0	Kick	Coleoptera	*Gyrinus*	Semiaquatic adults	5.391456
DP	t0	Emergence	Coleoptera	*Laccobius*	Semiaquatic adults	8.733027
M2	t1	Flight interception	Coleoptera	*Anacaena*	Semiaquatic adults	1.396883
M3	t1	Flight interception	Coleoptera	*Dryops*	Semiaquatic adults	-0.292852
M7	t1	Flight interception	Coleoptera	*Dryops*	Semiaquatic adults	16.27254

Site	Time	Trap	Group	Taxa	Biological category	$\delta^{15}N$
DP	t1	Emergence	Coleoptera	*Graptodytes*	Semiaquatic adults	972.0123
M2	t2	Mesocosm	Coleoptera	*Dryops*	Semiaquatic adults	7.754887
M2	t2	Mesocosm	Coleoptera	*Hydraena*	Semiaquatic adults	284.6151
M2	t2	Mesocosm	Coleoptera	*Anacaena*	Semiaquatic adults	-0.21643
M1	t2	Flight interception	Coleoptera	*Dryops*	Semiaquatic adults	231.5041
M1	t2	Flight interception	Coleoptera	*Hydraena*	Semiaquatic adults	414.2251
M1	t2	Flight interception	Coleoptera	*Limnebius*	Semiaquatic adults	0.734559
M1	t2	Flight interception	Coleoptera	*Bidessus minutissimus*	Semiaquatic adults	18.10536
M1	t2	Flight interception	Coleoptera	*Anacaena*	Semiaquatic adults	0.15483
M2	t2	Flight interception	Coleoptera	*Hydraena*	Semiaquatic adults	1587.225
M2	t2	Flight interception	Coleoptera	*Dryops*	Semiaquatic adults	5.374491
M2	t2	Flight interception	Coleoptera	*Anacaena*	Semiaquatic adults	179.5887
M3	t2	Flight interception	Coleoptera	*Hydraena*	Semiaquatic adults	69.75491
M3	t2	Flight interception	Coleoptera	*Dryops*	Semiaquatic adults	1.822557
M4	t2	Flight interception	Coleoptera	*Hydraena*	Semiaquatic adults	-3.483666
M7	t2	Flight interception	Coleoptera	*Anacaena*	Semiaquatic adults	0.888467
M7	t2	Flight interception	Coleoptera	*Hydraena*	Semiaquatic adults	-1.679532
M7	t2	Flight interception	Coleoptera	*Graptodytes*	Semiaquatic adults	4.809252
DP	t2	Emergence	Coleoptera	*Laccobius*	Semiaquatic adults	320.8137
DP	t2	Emergence	Coleoptera	*Hydraena*	Semiaquatic adults	775.6165
DP	t2	Emergence	Coleoptera	*Graptodytes*	Semiaquatic adults	882.6434
M1	t3	Mesocosm	Coleoptera	*Agabus brunneus*	Semiaquatic adults	12.36949
M2	t3	Mesocosm	Coleoptera	*Agabus brunneus*	Semiaquatic adults	8.293124
M2	t3	Mesocosm	Coleoptera	*Agabus bipustulatus*	Semiaquatic adults	210.8456
M4	t3	Mesocosm	Coleoptera	*Meladema coriacea*	Semiaquatic adults	0.369918
M8	t3	Mesocosm	Coleoptera	*Meladema coriacea*	Semiaquatic adults	1.408003
M1	t3	Flight interception	Coleoptera	*Hydraena*	Semiaquatic adults	358.1719
M2	t3	Flight interception	Coleoptera	*Hydraena*	Semiaquatic adults	421.2834
M2	t3	Flight interception	Coleoptera	*Anacaena*	Semiaquatic adults	-3.17288
M3	t3	Flight interception	Coleoptera	*Hydraena*	Semiaquatic adults	-1.242112
M3	t3	Flight interception	Coleoptera	*Dryops*	Semiaquatic adults	-4.548594
M3	t3	Flight interception	Coleoptera	*Meladema coriacea*	Semiaquatic adults	2.497454
M3	t3	Flight interception	Coleoptera	*Hydroporus*	Semiaquatic adults	0.257433
M7	t3	Flight interception	Coleoptera	*Graptodytes*	Semiaquatic adults	1.903305
M8	t3	Flight interception	Coleoptera	*Anacaena*	Semiaquatic adults	-0.22307
DP	t3	Emergence	Coleoptera	*Dryops*	Semiaquatic adults	609.0575
DP	t3	Emergence	Coleoptera	*Limnebius*	Semiaquatic adults	347.7568
DP	t3	Emergence	Coleoptera	*Hydraena*	Semiaquatic adults	2064.78
DP	t3	Emergence	Coleoptera	*Haliplus*	Semiaquatic adults	186.7037
DP	t3	Emergence	Coleoptera	*Agabus brunneus*	Semiaquatic adults	376.4174

Site	Time	Trap	Group	Taxa	Biological category	$\delta^{15}N$
DP	t3	Emergence	Coleoptera	*Agabus bipustulatus*	Semiaquatic adults	3327.172
DP	t3	Emergence	Coleoptera	*Graptodytes*	Semiaquatic adults	1434.232
M1	tf	Mesocosm	Coleoptera	*Agabus brunneus*	Semiaquatic adults	41.03636
M1	tf	Mesocosm	Coleoptera	*Graptodytes*	Semiaquatic adults	131.9152
M1	tf	Mesocosm	Coleoptera	*Hydraena*	Semiaquatic adults	215.407
M1	tf	Mesocosm	Coleoptera	*Haliplus lineatocollis*	Semiaquatic adults	340.4524
M2	tf	Mesocosm	Coleoptera	*Bidessus minutissimus*	Semiaquatic adults	32.07032
M2	tf	Mesocosm	Coleoptera	*Graptodytes*	Semiaquatic adults	147.7255
M2	tf	Mesocosm	Coleoptera	*Hydraena*	Semiaquatic adults	6.277888
M3	tf	Mesocosm	Coleoptera	*Graptodytes*	Semiaquatic adults	146.3634
M3	tf	Mesocosm	Coleoptera	*Hydraena*	Semiaquatic adults	133.1159
M4	tf	Mesocosm	Coleoptera	*Graptodytes*	Semiaquatic adults	26.82571
M4	tf	Mesocosm	Coleoptera	*Agabus brunneus*	Semiaquatic adults	1.310078
M4	tf	Mesocosm	Coleoptera	*Bidessus minutissimus*	Semiaquatic adults	14.06816
M4	tf	Mesocosm	Coleoptera	*Meladema coriacea*	Semiaquatic adults	0.315793
M4	tf	Mesocosm	Coleoptera	*Hydraena*	Semiaquatic adults	-1.447258
M5	tf	Mesocosm	Coleoptera	*Hydraena*	Semiaquatic adults	532.7496
M6	tf	Mesocosm	Coleoptera	*Graptodytes*	Semiaquatic adults	9.457573
M6	tf	Mesocosm	Coleoptera	*Hydraena*	Semiaquatic adults	1073.191
M7	tf	Mesocosm	Coleoptera	*Graptodytes*	Semiaquatic adults	4.891804
M7	tf	Mesocosm	Coleoptera	*Stictonectes*	Semiaquatic adults	3.052357
M7	tf	Mesocosm	Coleoptera	*Meladema coriacea*	Aquatic larvae	8.516947
M7	tf	Mesocosm	Coleoptera	*Hydraena*	Semiaquatic adults	989.241
M7	tf	Mesocosm	Coleoptera	*Limnebius*	Semiaquatic adults	0.78657
M8	tf	Mesocosm	Coleoptera	*Graptodytes*	Semiaquatic adults	7.114468
M8	tf	Mesocosm	Coleoptera	*Meladema coriacea*	Semiaquatic adults	5.891157
M8	tf	Mesocosm	Coleoptera	*Gyrinus*	Semiaquatic adults	3.982034
M8	tf	Mesocosm	Coleoptera	*Elodes*	Aquatic larvae	-2.198228
M8	tf	Mesocosm	Coleoptera	*Hydraena*	Semiaquatic adults	115.6558
DP	tf	Kick	Coleoptera	*Anacaena*	Semiaquatic adults	3055.567
DP	tf	Kick	Coleoptera	*Agabus*	Semiaquatic adults	885.4075
DP	tf	Kick	Coleoptera	*Stictonectes*	Semiaquatic adults	279.1326
DP	tf	Kick	Coleoptera	*Gyrinus*	Semiaquatic adults	322.6144
M4	tf	Kick	Coleoptera	*Meladema coriacea*	Semiaquatic adults	4.254766
M8	tf	Kick	Coleoptera	*Meladema coriacea*	Semiaquatic adults	9.325189
M2	tf	Flight interception	Coleoptera	*Hydraena*	Semiaquatic adults	733.1541
M3	tf	Flight interception	Coleoptera	*Hydraena*	Semiaquatic adults	-1.773871
M3	tf	Flight interception	Coleoptera	*Graptodytes*	Semiaquatic adults	141.3465
M7	tf	Flight interception	Coleoptera	*Hydraena*	Semiaquatic adults	3.606001
DP	tf	Emergence	Coleoptera	*Hydraena*	Semiaquatic adults	1431.924

Site	Time	Trap	Group	Taxa	Biological category	$\delta^{15}N$
DP	tf	Emergence	Coleoptera	*Graptodytes*	Semiaquatic adults	767.1779
M1	tf	Mesocosm	Copepoda	Copepoda	Aquatic obligates	4797.797
M2	tf	Mesocosm	Copepoda	Copepoda	Aquatic obligates	895.273
M3	tf	Mesocosm	Copepoda	Copepoda	Aquatic obligates	24.17963
M4	tf	Mesocosm	Copepoda	Copepoda	Aquatic obligates	5.895615
M7	tf	Mesocosm	Copepoda	Copepoda	Aquatic obligates	17.78371
M8	tf	Mesocosm	Copepoda	Copepoda	Aquatic obligates	8.075002
DP	t0	Kick	Diptera	Pentaneurinii	Aquatic larvae	0.931132
DP	t0	Kick	Diptera	Macropelopinii	Aquatic larvae	0.931132
DP	t0	Kick	Diptera	Chironominii	Aquatic larvae	-0.214115
DP	t0	Kick	Diptera	*Chaoborus*	Aquatic larvae	2.041309
DP	t0	Kick	Diptera	*Culex*	Aquatic larvae	-0.301286
DP	t0	Kick	Diptera	*Aedes*	Aquatic larvae	-0.301286
DP	t0	Kick	Diptera	*Oxycera*	Aquatic larvae	-0.362406
DP	t0	Kick	Diptera	*Dasyhelea*	Aquatic larvae	-0.468614
DP	t0	Emergence	Diptera	Chironominii	Aerial adults	1.848476
DP	t0	Emergence	Diptera	*Procladius*	Aerial adults	2.033899
DP	t0	Emergence	Diptera	*Chaoborus*	Aerial adults	4.40551
M4	t1	Flight interception	Diptera	*Dasyhelea*	Aerial adults	-0.730269
DP	t1	Emergence	Diptera	Culicidae	Aerial adults	9.1947
DP	t1	Emergence	Diptera	Orthocladiinae	Aerial adults	12.43509
DP	t1	Emergence	Diptera	Tanytarsinii	Aerial adults	11.02555
DP	t1	Emergence	Diptera	*Procladius*	Aerial adults	3.74756
DP	t1	Emergence	Diptera	Chironominii	Aerial adults	6.930643
DP	t1	Emergence	Diptera	*Chaoborus*	Aerial adults	9.207549
DP	t1	Emergence	Diptera	Orthocladiinae	Aerial adults	8.93551
DP	t1	Emergence	Diptera	*Dasyhelea*	Aerial adults	3.170339
M2	t2	Flight interception	Diptera	Chironominii	Aerial adults	8.52971
M8	t2	Flight interception	Diptera	Chironominii	Aerial adults	1.740147
DP	t2	Emergence	Diptera	Chironominii	Aerial adults	410.1434
DP	t2	Emergence	Diptera	*Procladius*	Aerial adults	875.696
DP	t2	Emergence	Diptera	Tanytarsinii	Aerial adults	2090.256
DP	t2	Emergence	Diptera	Culicidae	Aerial adults	22932.71
DP	t2	Emergence	Diptera	*Chaoborus*	Aerial adults	41.62237
DP	t2	Emergence	Diptera	Pentaneurinii	Aerial adults	2634.523
DP	t2	Emergence	Diptera	*Dasyhelea*	Aerial adults	3411.489
M1	t3	Flight interception	Diptera	Chironominii	Aerial adults	12.06183
M1	t3	Flight interception	Diptera	Culicidae	Aerial adults	1.780152
M3	t3	Flight interception	Diptera	Chironominii	Aerial adults	1.068153
M4	t3	Flight interception	Diptera	Chironominii	Aerial adults	-0.297231

Site	Time	Trap	Group	Taxa	Biological category	$\delta^{15}N$
DP	t3	Emergence	Diptera	Chironominii	Aerial adults	10562.45
DP	t3	Emergence	Diptera	*Procladius*	Aerial adults	2572.071
DP	t3	Emergence	Diptera	*Chaoborus*	Aerial adults	8302.031
DP	t3	Emergence	Diptera	Culicidae	Aerial adults	13056.77
DP	t3	Emergence	Diptera	Pentaneurinii	Aerial adults	9828.439
DP	t3	Emergence	Diptera	Orthocladiinae	Aerial adults	10988.2
DP	t3	Emergence	Diptera	*Dasyhelea*	Aerial adults	5667.974
M1	tf	Mesocosm	Diptera	Pentaneurinii	Aquatic larvae	170.6231
M1	tf	Mesocosm	Diptera	*Corynoneura*	Aquatic larvae	112.3393
M1	tf	Mesocosm	Diptera	Chironominii	Aquatic larvae	126.4827
M1	tf	Mesocosm	Diptera	*Culex*	Aquatic larvae	58.23789
M2	tf	Mesocosm	Diptera	Pentaneurinii	Aquatic larvae	46.20495
M2	tf	Mesocosm	Diptera	Chironominii	Aquatic larvae	37.45814
M2	tf	Mesocosm	Diptera	*Culex*	Aquatic larvae	136.4526
M2	tf	Mesocosm	Diptera	*Anopheles*	Aquatic larvae	50.13538
M2	tf	Mesocosm	Diptera	*Dasyhelea*	Aquatic larvae	5.146965
M3	tf	Mesocosm	Diptera	Pentaneurinii	Aquatic larvae	74.32786
M3	tf	Mesocosm	Diptera	*Corynoneura*	Aquatic larvae	25.75612
M3	tf	Mesocosm	Diptera	Chironominii	Aquatic larvae	26.34663
M3	tf	Mesocosm	Diptera	*Culex*	Aquatic larvae	30.75473
M4	tf	Mesocosm	Diptera	Pentaneurinii	Aquatic larvae	4.825155
M4	tf	Mesocosm	Diptera	*Corynoneura*	Aquatic larvae	6.592872
M4	tf	Mesocosm	Diptera	Chironominii	Aquatic larvae	0.484612
M4	tf	Mesocosm	Diptera	*Culex*	Aquatic larvae	13.49479
M4	tf	Mesocosm	Diptera	*Aedes*	Aquatic larvae	7.970382
M5	tf	Mesocosm	Diptera	Pentaneurinii	Aquatic larvae	80.03898
M5	tf	Mesocosm	Diptera	*Corynoneura*	Aquatic larvae	10.37593
M5	tf	Mesocosm	Diptera	Chironominii	Aquatic larvae	54.58249
M5	tf	Mesocosm	Diptera	*Culex*	Aquatic larvae	31.01634
M5	tf	Mesocosm	Diptera	*Aedes*	Aquatic larvae	14.19654
M5	tf	Mesocosm	Diptera	*Anopheles*	Aquatic larvae	59.49789
M6	tf	Mesocosm	Diptera	Pentaneurinii	Aquatic larvae	126.5022
M6	tf	Mesocosm	Diptera	Chironominii	Aquatic larvae	8.17653
M6	tf	Mesocosm	Diptera	*Culex*	Aquatic larvae	7.630071
M6	tf	Mesocosm	Diptera	*Aedes*	Aquatic larvae	10.67292
M7	tf	Mesocosm	Diptera	Pentaneurinii	Aquatic larvae	22.14657
M7	tf	Mesocosm	Diptera	*Corynoneura*	Aquatic larvae	15.72443
M7	tf	Mesocosm	Diptera	Chironominii	Aquatic larvae	15.79709
M7	tf	Mesocosm	Diptera	*Culex*	Aquatic larvae	17.12592
M7	tf	Mesocosm	Diptera	*Aedes*	Aquatic larvae	11.90917

Site	Time	Trap	Group	Taxa	Biological category	$\delta^{15}N$
M8	tf	Mesocosm	Diptera	Pentaneurinii	Aquatic larvae	12.93242
M8	tf	Mesocosm	Diptera	*Corynoneura*	Aquatic larvae	12.38894
M8	tf	Mesocosm	Diptera	Chironominii	Aquatic larvae	12.47554
M8	tf	Mesocosm	Diptera	*Culex*	Aquatic larvae	13.02797
M8	tf	Mesocosm	Diptera	*Aedes*	Aquatic larvae	10.13244
DP	tf	Kick	Diptera	Chironominii	Aquatic larvae	9032.699
M7	tf	Flight interception	Diptera	Chironominii	Aerial adults	22.98444
DP	tf	Emergence	Diptera	Chironominii	Aerial adults	516.7187
DP	tf	Emergence	Diptera	*Procladius*	Aerial adults	6614.755
DP	tf	Emergence	Diptera	*Chaoborus*	Aerial adults	8567.174
DP	tf	Emergence	Diptera	Pentaneurinii	Aerial adults	15621.3
DP	t0	Kick	Ephemeroptera	*Cloeon*	Aquatic larvae	-1.424489
DP	t0	Kick	Ephemeroptera	*Caenis*	Aquatic larvae	-1.28722
DP	t0	Emergence	Ephemeroptera	*Cloeon dipterum*	Aerial adults	1.111771
DP	t0	Emergence	Ephemeroptera	*Cloeon simile*	Aerial adults	1.073889
DP	t0	Emergence	Ephemeroptera	*Caenis luctuosa*	Aerial adults	0.218554
M3	t1	Flight interception	Ephemeroptera	*Cloeon dipterum*	Aerial adults	0.282074
M4	t1	Flight interception	Ephemeroptera	*Caenis*	Aerial adults	46.94099
M7	t1	Flight interception	Ephemeroptera	*Cloeon dipterum*	Aerial adults	1.94019
DP	t1	Emergence	Ephemeroptera	*Cloeon dipterum*	Aerial adults	4.384575
DP	t1	Emergence	Ephemeroptera	*Cloeon simile*	Aerial adults	269.5066
DP	t1	Emergence	Ephemeroptera	*Caenis luctuosa*	Aerial adults	11.26015
M3	t2	Flight interception	Ephemeroptera	*Cloeon dipterum*	Aerial adults	1.515269
M4	t2	Flight interception	Ephemeroptera	*Cloeon dipterum*	Aerial adults	0.092265
M7	t2	Flight interception	Ephemeroptera	*Cloeon dipterum*	Aerial adults	-0.394536
M8	t2	Flight interception	Ephemeroptera	*Cloeon dipterum*	Aerial adults	2.234777
DP	t2	Emergence	Ephemeroptera	*Cloeon dipterum*	Aerial adults	4296.836
DP	t2	Emergence	Ephemeroptera	*Cloeon simile*	Aerial adults	3875.601
DP	t2	Emergence	Ephemeroptera	*Caenis luctuosa*	Aerial adults	5659.058
M1	t3	Flight interception	Ephemeroptera	*Cloeon dipterum*	Aerial adults	1.482407
M2	t3	Flight interception	Ephemeroptera	*Cloeon dipterum*	Aerial adults	5.371021
M4	t3	Flight interception	Ephemeroptera	*Cloeon dipterum*	Aerial adults	-0.745392
M4	t3	Flight interception	Ephemeroptera	*Caenis*	Aerial adults	12.82827
M6	t3	Flight interception	Ephemeroptera	*Cloeon dipterum*	Aerial adults	-0.200164
M7	t3	Flight interception	Ephemeroptera	*Cloeon dipterum*	Aerial adults	6.043115
M8	t3	Flight interception	Ephemeroptera	*Cloeon dipterum*	Aerial adults	-0.72474
DP	t3	Emergence	Ephemeroptera	*Cloeon dipterum*	Aerial adults	3959.999
DP	t3	Emergence	Ephemeroptera	*Cloeon simile*	Aerial adults	4332.365
DP	t3	Emergence	Ephemeroptera	*Caenis luctuosa*	Aerial adults	4423.236
M1	tf	Mesocosm	Ephemeroptera	*Cloeon*	Aquatic larvae	57.33281

Site	Time	Trap	Group	Taxa	Biological category	$\delta^{15}N$
M2	tf	Mesocosm	Ephemeroptera	*Cloeon*	Aquatic larvae	36.84673
M3	tf	Mesocosm	Ephemeroptera	*Cloeon*	Aquatic larvae	28.37226
M4	tf	Mesocosm	Ephemeroptera	*Cloeon*	Aquatic larvae	3.89602
M5	tf	Mesocosm	Ephemeroptera	*Cloeon*	Aquatic larvae	16.86482
M6	tf	Mesocosm	Ephemeroptera	*Cloeon*	Aquatic larvae	6.238541
M7	tf	Mesocosm	Ephemeroptera	*Cloeon*	Aquatic larvae	20.43851
M8	tf	Mesocosm	Ephemeroptera	*Cloeon*	Aquatic larvae	13.93475
DP	tf	Emergence	Ephemeroptera	*Cloeon dipterum*	Aerial adults	4890.956
DP	tf	Emergence	Ephemeroptera	*Cloeon simile*	Aerial adults	5782.869
DP	tf	Emergence	Ephemeroptera	*Caenis luctuosa*	Aerial adults	7268.141
DP	t0	Kick	Gastropoda	*Ancylus fluviatilis*	Aquatic obligates	2.14852
DP	t0	Kick	Gastropoda	*Lymnaea stagnalis*	Aquatic obligates	2.14852
M1	tf	Mesocosm	Gastropoda	Gastropoda	Aquatic obligates	17.94132
M2	tf	Mesocosm	Gastropoda	*Potamopyrgus antipodarum*	Aquatic obligates	12.9839
M3	tf	Mesocosm	Gastropoda	*Physella*	Aquatic obligates	14.91978
M3	tf	Mesocosm	Gastropoda	*Potamopyrgus antipodarum*	Aquatic obligates	12.50981
M4	tf	Mesocosm	Gastropoda	*Potamopyrgus antipodarum*	Aquatic obligates	14.2468
DP	t0	Kick	Heteroptera	*Notonecta*	Semiaquatic adults	0.667615
DP	t0	Kick	Heteroptera	*Hydrometra stagnorum*	Semiaquatic adults	2.676556
DP	t0	Kick	Heteroptera	*Parasigara perdubia*	Semiaquatic adults	0.254805
DP	t1	Emergence	Heteroptera	*Hydrometra stagnorum*	Semiaquatic adults	4.672677
DP	t2	Emergence	Heteroptera	*Hydrometra stagnorum*	Semiaquatic adults	43.46083
M2	t3	Mesocosm	Heteroptera	*Notonecta maculata*	Semiaquatic adults	5.200016
M3	t3	Mesocosm	Heteroptera	*Notonecta*	Semiaquatic adults	0.733754
M4	t3	Mesocosm	Heteroptera	*Parasigara perdubia*	Semiaquatic adults	3.651458
M7	t3	Mesocosm	Heteroptera	*Notonecta maculata*	Semiaquatic adults	6.404768
M3	tf	Mesocosm	Heteroptera	*Parasigara perdubia*	Semiaquatic adults	43.12215
M4	tf	Mesocosm	Heteroptera	*Parasigara perdubia*	Semiaquatic adults	3.977206
DP	tf	Kick	Heteroptera	*Notonecta maculata*	Semiaquatic adults	77.95706
DP	tf	Kick	Heteroptera	*Parasigara*	Semiaquatic adults	4373.318
DP	tf	Emergence	Heteroptera	*Hydrometra stagnorum*	Semiaquatic adults	4528.285
DP	t0	Kick	Odonata	*Coenagrion puella*	Aquatic larvae	2.674552
DP	t0	Kick	Odonata	*Crocothemis erythraea*	Aquatic larvae	-0.988634
DP	t0	Kick	Odonata	*Orthetrum chrysostigma*	Aquatic larvae	-0.988634
DP	t0	Kick	Odonata	*Anax imperator*	Aquatic larvae	2.1896
DP	t0	Net	Odonata	*Crocothemis erythraea*	Aerial adults	1.08984
M1	t0	Net	Odonata	*Chalcolestes viridis*	Aerial adults	0.515629
M1	t3	Net	Odonata	*Aeshna cyanea*	Aerial adults	2.056827
PR	t3	Net	Odonata	*Chalcolestes viridis*	Aerial adults	2.933097
DP	tf	Kick	Odonata	*Anax*	Aquatic larvae	567.0777

Site	Time	Trap	Group	Taxa	Biological category	$\delta^{15}N$
DP	tf	Kick	Odonata	*Coenagrion*	Aquatic larvae	8527.073
DP	tf	Kick	Odonata	*Crocothemis erythraea*	Aquatic larvae	2427.605
DP	tf	Net	Odonata	*Chalcolestes viridis*	Aerial adults	17.83068
M1	tf	Mesocosm	Ostracoda	Ostracoda	Aquatic obligates	4797.797
M2	tf	Mesocosm	Ostracoda	Ostracoda	Aquatic obligates	895.273
M3	tf	Mesocosm	Ostracoda	Ostracoda	Aquatic obligates	24.17963
M4	tf	Mesocosm	Ostracoda	Ostracoda	Aquatic obligates	5.895615
M6	tf	Mesocosm	Ostracoda	Ostracoda	Aquatic obligates	32.18489
M4	t3	Flight interception	Trichoptera	*Polycentropus*	Aerial adults	5.961661
DP	tf	Emergence	Trichoptera	*Polycentropus*	Aerial adults	94.86268

Table S2. Percentage of taxa shared between the isotopic analyses and the total community for different sampling methods (Mesocosms and DPs, Emergence traps and Flight Interception traps). The tables compare the taxa identified in the isotopic analysis to the taxa present in the entire community across different sampling dates and sites.

Mesocosms and DPs samples		
Site	**Time**	**% of taxa in isotopic analysis**
M1	tf	83.33
M2	tf	83.33
M3	tf	64.28
M4	tf	86.66
M5	tf	88.88
M6	tf	70
M7	tf	92.3
M8	tf	91.66
DP	t0	34.14

Emergence traps samples	
Time	**% of taxa in isotopic analysis**
t1	83.33
t2	78.57
t3	76.47
tf	63.63

Flight Interception traps samples	
Time	**% of taxa in isotopic analysis**
t1	66.66
t2	77.77
t3	57.14
tf	50

Table S3. Model fitting results for both aerial and semiaquatic adult biological groups. The table presents the goodness of fit for the linear, exponential, quadratic polynomial, and cubic polynomial models, including the coefficient of determination (R^2) and the Akaike Information Criterion (AIC) for each model. The best-fitting model for each group, selected based on the lowest AIC, is marked with an asterisk (*).

Model	Biological group	R^2	AIC	p value
Linear	Aerial adults	0.69	192	< 0.001
Exponential	Aerial adults	0.56	209	< 0.001
Quadratic Polynomial	Aerial adults	0.8	175	< 0.001
Cubic Polynomial *	Aerial adults	0.85	164	< 0.001
Linear *	Semiaquatic adults	0.49	64	0.001
Exponential	Semiaquatic adults	0.45	65	0.006
Quadratic Polynomial	Semiaquatic adults	0.51	65	0.006
Cubic Polynomial	Semiaquatic adults	0.51	67	0.02

Figure S1. Temporal variation in log-transformed $\delta^{15}N$ values for aerial adult insects with fitted linear, exponential, quadratic, and cubic polynomial models. The time points are labeled as 0 (t_0), 1 (t_1), 2 (t_2), 3 (t_3), and 4 (tf). The cubic polynomial model provides the best fit, as indicated by the lowest AIC.

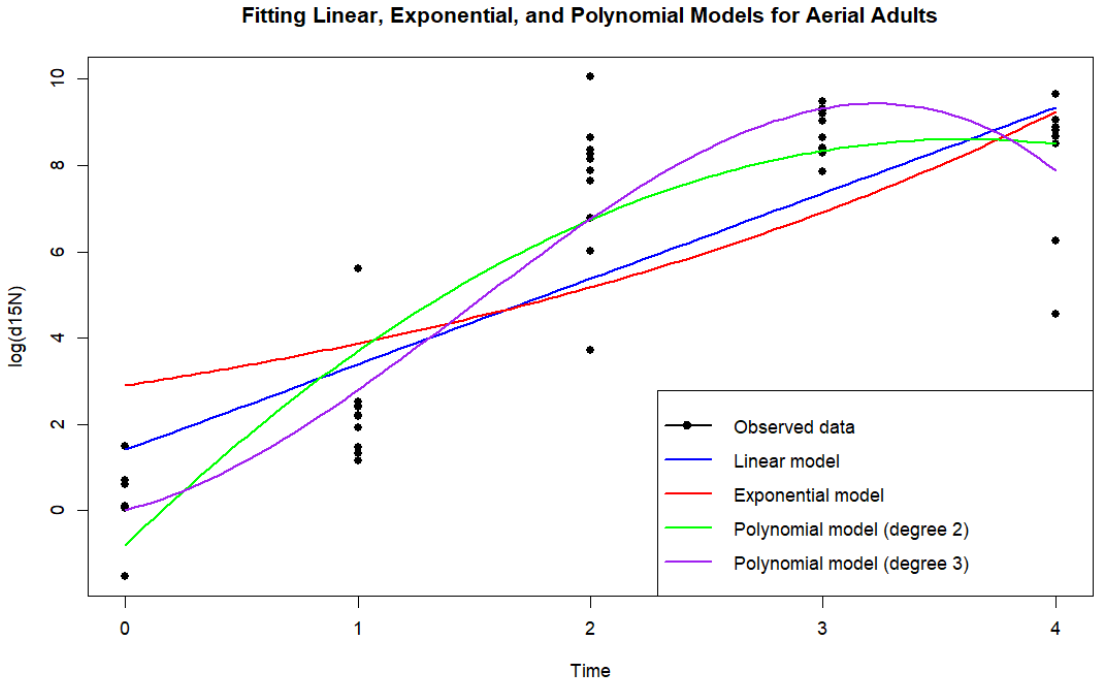


Figure S2. Temporal variation in log-transformed $\delta^{15}N$ values for semiaquactic adult insects with fitted linear, exponential, quadratic, and cubic polynomial models. The time points are labeled as 0 (t_o), 1 (t_1), 2 (t_2), 3 (t_3), and 4 (tf). The linear model provides the best fit, as indicated by the lowest AIC.

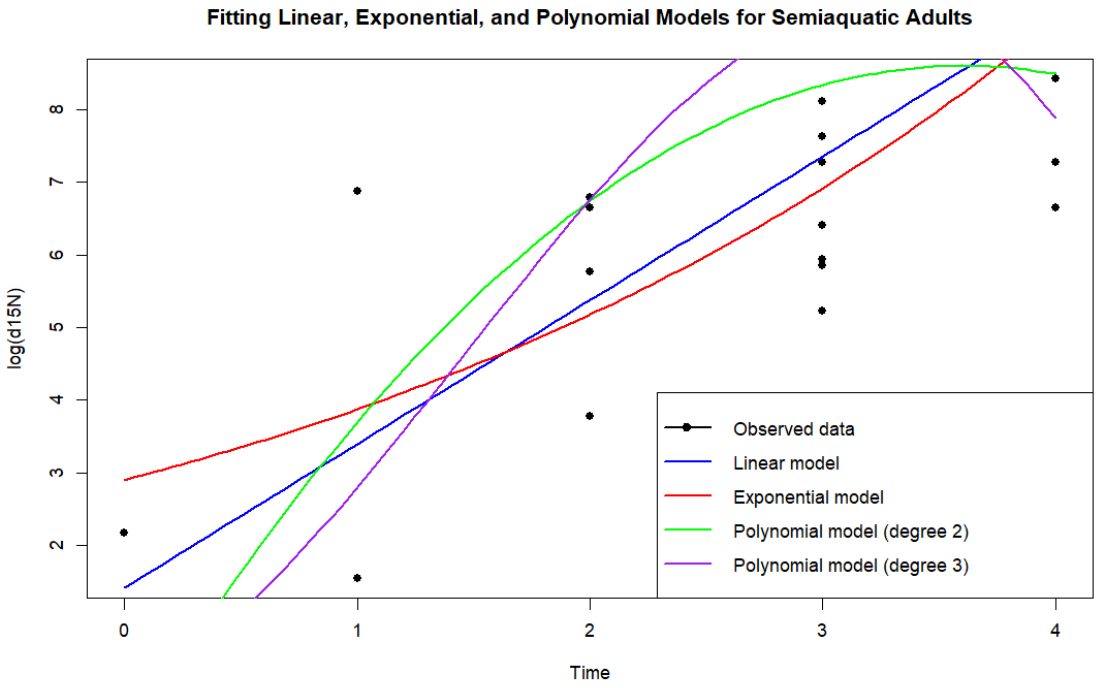
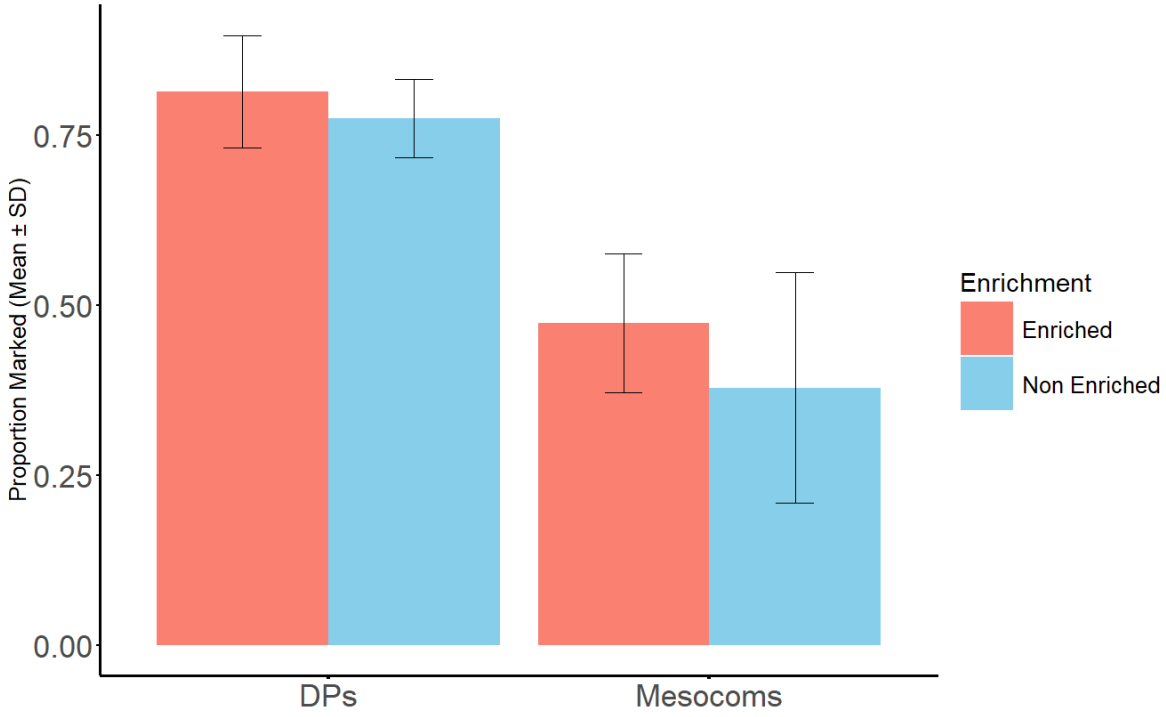


Figure S3. Proportion of marked Iso-samples by habitat (Disconnected pools (DPs) and mesocosms), with means and standard deviations. Bars represent the mean proportion of marked Iso-samples for each group, with error bars indicating ±1 standard deviation.



Supporting Information 3

Table S1. Abundance of macroinvertebrate taxa collected from enriched disconnected pools (DP_i and DP_f) and perennial reach (PR_i and PR_f) at initial and final times, as well as from mesocosms through kick sampling at study sites.

Code	Date	Aedes	Agabus bipustulatus	Agabus brunneus	Agabus didymus	Anacaena	Anax imperator	Ancylus fluviatilis	Anisus	Anopheles	Armiger crista	Atrichopogon	Bezzia
M1	Tf	0	0	2	0	0	0	0	0	0	0	0	0
M2	Tf	0	1	1	0	1	0	0	0	3	0	0	0
M3	Tf	1	0	0	0	0	0	0	0	0	0	0	0
M4	Tf	117	0	1	0	0	0	0	0	0	0	0	0
M5	Tf	82	0	0	0	0	0	0	0	1	0	0	0
M6	Tf	664	0	0	0	0	0	0	0	0	0	0	0
M7	Tf	35	0	0	0	0	0	0	0	0	0	0	0
M8	Tf	295	0	0	0	0	0	0	0	0	0	0	0
DPf	Tf	16	0	5	2	0	0	7	1	0	0	8	2
DPi	T0	261	1	2	1	1	3	3	0	0	0	1	0
PRf	Tf	2	0	0	0	0	0	52	0	8	0	0	14
PRi	T0	5	0	1	0	0	0	162	0	60	1	0	34

Code	Date	Bidessus minutissimus	Caenis	Chaoborus	Chironominii	Cladocera	Cloeon	Coenagrion puella	Copepoda	Cordulegaster boltonii	Corynoneura	Crocothemis erythraea
M1	Tf	0	0	0	398	0	1923	0	19	0	13	0
M2	Tf	1	0	0	304	0	1232	0	12	0	3	0
M3	Tf	0	0	0	6	0	800	0	22	0	3	0
M4	Tf	1	0	0	15	0	323	0	346	0	3	0
M5	Tf	0	0	0	960	0	928	0	0	0	11	0
M6	Tf	0	0	0	901	0	2316	0	0	0	0	0
M7	Tf	0	0	0	1056	0	2896	0	512	0	232	0
M8	Tf	0	0	0	1858	2	879	0	480	0	44	0
DPf	Tf	4	56	475	1027	0	2693	22	0	2	48	5
DPi	T0	0	27	744	671	0	1449	25	0	0	0	48
PRf	Tf	0	0	1	77	0	261	0	0	0	3	0
PRi	T0	0	0	19	142	0	691	0	0	0	22	0

Code	Date	Culex	Cyphon	Dasyhelea	Deronectes	Dixella	Dolichopeza	Dryops	Echinogammarus	Electrogena lateralis	Elodes	Erpobdella	Forcypomyiia	Gastropoda	Graptodytes
M1	Tf	159	0	0	0	0	0	0	0	0	0	0	0	5	7
M2	Tf	116	0	1	0	0	0	1	0	0	0	0	0	0	8
M3	Tf	2	0	0	0	0	0	0	0	0	0	0	0	0	23
M4	Tf	1	0	0	0	0	0	0	0	0	0	0	0	0	9
M5	Tf	10	0	0	0	0	0	0	0	0	0	0	0	0	0
M6	Tf	248	0	1	0	0	0	0	0	0	0	0	0	0	4
M7	Tf	32	0	0	0	0	0	0	0	0	0	0	0	0	24
M8	Tf	36	0	0	0	0	0	0	0	0	1	0	0	0	6
DPf	Tf	16	280	16	13	0	0	6	0	0	0	0	0	0	70
DPi	T0	157	1	863	3	0	0	4	0	0	0	0	0	0	56
PRf	Tf	1	0	0	0	17	0	0	11	0	11	9	0	0	11
PRi	T0	17	0	8	0	99	1	0	5	1	22	12	1	0	9

Code	Date	Gymnometriocnemus	Gyraulus	Gyrinus	Habrophlebia	Haliplus lineatocollis	Helobdella stagnalis	Helochares lividus	Hydrachnidiae	Hydraena	Hydrochus	Hydrocyphon
M1	Tf	0	0	0	0	1	0	0	3	23	0	0
M2	Tf	0	0	0	0	0	0	0	1	6	0	0
M3	Tf	0	0	0	0	0	0	0	1	52	0	0
M4	Tf	0	0	0	0	0	0	0	1	4	0	0
M5	Tf	0	0	0	0	0	0	0	2	1	0	0
M6	Tf	0	0	0	0	0	0	0	3	1	0	0
M7	Tf	0	0	0	0	0	0	0	3	1	0	0
M8	Tf	0	0	1	0	0	0	0	82	8	0	0
DPf	Tf	1	1	12	0	14	0	1	18	30	2	0
DPi	T0	0	0	11	1	4	0	3	74	23	0	0
PRf	Tf	0	0	14	0	0	5	0	4	8	0	1
PRi	T0	0	15	5	36	1	3	0	33	31	0	0

Code	Date	Hydrometra stagnorum	Limnebius	Lymnaea stagnalis	Lymnaeidae	Macropelopinii	Meladema coriacea	Nepa cinerea	Nilobezzia	Notonecta maculata	Oligochaeta
M1	Tf	0	0	0	0	0	0	0	0	0	0
M2	Tf	0	0	0	0	0	0	0	0	1	0
M3	Tf	0	0	0	0	0	0	0	0	1	0
M4	Tf	0	0	0	0	0	3	0	0	0	0
M5	Tf	0	0	0	0	0	0	0	0	0	0
M6	Tf	0	0	0	0	0	0	0	0	0	0
M7	Tf	0	1	0	0	0	2	0	0	1	0
M8	Tf	0	0	0	0	0	6	0	0	0	0
DPf	Tf	0	1	2	0	320	1	0	1	3	0
DPi	T0	11	1	4	0	312	0	0	0	27	0
PRf	Tf	0	0	0	1	9	0	1	0	1	3
PRi	T0	4	2	0	0	28	0	1	0	5	0

Code	Date	Orthetrum chrysostigma	Orthocladiinae	Ostracoda	Oulimnius	Oxycera	Parasigara perdubia	Pentaneurinii	Pericoma	Physella	Planorbis	Polycentropus
M1	Tf	0	1	3	0	0	0	79	0	0	0	0
M2	Tf	0	1	3	0	0	0	3	0	0	0	0
M3	Tf	0	0	2	0	0	3	126	0	1	0	0
M4	Tf	0	0	214	0	0	2	53	0	0	0	0
M5	Tf	0	0	0	0	0	0	18	0	0	0	0
M6	Tf	0	0	3	0	0	0	1	0	0	0	0
M7	Tf	0	0	0	0	0	0	127	0	0	0	0
M8	Tf	0	0	0	0	0	0	284	0	0	0	0
DPf	Tf	0	0	0	1	5	9	160	0	0	0	0
DPi	T0	2	0	0	3	13	33	115	0	0	0	0
PRf	Tf	0	0	0	6	18	0	42	11	129	18	23
PRi	T0	0	0	0	1	11	4	331	11	182	0	24

Code	Date	Potamopyrgus antipodarum	Psychoda	Radix	Siphonoperla torrentium	Sphaerium corneum	Stictonectes	Tanytarsinii	Thraulus bellus	Tipula
M1	Tf	0	0	0	0	0	0	0	0	0
M2	Tf	19	0	0	0	0	0	0	0	0
M3	Tf	72	0	0	0	0	0	9	0	0
M4	Tf	27	0	0	0	0	0	1	0	0
M5	Tf	0	0	0	0	0	0	0	0	0
M6	Tf	0	0	0	0	0	0	1	0	0
M7	Tf	0	0	0	0	0	1	0	0	0
M8	Tf	0	0	0	0	0	0	0	0	0
DPf	Tf	0	3	0	0	0	28	192	0	0
DPi	T0	0	2	0	0	0	57	8	0	0
PRf	Tf	0	0	0	0	33	19	6	10	0
PRi	T0	0	0	13	1	25	52	0	0	1

Code	Date	Tonnoiriella pulchra	Velia	Velia caprai	Yola bicarinata
M1	Tf	0	0	0	0
M2	Tf	0	0	0	0
M3	Tf	0	0	0	0
M4	Tf	0	0	0	0
M5	Tf	0	0	0	0
M6	Tf	0	0	0	0
M7	Tf	0	0	0	0
M8	Tf	0	0	0	0
DPf	Tf	0	1	0	0
DPi	T0	0	0	0	0
PRf	Tf	1	0	0	0
PRi	T0	48	0	6	1

Table S2. Abundance of macroinvertebrate taxa collected from emergence traps in disconnected pools (DPE) and perennial reach (PRE) over time.

Code	Trap	Date	Cloeon dipterum	Cloeon simile	Caenis luctuosa	Habrophlebia fusca	Electrogena lateralis	Hydrometra stagnorum	Laccobius	Graptodytes	Agabus brunneus
DPE1	Emergence	t0	62	96	36	0	0	0	3	0	0
DPE2	Emergence	t0	0	0	0	0	0	0	0	0	0
DPE1	Emergence	t1	155	168	60	1	0	1	0	0	0
DPE2	Emergence	t1	3	3	0	0	0	0	0	1	0
PRE	Emergence	t1	6	2	0	3	0	0	0	0	0
DPE1	Emergence	t2	105	155	59	0	0	4	1	0	0
DPE2	Emergence	t2	10	6	0	0	0	0	0	3	0
PRE	Emergence	t2	26	9	0	8	0	0	0	0	0
DPE1	Emergence	t3	27	91	27	0	0	0	0	0	0
DPE2	Emergence	t3	2	0	0	0	0	0	0	1	1
PRE	Emergence	t3	13	8	0	2	1	0	0	0	0
DPE1	Emergence	tf	7	26	2	0	0	2	0	0	0
DPE2	Emergence	tf	1	0	0	0	0	0	0	1	0
PRE	Emergence	tf	4	3	0	2	0	2	0	0	0

Code	Trap	Date	Agabus bipustulatus	Hydraena	Limnebius	Haliplus lineatocollis	Dryops	Polycentropodidae	Chironomini	Pentaneurini	Macropelopini	Procladius
DPE1	Emergence	t0	0	0	0	0	0	0	85	0	5	2
DPE2	Emergence	t0	0	0	0	0	0	0	43	0	3	3
DPE1	Emergence	t1	0	0	0	0	0	0	175	0	1	15
DPE2	Emergence	t1	0	0	0	0	0	0	80	0	2	8
PRE	Emergence	t1	0	0	0	0	0	23	33	59	0	1
DPE1	Emergence	t2	0	1	0	0	0	0	179	0	5	27
DPE2	Emergence	t2	0	0	0	0	0	0	69	3	0	9
PRE	Emergence	t2	0	1	0	0	0	20	102	91	0	0
DPE1	Emergence	t3	0	1	1	1	2	0	306	0	1	55
DPE2	Emergence	t3	1	4	0	0	0	0	84	1	2	13
PRE	Emergence	t3	0	0	0	0	0	10	79	60	24	7
DPE1	Emergence	tf	0	1	0	0	0	1	836	0	2	123
DPE2	Emergence	tf	0	2	0	0	0	0	36	3	1	16
PRE	Emergence	tf	0	0	0	0	0	7	79	32	27	0

Code	Trap	Date	Tanytarsinii	Orthocladiinae	Dasyhelea	Culicidae	Chaoborus flavicans	Chaoborus pallidus	Dixella graeca	Psychodidae
DPE1	Emergence	t0	0	0	0	0	1	0	0	0
DPE2	Emergence	t0	0	0	0	0	2	3	0	0
DPE1	Emergence	t1	1	1	0	3	2	4	0	0
DPE2	Emergence	t1	0	2	1	0	8	4	0	0
PRE	Emergence	t1	0	10	0	0	0	0	1	0
DPE1	Emergence	t2	1	0	0	8	1	4	0	0
DPE2	Emergence	t2	0	0	15	2	1	9	0	0
PRE	Emergence	t2	0	10	7	0	0	0	1	6
DPE1	Emergence	t3	0	0	0	4	1	3	0	0
DPE2	Emergence	t3	0	3	2	0	0	10	0	0
PRE	Emergence	t3	0	0	0	0	0	0	3	8
DPE1	Emergence	tf	0	0	0	0	0	3	0	0
DPE2	Emergence	tf	0	0	0	0	1	2	0	0
PRE	Emergence	tf	0	30	0	0	0	0	13	0

Code	Trap	Date	Opacifrons coxata	Phthitia plumosula	Pseudocollinella jorlii	Rachispoda lutosoidea	Acari
DPE1	Emergence	t0	0	1	0	2	25
DPE2	Emergence	t0	0	1	0	0	23
DPE1	Emergence	t1	28	14	4	3	18
DPE2	Emergence	t1	1	1	0	0	27
PRE	Emergence	t1	12	26	0	0	38
DPE1	Emergence	t2	0	0	0	0	14
DPE2	Emergence	t2	0	0	0	0	34
PRE	Emergence	t2	0	0	0	0	67
DPE1	Emergence	t3	0	0	0	0	8
DPE2	Emergence	t3	0	0	0	0	53
PRE	Emergence	t3	0	0	0	0	45
DPE1	Emergence	tf	0	0	0	0	20
DPE2	Emergence	tf	0	0	0	0	26
PRE	Emergence	tf	0	0	0	0	18

Table S3. Abundance of macroinvertebrate taxa collected from flight interception traps over time.

Code	Trap	Date	Cloeon dipterum	Caenis luctuosa	Graptodytes	Hydraena	Limnebius	Dryops	Bidessus minutissimus
M1	FI	t1	0	0	0	0	0	0	0
M1	FI	t2	0	0	0	1	1	1	1
M1	FI	t3	1	0	0	2	0	0	0
M1	FI	tf	0	0	0	0	0	0	0
M2	FI	t1	0	0	0	0	0	0	0
M2	FI	t2	0	0	0	2	0	1	0
M2	FI	t3	1	0	0	1	0	0	0
M2	FI	tf	0	0	0	2	0	0	0
M3	FI	t1	1	0	0	0	0	1	0
M3	FI	t2	1	0	0	9	0	3	0
M3	FI	t3	0	0	0	1	0	1	0
M3	FI	tf	0	0	1	2	0	0	0
M4	FI	t1	2	2	0	0	0	0	0
M4	FI	t2	1	0	0	1	0	0	0
M4	FI	t3	2	1	0	0	0	0	0
M4	FI	tf	0	0	0	0	0	0	0
M5	FI	t2	0	0	0	0	0	0	0
M5	FI	t3	0	0	0	0	0	0	0
M6	FI	t2	0	0	0	0	0	0	0
M6	FI	t3	1	0	0	0	0	0	0
M6	FI	tf	0	0	0	0	0	0	0
M7	FI	t1	7	0	0	0	0	1	0
M7	FI	t2	2	0	1	3	0	0	0
M7	FI	t3	1	0	4	0	0	0	0
M7	FI	tf	0	0	0	3	0	0	0
M8	FI	t2	1	0	0	0	0	0	0
M8	FI	t3	1	0	0	0	0	0	0
M8	FI	tf	0	0	0	0	0	0	0

Code	Trap	Date	Hydroporus	Meladema coriacea	Anacaena	Polycentropodidae	Chironomini	Dasyhelea	Culicidae
M1	FI	t1	0	0	0	0	0	0	0
M1	FI	t2	0	0	2	0	0	0	0
M1	FI	t3	0	0	0	0	1	0	1
M1	FI	tf	0	0	0	0	0	0	0
M2	FI	t1	0	0	1	0	0	0	0
M2	FI	t2	0	0	2	0	2	0	0
M2	FI	t3	0	0	2	0	0	0	0
M2	FI	tf	0	0	0	0	0	0	0
M3	FI	t1	0	0	0	0	0	0	0
M3	FI	t2	0	0	0	0	0	0	0
M3	FI	t3	1	1	0	0	1	0	0
M3	FI	tf	0	0	0	0	0	0	0
M4	FI	t1	0	0	0	0	0	1	0
M4	FI	t2	0	0	0	0	0	0	0
M4	FI	t3	0	0	0	1	1	0	0
M4	FI	tf	0	0	0	0	0	0	0
M5	FI	t2	0	0	0	0	0	0	0
M5	FI	t3	0	0	0	0	0	0	0
M6	FI	t2	0	0	0	0	0	0	0
M6	FI	t3	0	0	0	0	0	0	0
M6	FI	tf	0	0	0	0	0	0	0
M7	FI	t1	0	0	0	0	0	0	0
M7	FI	t2	0	0	1	0	0	0	0
M7	FI	t3	0	0	0	0	0	0	0
M7	FI	tf	0	0	0	0	1	0	0
M8	FI	t2	0	0	0	0	1	0	0
M8	FI	t3	0	0	3	0	0	0	0
M8	FI	tf	0	0	0	0	0	0	0

Table S4. List of taxa and corresponding sampling techniques (kicking includes mesocosms samples). Note that some taxa (e.g., *Cloeon*) were only collected as larvae through kicking, preventing species identification. In contrast, the emergence and flight interception traps collected adults, allowing for species-level identification.

Taxonomic classification					Sampling methods		
Order	**Family**	**Genus**	**Species**	**Taxa**	**Kicking**	**Emergence**	**Flight Interception**
Diptera	Culicidae	*Aedes*		*Aedes*	X		
Coleoptera	Dytiscidae	*Agabus*	*Agabus bipustulatus*	*Agabus bipustulatus*	X	X	
Coleoptera	Dytiscidae	*Agabus*	*Agabus brunneus*	*Agabus brunneus*	X	X	
Coleoptera	Dytiscidae	*Agabus*	*Agabus didymus*	*Agabus didymus*	X		
Coleoptera	Hydrophilidae	*Anacaena*		*Anacaena*	X		X
Odonata	Aeshnidae	*Anax*	*Anax imperator*	*Anax imperator*	X		
Basommatophora	Ancylidae	*Ancylus*	*Ancylus fluviatilis*	*Ancylus fluviatilis*	X		
Basommatophora	Planorbidae	*Anisus*		*Anisus*	X		
Diptera	Culicidae	*Anopheles*		*Anopheles*	X		
Basommatophora	Planorbidae	*Armiger*		*Armiger crista*	X		
Diptera	Ceratopogonidae	*Atrichopogon*		*Atrichopogon*	X		
Basommatophora				Basommatophora	X		
Diptera	Ceratopogonidae	*Bezzia*		*Bezzia*	X		
Coleoptera	Dytiscidae	*Bidessus*	*Bidessus minutissimus*	*Bidessus minutissimus*	X		X
Ephemeroptera	Caenidae	*Caenis*		*Caenis*	X		
Ephemeroptera	Caenidae	*Caenis*	*Caenis luctuosa*	*Caenis luctuosa*		X	X
Diptera	Chaoboridae	*Chaoborus*		*Chaoborus*	X		
Diptera	Chaoboridae	*Chaoborus*	*Chaoborus flavicans*	*Chaoborus flavicans*		X	
Diptera	Chaoboridae	*Chaoborus*	*Chaoborus pallidus*	*Chaoborus pallidus*		X	
Diptera	Chironomidae			Chironomini	X	X	X
Cladocera				Cladocera	X		
Ephemeroptera	Baetidae	*Cloeon*		*Cloeon*	X		
Ephemeroptera	Baetidae	*Cloeon*	*Cloeon dipterum*	*Cloeon dipterum*		X	X
Ephemeroptera	Baetidae	*Cloeon*	*Cloeon simile*	*Cloeon simile*		X	
Odonata	Coenagrionidae	*Coenagrion*	*Coenagrion puella*	*Coenagrion puella*	X		
Copepoda				Copepoda	X		
Odonata	Cordulegastridae	*Cordulegaster*	*Cordulegaster boltonii*	*Cordulegaster boltonii*	X		
Diptera	Chironomidae	*Corynoneura*		*Corynoneura*	X		
Odonata	Libellulidae	*Crocothemis*	*Crocothemis erythraea*	*Crocothemis erythraea*	X		
Diptera	Culicidae	*Culex*		*Culex*	X		
Diptera	Culicidae			Culicidae		X	X
Coleoptera	Scirtidae	*Cyphon*		*Cyphon*	X		
Diptera	Ceratopogonidae	*Dasyhelea*		*Dasyhelea*	X	X	X
Coleoptera	Dytiscidae	*Deronectes*		*Deronectes*	X		
Diptera	Dixidae	*Dixella*		*Dixella*	X		
Diptera	Dixidae	*Dixella*	*Dixella graeca*	*Dixella graeca*		X	
Diptera	Tipulidae	*Dolichopeza*		*Dolichopeza*	X		
Coleoptera	Dryopidae	*Dryops*		*Dryops*	X	X	X
Amphipoda	Gammaridae	*Echinogammarus*		*Echinogammarus*	X		
Ephemeroptera	Heptageniidae	*Electrogena*	*Electrogena lateralis*	*Electrogena lateralis*	X	X	
Coleoptera	Scirtidae	*Elodes*		*Elodes*	X		
Hirudinoidea	Erpobdellidae	*Erpobdella*		*Erpobdella*	X		
Diptera	Ceratopogonidae	*Forcypomyiia*		*Forcypomyiia*	X		
Coleoptera	Dytiscidae	*Graptodytes*		*Graptodytes*	X	X	X
Diptera	Chironomidae	*Gymnometriocnemus*		*Gymnometriocnemus*	X		
Basommatophora	Planorbidae	*Gyraulus*		*Gyraulus*	X		

Taxonomic classification					Sampling methods		
Order	**Family**	**Genus**	**Species**	**Taxa**	**Kicking**	**Emergence**	**Flight Interception**
Coleoptera	Gyrinidae	*Gyrinus*		*Gyrinus*	X		
Ephemeroptera	Leptophlebiidae	*Habrophlebia*		*Habrophlebia*	X		
Ephemeroptera	Leptophlebiidae	*Habrophlebia*	*Habrophlebia fusca*	*Habrophlebia fusca*		X	
Coleoptera	Haliplidae	*Haliplus*	*Haliplus lineatocollis*	*Haliplus lineatocollis*	X	X	
Rhynchobdellida	Glossiphoniidae	*Helobdella*	*Helobdella stagnalis*	*Helobdella stagnalis*	X		
Coleoptera	Hydrophilidae	*Helochares*	*Helochares lividus*	*Helochares lividus*	X		
Hydrachnidiae				Hydrachnidiae	X	X	
Coleoptera	Hydraenidae	*Hydraena*		*Hydraena*	X	X	X
Coleoptera	Hydrochidae	*Hydrochus*		*Hydrochus*	X		
Coleoptera	Scirtidae	*Hydrocyphon*		*Hydrocyphon*	X		
Heteroptera	Hydrometridae	*Hydrometra*	*Hydrometra stagnorum*	*Hydrometra stagnorum*	X	X	
Coleoptera	Dytiscidae	*Hydroporus*		*Hydroporus*			X
Coleoptera	Hydrophilidae	*Laccobius*		*Laccobius*		X	
Coleoptera	Hydraenidae	*Limnebius*		*Limnebius*	X	X	X
Basommatophora	Lymnaeidae	*Lymnaea*	*Lymnaea stagnalis*	*Lymnaea stagnalis*	X		
Basommatophora	Lymnaeidae			*Lymnaeidae*	X		
Diptera	Chironomidae			Macropelopini	X	X	
Coleoptera	Dytiscidae	*Meladema*	*Meladema coriacea*	*Meladema coriacea*	X		X
Heteroptera	Nepidae	*Nepa*	*Nepa cinerea*	*Nepa cinerea*	X		
Diptera	Ceratopogonidae	*Nilobezzia*		*Nilobezzia*	X		
Heteroptera	Notonectidae	*Notonecta*	*Notonecta maculata*	*Notonecta maculata*	X		
Oligochaeta				Oligochaeta	X		
Diptera	Sphaeroceridae	*Opacifrons*	*Opacifrons coxata*	*Opacifrons coxata*		X	
Odonata	Libellulidae	*Orthetrum*	*Orthetrum chrysostigma*	*Orthetrum chrysostigma*	X		
Diptera	Chironomidae			Orthocladiinae	X	X	
Ostracoda				Ostracoda	X		
Coleoptera	Elmidae	*Oulimnius*		*Oulimnius*	X		
Diptera	Stratiomyidae	*Oxycera*		*Oxycera*	X		
Heteroptera	Corixidae	*Parasigara*	*Parasigara perdubia*	*Parasigara perdubia*	X		
Diptera	Chironomidae			Pentaneurini	X	X	
Diptera	Psychodidae	*Pericoma*		*Pericoma*	X		
Diptera	Sphaeroceridae	*Phthitia*	*Phthitia plumosula*	*Phthitia plumosula*		X	
Basommatophora	Physidae	*Physa*		*Physa*	X		
Basommatophora	Planorbidae	*Planorbis*		*Planorbis*	X		
Trichoptera	Polycentropodidae			Polycentropodidae		X	X
Trichoptera	Polycentropodidae	*Polycentropus*		*Polycentropus*	X		
Basommatophora	Tateidae	*Potamopyrgus*	*Potamopyrgus antipodarum*	*Potamopyrgus antipodarum*	X		
Diptera	Chironomidae	*Procladius*		Procladius		X	
Diptera	Sphaeroceridae	*Pseudocollinella*	*Pseudocollinella jorlii*	*Pseudocollinella jorlii*		X	
Diptera	Psychodidae	*Psychoda*		*Psychoda*	X		
Diptera	Psychodidae			Psychodidae		X	
Diptera	Sphaeroceridae	*Rachispoda*	*Rachispoda lutosoidea*	*Rachispoda lutosoidea*		X	
Basommatophora	Lymnaeidae	*Radix*		*Radix*	X		
Plecoptera	Chloroperlidae	*Siphonoperla*	*Siphonoperla torrentium*	*Siphonoperla torrentium*	X		
Sphaeriida	Sphaeriidae	*Sphaerium*	*Sphaerium corneum*	*Sphaerium corneum*	X		
Coleoptera	Dytiscidae	*Stictonectes*		*Stictonectes*	X		
Diptera	Chironomidae			Tanytarsinii	X	X	
Ephemeroptera	Leptophlebiidae	*Thraulus*	*Thraulus bellus*	*Thraulus bellus*	X		
Diptera	Tipulidae	*Tipula*		*Tipula*	X		
Diptera	Psychodidae	*Tonnoiriella*	*Tonnoiriella pulchra*	*Tonnoiriella pulchra*	X		
Heteroptera	Veliidae	*Velia*		*Velia*	X		
Heteroptera	Veliidae	*Velia*	*Velia caprai*	*Velia caprai*	X		
Coleoptera	Dytiscidae	*Yola*	*Yola bicarinata*	*Yola bicarinata*	X		

CHAPTER 5:

Integrating spatiotemporal hydrological connectivity into conservation planning to protect temporary rivers

To access the Supplementary Information, please visit the publication's webpage at the following link: https://onlinelibrary.wiley.com/doi/10.1002/aqc.4139

Supporting Information 1

Table S1. Mann-Whitney-Wilcoxon test between seasons for macroinvertebrate data.

Mann-Whitney-wilcox test between seasons			
Macroinvertebrates			
	Autumn	**Spring**	**Summer**
Spring	0.002	-	-
Summer	0.002	0.958	-
Winter	0.002	0.958	0.958

Table S2. Results of beta regression models for LCBD and all predictor variables.

Beta regression models (LCBD vs Predictor variables)							
Diatom				Macroinvertebrates			
Predictor variables	**Std. Error**	**Z value**	**p value**	**Predictor variables**	**Std. Error**	**Z value**	**p value**
pH	0.02	0.56	0.572	**pH**	0.02	-0.55	0.579
DO mg/L	0.018	1.35	0.177	**DO mg/L**	0.02	0.99	0.322
T	0.019	-0.49	0.626	**T**	0.02	-0.78	0.435
TDS	0.029	1.40	0.163	**TDS**	0.032	-0.21	0.83
B1	0.02	0.71	0.479	**B1**	0.02	2.98	0.003 **
B2	0.022	0.15	0.882	**B2**	0.021	-2.62	0.008 **
B3	0.02	0.40	0.692	**B3**	0.02	3.08	0.002 **
B4	0.022	1.11	0.265	**B4**	0.021	-1.86	0.062 .
B5	0.025	0.40	0.687	**B5**	0.023	0.4	0.686
B7	0.02	-0.73	0.465	**B6**	0.023	-0.34	0.734
FP50	0.021	0.10	0.923	**B7**	0.02	-1.65	0.098 .
DirBin	0.021	-0.36	0.721	**FP50**	0.02	-1.06	0.287
DirWei	0.019	-0.95	0.344	**DirBin**	0.022	0.91	0.362
UndBin	0.04	-0.18	0.861	**DirWei**	0.019	2.27	0.023 *
UndWei	0.043	0.53	0.598	**UndBin**	0.02	-2.52	0.012 *
TotLeng	0.021	-0.12	0.903	**UndWei**	0.025	0.97	0.334
				TotLeng	0.022	-0.98	0.326

Table S3. Results of linear mixed-effects models (LMM) for MSF and all predictor variables.

Linear mixed-effect models (MSF vs Predictor variables)

Diatom			Macroinvertebrates		
Predictor variables	**F value**	**p value**	**Predictor variables**	**F value**	**p value**
pH	0.015	0.901	**pH**	3,052	0.085 .
DO mg/L	7.61	0.007 **	**DO mg/L**	2,001	0.161
T	7.81	0.007 **	**T**	0.328	0.568
TDS	0.879	0.351	**TDS**	2.48	0.119
B1	1,749	0.191	**B1**	0.416	0.521
B2	<0.001	0.984	**B2**	0.837	0.363
B3	0.073	0.787	**B3**	0.053	0.817
B4	0.003	0.96	**B4**	0.03	0.862
B5	0.223	0.638	**B5**	0.308	0.58
B7	0.194	0.661	**B6**	0.153	0.696
FP50	1,723	0.193	**B7**	10,374	0.001 **
DirBin	0.529	0.469	**FP50**	0.629	0.43
DirWei	0.808	0.371	**DirBin**	0.207	0.65
UndBin	3,021	0.087 .	**DirWei**	2,499	0.118
UndWei	3,559	0.064 .	**UndBin**	1,328	0.253
TotLeng	0.049	0.824	**UndWei**	1,038	0.312
			TotLeng	2,332	0.131

Supporting Information 2

Table S4. Percentage of flow permanence (FP) values for all sites.

Sites	Stream	Flow Permanence (FP) %
CA1	Castello	94.86679662
CA2	Castello	73.0994152
CA3	Castello	60.03898635
CA4	Castello	77.582846
H1	Vall d Horta	99.61013645
H2	Vall d Horta	99.70760234
H3	Vall d Horta	99.61013645
H4	Vall d Horta	88.59649123
H5	Vall d Horta	33.13840156
MU1	Mura	42.49512671
MU2	Mura	78.75243665
MU3	Mura	99.61013645
MU4	Mura	99.80506823
R1	Rellinars	55.75048733
R2	Rellinars	55.75048733
R3	Rellinars	62.76803119
R4	Rellinars	98.44054581
SA1	Sanana	81.54645874
SA2	Sanana	98.05068226
SA3	Sanana	86.7202729
SA4	Sanana	80.01949318
SA5	Sanana	87.26445744
SC1	Santa Creu	55.75048733
SC2	Santa Creu	33.91812865
SC3	Santa Creu	34.89278752
SC4	Santa Creu	53.28135153
T1	Talamanca	85.28265107
T2	Talamanca	51.4619883
T3	Talamanca	57.76478233
T4	Talamanca	69.00584795

Appendix 1

Table appendix 1. List of diatom species and their respective codes.

Taxa	Code	Taxa	Code
Achnanthidium caledonicum	ADCA	Diploneis krammeri	DKRA
Achnanthes trinodis	ATRI	Diploneis modica	DMOD
Achnanthidium minutissimum	ADMI	Diploneis parma	DPAR
Achnanthidium neomicrocephalum	ADNM	Diploneis separanda	DSEP
Achnanthidium pyrenaicum	ADPY	Encyonema lacustre	ELAC
Achnanthidium straubianum	ADSB	Encyonema prostratum	EPRO
Adlafia bryophila	ABRY	Encyonema silesiacum	ESLE
Adlafia minuscula	ADMS	Encyonema ventricosum	ENVE
Amphipleura pellucida	APEL	Encyonopsis cesatii	ECES
Amphora alpestris	AAPS	Encyonopsis falaisensis	ECFA
Amphora lange-bertalotii	AMLB	Encyonopsis minuta	ECPM
Amphora minutissima	AMNU	Encyonopsis subminuta	ESUM
Amphora pediculus	APED	Encyonopsis krammeri	ECKR
Amphora sp1	AMPH	Eolimna minima	EOMI
Aneumastus pseudotusculus	ANPT	Epithemia adnata	EADN
Aulacoseira granulata	AUGR	Epithemia frickei	EFRI
Brachysira styriaca	BSTY	Eucocconeis flexella	EUFL
Brachysira neglectissima	BNEG	Eucocconeis laevis	EULA
Brachysira neoexilis	BNEO	Eunotia arcubus	EARB
Brachysira vitrea	BVIT	Eunotia bilunaris	EBIL
Caloneis strelnikovae	CSNK	Eunotia minor	EMIN
Caloneis alpestris	CAPS	Fallacia pygmaea	FPYG
Caloneis bacillum	CBAC	Fallacia subhamulata	FSBH
Caloneis cf bacillum f. inflata	CBFI	Fistulifera pelliculosa	FPEL
Caloneis falcifera	CFAF	Fragilaria acidoclinata	FACD
Caloneis lancettula	CLCT	Fragilaria gracilis	FGRA
Caloneis schumanniana	CSHU	Fragilaria mesolepta	FMES
Caloneis silicula	CSIL	Pseudostaurosira parasitica	PPRS
Caloneis sp1	CALO	Fragilaria recapitellata	FRCP
Caloneis tenuis	CATE	Fragilaria sp1	FRGL
Cocconeis lineata	CLNT	Fragilaria tenera	FTEN
Cocconeis pediculus	CPED	Fragilaria bicapitata	FBSI
Cocconeis placentula	CPLA	Frustulia amosseana	FAMO
Cocconeis placentula var. euglypta	CPLE	Frustulia vulgaris	FVUL
Craticula buderi	CRBU	Frustulia vulgaris var. capitata	FVUC
Craticula cuspidata	CRCU	Gomphonema acidoclinatum	GADC
Cyclotella distinguenda	CDTG	Gomphonema acuminatum	GACU
Cyclotella meneghiniana	CMEN	Gomphonema auritum	GAUR
Cyclotella radiosa	CRAD	Gomphonema calcareum	GHCA
Cymatopleura elliptica	CELL	Gomphonema capitatum	GCAP
Cymatopleura solea	CSOL	Gomphonema clavatum	GCLA
Cymbella affinis var. procera	CAPR	Gomphonema cymbelliclinum	GCBC
Cymbella aspera	CASP	Gomphonema exilissimum	GEXL
Cymbella compacta	CCMP	Gomphonema gracile	GGRA
Cymbella excisa	CAEX	Gomphonema lateripunctatum	GLAT
Cymbella excisiformis	CEXF	Gomphonema micropus	GMIC
Cymbella helvetica	CHEL	Gomphonema minusculum	GMIS
Cymbella lancettula	CLTL	Gomphonema parvulum var. parvolum f. parvulum	GPAR
Cymbella neocistula	CNIS	Gomphonema subclavatum	GSCL
Cymbella neoleptoceros	CNLP	Gomphonema tergestinum	GTER
Cymbella parva	CPAR	Gomphonema truncatum	GTRU
Cymbella tropica	CTRO	Gomphonema vibrio	GVIB
Cymbella vulgata	CVUL	Gyrosigma acuminatum	GYAC
Cymbopleura amphicephala	CBAM	Gyrosigma attenuatum	GYAT
Cymbopleura diminuta	CBDM	Gyrosigma sciotoense	GSCI
Cymbopleura laeviformis	CLVF	Halamphora montana	HLMO
Cymbopleura subaequalis	CSAQ	Halamphora normanii	HNOR
Denticula tenuis	DTEN	Halamphora oligotraphenta	HOLI
Diadesmis contenta	DCOT	Halamphora veneta	HVEN
Diatoma moniliformis	DMON	Hantzschia amphioxys	HAMP

Taxa	Code
Hantzschia abundans	HABU
Luticola mutica	LMUT
Luticola nivalis	LNIV
Luticola ventriconfusa	LVCF
Mastogloia albertii	MALB
Mastogloia elliptica	MELL
Mastogloia grevillei	MGRE
Mastogloia lacustris	MLAC
Mastogloia smithii	MSMI
Mayamaea atomus	MAAT
Melosira varians	MVAR
Meridion circulare	MCIR
Navicula angusta	NAAN
Navicula antonii	NANT
Navicula brockmanni	NBRO
Navicula cincta	NCIN
Navicula cryptocephala	NCRY
Navicula cryptotenella	NCTE
Navicula cryptotenelloides	NCTO
Navicula dealpina	NDEA
Navicula gottlandica	NGOT
Navicula oblonga	NOBL
Navicula pseudolanceolata	NPSL
Navicula radiosa	NRAD
Navicula seibigiana	NSEB
Navicula subalpina	NSBN
Navicula tenelloides	NTEN
Navicula tripunctata	NTPT
Navicula trivialis	NTRV
Navicula veneta	NVEN
Neidium binodiforme	NBNF
Neidium ampliatum	NEAM
Neidium cuneatiforme	NECF
Neidium sp1	NEID
Nitzschia acicularis	NACI
Nitzschia acidoclinata	NACD
Nitzschia alpinobacillum	NAPB
Nitzschia amphibia	NAMP
Nitzschia bulnheimiana	NIBU
Nitzschia denticula	NDEN
Nitzschia dissipata var. dissipata	NDIS
Nitzschia dissipata var. media	NDME
Nitzschia filiformis	NFIL
Nitzschia fonticola	NFON
Nitzschia subtilis	NISU
Nitzschia fossilis	NIFS
Nitzschia linearis	NLIN
Nitzschia oligotraphenta	NIOG
Nitzschia paleacea	NPAE
Nitzschia palea	NPAL
Nitzschia paleaeformis	NIPF
Nitzschia perminuta	NIPM
Nitzschia pusilla	NIPU
Nitzschia regula	NIRE
Nitzschia sociabilis	NSOC
Nitzschia solita	NISO
Nitzschia solgensis	NSOL
Nitzschia tenuis	NITE
Nitzschia sigmoidea	NSIO
Nitzschia tabellaria	NTAB
Orthoseira roeseana	OROE
Peronia fibula	PFIB
Pinnularia borealis var. borealis	PBOR
Pinnularia brebissonii	PBRE
Pinnularia interrupta	PINT
Pinnularia rupestris	PRUP
Pinnularia subrupestris	PSRU
Placoneis paraelginensis	PPAE
Planothidium frequentissimum	PLFR
Planothidium lanceolatum	PTLA
Platessa conspicua	PTCO
Pseudostaurosira brevistriata	PSBR
Reimeria uniseriata	RUNI
Rhoicosphenia abbreviata	RABB
Rhopalodia gibba	RGIB
Rhopalodia operculata	ROPE
Rhopalodia parallela	RPAR
Rossithidium sp1	ROSS
Sellaphora capitata	SECA
Sellaphora lanceolata	SLCL
Sellaphora stroemii	SSTM
Stauroneis amphicephala	SAAP
Stauroneis gracilis	SGRC
Stauroneis kriegeri	STKR
Stauroneis phoenicenteron	SPHO
Stauroneis separanda	STSE
Stauroneis smithi	SSMI
Surirella angusta	SANG
Surirella iowensis	SUIW
Surirella ovalis	SOVI
Surirella terricola	STER
Tryblionella angustata	TANG
Tryblionella angustatula	TATU
Tryblionella brunoi	TBNO
Tryblionella calida	TCAL
Tryblionella constricta	TBCO
Tryblionella debilis	TDEB
Ulnaria capitata	UCAP
Ulnaria contracta	UCON
Ulnaria danica	UDAN
Ulnaria ulna	UULN
Ulnaria verhaegeniana	ULVH

***Appendix 2.** Table of Macroinvertebrate Taxa. This information can be found in Tables 4, 5, 6, and 7 of biological and environmental data (next section).

TABLES OF BIOLOGICAL AND ENVIRONMENTAL DATA

Table 1. Fluvial Habitat Index (IHF) developed by Pardo *et al*. (2002) for characterizing habitat heterogeneity and physical variables in Mediterranean streams.

Fluvial Habitat Index for Mediterranean streams. Index IHF



Sampling site	
Data	
Operator	

Parts			Score
1. Embeddedness in riffles and runs – sedimentation in pools			
Riffles	Stones, pebbles and gravel embebed in fine sediment in 0 - 30%.	10	
	Stones, pebbles and gravel embebed in fine sediment in 30 - 60%.	5	
	Stones, pebbles and gravel embebed in fine sediment in > 60%.	0	
Pools	Sedimentation 0 - 30%	10	
	Sedimentation 30 - 60%	5	
	Sedimentation > 60%	0	
	TOTAL (only one score from pools or from riffles)		
2. Riffle frequency			
	High frequency of riffles. Ratio: distance between riffles / stream witdh < 7	10	
	Medium. Ratio: distance between riffles / stream witdh 7 - 15	8	
	Ocassional. Ratio: distance between riffles / stream witdh 15 - 25	6	
	Scarce or null, laminar flow. Ratio: distance between riffles / stream witdh >25	4	
	Only pools	2	
	TOTAL (only one score)		
3. Substrate composition			
% Boulders and stones	1 - 10%	2	
	> 10%	5	
% Pebbles and gravels	1 - 10%	2	
	> 10%	5	
% Sand	1 - 10%	2	
	> 10%	5	
% Silt and clay	1 - 10%	2	
	> 10%	5	
	TOTAL (sum of scores of each class of substrate)		
4. Velocity/depth regime			
shallow:< 0.5 m	4 classes present. Slow-depth, slow-shallow, fast-depth and fast-shallow.	10	
slow:< 0.3 m/s	Only 3 of 4 regimes	8	
	Only 2 of 4 regimes	6	
	Only 1 regime	4	
	TOTAL (only one socre)		
5. Shading of river bed			
	Shaded with some open areas	10	
	Completely shaded	7	
	Large open areas	5	
	Not shaded	3	
	TOTAL (only one score)		
6. Heterogeneity components			
Leaf litter	> 10% or < 75%	4	
	< 10% or > 75%	2	
Presence of branches and wood in the stream		2	
Tree roots in the banks		2	
Natural dams		2	
	TOTAL (sum of scores of each class)		
7. Aquatic vegetation cover			
% Plocon + mosses	10 - 50%	10	
	< 10% or > 50%	5	
% Pecton	10 - 50%	10	
	< 10%or > 50%	5	
% Phanerogams + Charales	10 - 50%	10	
	< 10% or > 50%	5	
	TOTAL (sum of scores of each class)		
HID98-0323-C05 ; REN2001-3438-C07	**FINAL SCORE (Addition of all previous scores)**		

The original description of the IHF index was made by Pardo *et al* ., Limnética *in press*

Table 2. Fluvial Habitat Index (IHF) data for the MECODISPER project, presented by sampling point and season. Data are detailed by each index block (B1, B2, ...) along with the final IHF score.

Code	Reach	Stream	Season	B1	B2	B3	B4	B5	B6	B7	IHF
CA1W	CA1	Castello	Winter	10	8	12	8	10	6	10	64
CA1SP	CA1	Castello	Spring	10	8	9	8	3	6	10	54
CA1SU	CA1	Castello	Summer	5	8	11	8	5	4	20	61
CA1A	CA1	Castello	Autumn	5	4	10	6	3	6	20	54
CA2W	CA2	Castello	Winter	10	10	12	8	10	6	15	71
CA2SP	CA2	Castello	Spring	5	8	9	8	5	4	10	49
CA2SU	CA2	Castello	Summer	5	8	11	8	10	8	20	70
CA2A	CA2	Castello	Autumn	5	4	12	6	5	6	30	68
CA3W	CA3	Castello	Winter	10	10	9	8	10	10	15	72
CA3SP	CA3	Castello	Spring	5	8	9	8	5	4	20	59
CA3SU	CA3	Castello	Summer	5	8	11	8	5	8	25	70
CA3A	CA3	Castello	Autumn	5	4	17	6	3	6	30	71
CA4W	CA4	Castello	Winter	10	10	4	8	10	10	10	62
CA4SP	CA4	Castello	Spring	5	8	11	8	5	6	25	68
CA4A	CA4	Castello	Autumn	5	2	7	4	5	6	15	44
H1W	H1	Vall d Horta	Winter	5	6	9	8	10	10	10	58
H1SP	H1	Vall d Horta	Spring	5	0	17	8	10	10	15	65
H1SU	H1	Vall d Horta	Summer	10	8	17	8	10	10	15	78
H1A	H1	Vall d Horta	Autumn	5	8	20	10	10	10	20	83
H2W	H2	Vall d Horta	Winter	10	6	4	8	10	6	15	59
H2SP	H2	Vall d Horta	Spring	5	8	17	8	10	10	20	78
H2SU	H2	Vall d Horta	Summer	10	8	14	8	10	10	15	75
H2A	H2	Vall d Horta	Autumn	5	8	20	10	10	10	20	83
H3W	H3	Vall d Horta	Winter	5	10	9	10	5	8	20	67
H3SU	H3	Vall d Horta	Summer	10	6	17	8	10	10	15	76
H3A	H3	Vall d Horta	Autumn	5	10	20	10	10	10	20	85
H4W	H4	Vall d Horta	Winter	5	4	15	6	10	10	10	60
H5W	H5	Vall d Horta	Winter	10	6	15	6	7	6	10	60
H5SP	H5	Vall d Horta	Spring	5	6	12	8	10	6	20	67
H5SU	H5	Vall d Horta	Summer	10	2	9	4	10	6	15	56
H5A	H5	Vall d Horta	Autumn	5	4	17	6	5	4	15	56
MU1W	MU1	Mura	Winter	5	10	17	8	5	10	20	75
MU1SP	MU1	Mura	Spring	5	10	8	6	5	4	15	53
MU2W	MU2	Mura	Winter	10	8	11	8	10	8	10	65
MU2SP	MU2	Mura	Spring	10	6	17	8	10	10	20	81
MU2SU	MU2	Mura	Summer	5	2	17	6	10	10	25	75
MU2A	MU2	Mura	Autumn	10	6	17	8	10	8	20	79
MU3W	MU3	Mura	Winter	10	10	9	10	3	4	15	61
MU3SP	MU3	Mura	Spring	5	8	14	8	5	8	15	63
MU3SU	MU3	Mura	Summer	5	6	17	0	5	6	25	64

Table 2. Continued on next page.

Table 2. Continued from previous page.

Code	Reach	Stream	Season	B1	B2	B3	B4	B5	B6	B7	IHF
MU3A	MU3	Mura	Autumn	5	6	17	8	3	6	15	60
MU4W	MU4	Mura	Winter	10	10	5	6	5	8	15	59
MU4SP	MU4	Mura	Spring	10	10	11	8	3	4	15	61
MU4SU	MU4	Mura	Summer	5	6	14	8	0	6	25	64
MU4A	MU4	Mura	Autumn	5	6	14	8	3	8	15	59
R1W	R1	Rellinars	Winter	5	8	15	6	5	4	20	63
R2W	R2	Rellinars	Winter	10	6	12	6	5	4	20	63
R3W	R3	Rellinars	Winter	5	8	6	6	3	0	10	38
R4W	R4	Rellinars	Winter	5	6	6	6	3	2	5	33
R4SP	R4	Rellinars	Spring	10	2	14	4	3	6	0	39
R4SU	R4	Rellinars	Summer	10	2	6	4	3	4	10	39
R4A	R4	Rellinars	Autumn	10	4	12	8	3	4	10	51
SA1W	SA1	Sanana	Winter	10	8	14	10	10	8	20	80
SA1SP	SA1	Sanana	Spring	5	8	14	0	10	6	30	73
SA1SU	SA1	Sanana	Summer	5	6	17	8	10	4	30	80
SA1A	SA1	Sanana	Autumn	5	6	12	8	5	8	30	74
SA2W	SA2	Sanana	Winter	10	8	20	8	5	8	25	84
SA2SP	SA2	Sanana	Spring	5	6	14	8	5	6	20	64
SA2SU	SA2	Sanana	Summer	5	6	11	8	3	0	25	58
SA2A	SA2	Sanana	Autumn	5	6	20	8	3	8	25	75
SA3W	SA3	Sanana	Winter	5	10	11	8	10	8	15	67
SA3SP	SA3	Sanana	Spring	10	6	9	8	10	10	0	53
SA3SU	SA3	Sanana	Summer	10	8	14	8	10	10	25	85
SA3A	SA3	Sanana	Autumn	5	8	17	8	10	8	15	71
SA4W	SA4	Sanana	Winter	10	8	6	8	5	2	10	49
SA4SP	SA4	Sanana	Spring	5	6	11	6	3	2	20	53
SA4SU	SA4	Sanana	Summer	10	2	17	4	3	4	15	55
SA4A	SA4	Sanana	Autumn	5	6	12	8	3	6	20	60
SA5W	SA5	Sanana	Winter	10	8	6	8	5	2	10	49
SA5SP	SA5	Sanana	Spring	5	6	11	8	3	2	20	55
SA5SU	SA5	Sanana	Summer	10	4	15	8	3	2	15	57
SA5A	SA5	Sanana	Autumn	5	4	15	6	3	6	10	49
SC1W	SC1	Santa Creu	Winter	5	2	9	4	3	0	15	38
SC1SP	SC1	Santa Creu	Spring	15	2	14	4	3	0	15	53
SC1A	SC1	Santa Creu	Autumn	10	2	15	6	3	2	10	48
SC2W	SC2	Santa Creu	Winter	10	4	10	4	3	2	10	43
SC2SP	SC2	Santa Creu	Spring	15	2	12	4	3	0	5	41
SC2A	SC2	Santa Creu	Autumn	10	2	15	4	3	4	10	48
SC3W	SC3	Santa Creu	Winter	5	4	4	4	3	2	20	42
SC3SP	SC3	Santa Creu	Spring	15	2	14	4	3	0	15	53

Table 2. Continued on next page.

Table 2. Continued from previous page.

Code	Reach	Stream	Season	B1	B2	B3	B4	B5	B6	B7	IHF
SC3SU	SC3	Santa Creu	Summer	10	2	9	4	3	0	20	48
SC3A	SC3	Santa Creu	Autumn	5	0	20	4	3	4	10	46
SC4W	SC4	Santa Creu	Winter	10	2	4	4	3	2	20	45
SC4SP	SC4	Santa Creu	Spring	15	2	11	4	3	0	10	45
SC4SU	SC4	Santa Creu	Summer	10	2	12	4	3	2	10	43
SC4A	SC4	Santa Creu	Autumn	10	2	15	6	3	6	15	57
T1W	T1	Talamanca	Winter	10	10	8	8	5	6	15	62
T1SP	T1	Talamanca	Spring	5	8	14	6	3	4	15	55
T1SU	T1	Talamanca	Summer	5	6	14	6	3	2	20	56
T1A	T1	Talamanca	Autumn	10	6	14	8	3	6	20	67
T2W	T2	Talamanca	Winter	10	10	14	8	5	4	15	66
T2SP	T2	Talamanca	Spring	5	6	11	8	5	0	20	55
T2A	T2	Talamanca	Autumn	5	4	15	6	3	4	10	47
T3W	T3	Talamanca	Winter	10	10	11	10	3	6	10	60
T3SP	T3	Talamanca	Spring	5	8	8	8	3	2	20	54
T3SU	T3	Talamanca	Summer	10	2	12	4	3	0	15	46
T3A	T3	Talamanca	Autumn	10	4	15	6	3	4	20	62
T4W	T4	Talamanca	Winter	10	8	11	8	7	8	15	67
T4SP	T4	Talamanca	Spring	5	6	11	8	5	4	20	59
T4SU	T4	Talamanca	Summer	10	2	10	4	3	0	5	34
T4A	T4	Talamanca	Autumn	5	4	17	0	3	6	20	55

Table 3. Physico-chemical data for the MECODISPER project, measured at each sampling point and season using a YSI Professional Plus Multiparameter Probe. Parameters include aquatic states (AE) (following Gallart *et al.*, 2012), pH, dissolved oxygen (D.O. mg/L), oxygen saturation (D.O. %), water temperature (T ºC), conductivity (EC µS/cm), specific conductivity (SPC µS/cm), and total dissolved solids (TDS mg/L).

Code	Reach	Stream	Season	AE	pH	D.O. (mgL)	D.O. (%)	T (ºC)	EC	SPC	TDS
CA1W	CA1	Castello	Winter	Eurheic	8,74	5,82	43,5	3,2	360,4	638,6	418,6
CA1SP	CA1	Castello	Spring	Oligorheic	7,54	10,06	96,3	13,7	414,3	528,7	343,85
CA1SU	CA1	Castello	Summer	Eurheic	7,65	6,89	75,1	19,4	442,4	495,3	322,4
CA1A	CA1	Castello	Autumn	Oligorheic	8,25	11,27	89,1	5,4	427,6	690,3	448,5
CA2W	CA2	Castello	Winter	Eurheic	8,79	4,62	35,7	4,3	402,8	665,9	432,9
CA2SP	CA2	Castello	Spring	Oligorheic	8,05	10,87	111,5	17,7	505	586	383,5
CA2SU	CA2	Castello	Summer	Eurheic	8,01	7,57	80,4	17,9	462,9	524,3	340,6
CA2A	CA2	Castello	Autumn	Oligorheic	8,01	12,01	97,7	6,5	379,5	593,2	386,75
CA3W	CA3	Castello	Winter	Eurheic	8,73	4,9	31,7	4,6	415,2	681	442
CA3SP	CA3	Castello	Spring	Oligorheic	8,04	10,07	107	18,1	512	589	383,5
CA3SU	CA3	Castello	Summer	Eurheic	7,97	8,94	96	18,3	434	497	323,7
CA3A	CA3	Castello	Autumn	Oligorheic	7,88	11,26	92,3	6,8	450,3	696,6	453,7
CA4W	CA4	Castello	Winter	Eurheic	8,59	4,41	33,8	5,4	439,5	702,2	456,3

Table 3. Continued on next page.

Table 3. Continued from previous page.

Code	Reach	Stream	Season	AE	pH	D.O. (mgL)	D.O. (%)	T (ºC)	EC	SPC	TDS
CA4SP	CA4	Castello	Spring	Oligorheic	7,57	10,28	101,2	14,2	517	654	422,5
CA4A	CA4	Castello	Autumn	Arheic	8,16	13,86	108,7	5,1	329,1	527,8	343,85
H1W	H1	Vall d Horta	Winter	Eurheic	8,23	11,19	92,39	7,4	431,5	648,9	421,2
H1SP	H1	Vall d Horta	Spring	Eurheic	7,59	8,52	82,1	13,2	520	671	435,5
H1SU	H1	Vall d Horta	Summer	Eurheic	7,47	6,79	73,9	18,3	618	709	461,5
H1A	H1	Vall d Horta	Autumn	Eurheic	7,8	9,95	82	7	445,5	685,2	445,25
H2W	H2	Vall d Horta	Winter	Eurheic	8,28	11,2	92,42	8,1	444,5	656,5	427,05
H2SP	H2	Vall d Horta	Spring	Arheic	7,69	8,96	88,3	14,4	547	687	448,5
H2SU	H2	Vall d Horta	Summer	Eurheic	7,6	3,61	39,5	20,3	632	692	448,5
H2A	H2	Vall d Horta	Autumn	Eurheic	7,87	11,03	89,3	6,4	450	702,8	454,35
H3W	H3	Vall d Horta	Winter	Eurheic	8,23	11,2	94,99	8,8	448,7	649,5	421,85
H3SU	H3	Vall d Horta	Summer	Eurheic	7,38	8,27	96,1	20,8	642	698	455
H3A	H3	Vall d Horta	Autumn	Arheic	7,6	10,07	84,6	7,8	464,8	696	452,4
H4W	H4	Vall d Horta	Winter	Eurheic	7,37	8,51	82,39	12,8	483,6	630,7	440
H5W	H5	Vall d Horta	Winter	Oligorheic	8,24	10,92	96,27	7,7	371,6	55,7	361,4
H5SP	H5	Vall d Horta	Spring	Oligorheic	7,88	9,87	94,7	12,8	431,5	660,7	366,6
H5SU	H5	Vall d Horta	Summer	Arheic	7,87	3,83	46,6	24,6	462,2	466,6	302,9
H5A	H5	Vall d Horta	Autumn	Oligorheic	7,89	10,91	92,6	8,2	409,5	607,4	395,2
MU1W	MU1	Mura	Winter	Eurheic	8,73	8,67	75,8	9,6	433,3	610,7	401,7
MU1SP	MU1	Mura	Spring	Oligorheic	8,04	10,78	109	15,9	460,2	557,4	362,05
MU2W	MU2	Mura	Winter	Eurheic	8,73	8,08	68,55	9,1	417	606,6	388,27
MU2SP	MU2	Mura	Spring	Oligorheic	7,73	11,61	113,2	14	456,5	577,9	375,7
MU2SU	MU2	Mura	Summer	Eurheic	7,33	2,57	28	19,2	554	623	403
MU2A	MU2	Mura	Autumn	Arheic	7,94	10,68	88,2	7,1	381	584,4	380,25
MU3W	MU3	Mura	Winter	Eurheic	8,66	7,19	61	8,1	400,3	591,6	384,16
MU3SP	MU3	Mura	Spring	Oligorheic	8,14	10,54	107,7	13,9	438	556,5	362,05
MU3SU	MU3	Mura	Summer	Oligorheic	7,9	7,59	87,8	22,6	568	596	390
MU3A	MU3	Mura	Autumn	Eurheic	8,14	12,8	97	3,6	353,1	596,8	388,06
MU4W	MU4	Mura	Winter	Eurheic	8,65	10,4	85,7	6,9	381,7	583,1	378,95
MU4SP	MU4	Mura	Spring	Oligorheic	8,02	9,19	85,3	12,1	423,1	561	364,65
MU4SU	MU4	Mura	Summer	Oligorheic	7,81	7	78	20,3	531	586	377
MU4A	MU4	Mura	Autumn	Eurheic	7,93	12,4	94,4	3,9	347,4	586,2	380,25
R1W	R1	Rellinars	Winter	Eurheic	7,36	9,19	86,44	10,3	500	696	455
R2W	R2	Rellinars	Winter	Eurheic	8,27	11,37	92,77	6	418	679,3	440,7
R3W	R3	Rellinars	Winter	Eurheic	8,45	9,13	78,48	5,3	416,14	668,7	434,85
R4W	R4	Rellinars	Winter	Eurheic	8,34	11,95	96,85	5,1	430,2	681	442
R4SP	R4	Rellinars	Spring	Arheic	7,84	9,47	113,3	24,1	402,9	498,8	321,1
R4SU	R4	Rellinars	Summer	Arheic	8,04	5	57	21,9	394,8	419	272,36
R4A	R4	Rellinars	Autumn	Oligorheic	8,29	13,39	100	3,1	368	622,4	404,3
SA1W	SA1	Sanana	Winter	Eurheic	8,58	7,8	57,8	3	374,5	645,9	414,5
SA1SP	SA1	Sanana	Spring	Eurheic	7,75	8,64	83	13	442	638	414
SA1SU	SA1	Sanana	Summer	Oligorheic	7,8	8,87	104,1	22,4	668	704	455
SA1A	SA1	Sanana	Autumn	Eurheic	7,68	9,29	80,9	9,3	448,3	645,1	419,25
SA2W	SA2	Sanana	Winter	Eurheic	8,6	7,82	59,8	3,5	383,1	650,1	422,5
SA2SP	SA2	Sanana	Spring	Eurheic	7,66	9,23	94,3	16,4	138,8	170,5	344,8

Table 3. Continued on next page.

Table 3. Continued from previous page.

Code	Reach	Stream	Season	AE	pH	D.O. (mgL)	D.O. (%)	T (ºC)	EC	SPC	TDS
SA2SU	SA2	Sanana	Summer	Oligorheic	7,85	7,2	87,5	24	645	657	429
SA2A	SA2	Sanana	Autumn	Eurheic	7,97	10,9	93,6	8,7	447,3	655,725	424,45
SA3W	SA3	Sanana	Winter	Eurheic	8,55	9,68	71	3,7	386,9	651,2	423,8
SA3SP	SA3	Sanana	Spring	Eurheic	7,86	9,63	99,2	15,9	528	639	416
SA3SU	SA3	Sanana	Summer	Oligorheic	7,6	7,49	86	19,9	629	694	448,5
SA3A	SA3	Sanana	Autumn	Eurheic	8,02	11,51	95,5	7,3	436,3	664	431,6
SA4W	SA4	Sanana	Winter	Eurheic	8,57	6,88	52,1	4	391,5	653,4	424,5
SA4SP	SA4	Sanana	Spring	Oligorheic	7,91	9,3	109,3	22,9	594	620	403
SA4SU	SA4	Sanana	Summer	Arheic	7,98	8,37	108,7	30,3	582	528	344,5
SA4A	SA4	Sanana	Autumn	Eurheic	8	11,29	93,7	7,4	430,7	652,3	424,45
SA5W	SA5	Sanana	Winter	Eurheic	8,6	6,5	49,6	4	392,1	654,3	425,1
SA5SP	SA5	Sanana	Spring	Oligorheic	7,84	10,51	124,9	23,2	622	644	416
SA5SU	SA5	Sanana	Summer	Oligorheic	7,77	9,27	119,5	28,2	761	717	468
SA5A	SA5	Sanana	Autumn	Oligorheic	8,28	13,75	108,2	5,2	406,7	661,5	430,95
SC1W	SC1	Santa Creu	Winter	Oligorheic	8,73	7,05	58	0,2	421,6	803,9	520
SC1SP	SC1	Santa Creu	Spring	Arheic	7,46	7,17	74,2	16,5	680	813	526,5
SC1A	SC1	Santa Creu	Autumn	Arheic	7,78	8,67	70,9	6,7	505	792	513,5
SC2W	SC2	Santa Creu	Winter	Oligorheic	8,38	11,18	95,4	1,4	442,3	804,5	520,65
SC2SP	SC2	Santa Creu	Spring	Arheic	7,65	10,25	109	18,1	710	816	533
SC2A	SC2	Santa Creu	Autumn	Arheic	8,01	11,54	99,5	8,7	586	858	559
SC3W	SC3	Santa Creu	Winter	Oligorheic	8,49	8,42	75,1	4,8	544	886	578
SC3SP	SC3	Santa Creu	Spring	Arheic	8,31	13,17	155,4	23,4	799	824	533
SC3SU	SC3	Santa Creu	Summer	Arheic	8,39	7,05	89	26,8	972	939	611
SC3A	SC3	Santa Creu	Autumn	Arheic	8,07	11,5	92	5,7	542	877	572
SC4W	SC4	Santa Creu	Winter	Oligorheic	7,99	11	97,63	5,6	574	911	591
SC4SP	SC4	Santa Creu	Spring	Arheic	7,26	6,8	75,3	17	798	965	617,5
SC4SU	SC4	Santa Creu	Summer	Arheic	7,7	6,79	81	23,7	1082	1109	721,5
SC4A	SC4	Santa Creu	Autumn	Arheic	7,95	11,58	107	11,7	630	839	546
T1W	T1	Talamanca	Winter	Eurheic	8,61	6,39	50,1	5	391,5	632,8	411,45
T1SP	T1	Talamanca	Spring	Oligorheic	7,43	8,48	96	21,5	597	643	416
T1SU	T1	Talamanca	Summer	Oligorheic	7,44	6,43	73,4	20,9	685	746	481
T1A	T1	Talamanca	Autumn	Eurheic	8,01	12,72	100,6	5,3	378,4	610	396,5
T2W	T2	Talamanca	Winter	Eurheic	8,74	6,95	50,3	1,8	340,6	610,4	396,5
T2SP	T2	Talamanca	Spring	Oligorheic	7,87	10,01	97,9	14,1	413,7	527,6	343,2
T2A	T2	Talamanca	Autumn	Oligorheic	8,08	13,12	106,2	6,2	344,2	535,2	347,1
T3W	T3	Talamanca	Winter	Eurheic	8,69	8,66	63,8	1,8	339	607,7	401,05
T3SP	T3	Talamanca	Spring	Eurheic	8,27	11,09	126,9	20,8	463,1	473,2	308,1
T3SU	T3	Talamanca	Summer	Arheic	7,96	8,01	101	26,8	743	719	468
T3A	T3	Talamanca	Autumn	Oligorheic	8,19	13,52	101,2	3,3	324,4	558,8	362,7
T4W	T4	Talamanca	Winter	Eurheic	8,6	6,86	50,2	2,6	347,7	607,6	395,2
T4SP	T4	Talamanca	Spring	Oligorheic	7,2	6,3	61,6	13,5	408,6	523,1	338,65
T4SU	T4	Talamanca	Summer	Arheic	8	7,69	97,6	27,3	550	528	344
T4A	T4	Talamanca	Autumn	Oligorheic	7,65	7,79	62,5	6,2	358,9	561,5	364

Table 4. List of taxa and their abundances during the winter season. The genus* includes subfamily and tribe levels within the Chironomidae family. Site codes correspond to specific streams, as detailed in Tables 2 and 3.

Taxonomic classification			Winter Sites																												
Order/Group	Family	Genus	CA1	CA2	CA3	CA4	H1	H2	H3	H4	H5	MU1	MU2	MU3	MU4	R1	R2	R3	R4	SA1	SA2	SA3	SA4	SA5	SC1	SC2	SC3	SC4	T1	T2	T3	T4
Acari	Hydracarina		6	3	13	7	7	7	3	3	-	5	5	4	8	16	3	2	2	13	12	1	-	-	-	-	1	5	82	2	4	-
Bivalvia	Sphaeriidae	Sphaerium	1	1	-	-	18	3	1	-	-	-	-	-	-	-	1	-	3	3	14	1	-	-	-	-	-	1	5	1	-	-
Coleoptera	Dryopidae	Dryops	-	-	2	1	-	-	1	1	-	-	-	-	1	4	3	6	1	1	-	-	-	-	-	-	5	-	-	1	1	-
	Dytiscidae	Agabus	-	-	1	2	3	-	7	12	-	7	-	-	3	27	18	7	2	1	6	1	-	9	12	13	14	22	1	5	16	2
		Deronectes	-	-	-	-	-	1	8	37	2	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Graptodytes	-	-	-	-	-	-	-	3	-	4	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Hydroglyphus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
		Hydroporus	-	-	-	-	-	-	-	-	-	-	-	-	-	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Laccophilus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-
		Meladema	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
		Stictonectes	-	-	-	2	4	1	3	-	6	15	1	-	1	-	1	-	2	1	-	-	-	3	-	-	5	22	-	-	-	-
		Yola	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Elmidae	Oulimnius	-	-	-	-	1	7	1	-	-	-	1	-	-	-	-	-	-	8	26	-	-	-	-	-	-	-	1	-	-	-
		Riolus	-	-	-	2	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	2	-	1	-	-	-	-	-	-	-	-
	Gyrinidae	Gyrinus	-	-	-	-	1	1	1	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	15	-	-	7	-	-	-	-
	Haliplidae	Haliplus	-	-	-	-	-	-	-	-	-	29	-	-	2	13	3	-	-	2	5	-	-	2	-	-	-	1	-	12	-	-
		Peltodytes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-
	Hydraenidae	Hydraena	-	1	1	2	-	-	-	4	-	1	-	-	2	-	-	1	-	-	-	1	4	-	-	-	-	-	-	-	-	-
		Limnebius	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Ochthebius	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
	Hydrophilidae	Anacaena	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	1	-	-	2	-	-	-	-	-	-	-	-
		Berosus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	-	-	1	-	-	-	-
		Helochares	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Laccobius	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	2	1	-	1	7	-	-
	Scirtidae	Cyphon	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Elodes	1	4	3	28	1	2	6	12	4	-	-	-	-	2	1	2	-	-	1	7	-	-	-	-	-	5	-	-	-
		Hydrocyphon	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Crustacea	Cambaridae	Procambarus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	3	1
	Gammaridae	Echinogammarus	-	-	-	-	-	4	36	11	616	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diptera	Athericidae	Atherix	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Atrichops	-	-	-	-	1	3	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Ceratopogonidae	Atrichopogon	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	2	-	-	-	-
		Bezzia	68	44	48	62	16	68	20	2	1	3	5	1	2	17	10	6	44	6	-	-	52	4	96	-	32	128	52	44	32	6
		Brachypogon	-	-	-	-	8	-	4	-	-	1	-	-	-	-	-	-	2	-	11	-	-	-	-	-	-	-	1	-	-	-
		Culicoides	-	-	8	-	28	12	1	-	4	-	-	-	-	-	-	-	4	-	1	-	-	-	29	-	-	24	1	20	-	14
		Dasyhelea	12	6	-	3	16	4	4	-	-	-	-	-	-	-	3	5	16	4	28	-	152	63	144	1	254	19	16	84	84	6
		Forcypomyia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	1	-	1
		Nilobezzia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	43	-	-	-	-

Table 4. Continued on next page.

Table 4. Continued from previous page.

Taxonomic classification			Winter Sites																													
Order/Group	**Family**	**Genus***	**CA1**	**CA2**	**CA3**	**CA4**	**H1**	**H2**	**H3**	**H4**	**H5**	**MU1**	**MU2**	**MU3**	**MU4**	**R1**	**R2**	**R3**	**R4**	**SA1**	**SA2**	**SA3**	**SA4**	**SA5**	**SC1**	**SC2**	**SC3**	**SC4**	**T1**	**T2**	**T3**	**T4**
Diptera	Chironomidae	Chironomini*	24	24	28	-	48	-	-	-	24	13	-	-	-	-	-	-	20	-	-	-	-	-	334	1	18	27	-	64	-	56
		Macropelopini*	-	-	-	-	4	8	1	-	-	-	-	-	-	-	-	-	48	2	4	220	-	4	130	-	1	6	-	24	-	12
		Orthocladiinae*	528	224	272	141	56	116	360	540	96	68	132	408	1096	640	592	18	56	76	38	80	82	46	134	151	259	240	788	88	288	144
		Pentaneurini*	48	120	60	69	128	56	168	24	22	32	52	12	24	96	320	39	276	10	28	16	28	64	111	25	88	57	28	76	6	12
		Prodiamesa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
		Tanytarsini*	88	88	48	134	64	284	192	60	4	4	-	8	16	16	64	8	92	6	62	32	62	9	798	17	50	415	52	76	4	52
	Culicidae	Anopheles	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Dixidae	Dixa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Dixella	-	1	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
	Dolichopodidae	Tachytrechus	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	1	-	-	2	-	1	-	-	-	-
	Empididae	Chelifera	-	-	1	-	-	-	1	1	-	-	2	1	2	7	1	-	3	1	-	-	-	-	3	1	1	5	6	2	1	-
		Clinocera	-	-	-	-	1	-	-	9	-	-	-	3	4	11	-	-	1	-	-	-	-	-	-	-	-	5	-	-	1	-
		Hemerodromia	-	2	-	-	2	68	10	-	-	2	-	-	-	-	-	-	2	12	36	10	11	6	1	1	-	1	206	1	-	2
	Ephydridae	Hydrellia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	5	-	-	2	-	-
	Limoniidae	Chionea	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Dicranota	3	-	-	3	-	-	13	9	-	2	2	5	2	18	2	-	2	-	-	1	-	-	-	2	3	-	-	2	5	-
		Helius	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
		Molophilus	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
		Pilaria	1	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-
		Pseudolimnophila	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Muscidae	Limnophora	-	-	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Lispe	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
	Psychodidae	Paramormia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-
		Pericoma	1	1	1	5	1	38	76	8	2	3	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
		Psychoda	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Satchelliella	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
		Tonnoiriella	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
		Ulomyia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
	Ptychopteridae	Ptychoptera	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Rhagionidae	Chrysopilus	-	-	-	-	-	1	-	-	-	1	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
	Sciomyzidae		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
	Simuliidae	Simulium	2	1	1	8	22	56	464	456	-	3	6	3	2	1136	4	8	5	7	47	76	12	8	1	1	37	48	16	30	6	28
	Stratiomyidae	Nemotelus	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Oxycera	2	2	2	4	2	3	11	6	4	17	3	4	14	3	6	6	4	-	6	5	9	-	3	2	3	1	7	12	4	-
	Syrphidae		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
	Tabanidae	Chrysops	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-
		Tabanus	1	2	2	-	-	-	-	1	-	-	1	-	3	1	-	-	-	-	1	-	1	-	-	2	1	1	2	-	1	1
	Tipulidae	Tipula	-	-	-	-	-	3	-	-	-	1	-	4	-	-	-	-	3	1	-	1	-	-	5	3	18	2	9	3	4	2

Table 4. Continued on next page.

Table 4. Continued from previous page.

Taxonomic classification			Winter Sites																													
Order/Group	**Family**	**Genus**	**CA1**	**CA2**	**CA3**	**CA4**	**H1**	**H2**	**H3**	**H4**	**H5**	**MU1**	**MU2**	**MU3**	**MU4**	**R1**	**R2**	**R3**	**R4**	**SA1**	**SA2**	**SA3**	**SA4**	**SA5**	**SC1**	**SC2**	**SC3**	**SC4**	**T1**	**T2**	**T3**	**T4**
Ephemeroptera	Baetidae	Baetis	180	73	32	220	100	72	692	268	1	78	23	33	11	1538	52	16	17	35	90	26	28	62	9	21	64	71	24	20	-	4
		Centroptilum	18	26	148	55	-	-	-	-	-	-	-	-	-	-	-	1	1	14	7	-	-	17	-	-	-	-	-	-	-	-
		Cloeon	-	-	-	-	56	-	57	-	-	10	-	-	-	-	36	14	12	5	8	-	-	11	350	39	75	176	-	-	12	12
		Procloeon	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	5	3	-	-	-	-	-
	Caenidae	Caenis	28	8	26	63	14	16	46	9	-	12	9	4	11	25	144	25	168	36	66	5	9	102	202	134	168	256	168	136	52	50
	Ephemerellidae	Serratella	-	-	-	-	-	1	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Heptageniidae	Electrogena	-	-	-	-	-	1	3	5	34	-	-	1	-	-	-	-	-	-	-	7	2	30	-	-	-	-	-	-	-	-
	Leptophlebiidae	Habroleptoides	3	5	18	34	-	-	300	238	-	24	2	4	6	-	-	3	-	-	12	2	-	16	-	-	-	-	-	-	-	-
		Habrophlebia	-	-	-	42	-	144	271	102	18	200	96	41	32	2	24	-	12	-	-	-	-	-	4	-	6	137	6	-	12	6
		Thraulus	-	-	-	-	156	-	37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Heteroptera	Corixidae	Micronecta	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	7	-	-	-	-	2	-	1
		Parasigara	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	5	1	-	-	1	-	1	-	-
	Hydrometridae	Hydrometra	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
	Notonectidae	Notonecta	-	-	-	-	1	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	2	2	2	-	1	5	-	1	4	-
	Pleidae	Plea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
	Veliidae	Velia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Hirudinoidea	Erpobdellidae	Erpobdella	-	-	-	-	-	2	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
	Glossiphoniidae	Helobdella	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Megaloptera	Sialidae	Sialis	-	-	-	-	3	4	-	-	-	-	-	-	-	-	-	-	-	1	5	-	-	-	-	-	-	-	-	1	-	-
Mollusca	Ancylidae	Ancylus	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-
	Hydrobiidae	Bythinella	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-
		Potamopyrgus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	37	-	-	-	-	-	-	-	-	-	-	-
	Lymnaeidae	Galba	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-
		Lymnaea	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1	-	-	-	-	-	-	-	-	-	3	1	5	14	-	-
		Radix	-	-	-	-	-	2	1	-	-	-	1	-	-	-	-	-	-	2	35	-	-	-	-	1	11	4	-	-	-	-
		Stagnicola	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Physidae	Physella	1	-	-	-	6	3	3	-	-	-	-	1	-	2	5	-	10	7	34	4	7	11	24	3	30	9	2	6	5	6
	Planorbidae	Anisus	-	-	-	-	1	-	1	-	-	-	-	-	-	-	1	-	-	3	-	-	-	-	-	-	1	-	-	-	1	-
Odonata	Aeschnidae	Aeshna	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	2	-	-	1	-	-	-	-
		Anax	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	1	-	-	-	-	-	-	-	-	-
	Calopterygidae	Calopteryx	-	-	-	-	3	-	1	-	-	-	-	-	-	-	-	-	-	2	3	2	1	9	-	-	-	-	-	-	-	-
	Coenagrionidae	Ceriagrion	-	-	-	-	3	6	-	-	-	-	-	-	-	-	-	-	-	-	11	-	2	32	-	-	2	15	-	-	-	-
		Coenagrion	-	-	-	-	4	-	-	-	-	-	-	-	-	-	2	2	-	2	20	-	-	-	28	-	3	10	-	-	-	-
		Pyrrhosoma	1	-	2	-	7	3	6	-	-	-	-	1	-	-	2	-	3	7	12	4	4	30	-	3	1	20	-	-	-	-
	Cordulegasteridae	Cordulegaster	-	-	1	-	-	1	-	-	1	1	-	-	-	1	-	-	1	-	5	2	-	-	-	-	-	-	-	-	-	-
	Gomphidae	Onychogomphus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	4	7	-
	Libellulidae	Crocothemis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	-	2	43	-	-	-	-
		Orthetrum	2	1	2	-	1	1	2	-	-	-	-	-	-	-	2	2	4	12	30	3	1	21	21	5	14	50	5	8	-	5

Table 4. Continued on next page.

Table 4. Continued from previous page.

Taxonomic classification			Winter Sites																													
Order/Group	**Family**	**Genus**	**CA1**	**CA2**	**CA3**	**CA4**	**H1**	**H2**	**H3**	**H4**	**H5**	**MU1**	**MU2**	**MU3**	**MU4**	**R1**	**R2**	**R3**	**R4**	**SA1**	**SA2**	**SA3**	**SA4**	**SA5**	**SC1**	**SC2**	**SC3**	**SC4**	**T1**	**T2**	**T3**	**T4**
Oligochaeta			3	4	3	10	1	6	9	6	-	-	3	4	4	4	-	6	72	5	-	-	-	4	120	-	-	-	20	9	12	12
Plecoptera	Chloroperlidae	Siphonoperla	-	-	-	-	-	-	7	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Leuctridae	Tyrrhenoleuctra	-	-	-	-	-	-	-	-	-	-	-	-	-	19	-	-	-	-	-	-	-	-	9	45	1	4	-	-	-	-
	Nemouridae	Nemoura	46	68	89	465	5	180	541	364	-	120	3	-	9	722	232	36	70	163	153	402	208	111	4	9	42	85	180	232	184	144
	Perlodidae	Isoperla	-	-	-	-	1	6	13	-	-	35	-	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trichoptera	Beraeidae	Beraea	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	3	-	-	-
	Hydropsychidae	Hydropsyche	3	2	2	4	1	66	28	-	-	5	2	-	1	3	-	-	-	1	31	44	38	6	-	3	2	1	30	-	-	-
	Hydroptilidae	Hydroptila	2	-	-	1	-	-	14	2	-	1	5	-	2	4	1	-	2	3	30	-	1	3	-	-	6	8	38	-	-	-
		Oxyethira	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	7	-	-	-	-	-	-	-	-	-	-	-
		Stactobia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	30	-	-	-
	Leptoceridae	Adicella	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Mystacides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	11	-	-	-	-	-	-	-	2	2	-	2
	Limnephilidae		-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	3	5	5
		Limnephilus	-	-	-	-	11	7	5	1	1	-	-	-	-	6	4	-	-	5	21	-	-	-	15	10	100	117	3	-	-	-
		Mesophylax	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	15	2	-	4	-	-
	Philopotamidae	Wormaldia	-	7	16	129	-	60	68	2	-	1	-	-	-	2	-	-	-	-	-	15	4	-	-	-	-	-	-	-	-	-
	Polycentropodidae	Plectrocnemia	-	-	-	-	2	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-
		Polycentropus	-	-	-	-	-	3	28	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-
	Psychomyiidae	Tinodes	2	6	30	4	-	-	2	-	-	-	-	-	-	-	1	2	11	1	4	-	3	2	-	1	6	2	12	-	7	-

Table 5. List of taxa and their abundances during the spring season. The genus* includes subfamily and tribe levels within the Chironomidae family. Site codes correspond to specific streams, as detailed in Tables 2 and 3.

Taxonomic classification			Spring Sites																								
Order/Group	Family	Genus	CA1	CA2	CA3	CA4	H1	H2	H5	MU1	MU2	MU3	MU4	R4	SA1	SA2	SA3	SA4	SA5	SC1	SC2	SC3	SC4	T1	T2	T3	T4
Acari	Hydracarina		21	7	4	2	16	38	-	1	6	2	12	4	12	64	11	12	21	-	-	-	4	-	2	128	-
Bivalvia	Sphaeriidae	Sphaerium	6	6	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Coleoptera	Dryopidae	Dryops	-	-	-	-	1	-	-	1	-	1	-	-	3	-	-	4	-	2	-	1	3	-	1	-	2
	Dytiscidae	Agabus	3	4	4	4	2	15	11	4	39	20	10	-	5	12	-	-	3	-	1	-	2	2	1	-	4
		Bidessus	-	1	-	-	-	-	-	1	-	-	-	2	-	-	-	1	1	4	6	2	-	-	-	12	2
		Deronectes	-	-	-	1	1	3	11	1	-	-	-	-	7	-	-	1	1	-	-	-	-	-	-	-	-
		Dytiscus	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
		Graptodytes	-	1	-	1	-	2	1	2	-	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	1
		Hydroporus	-	-	-	-	-	-	2	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
		Laccophilus	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
		Stictonectes	1	20	-	39	84	117	66	200	2	-	-	-	1	11	1	2	53	4	-	-	47	-	-	-	1
	Elmidae	Oulimnius	-	-	-	-	14	19	-	-	-	-	-	-	22	-	2	-	-	-	-	-	-	1	3	1	-
		Riolus	-	-	-	1	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-
	Gyrinidae	Gyrinus	-	-	-	-	2	1	-	-	-	-	-	-	2	5	-	1	-	7	-	-	7	-	-	2	1
	Haliplidae	Haliplus	-	-	-	-	5	14	2	3	-	1	12	1	5	30	2	3	-	-	-	-	1	-	-	-	-
		Peltodytes	-	-	-	-	-	-	-	-	-	-	-	-	2	9	-	-	4	-	-	-	-	-	-	-	-
	Helophoridae	Helophorus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
	Hydraenidae	Hydraena	-	1	1	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-
		Limnebius	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
		Ochthebius	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
	Hydrophilidae	Anacaena	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
		Berosus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-
		Helochares	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-
		Hydrophilus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
		Laccobius	8	8	7	3	1	3	5	44	11	30	28	4	4	1	1	11	7	27	1	-	14	9	88	37	61
		Paracymus	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	Noteridae	Noterus	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
	Scirtidae	Cyphon	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-
		Elodes	-	-	6	-	-	3	3	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Crustacea	Cambaridae	Procambarus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	Gammaridae	Echinogammarus	-	-	-	1	1	-	364	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diptera	Athericidae	Atrichops	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Ceratopogonidae	Atrichopogon	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Bezzia	87	47	11	36	50	160	2	1	3	3	8	8	5	37	12	19	11	22	7	1	3	38	28	5	18
		Brachypogon	-	1	-	-	-	77	-	-	-	2	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-
		Culicoides	1	2	1	-	-	8	-	-	-	2	-	-	-	1	-	-	-	-	-	1	14	-	2	2	2

Table 5. Continued on next page.

Table 5. Continued from previous page.

Taxonomic classification			Spring Sites																							
Order/Group	**Family**	**Genus***	**CA1**	**CA2**	**CA3**	**CA4**	**H1**	**H2**	**H5**	**MU1**	**MU2**	**MU3**	**MU4**	**R4**	**SA1**	**SA2**	**SA3**	**SA4**	**SA5**	**SC1**	**SC2**	**SC3**	**SC4**	**T1**	**T2**	**T3**	**T4**
Diptera	Ceratopogonidae	Dasyhelea	6	2	1	3	-	-	-	-	-	-	-	-	3	13	3	23	29	10	-	9	-	2	10	8	12
		Forcypomyia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
		Nilobezzia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
	Chironomidae	Chironomini	1392	672	176	39	968	864	112	22	96	192	119	77	-	104	212	136	64	7	-	6	134	296	155	435	20
		Macropelopini	96	32	16	3	352	32	144	5	32	16	31	2	-	32	64	16	16	7	-	24	27	80	91	51	2
		Orthocladiinae	576	800	1000	55	304	1456	1600	29	2070	2880	433	16	456	160	364	312	368	13	19	19	166	152	26	729	50
		Pentaneurini	336	400	152	41	200	432	800	23	43	32	113	85	184	208	92	184	256	-	1	22	80	104	214	230	58
		Prodiamesa	-	16	-	1	16	80	16	-	21	16	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Tanytarsini	5856	1792	640	4	224	880	608	14	555	592	452	12	136	200	60	240	240	5	-	11	38	560	528	1088	68
	Culicidae	Anopheles	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
		Culex	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	3	8	1	-	2	68	1	-	1	13
	Dixidae	Dixa	-	1	2	1	-	-	3	-	4	2	1	-	-	-	-	-	-	-	-	-	-	1	2	-	-
		Dixella	2	4	4	-	2	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	19	-	1	1	-
	Dolichopodidae	Tachytrechus	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11	1	-	-	-	-	-	-
	Empididae	Clinocera	-	-	-	1	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Hemerodromia	-	1	2	1	14	16	-	-	-	-	-	-	-	3	17	3	-	-	-	-	-	2	3	-	-
	Limoniidae	Dicranota	-	-	8	-	1	14	6	-	1	-	1	-	1	-	-	-	-	-	-	-	-	1	-	-	-
		Helius	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
		Hexatoma	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
		Molophilus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
	Muscidae	Limnophora	1	2	4	-	-	-	1	-	3	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Lispe	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-
	Psychodidae	Berdeniella	-	-	-	-	-	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Pericoma	-	-	1	-	1	5	5	-	2	2	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
		Psychoda	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
		Tonnoiriella	-	-	-	-	-	1	2	1	3	5	-	-	5	-	-	-	-	-	-	-	-	1	1	2	-
	Rhagionidae	Chrysopilus	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Simuliidae	Simulium	1	3	96	3	2	118	254	1	661	672	27	-	4	-	14	90	1	-	-	-	1	4	-	87	-
	Stratiomyidae	Oxycera	-	2	3	-	4	2	5	9	1	5	4	-	-	1	2	22	12	4	-	-	-	7	2	14	1
		Stratiomys	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-
	Tabanidae	Chrysops	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Tipulidae	Tipula	2	1	-	-	-	1	-	-	-	2	2	-	2	-	-	1	-	6	-	1	3	1	1	-	1
Ephemeroptera	Baetidae	Baetis	-	74	173	2	4	1280	144	2	276	666	35	-	34	5	28	42	18	-	-	-	4	38	-	162	-
		Centroptilum	3	14	11	-	-	-	-	-	-	-	-	-	11	-	-	-	-	-	-	-	-	-	-	-	-
		Cloeon	-	-	-	-	15	-	-	-	-	-	-	-	-	21	-	12	7	-	2	114	35	-	-	-	-
		Procloeon	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	27	76	36	7

Table 5. Continued on next page.

Table 5. Continued from previous page.

Taxonomic classification			Spring Sites																								
Order/Group	**Family**	**Genus**	**CA1**	**CA2**	**CA3**	**CA4**	**H1**	**H2**	**H5**	**MU1**	**MU2**	**MU3**	**MU4**	**R4**	**SA1**	**SA2**	**SA3**	**SA4**	**SA5**	**SC1**	**SC2**	**SC3**	**SC4**	**T1**	**T2**	**T3**	**T4**
	Caenidae	Caenis	58	2	25	5	56	46	-	1	1	-	2	6	16	32	54	19	30	-	4	106	17	32	90	54	30
	Ephemerellidae	Serratella	-	-	-	-	164	296	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Heptageniidae	Electrogena	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Leptophlebiidae	Habrophlebia	107	174	368	60	560	613	72	60	47	22	25	1	112	136	133	36	22	-	-	-	26	15	17	57	409
		Thraulus	-	-	-	-	224	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13
Heteroptera	Corixidae	Corixidae	-	-	-	-	74	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-
		Micronecta	10	-	-	-	-	-	-	4	-	-	-	1	15	-	1	-	10	1	-	-	-	-	1	-	-
		Parasigara	-	-	-	-	-	-	-	-	-	-	-	-	3	49	-	-	-	-	-	-	-	-	-	-	-
	Gerridae	Aquarius	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	12	-	-
		Gerris	-	2	-	2	-	-	-	-	-	-	4	-	10	3	-	16	6	3	-	-	10	55	11	1	2
	Hydrometridae	Hydrometra	-	-	-	-	-	-	-	1	2	2	5	-	1	1	-	6	2	3	-	-	1	7	1	-	-
	Naucoridae	Naucoris	-	-	-	-	-	-	-	-	-	-	-	-	-	6	-	1	-	-	-	-	-	-	-	-	-
	Nepidae	Nepa	-	1	-	1	1	-	-	-	2	-	3	-	-	-	-	-	1	-	1	2	-	-	-	-	-
	Notonectidae	Notonecta	3	4	11	7	2	8	1	1	1	1	1	11	19	23	4	16	94	4	-	8	25	3	10	3	13
	Pleidae	Plea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
	Veliidae	Microvelia	-	-	-	-	-	-	-	-	-	-	-	-	-	2	3	4	-	-	2	-	-	-	-	-	-
		Velia	-	2	4	8	-	5	-	7	7	3	2	-	2	-	-	-	-	-	-	-	-	4	-	3	-
Hirudinoidea	Erpobdellidae	Erpobdella	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Glossiphoniidae	Glossiphonia	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Megaloptera	Sialidae	Sialis	-	-	-	-	6	3	-	-	-	-	-	-	-	13	-	1	-	-	-	-	-	-	-	-	-
Mollusca	Ancylidae	Ancylus	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Hydrobiidae	Potamopyrgus	-	-	-	-	-	-	-	-	-	-	-	-	8	1	-	-	-	-	-	-	-	-	-	-	-
	Lymnaeidae	Lymnaea	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	2	8	-	-	-	2	3	-	6
		Radix	5	10	-	-	4	2	-	2	7	5	8	3	16	164	2	2	-	5	2	6	2	1	1	1	2
	Physidae	Physella	1	-	-	-	1	2	-	-	-	-	-	1	11	28	3	3	2	30	3	4	2	3	6	34	9
	Planorbidae	Anisus	-	-	-	-	-	-	1	-	-	2	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Odonata	Aeschnidae	Aeshna	-	-	8	3	6	1	19	-	-	-	-	-	10	-	-	-	-	-	-	-	6	-	-	-	2
		Anax	-	-	-	-	-	-	-	-	-	-	-	-	4	5	5	-	6	-	-	-	-	-	1	-	-
		Boyeria	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-
	Calopterygidae	Calopteryx	-	-	-	-	1	-	-	-	-	-	-	-	3	2	4	3	6	-	-	-	-	-	-	-	-
	Coenagrionidae	Ceriagrion	-	-	-	-	-	-	-	-	-	-	-	-	2	35	9	13	3	-	-	-	-	-	-	-	-
		Coenagrion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
		Ischnura	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
		Pyrrhosoma	-	-	2	-	4	-	-	-	-	-	-	-	9	17	8	9	11	1	-	-	4	-	-	-	-
	Cordulegasteridae	Cordulegaster	-	-	2	-	-	2	-	-	1	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-

Table 5. Continued on next page.

Table 5. Continued from previous page.

Taxonomic classification			Spring Sites																								
Order/Group	**Family**	**Genus**	**CA1**	**CA2**	**CA3**	**CA4**	**H1**	**H2**	**H5**	**MU1**	**MU2**	**MU3**	**MU4**	**R4**	**SA1**	**SA2**	**SA3**	**SA4**	**SA5**	**SC1**	**SC2**	**SC3**	**SC4**	**T1**	**T2**	**T3**	**T4**
	Gomphidae	Gomphus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
		Onychogomphus	5	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	4	2	6
	Lestidae	Chalcolestes	2	122	55	7	92	68	64	3	1	1	-	1	22	51	22	10	26	-	-	2	33	1	2	3	96
	Libellulidae	Crocothemis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
		Orthetrum	-	-	-	-	-	-	-	-	-	-	-	2	-	1	1	4	11	-	-	-	-	-	-	1	-
		Sympetrum	10	2	7	1	-	-	-	3	-	-	1	4	4	6	-	4	-	-	-	23	-	1	8	4	9
Oligochaeta			-	-	-	-	-	1	-	1	-	81	14	-	-	4	-	3	-	696	-	-	-	-	-	1	-
Plecoptera	Leuctridae	Leuctra	-	-	-	-	-	-	-	-	-	-	-	-	5	-	4	6	3	-	-	-	-	-	-	-	-
	Nemouridae	Nemoura	-	4	13	5	-	-	-	-	-	-	3	-	20	3	1	5	1	-	-	-	-	-	-	-	-
	Perlodidae	Isoperla	-	-	-	-	7	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trichoptera	Beraeidae	Beraea	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
	Hydropsychidae	Hydropsyche	-	1	1	-	1	16	-	-	-	-	1	-	-	-	-	6	1	-	-	-	-	-	-	-	-
	Hydroptilidae	Hydroptila	-	2	-	-	-	8	4	3	56	94	22	-	13	-	2	3	5	-	-	-	-	5	-	6	-
		Oxyethira	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-
		Stactobia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	-	-	-
	Leptoceridae	Mystacides	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
		Setodes	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-
	Limnephilidae	Halesus	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Limnephilus	-	-	1	-	6	1	-	-	-	-	-	-	5	3	-	-	1	-	-	-	-	-	-	-	-
	Philopotamidae	Philopotamus	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
		Wormaldia	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Polycentropodidae	Plectrocnemia	-	-	-	-	4	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
		Polycentropus	-	-	-	-	5	4	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Psychomyiidae	Tinodes	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2	-	-	-	-	6	4	2	-
Tricladida	Dugesiidae	Dugesia	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 6. List of taxa and their abundances during the summer season. The genus* includes subfamily and tribe levels within the Chironomidae family. Site codes correspond to specific streams, as detailed in Tables 2 and 3.

Taxonomic classification			Summer Sites																				
Order/Group	**Family**	**Genus**	**CA1**	**CA2**	**CA3**	**H1**	**H2**	**H3**	**H5**	**MU2**	**MU3**	**MU4**	**R4**	**SA1**	**SA2**	**SA3**	**SA4**	**SA5**	**SC3**	**SC4**	**T1**	**T3**	**T4**
Acari	Hydracarina		10	11	12	30	15	57	1	21	5	28	-	16	15	14	-	24	2	3	14	1	-
Bivalvia	Sphaeriidae	Sphaerium	2	1	3	-	2	1	-	-	-	-	-	-	3	1	-	5	-	-	3	-	-
Coleoptera	Dryopidae	Dryops	-	2	2	3	-	-	-	1	8	4	-	-	-	1	-	5	2	2	6	1	-
	Dytiscidae	Agabus	-	1	1	16	1	3	-	4	8	11	-	2	-	-	-	-	-	-	2	-	-
		Bidessus	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	1	-	-	-	-	-
		Deronectes	-	-	1	1	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Dytiscus	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Graptodytes	-	-	-	-	1	5	3	-	-	1	-	-	-	-	-	-	-	-	4	-	-
		Hydroglyphus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
		Ilybius	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
		Laccophilus	-	-	-	-	-	-	-	-	-	-	-	-	6	6	-	3	-	-	-	-	-
		Stictonectes	-	-	3	20	8	8	20	1	6	18	-	3	-	-	1	6	1	3	-	-	-
		Yola	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	10	-	1
	Elmidae	Elmis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-
		Oulimnius	-	-	-	165	3	4	-	-	-	-	-	4	11	4	-	-	-	-	8	-	-
		Riolus	-	1	1	-	-	-	-	-	1	-	-	-	2	-	-	-	-	-	-	-	-
	Gyrinidae	Gyrinus	-	-	-	-	1	6	-	2	-	-	-	5	-	-	-	-	-	3	-	-	-
	Haliplidae	Haliplus	-	-	-	2	3	17	-	-	1	8	-	2	15	-	-	1	-	1	3	-	-
		Peltodytes	-	-	-	-	-	-	-	-	-	3	-	1	41	2	-	-	-	-	-	-	-
	Hydraenidae	Hydraena	-	-	1	16	-	13	2	4	-	3	2	-	6	2	-	-	-	1	6	-	-
		Limnebius	-	-	-	-	-	1	2	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Hydrophilidae	Anacaena	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	5	-	-
		Berosus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-
		Helochares	1	-	-	-	-	-	-	-	1	3	-	-	-	-	-	-	2	1	-	-	-
		Hydrophilus	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-
		Laccobius	2	-	5	-	2	-	2	-	3	10	-	-	3	-	-	2	5	-	22	-	-
	Scirtidae	Cyphon	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-
		Elodes	-	-	1	6	1	20	1	-	-	-	-	-	3	1	-	-	-	-	2	-	-
Crustacea	Gammaridae	Echinogammarus	-	-	-	1	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 6. Continued on next page.

Table 6. Continued from previous page.

Taxonomic classification			Summer Sites																			
Order/Group	**Family**	**Genus***	**CA1**	**CA2**	**CA3**	**H1**	**H2**	**H3**	**H5**	**MU2**	**MU3**	**MU4**	**R4**	**SA1**	**SA2**	**SA3**	**SA4**	**SA5**	**SC3**	**SC4**	**T1**	**T3**	**T4**
Diptera	Athericidae	Atherix	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Ceratopogonidae	Atrichopogon	1	2	-	1	1	-	-	-	-	1	-	-	-	-	-	-	-	-	4	-	-
		Bezzia	54	9	24	22	1	7	9	2	1	72	1	2	32	48	34	56	46	124	162	21	85
		Brachypogon	1	-	-	6	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-
		Culicoides	42	-	18	3	-	-	-	-	-	8	-	-	-	-	-	-	10	5	-	-	-
		Dasyhelea	6	7	20	143	1	-	35	-	-	8	93	3	12	44	236	156	52	1144	255	62	750
		Forcypomyia	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
	Chaoboridae	Chaoborus	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
	Chironomidae	Chironomini	68	252	304	848	652	440	28	215	62	704	159	13	8	57	8	24	5	56	144	3	-
		Macropelopini	24	80	120	112	44	15	4	36	3	16	11	40	49	46	38	24	80	166	123	8	413
		Orthocladiinae	40	148	264	1312	84	1315	52	144	2	496	16	5	143	103	52	4	1	8	49	9	445
		Pentaneurini	110	252	640	416	148	491	252	96	61	784	9	82	147	172	92	80	18	168	319	4	639
		Tanytarsini	74	104	464	944	8	170	122	59	-	304	-	30	30	81	82	52	28	92	391	5	2257
	Culicidae	Aedes	-	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
		Anopheles	-	-	-	14	1	2	-	-	-	-	-	-	1	-	-	-	-	-	2	-	-
		Culex	3	7	2	38	13	47	1	1	2	31	2	6	-	-	1	1	-	-	18	1	-
	Dixidae	Dixa	-	-	8	6	-	-	-	-	-	-	-	4	2	-	-	-	-	-	-	-	-
		Dixella	-	12	-	44	9	15	-	-	1	3	-	-	2	2	-	-	-	1	2	-	-
	Dolichopodidae	Tachytrechus	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	1	-	-
	Empididae	Chelifera	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
		Hemerodromia	1	3	3	60	-	-	-	-	-	-	-	-	-	24	-	1	-	-	-	-	-
	Ephydridae	Ephydra	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
	Limoniidae	Limoniidae	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-
		Molophilus	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Pilaria	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Muscidae	Limnophora	-	-	-	4	-	27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Lispe	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
	Psychodidae	Pericoma	-	-	2	25	3	123	-	-	-	1	-	-	-	2	-	-	-	-	1	-	-
		Peripsychoda	-	-	8	-	-	-	-	-	-	64	-	-	-	-	-	-	-	-	-	-	-
		Psychoda	-	-	10	8	-	96	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-
		Satchelliella	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Tonnoiriella	-	-	4	33	2	55	-	3	2	-	-	-	1	-	-	-	-	-	1	-	-

Table 6. Continued on next page.

Table 6. Continued from previous page.

Taxonomic classification			Summer Sites																			
Order/Group	**Family**	**Genus**	**CA1**	**CA2**	**CA3**	**H1**	**H2**	**H3**	**H5**	**MU2**	**MU3**	**MU4**	**R4**	**SA1**	**SA2**	**SA3**	**SA4**	**SA5**	**SC3**	**SC4**	**T1**	**T3**	**T4**
Diptera	Ptychopteridae	Ptychoptera	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
	Rhagionidae	Chrysopilus	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Simuliidae	Simulium	1	-	14	67	2	196	-	-	3	-	-	-	4	10	3	-	-	-	2	-	-
	Stratiomyidae	Oxycera	4	-	-	28	2	16	6	4	3	48	1	2	-	-	11	12	7	2	168	1	1
		Stratiomys	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-
	Tabanidae	Chrysops	-	2	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Tabanus	1	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	5	1	-	-
	Tipulidae	Prionocera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
		Tipula	-	-	1	1	1	-	-	1	1	-	-	-	-	-	-	-	-	-	1	-	-
Ephemeroptera	Baetidae	Baetis	14	3	7	10	17	84	-	-	26	-	-	16	43	32	-	-	-	-	48	-	-
		Centroptilum	-	12	356	-	-	-	-	-	-	-	-	127	-	-	-	-	-	-	-	-	-
		Cloeon	-	3	32	94	40	260	62	-	45	147	9	133	106	14	478	58	31	150	111	45	60
		Procloeon	1	-	-	-	-	-	-	-	-	-	-	1	-	-	584	-	-	-	100	35	146
	Caenidae	Caenis	225	76	1424	25	3	3	41	3	48	2416	34	10	98	10	312	46	58	326	206	444	1246
	Ephemerellidae	Serratella	-	-	-	64	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Leptophlebiidae	Habrophlebia	-	3	24	73	55	54	-	2	12	4	-	7	13	3	-	1	-	-	-	-	-
		Thraulus	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Heteroptera	Corixidae	Corixidae	-	-	-	2	19	1	-	-	-	-	-	12	1	1	-	-	-	-	-	-	-
		Micronecta	1	7	-	2	-	1	-	-	-	-	-	-	3	-	-	-	-	-	1	1	3
		Sigara	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Gerridae	Aquarius	-	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-
		Gerris	-	-	6	-	-	1	8	-	1	4	1	3	-	2	1	1	-	-	8	-	4
	Hydrometridae	Hydrometra	-	-	-	4	2	1	-	1	1	2	-	-	-	2	1	-	-	-	7	-	1
	Naucoridae	Naucoris	-	-	-	-	-	-	-	-	-	-	-	-	5	4	1	8	-	-	1	-	-
	Nepidae	Nepa	1	-	2	4	-	3	-	-	1	2	2	-	1	-	-	-	3	-	-	-	1
	Notonectidae	Notonecta	-	1	4	2	4	2	-	-	2	1	3	12	2	1	1	1	-	2	3	5	4
	Veliidae	Velia	-	-	1	2	-	-	-	2	-	1	-	-	-	1	-	-	-	-	-	-	-
			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-
Hirudinoidea	Erpobdellidae	Erpobdella	-	-	-	-	-	7	-	-	-	1	-	-	-	-	-	-	-	-	2	-	-
	Glossiphoniidae	Helobdella	-	-	5	1	1	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Megaloptera	Sialidae	Sialis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-

Table 6. Continued on next page.

Table 6. Continued from previous page.

Taxonomic classification			Summer Sites																			
Order/Group	**Family**	**Genus**	**CA1**	**CA2**	**CA3**	**H1**	**H2**	**H3**	**H5**	**MU2**	**MU3**	**MU4**	**R4**	**SA1**	**SA2**	**SA3**	**SA4**	**SA5**	**SC3**	**SC4**	**T1**	**T3**	**T4**
Mollusca	Ancylidae	Ancylus	-	-	-	-	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Hydrobiidae	Potamopyrgus	-	-	-	-	-	-	-	-	-	-	-	-	12	1	-	-	-	-	-	-	-
	Lymnaeidae	Galba	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-
		Lymnaea	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-
		Radix	-	-	4	54	19	68	1	32	25	84	-	1	36	13	-	4	-	8	11	-	-
	Physidae	Physella	6	-	3	12	3	72	-	1	-	2	-	11	12	8	-	38	-	2	34	2	2
Odonata	Aeschnidae	Aeshna	-	4	9	5	6	2	2	-	-	6	1	1	3	-	2	12	-	30	-	-	-
		Anax	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-
		Boyeria	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
	Calopterygidae	Calopteryx	-	-	-	5	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Coenagrionidae	Ceriagrion	-	-	-	22	-	53	-	-	-	-	1	-	14	-	19	60	-	42	6	-	-
		Coenagrion	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-
			1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Erythromma	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-
		Pyrrhosoma	-	17	44	-	29	-	-	-	3	9	-	11	6	45	4	64	2	82	-	-	-
	Cordulegasteridae	Cordulegaster	-	-	10	1	-	-	8	1	1	5	-	-	-	-	-	-	-	1	-	-	-
	Gomphidae	Onychogomphus	3	-	-	-	-	-	-	-	-	1	-	-	-	1	12	1	-	-	4	3	7
	Lestidae	Chalcolestes	-	2	2	32	39	11	4	-	9	8	-	8	4	2	-	3	-	2	-	-	-
	Libellulidae	Orthetrum	5	-	-	-	-	-	-	-	-	7	4	3	4	1	6	1	-	3	12	-	1
		Sympetrum	1	8	2	1	3	3	-	-	2	3	-	-	-	1	-	3	5	5	5	-	1
	Platycnemididae	Platycnemis	-	8	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-
Oligochaeta			72	-	-	-	-	9	-	6	-	-	-	16	1	8	-	-	15	-	134	-	-
Plecoptera	Leuctridae	Leuctra	-	-	-	-	1	-	-	-	-	-	-	3	-	1	-	1	-	-	-	-	-

Table 6. Continued on next page.

Table 6. Continued from previous page.

Taxonomic classification			Summer Sites																			
Order/Group	**Family**	**Genus**	**CA1**	**CA2**	**CA3**	**H1**	**H2**	**H3**	**H5**	**MU2**	**MU3**	**MU4**	**R4**	**SA1**	**SA2**	**SA3**	**SA4**	**SA5**	**SC3**	**SC4**	**T1**	**T3**	**T4**
Trichoptera	Beraeidae	Beraea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
	Hydropsychidae	Hydropsyche	-	-	12	50	-	45	-	-	-	4	-	-	32	48	1	9	-	-	25	-	-
	Hydroptilidae	Hydroptila	7	1	6	70	2	315	1	5	3	133	-	-	7	26	-	2	-	-	23	-	-
		Oxyethira	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	4	-	-
		Stactobia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	21	-	-
	Leptoceridae	Mystacides	-	2	16	3	-	1	-	-	-	4	-	-	-	7	-	-	-	-	3	-	-
	Limnephilidae	Limnephilus	1	-	-	3	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-
	Philopotamidae	Philopotamus	-	-	-	3	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
		Wormaldia	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Polycentropodidae	Plectrocnemia	-	2	-	15	-	15	-	-	-	-	-	-	-	-	1	1	-	-	3	-	-
		Polycentropus	-	-	2	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Psychomyiidae	Tinodes	1	3	1	-	6	1	-	-	-	4	-	-	6	18	2	2	-	2	33	-	-
Tricladida	Dugesiidae	Dugesia	16	9	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 7. List of taxa and their abundances during the autumn season. The genus* includes subfamily and tribe levels within the Chironomidae family. Site codes correspond to specific streams, as detailed in Tables 2 and 3.

Taxonomic classification			Autumn Sites																								
Order/Group	Family	Genus	CA1	CA2	CA3	CA4	H1	H2	H3	H5	MU2	MU3	MU4	R4	SA1	SA2	SA3	SA4	SA5	SC1	SC2	SC3	SC4	T1	T2	T3	T4
Acari	Hydracarina		25	3	2	4	19	10	44	3	1	4	-	1	-	2	-	1	-	-	-	-	-	-	-	-	1
Bivalvia	Sphaeriidae	Sphaerium	-	-	5	1	16	1	-	-	-	-	-	-	1	2	-	-	-	-	-	-	-	-	-	-	-
Coleoptera	Dryopidae	Dryops	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	1	-
	Dytiscidae	Agabus	-	-	-	1	2	3	5	3	-	2	2	-	15	2	-	-	2	-	-	-	-	1	-	-	-
		Deronectes	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Dytiscus	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Graptodytes	-	-	2	14	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Laccophilus	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Stictonectes	-	-	3	13	3	-	-	-	-	-	-	-	2	2	-	-	2	-	-	-	-	-	-	-	-
	Elmidae	Elmis	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
		Oulimnius	-	-	-	-	27	10	3	-	-	-	-	-	12	-	-	-	-	-	-	-	-	-	-	-	-
		Riolus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
	Gyrinidae	Gyrinus	-	-	-	-	10	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-	2	-	-	-	-
	Haliplidae	Haliplus	-	-	1	4	2	-	-	-	-	-	-	1	2	2	-	-	1	-	-	-	-	-	-	-	-
		Peltodytes	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
	Hydraenidae	Hydraena	1	-	-	2	-	-	-	-	1	-	-	-	1	-	-	-	1	-	-	-	-	2	-	-	-
		Limnebius	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
	Hydrophilidae	Anacaena	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Laccobius	1	-	-	-	-	-	-	-	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Paracymus	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Scirtidae	Cyphon	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-
		Elodes	1	2	1	-	1	2	4	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Crustacea	Cambaridae	Procambarus	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Gammaridae	Echinogammarus	-	-	-	-	1	-	3	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diptera	Athericidae	Atherix	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Atrichops	-	5	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 7. Continued on next page.

Table 7. Continued from previous page.

Taxonomic classification			Autumn Sites																								
Order/Group	**Family**	**Genus***	**CA1**	**CA2**	**CA3**	**CA4**	**H1**	**H2**	**H3**	**H5**	**MU2**	**MU3**	**MU4**	**R4**	**SA1**	**SA2**	**SA3**	**SA4**	**SA5**	**SC1**	**SC2**	**SC3**	**SC4**	**T1**	**T2**	**T3**	**T4**
Diptera	Ceratopogonidae	Atrichopogon	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
		Bezzia	27	9	7	5	11	17	3	-	1	4	2	-	8	5	1	2	-	-	-	-	-	7	-	-	5
		Brachypogon	-	-	-	-	-	-	-	-	-	-	-	-	4	1	-	-	-	-	-	-	-	-	-	-	-
		Culicoides	44	-	6	-	2	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
		Dasyhelea	17	32	10	1	2	2	-	6	-	5	1	1	6	40	2	1	6	-	-	-	2	14	4	52	-
	Chironomidae	Chironomini	59	70	14	4	81	-	22	-	2	-	-	3	4	7	-	1	5	43	1	35	60	-	7	-	2
		Macropelopini	65	46	28	3	6	-	-	-	-	-	-	-	1	6	-	-	-	3	-	1	-	-	3	-	-
		Orthocladiinae	202	107	18	2	246	161	390	153	6	465	247	19	165	59	20	34	26	5	10	6	35	170	21	81	102
		Pentaneurini	40	11	40	20	41	6	24	20	-	1	2	13	9	16	2	3	2	-	-	2	5	6	1	3	3
		Tanytarsini	28	9	20	1	15	36	24	1	-	-	9	-	24	22	5	11	11	3	11	7	25	12	3	2	4
	Culicidae	Anopheles	1	-	7	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Culex	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
	Dixidae	Dixella	40	7	6	-	27	-	1	-	-	-	1	-	1	1	1	-	-	-	-	-	-	8	-	-	-
	Empididae	Hemerodromia	-	1	2	-	5	2	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	2	-	-	-
	Limoniidae	Limoniidae	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Pilaria	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Muscidae	Limnophora	1	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
	Psychodidae	Berdeniella	-	-	-	-	-	2	95	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Mormia	-	-	-	-	-	-	12	-	-	4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Pericoma	1	-	-	-	3	-	18	-	-	6	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-
		Satchelliella	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Threticus	-	-	-	-	-	2	47	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Tonnoiriella	-	-	-	-	-	-	6	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
	Sciomyzidae		1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-
	Simuliidae	Simulium	2	-	-	-	8	19	125	7	-	1	-	-	183	3	3	-	-	-	11	1	2	37	1	-	-
	Stratiomyidae	Oxycera	2	1	1	1	3	2	11	3	4	7	2	-	4	1	-	-	2	-	-	-	1	4	-	-	-
	Syrphidae		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	45	-	-	-	-	-
	Tabanidae	Tabanus	-	-	1	-	1	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-
	Tipulidae	Tipula	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1

Table 7. Continued on next page.

Table 7. Continued from previous page.

Taxonomic classification			Autumn Sites																									
Order/Group	**Family**	**Genus**	**CA1**	**CA2**	**CA3**	**CA4**	**H1**	**H2**	**H3**	**H5**	**MU2**	**MU3**	**MU4**	**R4**	**SA1**	**SA2**	**SA3**	**SA4**	**SA5**	**SC1**	**SC2**	**SC3**	**SC4**	**T1**	**T2**	**T3**	**T4**
Ephemeroptera	Baetidae	Baetis	8	-	-	-	-	6	5	-	-	1	10	-	18	-	2	-	-	2	6	-	7	-	1	-	-
		Centroptilum	34	46	150	3	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-
		Cloeon	42	23	128	27	38	17	40	2	-	-	-	7	48	31	4	16	57	-	5	-	-	13	10	30	19
	Caenidae	Caenis	196	47	128	38	61	49	54	2	-	4	11	-	164	28	21	30	37	4	20	-	6	20	2	2	2
	Heptageniidae	Electrogena	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Leptophlebiidae	Habroleptoides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-
		Habrophlebia	1	-	3	2	2	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-
Heteroptera	Corixidae	Micronecta	38	37	2	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
		Parasigara	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Hydrometridae	Hydrometra	3	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Naucoridae	Naucoris	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
	Notonectidae	Notonecta	-	1	1	5	1	1	-	-	-	-	-	-	1	-	-	-	1	-	-	1	1	-	-	-	-
	Veliidae	Velia	6	-	-	1	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-
Megaloptera	Sialidae	Sialis	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Mollusca	Lymnaeidae	Lymnaea	1	2	12	10	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Radix	-	-	7	-	-	-	-	-	-	2	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-
	Physidae	Physella	-	2	24	20	-	8	2	-	-	-	1	-	3	-	-	-	3	-	-	-	-	-	-	2	1
	Planorbidae	Anisus	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Gyraulus	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Odonata	Aeschnidae	Aeshna	-	-	-	-	-	1	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
		Anax	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Boyeria	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Calopterygidae	Calopteryx	-	-	4	-	1	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-
	Coenagrionidae	Ceriagrion	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
		Coenagrion	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
		Pyrrhosoma	-	2	17	6	2	1	-	-	-	1	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-
	Cordulegasteridae	Cordulegaster	-	1	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Gomphidae	Onychogomphus	2	4	3	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
	Libellulidae	Orthetrum	11	9	6	9	-	-	2	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
		Sympetrum	3	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Platycnemididae	Platycnemis	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 7. Continued on next page.

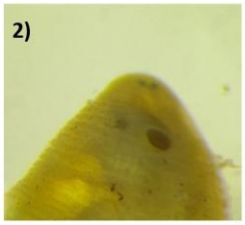
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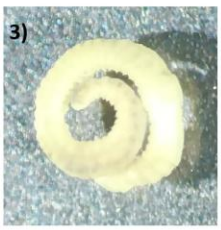
Taxonomic classification			Autumn Sites																								
Order/Group	**Family**	**Genus**	**CA1**	**CA2**	**CA3**	**CA4**	**H1**	**H2**	**H3**	**H5**	**MU2**	**MU3**	**MU4**	**R4**	**SA1**	**SA2**	**SA3**	**SA4**	**SA5**	**SC1**	**SC2**	**SC3**	**SC4**	**T1**	**T2**	**T3**	**T4**
Oligochaeta			13	15	10	2	-	7	-	13	-	1	-	-	19	13	-	1	-	-	-	-	-	-	1	5	1
Plecoptera	Nemouridae	Nemoura	8	7	47	31	5	11	8	-	-	4	6	1	376	8	13	21	10	-	2	-	-	85	1	34	28
Trichoptera	Hydropsychidae	Hydropsyche	-	-	-	-	3	2	1	-	-	-	-	-	5	1	-	-	-	-	-	-	-	-	-	-	-
	Hydroptilidae	Hydroptila	-	-	2	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
		Stactobia	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	Leptoceridae	Mystacides	6	4	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Limnephilidae	Limnephilus	1	-	6	10	-	2	5	1	-	-	2	-	-	-	-	-	-	-	3	-	2	3	-	1	1
	Philopotamidae	Philopotamus	-	-	-	-	4	1	11	3	-	-	-	-	8	1	2	-	-	-	-	-	-	1	-	-	-
	Polycentropodidae	Polycentropus	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Psychomyiidae	Tinodes	-	-	5	-	-	-	1	-	-	-	-	-	4	2	-	-	-	-	-	-	-	4	-	-	-
	Sericostomatidae	Sericostoma	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tricladida	Dugesiidae	Dugesia	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PHOTOGRAPHIC CATALOGUE

- **Photographic catalogue of aquatic macroinvertebrates**
- **Photographic catalogue of stream sites**

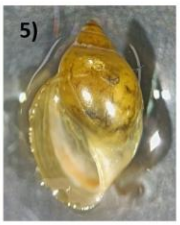






PHYL. ANNELIDA







PHYL. MOLLUSCA

PHYL. ARTHROPODA









Cl. Arachnida (Subph. Chelicerata)









Cl. Malacostraca (Subph. Crustacea)

Non-insect macroinvertebrtes groups. 1) *Helobdella stagnalis* (Fam. Glossiphonidae); **2)** Detailed view of the head of *H. stagnalis*; **3)** Cl. Oligochaeta; **4)** *Potamopyrgus antipodarum* (Fam. Hydrobiidae); **5)** *Physella acuta* (Fam. Physidae); **6)** Fam. Sphaeriidae; **7)** and **8)** *Hydrachnidiae ventral view; **9)** and **10)** *Hydrachnidiae dorsal view; **11)** *Procambarus clarkii* (Fam. Cambaridae); **12)** Dorsal view of the head of *P. clarkii*; **13)** *Echinogammarus* sp. (Fam. Gammaridae); **14)** Details of the uropods of *Echinogammarus* sp.

* Hydrachnidiae is not a formally recognized taxonomic entity but a general term for aquatic mites from diverse taxonomic lineages.

Cl. Insecta (Subph. Hexapoda)

Ord. Ephemeroptera







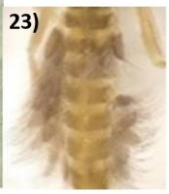












Order Ephemeroptera. 15) *Baetis* sp. (Fam. Baetidae); **16)** *Baetis* sp.; **17)** *Caenis* sp. (Fam. Caenidae); **18)** *Electrogena* sp. (Fam. Heptageniidae); **19)** *Ephemerella* sp. (Fam. Ephemerellidae); **20)** *Habroleptoides* sp. (Fam. Lepthophlebiidae); **21)** *Habrophlebia* sp. (Fam. Lepthophlebiidae); **22)** *Thraulus bellus* (Fam. Lepthophlebiidae); **23)** Detailed view of the gills of *T. bellus.*

Ord. Plecoptera



Order Plecoptera. 24) *Isoperla* sp. (Fam. Perlodidae); **25)** *Leuctra* sp. (Fam. Leuctridae); **26)** Ventral view of *Tyrrhenoleuctra* sp. (Fam. Leuctridae); **27)** Dorsal view of *Tyrrhenoleuctra* sp.; **28)** *Siphonoperla torrentium* (Fam. Chloroperlidae).

Ord. Odonata



Order Odonata. 29) *Anax imperator*, *Aeshna* sp. and *Boyeria irene* (Fam. Aeshnidae) from left to right; **30)** *A. imperator*; **31)** *Aeshna* sp.; **32)** *B. irene*; **33)** *Calopteryx* sp. (Fam, Calopterygidae); **34)** Detailed view of the head of *Calopteryx* sp.; **35)** Fam. Lestidae; **36)** Detailed view of the mask of *Chalcolestes viridis* (Fam. Lestidae); **37)** *Cordulegaster boltonii* (Fam. Cordulegasteridae); **38)** Detailed view of the mask of *C. boltonii*; **39)** *Crocothermis erythraea* (Fam. Libellulidae); **40)** *Platycnemis* sp. (Fam. Platycnemididae); **41)** Detailed view of the caudal lamellae of *Platycnemis* sp.; **42)** Fam. Gomphidae.

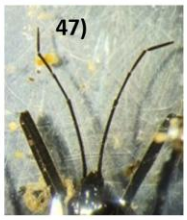
Ord. Hemiptera

















Order Hemiptera. 43) *Nepa cinerea* (Fam. Nepidae); **44)** *Parasigara perdubia* (Fam. Corixidae); **45)** *Micronecta* sp. (Fam. Corixidae); **46)** *Plea minutissima* (Fam. Pleidae); **47)** Detailed view of the antennae of *Aquaris* sp. (Fam. Gerridae); **48)** *Aquaris* sp.; **49)** Ventral view of *Velia* sp. (Fam. Veliidae); **50)** Dorsal view of *Velia* sp.

Ord. Megaloptera



Order Megaloptera. 51) *Sialis* sp. (Fam. Sialidae).

Ord. Coleoptera (Fam. Dytiscidae)











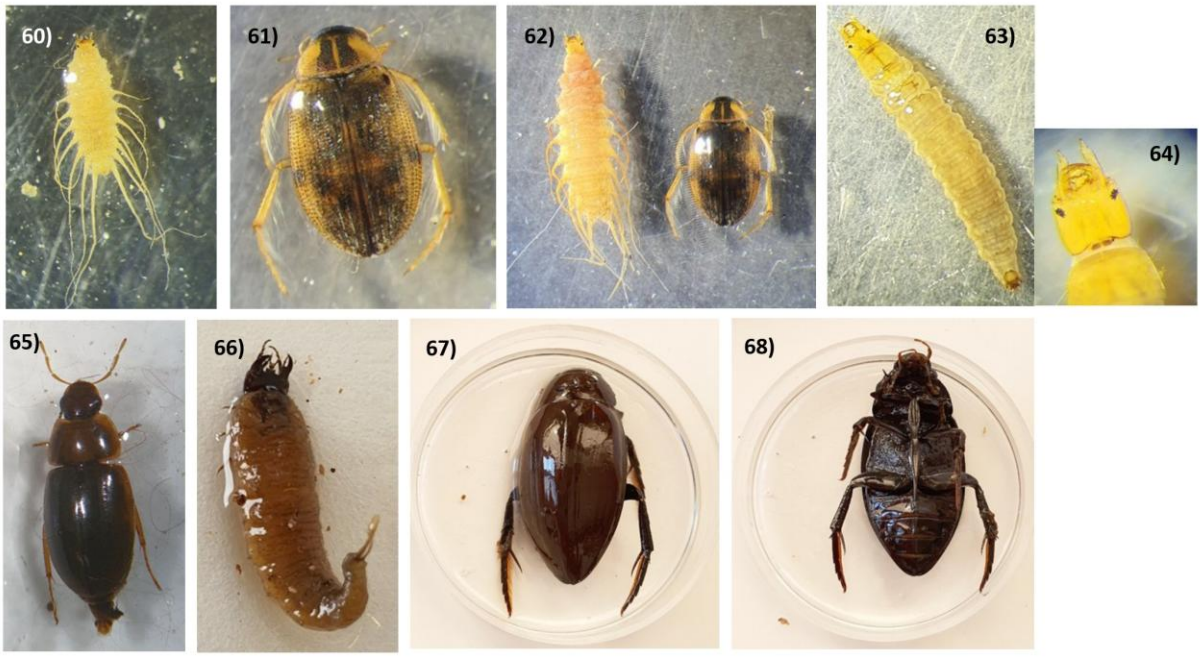






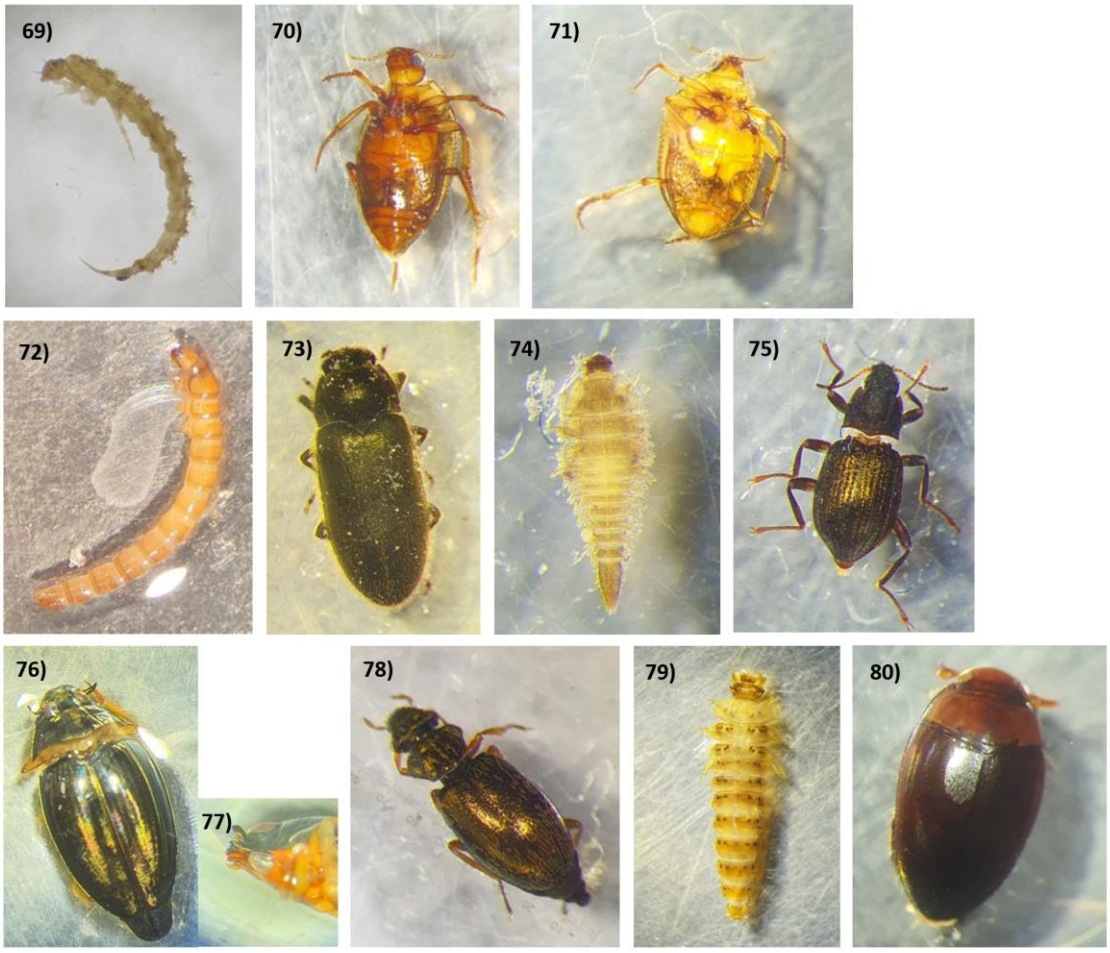
Order Coleoptera (Family Dytiscidae). 52) *Agabus biguttatus*; **53)** *Agabus brunneus*; **54)** *Agabus didymus*; **55)** *Ilybius meridionalis*; **56)** *Deronectes* sp.; **57)** *Laccophilus* sp.; **58)** *Yola bicarinata*; **59)** *Bidessus* sp. and *Y. bicarinata*.

Ord. Coleoptera (Fam. Hydrophilidae)



Order Coleoptera (Family Hydrophilidae). 60) *Berosus* sp. (larvae); **61)** *Berosus* sp. (adult); **62)** *Berosus* sp. (larvae and adult, respectively); **63)** *Helochares* sp. (larvae); **64)** Detailed view of the clypeus of *Helochares* sp.; **65)** *Helochares* sp. (adult); **66)** *Hydrophilus* sp. (larvae); **67)** Dorsal view of *Hydrophilus* sp. (adult); **68)** Ventral view of *Hydrophilus* sp. (adult).

Ord. Coleoptera (Other Families)

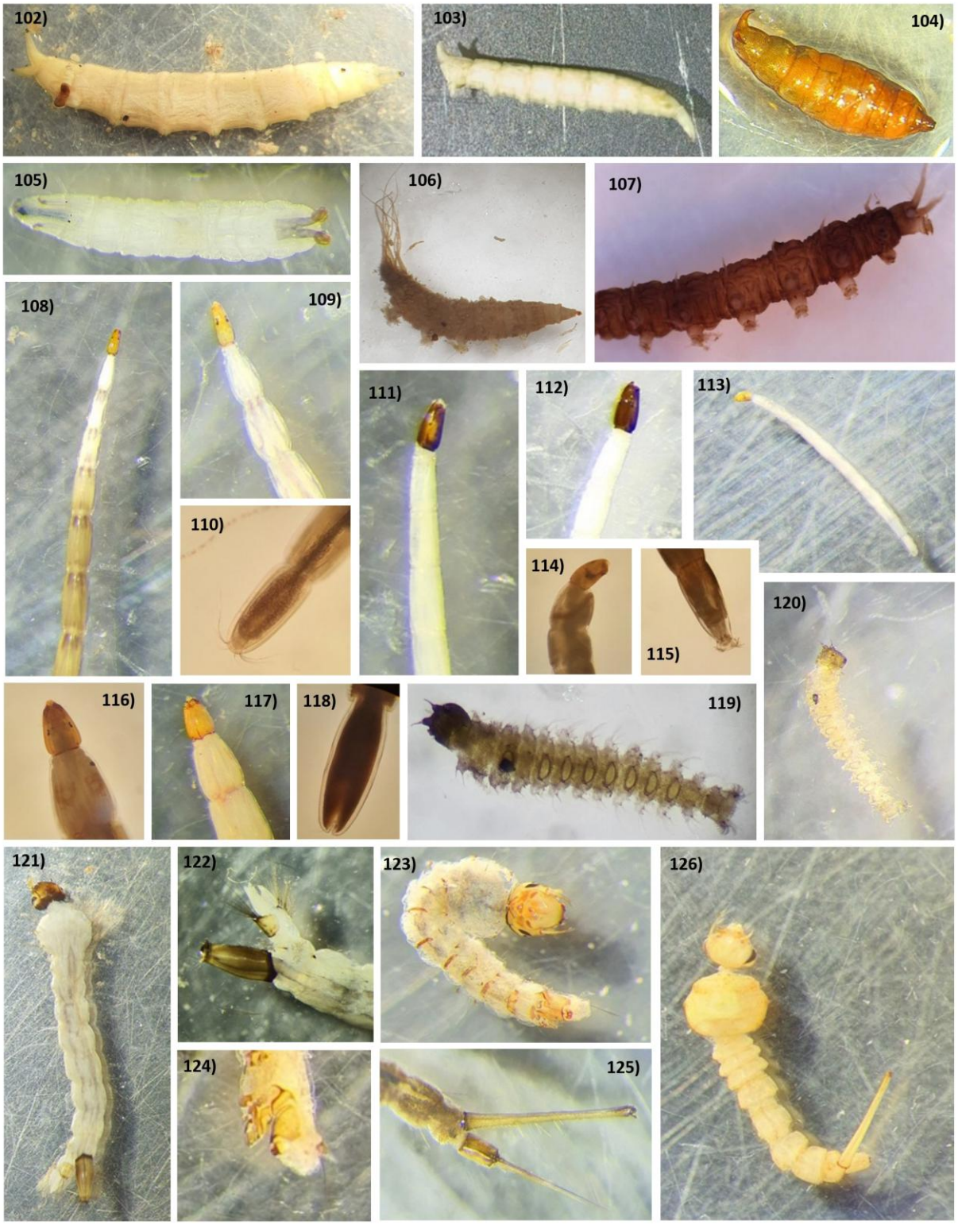


Order Coleoptera (Other Families). 69) *Haliplus* sp. (larvae) (Fam. Haliplidae); **70)** *Haliplus* sp. (adult); **71)** *Peltodytes* sp. (Fam. Haliplidae); **72)** *Dryops* sp. (larvae) (Fam. Dryopidae); **73)** *Dryops* sp. (adult); **74)** *Elmis* sp. (larvae) (Fam. Elmidae); **75)** *Elmis* sp. (adult); **76)** *Gyrinus* sp. (Fam. Gyrinidae); **77)** Detailed view of the head of *Gyrinus* sp.; **78)** *Ochthebius* sp. (Fam. Hydraenidae); **79)** *Elodes* sp. (Fam. Scirtidae); **80)** *Noterus clavicornis* (Fam. Noteridae).



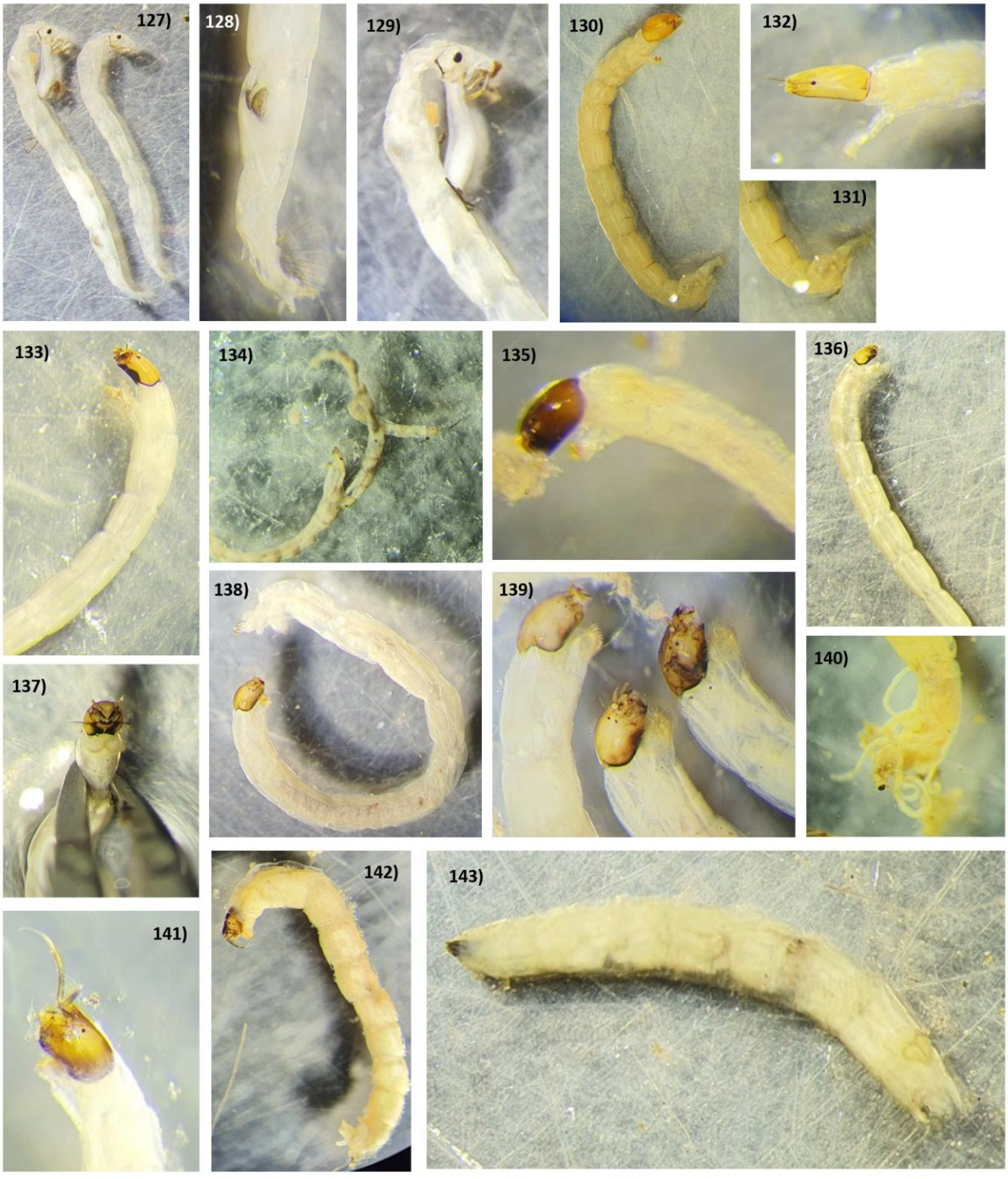
Order Trichoptera. 81) *Adicella reducta* (Fam. Leptoceridae); **82)** Case of *A. reducta*; **83)** Case of *Mystacides* sp. (Fam. Leptoceridae); **84)** *Mystacides* sp.; **85)** *Beraea* sp. (Fam. Beraeidae); **86)** Case of *Beraea* sp.; **87)** *Hydroptila* sp. (Fam. Hydroptilidae); **88)** Case of *Hydroptila* sp.; **89)** *Oxyethira* sp. (Fam. Hydroptilidae); **90)** *Stactobia* sp. (Fam. Hydroptilidae); **91)** Case and larvae of *Stactobia* sp.; **92)** *Tinodes* sp. (Fam. Psychomyiidae); **93)** *Hydropsyche* sp. (Fam. Hydropsychidae); **94)** Detailed view of the trochantin of *Hydropsyche* sp.; **95)** Fam. Philopotamidae; **96)** *Wormaldia* sp. (Fam. Philopotamidae); **97)** *Polycentropus kingi* (Fam. Polycentropodidae); **98)** Fam. Limnephilidae; **99)** Larvae of Limnephilidae; **100)** Case of *Limnephilus guadarramicus* (Fam. Limnephilidae); **101)** Frontal view of the case of *L. guadarramicus* (Fam. Limnephilidae).

Ord. Diptera
(Fam. Muscidae, Athericidae, Ceratopogonidae and Culicidae)



Order Diptera (Families Muscidae, Athericidae, Ceratopogonidae and Culicidae). 102) *Limnophora* sp. (Fam. Muscidae); **103)** *Limnophora* sp.; **104)** Pupa of *Limnophora* sp.; **105)** *Lispe* sp. (Fam. Muscidae); **106)** *Atrichops crassipes* (Fam. Athericidae); **107)** *Atherix* sp. (Fam Athericidae); **108)** *Bezzia* sp. (Fam. Ceratopogonidae); **109)** Detailed view of the head of *Bezzia* sp.; **110)** Detailed view of the posterior setae of *Bezzia* sp.; **111)** *Brachypogon* sp. (Fam. Ceratopogonidae); **112)** Detailed view of the head of *Brachypogon* sp.; **113)** *Dasyhelea* sp. (Fam. Ceratopogonidae); **114)** Detailed of the head of *Dasyhelea* sp.; **115)** Detailed view of the posterior setae of *Dasyhelea* sp.; **116)** Dorsal view of the head of *Nilobezzia* sp. (Fam. Ceratopogonidae); **117)** Ventral view of the head of Nilobezzia sp.; **118)** Detailed view of the posterior part of the abdomen of *Nilobezzia* sp.; **119)** *Atrichopogon* sp. (Fam. Ceratopogonidae); **120)** *Atrichopogon* sp.; **121)** *Aedes* sp. (Fam. Culicidae); **122)** Detailed view of the posterior part of the abdomen of *Aedes* sp.; **123)** *Anopheles* sp. (Fam. Culicidae); **124)** Detailed view of the posterior part of the abdomen of *Anopheles* sp.; **125)** Detailed view of the posterior part of the abdomen (siphon) of *Culex* sp. (Fam. Culicidae); **126)** *Culex* sp.

Ord. Diptera
(Fam. Chaoboridae, Chironomidae and Dolichopodidae)



Order Diptera (Families Chaoboridae, Chironomidae and Dolichopodidae). 127) *Chaoborus* sp. (Fam. Chaoboridae); **128)** Detailed view of the posterior part of the abdomen of *Chaoborus* sp.; **129)** Detailed view of the head of *Chaoborus* sp.; **130)** Tr. Macropelopini (Fam. Chironomidae; Subfam. Tanypodinae); **131)** Detailed view of the posterior part of the abdomen of Tr. Macropelopini; **132)** Tr. Pentaneurini (Fam. Chironomidae; Subfam. Tanypodinae); **133)** *Brillia* sp. (Fam. Chironomidae; Subfam. Orthocladiinae); **134)** *Corynoneura* sp. (Fam. Chironomidae; Subfam. Orthocladiinae); **135)** *Eukiefferiella* sp. (Fam. Chironomidae; Subfam. Orthocladiinae); **136)** *Prodiamesa olivacea* (Fam. Chironomidae; Subfam. Prodiamesinae); **137)** Detailed view of the setae of *P. olivacea*; **138)** Tr. Chironomini (Fam. Chironomidae; Subfam. Chironominae); **139)** Detailed view of the heads of members of the Tr. Chironomini; **140)** Detailed of the posterior part of the abdomen of Tr. Chironomini; **141)** Detailed view of the head of Tr. Tanytarsini (Fam. Chironomidae; Subfam. Chironominae); **142)** Tr. Tanytarsini; **143)** Fam. Dolichopodidae.

Ord. Diptera
(Fam. Dixidae, Empididae, Ephydridae and Limoniidae)



Order Diptera (Families Dixidae, Empididae, Ephydridae and Limoniidae). 144) *Dixa* sp. (Fam. Dixidae); **145)** *Dixa* sp.; **146)** Detailed view of the prothoracic setae of *Dixa* sp.; **147)** Detailed view of the posterior part of the abdomen of *Dixa* sp.; **148)** *Dixella* sp. (Fam. Dixidae); **149)** Detailed view of the prothoracic setae of *Dixella* sp.; **150)** *Clinocera* sp. (Fam. Empididae); **151)** Detailed view of the posterior part of the abdomen of *Clinocera* sp.; **152)** *Hemerodromia* sp. (Fam. Empididae); **153)** Detailed view of the posterior part of the abdomen of *Hemerodromia* sp.; **154)** *Hydrellia* sp. (Fam. Ephydridae); **155)** Detailed view of the posterior part of *Hydrellia* sp.; **156)** *Parydra* sp. (Fam. Ephydridae); **157)** Detailed view of the posterior part of *Parydra* sp.; **158)** *Dicranota* sp. (Fam. Limoniidae); **159)** Detailed view of the head of *Dicranota* sp.; **160)** Detailed view of the posterior part of the abdomen of *Dicranota* sp.; **161)** Detailed view of the posterior part of the abdomen of *Helius* sp. (Fam. Limoniidae); **162)** *Pilaria* sp. (Fam. Limoniidae); **163)** Detailed view of the posterior part of *Pilaria* sp.; **164)** *Pseudolimnophila* sp. (Fam. Limoniidae); **165)** Detailed view of the ventroposterior part of the abdomen of *Pseudolimnophila* sp.; **166)** Detailed view of the dorsoposterior part of the abdomen of *Pseudolimnophila* sp..

Ord. Diptera
(Fam. Psychodidae)



Order Diptera (Family Psychodidae). 167) *Berdeniella* sp.; **168)** Detailed view of the flabellate lamellae of *Berdeniella* sp.; **169)** Paramormia sp.; **170)** Detailed view of the posterior part of the abdomen of *Paramormia* sp.; **171)** Detailed view of the head of *Paramormia* sp.; **172)** *Pericoma* sp.; **173)** Detailed view of the flabellate lamellae of *Pericoma* sp.; **174)** *Peripsychoda* sp.; **175)** Detailed view of the head of *Peripsychoda* sp.; **176)** Detailed view of the posterior part of the abdomen of *Peripsychoda* sp.; **177)** *Psychoda* sp.; **178)** Dorsal view of *Psychoda* sp.; **179)** *Tonnoiriella pulchra*; **180)** Detailed view of the posterior part of the *T. pulchra*; **181)** *Ulomyia* sp.; **182)** Detailed view of the posterodorsal part of the abdomen of *Ulomyia* sp.; **183)** Detailed view of the lateroposterior part of the abdomen of *Ulomyia* sp..

Ord. Diptera
(Fam. Ptychopteridae, Simulidae, Rhagionidae, Stratiomyidae, Tabanidae and Tipulidae)



Order Diptera (Family Ptychopteridae, Simulidae, Rhagionidae, Stratiomyidae, Tabanidae and Tipulidae). 184) *Ptychoptera* sp. (Fam. Ptychopteridae); **185)** Detailed view of the posterior part of the *Ptychoptera* sp.; **186)** *Simulium* sp. (Fam. Simulidae); **187)** Pupa of *Simulium* sp.; **188)** Fam. Rhagionidae; **189)** Detailed view of the posteroventral part of the Fam. Rhagionidae; **190)** *Nemotelus* sp. (Fam. Stratiomyidae); **191)** Detailed view of the posterior part of the abdomen of *Nemotelus* sp.; **192)** Dorsal view of *Oxycera* sp. (Fam. Stratiomyidae); **193)** Ventral view of *Oxycera* sp.; **194)** Detailed view of the head of *Oxycera* sp.; **195)** Detailed view of the posterior part of the abdomen of *Oxycera* sp.; **196)** Dorsal view of *Stratiomys* sp. (Fam. Stratiomyidae); **197)** Ventral view of *Stratiomys* sp.; **198)** Detailed view of the head of *Stratiomys* sp.; **199)** Detailed view of the posterior part of the abdomen of *Stratiomys* sp.; **200)** *Tabanus* sp. (Fam. Tabanidae); **201)** Fam. Syrphidae; **202)** *Tipula* sp. (Fam. Tipulidae); **203 and 204)** Detailed view of the posterior part of the abdomen of *Tipula* sp..



Figure 1. Images of Castelló stream.



Figure 2.1. Images of Vall d'Horta stream.



Figure 2.2. Images of Vall d'Horta stream.



Figure 2.3. Image of Vall d'Horta stream.



Figure 3.1. Images of Mura stream.



Figure 3.2. Images of Mura stream.



Figure 3.3. Images of Mura stream.



Figure 4. Images of Rellinars stream.



Figure 5.1. Images of Sanana stream.





Figure 5.2. Images of Sanana stream.



Figure 6.1. Images of Santa Creu stream.



Figure 6.2. Images of Santa Creu stream.



Figure 7.1. Images of Talamanca stream.



Figure 7.2. Images of Talamanca stream.

PUBLISHED CHAPTERS

- **Chapter 1:** Navigating through space and time: a methodological approach to quantify spatiotemporal connectivity using stream flow data as a case study
- **Chapter 5:** Integrating spatiotemporal hydrological connectivity into conservation planning to protect temporary rivers

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Methods in Ecology and Evolution

RESEARCH ARTICLE

Navigating through space and time: A methodological approach to quantify spatiotemporal connectivity using stream flow data as a case study

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Handling Editor: Brenda Pracheil

Abstract

1. The growing interest in combining spatial and temporal patterns in nature has been fostered by the current availability of high-frequency measurements. However, we still lack a methodological framework to process and interpret spatiotemporal datasets into meaningful values, adaptable to different time windows and/or responding to different spatial structures. Here, we developed and tested a framework to evaluate spatiotemporal connectivity using two new measures: the spatiotemporal connectivity (*STcon*) and the spatiotemporal connectivity matrix (*STconmat*).
2. To obtain these measures, we consider a set of spatially connected sites within a temporally dynamic network. These measures are calculated from a spatiotemporal matrix where spatial and temporal connections across sites are captured. These connections respond to a determined network structure, assign different values to these connections and generate different scenarios from which we obtain the spatiotemporal connectivity. We developed these measures by using a dataset of stream flow state spanning a 513-day period obtained from data loggers installed in seven temporary streams. These measures allowed us to characterise connectivity among stream reaches and relate spatiotemporal patterns with macroinvertebrate community structure and composition.
3. Spatiotemporal connectivity differed within and among streams, with *STcon* and *STconmat* capturing different hydrological patterns. Macroinvertebrate richness and diversity were higher in more spatiotemporally connected sites.

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Community dissimilarity was related to *STconmat* showing that more spatiotemporally connected sites had similar communities for active and passive dispersers. Interestingly, both groups were related to spatiotemporal connectivity patterns for some of the analysed scenarios, highlighting the relevance of spatiotemporal connectivity in dynamic systems.

4. As we exemplified, the proposed framework can help to disentangle and quantify spatiotemporal dynamics or be applied in the conservation of dynamic systems such as temporary streams. However, the current framework is not limited to the temporal and spatial features of temporary streams. It can be extended to other ecosystems by including different time windows and/or consider different network structures to assess spatiotemporal patterns. Such spatiotemporal measures are especially relevant in a context of global change, with the spatiotemporal dynamics of ecosystems being heavily disrupted by human activities.

KEYWORDS
ephemeral streams, intermittent rivers, network structure, spatial connectivity, spatiotemporal graphs, temporal connectivity

1 | INTRODUCTION

The interaction between populations, communities and ecosystems within a landscape has a prominent role in ecology (Gounand et al., 2020; Hanski, 1999; McArthur & Wilson, 1967). To better understand this interaction, the so-called meta-system framework has been developed (Cid et al., 2022; Gounand et al., 2018; Schiesari et al., 2019). This framework asserts that landscape connectivity drives exchanges of matter, energy and organisms among localities within a landscape, affecting ecosystem structure, functioning and dynamics (Battin et al., 2009; Dale, 2017; Larsen et al., 2012). For example, several studies have highlighted the relevance of connectivity in driving species' diversity and abundance (Borthagaray et al., 2020; Economo & Keitt, 2010), community turnover (Epele et al., 2021; Horváth et al., 2016), population synchrony (Larsen et al., 2021; Sarremejane et al., 2021), mutualistic interactions (Ferreira et al., 2020; Monteiro et al., 2022), species' extinctions (Horváth et al., 2019), vulnerability to species invasions (Drake et al., 2017) and ecosystem services (Mitchell et al., 2013). Therefore, connectivity is emerging as one of the main drivers of community assembly and ecosystem functioning as it varies the strength of dispersal (Leibold & Chase, 2018; Thompson et al., 2020; Vellend, 2016) and affects biodiversity, which is linked to ecosystem properties such as resistance or productivity (Thompson et al., 2017; Tonkin, Heino, et al., 2018). Nevertheless, most of these examples are focused on spatial connectivity, while the temporal facet of connectivity remains relatively understudied (Uroy et al., 2021).

Metapopulations, metacommunities and meta-ecosystems have been generally assessed using a snapshot approach that assumes temporal stability (Holyoak et al., 2020; Uroy et al., 2021). In the specific case of metacommunities, most studies have considered spatial or temporal connectivity individually while communities experience both temporal and spatial variation in connectivity affecting populations (Kurihara, 2007), communities (Cañedo-Argüelles et al., 2020; Dong et al., 2017) and ecological processes (Ding et al., 2013; Sadchatheeswaran et al., 2021). Whereas spatial connectivity refers to how communities are connected within a landscape (Moritz et al., 2013), temporal connectivity refers to how communities are connected in time, for example, across multiple years (Holyoak et al., 2020). Within this context, considering only one facet of connectivity can lead to misleading conclusions about the importance of landscape connectivity for the exchange of species between local communities (Castillo-Escrivà et al., 2020; Huang et al., 2020; Rasmussen et al., 2013). To date, few studies have assessed how connectivity changes simultaneously in space and time, defined here as spatiotemporal connectivity, and how these can affect natural systems (Fullerton et al., 2010; Uroy et al., 2021).

Nowadays, new technologies such as satellite high-frequency sensors (Burel & Baudry, 2005; Ducklow et al., 2009; Pekel et al., 2016), high time frequency measuring devices such as GPS (Bischof et al., 2019; Fattebert et al., 2015; Foley & Sillero-Zubiri, 2020) or data loggers (Crabot et al., 2020; Jensen et al., 2019; Vander Vorste et al., 2016) offer good opportunities to characterise spatiotemporal connectivity because they capture high spatial and temporal resolution data that can be later combined into spatiotemporal measures (Martensen et al., 2017; Uroy et al., 2021). Some studies have used long-term datasets to assess the temporal variation of spatial processes through consecutive snapshot analyses (Cañedo-Argüelles et al., 2020; Leppänen et al., 2019; Rouissi et al., 2014), but in these studies, the interaction between different time steps is not considered. In contrast, other studies (Huang et al., 2020; Martensen et al., 2017; Thompson et al., 2017) simultaneously consider the interaction between spatial and temporal dimensions by including the effect of connectivity through more

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than one time step (e.g. the connectivity of a given time point has an effect on the next time points and this effect propagates in time). However, few examples exist where spatial and temporal variation are simultaneously considered to estimate spatiotemporal connectivity (Uroy et al., 2021). The lack of a clear framework from which to calculate spatiotemporal connectivity values or assess different time windows (i.e. number of time steps considered to impact connectivity) is limiting the development of such approximations. Even though technologies to capture spatiotemporal connectivity changes are nowadays available, we still need a methodological framework for obtaining connectivity measures that integrate spatial and temporal patterns (Uroy et al., 2021).

Temporary river networks are suitable systems to analyse and develop a framework to assess spatiotemporal connectivity due to their high hydrological variability in space and time (Datry et al., 2014). Spatially, fluvial systems are driven by an up- to downstream unidirectional flow following a dendritic structure which affects the distribution and presence of species (Altermatt, 2013; Altermatt et al., 2020; Borthagaray et al., 2020; Schmera et al., 2018). Temporally, they are subjected to a strong seasonal and/or interannual variation, with recurrent drying and flowing periods determining habitat availability (Bonada et al., 2020; Datry et al., 2016; Valente-Neto et al., 2020). Finally, species inhabiting these systems have two different strategies to withstand drying and thus coping with spatiotemporal variability: resisting in situ as dormant stage (temporal dispersal) or recolonising from nearby refuges after flow resumption (spatial dispersal; He et al., 2020; Heino, 2013; Sarremejane et al., 2020). These different ways of interacting with the landscape can be related to different network structures or dispersal abilities, thereby informing about changes in network connectivity (Cañedo-Argüelles et al., 2020; Pineda-Morante et al., 2022; Tonkin, Altermatt, et al., 2018). Consequently, in temporary streams, spatiotemporal connectivity among communities is promoted by unidirectional or by multidirectional dispersal (Borthagaray et al., 2015; Seymour et al., 2015; Tonkin, Altermatt, et al., 2018). A unidirectional or directed network would be related to species that are aquatic obligates and that follow the stream dendritic structure to move from one site to another, hereafter passive dispersers. A multidirectional or undirected network would be related to species that can fly in all directions, with no need to follow the stream course, hereafter aerial dispersers. So far, flow data obtained using high-frequency temperature data loggers have been used to characterise the local hydrological conditions of temporary streams (e.g. drying duration, number of consecutive dry days, days with disconnected pools, days after flow return) and its effects on aquatic communities (Arias-Real et al., 2021; B-Béres et al., 2019; Beesley & Prince, 2010; Crabot et al., 2020). However, this information has not yet been used to develop spatiotemporal connectivity measures.

In this study, we developed a methodological framework to assess spatiotemporal connectivity focusing on Mediterranean streams as a case study. Using high-frequency data on water presence/absence from a set of temporary streams, we propose two measures of connectivity (Figure 1): spatiotemporal connectivity (*STcon*) and spatiotemporal connectivity matrix (*STconmat*). *STcon* measures average

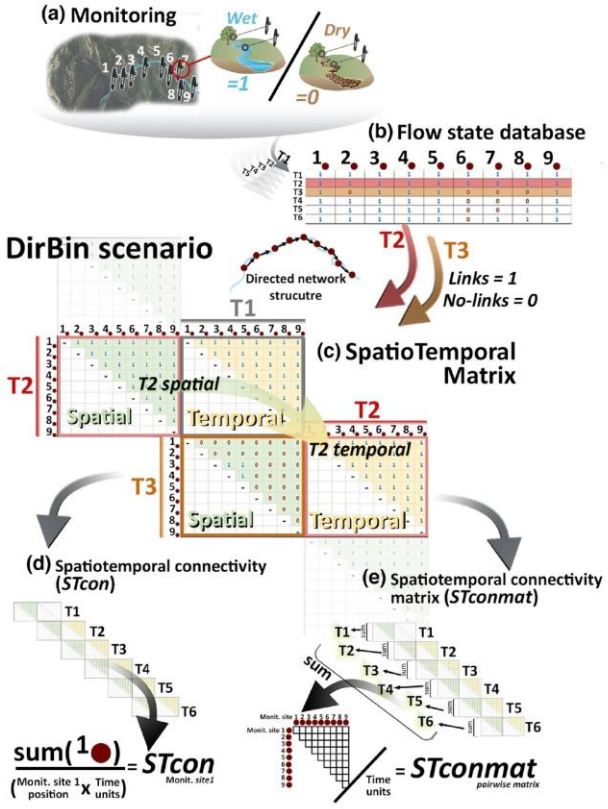


FIGURE 1 Conceptual graphical representation of how the *STcon* and *STconmat* are calculated using the example of Vall d'Horta stream (VH). (a) The data loggers are deployed in the field and monitor the presence (i.e. wet) or absence (i.e. dry) of water. (b) These values are then presented in a flow state database where rows represent each time unit (i.e. 6 days, from T1 to T6) and columns every monitored site (from 1 to 9). Each cell of the flow state database is filled with a value of '1' if the monitored site was 'wet' that day (e.g. all sites in day 1, T2, were defined as 1) or with a value of '0' if the monitored site was 'dry' that day (e.g. in day 2, T3, monitored site 2, 6, 7 and 8 were defined as 0). (c) Using the flow state database, the spatiotemporal matrix is built based on a defined scenario, in this case, we used a directed network structure and a binary quantification of links and no-links (DirBin scenario). For each row of the flow state matrix (e.g. T2), a pairwise matrix is constructed where all the possible spatial links, based on which monitored sites are 'wet' and which are 'dry', are assessed. This defines the spatial connections for a specific time (green part of the matrix). Temporal connections are filled based on the same connections of that time (T2) but introduced in the spatiotemporal matrix in the following time step (T3). (d) *STcon* is calculated for each site individually (e.g. Site 1) and is the average of how often a site has been connected to all its potential neighbours (e.g. Site 1 has eight potential neighbours) during the studied time (6 units of time). (d) *STconmat* corresponds to the sum of all the spatiotemporal matrices into one unique matrix later divided by the studied time (6 units of time).

site connectivity for each individual site and thus can be used as a predictor of community structural and functional metrics such as local species richness or Shannon–Wiener diversity. *STconmat* represents

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these connectivity values by pairs of sites so as a spatiotemporal connectivity matrix and allows to assess changes in community composition (e.g. Jaccard dissimilarity, Bray–Curtis dissimilarity). Here, we used these two measures to explain changes in the structure of macroinvertebrate communities at the local and regional scales to illustrate their relevance for capturing ecological processes.

2 | MATERIALS AND METHODS

2.1 | Study sites and sampling

The dataset used for developing and testing the current framework comes from a study carried out in Sant Llorenç del Munt i l'Obac Natural Park, a protected area in the Vallès Occidental region (Catalonia, NE Spain). The area has a Mediterranean climate and the underlying geology is dominated by karst limestone. These characteristics make surface flows heavily linked to rainfall events and thus streams in the region have marked flowing/drying periods, especially during the summer. We selected seven temporary streams that were sampled in November 2019 as part of a yearly seasonal monitoring where streams were monitored from July 2018 until November 2019. All streams had similar instream characteristics and aquatic macroinvertebrates were sampled following a multihabitat design and identified at the lowest possible taxonomic level, mostly species or genus. Information on sampling location, procedure, characteristics and specific taxonomic identification can be found in Pineda-Morante et al. (2022).

2.2 | Flow state monitoring and database building

A total of 69 temperature data loggers (HOBO Pendant® Temperature/Light logger, hereafter loggers) were used to assess flow state in the seven selected streams (Figure 1a), with between 10 and 15 loggers per stream (Figure S1; Pineda-Morante et al., 2022). These data loggers were installed in July 2018 and kept in the streams until December 2019. At each study site, a logger was submersed into the water to record water temperature (°C) and, for each stream, a logger was placed outside the water (e.g. hanging from a tree) to record air temperature (°C). The loggers recorded temperature at hourly intervals for 513 days. We calculated the daily mean temperature and compared the diurnal temperature variation of the installed loggers to assess if each site had enough water to cover the data logger (hereafter wet) or no surface water (hereafter dry) comparing air and instream temperaturesfollowing Gungle (2006). Those estimations were validated using field observations and time-lapse images taken for some streams. Finally, we used a logistic regression model including stream characteristics to correct the missing values generated due to loggers malfunctioning or loss due to climatic events (e.g. temperatures below 0°C, floods). Specific details about the model and the process of predicting missing data can be found in Pineda-Morante et al. (2022). The final dataset resulted in a table with the daily wet/dry values (rows) for each logger (columns) for each one of the monitored streams. In this table, a wet day with the presence of surface water flowing or as disconnected pools is represented by '1' and a dry day by '0'. This dataset constitutes the basic information from which we later calculated all the spatiotemporal indices. In this work, we used the entire 513-day period to calculate all the spatiotemporal indices. Using the same flow state database obtained with the data loggers, we also calculated several local hydrological metrics commonly used to characterise surface flow state in temporary streams (Arias-Real et al., 2021; Crabot et al., 2020; Pineda-Morante et al., 2022), such as the total number of dry days (TotDur), the number of changes from wet to dry days (TotNum) and the average number of dry days for each drying event (TotLeng).

2.3 | Building spatiotemporal matrices based on logger data

Spatiotemporal connectivity was obtained after building a spatiotemporal matrix, hereafter ST matrix, for each stream. Each row and column of the ST matrix corresponded to a monitored site with a data logger deployed at a specific time (Figure 1b). Therefore, the matrix was as big as many sites and times were monitored. Then, the ST matrix was filled with information regarding the two levels of connectivity that we assessed: spatial and temporal.

2.3.1 | Spatial connection

Spatial connections were built based on a defined stream network structure (Figure 1c). A directed structure follows an upstream–downstream direction (Figure S2) and an undirected structure implies that all nodes can be reached from everywhere (Figure S2). This structure was related to the dispersal modes of the organisms that we wanted to study. We assumed that passive dispersers would disperse mostly downstream by drift so they would mainly respond to a directed network. Contrarily, active dispersers that disperse by flight would mostly respond to an undirected network. In the two networks used to create the ST matrix, each node represented a monitored site and each link an effective connection between sites. If the node's flow state was considered as wet, the connection with other nodes was possible. Following the paths defined by the network, the number of reachable nodes from each monitored site was assessed (Figure 1c; Spatial). In the case that a monitored site was dry for that day, the connection with this dry site or throughout it was not possible.

2.3.2 | Temporal connections

Temporal connections were built in the same way as spatial links (Figure 1c; Temporal). All the possible connections within the network for a specific time were assessed based on the same network structure of the spatial ones. However, the temporal links were entered in the ST matrix on the following time step. In other words, we

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considered that the connection between the nodes on a given day would influence their connectivity on the following day (Figure 1c; T2 Temporal connections).

2.3.3 | Link and no-link values

For each node, all the spatial and temporal connections with its neighbours were filled with the values corresponding to what we defined as the *link* or *no-link* value (Figure 1c). The *link* value is the value that we give when dispersal can occur between two nodes. In this study, this occurs when the flow state is 1 in a pair of nodes and the path to reach them. Contrarily, the *no-link* value is the value given when dispersal is not possible or in our case, there is at least one 0 in the path between a given pair of nodes. *Link* and *No-link* values can be modified depending on how connected and unconnected sites are defined. These values can be modified in the function *spat_temp_index*, an R function created to calculate the spatiotemporal connectivity metrics (Cunillera-Montcusí, 2023), to define different scenarios. Therefore, the current framework allows for calibrating spatiotemporal connections between sites and can include any ecologically meaningful network structure or link interpretation (Cañedo-Argüelles et al., 2015; Phillipsen & Lytle, 2013).

2.3.4 | Binary and weighted scenarios

In this study, we designed four different scenarios from which we calculated *STcon* and *STconmat* to exemplify different possibilities of the current framework. Each scenario combined a different network structure, directed or undirected, and a different way of quantifying the *links* and *no-links* (binary and weighted). For the two **binary scenarios**, the directed binary (hereafter DirBin) and the undirected binary (hereafter UndBin), the *link* value was defined as 1 and the *no-link* value as 0. Therefore, *STcon* and *STconmat* were quantifying the number of times that each site was connected to its neighbours. For these scenarios, high values meant that a site was highly connected to its potential neighbours during the considered time.

For the other two **weighted scenarios**, the directed weighted (DirWei) and the undirected weighted (UndWei), we added an additional variable: the Euclidean distance between sites. Here, the *link* value was 0.1 and the *no-link* value was 1, and these values were multiplied by the distance between the two nodes. Thus, for the weighted scenarios, we assumed that the presence of water between two sites was facilitating dispersal. Consequently, when connection was possible, the Euclidean distance between two sites was reduced by 90% and when connection was not possible, the Euclidean distance between these two sites was preserved. Therefore, *STcon* and *STconmat* calculated for the DirWei and UndWei scenarios were assessing dispersal resistance. For these scenarios, high and low resistance values indicate that the connectivity between two sites is low and high respectively.

2.4 | Spatiotemporal connectivity measures

Once the ST matrix was filled with the corresponding *link* and *no-link* values, *STcon* and *STconmat* were calculated. Depending on the desired output, connectivity can be either calculated for each site individually resulting on the *STcon* measure, or as a pairwise matrix that incorporates the relation of each site with all the other pairs within the network, resulting in the *STconmat*.

The *STcon* corresponds to the average of all the spatial and temporal links for each individual node (Figure 1d). To calculate it, we summed all the times that a node had been connected to its neighbours both spatially and temporally, and later, we divided that value by the amount of time considered and the total potential neighbours that the individual node can have: $STcon = \sum_{Tx}(S_i + T_i)/Nneigh_i \times TTU$. Where $\sum_{Tx}$ is the sum of values through all considered times, *Si* and *Ti* are the spatial and temporal connections of a specific node *i* with its neighbours, *Nneigh* corresponds to all the potential neighbours that node *i* has and TTU is the total time units considered. Therefore, *STcon* represents an average value of how often an individual node has been connected to all their potential neighbours (Figure 1c). In the case of a directed network structure, the value is calculated considering each site's outflow path, which identifies the relevance of each site as a source of colonists for all their neighbours. As *STcon* is averaged by the total potential neighbours of each site, *STcon* can be compared within sites of the same system and among systems. For the *STconmat*, we summed the ST matrix in a unique pairwise matrix including all stream sites (Figure 1e). We then divided this pairwise matrix by the amount of time considered being $STconmat = [(S(i_1 - n_1)...n) + (T(i_1 - n_1)\cdots n)]/TTU$, where *S* and *T* are the spatial and temporal links for each pair of nodes, and TTU the total time units considered. Therefore, *STconmat* is averaging daily spatial and temporal connections for each pair of nodes. Both *STcon* and *STconmat* capture the spatiotemporal connectivity of each site during the studied period and their values are directly related to the values assigned to *link* and *no-link*.

2.5 | Testing the spatiotemporal connectivity measures

For each one of the four scenarios, DirBin, DirWei, UndBin and UndWei, we calculated *STcon* and *STconmat* for the entire monitored period of 513 days. We conducted a principal components analysis, PCA, including the *STcon* values for each scenario and the three local hydrological variables (TotNum, TotDur and TotLeng) to analyse how they were correlated based on the Euclidean distances between them, using *prcomp* function from STATS package (R-Core Team, 2019). We used the *adonis* function from VEGAN (Oksanen et al., 2010) to test if upstream–downstream position or stream ID were presenting similar values in this multivariate space using a permutational analysis of variance (PERMANOVA) and expecting that the high hydrological variability in the sampled streams would result in significant differences among them. In addition, we used

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Spearman correlations for the same set of variables to test their correlation and quantify the strength of their relationship.

As *STcon* and *STconmat* differ in their structure, the approach to characterise each stream was also different. First, we related *STcon* to the relative position of each monitored site within each stream network in an up- to downstream direction. Second, we analysed the differences in *STconmat* for each stream in a multivariate space through a non-metric multidimensional scaling (NMDS). As *STconmat* is a pairwise matrix, the values are equivalent to spatiotemporal distances between sites and can be represented in a bidimensional space.

Finally, to test *STcon* and *STconmat* against macroinvertebrate community's metrics, we used the period of 480 days between the start of the monitoring (26/07/2018) until the biological sampling campaign (18–21/11/2019) to build the spatiotemporal connectivity metrics. We considered this period for the four different scenarios (DirBin, DirWei, UndBin and UndWei). As the two network structures were related to different dispersal abilities (i.e. directed for aquatic passive and undirected for aerial active), we split the community matrix in these two dispersal groups based on available biological trait data (Pineda-Morante et al., 2022; Sarremejane et al., 2020; Tachet et al., 2010). We did not account for aquatic active and aerial passive dispersers because of their low abundance representing both around 12% of sample's total abundance. For both aquatic passive and aerial active dispersers, we calculated taxonomic richness, Shannon–Wiener diversity, relative dispersal traits abundance and compared them against STcon. We also calculated two beta diversity proxies: Jaccard, based on presence–absence, and Bray–Curtis, based on abundance data and compared them against STconmat. These metrics were calculated using the VEGAN package (Oksanen et al., 2010). Finally, linear mixed models were used to analyse the relationship between *STcon* and the calculated community metrics (taxonomic richness, Shannon–Wiener diversity, relative dispersal traits abundance) using the package LME4 (Wood & Scheipl, 2013) and considering stream identity as a random factor to control for variability between the different streams. We analysed all pairwise distance matrices with Mantel tests relating *STconmat* to the two community dissimilarity metrics (Bray–Curtis and Jaccard indices) using the VEGAN package (Oksanen et al., 2010).

3 | RESULTS

3.1 | Spatiotemporal connectivity and network structure

The selected streams showed strong differences in their spatiotemporal patterns in relation to network structure. In the binary scenarios DirBin and UndBin, where direct connectivity is quantified, both *STcon* (Figure 2a) and *STconmat* (Figure 2b) captured wet/dry patterns for each monitored site. For example, sites that dry more often and for longer durations had lower values of *STcon* for the DirBin and UndBin scenarios (Figure 2; site highlighted in red). When the network structure was directed (DirBin), the sites that were dry more often were impacting other sites located above them by reducing their *STcon* and *STconmat* values as these sites cannot connect to their downstream neighbours when an intermediate site is dry (Figure 2; DirBin, sites above the one highlighted in red). On the contrary, this effect was not observed for the undirected network structure (Figure 2; UndBin). In the weighted scenarios DirWei and UndWei (Figure 2), where *STcon* and *STconmat* quantified dispersal resistance, the highest values were found at upstream sites when there was a directed structure, DirWei, whereas the drier sites had the greatest values of *STcon* and *STconmat* in the undirected network UndWei (Figure 2; site highlighted in red). In weighted scenarios, *STcon* and *STconmat* captured the interaction between distance and drying so when a site contained water the distance between two sites decreased concomitantly with *STcon* and *STconmat* values. Contrarily, when a site was dry, the distance corresponded to the Euclidean distance between the sites leading to an increase of *STcon* and *STconmat* values. The complete dataset with *STcon* and *STconmat* values for the four scenarios and for all the monitored streams can be seen in Figure S3 and Table S1.

3.2 | Spatiotemporal connectivity and local flow state

When comparing the spatiotemporal indices with previously existing indices based on flow state data (i.e. TotDur, TotNum and TotLeng), we observed that there were negative correlations between the *STcon* and the TotDur (DirBin = −0.69 and UndBin = −0.65) and TotNum (DirBin = −0.51 and UndBin = −0.53) for both directed and undirected networks (Figure 3). Thus, higher values of *STcon* (i.e. high spatiotemporal connectivity) were associated with lower values of TotDur (i.e. total duration of drying events) and TotNum (i.e. frequency of drying events). Spearman correlation analysis highlighted these negative correlations (Figure S4). Finally, for the PERMANOVA analysis, we found significant differences across sites position in the PCA between streams and between site position within streams (i.e. upstream–downstream), and a significant interaction of both factors as expected (Figure 3).

3.3 | Stream spatiotemporal characterisation

STcon differed between streams and between upstream and downstream sections. For the binary non-weighted scenarios (Figure 4a; DirBin and UndBin), there was a general increase in *STcon* towards downstream sites meaning that downstream sites presented higher spatiotemporal connectivity (Figure 4a; DirBin), except for the M and SA streams (where no clear tendency was found). The indices followed an inverse trend in the weighted scenarios as dispersal resistance increased towards upstream reaches (Figure 4a; DirWei and UndWei), especially in the directed scenario (Figure 4a; DirWei). *STcon* captured drier sites impact on connectivity for directed networks (Figure 4a; DirBin and DirWei), which disappeared for

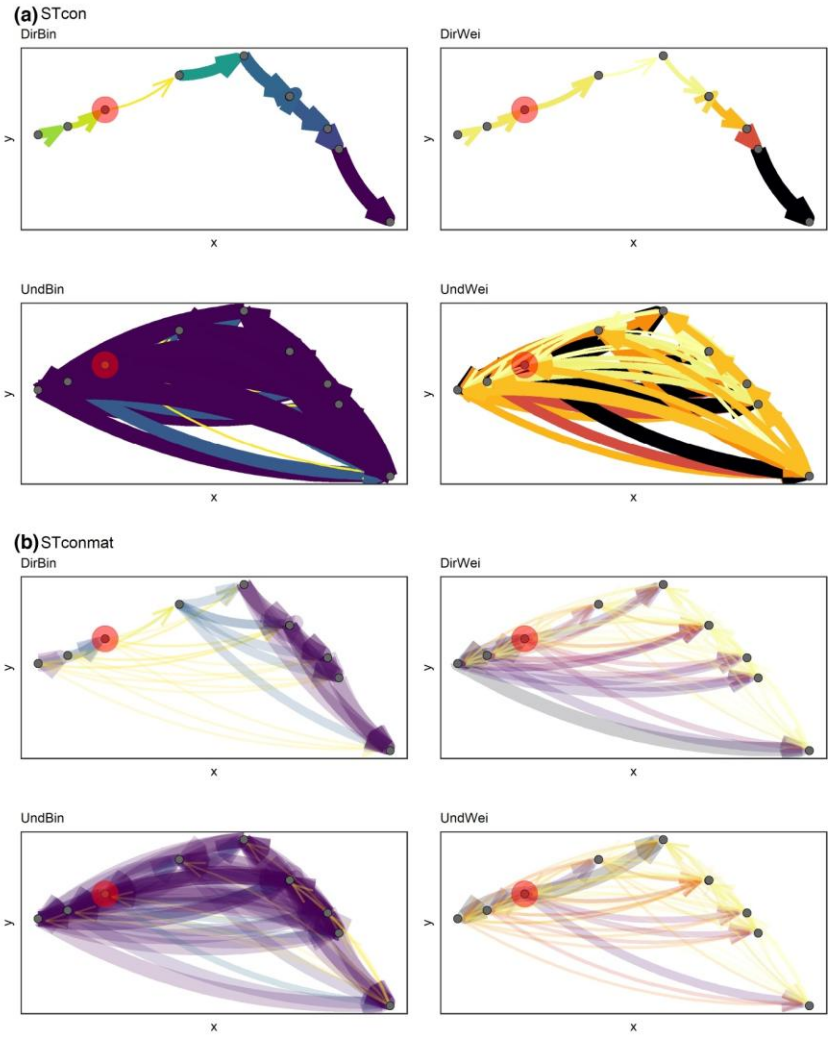


FIGURE 2 (a) *STcon* and (b) *STconmat* values for the Vall d'Horta stream (VH). Colour viridis gradient and size correspond to *STcon* and *STconmat* values. For DirBin and UndBin scenarios, purple/blue and bigger sized arrows indicate higher connectivity while yellow and smaller arrows indicate isolation. For DirWei and UndWei, grey/red and bigger sized arrows indicate higher dispersal resistance while orange/yellow and smaller arrows indicate less resistance. We highlighted in red the monitored site 3 just to ease its location in the network. All the data are calculated based on the whole monitored time window (513 days). See the other studied streams in Figure S3.

undirected scenarios (Figure 4a; UndBin and UndWei). The NMDS performed using the *STconmat* values (Figure 4b) illustrated the differences in spatiotemporal connectivity between sites within each of the streams. Widely dispersed sites (Figure 4b; VH stream) experienced a higher temporal variation in connectivity than clustered sites (Figure 4b; SC and C streams). STcon values for each individual stream and scenario are available in Figure S5.

3.4 | Testing spatiotemporal connectivity against macroinvertebrates communities

The linear mixed models showed several significant relationships between *STcon* calculated for each of the four scenarios and the three site-specific biodiversity metrics (taxonomic richness, Shannon–Wiener diversity and dispersal traits abundance) for the two dispersal groups (Figure 5a–c). *STcon* was negatively related to the richness of passive dispersers in the DirWei scenario, whereas *STcon* was positively related to the taxonomic richness of both passive and active dispersers in the UndBin scenario. Shannon–Wiener diversity of passive and active dispersers as well as the dispersal traits abundance of passive dispersers were positively related to *STcon* in the UndBin scenario. We also identified significant relationships between *STconmat* and community dissimilarity (Figure 5d,e). Passive and active dispersers responded significantly to both directed and undirected weighted scenarios, with Bray–Curtis dissimilarity positively related to *STconmat* in the DirWei and UndWei scenarios. Jaccard dissimilarity for passive dispersers was positively related to *STconmat* in the DirBin and UndBin scenarios. Contrastingly, Jaccard dissimilarity for active dispersers was negatively related to *STconmat* in the DirBin scenario. Finally, Jaccard dissimilarity for active dispersers was positively related to *STconmat* in the undirected weighted scenario (UndWei). All community metrics and model effect sizes can be found in Figure S6 and Table S2.

4 | DISCUSSION

We present a new methodological framework from which spatiotemporal connectivity can be calculated combining both spatial

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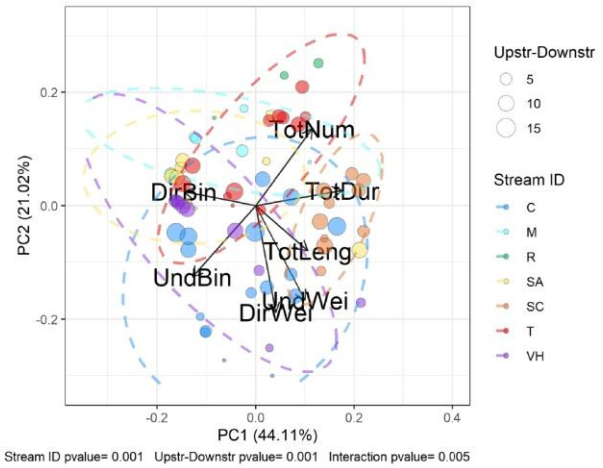


FIGURE 3 PCA plot for *STcon* indices for each one of the four scenarios: directed binary (DirBin), directed weighted (DirWei), undirected binary (UndBin), undirected weighted (UndWei) and the hydrological variables: the total duration of drying events (TotDur), the frequency of drying events (TotNum) and the average length of each drying event (TotLeng). All the data are calculated based on the whole monitored time window (513 days).

and temporal dimensions in one unique value. Our spatiotemporal connectivity measures use data obtained at a continuous time interval (e.g. minutes, days, months, years) and consider a particular spatial structure (i.e. dendritic network or undirected network in our case). We developed this approach using a high-frequency database of flow state where daily wet/dry values of seven streams were recorded for 513 days (Pineda-Morante et al., 2022). Past studies used daily monitoring to assess the spatial variation (i.e. wet or dry reaches) and/or to characterise temporal variation (e.g. seasonal changes) of river networks following a snapshot approach (Crabot et al., 2020; Pineda-Morante et al., 2022). Our framework allows merging spatial and temporal dynamics within the same connectivity value, while being adaptable to different temporal windows, monitoring studies and systems (e.g. months or years with datasets like Mouton et al., 2022; Perera et al., 2020; Ridl et al., 2018). In addition, the framework can incorporate non-binary connections based on, for example, distances, resistance (e.g. non-transitable areas), barriers (e.g. dams, forests) or dispersal pathways (e.g. wind or flow). Therefore, the proposed framework can be extended to any system presenting a spatial structure that has been monitored through time and with enough detail to build a network structure that captures fluxes of individuals, species, energy or matter.

The spatiotemporal connectivity measures developed here captured distinct network connectivity patterns. For example, they showed differences among headwaters and downstream reaches and accounted for how dry reaches acted as dispersal barriers. From a management point of view, such information could prove useful in defining stream typologies according to their spatiotemporal trends and associate different management practices to them (Bonada et al., 2020; Datry et al., 2014; Gallart et al., 2017). For example, when assessing temporary stream biological quality, one could assign relevance to the terrestrial and/or the aquatic biota according to stream spatiotemporal connectivity patterns (Steward et al., 2022). Furthermore, these spatiotemporal measures can be useful to understand how network connectivity determines the fluxes of energy, matter and organisms within the river (Cid et al., 2022; Gounand et al., 2018; Martensen et al., 2017; Uroy et al., 2021). Although we used high time frequency data loggers to obtain flow state data, the current framework could be applied to data coming from gauging stations (Monk et al., 2008; Stocks et al., 2021; Walker et al., 2016) or from hydrological models (Costigan et al., 2017; Döll & Schmied, 2012; Yu et al., 2020), which could be used to define the values assigned to the 'links' and 'no links' to assess connectivity. In this sense, instead of binary values of connectivity, we could incorporate flow, wind velocity or even permeability values between sites as far as these data would be available at the same temporal resolution. In terms of drying patterns, we found that *STcon* values showed negative correlations with TotNum, TotDur and TotLeng in binary scenarios. These correlations were already expected because both *STcon* and hydrological metrics are quantifying intermittence similarly. For example, high values of *STcon* for the scenario DirBin were negatively correlated with the duration of dry events (TotDur), meaning that highly spatiotemporally connected sites are also the ones which generally have shorter dry events. Nevertheless, these results also suggest that by only using the *STcon*, we would be already capturing drying patterns and the usage of local hydrological metrics such as TotNum, TotDur and TotLeng would not be necessary. Overall, *STcon* and *STconmat* present a great potential to characterise spatiotemporal patterns in streams, representing a powerful tool to guide conservation and restoration actions given the importance of spatiotemporal connectivity for population/community dynamics and ecosystem functioning (Chase et al., 2020; Cid et al., 2020).

Macroinvertebrate communities significantly responded to *STcon*, with different responses for active aerial and passive dispersers, thereby suggesting that our indices captured their dispersal dynamics. Species richness and Shannon diversity increased with greater spatiotemporal connectivity in the UndBin scenario, which seems to quantify the impacts of drying on aquatic communities (Crabot et al., 2020; Pineda-Morante et al., 2022). Furthermore, passive dispersers responded negatively to the DirWei scenario, with the communities experiencing higher resistance to dispersal showing lower richness. Thus, drier and more distant sites had fewer taxa, as seen in other studies (Brooks et al., 2018; Elliott, 2002; Fonseca, 1999; Kappes & Haase, 2012; O'Hop & Wallace, 1983). The differences between active and passive dispersers regarding dispersal resistance highlighted the relevance of overland pathways for active dispersers (Bogan & Boersma, 2012; Cañedo-Argüelles et al., 2015; Phillipsen & Lytle, 2013). Passive dispersers abundance responded positively to *STcon* in the UndBin scenario, indicating that perennial sites would tend to accumulate higher abundances of this dispersal group (Hershkovitz & Gasith, 2013). Interestingly, the fact that passive dispersers appear related to undirected scenarios could be linked to the small distances between sites, which could be overcome during

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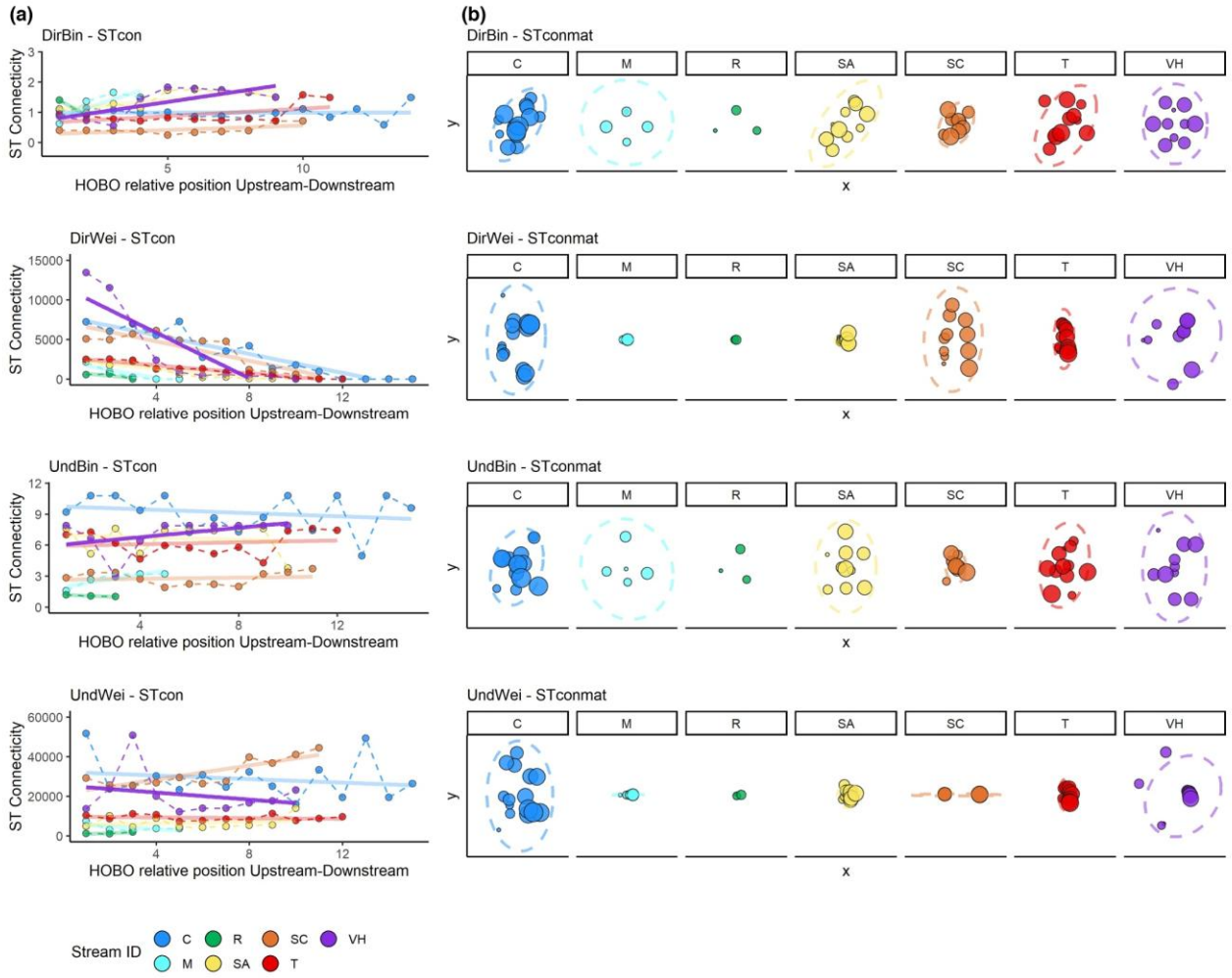


FIGURE 4 (a) Monitored streams *STcon* values (*y*-axis) ordered along their relative position from upstream to downstream (*x*-axis). (b) NMDS with *STconmat* values of each monitored stream. More compact clouds of points mean a greater similarity between sites in terms of spatiotemporal connectivity (i.e. all sites dry similarly). More dispersed clouds indicate a greater variability within the stream in terms of drying. Circle size indicates upstream (smaller) or downstream (bigger) site position. Each plot row corresponds to one of the four scenarios: directed binary (DirBin), directed weighted (DirWei), undirected binary (UndBin), undirected weighted (UndWei). All the data are calculated based on the whole monitored time window (513 days). Individual stream *STcon* plots can be found in Figure S5.

wet conditions (Kappes & Haase, 2012; Razeng et al., 2016) and/or through dispersal vectors such as wind (Cáceres & Soluk, 2002; Epele et al., 2021; Vanschoenwinkel, Gielen, et al., 2008) or animals such as birds and terrestrial mammals (Figuerola & Green, 2002; Frisch et al., 2007; Vanschoenwinkel, Waterkeyn, et al., 2008).

Regarding *STconmat*, both weighted scenarios (DirWei and UndWei) had positive relationships with dissimilarity indices suggesting that dryer and more distant sites harboured more unique communities. Such pattern was probably linked to a combination of dispersal limitation and harsher environmental conditions imposed by drying events leading to higher dispersal resistance and the development of distinct communities (Bonada et al., 2020; Steward et al., 2022; Valente-Neto et al., 2020). On the contrary, the binary scenarios (DriBin and UndBin) resulted in contrasting patterns depending on the dispersal group for presence–absence data. In this scenario, passive and active dispersers were positively and negatively (respectively) related to spatiotemporal connectivity. This suggests that more connected sites tend to have passive dispersers' communities with different species composition while active dispersers' communities tend to be more similar. Thus, when sites are hydrologically connected for longer periods of time the communities of passive dispersers tend to diverge in their composition. Since many passive dispersers are lost with drying and it takes time for them to recolonise rewetted streams (Bogan & Boersma, 2012; Kappes & Haase, 2012), more perennial sites tend to diverge in their composition as greater species number promotes niche partitioning between habitat patches. In the case of active dispersers, as the spatiotemporal connection between sites increases the communities become more homogeneous due to mass effects (Gansfort et al., 2021; Heino et al., 2015; Sarremejane et al., 2017).

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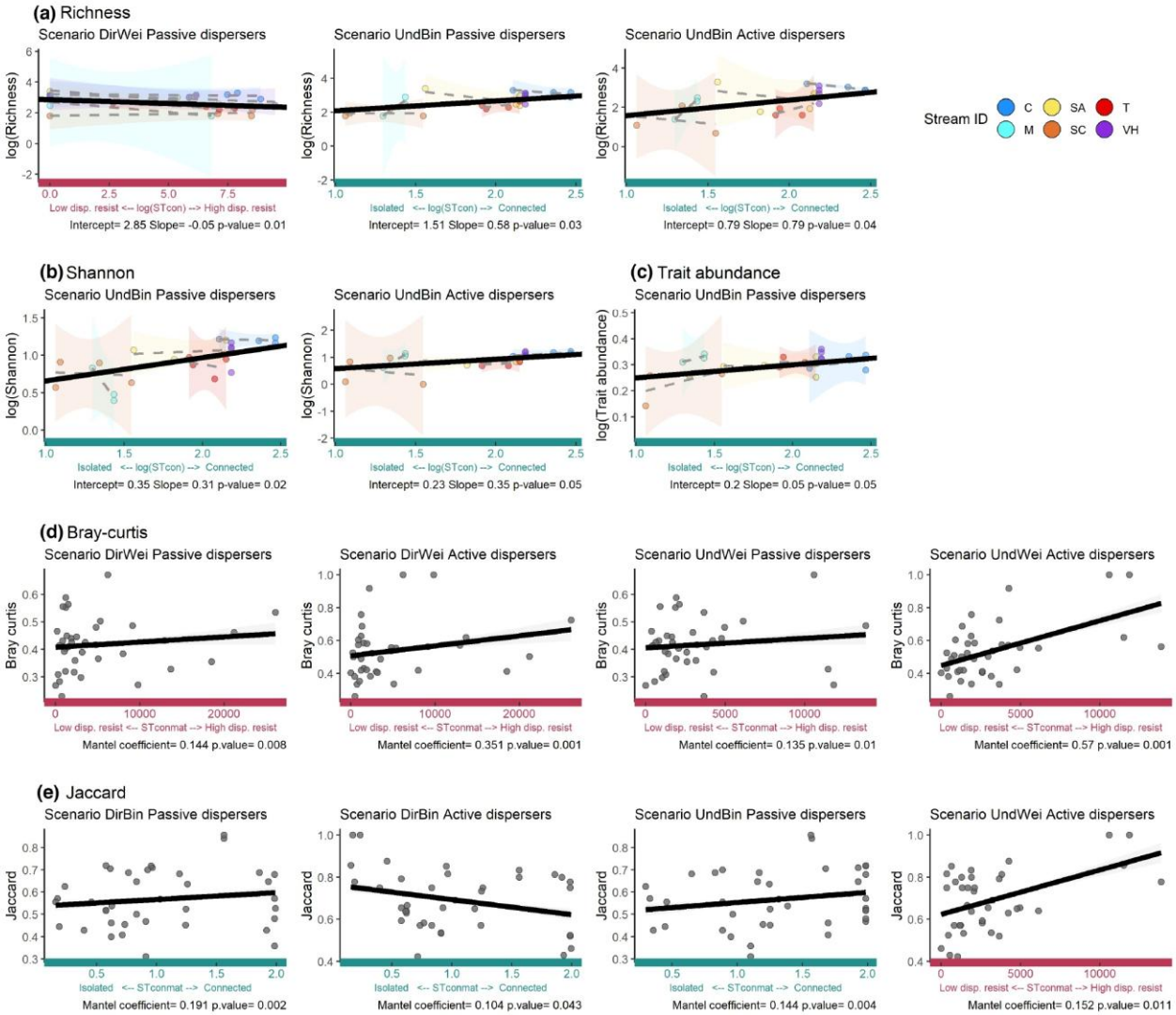


FIGURE 5 Significant linear mixed models between *STcon* (a–c) and *STconamt* (d, e) and biological metrics calculated for passive aquatic and active aerial dispersers. (a) Richness, (b) Shannon, (c) Trait abundance, (d) Pairwise metrics (i.e. Jaccard and Bray–Curtis indices). Note that *STcon* values for DirBin and UndBin scenarios (green *x* axes) quantify how much a site has been connected, whereas values for UndWei and DirWei scenarios (red *x* axes) quantify the dispersal resistance of each site. All the data are calculated based on the time window between the beginning and the sampling date (480 days). See all other results for all the metrics and scenarios in Figure S6.

Overall, although the study sites were close to each other (mean distance between sites within a stream was around 1 km and all the streams were located within an approximate range of 100 km^2), the macroinvertebrate communities showed significant responses to spatiotemporal connectivity. This highlights the importance of quantifying spatiotemporal connectivity in highly dynamic systems such as temporary streams (Cid et al., 2020; Datry et al., 2016). Finally, *STconmat* proved useful to incorporate pairwise metrics (e.g. beta diversity calculated with Jaccard or Bray–Curtis indices) within spatiotemporal analysis of temporary systems (Khattar et al., 2021; Legendre & De Cáceres, 2013), which is key to better capture assembly processes or improve management of spatiotemporally variable ecosystems (Bo et al., 2020; Boyé et al., 2019; Lamy et al., 2015; Ruhí et al., 2017; Sobek et al., 2009).

In dendritic networks such as fluvial systems, connectivity has been acknowledged for a long time as a key driver of diversity and functioning at population, community and/or ecosystem levels (Fullerton et al., 2010; Vannote et al., 1980). Indeed, several indices have been developed to assess the degree of fragmentation generated by dams (Baldan et al., 2022; Cote et al., 2009) or connectivity indices accounting for longitudinal and lateral connectivity (Rivers-Moore et al., 2016). However, the inclusion of both spatial and temporal connectivity facets has been poorly explored so far (Fullerton et al., 2010; Uroy et al., 2021). We believe that the proposed framework represents a first step towards a better inclusion

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of spatiotemporal connectivity in general ecology, extending beyond our study case and being potentially applied to any ecosystem. For example, our measures could be useful to incorporate network connectivity into biodiversity conservation using systematic planning approaches (Hermoso et al., 2012). Also, *STcon* and *STconmat* can serve to assess the effectiveness of restoration measures as the community response to habitat restoration will depend on spatiotemporal connectivity to some extent (Cid et al., 2022). At the same time, it complements other methods like remote sensing (Bishop-Taylor et al., 2017) by specifically quantifying spatiotemporal dynamics and incorporating multiple time windows.

Overall, the proposed framework has a strong potential to be used at several temporal scales (e.g. days, months, years) and across different levels of biological organisation (e.g. populations, communities, ecosystems). Besides flow state, other metrics that vary spatiotemporally and define connectivity such as land use could be used (Firmiano et al., 2021). Furthermore, the current function could be extended to include other network structures by defining the number of neighbours at which dispersal is considered effective, either setting distance thresholds or dispersal decay with distance (Borthagaray et al., 2015; Muneepeerakul et al., 2008; Radinger & Wolter, 2014). In addition, another aspect that could be further developed within this framework is related to antecedent spatiotemporal connectivity (i.e. the temporal scale considered by *STcon* and *STconmat*). In the approach presented here, the ST matrix considers the spatial structure from the previous day, thereby considering two time steps (i.e. what happened on day 1 has an impact on day 2 but not on day 3; Uroy et al., 2021). Both the defined network structure and the time span are case-specific and could be adjusted to the characteristics of the studied system and the focal organism (e.g. organism life span, a pollution event). So far, the main limitation of this framework is still the lack of databases that include high spatial and temporal resolutions at relevant scales. Furthermore, the adaptation of the framework to different ecosystems and network structures should be made with caution. For example, the parameters used to calculate *STcon* and *STconmat* need to be defined for each system, because the results can be considerably different depending on the designed scenario. Nonetheless, the adaptability of our framework suggests that it can still be expanded to incorporate more facets of the interaction between spatial and temporal processes. In this regard, we provide the R code (see Supporting Information, Cunillera-Montcusí, 2023) to facilitate and promote future studies.

The quantification of spatiotemporal connectivity is key for any ecosystems subjected to high seasonal and/or interannual variability, or affected by natural (e.g. wildfires, hurricanes, floods, droughts) or human-driven disturbances (e.g. invasive species, point source pollution). Moreover, environmental conditions are becoming globally harsher and more variable, with an expected increase in the frequency, duration and variability of natural and human-driven disturbances (IPCC, 2022). Consequently, we need to further explore and develop frameworks that can capture such variability in space and time (Uroy et al., 2021). To guide future conservation and management actions (Chase et al., 2020; Cid et al., 2020; Schiesari et al., 2019). We encourage the inclusion of spatiotemporal connectivity in future studies in the field of meta-system ecology, restoration ecology and biodiversity conservation to test and challenge our method so we can gain insights into spatiotemporal processes.

AUTHOR CONTRIBUTIONS

David Cunillera-Montcusí conceptualised the framework and leaded the paper in the analysis, writing, interpretation and edition. Miguel Cañedo-Argüelles and Núria Bonada conceptualised the study, collected the data, interpreted the results, wrote and edited the manuscript. Núria Cid collected the data, interpreted the results, wrote and edited the manuscript. José María Fernández-Calero, Sebastian Pölster, Roger Argelich and Pau Fortuño collected the data and edited the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare none.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

All data used in this work as well as the corresponding code and the function to calculate STcon and STconmat values are available at Cunillera-Montcusí (2023) and a tutorial to illustrate the usage of the function *spat_temp_index* is available at https://cunillera-montcusi.github.io/Quantifyinig-SpaTem-connectivity/.

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SUPPORTING INFORMATION

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Figure S1: Maps of the main study area located in Sant Llorenç del Munt i l'Obac natural park and of each one of the monitored streams with the sampling locations and data loggers deployed to monitor flow states. Castelló (C), Mura (M), Rellinars (R), San Ana (SA), Santa Creu (SC), Talamanca (T) and Vall d'horta (VH). Note that the number of data loggers deployed in each stream varied due to differences in stream morphology and the number of reaches that were normally dry in that stream.

Figure S2: Network representation of all monitored streams C, M, R, SA, SC, T and VH respectively. Two network structure were considered: (a) directed, which was used for the DirBin and DirWei scenarios and (b) undirected, which was used for the UndBin and UndWei scenarios.

Figure S3: STcon and STconmat graphical representation for the seven monitored streams: C, M, R, SA, SC, T and VH respectively. The four calculated scenarios are represented in each part: DirBin; DirWei; UndBin; UndWei. The viridis colour gradient and arrow size illustrates the corresponding values of STcon and STconmat. For DirBin and UndBin scenarios a gradient from purple/blue to green/yellow indicates high to low spatiotemporal connectivity respectively. For DirWei and UndWei a gradient from grey/purple to red/yellow indicates greater or lower spatiotemporal resistance to dispersal. Binary and Weighted scenarios quantify differently connectivity as they focus on only number of connections or how big is the distance to be connected.

Figure S4: Spearman correlation matrix including STcon values calculated for the four scenarios DirBin, DirWei, UndBin, and UndWei. Spearman rho values for each pair are represented in the upper triangle section of the plot. Pairs with a significant correlation were indicated with a black asterisk next to Spearmen correlation rho.

Figure S5: STcon values for each stream and for each one of the four scenarios. (a) DirBin, (b) DirWei, (c) UndBin, (d) UndWei. Note that these plots represent the same values as Figure 4 but here they are shown for each individual stream.

Figure S6: All community metrics results for the six monitored streams from which enough biological data was obtained (the R stream was not considered here). Each box corresponds to an individual community metric: (a) Taxonomic Richness, (b) Shannon Wiener-diversity, (c) Trait abundance, (d) Jaccard dissimilarity, and (d) Bray Curtis dissimilarity. In each box, the first row of plots correspond to active dispersers (group of trait f4) and the second row of plots to passive dispersers (group of trait f1). Note the *X* axes of plots must be read carefully for each scenario: for DirBin and UndBin higher STcon values mean higher spatiotemporal connectivity whereas for DirWei and UndWei higher STcon values mean greater spatiotemporal resistance to dispersal.

Table S1: STcon and STconmat values for each one of the monitored sites (Codi_HOBO) with their corresponding coordinates, the ID of the stream, the position in following an Uo- to Downstream (DtoU) for each of thef four scenarios considered: DriBin, DirWei, UndBin and UndWei.

Table S2: Linear mixed models results for each one of the community metrics calculated: (a) Taxonmic richness; (b) Shannon-Wiener index; (c) Trait abundance; (d) Jaccard dissimilarity and (e) Bray–Curtis dissimilarity. For each metric, the results for each one of the four scenarios (DirBin, DirWei, UndBin, UndWei) is reported together with the dispersal group (Active and Passive dispersers). Note that significant results are highlighted in bold.

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RESEARCH ARTICLE

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Integrating spatiotemporal hydrological connectivity into conservation planning to protect temporary rivers

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Abstract

1. Temporary rivers (TRs) have been largely overlooked in conservation assessments. Because TRs are such dynamic ecosystems, spatial and temporal aspects of their hydrology and ecology need to be taken into account when designing conservation plans.
2. The aim of this paper is to propose a set of recommendations that could be useful for managers to do this, using seasonal diatom and macroinvertebrate data from north-eastern Spain as a case study. Beta diversity was partitioned into local and species contributions to beta diversity (LCBD and SCBD). Additionally, priority conservation sites covering the spatial distribution of all species were identified using Marxan and the selection frequency (MSF) of the sites served as a measure of the relative irreplaceability of each site.
3. Using both approaches (beta diversity and Marxan), the effects of changing spatiotemporal connectivity and habitat heterogeneity on the selection and prioritization of sites to be conserved were assessed.
4. It was found that LCBD and MSF ranged widely both in space and time. However, LCBD and MSF were weakly related. Marxan adequately represented all taxa by selecting a few sites, while LCBD selected communities with higher differentiation but not necessarily those with rare species. In addition, SCBDs assigned low values to rare taxa, thus care must be taken when using this index for conservation planning.
5. Spatiotemporal connectivity and local habitat heterogeneity played a critical role at the regional and local scales, driving site prioritization.
6. Overall, we recommend: 1) monitoring multiple hydrological phases to encompass the different community types and capturing total diversity; 2) using Marxan and LCBD in combination, to benefit from their complementary insights; and 3) integrating spatiotemporal isolation and habitat heterogeneity into conservation

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plans, since they are the main drivers of community variation over space and time in TRs.

KEYWORDS

aquatic macroinvertebrates, conservation planning, diatoms, habitat connectivity, habitat heterogeneity, local contribution to beta diversity, Marxan, species contribution to beta diversity, temporary rivers

1 | INTRODUCTION

Preserving freshwater biodiversity is one of the most pressing global challenges of our time (IPBES, 2019). Pressures on freshwater biodiversity and freshwater habitats have exponentially increased during the last decades, mainly due to habitat destruction, fragmentation, pollution, invasive species, and overexploitation (Albert et al., 2021; Harrison et al., 2018). Consequently, freshwater species are disappearing at alarming rates (over 83% have become extinct since 1970), faster than their marine and terrestrial counterparts (Harrison et al., 2018). This is expected to worsen in the future, as a result of climate change and increasing human water demands (Dudgeon, 2019). Within this context, there is an urgent need to improve current conservation efforts to address the particular management needs of freshwater ecosystems, mostly overlooked under current conservation priorities (Hermoso et al., 2016).

Temporary rivers (hereafter TRs) are generally described as watercourses that stop flowing in both space and time (Acuña et al., 2014; Datry, Bonada & Boulton, 2017), and are also known as non-perennial, intermittent, ephemeral, or episodic. TRs are one of the most common freshwater habitats in the planet (Messager et al., 2021), and they are expected to become even more widespread in the future because of water scarcity (Kumar et al., 2014; Mekonnen & Hoekstra, 2016; van Vliet et al., 2021). TRs are characterised by shifting wet and dry phases (Gallart et al., 2012), which change spatiotemporal hydrological connectivity (Cunillera-Montcusí et al., 2023), constraint species dispersal, and affect metacommunity dynamics (Cañedo-Argüelles et al., 2020). This dynamism can have challenging implications for the conservation and biomonitoring of TRs (Datry et al., 2023) because available habitats and species to be potentially protected and/or used as bioindicators change dramatically in space and through time. Additionally, TRs are among the most damaged and vulnerable freshwater habitats in the world (Datry et al., 2014; Fovet et al., 2021; Skoulikidis et al., 2017). Despite some efforts have been done in some parts of the world (e.g., partial recognition in the EU Water Framework Directive and Habitats Directive; Fritz et al., 2017), they still remain neglected by policy-makers (Datry et al., 2023; Leigh et al., 2015), and conservation plans have largely ignored them (Bruno et al., 2022). The few existing studies on the conservation of TRs argue that priority sites for conservation can vary over time in these ecosystems (Rodríguez-Lozano et al., 2023; Ruhí et al., 2017). For example, when present, disconnected pools might play a vital role as biodiversity refugia during the dry season (e.g., fish or amphibians) (Bonada et al., 2020; Hermoso et al., 2013), or can be used as stepping-stones habitats, favouring connections across the disconnected river network (Datry, Boulton, et al., 2017; Sánchez-Montoya et al., 2022). However, the integration of spatiotemporal changes of TRs into conservation plans remains neglected.

There are different conservation approaches that could be used to select and prioritise sites to be preserved. One of them is to partition beta diversity to obtain the relative contribution of sites or species as in Legendre & De Cáceres (2013). This framework allows assessing the relative contribution of each sampling unit to beta diversity, that is, local contribution to beta diversity (LCBD), and the relative contribution of each species to beta diversity (SCBD). Considering a set of sites (i.e., regional scale), LCBD is an indicator of the biological uniqueness of each site, that is, a very different community composition compared with the regional mean. Thus, large values of LCBD could be considered as indicators of priority sites for conservation (high LCBD and high alpha diversity) or species-poor sites with restoration needs (high LCBD and low alpha diversity) (Legendre, 2014; Legendre & De Cáceres, 2013). The LCBD value identifies relatively unique communities in terms of their community differentiation, although not necessarily the communities with the rarest species. Therefore, setting conservation priorities based only on LCBD could lead to underrepresenting these rare species, which are often the most threatened ones. Importantly, LCBD values can change over time, reflecting metacommunity dynamics processes (i.e., habitats that may operate as sources, or sinks, to whole river networks) (Ruhí et al., 2017). In turn, SCBD indicates species with high variation in abundance between different sites in a region. Usually, species with high SCBD values have a large, or moderate, occupancy across sites and high abundance, resulting in the most important species contributing to the uniqueness (LCBD) of the different sites (Heino & Grönroos, 2017). These beta partitioning approaches have increasingly been used for identifying conservation sites based on different organisms, such as diatoms and macroinvertebrates (Heino & Grönroos, 2017; Rodríguez-Lozano et al., 2023; Vilmi et al., 2017), fish (Xia et al., 2022), terrestrial arthropods (Sánchez-Montoya et al., 2020), and terrestrial plants (Kirk et al., 2021).

An alternative approach for selecting and prioritising sites to be conserved are systematic planning (Margules & Pressey, 2000), which have been used in rivers (Linke et al., 2011) and, only recently, in TRs (Bruno et al., 2022). Among these tools, one of the most widely used

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is Marxan (Ball et al., 2009), which relies on the principle of complementarity to find the best combination of sites to be protected to meet a set of conservation targets at the minimum cost. Unlike LCBD-based approaches, Marxan may be used to identify priority sites based on a specific predefined target, rather than just considering sites very far from the median community composition in a regional pool. Thus, Marxan-based conservation planning may be particularly beneficial when community composition (and thus centroids) vary strongly over space and time and the main conservation target is total regional gamma diversity.

In this study, we aim to propose a set of recommendations that can be useful for managers when designing conservation plans in TRs. To do so, the above-mentioned conservation prioritisation approaches were used to identify priority conservation sites in TRs, and to assess the effects of changing spatiotemporal connectivity on the selection and prioritisation of sites. Since connectivity plays an important role in structuring aquatic communities in TRs (Cid et al., 2022), the focus was on two groups of organisms with contrasting dispersal abilities: diatoms and macroinvertebrates. Diatoms are widely dispersed (Bogan et al., 2017; Kristiansen, 1996) by water flow, wind, or animal vectors such as mammals and birds (Leone et al., 2014; Liu et al., 2013; Manning et al., 2021; Romero et al., 2003). Macroinvertebrates have two main dispersal modes (Bilton et al., 2001): i) active dispersers (e.g., adult dragonflies) can reach very long distances and they usually fly oriented upstream (Bogan & Boersma, 2012; French & McCauley, 2019); whereas ii) passive dispersers (e.g., larvae of non-biting midges) might drift downstream (Brown et al., 2011; Sarremejane et al., 2020) or restrict their movements to the benthic zone (Kappes & Haase, 2012).

Firstly, we hypothesised that priority conservation sites and species should change across seasons in response to changes in hydrological conditions, because drying determines biodiversity and habitat availability (Leigh & Datry, 2017; Ruhí et al., 2017). This would suggest that conservation actions should be adaptive and priority sites to be conserved should vary in space and time. Secondly, we hypothesised that both conservation approaches mentioned above are complementary. Whereas LCBD can identify sites with unique species composition, systematic planning tools are based on the complementarity principle and, therefore, these sites might not necessarily be selected. In addition, SCBD is not designed to select rare species but those that occur in a moderate number of sites in high abundances or have narrow niche breadth (Pozzobom et al., 2020; Xia et al., 2022), whereas rare species could be used as a selection criteria in systematic planning tools. Thirdly, we hypothesised that environmental drivers behind selected sites could inform us whether the conservation of local habitat or spatiotemporal connectivity should be preserved (or enhanced) to ensure current conservation status. For example, when local habitat variables are relevant, measures to enhance habitat diversity or to improve water quality should be implemented, whereas if drying is important, measures should consider solutions that reduce its effects and increase connectivity (Datry et al., 2015).

2 | METHODS

2.1 | Study area

The study was carried out in the Sant Llorenç del Munt i l'Obac Natural Park, a 140 km^2 protected area in northeast Spain with a typical Mediterranean climate (i.e., dry summers and heavy rains during autumn). The geology of the area is mainly karstic with permeable substrates (Anglés et al., 2017), but with abundant exposed bedrock in some areas. The flora of the natural park is typically Mediterranean, dominated by evergreen oaks (*Quercus ilex*) and several species of pines (*Pinus halepensis*, *P. nigra*, and *P. sylvestris*). Some of the main species of shrubs are the common box (*Buxus sempervirens*) or heath species (*Erica* spp.). Two species of native barbels (*Barbus haasi* or *B. meridionalis*, depending on river basin) inhabit the study area, as well as the native *Squalius laietanus*, and the invasive exotic cyprinid (*Phoxinus septimaniae* x *P. dragarum)* (Corral-Lou et al., 2019). Thirty sites were sampled distributed among seven TRs: Vall d'Horta (H), Sanana (SA), Santa Creu (SC), Rellinars (R), Talamanca (T), Castelló (CA) and Mura (MU). These TRs covered a wide hydrological gradient, with Flow Permanence values (i.e., percentage of days with wet reaches in each site, FP) ranging from 33.14% to 99.80% for the 513 monitored days (from July 2018 to December 2019) (see Supporting information S2 for all FP values) (Pineda-Morante et al., 2022). The samples were collected once per season during 2019: winter (January), spring (May), summer (July), and autumn (November).

2.2 | Diatom and macroinvertebrate data

Diatoms were sampled by brushing the surface of three submerged stones with a toothbrush. The samples were preserved in the freezer using 70% ethanol. Then, organic matter was removed with hydrogen peroxide at 100 °C, rinsed with distilled water and prepared permanent slides with Naphrax ®. Afterwards, diatoms were identified to species level with a DIC Polyvar Zeiss microscope with a built-in camera, counting a minimum of 500 diatom valves per sample. Macroinvertebrates were collected along 100 m reaches using a 250 μm mesh size hand net by kicking all the available habitats. Before sampling, each studied reach was carefully inspected to identify all the available habitats based on expert judgement (including different items, such as substrate type, flow conditions, and aquatic vegetation). The samples were preserved in 96% ethanol and stored them in the freezer until their sorting and identification. Likewise, all macroinvertebrates were identified and counted using a binocular stereoscope to the lowest possible taxonomic resolution (usually genus). Once all diatoms and macroinvertebrates were identified and counted, a logarithmic transformation was applied (log x + 1) to abundances values of each taxon.

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2.3 | Environmental and hydrological data

The abiotic variables were measured once per reach in each season. Dissolved oxygen (DO mg/L), oxygen saturation (DO %), water temperature (T °C), pH, conductivity (EC µS/cm), specific conductivity (SPC µS/cm) and total dissolved solids (TDS mg/L) were measured using a multiparameter probe (YSI Professional plus Multiparameter Probe). A characterisation of the habitat heterogeneity was also conducted by calculating the "Fluvial Habitat Index" (IHF) (Pardo et al., 2002), which accounts for the embeddedness in riffles and sedimentation in pools (B1), the frequency of riffles (B2), the substrate composition (B3), the flow velocity and depth regimes (B4), the percentage of shade in the channel (B5), the presence of habitat heterogeneity elements (e.g., leaf litter, branches and woods, or roots) (B6) and the aquatic vegetation coverage (B7). Presence/absence of water was registered from July 2018 to December 2019 using temperature data loggers deployed at each sampling site (Pineda-Morante et al., 2022). This information was used to calculate different hydrological metrics: the total number of dry days or duration of drying events (hereafter TotDur), the frequency of drying events or number of drying events (hereafter TotNum) and the average length of each drying event (hereafter TotLeng) (Arias-Real et al., 2021; Crabot et al., 2020; Pineda-Morante et al., 2022). In addition, the flow permanence was calculated as the percentage of the days that reaches were wet along two different time windows: 50 days before each sampling campaign (FP50), and 100 days before each sampling campaign (FP100). Two different time windows were considered, since organisms with different life cycles will be affected differently depending on when and how long sites were dried.

A presence/absence of water dataset was utilized to calculate spatiotemporal hydrological connectivity variables (hereafter STcon) for each sampling site, and considering the two scenarios proposed in Cunillera-Montcusí et al. (2023): a binary scenario (i.e., connection between sites as 1 and no connection as 0) and a weighted scenario (i.e., connection between sites weighted by the Euclidean distance between them). In addition, two types of network structures were considered for each of these two scenarios: directed (i.e., with an upstream to downstream directionality) and undirected (i.e., where all sites are connected between each other). Four STcon values were finally obtained: directed and binary, undirected binary, directed weighted and undirected weighted (DirBin, UndBin, DirWei, and UndWei, respectively). For binary scenarios, higher STcon values imply that a site has been highly connected with all its potential neighbours during the monitored time (i.e., high connectivity), whereas weighted scenarios with higher values imply that a site has been highly disconnected during the monitored time and it is located in an isolated position from its neighbours (i.e., high dispersal resistance). As distances between sampling sites for each stream were small, and streams were relatively straight, Euclidean distances could be used to weight both Binary and Undirected scenarios. See more details about STcon in Cunillera-Montcusí et al. (2023).

2.4 | Beta diversity partitioning: LCBD and SCBD

Community uniqueness results from partitioning beta diversity indices between sites (LCBD) and species (SCBD). LCBD calculates the uniqueness of the community across several sites and indicates how far each individual site is with respect to the mean centroid sample. The sum of the LCBDs across all sites equals 1, being the highest values of LCBD corresponding to communities with different species composition (Legendre, 2014). Then, sites with significantly different LCBD values were identified (p-value < 0.05 after Holm correction for multiple comparisons; Borcard et al., 2011; Holm, 1979). In order to identify sites with significant LCBD values, a Holm-corrected test was used (Holm, 1979). To explore the temporal variation of selected sites with high LCBD, LCBD values for each season were plotted. Moreover, SCBD calculates the species contribution to beta diversity, which represents the relative contribution of each taxa to beta diversity. For this, a "rose plot" was plotted to visually explore the different contributions of each taxa to beta diversity and the relationship with occupancy and abundance. The "beta.div" function (Legendre & De Cáceres, 2013) in the R package "Adespatial" (Dray et al., 2022) was used to calculate LCBD and SCBD, and the "ggplot" function in the ggplot2 v. 3.4.1 (Wickham, 2016) to plot the results of the analyses.

2.5 | Systematic planning tools: MSF

To identify a minimum set of sites that collectively achieve a coverage target, the systematic planning tool Marxan was used (Ball et al., 2009). Marxan relies on a simulated annealing optimization algorithm to identify a minimum set of sites that meet user-defined targets at a minimum cost. A representation target of 20% of the sites where each species was found was set (i.e., occupancy of each specie*0.2), keeping a minimum target of 1 for those taxa that were present in less than five sites. Given the high rate of taxa turnover in our dataset, with most taxa restricted to a few sites, high representation targets could only be achieved for all taxa at the same time by selecting almost all sites simultaneously. To address this problem, a bootstrap analysis was conducted (Bruno et al., 2022), by attempting to achieve the targets for a given proportion of all taxa each time. For this, 1,000 random samples were selected containing 50% of the taxa from the total pool and identified the minimum set of locations to cover the representation targets explained above. This decision was based on the work of Bruno et al. (2022), who tested three different thresholds (50%, 75%, 90%) and found that the results were consistent across the different bootstrap thresholds used. Given the lack of estimates of conservation costs across the study area, a constant cost across all locations were used (e.g., Hermoso et al., 2021) and no connectivity penalty measure was included, similar to Bruno et al. (2022). With this configuration, Marxan was ran 100 times, using a million iterations each, and kept the solution with the lowest objective function score across those runs as our best solution, for each of the bootstrap selection of taxa. All best solutions

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were summarised by calculating the selection frequency of each location across the 1,000 solutions, calling this variable Marxan Selection Frequency (MSF). In this way, the irreplaceability of each site at representing the taxa was measured, regardless of the particular combination of taxa selected in each of the 1,000 bootstraps. As previously described above for LCBD, MSF values for each season were plotted to explore changes in space and time.

Finally, in order to compare results obtained by LCBD and MSF approaches, taxa richness per site was calculated using the "vegan" package (Oksanen et al., 2022), and compared values among seasons using a Kruskal-Wallis test. In case of differences between seasons, a Mann–Whitney-Wilcoxon test was conducted.

2.6 | Environmental and hydrological models

Finally, all predictor variables were normalised (i.e., water chemistry, hydrology, habitat, spatiotemporal connectivity) using the "scale" function from R Base Package and tested their correlation between them using the function "findCorrelation" from "caret" R package (Kuhn, 2008). The Spearman correlation cut-off was set at 0.7 and then calculated the mean absolute correlation of each variable with the rest of the variables in the dataset, eliminating those with the highest mean absolute correlation. For the diatom data, a total of 16 variables were considered to test with LCBD and MSF. Previously, seven variables were deleted (TotDur, TotNum, FP100, B6, EC, SPC and DO %) that had a high Spearman correlation. For the macroinvertebrate dataset, six of the 23 variables were eliminated because of their high Spearman correlation (TotDur, TotNum, FP100, EC, SPC and DO %), resulting in a total of 17 variables to test with LCBD and MSF.

In order to analyse the complementarity of LCBD and MSF, we compared data obtained by the two approaches and with richness using beta regression models for LCBD, and linear mixed-effect models (LMM) for MSF. All models included streams and sites as random effects, and season as fixed effect. For LMM, the "lme" function in the package "nlme" was used (Pinheiro et al., 2022), whereas for beta regression models, the "glmmTMB" function in the glmmTMB v. 1.1.5 (Brooks et al., 2017) was employed. Finally, in order to determine the environmental drivers behind selected sites by LCBD and MSF, similar models were run between each approach and the predictor variables. Two different coefficients of determination were reported, denoted R^2. Marginal R^2 (R^2m) describes the proportion of variance of fixed factors and conditional R^2 (R^2c), that takes into account fixed and random effects (i.e., the total model) (Nakagawa & Schielzeth, 2013).

Rstudio 4.3.0 was used for all the analyses and Muff et al. (2022) was followed to report the results in the language of evidence.

3 | RESULTS

A total of 55,654 diatoms belonging to 222 species and 144,846 macroinvertebrates belonging to 178 different taxa were collected (diatom species and macroinvertebrate taxa can be found in Supporting Information S3). Diatoms richness ranged between 14 and 65, with an average of 34. The Kruskal–Wallis test showed no significant difference in richness among seasons ($p = 0.141$). Macroinvertebrates richness ranged between 6 and 62, with an average of 34. The Kruskal-Wallis test showed strong differences in richness among seasons ($p < 0.001$). The strongest differences were found between autumn and the rest of the seasons (see Mann–Whitney–Wilcoxon test for further information in Supporting Information S1).

According to the Holm-corrected LCBD values, four sites strongly contributing to beta diversity which belonged to the same stream and two different seasons for diatoms were found (H1W, $p = 0.009$; H2W, $p = 0.009$; H3A, $p = 0.037$; H5A, $p = 0.037$). Four sites were also found for macroinvertebrates, belonging to three different streams and three different seasons (H5W, $p = 0.039$; MU2A, $p = 0.01$; SC2SP, $p = 0.01$; SC3A, $p = 0.03$). A high spatiotemporal variability in LCBD for both diatoms and macroinvertebrates was found (Figure 1). For example, some ephemeral sites (e.g., R1 and R2) were completely dry for most of the year and LCBD ranged from 0 to 0.011, whereas some perennial reaches (e.g., H1) maintained very similar LCBD values throughout the seasons for macroinvertebrates (Figure 1c,d). MSF also showed a very strong seasonal variation for both diatoms and macroinvertebrates. For example, for diatoms, only two sites were selected by MSF during summer (T3 and CA1) (Figure 1b).

No strong relationship was found between LCBD and MSF (diatoms: $p = 0.641$; $R^2m = 0.002$; $R^2c = 0.125$; macroinvertebrates: $p = 0.264$; $R^2m = 0.012$; $R^2c = 0.211$). LCBD showed a negative relationship with species richness, which was weak for diatoms ($p = 0.026$; $R^2m = 0.077$; $R^2c = 0.445$) and very strong for macroinvertebrates ($p < 0.0001$; $R^2m = 0.355$; $R^2c = 0.411$). Contrarily to LCBD, MSF showed a strong positive relationship with richness for both, diatoms ($p < 0.0001$; $R^2m = 0.298$; $R^2c = 0.304$) and macroinvertebrates; ($p < 0.0001$; $R^2m = 0.443$; $R^2c = 0.46$) (Figure 2).

SCBD values ranged from 0 to 0.024 for diatoms, and from 0.0001 to 0.035 for macroinvertebrates. SCBD showed an unimodal relationship with species occupancy when presence/absence data was used (Figure 3b–d), whereas it increased exponentially with occupancy when using abundances (Figure 3a–c).

A great seasonal variability was found in the contribution of each taxa to beta diversity (Figure 4). For diatoms, several species showed high SCBD values in three seasons (e.g., *Brachysira neoexilis*, *B. neglectissima*, *Encyonopsis subminuta*, *Gomphonema lateripunctatum*, *Gomphonema tergestinum*, *Amphora pediculus and Achnanthidium pyrenaicum)* whereas other species showed high SCBD values for only one season (e.g., few *Fragilaria* spp. in winter, *Cocconeis placentula* in spring or *Navicula antonii* in autumn). In the case of macroinvertebrates there were seasonal variations in SCBD. For example, *Bidessus* exclusively contributed to beta diversity in spring, whereas *Habroleptoides* greatly contributed in winter. However, some taxa (e.g., *Dasyhelea* or *Cloeon*) greatly contributed

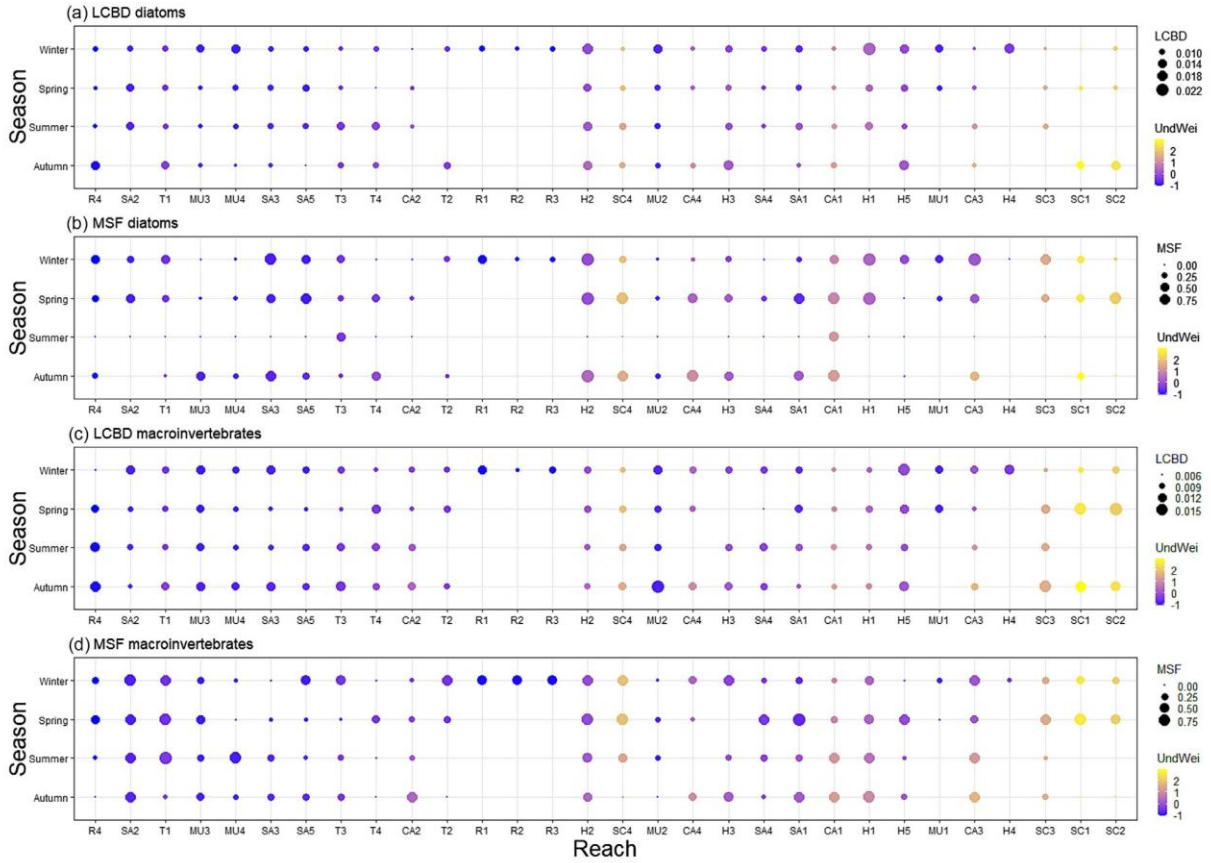


FIGURE 1 Bubble plots showing Local Contribution to Beta Diversity (LCBD) values and Marxan's Selection Frequency (MSF) for the four seasons and across sites. (a,b) Diatoms and (c,d) macroinvertebrates values. Bubble plot size represents LCBD and MSF values. The order of the reaches is in increasing order of dispersal resistance (UndWei) values.

to beta diversity across seasons (Figure 4). SCBD did not seem to be related to the rarity of the taxa, since some taxa that were exclusively found in ephemeral streams (e.g., *Tyrrhenoleuctra* and *Mesophylax*), showed very low SCBD values (0.003 and 0.001, respectively).

According to beta regression models using diatoms, LCBD did not show any relationship with the predictor variables, whereas MSF showed a strong positive relationship with two variables: dissolved oxygen (DO mg/L) ($F = 7.61$; $p = 0.007$) and temperature ($F = 7.81$; $p = 0.007$); and a weak relationship with two spatiotemporal connectivity metrics: connectivity (UndBin) ($F = 3.021$; $p = 0.087$) and dispersal resistance (UndWei) ($F = 3.559$; $p = 0.064$) (Figure 5) (for more information about the rest of the variables see Supporting Information S1). For macroinvertebrates, there was a strong relationship between LCBD and three habitat heterogeneity variables: embeddedness in riffles and sedimentation in pools (B1) ($Z = 2.98$; $p = 0.003$), frequency of riffles (B2) ($Z = -2.62$; $p = 0.008$) and the substrate composition (B3) ($Z = 3.08$; $p = 0.002$). Other two variables related to spatiotemporal connectivity showed a moderate relationship with LCBD: directed weighted scenario (DirWei: $Z = 2.27$; $p = 0.023$) and undirected binary scenario (UndBin: $Z = -2.52$; $p = 0.012$). Finally, two habitat heterogeneity variables showed a weak relationship with LCBD: flow velocity and depth regimes (B4) ($Z = -1.86$; $p = 0.062$) and aquatic vegetation cover (B7) ($Z = -1.65$; $p = 0.098$). For MSF using macroinvertebrates models only showed a strong relationship with aquatic vegetation cover (B7) ($F = 10.37$; $p = 0.02$) (for more information about the rest of the variables see Supporting Information S1).

The relationship of LCBD with the variables selected by the beta regression models experienced seasonal variations (Figure 6). For example, DirWei (i.e., the dispersal resistance, or spatiotemporal isolation, between reaches following a downstream direction) was positively related with LCBD in all seasons, whereas UndBin (i.e., the connectivity between each pair of reaches without any given direction) was negatively correlated with LCBD in all seasons except for winter (Figure 6e).

4 | DISCUSSION

In spite of covering a small study area with similar climatic, geological, and habitat characteristics, selected sites for conservation using LCBD

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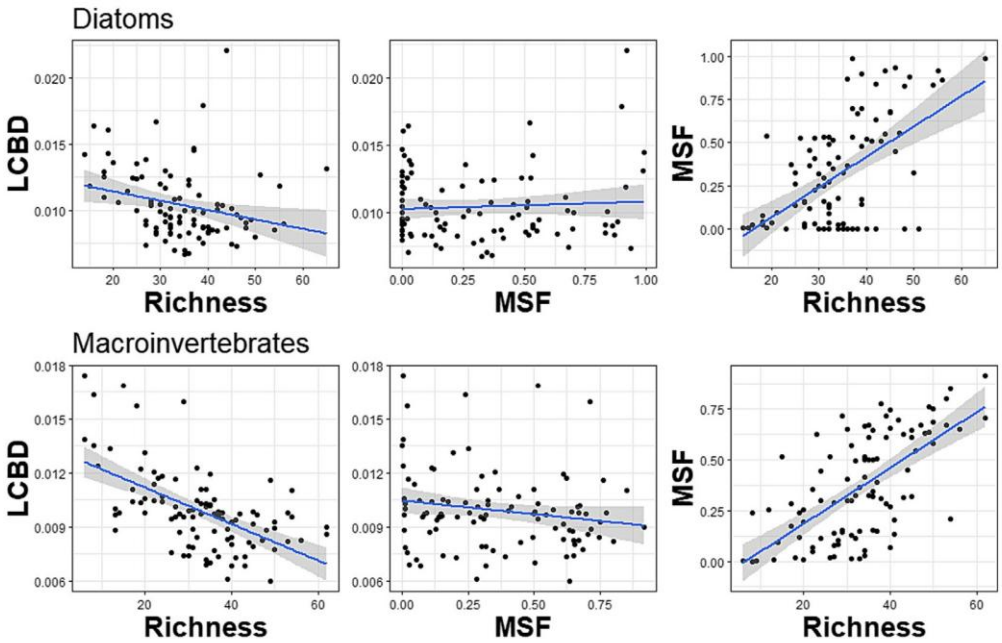


FIGURE 2 Relationship between Local Contribution to Beta Diversity (LCBD), Marxan's Selection Frequency (MSF) and taxa richness for diatoms and macroinvertebrates.

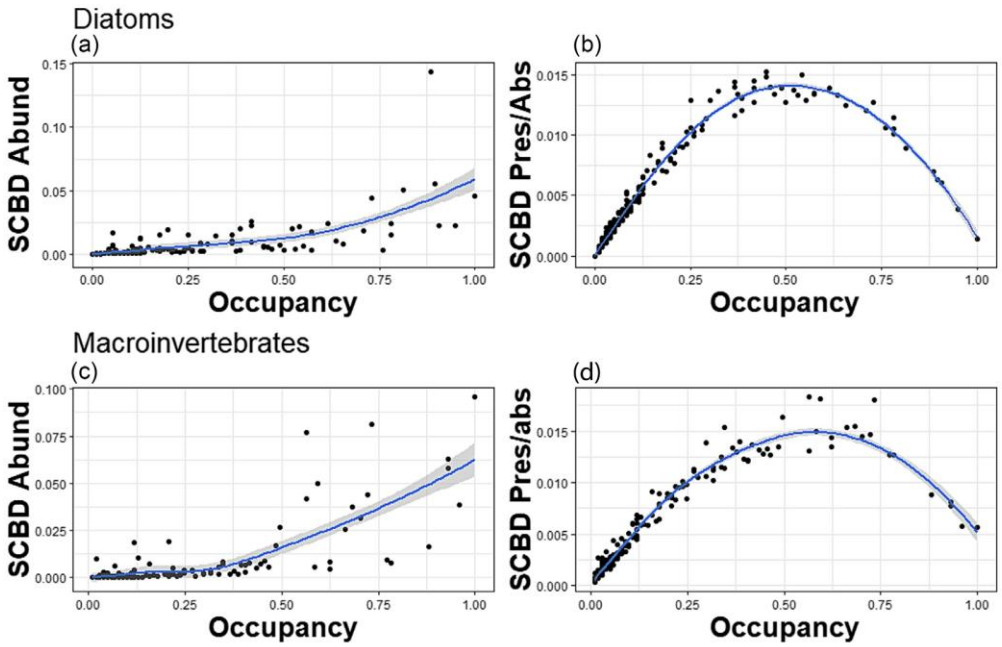


FIGURE 3 Relationship between occupancy and abundance or presence/absence data for diatoms (a,b) and macroinvertebrates (c,d).

and MSF varied widely in space and time, which agrees with our first hypothesis. Given our results, we advise conservation plans to properly capture the spatiotemporal hydrological variability of TRs and be able to accommodate the changing conditions. As we have hypothesised, TRs are highly dynamic systems that create a patchy landscape in which flowing reaches can co-occur with disconnected pools and dry riverbeds within a single network. This favours the co-existence of different communities (e.g., aquatic and terrestrial) because of multiple factors, such as habitat heterogeneity or the existence of spatially isolated sites that serve as a refugia for weak competitors (Bonada et al., 2020; Larned et al., 2010). Moreover, this landscape drastically changes over time. For example, in late summer,

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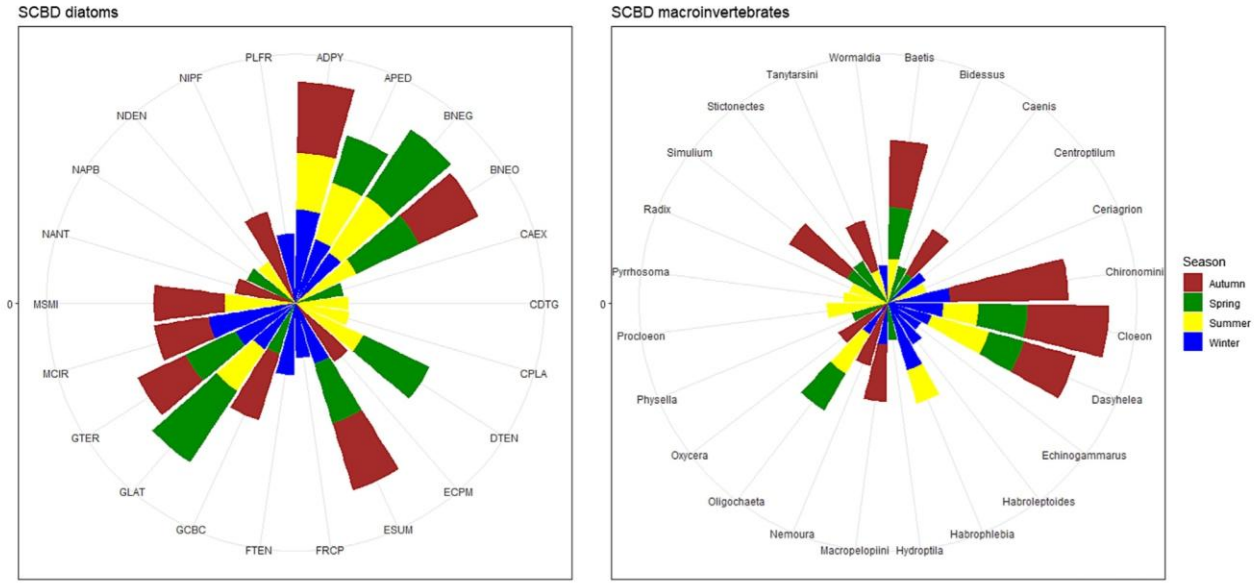


FIGURE 4 Rose plot of the most contributing taxa to beta diversity (SCBD) across seasons for diatoms and macroinvertebrates. Diatom species codes can be found in Supporting Information S3.

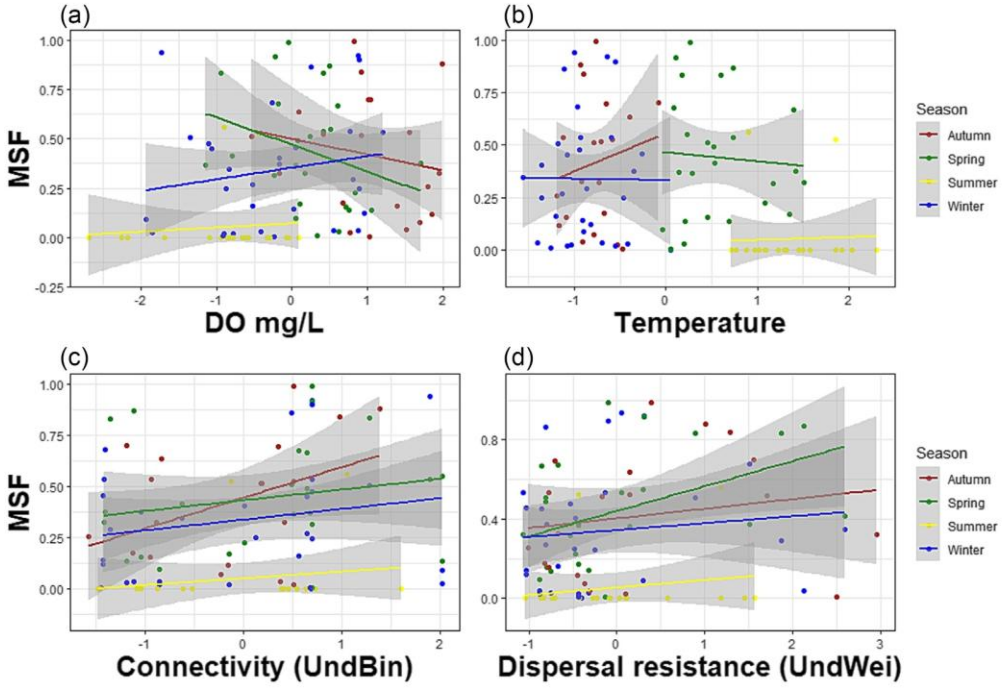


FIGURE 5 Relationships between predictor variables and Marxan's selection frequency (MSF) for diatoms with p-values < 0.1 in each season. DO mg/L refers to dissolved oxygen, temperature to the temperature on water, UndBin to the connectivity between each pair of reaches without any given direction, and UndWei to the dispersal resistance (or spatiotemporal isolation) between reaches not following a downstream direction.

a dry fragmented stream network can be rapidly transformed into a fully connected network after heavy precipitation events (Pineda-Morante et al., 2022). Taking this into account, planning conservation on the basis of snap-shot aquatic biodiversity surveys would lead to strongly biased and misguided decisions that could hamper conservation efforts.

MSF and LCBD showed a strong spatiotemporal variability, but they were weakly related. In agreement with our second

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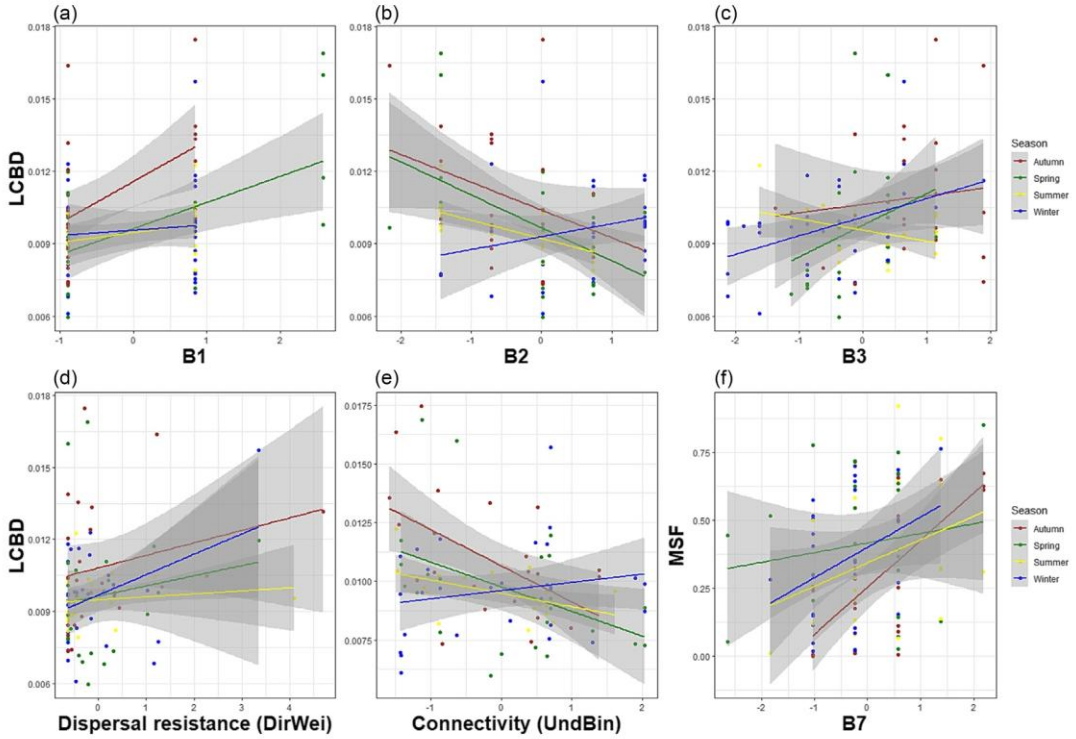


FIGURE 6 Relationships between predictor variables and Local Contribution to Beta Diversity (LCBD) or Marxan's Selection Frequency (MSF) values for macroinvertebrates with p-values < 0.05 in each season. "B1" refers to embeddedness in riffles and sedimentation in pools, "B2" to the frequency of riffles, "B3" to the substrate composition, "DirWei" to the dispersal resistance (or spatiotemporal isolation) between reaches following a downstream direction, "UndBin" to the connectivity between each pair of reaches without any given direction, and "B7" to the aquatic vegetation cover.

hypothesis, MSF usually selects a combination of sites with high local richness and very rare taxa (Epele et al., 2021). In our study, under some circumstances, MSF was able to adequately represent all taxa by selecting a few sites (e.g., only two sites for diatoms in summer). On the contrary, LCBD selected the communities with a higher relative weight at the level of community differentiation but not necessarily the communities with rare or endemic species. Thus, this approach does not seem adequate to design conservation strategies in rivers when these rare or endemic species occur and have a high conservation value, such as in TRs (Bogan, 2017; Fernández-Calero et al., 2022; Stubbington et al., 2017). In these cases, MSF with its principle of complementarity and its flexibility in the application of specific targets, would be a more reliable tool. However, LCBD selected communities with higher differentiation and low richness that were not necessarily selected by MSF (e.g., site R4 in autumn or H5 in winter (Figure 1)). This highlights the conservation value of TRs and specially of those with low richness as a result of low flow permanence (Datry et al., 2014).

According to previous studies (Heino & Grönroos, 2017; Siqueira et al., 2009) taxa with high abundances and occupancy across sites, which are often the least threatened taxa, present a greater contribution to beta diversity. Concordantly, we found a very strong relationship between species occupancy and abundance with SCBD for diatoms and macroinvertebrates. This suggests that SCBD alone is not a suitable metric to assess the conservation value of a given taxa, since it is strongly driven by locally abundant and regionally common species, rather than rare or endemic species with high conservation value (Rodríguez-Lozano et al., 2023). For example, the genus *Tyrrhenoleuctra*, a specialist taxon of ephemeral rivers, whose nymphal presence only occurs in winter and early spring, did not strongly contribute to SCBD in our study. However, this is a rare and vulnerable taxa with restricted geographical distribution (Fernández-Calero et al., 2022; Fochetti et al., 2009) that should be considered in conservation plans and probably requires specific conservation actions.

Despite the use of SCBD is not recommended for conservation, we found different diatom and macroinvertebrate taxa strongly contributing to beta diversity depending on the season. In the case of diatoms, species characteristic of oligotrophic calcareous rivers contributed the most to beta diversity (e.g., *Brachysira neoexilis*, *Gomphonema lateripunctatum*, *G. tergestinum* and *Encyonema subminuta*), as well as generalist species (e.g., *Achnanthidium pyrenaicum* and *A. pediculus*). This aligns with previous studies showing that commonly occurring diatom taxa greatly contribute to beta diversity (Szabó et al., 2019; Vilmi et al., 2017). For

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macroinvertebrates, the differential seasonal contribution of taxa might be explained by changes in habitat variability and flow permanence (Bonada et al., 2007), which influences habitat preference and phenology (Porst et al., 2012; Woods et al., 2022). For example, in agreement with previous studies (Bogan et al., 2017; Bonada et al., 2007), Ephemeroptera, Plecoptera, and Trichoptera (EPT) were more abundant in winter and autumn, during flowing conditions and mild temperatures, whereas Odonata, Coleoptera, and Hemiptera (OCH) dominated in spring and summer, when many stream reaches might have disconnected pools and temperatures are high. Thus, riffle-adapted taxa, such as *Simulium* and *Habroleptoides* (Horne et al., 1992), showed high SCBD values in winter and autumn, whereas in spring and summer lentic-adapted taxa, such as *Bidessus*, *Stictonectes*, *Gerris*, or *Sympetrum* (Buffagni, 2021; Letsch et al., 2016), showed the highest SCBD values. However, there were many generalist taxa that contributed significantly to beta diversity throughout most of the year (e.g., *Cloeon* and *Dasyhelea*).

Concerning our third hypothesis, local conditions largely determined the conservation importance of our study sites. Physicochemical and habitat variables were the most important drivers of LCBD and MSF for diatoms and macroinvertebrates, respectively. We found that dissolved oxygen and temperature drove diatom MSF, in line with previous works showing that local physicochemical conditions shaped diatom communities (Soininen & Teittinen, 2019). Diatoms also responded to environmental heterogeneity, but this might be directly related to the species-area relationship (SARs) (i.e., larger samples, more richness) (Soininen & Teittinen, 2019). For macroinvertebrates, embeddedness in riffles and sedimentation in pools (B1), along with substrate composition (B3), and aquatic vegetation cover (B7), determined LCBD across seasons, suggesting that, in general, increasing habitat heterogeneity might promote community turnover through a niche partitioning. For example, rock characteristics can determine caddisfly oviposition patterns and influence population dynamics (Reich et al., 2011). Overall, our findings illustrate the importance of preserving local habitat heterogeneity to support diverse macroinvertebrate communities.

Our results agree with that more spatiotemporally isolated sites in TRs showed a stronger contribution to LCBD (Crabot et al., 2020; Ruhí et al., 2017). However, there were important temporal variations in the relationship between LCBD and hydrological connectivity. When the network was fully connected in winter (after the rewetting), LCBD values linearly increased with spatiotemporal connectivity, most likely because there are fewer dispersal restrictions and each species occupies its preferential niche (Fletcher et al., 2016; Leibold & Chase, 2017; Thompson et al., 2017; Warfe et al., 2012). In the remaining seasons, the communities with highest LCBD values were located in the most spatiotemporally isolated sites, suggesting that isolation promotes beta diversity (e.g., by allowing rare species with low competitive advantage to persist), which has already been shown for high mountain streams (Finn et al., 2011). Hydrological connectivity also played a role in the selection of sites by MSF, with spatially isolated sites being often selected to represent rare taxa. Overall, our results suggest that conservation plans should adequately capture the natural hydrological variability of drying river networks, because the networks can also have temporary reaches with high conservation value (Hamdhani et al., 2020; Hermoso et al., 2013; Naia et al., 2021; Vander Vorste et al., 2020).

TRs are one of the most widespread and vulnerable river systems on the planet (Chiu et al., 2017; Datry et al., 2023; Messager et al., 2021), but host unique freshwater biodiversity adapted to these ecosystems (e.g., *Mesophylax*, *Tyrrhenoleuctra*) (Bonada et al., 2020; Magoulick & Kobza, 2003; Vander Vorste et al., 2020; Yu et al., 2022). In addition, TRs also support semi aquatic and terrestrial taxa (i.e., invertebrates and vertebrates) that use dry channels and riparian habitats as new habitats and migration corridors (Sánchez-Montoya et al., 2017). These singular river ecosystems should have specific conservation strategies to ensure their biodiversity and functionality. However, specific protection plans including TRs are still lacking (Datry et al., 2023). For example, in Europe, even though the Annex I of the Habitats Directive (Council Directive 92/43/EEC, 1992) describes habitat types that refer to TRs (i.e., Intermittently flowing Mediterranean rivers of the Paspalo-Agrostidion, and Riparian formations on intermittent Mediterranean watercourses with *Rhododendron ponticum*, *Salix*, and other species), this has resulted in limited conservation and policy actions related to the protection of these ecosystems (Datry et al., 2023; Fritz et al., 2017; Magand et al., 2020). In the United States, many temporary waterbodies are protected under a range of policy instruments at the local, State, and Federal levels. However, protections for TRs at the Federal level have been increasingly threatened over the past decades, owing to Supreme Court rulings (Sulliván & Gardner, 2023). A recent Supreme Court decision (Sackett v. US Environmental Protection Agency) has ruled that for a "wetland" (including streams and rivers) to receive protection under the Clean Water Act of 1972, it must have a continuous surface connection with a larger body of water (i.e., an ocean, a lake, or a larger perennial river). This decision threatens protection to TRs, which strongly dominate the subarid and arid regions of the U.S. Southwest (Levick et al., 2008). Moreover, as many rivers across the U.S. Southwest continues to lose flow because of climate change and overallocation of freshwater resources (Ruhí et al., 2016), often shifting from perennial to intermittent flow regimes, using physical but not biological connectivity as a condition for stream protection may enable further degradation in the near future.

In addition, many rivers around the world are expected to become temporary and unpredictable during the next decades because of climate change (Döll & Schmied, 2012). Thus, conservation plans that incorporate the spatiotemporal variability of TRs, will become increasingly needed to effectively protect freshwater biodiversity. Therefore, based on our results, we suggest a set of recommendations to guide future conservation plans for TRs that help to ensure their biodiversity and functionality. Firstly, we strongly recommend regular assessments across seasons or, at least, during the dry season (including disconnected pools), the

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rewetting period, and the fully connected period to have a full characterization of biodiversity. Secondly, we recommend the use of beta diversity components such as LCBD and conservation planning methods (MSF) as complementary approaches to determine and prioritize sites to be conserved. LCBD is able to detect unique species composition even in sites with low richness as a result of low flow permanence (Datry et al., 2014), whereas MSF could be used as a tool to design specific criteria, for example, to include rare or singular species. Thirdly, focusing on a local perspective, we encourage the preservation of local habitat heterogeneity such as the substrate composition or instream aquatic vegetation. Fourthly, in a regional perspective, we encourage the conservation of spatiotemporal isolated communities with low species richness because these sites usually host unique communities with high LCBD values. Fifthly, we argue that natural spatiotemporal connectivity should be enforced and be case specific (Cid et al., 2022). For example, in historically isolated populations and communities such as the ones in our study, anthropogenic water discharges that could increase hydrological connectivity but decrease biodiversity of the TRs should be avoided.

Although our study provides valuable insights about the spatiotemporal dynamics of biodiversity patterns in TRs and how it can influence conservation decisions, it also has limitations that should be consider if our approach is applied to other areas. Firstly, our study area covers a small spatial scale with very homogeneous environmental conditions, which could potentially result in homogeneous community dynamics. However, we showed that even under similar environmental conditions community dynamics differ because of differences in spatiotemporal hydrological dynamics. Secondly, the temporal duration of the study was only one year, and the patterns observed may not reflect long-term trends. Although the year of the study was a normal hydrological year in the area, in a drier year, communities can behave completely different (Cid et al., 2017). This highlights the need to incorporate data coming from multiple years in conservation planning studies, especially in areas with high interannual variability, such the Mediterranean region (Bonada et al., 2020; Cid et al., 2017). Thirdly, our study only considers diatoms and macroinvertebrates, which may not represent the full complexity of the ecosystem. Data on other aquatic or even terrestrial organisms (e.g., fish, amphibians) would be recommended if the approach needs to be applied to other areas. Future research should address these limitations when applying our approach to other TRs (and even perennial) in order to increase the performance of conservation strategies.

Finally, conservation plans need to be flexible and adapted to each hydrological scenario. For example, in the wet season the connectivity of the river network and microhabitat heterogeneity (riffles and pools) should be maintained at the reach scale. However, during the dry season, maintaining disconnected pools is key to sustaining refuges for aquatic biodiversity. All these recommendations stand as broad guidelines that should be specifically adapted to the particular characteristics of each TR but keeping in mind the relevance of these ecosystems at the global scale (Messager et al., 2021), especially considering future global change scenarios.

AUTHOR CONTRIBUTIONS

José María Fernández-Calero leaded the paper in the contextualization, analysis, writing, interpretation and edition. He also collected the data, sorted and identified macroinvertebrates. Núria Bonada conceptualised the paper, interpreted the results, wrote and edited the manuscript and obtained funding. Miguel Cañedo-Argüelles collected the data, conceptualised the paper, interpreted the results, wrote and edited the manuscript and obtained funding. David Cunillera-Montcusí calculated spatiotemporal connectivity metrics, interpreted the results, wrote and edited the manuscript. Núria Cid collected the data, interpreted the results, edited the manuscript and obtained funding. Albert Ruhí conceptualised the paper, interpreted the results and edited the manuscript. Dolors Vinyoles collected the data and edited the manuscript. Guillermo Quevedo-Ortiz identified diatom and edited the manuscript. Joan Gomà interpreted diatom results and edited the manuscript. Virgilio Hermoso interpreted the results, wrote and edited the manuscript. Pau Fortuño and Raúl Acosta collected the data, identified macroinvertebrates and edited the manuscript.

CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Check list of taxa available as Supporting Information S3.
Models results available as Supporting information S1 and S2.
Code used is available on Github: https://github.com/Josefernandezcalero/Conservation-paper-from-the-MECODISPER-project.

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