



# Local environment and fragmentation by drought and damming shape different components of native and non-native fish beta diversity across pool refuges

Joana Martelo<sup>a,b,\*</sup>, Christos Gkenas<sup>b</sup>, Diogo Ribeiro<sup>b</sup>, M. Judite Alves<sup>a,f</sup>, Filipe Ribeiro<sup>b</sup>, Julien Cucherousset<sup>c</sup>, João Gago<sup>b,d,e</sup>, M. Filomena Magalhães<sup>a</sup>

<sup>a</sup> cE3c- Centre for Ecology, Evolution and Environmental Changes & CHANGE - Global Change and Sustainability Institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal

<sup>b</sup> MARE, Marine and Environmental Sciences Centre & ARNET - Aquatic Research Network Associated Laboratory, Faculty of Sciences, University of Lisbon, Lisbon, Portugal

<sup>c</sup> Laboratoire EDB (UMR 5174), CNRS, University Toulouse III, Paul Sabatier, France

<sup>d</sup> Escola Superior Agrária de Santarém, Instituto Politécnico de Santarém, Santarém, Portugal

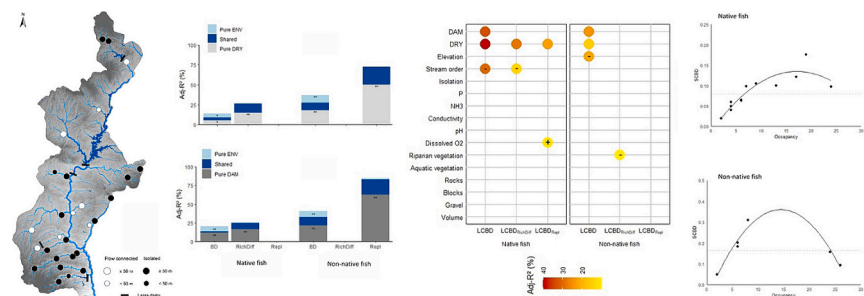
<sup>e</sup> CERNAS, Research Centre for Natural Resources, Environment and Society, Portugal

<sup>f</sup> Museum of Natural History and Sciences, University of Lisbon, Lisbon, Portugal

## HIGHLIGHTS

- Richness difference drives native and non-native fish beta diversity in pool refuges.
- Stream fragmentation shapes native species loss and non-native species replacement.
- Environment filtering remains influential for non-native beta diversity.
- Pools in low order streams should be prioritized in conserving native fish.
- Pools at low elevations should be targeted for control of non-native fish.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

Editor: Sergi Sabater

### Keywords:

Intermittent streams  
Metacommunities  
Dispersal limitation  
Environmental filtering  
Biodiversity conservation

## ABSTRACT

Pool refuges are critical for maintaining stream fish diversity in increasingly intermittent streams. Yet, the patterns and drivers of beta diversity of native and non-native fish in pool refuges remain poorly known. Focusing on Mediterranean streams, we decomposed beta diversity of native and non-native fish into richness difference (RichDiff) and species replacement (Repl), and local (LCBD, LCBD<sub>RichDiff</sub> and LCBD<sub>Repl</sub>) and species (SCBD) contributions. We assessed the influence of environmental and spatial factors associated with drought and damming fragmentations on beta diversity components and LCBDs, and of local species richness and occupancy on LCBDs and SCBD, respectively. Overall, non-native species showed a more limited occupancy of pool refuges than native fish. RichDiff dominated beta diversity, though it was influenced by drought and damming fragmentations for native fish and local environment for non-native fish. Repl for native fish was slightly influenced by local environment, but for non-native fish was largely driven by drought and damming, albeit with a contribution of local environment as well. LCBD and LCBD<sub>RichDiff</sub> increased in pools in low order streams for

\* Corresponding author.

E-mail address: [jmmartelo@fc.ul.pt](mailto:jmmartelo@fc.ul.pt) (J. Martelo).

<https://doi.org/10.1016/j.scitotenv.2024.172517>

Received 24 November 2023; Received in revised form 12 April 2024; Accepted 14 April 2024

Available online 17 April 2024

0048-9697/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

native fish and at low elevations for non-native fish, and with high or low species richness. SCBD was higher for native species with intermediated pool occupancy, but for non-native species with low occupancy. Our results suggest that stream fragmentation may drive native species loss and non-native species replacement in pool refuges, and that environmental filtering may shape non-native species loss. Pools in lower order streams harbouring unique species-rich or species-poor assemblages should be prioritize for conservation and restoration, respectively, and pools at low elevation with unique non-native assemblages should deserve control efforts. We encourage the partitioning of beta diversity and individual analysis of native and non-native fish in intermittent streams, which may be key in stressing the importance of pool refuges in safeguarding native fish diversity.

## 1. Introduction

Freshwater ecosystems cover <1 % of Earth's surface but harbour substantial and irreplaceable biodiversity (Allen and Pavelsky, 2018; Tickner et al., 2020). However, multiple interacting threats increasingly impact freshwater ecosystems, including changes in hydrological regimes, habitat fragmentation and destruction, and biological invasions (Reid et al., 2019; Tickner et al., 2020). Of particular concern is the fragmentation and loss of stream habitats driven by droughts of increasing frequency and intensity, associated with climate change and rising water demands (Datry et al., 2018; Gido et al., 2023). Severe droughts are causing formerly perennial streams to become intermittent and intermittent streams to dry up permanently (Messenger et al., 2021; Pumo et al., 2016). These changes are leading to unprecedented declines in native species diversity and alterations in community composition (e.g., Carey et al., 2023; Gido et al., 2023). The situation is further complicated by escalating dam construction, which exacerbates stream fragmentation and flow regime changes, while also facilitating non-native species invasion (Freyhof et al., 2020; Perkin et al., 2015; Su et al., 2021). Addressing this crisis requires advancing understanding about the way biodiversity is spatially organized in intermittent streams (Rogosch and Olden, 2019), and how it is affected by fragmentations associated with droughts and damming (Gauthier et al., 2020, 2021).

Studies of beta diversity in intermittent streams increasingly adopted a metacommunity perspective which has proven beneficial in understanding the relative roles of niche-based and dispersal processes in driving beta diversity and in conservation management (Datry et al., 2016b; Gauthier et al., 2020; Rogosch and Olden, 2019; Vardakas et al., 2020). Distance and network connectivity metrics consistently surpass the influence of local environment in structuring community assembly, which suggests a significant effect of dispersal processes, even where strong environmental filters are in place (Gauthier et al., 2020; Sarremejane et al., 2020). In addition, richness difference rather than species replacement appears prevalent along intermittency gradients, with species-poor communities often being subsets of species-rich communities (Datry et al., 2014, 2016a; Gauthier et al., 2020). This implies that conservation management should prioritize species rich communities that may act as sources within the metacommunity, instead of focusing on a range of communities with variable compositions as when species replacement prevails (Rogosch and Olden, 2019; Socolar et al., 2016). However, beta diversity studies mainly centred on macroinvertebrates and other organisms in the basis of food webs, and is uncertain whether these assembly patterns apply to fish (Gavioli et al., 2019, 2022; Rogosch and Olden, 2019; Vardakas et al., 2020). Moreover, the extent to which patterns in beta diversity may differ between native and non-native fish has been overlooked (but see Gavioli et al., 2019), though their local richness and abundance is often driven by different factors (Anas and Mandrak, 2021; Filipe et al., 2010; Radinger et al., 2019). Clarifying this issue is particularly important given the increasing introduction and spread of non-native fish and the associated risk of faunal homogenization in intermittent streams (Clavero and Hermoso, 2011; Clavero et al., 2013; Freyhof et al., 2020; Gavioli et al., 2019).

Beta diversity research can be further complemented by recent approaches that assess variability in the way sites and species in the landscape contribute to beta diversity (Legendre, 2014; Legendre and De

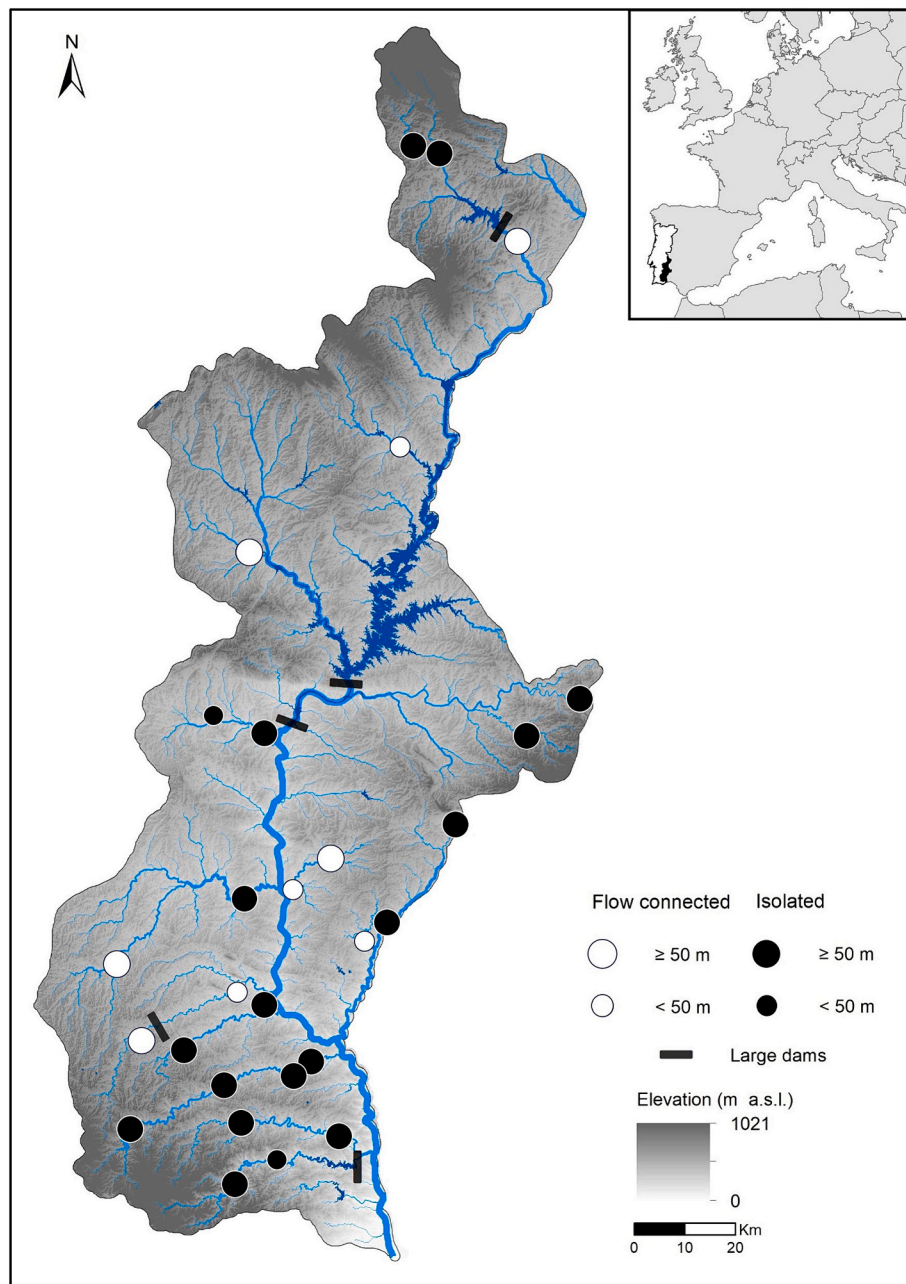
Cáceres, 2013; Leibold et al., 2022). The ecological uniqueness of individual sites in terms of species composition, richness difference and species replacement, often depends on environmental and dispersal processes but also on local species richness (Legendre, 2014; Leibold et al., 2022). Likewise, the relative importance of individual species in affecting beta diversity may associate with occupancy, abundance, niche requirements and functional traits (da Silva et al., 2018; Heino and Grönroos, 2017; Xia et al., 2022). To date, site and species contributions to beta diversity for native and non-native fish in intermittent streams remain poorly examined (but see Gavioli et al., 2019, 2022; Rogosch and Olden, 2019), though they will be key to inform conservation management. For instance, high scoring sites may harbour rich or unique native communities suitable for conservation or species-poor communities suitable for restoration (Legendre, 2014), and species with intermediate occupancy rather than rare species may contribute the most to beta diversity (Xia et al., 2022).

Intermittent streams in Mediterranean climate regions (INTMED) are an ideal model system to explore the influence of drought and damming fragmentations in driving beta diversity of native and non-native fish communities. Drought is seasonally predictable in Mediterranean streams, occurring each year in summer-early fall, but varying markedly in intensity on a multi-annual scale (Gasith and Resh, 1999). Flow cessation and surface drying result in significant habitat fragmentation and loss, with water restricting to chains of residual pools embedded in dry channels during severe droughts (Magalhães et al., 2002a, 2007; Vander Vorste et al., 2020). Fish are strictly dependent upon such remnant pools, which act as refugia and function as sources of recruits and colonists for the entire stream networks after flow resumption (Magalhães et al., 2002a; Pires et al., 2014). Refuge pools are thus recognized to play a central role in shaping local richness and community composition, and maintain local and regional stream biodiversity (Bonada et al., 2020). However, the suitability of refuge pools for fish is variable, with species richness increasing with pool size and the addition of rare native species and of non-native species reflecting environmental tolerances but also differential colonization and extinction processes (Magalhães et al., 2002a). Furthermore, fish community structure after flow resumption largely associates with spatial factors rather than local environment (Magalhães et al., 2002b; Vardakas et al., 2020), with similarity between local communities being associated with the distribution of pool refuges and dispersal opportunities (Magalhães et al., 2002b). These are also significantly limited by damming, which impairs fish movements, impacts natural flow regime, and often favours non-native species introductions (Clavero et al., 2013; Clavero and Hermoso, 2011; Freyhof et al., 2020). Therefore, clarifying how local environment and fragmentations associated with drought and damming cascade into beta diversity patterns of native and non-native species in refuge pools is key for the development of a framework to better conceptualize, conserve, and manage INTMED (but see Bonada et al., 2020).

In this study, we integrated two partitioning approaches to disentangle the contributions of local environmental and spatial factors to beta diversity of native and non-native fish in pool refuges in INTMED. Specifically, we partitioned beta diversity into richness difference and species replacement as well as local and species contributions, and we explored results to inform community assembly and conservation

management. We predicted that:

- i) richness difference is the main component of beta diversity for both native and non-native fish, and for the former mainly relates to dispersal limitation associated with drought and damming fragmentations (Datry et al., 2014), whereas for the later mainly relates to damming promoting species additions and colonization opportunities (Clavero et al., 2013; Clavero and Hermoso, 2011; Liew et al., 2016);
- ii) replacement of both native and non-native fish mainly associates with environmental gradients in pool refuges (Legendre, 2014; López-Delgado et al., 2020), but should be increasingly influential for non-native fish, which have not been filtered to cope with prevailing drought conditions, thus are more constrained by local environment (Lennox et al., 2019; Radinger et al., 2019);
- iii) local contributions to beta diversity are influenced by local environmental gradients in pool refuges and dispersal limitation associated with fragmentations for native and non-native fish, and also negatively related to local species richness (Borges et al., 2020; Xia et al., 2022);
- iv) species contributions to beta diversity relate to occupancy, showing a hump-shaped relationship (Heino and Grönroos, 2017; Xia et al., 2022).



**Fig. 1.** Map of the Lower Guadiana basin and the 28 pools sampled in July 2019. The pools are categorized as flow-connected (white circles) and isolated (black circles), and further differentiated in below (small circles) and equal or over 50 m (large circles) in total length. Strahler's stream order is indicated by the width of the watercourse line, ranging from 1 (narrow blue line) to 4 (broad blue line), and elevation denoted by the grey gradient, ranging from 0 to 1021 m (a.s.l.). The map also features the location of large dams  $\geq 15$  m high or 10–15 m high and impounding  $\geq 3$  hm<sup>3</sup> (black rectangle).

2. Methods

2.1. Study area

Our study was conducted in the lower part of the Guadiana River basin (37°33'20" N, 7°31'21" O), in Portugal (Fig. 1). The Guadiana River originates in Spain at 1700 m a.s.l., flows east to west for 810 km, ultimately discharging into the Atlantic Ocean. The Lower Guadiana flows for 150 km between 0 and 650 m a.s.l., draining 11,700 km<sup>2</sup>, mainly of schist-derived and highly impermeable soils with little groundwater. Human occupation in the region is low (15 inhabitants/km<sup>2</sup>), and land cover is dominated by dry and irrigated annual crops and pastures, holm and oak woodlands, olive and almond groves and vineyards (Aguilar et al., 2019). Riparian vegetation typically includes evergreen oleander *Nerium oleander*, African tamarisk *Tamarix africana* and tamujo *Flueggea tinctoria*, but there are also ash *Fraxinus angustifolia* and diverse willows *Salix* spp. (Aguilar et al., 2019).

The climate is typically Mediterranean, with mean monthly temperature ranging from 9 °C (January) to 25 °C (July–August) (SNIRH, 2023), and annual rainfall varying markedly from year to year (34–871 mm; 1990–2019 yr.) but following a predictable seasonal pattern (SNIRH, 2023). About 85 % of annual rain occurs in October–April and virtually none in the hottest months. The mainstem is permanent but flow regimes in the tributaries are highly dependent on rainfall patterns, with floods occurring in winter-early spring and low or no flows prevailing in summer-early fall. In wet years, there may be major floods and flow may persist in some tributaries. In dry years, there are no significant floods, the drying period is extended, and surface water restricts to a few pools scattered throughout the stream network. There are 20 large dams ≥15 m high or 10–15 m high and impounding ≥3 hm<sup>3</sup>, including the Alqueva in the mainstem which is the largest dam (4150 hm<sup>3</sup>) in Europe (SNIRH, 2023).

Typical of INTMED, the regional pool of native resident fish is reduced, including 12 resident species, 7 of which are threatened Iberian endemics (Magalhães et al., 2023). There are also 13 non-native resident species, 10 of which have been introduced in the region >20 years ago, and 3 anadromous and 4 catadromous species may also be found locally (Collares-Pereira et al., 2021).

2.2. Fish and environment sampling

We sampled fish and environment in 28 residual pools in the tributaries of the Lower Guadiana during July 2019, after flow among pools had ceased or significantly reduced (Fig. 1). We exclusively sampled residual pools, as they constituted the main refuges for fish during drought in INTMED (Bonada et al., 2020), and specifically the only that persisted in our study system. The study year was dry relative to the long-term median (139 mm vs. 453 mm; 1990–2019) and to the previous two years (310 and 239 mm in 2017 and 2018, respectively). Sampling was largely constrained by the natural distribution of pools and accessibility, but an effort was made to cover variability in fragmentation by drought and large dams across the stream network. Sampling pools were 130 ± 105 m (mean ± SD) (range: 25–402 m), 64 ± 24 cm (25–150 cm) deep, located at a nearest distance of 12 ± 8 km (4–37 km) from each other, and only nine remained flow connected (Fig. 1).

We sampled fish using single pass electrofishing (300–600 V, 4–6 A, pulsed DC), wading upstream in areas up to <150 cm deep and encompassing distinct microhabitats. Sampling covered the total length of pools up to 50 m long, and ranged from 50 to 80 m in the remaining pools, contingent on its length. Sample lengths were relatively small but similar lengths have been shown to adequately capture responses of fish communities to environmental variation in INTMED (Magalhães et al., 2007; Mota-Ferreira et al., 2021). Sampling was conducted from 20 to 50 min, with longer surveys in wider and deeper pools to enhance detectability of species in local communities (Ferreira et al., 2016). We identified fish to species, except small *Luciobarbus* (<10 cm TL) and

*Squalius* (<4 cm TL), which are not readily identified from external morphology (Table S1). Native fish were returned to the pools of origin, and non-native fish were euthanized with a lethal dose of anaesthetic (MS-222). No correlations were found between species number and sample length (Pearson's  $r = 0.03$ ,  $p = 0.86$ ) and time (Pearson's  $r = 0.15$ ,  $p = 0.43$ ).

We characterized the environment at each pool using 25 variables (Table 1), known to influence fish communities in INTMED (Ilhéu et al., 2020; Magalhães et al., 2007; Mota-Ferreira et al., 2021). At the mid-points of three to five cross transects distributed across each pool, we measured depth, temperature, dissolved O<sub>2</sub>, pH, conductivity, total dissolved solids, and the proportions of substrata, instream cover and riparian cover, and we collected water samples for inorganic nutrients (NH<sub>3</sub>, NO<sub>3</sub>, P, PO<sub>4</sub>) and chlorophyll-a analyses. Substrata was categorized as mud (0–1 mm), sand (>1–5 mm), gravel (>5–100 mm), block (>100–1000 mm) and rock (>1000 mm). Instream cover was categorized as debris (i.e., leaves, limbs and sticks), roots and aquatic vegetation. Inorganic nutrients and chlorophyll-a were analysed using UV/Vis spectrometry and HPLC, respectively (Cereja et al., 2022 and references therein). From Google Earth images (ca 0.15 m resolution; July 2019) we retrieved the area of each pool and characterized its isolation using a dummy variable, coding 0 for flow connected and 1 for isolated pools. Finally, we calculated the volume of each pool by approximation to an ellipsoid, and determined Strahler's stream order and elevation from CCM 2.1 geodatabase based on a 100 m digital elevation model (Vogt et al., 2008) in QGIS v3.12.

2.3. Data analysis

We primarily analysed variation in native and in non-native fish diversity across the stream network using two decomposition approaches to partition beta diversity (BD) into richness difference (RichDiff) and replacement (Repl) components, as well as local (LCBD, LCBD<sub>RichDiff</sub> and LCBD<sub>Repl</sub>) and species (SCBD) contributions. Additionally, we evaluated the relationships of BD and LCBD and their components with environmental and spatial predictors. Finally, we analysed the relationships of LCBD and SCBD with the local number of species and occupancy (i.e., the number of sites in which a species was

**Table 1**  
Environmental conditions in pools in the Lower Guadiana basin, in July 2019. Values are the mean ± standard deviation (SD) and range (minimum–maximum). Variables used in beta diversity analyses are highlighted in bold.

Environmental predictors	Mean ± SD	Range
Depth cm	64.2 ± 24.5	25.0–150.0
Area cm <sup>2</sup>	1815 ± 1976	105–7000
<b>Volume m<sup>3</sup></b>	<b>3317.2 ± 3625.1</b>	<b>157.6–13,321.6</b>
Mud %	9.4 ± 20.9	0.0–76.7
Sand %	13.0 ± 22.8	0.0–83.3
<b>Gravel %</b>	<b>6.7 ± 9.3</b>	<b>0.0–30.0</b>
<b>Blocks %</b>	<b>26.4 ± 21.6</b>	<b>0.0–76.7</b>
<b>Rocks %</b>	<b>19.7 ± 20.3</b>	<b>0.0–68.3</b>
Debris %	1.6 ± 3.8	0.0–15.0
Roots %	3.0 ± 5.5	0.0–20.0
<b>Aquatic vegetation %</b>	<b>6.2 ± 10.9</b>	<b>0.0–38.0</b>
<b>Riparian cover %</b>	<b>19.1 ± 23.3</b>	<b>0.0–80.0</b>
Temperature °C	25.5 ± 3.5	19.8–32.1
<b>Dissolved O<sub>2</sub> %</b>	<b>11.2 ± 5.6</b>	<b>2.3–24.2</b>
<b>pH</b>	<b>8.2 ± 0.6</b>	<b>7.2–9.3</b>
<b>Conductivity µS/cm</b>	<b>803.8 ± 436.3</b>	<b>191.7–1794.3</b>
TDS mg/L	0.5 ± 0.3	0.1–1.1
<b>NH<sub>3</sub> µmol/L</b>	<b>18.0 ± 40.2</b>	<b>1.3–157.3</b>
<b>NO<sub>3</sub> µmol/L</b>	<b>15.7 ± 46.0</b>	<b>0.6–185.5</b>
<b>P µmol/L</b>	<b>3.7 ± 5.6</b>	<b>0.6–22.8</b>
<b>PO<sub>4</sub> µmol/L</b>	<b>2.1 ± 4.6</b>	<b>0.1–18.6</b>
Chlorophyll a mg/L	30.6 ± 47.7	0.4–215.4
<b>Isolation 0/1</b>	<b>0.7 ± 0.5</b>	<b>0.0–1.0</b>
<b>Strahler's stream order</b>	<b>2.7 ± 0.7</b>	<b>1.0–4.0</b>
<b>Elevation m</b>	<b>137.2 ± 70.9</b>	<b>17.0–280.0</b>



recorded), respectively. All analyses were performed using R (R Core Team, 2021) and significance of statistical tests was assessed at  $p < 0.05$ .

### 2.3.1. Richness difference and species replacement components of beta diversity

We determined BD as the total variance in the community composition matrix using Jaccard-based dissimilarity coefficient, and decomposed it into RichDiff and Repl components, based on indices in the Podani family for presence-absence data (Legendre, 2014; Legendre and De Cáceres, 2013; Podani and Schmera, 2011), using the function *beta.div.comp* of *adespatial* (Dray et al., 2021). Richness difference describes the fact that one community may include a larger number of species than another, and may reflect the diversity of niches available at different sites across the study area and result from species loss or other ecological processes, including barriers to dispersal (Legendre, 2014). Species replacement refers to the fact that species tend to replace each other along spatial and environmental gradients, reflecting the simultaneous gain and loss of species due to environmental filtering, biotic interactions and historical factors (Legendre, 2014).

### 2.3.2. Local and species contributions to beta diversity

We decomposed BD into LCB and SCBD, following Legendre and De Cáceres (2013). LCB indicate the degree of ecological uniqueness of the sites, with large values corresponding to sites that have strongly different species compositions compared with a mean site (Legendre, 2014; Legendre and De Cáceres, 2013). We computed LCB from the community composition matrix and determined the statistical significance of individual LCB values through random, independent permutations within the columns of the community composition matrix (Legendre and De Cáceres, 2013), using the function *beta.div* in *adespatial* (Dray et al., 2021). Local contributions to richness difference ( $LCB_{RichDiff}$ ) and species replacement ( $LCB_{Repl}$ ) were calculated from the Jaccard-based dissimilarity matrices of RichDiff and Repl, respectively, using the function *LCB.comp* also in *adespatial* (Dray et al., 2021). LCB,  $LCB_{RichDiff}$  and  $LCB_{Repl}$  were mapped using QGIS v3.12.

SCBD describe the degree of variation in individual species contributions to beta diversity, with large values indicating species that vary more than the mean (Legendre and De Cáceres, 2013). We calculated SCBD from the community composition matrix, using the *beta.div* function (Dray et al., 2021).

### 2.3.3. Environmental and spatial predictors

As environmental predictors (ENV) we selected 14 out of the 25 variables measured, for having <45 % zeros and reduced collinearity (Table 1). Prior to variable selection, we replaced oxygen and temperature by their residuals on time of day, thus avoiding confounding influences in the analyses. We transformed skewed variables, using the angular and the  $\log_{10}$  transforms for proportional and continuous data, respectively, and we standardized all variables using z-score. We computed Pearson's correlation between each pair of variables and removed, from each pair of strongly correlated variables ( $|r| > 0.60$ ), the one least associated with the response variables (BD and its components and LCB,  $LCB_{RichDiff}$  and  $LCB_{Repl}$ ) as judged from univariate linear regressions.

We derived multi-scale spatial predictors using graph-based Moran's eigenvector maps (gb-MEM), which eigenvalues are directly proportional to the Moran's index of spatial autocorrelation. Eigenvalues were generated from a spatial weighting matrix (SWM) describing the relations between sites, obtained by the product of a connectivity matrix defining the pairs of connected and unconnected sites by a weighting matrix defining the strength of the connections between sites (Dray et al., 2006). Because the specification of SWM is a crucial step in the analysis, we built a set of candidate SWMs, as recommended by Bauman et al. (2018). Specifically, we built four connectivity matrices based on the Delaunay triangulation, Gabriel's graph, relative neighbourhood graph and minimum spanning tree schemes, using the function *nb* of

*spdep* (Bivand et al., 2022), and removed unrealistic spatial links (i.e., crossing river basins in CCM 2.1, QGIS v3.12) manually. We used graph-based rather than distance-based connectivity matrices because our sites were irregularly distributed within the study area (Bauman et al., 2018). We built two weighting uncorrelated matrices ( $r = -0.162$ ,  $p \leq 0.05$ ) to describe fragmentation by drought (DRY) and damming (DAM). DRY was based on the inverse dry distance ( $1/[d/d_{max}]$ , where  $d$  is length of the channel between sites that was dry as depicted from google Earth images (ca 0.15 m resolution; July 2019) and QGIS v3.12, and  $d_{max}$  is the maximum value of  $d$  in the dataset). DAM was based on the inverse number of dams ( $1/[N/N_{max}]$ , where  $N$  is the number of large dams between consecutive sites, and  $N_{max}$  is the maximum number of large dams in the dataset. SWMs obtained by the product of each connectivity matrix by each weighting matrix with the function *list.candidates* of *adespatial*, were double-centred and diagonalized to generate gb-MEMs, using *scores.list* also in *adespatial* (Dray et al., 2021). We only considered gb-MEMs with positive eigenvalues because we were primarily interested in dispersal, which is a contagious process, displaying positive spatial autocorrelation (Bauman et al., 2018; Borcard et al., 2018).

### 2.3.4. Variation in beta diversity, richness difference and species replacement

We analysed variation in BD and in RichDiff and Repl in relation to environmental (ENV) and spatial predictors (i.e., DRY or DAM gb-MEMs) with distance-based redundancy analysis (db-RDA), using *dbrda* function of *vegan* (Legendre and Anderson, 1999; Oksanen et al., 2020). We used a linear model of ordination because preliminary detrended correspondence analysis, not presented here, showed turn-overs <2 SD, which is the recommended criterion for choosing linear versus uni-modal ordination models (Borcard et al., 2018). Prior to analysis, dissimilarities for BD, RichDiff and Repl were square rooted to account for their non-Euclidean components (Legendre, 2014; Legendre and De Cáceres, 2013). We conducted separated db-RDAs for ENV, DRY and DAM using the following procedures. For ENV, we first test all variables using *anova.cca* function ( $10^4$  permutations) of *vegan* (Oksanen et al., 2020) and carried to subsequent forward selection only the subset producing significant full models. We performed forward selection using the double stop criterion of Blanchet et al. (2008), using function *ordir2step* of *vegan* (Oksanen et al., 2020). When lack of significance of the full model precluded the use of forward selection, we built db-RDAs using the ENV predictors yielding the best univariate relationships. For DRY and DAM, we followed the optimization procedure by Bauman et al. (2018). We first performed a test of significance on each of the four candidate SWMs for DRY and on the four candidate SWMs for DAM, using the entire set of positive gb-MEMs, with *anova.cca* and p-value corrected by the Sidak correction, where  $p_s = 1 - (1 - p)^k$  with  $p_s$  = the corrected p-value,  $p$  = the uncorrected p-value, and  $k$  = the number of SWMs. After, we conducted a forward selection with double stopping criterion on the significant SWMs to define the best subset of gb-MEMs for DRY and for DAM. Finally, we selected as optimal SWMs for DRY and for DAM the ones for which the best subsets of gb-MEMs yielded the highest  $adj-R^2$  (Table S2).

We analysed variation in local contributions to LCB,  $LCB_{RichDiff}$  and  $LCB_{Repl}$  in relation to ENV and DRY or DAM. Modelling of ENV was conducted using multivariate regression analysis and applying forward selection with the two stopping criteria (Blanchet et al., 2008) or using univariate regression analysis in case of lack of significance of the full model, as referred above. Following Bauman et al. (2018), we optimized the choice of SWMs for DRY and DAM based on the four candidates for each, using multivariate regression analysis and double stop forward selection (Table S3).

We used variance partitioning to isolate the relative contributions of ENV and each of DRY or DAM for variation in beta diversity and their components, using the function *varpart* in *vegan* (Oksanen et al., 2020). Following Borcard et al. (1992), we partitioned variation into four independent fractions: i) pure environmental, which is the fraction of

variation explained by environmental predictors independently of any spatial structure; ii) pure spatial, which is the spatial fraction not related to environmental predictors; iii) spatial component of environmental variation, or variation shared by environmental and spatial predictors, and iv) unexplained variation. Partial analyses for BD, RichDiff and Repl were conducted based on db-RDA, and we reported the adj-R<sup>2</sup> values (Peres-Neto et al., 2006), and tested the statistical significance of the pure fractions with *anova.cca* (10<sup>4</sup> permutations). For LCB<sub>D</sub>, LCB<sub>D</sub><sub>RichDiff</sub> and LCB<sub>D</sub><sub>Repl</sub> and its components, we conducted multiple linear regression analyses using *lm* function, reported adj-R<sup>2</sup>, and tested the statistical significance using *anova*.

We related LCB<sub>D</sub>, LCB<sub>D</sub><sub>RichDiff</sub> and LCB<sub>D</sub><sub>Repl</sub> and its components to the local number of both native and non-native species and we related SCBD to the number of sites in which the species were recorded using regression analysis, and the *lm* function. In both cases we screened models including linear and quadratic terms (Heino and Grönroos, 2017), and retained the one that provided the best fit to the data, as judged from adj-R<sup>2</sup> and the Akaike Information Criteria corrected for small sample sizes (AICc) (Murtaugh, 2009). We analysed associations of LCB<sub>D</sub>, LCB<sub>D</sub><sub>RichDiff</sub> and LCB<sub>D</sub><sub>Repl</sub> between native and non-native fish using the Pearson correlation coefficient.

### 3. Results

In total, 12 native and only 6 from 13 non-native species were among 9399 fish. The local number of species ranged from 1 to 8 for native fish (mean ± SD, 4 ± 2), and 1 to 5 for non-native fish (3 ± 1). Three native species were recorded in a high number of pools, including *Cobitis paludica* (24), *Luciobarbus steindachneri* (19) and *Squalius alburnoides* (17), as well as two non-natives, namely *Gambusia holbrooki* (26) and *Lepomis gibbosus* (24). The remaining native and non-native species were recorded in only 13 to 2 and 8 to 2 pools, respectively (Table S1).

#### 3.1. Variation in beta diversity, richness difference and species replacement

BD was higher for native (0.33) than non-native fish (0.21), but mainly driven by RichDiff (61 % and 65 %, respectively). For native fish, BD was associated with DRY (9 %), DAM (13 %) and ENV, specifically conductivity (3 %), isolation (2 %) and stream order (2 %) (Fig. 2, Table S2). Accordingly, more variation was explained by pure DAM (12 %) than pure ENV (7 %) whereas similar amounts were accounted for by pure DRY (5 %) and pure ENV (4 %), but approximately 80 % of

variation remained unexplained (86–80 %) (Fig. 3). For non-native fish, BD was associated with DRY (28 %), DAM (33 %) and ENV, specifically with elevation (13 %) and phosphorous (6 %). Correspondingly, more variation was explained by pure DAM (21 %) and pure DRY (18 %) than pure ENV (10–8 %) (Fig. 2).

For native fish, RichDiff was associated with DRY (27 %), DAM (25 %) and ENV, specifically with stream order (9 %) (Fig. 2, Table S2). However, variation in RichDiff was accounted for by pure DRY (15 %) and pure DAM (17 %) only, with pure ENV being not significant (Fig. 3). In contrast, Repl was solely related to ENV, namely aquatic vegetation (6 %) (Fig. 2). For non-native fish, RichDiff was only associated with ENV, specifically with elevation (13 %) (Fig. 2). Conversely, Repl was associated with both DRY (73 %) and DAM (83 %) and in addition with phosphorus (22 %) (Fig. 2, Table S2). However, only pure DAM and pure DRY were significant ( $p < 0.001$ ), explaining large amounts of variation (63 and 50 %, respectively), with contributions of approximately 20 % of shared effects (Fig. 3).

#### 3.2. Variation in local contributions to beta diversity

There were no significant associations between local contributions to beta diversity of native and non-native fish. Pools with contributions above the mean to BD, RichDiff and Repl of native (Fig. 4a–c) and non-native fish (Fig. 4d–f) were spread across the stream network. The two pools contributing significantly to BD of native fish, recorded the highest species richness difference and harboured a single species (*L. steindachneri*) (Fig. 4a). In contrast, the pool contributing significantly to BD of non-native fish, recorded the highest species replacement and harboured a single species (*Australoheros facetus*) (Fig. 4d).

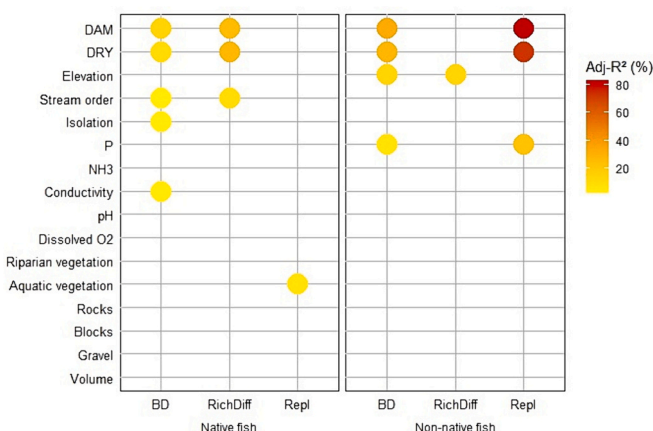
For native fish, LCB<sub>D</sub> were associated with DRY (40 %), DAM (33 %) and ENV, decreasing with stream order (30 %) (Fig. 5, Table S3). Variation in LCB<sub>D</sub> was however only explained by pure DRY (16 %) and shared effects (25 %), with both pure ENV and DAM being not significant (Fig. 6). For non-native fish, LCB<sub>D</sub> were associated with DRY (16 %), DAM (25 %) and ENV, decreasing with elevation (22 %) (Fig. 5, Table S3). Correspondingly, pure DAM (15 %) explained approximately the same amount of variation than pure ENV (13 %), while pure DRY (11 %) was less important than pure ENV (17 %) (Fig. 6).

LCB<sub>D</sub><sub>RichDiff</sub> and LCB<sub>D</sub><sub>Repl</sub> of native fish, showed no association with DAM, but similar to LCB<sub>D</sub>, were associated with DRY (28 and 23 %, respectively) and ENV, decreasing with stream order (14 %) and increasing with dissolved oxygen (11 %), respectively (Fig. 5, Table S3). However, variation in LCB<sub>D</sub><sub>RichDiff</sub> and LCB<sub>D</sub><sub>Repl</sub> was accounted for by pure DRY (14 and 19 %, respectively), and in the former also by shared effects (15 %), with pure ENV being not significant (Fig. 6). In contrast, for non-native fish, LCB<sub>D</sub><sub>RichDiff</sub> were solely associated with ENV, decreasing with riparian vegetation (10 %) (Fig. 5), whereas LCB<sub>D</sub><sub>Repl</sub> were not associated with predictors (Fig. 5, Table S3).

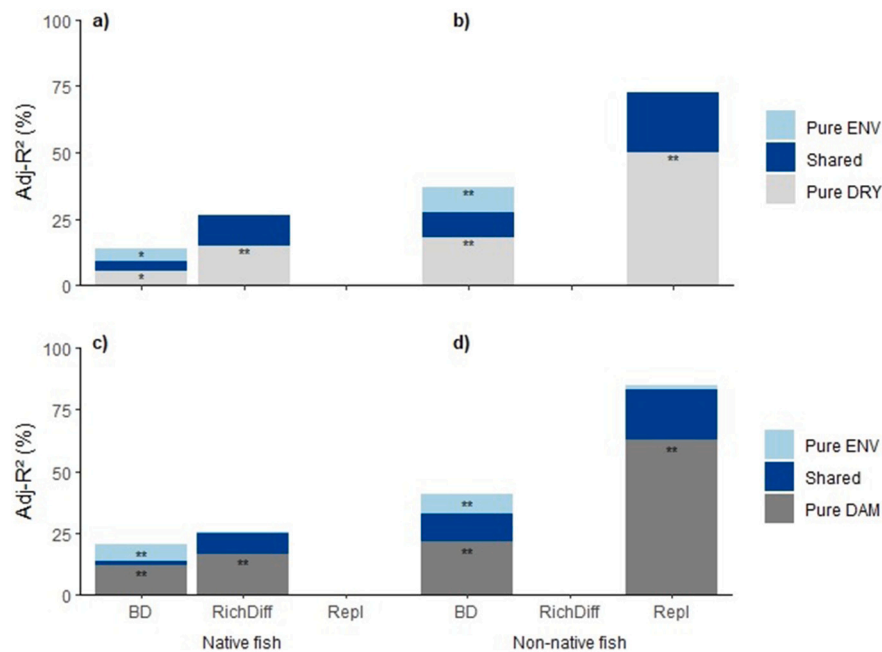
The best relationships between LCB<sub>D</sub>, LCB<sub>D</sub><sub>RichDiff</sub> and LCB<sub>D</sub><sub>Repl</sub> of native and non-native fish and the local number of corresponding species were quadratic ( $\Delta AIC_c = 2.5\text{--}60.8$ ; Table S4), and strong for LCB<sub>D</sub><sub>RichDiff</sub> (adj-R<sup>2</sup> = 92 and 89 %, respectively), which peaked in pools with either low or high species numbers, but weak or not significant for LCB<sub>D</sub><sub>Repl</sub> (Table 2). There were no significant relationships between LCB<sub>D</sub>, LCB<sub>D</sub><sub>RichDiff</sub> and LCB<sub>D</sub><sub>Repl</sub> of native and the local number of non-native, nor between LCB<sub>D</sub>, LCB<sub>D</sub><sub>RichDiff</sub> and LCB<sub>D</sub><sub>Repl</sub> of non-native and the local number of native species (Table S4).

#### 3.3. Variation in species contributions to beta diversity

Contributions above the mean to beta diversity of native fish were found for *L. steindachneri* (0.18) and also for *S. alburnoides* (0.12), *Squalius pyrenaicus* (0.11), *Luciobarbus sclateri* (0.10), *C. paludica* (0.10) and *Anguilla anguilla* (0.10). Contributions above the mean to beta diversity of non-native fish were observed for *A. facetus* (0.31) and also for *Micropterus nigricans* (0.20) and *Alburnus alburnus* (0.18) (Table S5). The



**Fig. 2.** Associations of beta diversity (BD), richness difference (RichDiff) and replacement (Repl) of native and non-native fish with environmental variables and spatial gb-MEM eigenvectors expressing the dry channel length between pools (DRY) and the number of large dams between pools (DAM), in pools in the Lower Guadiana basin, in July 2019. Adjusted-R<sup>2</sup> values (%) are indicated for each variable.



**Fig. 3.** Relative contributions of environmental variables (ENV) and of spatial gb-MEM eigenvectors expressing the dry channel length between pools (DRY) or number of large dams between pools (DAM) to beta diversity (BD), richness difference (RichDiff) and replacement (Repl) of native (a, c) and non-native fish (b, d), in pools in the Lower Guadiana basin, in July 2019. Adjusted- $R^2$  (%) are indicated, statistical significance of pure fractions is coded as \* for p-value < 0.05 and \*\* for p-value < 0.01, and negative values are omitted.

best relationships between SCBD and the number of pools in which species were recorded were quadratic ( $\Delta AIC_c = 4.1\text{--}14.0$ ; Table S6) and strong for both native (adj- $R^2 = 78\%$ ) and non-native fish (adj- $R^2 = 89\%$ ), with species recorded in an intermediated number of pools being predicted to contribute the most to beta diversity (Fig. 7; Table S7).

#### 4. Discussion

Understanding how beta diversity in pool refuges is structured is increasingly important to advance understanding of metacommunity organization and address conservation management in intermittent streams. Here, by examining individual beta diversity components in relation to local environment and fragmentations by drought and damming and distinguishing between native and non-native fish in INTMED, we exposed patterns and underlying processes that would otherwise not have been apparent. We found that: i) drought and damming fragmentations significantly influenced richness difference for native fish and were key drivers of species replacement for non-native fish; (ii) local environment significantly influenced richness difference and replacement for non-native fish; (iii) native assemblages were more unique in pools in low order streams whereas non-native assemblages were more unique in low elevation pools; and (iv) species with intermediated and low occupancy contributed significantly to beta diversity of native and non-native fish, respectively. Our results confirm the primary role of stream fragmentation in driving fish metacommunities in pool refuges and advance that beta diversity of native and non-native fish is differently influenced by drought and damming fragmentations.

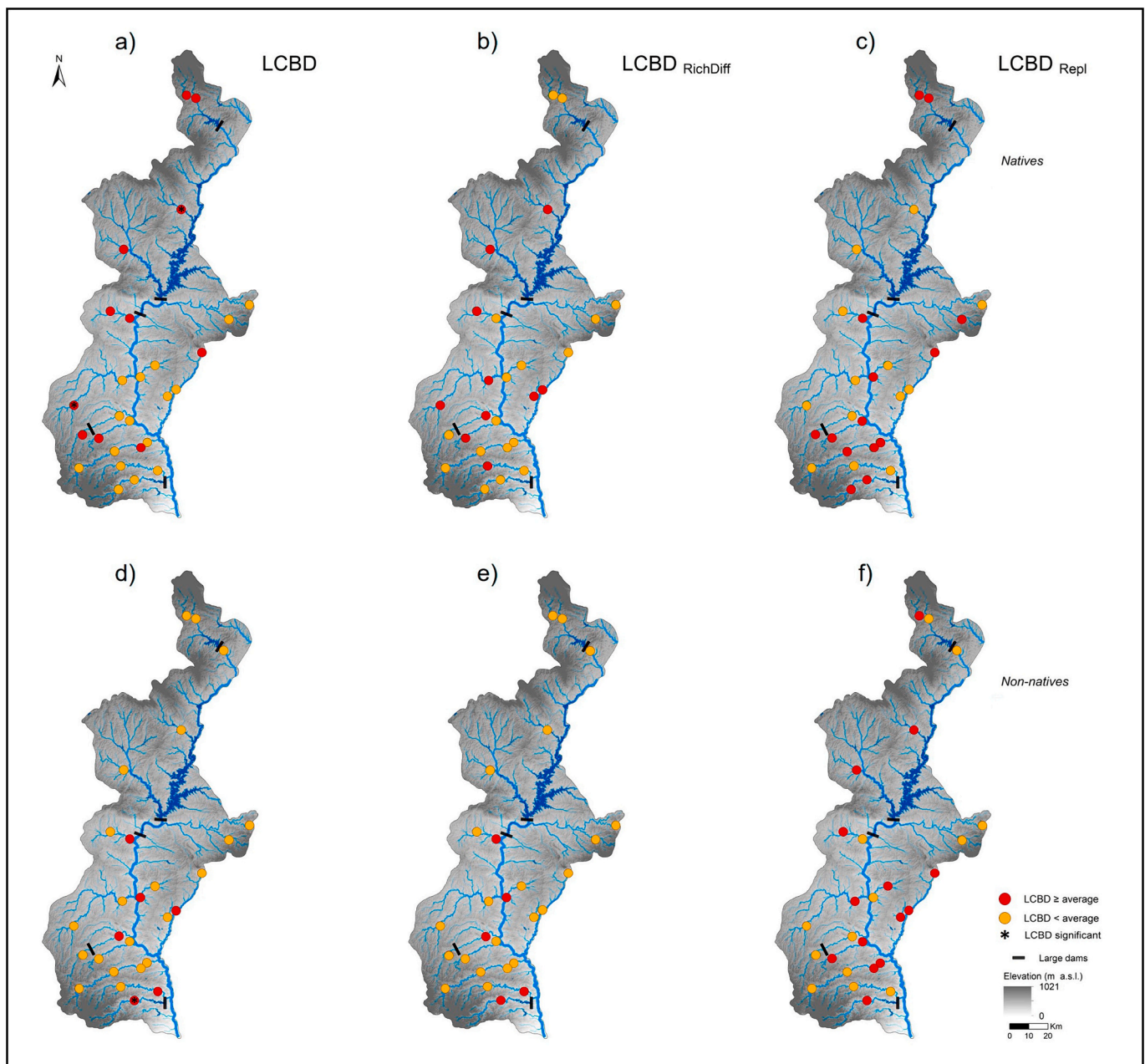
##### 4.1. Towards an improved assessment of beta diversity

Although the amounts of unexplained variation in beta diversity of native and non-native fish in refuge pools were similar to those found in prior empirical studies (e.g. Li et al., 2023; Xia et al., 2023) it would be important to further address the contributions of potentially important unmeasured factors. Climate, large-scale catchment characteristics (e.g., topography), and land use have been related to fish assemblage structure in INTMED as they typify aridity, stream size and location, and

human pressure (Filipe et al., 2010; Magalhães et al., 2002b). While these factors may hold relevance here, except for climate, the remaining influences might have been partially accounted for by stream order and elevation and phosphorous, respectively (Magalhães et al., 2002a; Matono et al., 2013). Furthermore, it would be important to consider the contributions of historical processes (e.g., large-scale dispersal and immigration-extinction events) to structuring native assemblages, along with stochastic processes, given both native and non-native fish assemblages are unsaturated in INTMED (Filipe et al., 2010). Acknowledging biotic interactions would also improve beta diversity assessment (Aspin and House, 2022; García-Girón et al., 2020), as species competition and predation are expected to intensify in refuge pools, particularly during severe droughts (Magalhães et al., 2002b; Bonada et al., 2020). In particular, a judicious analysis would be required to assess whether non-native species influence beta diversity of native fish, avoiding incorrectly link variations in species patterns to a priori expectations of species interactions. Moreover, it would be important to improve damming characterization by including small dams and weirs, which have been shown to affect the structure of non-native fish assemblages (Alexandre and Almeida, 2010), and complementing both drought and damming fragmentations with explicit fish movement modelling (Radinger et al., 2018). This may be particularly important, given we have not captured the full diversity of non-native species reported for the Lower Guadiana River basin (Collares-Pereira et al., 2021), missing species with restricted distributions or recently introduced that are typically associated with reservoirs (Banha et al., 2017; Martelo et al., 2021; Ribeiro et al., 2015).

A more complete appraisal of beta diversity is often achieved through the application of abundance-based dissimilarity measurements that may detect nuanced variations in richness difference and replacement, as well as local and species contributions to beta diversity (Heino and Grönroos, 2017; Dai et al., 2020). However, focusing on local fish abundances in INTMED which are more yearly fluctuating than species richness, would likely result in context specific patterns and decrease study generality and transferability (Magalhães et al., 2007). Indeed, there is a clear need for increasing the temporal frame of analysis, to account for the effects of inter- and intra- annual variation on assembly





**Fig. 4.** Maps of local contributions to beta diversity (LCBD), to richness difference ( $LCBD_{RichDiff}$ ) and to replacement ( $LCBD_{Repl}$ ) of native (a, b, c) and non-native fish (d, e, f), in pools in the Lower Guadiana basin, in July 2019. Values of LCBD,  $LCBD_{RichDiff}$  and  $LCBD_{Repl}$  above or equal and below the mean are differentiated, respectively, as red and orange, and indicated with an asterisk when significant.

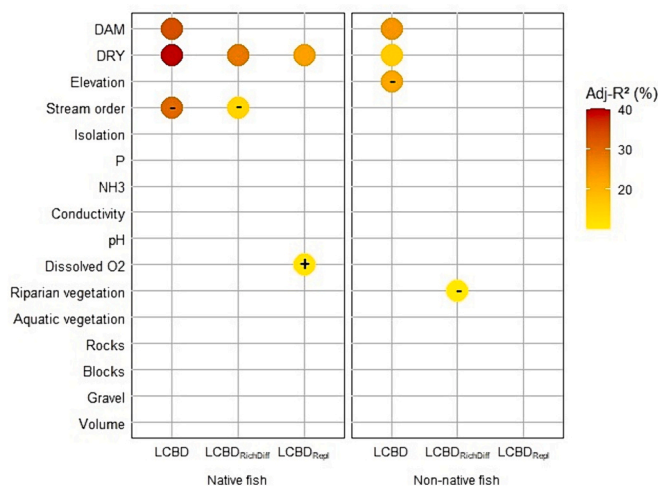
processes (Datry et al., 2016a; Ruhí et al., 2017; Xia et al., 2023). Nevertheless, by focusing on a severe drought, which may be a primary determinant of stream community structure (Leigh and Datry, 2017), it was possible to gain new insights into the beta diversity patterns and processes of fish community assembly in INTMED.

#### 4.2. Contrasting influences in richness difference and species replacement of native and non-native fish

As we predicted, the proportion of beta diversity accounted for by richness difference was remarkably high for native and non-native fish, indicating that differences in species composition between refuge pools were primarily a result of species loss. For native fish, richness difference was better accounted for by drought and damming fragmentations than local environment, pointing to the role of dispersal limitation, and

suggesting that the distribution of pool refuges and dams across the stream network combined with species-specific dispersal abilities to prevent some species to colonize pool refuges. These findings build on an existing body of evidence emphasizing the concurrent contributions of drought and damming, and the key role of spatial processes, in shaping metacommunities of fish (Marshall et al., 2021; Perkin et al., 2015; Vardakas et al., 2020) and other taxonomic groups in intermittent streams (Crabot et al., 2020; Gauthier et al., 2020, 2021). However, this contrasts with the dominance of environmental-related processes in shaping fish assembly in large sub-tropical rivers during the dry season (Xia et al., 2023). Moreover, our findings diverge from current conceptual models, which propose that communities in intermittent streams are primarily shaped by environmental filtering during drought (Datry et al., 2016a; Perkin et al., 2021). This context-dependency supports the need to expand the scope of intermittent streams and taxonomic groups





**Fig. 5.** Associations of local contributions to beta diversity (LCBD), to richness difference (LCBD<sub>RichDiff</sub>) and to replacement (LCBD<sub>Repl</sub>) of native and non-native fish with environmental variables and spatial gb-MEM eigenvectors expressing the dry channel length between pools (DRY) and the number of large dams between pools (DAM), in pools in the Lower Guadiana basin, in July 2019. Adjusted-R<sup>2</sup> values (%) are indicated for each variable. Adjusted-R<sup>2</sup> values (%) and direction of the association (+/-) are shown for each variable.

upon which current conceptual metacommunity models are based, as recently emphasized by [Aspin and House \(2022\)](#).

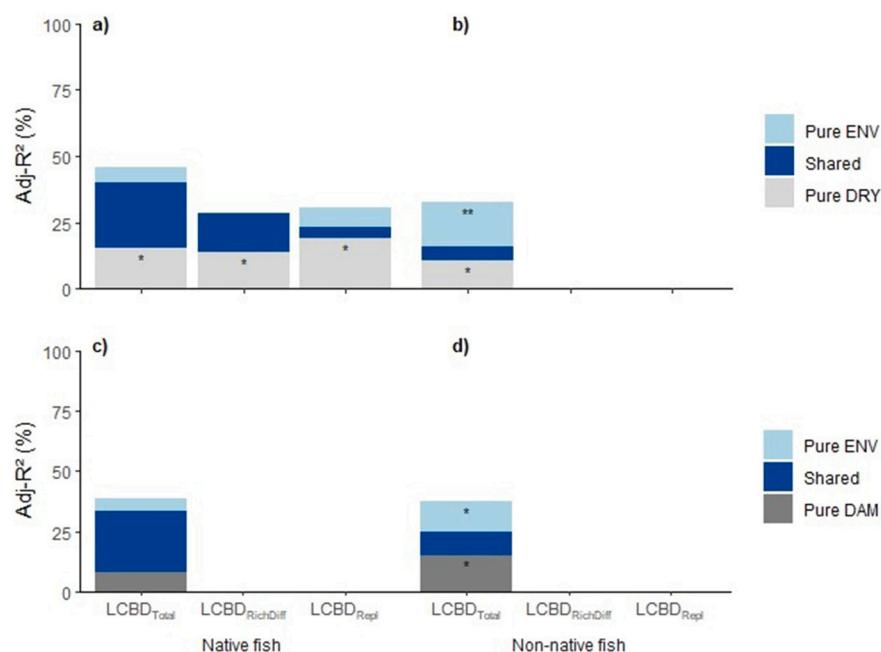
Contrary to our prediction, richness difference of non-native fish was influenced by local environment only. The influence of elevation may reflect the prevalence of less severe and unstable conditions in lowlands, which may be more suitable for non-native species in INTMED, considering their preference for lentic habitats ([Ilhéu et al., 2020](#); [Magalhães et al., 2002a](#); [Pires et al., 2010](#)). Furthermore, influences of other factors that covary with elevation and have been documented to influence local non-native species richness (e.g., land use) should not be disregarded ([Radinger et al., 2019](#)). Nevertheless, it is also possible that

human-driven processes, such as local species introductions, including deliberate stockings of species such as *A. alburnus* and *M. nigricans* for recreational fishing ([Anastácio et al., 2019](#); [Martelo et al., 2021](#)) have obscured the influences of drought and damming fragmentations in richness difference, as previously suggested for local richness and abundance of non-native fish in INTMED ([Radinger et al., 2019](#)).

Patterns of replacement for native fish partially also deviated from our initial prediction, being only slightly influenced by local environment, whereas patterns for non-native fish showed an important association with phosphorous. The influence of aquatic vegetation on native assemblages in INTMED has been previously documented ([Mesquita et al., 2006](#); [Martelo et al., 2014](#)), and likely reflects increased pool persistence, habitat quality and protection conditions ([Sabater et al., 2017](#); [Vardakas et al., 2017](#)). However, our results suggest that local environment may have lower importance in driving native species replacement in INTMED than in other riverine systems, where dispersal is not limited, and fish may track suitable habitat conditions ([Xia et al., 2023](#)). Drought is likely a strong filtering pressure that has been at work throughout the ecological history of native fish, selecting species with adequate traits to cope with the range of environmental conditions prevailing in pool refuges ([Magalhães et al., 2002a](#)). The influence of phosphorus on non-native fish replacement may be related to gradients in agricultural practices, including intensity and type, in the Lower Guadiana ([Matono et al., 2013](#)). This adds to previous evidence highlighting the influence of human pressures in shaping the distribution and abundance of non-native fish across INTMED ([Ilhéu et al., 2014](#)).

Contrary to our expectations, there were remarkably high contributions of drought and damming fragmentations to replacement of non-native fish. This likely reflects dispersal limitation imposed by drought and damming especially to recently established non-natives species, such as *A. alburnus*, which despite its broad environmental tolerance remains restricted to specific locations ([Martelo et al., 2021](#)). However, the association with fragmentation by damming may also reflect the facilitation of species additions and colonization opportunities, aligning with previous evidence on the role of human-mediated dispersal in shaping spatial turnover in non-native fish ([Leprieur et al., 2009](#)).

Taken together, our results suggest that during severe drought, beta



**Fig. 6.** Relative contributions of environmental variables (ENV) and spatial MEM eigenvectors expressing the dry channel length between pools (DRY) and number of large dams between pools (DAM) to local contributions to beta diversity (LCBD), to richness difference (LCBD<sub>RichDiff</sub>) and to replacement (LCBD<sub>Repl</sub>) of native (a, c) and non-native fish (b, d), in pools in the Lower Guadiana basin, in July 2019. Adjusted-R<sup>2</sup> (%) are indicated, statistical significance of pure fractions is coded as \* for p-value < 0.05 and \*\* for p-value < 0.01, and negative values are omitted.

Table 2

Summary results of regression models relating local contributions to beta diversity (LCBD), to species richness difference (LCBD<sub>RichDiff</sub>) and to species replacement (LCBD<sub>Repl</sub>) and local number of species for native and non-native species in pools in the Lower Guadiana basin, in July 2019. Regression coefficient estimates, standard errors of coefficient estimates and corresponding t, p and adjusted-R<sup>2</sup> values (%) are presented for the intercept and variables of each model.

	Estimate	SE	t	p-Value	Adj-R <sup>2</sup>
Natives					
LCBD					
Intercept	0.06	0.00	16.52	<0.001	66.87
Species richness	−0.01	0.00	−5.90	<0.001	
Species richness <sup>2</sup>	0.00	0.00	4.70	<0.001	
LCBD <sub>RichDiff</sub>					
Intercept	0.11	0.00	24.81	<0.001	92.07
Species richness	−0.04	0.00	−17.24	<0.001	
Species richness <sup>2</sup>	0.00	0.00	16.17	<0.001	
LCBD <sub>Repl</sub>					
Intercept	−0.02	0.01	−1.20	0.24	47.28
Species richness	0.03	0.01	4.71	<0.001	
Species richness <sup>2</sup>	0.00	0.00	−4.99	<0.001	
Non-natives					
LCBD					
Intercept	0.13	0.03	4.99	<0.001	34.16
Species richness	−0.07	0.02	−3.84	<0.001	
Species richness <sup>2</sup>	0.01	0.00	3.94	<0.001	
LCBD <sub>RichDiff</sub>					
Intercept	0.19	0.01	16.15	<0.001	89.37
Species richness	−0.12	0.01	−14.32	<0.001	
Species richness <sup>2</sup>	0.02	0.00	14.84	<0.001	
LCBD <sub>Repl</sub>					
Intercept	0.01	0.07	0.09	0.93	<0.001
Species richness	0.03	0.05	0.51	0.61	
Species richness <sup>2</sup>	0.00	0.01	−0.58	0.57	

diversity patterns for native and non-native fish in INTMED may be driven by different processes. Beta diversity for native fish may result from dispersal limitation by drought and damming leading to differences in species richness between pool refuges, but also from other unmeasured processes influencing species replacement. In contrast, beta diversity for non-native fish may result from environmental filtering influencing both richness difference and species replacement, and by dispersal limitation and species additions associated with drought and damming further influencing replacement. Nevertheless, perceived patterns for non-native fish should be considered cautiously, in light of the reduced number of species detected in pool refuges, underscoring the need to extend assessments to other permanent habitats.

4.3. Acknowledging the importance of local and species contributions to beta diversity

As predicted, local contributions to beta diversity for native and non-native fish were significantly explained by drought and damming fragmentations, but also by local environment, respectively by stream order and elevation. This underscores the role of dispersal limitation and species additions and colonization opportunities, but also of environmental filtering in shaping ecological uniqueness of individual pool refuges, as previously found elsewhere (Borges et al., 2020; Gavioli et al., 2019; Xia et al., 2022). Specifically, pools contributing most to beta diversity and to richness difference of native fish were located in low order streams and some harboured depauperate native assemblages. This was probably because headwaters typically experience higher isolation, with more restricted access of fish to pools, resulting in high differentiation of assemblages through species loss (Tonkin et al., 2018) and low species richness (Filipe et al., 2010; Ilhéu et al., 2020). Conversely, pools contributing the most to beta diversity of non-native fish were located at low elevations, possibly due to less harsh and

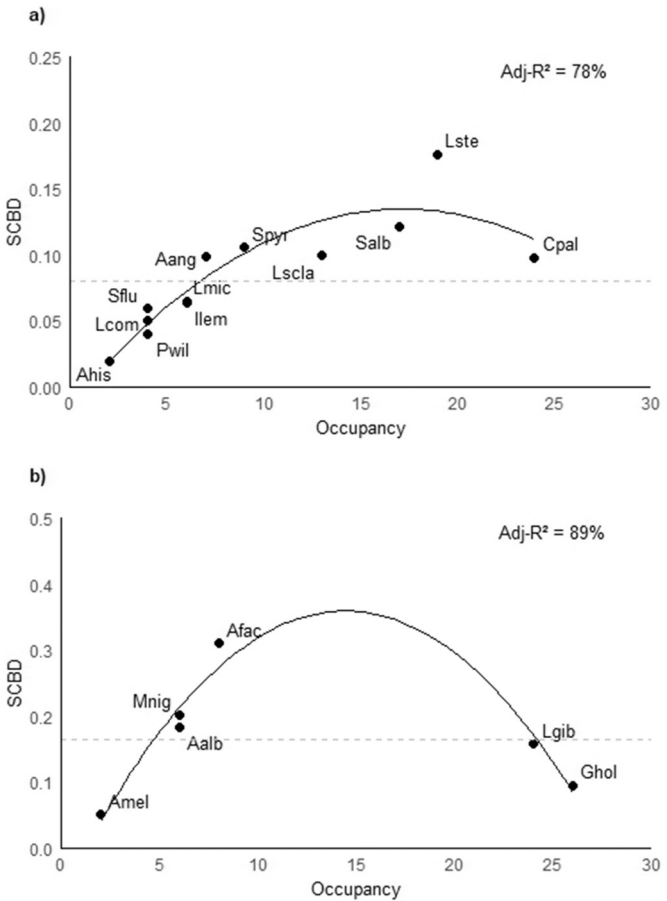


Fig. 7. Relationships between species contributions to beta diversity (SCBD) and the number of pools where native (a) and non-native species (b) were recorded in the Lower Guadiana basin, in July 2019. Adjusted-R<sup>2</sup> (%) values are indicated. Dashed grey line denotes the mean SCBD. The y-axes differ between (a) and (b).

more stable conditions at lowlands, to which non-native species are typically associated (Ilhéu et al., 2020; Magalhães et al., 2002a; Pires et al., 2010).

Contrary to our expectations, local contributions to beta diversity and especially to richness difference, exhibited U-shaped relationships with the local number of species for both native and non-native fish. The initial decrease of ecological uniqueness from single to multiple species assemblages was associated with the addition of common species (e.g. *C. paludica* and *S. alburnoides* in native assemblages and *G. holbrooki* and *A. facetus* in non-native assemblages), while the positive relationship with local richness was driven by rare native (e.g. *Anaocypris hispanica*, *Salariopsis fluviatilis* and *Pseudochondrostoma willkommii*) and non-native species (e.g. *A. melas* and *A. alburnus*). Although negative relationships between ecological uniqueness and local fish species richness appear to prevail in the literature (Borges et al., 2020; Ngor et al., 2018; Xia et al., 2022), our finding builds on previous evidence on the concurrent effects of additions of common and of rare species to communities (da Silva et al., 2018; Qiao et al., 2015).

As expected, species contributions to beta diversity were related to the number of pools where species were recorded, with a peak for native species at intermediate occupancy. This aligns with previous studies indicating that species contributions to beta diversity can be predictable from species occupancy (Gavioli et al., 2019; Heino and Grönroos, 2017; Xia et al., 2022). While a similar relationship was predicted for non-native species, no species were found to occupy an intermediate number of pools, with those contributing to beta diversity above the mean exhibiting low occupancy (<30 %). Species niche and biological traits

may affect species occupancy, and have been highlighted as potential correlates of species contributions to beta diversity (Heino and Grönroos, 2017), though little evidence supports this view. Xia et al. (2022) have associated species contributions to beta diversity with niche position but also with functional traits, with fish species only occurring in certain habitats contributing the less to beta diversity. Here, native species and non-native species with high contributions to beta diversity (e.g., *L. steindachneri* and *S. pyrenaicus* and *A. facetus* and *M. nigricans*, respectively) differed in habitat preferences, body size, feeding mode, and dispersal capacity (Alexandre et al., 2016; Magalhães, 1993; Pires et al., 2014; Ribeiro et al., 2007, 2008). Therefore, life-traits may not significantly influence species contributions to beta diversity, though this requires dedicated analyses, and particularly covering a higher diversity of non-native species in INTMED.

#### 4.4. Implications for conservation management

Our results have important implications for the conservation of native species diversity and the management of non-native species in pool refuges in INTMED, particularly in the face of increasingly drought and damming. The dominance of species richness difference in beta diversity patterns, indicates that pools supporting species-rich assemblages may act as sources for native species and should be prioritized for conservation, and that non-native species occurring there should be considered for control actions. Moreover, local contributions to beta diversity of native and non-native fish were independent, indicating there will be little or no conflicts addressing and implementing conservation and management goals.

For native fish, pools with high uniqueness in assemblage composition, located in low order streams, and harbouring species with high contributions to beta diversity (e.g. *L. steindachneri* and *S. pyrenaicus*), as well as rare species of conservation concern (e.g. *A. hispanica*, *S. fluviatilis* and *P. willkommii*) (Magalhães et al., 2023) should be assigned high conservation priority. Maintaining the environmental conditions, heterogeneity and distribution of these pools is particularly important, given more severe droughts and damming may exceed species resistance and resilience to habitat fragmentation and loss (Driver and Hoeinghaus, 2015). Moreover, pools with high uniqueness but comprising single species with significant contributions to beta diversity (e.g., *L. steindachneri*) should be the focus of restoration efforts. For non-native fish, species-rich pools located at low elevations, and harbouring species with high contributions to beta diversity (e.g. *A. facetus*, *M. nigricans* and *A. alburnus*) should be targeted for control and containment efforts to help mitigate their spread across the stream network.

Overall, our results highlight that accounting for the different components of beta diversity and individually analysing native and non-native fish across pool refuges reveal distinct patterns that must be considered in conservation management actions. Notably, in the Lower Guadiana River basin, despite non-native species exhibited a limited and marginal occupancy, all native species sought refuge in pools at tributaries. Therefore, by implementing measures to protect and restore these pools, as well as control non-native species, it may be possible to safeguard the whole diversity of native fish species.

#### Funding

Funding was granted by Foundation for Science and Technology FCT through project ISOINVA (PTDC/CTA-AMB/29105/2017), Centre for Ecology, Evolution and Environmental Changes cE3c and - Global Change and Sustainability Institute CHANGE (UIDB/00329/2020, doi:10.54499/UIDB/00329/2020), Marine and Environmental Sciences Centre MARE (UIDB/04292/2020 and UIDP/04292/2020), Associate Laboratory ARNET (LA/P/0069/2020), 10.54499/UIDB/00329/2020 and individual contracts for C. Gkenas (DL57/2016/CP1479/CT0036) and F. Ribeiro (CEEC/0482/2020). The funder had no role in study

design, collection, analysis and interpretation of data, and writing and decision to submit the article for publication.

#### CRedit authorship contribution statement

**Joana Martelo:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Christos Gkenas:** Writing – review & editing, Visualization, Investigation, Funding acquisition. **Diogo Ribeiro:** Writing – review & editing, Visualization, Investigation. **M. Judite Alves:** Writing – review & editing, Investigation, Funding acquisition. **Filipe Ribeiro:** Writing – review & editing, Investigation, Funding acquisition. **Julien Cucherousset:** Writing – review & editing, Investigation, Funding acquisition. **João Gago:** Writing – review & editing, Investigation. **M. Filomena Magalhães:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Conceptualization.

#### Declaration of competing interest

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

#### Data availability

Data will be made available on request.

#### Acknowledgment

We thank Gisela Cheoo, Loic Tudesque and Laetitia Lassalle for assistance in fieldwork, and to David Bauman for statistical advice on spatial Moran's eigenvector-maps. The manuscript benefited from the meaningful comments from two anonymous reviewers.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.172517>.

#### References

- Aguiar, F.C., Fernandes, M.R., Martins, M.J., Ferreira, M.T., 2019. Effects of a large irrigation reservoir on aquatic and riparian plants: a history of survival and loss. *Water* 11, 2379. <https://doi.org/10.3390/w11112379>.
- Alexandre, C.M., Almeida, P.R., 2010. The impact of small physical obstacles on the structure of freshwater fish assemblages. *River Res. Appl.* 26, 977–994. <https://doi.org/10.1002/rra.1308>.
- Alexandre, C.M., Almeida, P.R., Neves, T., Mateus, C.S., Costa, J.L., Quintella, B.R., 2016. Effects of flow regulation on the movement patterns and habitat use of a potamodromous cyprinid species. *Ecohydrology* 9, 326–340. <https://doi.org/10.1002/eco.1638>.
- Allen, G.H., Pavelsky, T.M., 2018. Global extent of rivers and streams. *Science* 361, 585–588.
- Anas, M.U.M., Mandrak, N.E., 2021. Drivers of native and non-native freshwater fish richness across North America: disentangling the roles of environmental, historical and anthropogenic factors. *Glob. Ecol. Biogeogr.* 30, 1232–1244. <https://doi.org/10.1111/geb.13298>.
- Anastácio, P.M., Ribeiro, F., Capinha, C., Banha, F., Gama, M., Filipe, A.F., Rebelo, R., Sousa, R., 2019. Non-native freshwater fauna in Portugal: a review. *Sci. Total Environ.* 650, 1923–1934. <https://doi.org/10.1016/j.scitotenv.2018.09.251>.
- Aspin, T., House, A., 2022. Alpha and beta diversity and species co-occurrence patterns in headwaters supporting rare intermittent-stream specialists. *Freshw. Biol.* 67, 1188–1202. <https://doi.org/10.1111/fwb.13910>.
- Banha, F., Veríssimo, A., Ribeiro, F., Anastácio, P.M., 2017. Forensic reconstruction of *Ictalurus punctatus* invasion routes using on-line fishermen records. *Knowl. Manag. Aquat. Ecosyst.* 56 <https://doi.org/10.1051/kmae/2017045>.
- Bauman, D., Drouet, T., Fortin, M.-J., Dray, S., 2018. Optimizing the choice of a spatial weighting matrix in eigenvector-based methods. *Ecology* 99, 2159–2166. <https://doi.org/10.1002/ecy.2469>.
- Bivand, R., Altman, M., Anselin, L., Assunção, R., Berke, O., Blanchet, G., Carvalho, M., Christensen, B., Chun, Y., Dormann, C., Dray, S., Gómez-Rubio, V., Krainski, E., Legendre, P., Lewin-Koh, N., Li, A., Mollo, G., Mueller, W., Ono, H., Parry, J., Peres-



- Neto, P., Piras, G., Reder, M., Sauer, J., Tiefseldorf, M., Westerholt, R., Wolf, L., Yu, D., 2022. *spdep: Spatial Dependence: Weighting Schemes, Statistics*.
- Blanchet, F.G., Legendre, P., Borcard, D., 2008. Forward selection of explanatory variables. *Ecology* 89, 2623–2632. <https://doi.org/10.1890/07-0986.1>.
- Bonada, N., Cañedo-Argüelles, M., Gallart, F., von Schiller, D., Fortuño, P., Latron, J., Llorens, P., Múria, C., Soria, M., Vinyoles, D., Cid, N., 2020. Conservation and management of isolated pools in temporary rivers. *Water* 12, 2870. <https://doi.org/10.3390/w12102870>.
- Borcard, D., Legendre, P., Drapeau, P., 1992. Partialling out the spatial component of ecological variation. *Ecology* 73, 1045–1055. <https://doi.org/10.2307/1940179>.
- Borcard, D., Gillet, F., Legendre, P., 2018. Spatial analysis of ecological data. In: Borcard, D., Gillet, F., Legendre, P. (Eds.), *Numerical Ecology With R, Use R!* Springer International Publishing, Cham, pp. 299–367. [https://doi.org/10.1007/978-3-319-71404-2\\_7](https://doi.org/10.1007/978-3-319-71404-2_7).
- Borges, P.P., Dias, M.S., Carvalho, F.R., Casatti, L., Pompeu, P.S., Cetra, M., Tejerina-Garro, F.L., Suárez, Y.R., Nabout, J.C., Teresa, F.B., 2020. Stream fish metacommunity organisation across a Neotropical ecoregion: the role of environment, anthropogenic impact and dispersal-based processes. *PLoS ONE* 15, e0233733. <https://doi.org/10.1371/journal.pone.0233733>.
- Carey, N., Chester, E.T., Robson, B.J., 2023. Loss of functionally important and regionally endemic species from streams forced into intermittency by global warming. *Glob. Chang. Biol.* 29, 3019–3038. <https://doi.org/10.1111/gcb.16650>.
- Cereja, R., Brotas, V., Nunes, S., Rodrigues, M., Cruz, J.P.C., Brito, A.C., 2022. Tidal influence on water quality indicators in a temperate mesotidal estuary (Tagus Estuary, Portugal). *Ecol. Indic.* 136, 108715. <https://doi.org/10.1016/j.ecolind.2022.108715>.
- Clavero, M., Hermoso, V., 2011. Reservoirs promote the taxonomic homogenization of fish communities within river basins. *Biodivers. Conserv.* 20, 41–57. <https://doi.org/10.1007/s10531-010-9945-3>.
- Clavero, M., Hermoso, V., Aparicio, E., Godinho, F.N., 2013. Biodiversity in heavily modified waterbodies: native and introduced fish in Iberian reservoirs. *Freshw. Biol.* 58, 1190–1201. <https://doi.org/10.1111/fwb.12120>.
- Collares-Pereira, M.J., Alves, M.J., Ribeiro, F., Domingos, I., Almeida, P.R., Costa, L., Gante, H., Filipe, A.F., Aboim, M.A., Rodrigues, P.M., Magalhães, M.F., 2021. *Guia dos peixes de água doce e migradores de Portugal Continental*. Edições Afrontamento, Lisboa.
- Crabot, J., Heino, J., Launay, B., Detry, T., 2020. Drying determines the temporal dynamics of stream invertebrate structural and functional beta diversity. *Ecography* 43, 620–635. <https://doi.org/10.1111/ecog.04835>.
- da Silva, P.G., Hernández, M.I.M., Heino, J., 2018. Disentangling the correlates of species and site contributions to beta diversity in dung beetle assemblages. *Divers. Distrib.* 24, 1674–1686. <https://doi.org/10.1111/ddi.12785>.
- Dai, B., Jiang, Z., Wang, C., Matsuzaki, S.-I.S., Zhou, L., 2020. Abundance-based dissimilarity measurements reveal higher heterogeneity of fish communities in the lotic habitats of the Yangtze-Caizi transitional floodplain. *Ecol. Indic.* 112, 106122. <https://doi.org/10.1016/j.ecolind.2020.106122>.
- Detry, T., Boulton, A.J., Bonada, N., Fritz, K., Leigh, C., Sauquet, E., Tockner, K., Huguency, B., Dahm, C.N., 2018. Flow intermittence and ecosystem services in rivers of the Anthropocene. *J. Appl. Ecol.* 55 (1), 353–364. <https://doi.org/10.1111/1365-2666.12941>.
- Detry, T., Larned, S.T., Fritz, K.M., Bogan, M.T., Wood, P.J., Meyer, E.I., Santos, A.N., 2014. Broad-scale patterns of invertebrate richness and community composition in temporary rivers: effects of flow intermittence. *Ecography* 37, 94–104. <https://doi.org/10.1111/j.1600-0587.2013.00287.x>.
- Detry, T., Bonada, N., Heino, J., 2016a. Towards understanding the organisation of metacommunities in highly dynamic ecological systems. *Oikos* 125, 149–159. <https://doi.org/10.1111/oik.02922>.
- Detry, T., Moya, N., Zubieta, J., Oberdorff, T., 2016b. Determinants of local and regional communities in intermittent and perennial headwaters of the Bolivian Amazon. *Freshw. Biol.* 61, 1335–1349. <https://doi.org/10.1111/fwb.12706>.
- Dray, S., Legendre, P., Peres-Neto, P.R., 2006. Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). *Ecol. Model.* 196, 483–493. <https://doi.org/10.1016/j.ecolmodel.2006.02.015>.
- Dray, S., Bauman, D., Blanchet, G., Borcard, D., Clappe, S., Guenard, G., Jombart, T., Larocque, G., Legendre, P., Madi, N., Wagner, H.H., 2021. *adespatial: Multivariate Multiscale Spatial Analysis*.
- Driver, L.J., Hoetinghaus, D.J., 2015. Spatiotemporal dynamics of intermittent stream fish metacommunities in response to prolonged drought and reactivity. *Mar. Freshw. Res.* 67, 1667–1679. <https://doi.org/10.1071/MF15072>.
- Ferreira, M., Filipe, A.F., Bardos, D.C., Magalhães, M.F., Beja, P., 2016. Modeling stream fish distributions using interval-censored detection times. *Ecol. Evol.* 6, 5530–5541. <https://doi.org/10.1002/ecs3.2295>.
- Filipe, A.F., Filomena Magalhães, M., Collares-Pereira, M.J., 2010. Native and introduced fish species richness in Mediterranean streams: the role of multiple landscape influences. *Divers. Distrib.* 16, 773–785. <https://doi.org/10.1111/j.1472-4642.2010.00678.x>.
- Freyhof, J., Bergner, L., Ford, M., 2020. Threatened Freshwater Fishes of the Mediterranean Basin Biodiversity Hotspot: Distribution, Extinction Risk and the Impact of Hydropower. *EuroNatur and RiverWatch i–viii*, pp. 1–348. <https://doi.org/10.7479/c6d4-2f73>.
- García-Girón, J., Heino, J., García-Criado, F., Fernández-Aláez, C., Alahueta, J., 2020. Biotic interactions hold the key to understanding metacommunity organisation. *Ecography* 43, 1180–1190. <https://doi.org/10.1111/ecog.05032>.
- Gasith, A., Resh, V.H., 1999. Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. *Annu. Rev. Ecol. Syst.* 30, 51–81. <https://doi.org/10.1146/annurev.ecolsys.30.1.51>.
- Gauthier, M., Launay, B., Le Goff, G., Pella, H., Douady, C.J., Detry, T., 2020. Fragmentation promotes the role of dispersal in determining 10 intermittent headwater stream metacommunities. *Freshw. Biol.* 65, 2169–2185. <https://doi.org/10.1111/fwb.13611>.
- Gauthier, M., Goff, G.L., Launay, B., Douady, C.J., Detry, T., 2021. Dispersal limitation by structures is more important than intermittent drying effects for metacommunity dynamics in a highly fragmented river network. *Freshw. Sci.* 40, 302–315. <https://doi.org/10.1086/714376>.
- Gavioli, A., Milardi, M., Castaldelli, G., Fano, E.A., Soininen, J., 2019. Diversity patterns of native and exotic fish species suggest homogenization processes, but partly fail to highlight extinction threats. *Divers. Distrib.* 25, 983–994. <https://doi.org/10.1111/ddi.12904>.
- Gavioli, A., Milardi, M., Soininen, J., Soana, E., Lanzoni, M., Castaldelli, G., 2022. How does invasion degree shape alpha and beta diversity of freshwater fish at a regional scale? *Ecol. Evol.* 12, e9493. <https://doi.org/10.1002/ecs3.9493>.
- Gido, K.B., Osborne, M.J., Propst, D.L., Turner, T.F., Olden, J.D., 2023. Megadroughts pose mega-risk to native fishes of the American Southwest. *Fisheries* 48, 204–214. <https://doi.org/10.1002/fsh.10912>.
- Heino, J., Grönroos, M., 2017. Exploring species and site contributions to beta diversity in stream insect assemblages. *Oecologia* 183, 151–160. <https://doi.org/10.1007/s00442-016-3754-7>.
- Ilhéu, M., Matono, P., Bernardo, J.M., 2014. Invasibility of Mediterranean-climate rivers by non-native fish: the importance of environmental drivers and human pressures. *PLoS ONE* 9, e109694. <https://doi.org/10.1371/journal.pone.0109694>.
- Ilhéu, M., da Silva, J., Morais, M., Matono, P., Bernardo, J.M., 2020. Types of dry-season stream pools: environmental drivers and fish assemblages. *Inland Waters* 10, 516–528. <https://doi.org/10.1080/20442041.2020.1843931>.
- Legendre, P., 2014. Interpreting the replacement and richness difference components of beta diversity. *Glob. Ecol. Biogeogr.* 23, 1324–1334. <https://doi.org/10.1111/geb.12207>.
- Legendre, P., Anderson, M.J., 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. *Ecol. Monogr.* 69, 1–24. <https://doi.org/10.1890/0012-9615>.
- Legendre, P., De Cáceres, M., 2013. Beta diversity as the variance of community data: dissimilarity coefficients and partitioning. *Ecol. Lett.* 16, 951–963. <https://doi.org/10.1111/ele.12141>.
- Leibold, M.A., Rudolph, F.J., Blanchet, F.G., De Meester, L., Gravel, D., Hartig, F., Peres-Neto, P., Shoemaker, L., Chase, J.M., 2022. The internal structure of metacommunities. *Oikos* 2022. <https://doi.org/10.1111/oik.08618>.
- Leigh, C., Detry, T., 2017. Drying as a primary hydrological determinant of biodiversity in river systems: a broad-scale analysis. *Ecography* 40, 487–499. <https://doi.org/10.1111/ecog.02230>.
- Lennox, R.J., Crook, D.A., Moyle, P.B., Struthers, D.P., Cooke, S.J., 2019. Toward a better understanding of freshwater fish responses to an increasingly drought-stricken world. *Rev. Fish Biol. Fish.* 29, 71–92. <https://doi.org/10.1007/s1160-018-09545-9>.
- Leprieux, F., Olden, J.D., Lek, S., Brosse, S., 2009. Contrasting patterns and mechanisms of spatial turnover for native and exotic freshwater fish in Europe. *J. Biogeogr.* 36, 1899–1912. <https://doi.org/10.1111/j.1365-2699.2009.02107.x>.
- Li, Z., Heino, J., Zhang, J., Ge, Y., Liu, Z., Xie, Z., 2023. Unravelling the factors affecting multiple facets of macroinvertebrate beta diversity in the World's Third Pole. *J. Biogeogr.* 50, 792–804. <https://doi.org/10.1111/jbi.14574>.
- Liew, J.H., Tan, H.H., Yeo, D.C.J., 2016. Dammed rivers: impoundments facilitate fish invasions. *Freshw. Biol.* 61, 1421–1429. <https://doi.org/10.1111/fwb.12781>.
- López-Delgado, E.O., Winemiller, K.O., Villa-Navarro, F.A., 2020. Local environmental factors influence beta-diversity patterns of tropical fish assemblages more than spatial factors. *Ecology* 101. <https://doi.org/10.1002/ecy.2940>.
- Magalhães, M.F., 1993. Effects of season and body-size on the distribution and diet of the Iberian chub *Leuciscus pyrenaicus* in a lowland catchment. *J. Fish Biol.* 42, 875–888. <https://doi.org/10.1111/j.1095-8649.1993.tb00397.x>.
- Magalhães, M.F., Beja, P., Canas, C., Collares-Pereira, M.J., 2002a. Functional heterogeneity of dry-season fish refugia across a Mediterranean catchment: the role of habitat and predation. *Freshw. Biol.* 47, 1919–1934. <https://doi.org/10.1046/j.1365-2427.2002.00941.x>.
- Magalhães, M.F., Batalha, D.C., Collares-Pereira, M.J., 2002b. Gradients in stream fish assemblages across a Mediterranean landscape: contributions of environmental factors and spatial structure. *Freshw. Biol.* 47, 1015–1031. <https://doi.org/10.1046/j.1365-2427.2002.00830.x>.
- Magalhães, M.F., Beja, P., Schlosser, I.J., Collares-Pereira, M.J., 2007. Effects of multi-year droughts on fish assemblages of seasonally drying Mediterranean streams. *Freshw. Biol.* 52, 1494–1510. <https://doi.org/10.1111/j.1365-2427.2007.01781.x>.
- Magalhães, M.F., Amaral, S.D., Sousa, M., Alexandre, C.M., Almeida, P.R., Alves, M.J., Cortes, R., Farrobo, A., Filipe, A.F., Franco, A., Jesus, J., Oliveira, J.M., Pereira, J., Pires, D., Reis, M., Ribeiro, F., Robalo, J.L., Sá, F., Santos, C.S., Teixeira, A., Domingos, I., 2023. *Livro vermelho dos peixes dulciaquícolas e diádomos de Portugal continental*. FCiências.ID & ICNF, I.P. Lisboa.
- Marshall, J.C., Lobegeiger, J.S., Starkey, A., 2021. Risks to fish populations in dryland rivers from the combined threats of drought and instream barriers. *Front. Environ. Sci.* 9, 325. <https://doi.org/10.3389/fenvs.2021.671556>.
- Martelo, J., Grossman, G.D., Porto, M., Filomena Magalhães, M., 2014. Habitat patchiness affects distribution and microhabitat use of endangered Mira chub *Squalius torgalensis* (Actinopterygii, Cypriniformes). *Hydrobiologia* 732, 93–109. <https://doi.org/10.1007/s10750-014-1850-4>.
- Martelo, J., Costa, L.M., Ribeiro, D., Gago, J., Magalhães, M.F., Gante, H.F., Alves, M.J., Cheoo, G., Gkenas, C., Banha, F., Gama, M., Anastácio, P.M., Tiago, P.M., Ribeiro, F., 2021. Evaluating the range expansion of recreational non-native fishes in Portuguese

- freshwaters using scientific and citizen science data. *BioInvasions Rec.* 10, 378–389. <https://doi.org/10.3391/bir>.
- Matono, P., Sousa, D., Ilhéu, M., 2013. Effects of land use intensification on fish assemblages in Mediterranean climate streams. *Environ. Manag.* 52, 1213–1229. <https://doi.org/10.1007/s00267-013-0152>.
- Mesquita, N., Coelho, M.M., Filomena, M.M., 2006. Spatial variation in fish assemblages across small Mediterranean drainages: effects of habitat and landscape context. *Environ. Biol. Fish.* 77, 105–120. <https://doi.org/10.1007/s10641-006-9058-8>.
- Message, M.L., Lehner, B., Cockburn, C., Lamouroux, N., Pella, H., Snelder, T., Tockner, K., Trautmann, T., Watt, C., Datry, T., 2021. Global prevalence of non-perennial rivers and streams. *Nature* 594, 391–397. <https://doi.org/10.1038/s41586-021-03565-5>.
- Mota-Ferreira, M., Filipe, A.F., Filomena Magalhães, M., Carona, S., Beja, P., 2021. Spatial modelling of temporal dynamics in stream fish communities under anthropogenic change. *Divers. Distrib.* 27, 313–326. <https://doi.org/10.1111/ddi.13189>.
- Murtaugh, P.A., 2009. Performance of several variable-selection methods applied to real ecological data. *Ecol. Lett.* 12, 1061–1068. <https://doi.org/10.1111/j.1461-0248.2009.01361.x>.
- Ngor, P.B., Legendre, P., Oberdorff, T., Lek, S., 2018. Flow alterations by dams shaped fish assemblage dynamics in the complex Mekong-3S river system. *Ecol. Indic.* 88, 103–114. <https://doi.org/10.1016/j.ecolind.2018.01.023>.
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2020. *vegan: Community Ecology Package*.
- Peres-Neto, P.R., Legendre, P., Dray, S., Borcard, D., 2006. Variation partitioning of species data matrices: estimation and comparison of fractions. *Ecology* 87, 2614–2625. <https://doi.org/10.1890/0012-9658>.
- Perkin, J.S., Gido, K.B., Cooper, A.R., Turner, T.F., Osborne, M.J., Johnson, E.R., Mayes, K.B., 2015. Fragmentation and dewatering transform Great Plains stream fish communities. *Ecol. Monogr.* 85, 73–92. <https://doi.org/10.1890/14-0121.1>.
- Perkin, J.S., Papraniku, I.F., Gibbs, W.K., Hoeinghaus, D.J., Walker, D.M., 2021. Temporal trajectories in metacommunity structure: insights from interdisciplinary research in intermittent streams. *Water* 8. <https://doi.org/10.1002/wat2.1531>.
- Pires, D.F., Pires, A.M., Collares-Pereira, M.J., Magalhães, M.F., 2010. Variation in fish assemblages across dry-season pools in a Mediterranean stream: effects of pool morphology, physicochemical factors and spatial context. *Ecol. Freshw. Fish* 19, 74–86. <https://doi.org/10.1111/j.1600-0633.2009.00391.x>.
- Pires, D.F., Beja, P., Magalhães, M.F., 2014. Out of pools: movement patterns of Mediterranean stream fish in relation to dry season refugia. *River Res. Appl.* 30, 1269–1280. <https://doi.org/10.1002/rra.2776>.
- Podani, J., Schmera, D., 2011. A new conceptual and methodological framework for exploring and explaining pattern in presence – absence data. *Oikos* 120, 1625–1638. <https://doi.org/10.1111/j.1600-0706.2011.19451.x>.
- Pumo, D., Caracciolo, D., Viola, F., Noto, L.V., 2016. Climate change effects on the hydrological regime of small non-perennial river basins. *Sci. Total Environ.* 542, 76–92. <https://doi.org/10.1016/j.scitotenv.2015.10.109>.
- Qiao, X., Li, Q., Jiang, Q., Lu, J., Franklin, S., Tang, Z., Wang, Q., Zhang, J., Lu, Z., Bao, D., Guo, Y., Liu, H., Xu, Y., Jiang, M., 2015. Beta diversity determinants in Badagongshan, a subtropical forest in central China. *Sci. Rep.* 5, 17043. <https://doi.org/10.1038/srep17043>.
- R Core Team, 2021. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing.
- Radinger, J., Hölker, F., Horký, P., Slavík, O., Wolter, C., 2018. Improved river continuity facilitates fishes' abilities to track future environmental changes. *J. Environ. Manag.* 208, 169–179. <https://doi.org/10.1016/j.jenvman.2017.12.011>.
- Radinger, J., Alcaraz-Hernández, J.D., García-Berthou, E., 2019. Environmental filtering governs the spatial distribution of alien fishes in a large, human-impacted Mediterranean river. *Divers. Distrib.* 25, 701–714. <https://doi.org/10.1111/ddi.12895>.
- Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T.J., Kidd, K.A., MacCormack, T.J., Olden, J.D., Ormerod, S.J., Smol, J.P., Taylor, W.W., Tockner, K., Vermaire, J.C., Dudgeon, D., Cooke, S.J., 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biol. Rev.* 94, 849–873. <https://doi.org/10.1111/bvr.12480>.
- Ribeiro, F., Orjuela, R.L., Magalhães, M.F., Collares-Pereira, M.J., 2007. Variability in feeding ecology of a South American cichlid: a reason for successful invasion in mediterranean-type rivers? *Ecol. Freshw. Fish* 16, 559–569. <https://doi.org/10.1111/j.1600-0633.2007.00252.x>.
- Ribeiro, F., Elvira, B., Collares-Pereira, M.J., Moyle, P.B., 2008. Life-history traits of non-native fishes in Iberian watersheds across several invasion stages: a first approach. *Biol. Invasions* 10, 89–102. <https://doi.org/10.1007/s10530-007-9112-2>.
- Ribeiro, F., Rylková, K., Moreno-Valcárcel, R., Carrapato, C., Kalous, L., 2015. Prussian carp *Carassius gibelio*: a silent invader arriving to the Iberian Peninsula. *Aquat. Ecol.* 49, 99–104. <https://doi.org/10.1007/s10452-015-9508-5>.
- Rogosch, J.S., Olden, J.D., 2019. Dynamic contributions of intermittent and perennial streams to fish beta diversity in dryland rivers. *J. Biogeogr.* 46, 2311–2322. <https://doi.org/10.1111/jbi.13673>.
- Ruhi, A., Datry, T., Sabo, J.L., 2017. Interpreting beta-diversity components over time to conserve metacommunities in highly dynamic ecosystems. *Conserv. Biol.* 31, 1459–1468. <https://doi.org/10.1111/cobi.12906>.
- Sabater, S., Timoner, X., Bornette, G., De Wilde, M., Stromberg, J.C., Stella, J.C., 2017. Chapter 4.2 - the biota of intermittent rivers and ephemeral streams: algae and vascular plants. In: Datry, T., Bonada, N., Boulton, A. (Eds.), *Intermittent Rivers and Ephemeral Streams*. Academic Press, pp. 189–216. <https://doi.org/10.1016/B978-0-12-803835-2.00016-4>.
- Sarremejane, R., England, J., Sefton, C.E.M., Parry, S., Eastman, M., Stubbington, R., 2020. Local and regional drivers influence how aquatic community diversity, resistance and resilience vary in response to drying. *Oikos* 129, 1877–1890. <https://doi.org/10.1111/oik.07645>. <https://doi.org/10.1016/j.scitotenv.2016.10.147>.
- SNIRH, 2023. Portuguese national water resources information system. <https://snirh.apambiente.pt>. (Accessed 15 January 2023).
- Socolar, J.B., Gilroy, J.J., Kunin, W.E., Edwards, D.P., 2016. How should beta-diversity inform biodiversity conservation? *Trends Ecol. Evol.* 31, 67–80. <https://doi.org/10.1016/j.tree.2015.11.005>.
- Su, G., Logez, M., Xu, J., Tao, S., Villéger, S., Brosse, S., 2021. Human impacts on global freshwater fish biodiversity. *Science* 371, 835–838. <https://doi.org/10.1126/science.abd3369>.
- Tickner, D., Opperman, J.J., Abell, R., Acreman, M., Arthington, A.H., Bunn, S.E., Cooke, S.J., Dalton, J., Darwall, W., Edwards, G., Harrison, I., Hughes, K., Jones, T., Leclère, D., Lynch, A.J., Leonard, P., McClain, M.E., Murruven, D., Olden, J.D., Ormerod, S.J., Robinson, J., Tharme, R.E., Thieme, M., Tockner, K., Wright, M., Young, L., 2020. Bending the curve of global freshwater biodiversity loss: an emergency recovery plan. *BioScience* 70, 330–342. <https://doi.org/10.1093/biosci/biaa002>.
- Tonkin, J.D., Altermatt, F., Finn, D.S., Heino, J., Olden, J.D., Pauls, S.U., Lytle, David A., 2018. The role of dispersal in river network metacommunities: patterns, processes, and pathways. *Freshw. Biol.* 63, 141–163. <https://doi.org/10.1111/fwb.13037>.
- Vander Vorste, R., Obedzinski, M., Nossaman Pierce, S., Carlson, S.M., Grantham, T.E., 2020. Refuges and ecological traps: extreme drought threatens persistence of an endangered fish in intermittent streams. *Glob. Chang. Biol.* 26, 3834–3845. <https://doi.org/10.1111/gcb.15116>.
- Vardakas, L., Kalogianni, E., Papadaki, C., Vavalidis, T., Mentzafou, A., Koutsoubas, D., Skoulikidis Th., N., 2017. Defining critical habitat conditions for the conservation of three endemic and endangered cyprinids in a Mediterranean intermittent river before the onset of drought. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 27, 1270–1280. <https://doi.org/10.1002/aqc.2735>.
- Vardakas, L., Kalogianni, E., Smeti, E., Economou, A.N., Skoulikidis, N.Th., Koutsoubas, D., Dimitriadis, C., Datry, T., 2020. Spatial factors control the structure of fish metacommunity in a Mediterranean intermittent river. *Ecohydrol. Hydrobiol.* 20, 346–356. <https://doi.org/10.1016/j.ecohyd.2020.04.005>.
- Vogt, J., Soille, P., De Jager, A., Rimaviciute, E., Mehl, W., Foisneau, S., Bodis, K., Dusart, J., Paracchini, M.-L., Haastrup, P., Bamps, C., 2008. A Pan-European River and Catchment Database. JRC Publications Repository. <https://doi.org/10.2788/35907>.
- Xia, Z., Heino, J., Yu, F., He, Y., Liu, F., Wang, J., 2022. Spatial patterns of site and species contributions to  $\beta$  diversity in riverine fish assemblages. *Ecol. Indic.* 145, 109728. <https://doi.org/10.1016/j.ecolind.2022.109728>.
- Xia, Z., Heino, J., Yu, F., Xu, C., Lin, P., He, Y., Liu, F., Wang, J., 2023. Local environmental and spatial factors are associated with multiple facets of riverine fish  $\beta$ -diversity across spatial scales and seasons. *Freshw. Biol.* n/a, 1–16. <https://doi.org/10.1111/fwb.14187>.