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Freshwater biodiversity crisis: multidisciplinary approaches as tools for conservation, volume II

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Freshwater biodiversity crisis: multidisciplinary approaches as tools for conservation, volume II

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Editorial: Freshwater biodiversity crisis: multidisciplinary approaches as tools for conservation Volume II

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Editorial on the Research Topic
Freshwater biodiversity crisis: multidisciplinary approaches as tools for conservation Volume II

This editorial extends comments by Ottoni et al. entitled "*Freshwater biodiversity crisis: Multidisciplinary approaches as tools for conservation*". As previously reported, the "*freshwater biodiversity crisis*" (e.g., Darwall et al., 2018; Harrison et al., 2018; Albert et al., 2020) is part of the emerging planetary emergency and sixth mass extinction event in Earth history arising from anthropogenic impacts (Ripple et al., 2020; Meier et al., 2025).

Although freshwater ecosystems cover a tiny fraction of Earth's surface, they comprise an astonishing diversity of species and ecological traits. According to Kopf et al. (2015), p. 799, rivers, lakes, streams and other freshwater habitats, collectively "*(. . .) make up less than 2% of the Earth's surface but are home to approximately 10% of all described species of fungi, plants, invertebrates, and vertebrates (...)*." In other words, the species richness presented consider only named species. Numerous species descriptions are still being published today. This suggests that it is likely that this freshwater species' richness will increase considerably in the next years, especially for groups such as invertebrates and fishes. Freshwater ecosystems also provide numerous services, especially in the group of provision (e.g., animal protein). However, they also provide other services, such as cultural (e.g., tourism), regulation (e.g., seed dispersal), support (nutrient cycling), and others (Albert et al., 2020; Pelicice et al., 2022).

It is important to emphasize that freshwater ecosystems and faunas face more risks than terrestrial and marine ones (Darwall et al., 2018; Harrison et al., 2018; Reid et al., 2019; Tickner et al., 2020; Ottoni et al.). Some examples of major threats are shown in Figure 1. Now, it is known that about one-quarter of all freshwater species are currently threatened with extinction, based on a study which investigated decapod crustaceans, fishes and odonates (Sayer et al.,

FIGURE 1
Examples of major threats to freshwater ecosystems: **(a)** Invasive species "Giant river prawn" introduced into the Lençóis Maranhenses National Park, northeastern Brazil (photo: Felipe Ottoni). **(b)** Polluted and channelized river heavily affected by urbanization, Rio de Janeiro municipality, southeastern Brazil (photo: Paulo Vilardo). **(c)** Polluted and channelized river receiving sewage effluents in the Codó municipality, northeastern Brazil (photo: Carlos Filgueira). **(d)** Polluted stream in the Adolpho Ducke Forest Reserve, Manaus municipality, northern Brazil (photo: Douglas Bastos). **(e)** Tributary of the Amazon River contaminated with plastic waste, Manaus municipality, northern Brazil (photo: Ricardo Oliveira). **(f)** River receiving pollution from industry, Sidoarjo Regency, East Java, Indonesia (photo: Prigi Arisandi). **(g)** Marimbondo hydroelectric power plant and reservoir, southeastern Brazil (photo: Valter Azevedo-Santos).

2025). The main threats are from water pollution (plastic, urban, industrial or agricultural pollution) (Figures 1b–f), dams (Figure 1g) that cause habitat degradation and loss as well as block migration routes of fish species, overharvesting, water diversion and extraction infrastructure, agribusiness induced land-use changes (i.e., deforestation and forest degradation), non-native invasive species (Figure 1a), and diseases, with 84% of the species affected by more than one threat (Sayer et al., 2025). These factors directly impact freshwater ecosystems, causing their degradation, modification and/or total destruction (Figures 1b–g) (Ottoni et al.; Sayer et al., 2025). Different taxa are impacted in distinct ways: odonates (dragonflies and damselflies) are mostly imperilled by habitat loss, while 60% of the studied decapod crustaceans (shrimps and crabs) are mostly affected by pollution, which is also a main threat for fishes, along with damming and the modification and degradation of aquatic ecosystems (Sayer et al., 2025).

Lack of information on the abundances and distributions of freshwater species is an impediment to scientific conservation and sustainable management (Edgar, 2025). While the International Union for Conservation of Nature (IUCN) Red List of Threatened Species provides a rigorous methodology for assessing conservation status, the enterprise is hampered by the sheer scale of the task and limited information on the ecology and biogeography of most species (Edgar, 2025). As a result, the IUCN Red List is strongly biased towards species with large body sizes or commercial importance (Edgar, 2025), obscuring the actual magnitude of the global biodiversity crisis. The Red List under-reports the conservation status of species with small body sizes (e.g., invertebrates and small-sized fish) and inconspicuous habits (e.g., nocturnal, underwater or underground). Consequently, these species often exhibit undetected population declines but are categorized as Data Deficient rather than receiving a higher threat category (Edgar, 2025). Population trends, one of the main evaluation criteria for the IUCN Red List, are easier to assess in large terrestrial vertebrates, but difficult to evaluate in inconspicuous species.

Although inconspicuous and cryptic species may benefit from measures that target charismatic taxa, many lesser-known species require specific conservation measures. Many species are still unknown to science and face accelerated extinction rates, creating an eternal information gap on species that will disappear before being described or known. Modern approaches such as environmental DNA (eDNA), integrative taxonomy, revisions at small geographic scales, and "dark taxonomy" protocols (Meier et al., 2025) can help us accurately recognize species. This recognition is crucial for adopting efficient conservation policies and for providing stakeholders with accurate information. This is urgent, given the risk of losing species before we even know them.

In this research topic, 10 papers explore various aspects related to freshwater biodiversity conservation. We provide a brief overview of

these publications. Recently, eDNA metabarcoding has become a powerful method for estimating species richness in local communities by employing high-throughput sequencing to detect species from environmental materials. Wu et al. developed a new portable eDNA collector that performs similarly to other commercial kits. Yet, to improve eDNA extraction, Zhao et al. experimentally evaluated the impacts of biotic and abiotic factors on eDNA degradation and found that bacterial abundance and pH are the main causes behind this decay. Kanjuh et al. utilized microsatellite loci to assesss the structure of 15 brown trout populations and non-native genetic material introgression into native populations. Substantial genetic similarities among populations were found, owing to stocks from fish farms that included non-native phylogenetic lineages.

This editorial also approached the effects of damming on biotic and abiotic factors. Novitskyi et al. examined the fishing losses in an exploded reservoir in the Ukraine, which caused long-term socioeconomic impacts and means a challenge for water supply and fishery in a post-war recovery. Pompeu et al. focused on the consequences of damming for irrigation on freshwater communities of mountain streams, concluding that dams strongly affect the riverine flow regime and diatom communities. López-Casas et al. modeled spawning areas for potamodromous freshwater fish in the Magdalena basin in Colombia and found that these areas strongly overlap with hydropower projects, emphasizing the importance of water management measures and promoting habitat connectivity.

Although the impacts of environmental change on the spread of diseases are often overlooked, Costa et al. analyzed the connection between pollutants and host-parasite interactions, highlighting how freshwater pollution significantly increases the transmission risk of schistosomiasis. Qu and Zhou explored the relationship between freshwater lake water quality and the phytoplankton community, stressing the need for monitoring to ensure the reduction of eutrophication. Ye et al. studied the relationship between water quality and watershed land cover at the Three Gorges Reservoir (TGR), advising long-term management to improve water quality. Lastly, Ma et al. discovered a direct link between the reduction of freshwater biodiversity and threats to ecosystem functioning, finding that in urbanized areas strongly affected by water pollution, phytoplankton community evenness contributes more to ecosystem functionality than environmental factors.

We conclude with a gentle reminder of the need to conserve aquatic ecosystems on different fronts, including new taxonomic studies, programs to prevent negative impacts on areas relevant to threatened freshwater biodiversity, the implementation of adequate protected areas, and engagement of scientists in policy formulation to protect freshwater biodiversity (Azevedo-Santos et al., 2021; Azevedo-Santos and Ottoni, 2025). For example, enhancing investments in new studies on taxonomic diversity (especially using integrative approaches), ecology, and regional species estimates (e.g., inventories and DNA-barcoding) are urgent, aiming to understand ecological aspects of species, estimate our freshwater biodiversity, as well as reveal and describe undescribed species. This is justified by the fact that undescribed species (i.e., not named) are frequently left out of legal protections, biodiversity assessments, and conservation planning. This invisibility means they may be lost to extinction before they are even discovered, particularly in fragile freshwater habitats threatened by deforestation, climate change, and other human activities. On the other hand, for example, the ecological roles of inconspicuous and cryptic freshwater species, which maintain water quality and support aquatic food webs, remain poorly known—limiting our understanding of aquatic ecosystem processes, and undermining efforts to conserve the rich biodiversity and ecosystem services of these vital habitats.

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Ecological water quality of the Three Gorges Reservoir and its relationship with land covers in the reservoir area: implications for reservoir management

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In this study, we evaluated the ecological water quality of the entire Three Gorges Reservoir (TGR) and further examined the relationship with changes in watershed land covers. Using the phytoplankton functional group-based Q index, we found that the ecological water quality in the mainstream (previously known as the Yangtze River) of TGR is good, with 84% of sites in the status above good. While the poor ecological water quality was generally observed in the backwater regions of TGR's tributaries, with 79% of sites below the good status. Further investigating the potential impacts of the changes in land covers within the watershed on the tributary ecological water quality, we found that the percentage of urban and farmland areas had a significant ($p < 0.05$) negative correlation with the Q index-based ecological water quality of the tributary bays, and the forest cover had a marginally significant ($p = 0.058$) positive correlation with the ecological water quality. As a comparison, total nitrogen and total phosphorus in the tributary backwater regions of TGR had no reasonable correlation with the land covers within the watershed. Our study highlights that watershed management can enhance the ecological water quality in the backwater regions of TGR's tributaries, but it likely to be a long-term process. This implies considerations of other rapid measures, such as the water level regulation approach, should also be considered in reservoir management. Our study underscores the importance of ecological water quality assessment in reservoir management and provides insights into the impacts of changes in watershed land covers on ecological water quality in backwater regions of TGR's tributaries.

KEYWORDS

watershed management, biological assessment, ecological status, phytoplankton, reservoir

1 Introduction

Constructing dams and reservoirs is a significant approach for human beings to utilize and manage water resources (Wang et al., 2022). Undoubtedly, reservoir provides essential functions in providing water supply, irrigation, regulating floods, and clean hydropower generation, thereby supporting the developments of society and economy (Winton et al.,

2019; Hanazaki et al., 2022). Dammed reservoirs contribute to about 30–40% of global agriculture irrigation water, and reservoir-related hydropower accounts for more than 16% of global power generation (Maavara et al., 2020). Moreover, approximately 70% of rivers on Earth are dammed and formed reservoirs according to relevant statistics (Kummu and Varis, 2007). Furthermore, globally, the construction of reservoirs is still in a rapid development period owing to the increased demands for water resource (Lehner et al., 2011; Mulligan et al., 2020).

However, the building of reservoirs is likely to lead various ecological and environmental issues including eutrophication and algal blooms, caused by alterations in hydrological situations (Fu et al., 2010; Gamez et al., 2019). Such problems are particularly pronounced in backwater areas of reservoir tributaries, where nutrients can have an extended residence time (Ye et al., 2014; Li et al., 2020). The recognition of excessive nutrient flux from upstream watershed as a primary contributor to downstream water quality degradation has been long-established in the literature (Carpenter et al., 1998; Li et al., 2021). Watershed land covers are considered a critical factor in determining nutrient export to downstream water bodies (Conley et al., 2009; Wei et al., 2020; Yin et al., 2021; Su et al., 2022; Zhang et al., 2022). Conversion of natural land covers to farmland or urban areas will increase nutrient export, which can degrade the water quality of the downstream water bodies (Huang et al., 2022; Su et al., 2022). Therefore, accurately evaluating the water quality of reservoirs and examining relationships among water quality and watershed land covers are essential for effective management of watersheds in reservoir regions.

The improvement of reservoir water quality through current knowledge has primarily relied on watershed management techniques (Komatsu et al., 2010; Shen et al., 2022). However, recent research has highlighted the effects of water level fluctuations and reservoir ecosystem characteristic itself on the water quality (Akongyuure and Alhassan, 2021; He et al., 2023). For instance, Naselli-Flores and Barone (2005) reported that the water level fluctuation is a significant factor driving water quality in Mediterranean reservoirs. Meanwhile, plankton and fish in reservoir ecosystems also have a significant impact on the biogeochemical cycle of biogenic elements, thereby influencing the water quality of reservoirs (Akongyuure and Alhassan, 2021; Xu et al., 2022). Given this, we pose the question that whether watershed land cover management alone is sufficient to enhance the water quality of reservoirs.

Beyond the chemical parameters-based water quality indices (e.g., nitrogen, phosphorus), there has been increasing focus on ecological water quality and its significance in environmental management (Karr, 1993; Katsiapi et al., 2016; Çelekli and Lekesiz, 2021). Phytoplankton, which is sensitive to environmental changes and forms the basis of aquatic ecosystems (Winder and Sommer, 2012; Ye et al., 2019), is a kind of biota widely used for evaluating ecological water quality (Padisak et al., 2006; Katsiapi et al., 2016). As the ecological status of water body gains more attention, several indices based on different groups of aquatic organisms have been established for the assessment of ecological water quality in recent years. Among them, the Q index (Padisak et al., 2006), rooted on the functional structure of phytoplankton community, is one of the most widely used indexes in assessing the ecological water quality for different water bodies with no geographic limitations (Çelekli and Lekesiz, 2021; Korneva and Solovyeva, 2021; Wu et al., 2023). Despite the growing interest in assessing the ecological water quality of lakes and reservoirs (European Environment Agency, 2000; Katsiapi et al., 2016), there has been little attention paid to the potential impacts of watershed land covers changes on ecological water quality.

The Three Gorges Reservoir (TGR) represents the largest strategic freshwater resource pool in China with a reservoir capacity of 39.3×10^9 m^3 (Zhang and Lou, 2011; Ye et al., 2014). Unfortunately, most backwater regions in the TGR's tributaries are facing the problems of eutrophication and phytoplankton blooms after the reservoir impoundment, as a results of reduced water velocity and the influx of excessive nutrients from the upstream watershed (Fu et al., 2010; Ye et al., 2014; Luo et al., 2022). Although watershed land covers management has long been considered a fundamental measure for water quality improvement, the impacts of watershed land cover changes in the TGR region on the water quality of backwater regions of tributaries is seldom addressed. Moreover, the evaluation of water quality for the TGR was largely relied on chemical parameters (e.g., total nitrogen and total phosphorus) (e.g., Zhang et al., 2019; He et al., 2023). Yet, the ecological water quality based on biological indicators was seldom addressed in the TGR.

The present study aims to fill the above gaps by 1) investigating the ecological water quality of tributaries and mainstream (former Yangtze River) of the whole TGR from the Three Gorges Dam to the upstream; 2) examining potential effects of changes in land covers withing watershed on ecological water quality of the backwater regions of TGR's tributaries by testing the hypotheses that increase of anthropologic activities (e.g., urbanization, farming) will deteriorate the ecological water quality (H1) and restoration of natural land cover (e.g., forest, grassland) can enhance the ecological water quality in the backwater regions of TGR's tributaries (H2); 3) discussing potential management measures that could enhance the ecological water quality of TGR and other similar reservoirs.

2 Materials and methods

2.1 Study area

The TGR is a crucial project for the developing and utilizing of water resource in the Yangtze River. From a geographical perspective, TGR is suited upstream of Yangtze River from Sandouping in Hubei province to Jiangjin County in Chongqing municipality (Xiang et al., 2021). The climate of the TGR area is the subtropical monsoon climate. The average annual air temperature of this area is 17.4°C, and the mean annual precipitation is 1204 mm (Cui et al., 2022). And the mean annual runoff of the TGR is 4.0×10^{11} m^3 (Yan et al., 2021).

Since the completion of the final impounding stage in October 2010, the TGR reached the design water level of 175 m, creating a massive reservoir spanning approximately 660 km in the mainstream of the Yangtze River, with a flood control capacity of 2.215×10^{10} m^3 (Cui et al., 2022). Typically, the lowest water levels (around 145 m) occur in late May for flood control, while the highest water levels (around 175 m above sea level) are usually observed in October (Ye et al., 2022).

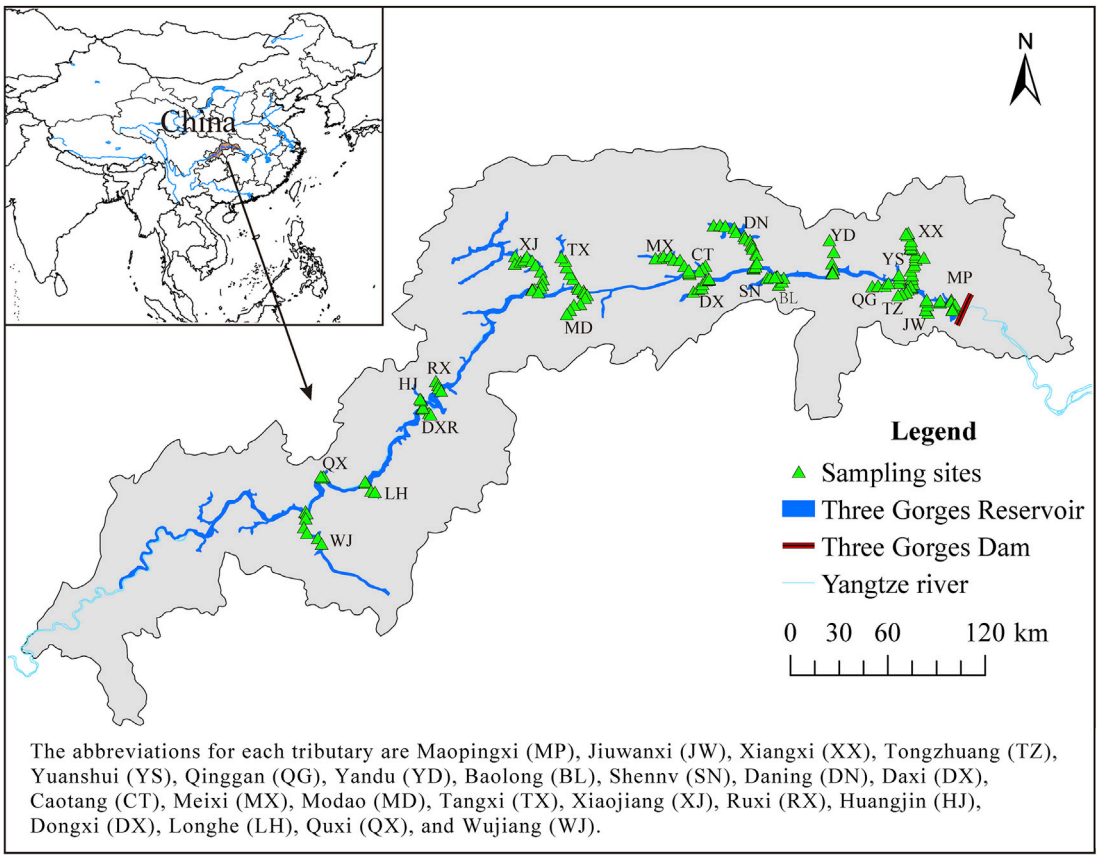

FIGURE 1
Locations of tributary bays and sites in the Three Gorges Reservoir. The polygon with grey color is the geological boundary of the Three Gorges Reservoir Region.

Because of the increased water level, downstream areas of the tributaries to the main channel of TGR in this region were flooded as the bay areas. Consequently, the watersheds for the flood tributaries formed by the impoundment of the TGR were divided into the Three Gorges Reservoir Region (Figure 1), which is the priority area for watershed management of the TGR (Zhang and Lou, 2011).

2.2 Field sampling and data analysis

In order to conduct a thorough evaluation for the ecological water quality of TGR, a total of 173 sampling sites were selected to cover 22 tributary bays and 17 transects in the mainstream of TGR, providing a full coverage of the major tributaries and mainstream of former Yangtze River of the TGR (Figure 1). Specifically, in the reservoir area in Hubei province, there are 7 tributaries and 6 transects in the mainstream of TGR (Figure 1). While the reservoir area in Chongqing province contains 15 tributaries and 11 transects in the mainstream of TGR. The field sampling was carried out on 14–25 April 2015. Due to the loss of a few phytoplankton and water quality samples, a total of 164 sampling sites were ultimately used in our study.

All field sampling and lab analyses strictly followed the standard protocols of the Chinese Ecosystem Research Network (Cai, 2007). Prior to laboratory analysis, the samples for water quality and phytoplankton were collected using a 5-L Van Dorn sampler at a depth of 0.5 m underwater. Water chemistry samples were obtained using polyethylene bottles that had been pre-cleaned and stored in dark and cool environment before lab analyses. Total nitrogen (TN) and total phosphorus (TP) used in this study were analyzed using a segmented flow analyzer (Skalar San^{++}, Netherlands). A 600 ml water sample was filtered through Whatman filter (−1.2 μm, GF/C) to measure the concentration of Chlorophyll-*a* (Chl-*a*), using by the trichromatic method from APHA (1998).

For phytoplankton enumeration and identification, 1.2-L water sample was collected and preserved with neutral Lugol's solution immediately in each site after sampling. The phytoplankton sample for each site was concentrated by the sedimentation method and then preserved with 4% formalin. Taxonomic identification of phytoplankton samples was carried out using the Fuchs-Rosenthal slide with an Olympus CX21 microscope at 10 × 40 magnification, with references to the works of Hu and Wei (2006) and John et al. (2002).

2.3 Ecological water quality assessment

The Q index, developed by Padisak et al. (2006), was used to evaluate the ecological water quality in the present study. The Q

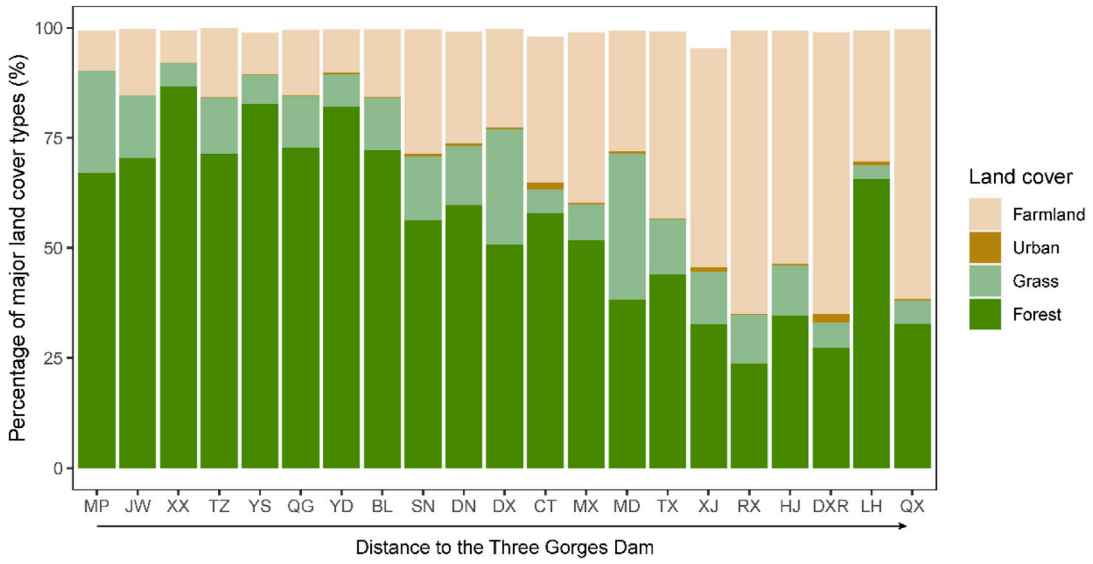

FIGURE 2
Percentage of farmland, urban, grass, and forest area of different tributary watersheds in the Three Gorges Reservoir Area. The tributaries were sorted according to their distances from the Three Gorges Dam.

index is rooted on the functional groups of the phytoplankton community and is calculated as follows:

$$Q = \sum_{j=1}^{n} p_j F$$

Here, p_j is the proportion of the biomass of the j-th functional group to the entire phytoplankton biomass for the sample; and F is the factor number for the functional group in a given water body. The resulting Q index values can be ranged from 0 to 5 and can be divided into 5 grades: bad ($0<Q\leq1$), poor ($1<Q\leq2$), moderate ($2<Q\leq3$), good ($3<Q\leq4$), and excellent ($4<Q\leq5$). The phytoplankton functional group-based Q index is a widely accepted index for the evaluation of reservoir ecological water quality worldwide (Becker et al., 2010; Wang et al., 2011; Shen et al., 2014; Korneva and Solovyeva, 2021; Çelekli and Lekesiz, 2021; Wu et al., 2023).

Here, the determinations of functional groups and the F factor mainly referred to the previous research carried out in the TGR by Wang et al. (2011). The details of phytoplankton species and the corresponding functional groups and the values of F factor could be found in Supplementary Table S1 in the supporting material. By utilizing the Q index, we are able to perform a comprehensive assessment of the ecological water quality of TGR and examine potential effects of changes in land covers on the ecological water quality of the tributary bays of TGR.

2.4 Land cover

The land cover data were obtained from the Resource and Environment Science and Data Center (RESDC) (https://www.resdc.cn). Specifically, the data of land covers were interpreted from the Landsat images by specialists in RESDC. These images were captured around the same time as our field sampling. The land cover data were aggregated into 5 categories including forest, farmland, grassland, urban area, and others, to align with our research aims.

To determine the specific land cover information for each tributary bay of TGR, we extracted the watershed outline for each tributary of TGR using QSWAT (Dile et al., 2022). Then, the area of each type of land covers in the watershed was counted to investigate potential effects of changes in watershed land covers on the ecological water quality.

3 Results

3.1 Land cover

Our study revealed that forest and farmland were the predominant land cover types in all watersheds of the tributaries located within the Three Gorges Reservoir Region (Figure 2). Specifically, the forest area ratios in all tributary watershed of TGR ranged from 23.84% to 86.72%, while the farmland ratios ranged from 7.36% to 64.25%. Notably, we observed a higher proportion of forest area in the watersheds of tributaries near the Three Gorges Dam, which decreased as the distance from the Three Gorges Dam increased (Figure 2). Conversely, the ratios of farmland area exhibited an opposite pattern, with lower percentages in the tributary watersheds near the dam and higher ratios in the tributary watersheds away from the dam. The coverage of grass was lower than that of forest and farmland, with values ranging from 3.16% to 33.31%.

TABLE 1 Statistics summary of the main water chemistry parameters in the tributary bays (n = 42) and mainstream (n = 122) in the Three Gorges Reservoir.

Category	Parameter	Mean	Range	Standard deviation
Tributary Bays	TN (mg/L)	1.689	0.687–3.519	0.501
	TP (mg/L)	0.094	0.011–0.339	0.065
	Chl*a* (μg/L)	12.47	0.17–125.60	19.69
Mainstream	TN (mg/L)	1.911	1.440–2.185	0.168
	TP (mg/L)	0.122	0.100–0.148	0.016
	Chl*a* (μg/L)	0.78	0.03–2.16	0.44

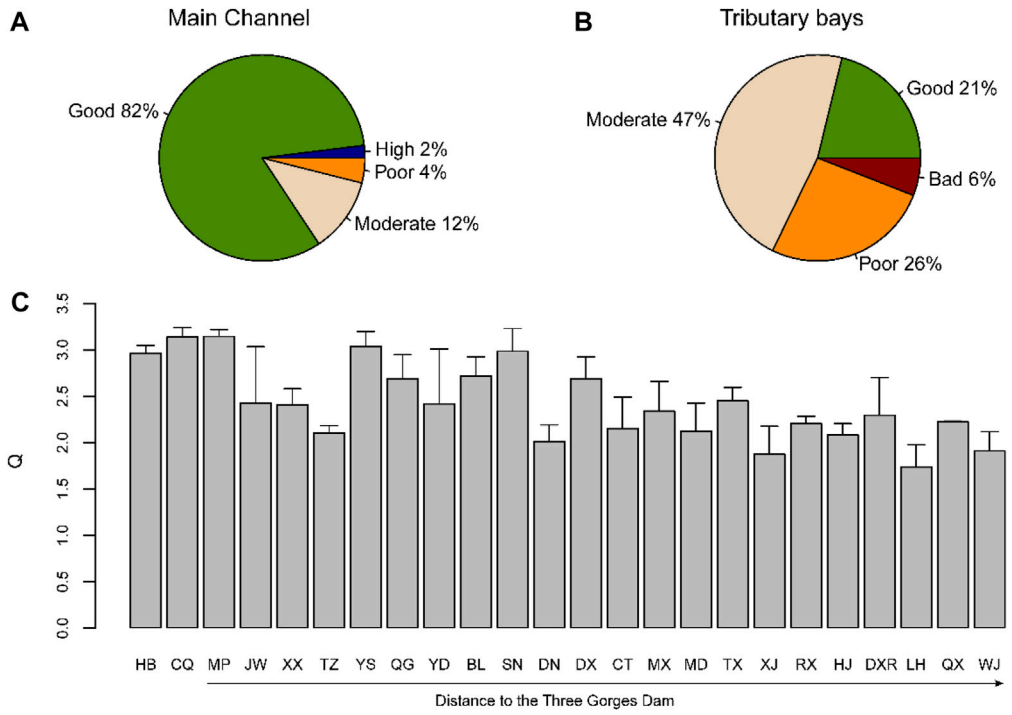

FIGURE 3
Q index-based ecological water quality assessment of the tributary bays and mainstream of Three Gorges Reservoir in Hubei province (HB) and Chongqing municipality (CQ). **(A)**: the overall ecological water quality of the main channel of TGR; **(B)**: the overall ecological water quality of the tributary bays of TGR; **(C)**: the bar plot of Q index for the mainstream and tributary bays. The tributaries were sorted according to their distances from the Three Gorges Dam. The error bar is 1 standard error.

Furthermore, the proportion of urban area in the tributary watersheds of TGR was extremely low, ranging from 0.02% to 1.81%.

3.2 Chemical characteristics

The statistical summaries for the main chemical parameters were presented in Table 1. The average concentrations of TN and TP within the backwater regions of TGR's tributaries were 1.689 and 0.094 mg/L, both of which are marginally lower than the corresponding averaged values observed in the mainstream of TGR. The averaged concentration of Chl-*a* in the tributary area was 12.47 μg/L, which is significantly higher than the mean value (0.78 μg/L) in the mainstream of TGR. Moreover, for the tributaries, we found the variations of water quality parameters in tributary bays are higher than the values in the main channel (Table 1).

3.3 Ecological water quality

The Q index-based assessment showed that the ecological water quality of most sites in the main channel of TGR are good, while most sites in the backwater regions of TGR's tributaries exhibit a status with moderate or bad ecological water quality (Figure 3). Specifically, the ecological water quality assessment found that 84% of the sampling sites in the main channel of TGR exhibited good or high ecological water quality, whereas 12% and 4% of the sites falling into moderate and poor status, respectively (Figure 3A). In contrast, only 21% of the sites in the tributary bays were in good status, with

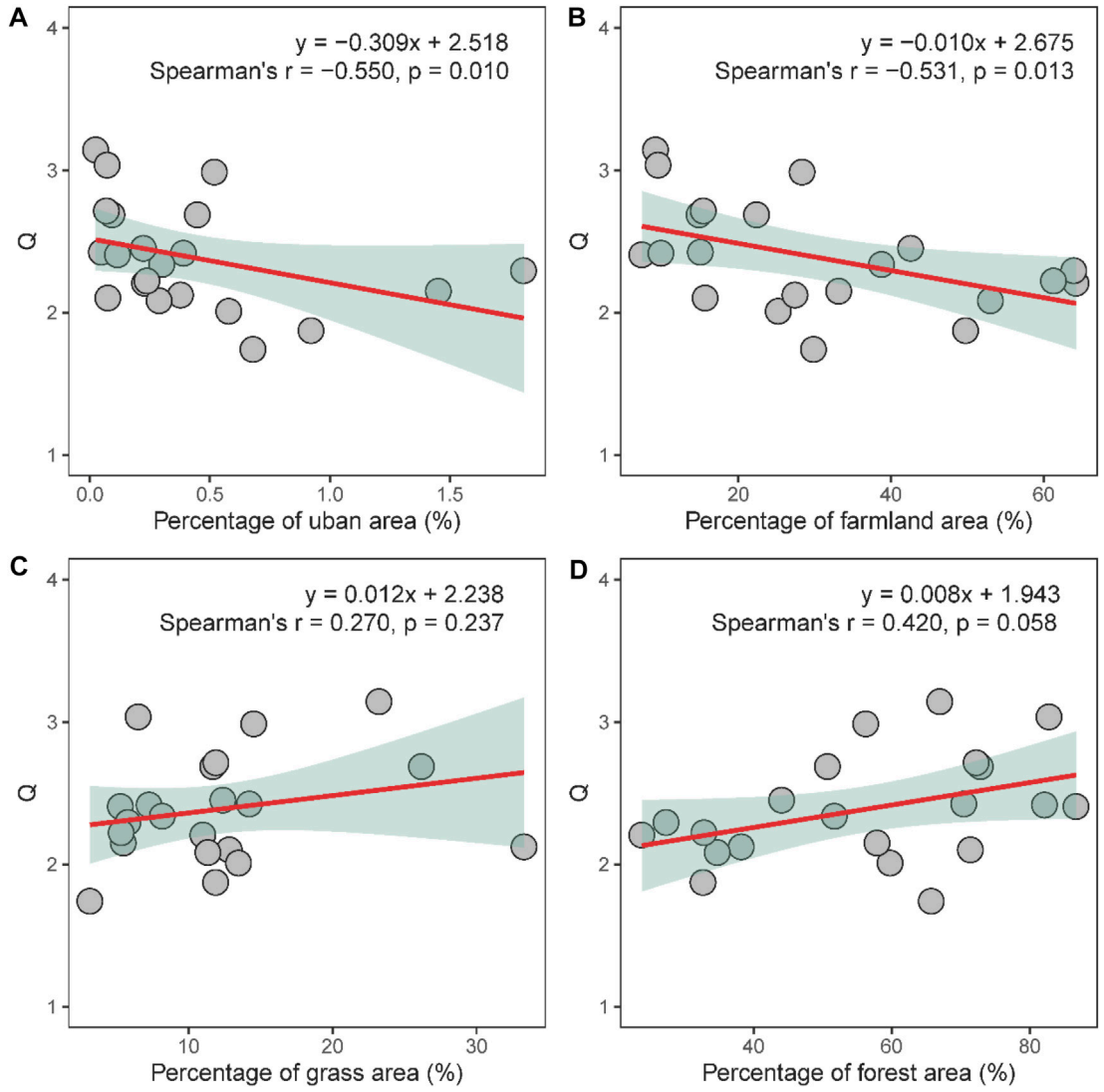

FIGURE 4
Relationships between the ecological water quality (Q) of the tributary bays and their watershed land covers [**(A)**: Urban area, **(B)** Farmland area, **(C)** Grass area, **(D)** Forest area]. The red lines denote the regression line, while the shaded areas represent the 95% confidence interval.

47%, 26%, and 6% of sites exhibiting moderate, poor, and bad ecological water quality, respectively (Figure 3B). Notably, we found that the tributary bays close to the Three Gorges Dam exhibit a better ecological water quality than the bays in the upstream (Figure 3C). Overall, the ecological water quality of tributary bays degraded with the distance from the Three Gorges Dam.

3.4 Relation between land cover and tributary ecological water quality

We found that the percentages of farmland and urban areas had significant negative correlations with the Q index-based ecological water quality of the tributary bays (Figure 4A, B). This finding supports our hypothesis that an increase in the urban and farmland area will degrade the ecological water quality in the backwater regions of TGR's tributaries (H1). On the other hand, our study did not fully support the hypothesis based on the natural land cover (H2), as the percentage of grass area had a nonsignificant positive correlation with the ecological water quality (Figure 4C). However, the percentage of forest area had a marginally significant ($p = 0.058$) positive correlation with the ecological water quality (Figure 4D), suggesting increasing forest area may improve the ecological water quality in the backwater regions of TGR's tributaries.

4 Discussion

4.1 Tributary bays are key areas for the reservoir water quality management

Here, we evaluated the ecological water quality of TGR and examined the effects of changes in watershed land covers on ecological water quality in the backwater regions of TGR's

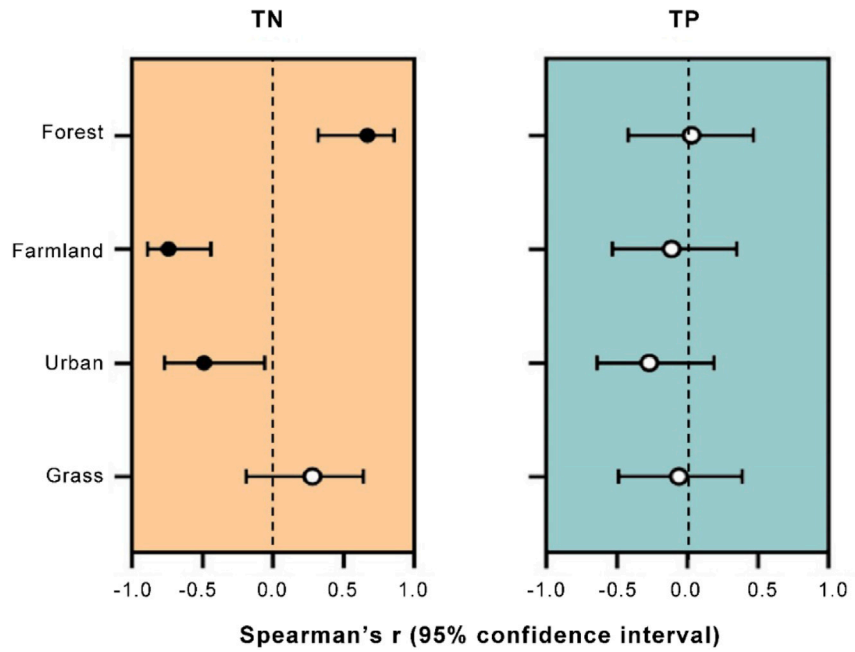

FIGURE 5
Spearman correlation between watershed land cover and the concentration of TN and TP in tributary bays of Three Gorges Reservoir. Solid circle denotes a significant correlation, while open cycle indicates a non-significant correlation.

tributaries. With the aid of the Q index, we have gained a comprehensive understanding of the ecological water quality of the entire TGR. Our investigation indicated that the ecological water quality of most regions within the mainstream (previously known as the Yangtze River) of TGR is good, while the water quality problem primarily manifests in the backwater regions of tributary bays. This finding is in accordance with the previous study based on Carlson's trophic state index (Xu et al., 2010; Tan et al., 2014) as well as the reported algal blooms in the Three Gorges Reservoir Region (Ouyang et al., 2021; Xu et al., 2021; Ye et al., 2022). From the perspective of ecological water quality, our study highlights the significance of managing the reservoir water quality in the backwater regions of TGR's tributaries.

4.2 Water quality and watershed land cover relationships

Water quality is a crucial aspect of ensuring the health and safety of our environment and public health (Su et al., 2022). The accurate assessment of water quality of a given water body is an essential concern for the successful management and protection of water resources (European Environment Agency, 2000; Cosgrove and Loucks, 2015; Bhateria and Jain, 2016; Zhang et al., 2022). Compared to the traditional chemical water quality indices such as TN and TP, our study found that Q index-based ecological water quality can better indicate the land cover changes in the watersheds of TGR's tributaries.

In our study, we observed a robust association among ecological water quality in the backwater regions of TGR's tributaries and the land covers within the corresponding watershed. However, we did not observe a reasonable correlation between the concentrations of TN or TP in the tributary bays of TGR and the watershed land cover (Figure 5). Specifically, the Spearman correlation analyses indicated that the concentrations of TP had nonsignificant relationships with watershed land cover (Figure 5). While, TN was significantly negatively correlated with the urban and farmland area, and had a significant positive correlation with the forest area. Nevertheless, the relationship between TN and watershed land cover is a spurious correlation as it is inconsistent with the common relationship observed in the TGR region, where watershed with high farmland and urban development tends to have more exports of TN and TP (Ye et al., 2009; Zhang et al., 2019; Huang et al., 2022).

The lack of a reasonable correlation between the concentrations of TN and TP and watershed land cover in the tributary bays of TGR (Figure 5) indicates that these chemical water quality indices may be susceptible to be affected by other factors, such as water level fluctuation in the TGR. Recent studies from the field observation (Xiang et al., 2021) and the hydrodynamic model (Luo et al., 2022) have supported this point, showing that water quality in the backwater regions of TGR's tributaries is mainly dominated by the backwater from the mainstream of the TGR. In contrast to traditional water quality indices, the Q index developed from phytoplankton functional composition (Padisak et al., 2006), can provide a more comprehensive and integrated assessment of ecological water quality in aquatic ecosystems because the biological assessment can measure the long-term effects of environmental changes on ecosystems (Prasse et al., 2015; Gecheva et al., 2023). Our study underscores the utility of the Q index in assessing ecological water quality in reservoir ecosystems.

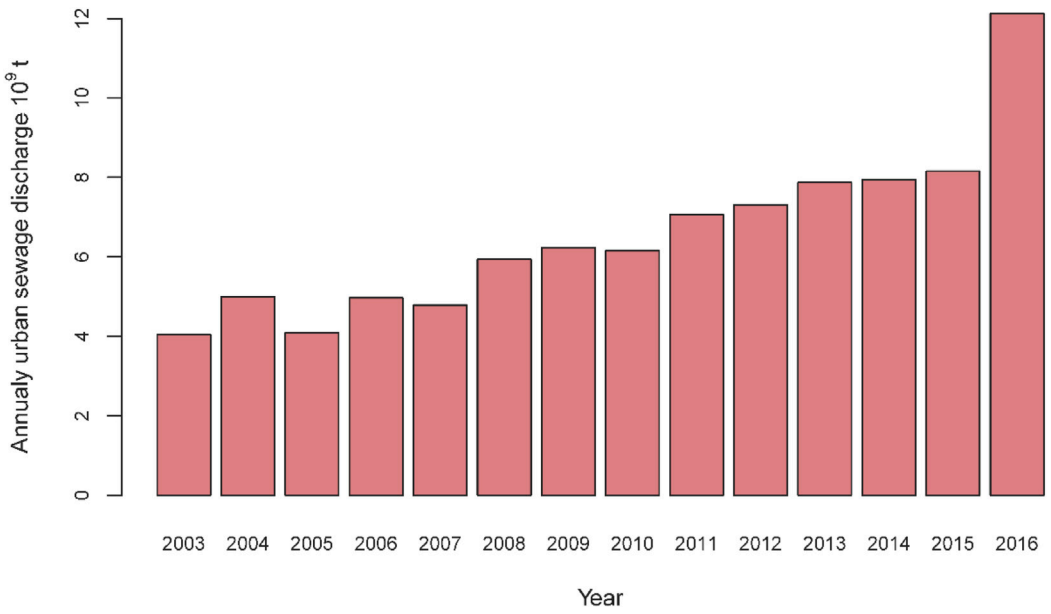

FIGURE 6
Annually urban sewage discharge in the Three Gorges Reservoir Region from 2003 to 2016.

4.3 Implication for reservoir management

Our study has several implications for the management of water quality in reservoirs. One essential implication for reservoir management is that our study revealed that appropriate watershed management can improve the ecological water quality in the backwater regions of TGR's tributaries. Specifically, the control the development of farmland and urban and increase forest area can improve the ecological water quality in the backwater regions of TGR's tributaries (Figure 4). The Three Gorges Reservoir Region is a key area for the socio-economic development of the Yangtze River Economic Belt. However, this region is still undergoing high-speed development. According to the public bulletins from the government (China National Environmental Monitoring Centre, 1998-2016), the total yearly urban sewage discharge in the TGR Region ranged from 4.04×10^9 t to 12.12×10^9 t from the year 2003–2016 (Figure 6). Meanwhile, this region is also the key area for ecological and environmental protection for the backwater regions of TGR's tributaries. In light of these facts, it is essential to implement appropriate watershed management measures to improve the ecological water quality in the backwater regions of TGR's tributaries. Our study found that watershed management is an effective approach to enhance the ecological water quality in the backwater regions of TGR's tributaries, but given the flat slope (Figure 4), this is likely to be a long-term process. Therefore, our study recommends exploring both short and middle-term approaches to improve the ecological water quality of TGR, in addition to the long-term watershed management measures.

Another implication is that the reservoir management practices should take into account the potential environmental and ecological impacts associated with fluctuations in the water levels of the reservoir. Our study showed that the land cover in the tributary watershed can significantly affect the ecological water quality in the tributary bay (Figure 4); however, the explanatory power of land cover changes to the ecological water quality appears to be limited, suggesting backwater from the mainstream of TGR would affect the ecological water quality in the tributary bays. This point was also supported by Xiang et al. (2021), which reported that the water quality (e.g., TN, TP) in the tributaries of TGR was mainly affected by the backwater movement from the mainstream of TGR, which was driven by the water level fluctuations. Meanwhile, research also showed that the water level fluctuation in reservoirs will drive the development of phytoplankton blooms in the backwater region in the Xaingxi Bay of TGR (Ye et al., 2022), which might pose a series of environmental and ecological problems (Anderson et al., 2012; Amorim and Moura, 2021). Given all the above concerns, our study suggests that effective reservoir management should also consider the impacts of water level fluctuations.

Finally, our study highlights the practicality of biological assessment in effective reservoir management. Compared to the classical water chemical indexes (e.g., TN, TP), we found that the phytoplankton functional group-based Q index can better indicate the watershed land cover changes. This result presents the advantages of biological assessment, which is sensitive to environmental changes and measures long-term effects (Prasse et al., 2015; Gecheva et al., 2023). In light of the above facts, the biotic index has been suggested as a fundamental measurement in assessing the ecological water quality by the Water Framework Directive of Europe (European Environment Agency, 2000) and the US Environmental Protection Agency (Barbour et al., 1999). And our study indicates Q index is a useful biotic index in assessing reservoir ecological water quality and has important value in water resources management.

5 Conclusion

In this study, we have investigated the ecological water quality of the whole TGR and further examined the effects of changes in watershed land covers on the ecological water quality in the backwater regions of tributary bays of TGR. The major findings of our research are as follows:

1) The ecological water quality in the mainstream (previously known as the Yangtze River) of TGR is good, while the bad ecological water quality was generally observed in the tributary bays.
2) Increase of urban or farmland area in the tributary watershed will degrade the ecological water quality in the backwater regions of the tributary bays. On the contrary, the concentrations of TP and TN in the tributary bays of TGR had no reasonable correlation with the watershed land cover.
3) Watershed management can improve the ecological water quality in the backwater regions of TGR's tributaries to some extent, but it is a long-term process based on the relationships between land cover and ecological water quality. For this reason, we suggest that effective reservoir management should also consider other rapid approaches, such as the water level regulation approach.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

LY: Conceptualization, Methodology, Formal analysis, Writing, Funding acquisition KC: Data Curation, Formal analysis JC: Data Curation, Visualization LT: Investigation, Data Curation MZ: Investigation XZ: Data Curation, Visualization QC: Conceptualization, Funding acquisition.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2023.1196089/full#supplementary-material

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Bacterial abundance and pH associate with eDNA degradation in water from various aquatic ecosystems in a laboratory setting

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Environmental DNA (eDNA) has been widely used in biomonitoring and has major advantages compared to traditional methods such as counting observations. However, the persistence of eDNA within an ecosystem can lead to false-positive results on the presence of organisms. To improve the accuracy of the interpretation of eDNA results, the present study aimed to enhance the understanding of the connection between environmental factors and eDNA persistence. Here, we set up tank experiments using freshwater from 16 field locations involving four ecosystem types and Milli-Q water as control to cultivate zebrafish, and monitor eDNA degradation over time after removing the organisms. Bacterial abundance, nitrate, phosphate, dissolved oxygen and pH were analyzed to evaluate their impacts on eDNA degradation. We found that bacterial abundance and pH were positively related to eDNA degradation. The eDNA at the size range of 1.2–5 μm (extracellular but intro-mitochondrial) decreased faster than at the >5 μm (intracellular) size range, leading to changes in the eDNA particle size proportion (PSP) with degradation. eDNA particle size proportion in the field water was different from in Milli-Q water. In conclusion, our findings help understand how eDNA persistence is connected with both abiotic and biotic environmental factors, and thereby will improve the accuracy of eDNA methods in aquatic biomonitoring.

KEYWORDS

ddPCR, dissolved oxygen, nitrate, particle size proportion, phosphate

1 Introduction

Analyzing environmental DNA (eDNA), i.e., DNA extracted from environmental samples such as soil, water and air (Taberlet et al., 2012), has developed into an efficient non-invasive method for biomonitoring ever since it first emerged about 2 decades ago (Willerslev et al., 2003; Ficetola et al., 2008; Thomsen and Willerslev, 2015; Salter et al., 2019; Murchie et al., 2022). In aquatic systems, macroorganisms release their eDNA through the continuous shedding of skin cells and metabolic products (Maruyama et al., 2014; Fremier et al., 2019; Wood et al., 2020). This released eDNA enables detection of species presence around the sampling site from water samples, using well-developed molecular techniques such as PCR (Taberlet et al., 2012). Owing to the ease of field sampling, its non-invasiveness and blind detection of species, eDNA methods have been demonstrated to be superior in

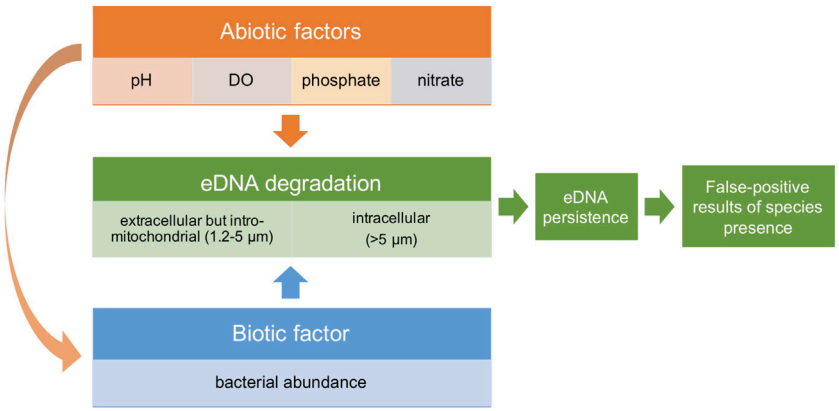

FIGURE 1
Conceptual framework figure showing the hypothesis that abiotic factors and bacterial abundance synergistically impact eDNA degradation, and therefore eDNA persistence. These factors potentially affect the chance of false-positive results of species presence using eDNA-based methods.

terms of efficiency and species detection, where eDNA often detects more species compared to traditional methods, such as trawl surveys (Salter et al., 2019) and counting observations (Katano et al., 2017). It has been successfully applied in analyzing community composition (Deiner et al., 2016; Djurhuus et al., 2018; Beentjes et al., 2021), investigating rare and invasive species (Alzaylaee et al., 2020; Brys et al., 2021), and surveying biodiversity (Carraro et al., 2020; Loewen et al., 2022).

Despite the benefits and increasing applications, interpretation of eDNA data is often hampered by uncertainties in eDNA persistence and its fate within environments. After eDNA is being shed off from the host organism, it can persist in aquatic systems from hours to years depending on the conditions (Joseph et al., 2022). Long persistence is usually caused by slow degradation due to adverse biotic (bacterial abundance and activity) and abiotic environmental factors, such as low temperature and pH (Andruszkiewicz et al., 2017; Mächler et al., 2018; Allan et al., 2021). For instance, eDNA persisted for over 2 weeks at low temperatures (≤10°C) but for a week or less at ≥20°C in one study (McCartin et al., 2022). Non-neutral pH (both acidic and alkaline) has been proven to accelerate eDNA degradation (Strickler et al., 2015; Jo et al., 2020). The effect of some other factors such as UV has shown contradictory effects on eDNA degradation. Mächler et al. declared that UV radiation does not affect eDNA-based detection rates (Mächler et al., 2018), yet Strickler et al. inferred that moderately high UV-B contributes to favorable environments for microbial growth which is associated with a higher eDNA degradation (Strickler et al., 2015). A long period in which eDNA persists in the environment could lead to false-positive results of species presence; while eDNA may be detected at a site the organism may have already left, or the organism was never there in the first place and this exogenous eDNA was transferred to the sample location from somewhere else (Mauvisseau et al., 2022). In biomonitoring studies, this situation can further culminate into incorrect inferences about aquatic biodiversity and corresponding water management measures (Deutschmann et al., 2019; Bedwell and Goldberg, 2020; Mauvisseau et al., 2022). Therefore, a deeper understanding of eDNA degradation in the environment is important to optimize eDNA methods to be reliable and accurate for biomonitoring. Specifically, we need to understand the influences of environmental factors and eDNA size (1.2–5 μm for intro-mitochondrial and >5 μm for intracellular particles) on eDNA degradation, and thereby on the persistence of eDNA.

eDNA degradation is most likely a biotic process, driven by the microbial composition, abundance (as actors) and their activity (Mentzer et al., 2006; Delita et al., 2007; van Bochove et al., 2020). Enzymes or other active products released by microorganisms, such as nucleases and hydrolases, can decompose extracellular DNA and organic particles containing eDNA in the environment. The impacts of abiotic factors on eDNA degradation most likely play out through microorganisms and their enzyme production (Fabian et al., 2016; Joseph et al., 2022). Therefore, microbial indicators might be seen as a proxy for the amount of eDNA degradation (Strickler et al., 2015; Salter, 2018). Few studies have directly linked bacterial abundance to eDNA degradation in aquatic systems. One study (Tsuji et al., 2017) indicated that bacterial abundance did not have a significant effect on extracellular DNA degradation, but this was very likely caused by the laboratory culturing water which contained insufficient amounts of nutrients for bacterial growth and activity. Another study confirmed that temperature and bacterial abundance had significant positive effects on extracted DNA degradation in sediments under laboratory conditions (Zulkefli et al., 2019). Yet it is still uncertain how degradation of naturally released eDNA is related to bacterial abundance under field conditions in natural aquatic systems. Subsequently, it is still largely unclear how varying abiotic conditions in the field might affect the degradation of eDNA directly or indirectly by impacting bacterial abundance and activity.

The microbial degradation of eDNA is also likely affected by the eDNA particle size (Jo and Minamoto, 2021; Zhao et al., 2021). eDNA obtained from environmental samples is usually a mix of eDNA at various sizes reflecting the multiple sources of eDNA, varying from extracellular but intro-organellar to intracellular, and up to tissue particles consisting of multiple cells (Turner et al., 2014; Sassoubre et al., 2016; Jo et al., 2019a). The particle size range of each eDNA state depends on the cell size of the target species and the size

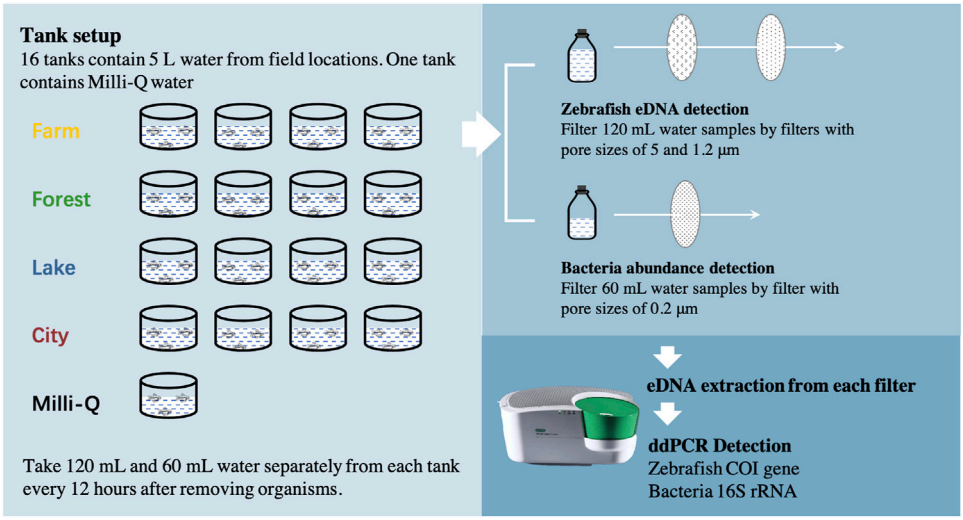

FIGURE 2
Flow chart of the experiment. The whole experiment included three steps; eDNA release, sampling and detecting. First, 16 tanks each containing 5 L water from one field location (Four locations of each ecosystem) and another tank containing Milli-Q water were set up. Around 100 zebrafish (D. rerio) larvae were added to each tank to let them release eDNA. No larvae died during the whole set-up. Second, between 0 and 84 h after removing the organisms, 120 mL and 60 mL water samples were taken from each tank separately after gently mixing the water every 12 h. The 120 ml water sample was filtered using two polyethersulfone (PES) membrane filters with different pore sizes (5, 1.2 μm) sequentially for zebrafish eDNA detection. The 60 ml water sample was filtered using PES membrane filters with pore sizes of 0.2 μm for bacteria abundance detection. Last, DNA was extracted from every filter separately to evaluate the copy concentration of zebrafish Cytochrome c Oxidase subunit 1 (COI) gene region or the copy number of 16S rRNA gene region through ddPCR species-special detection, using corresponding primers and probes.

of organelles containing the target DNA fragment, which can influence the sensitivity of eDNA to enzymatic and chemical degradation of eDNA in the environment. For example, eDNA protected by cell membranes or organelle barriers might persist for a longer period. Increased eDNA persistence might be even more pronounced in tissue particles, where eDNA is additionally protected by an outer barrier of cells. Subsequently, microorganisms may need different eDNA degradation strategies depending on the eDNA particle size (e.g., release different enzymes on cell or organelle membranes), thereby potentially leading to different eDNA decay rates and therefore persistence (Jo et al., 2019a). Additionally, eDNA particle size distribution (PSD) or PSP has been found to be different between species (Zhao et al., 2021). Therefore, studying the connection between eDNA particle size and eDNA degradation might also be important to increase our understanding of eDNA persistence differences between species (Zhao et al., 2021).

We hypothesize that bacterial abundance and abiotic factors synergistically impact the degradation of both extracellular and intracellular eDNA. Additionally, abiotic factors might also indirectly affect eDNA degradation by impacting bacteria abundance and activity (Figure 1). Resulting changes in eDNA persistence affect the likelihood of getting false-positive results on species presence. To test this hypothesis, freshwater from four ecosystems was collected as culturing water, while using Milli-Q water as control to see how eDNA decays in a situation without bacteria. Phosphate, nitrate, pH and Dissolved Oxygen (DO) were quantified to represent abiotic factors. Using zebrafish (*Danio rerio*) as model species, eDNA concentrations at both 1.2–5 (extracellular but intro-mitochondrial) and >5 μm (intracellular) size ranges were monitored by droplet digital PCR (ddPCR) detection within 84 h after removing the zebrafish. The decay rate of different sizes and for different ecosystems was determined and the impact of bacterial abundance and each abiotic factor on the estimated eDNA decay rate was analyzed.

2 Materials and methods

2.1 Tank experimentations

A total of 16 field locations (Supplementary Figure S1) in the Netherlands were chosen as freshwater sources in this study to create a field realistic range of both bacterial abundance and abiotic factors. Four different ecosystem types were involved, farmland ditch, river in the forest, lake and river in the city, each including four locations. 5 L water was taken from each location, put in a separate tank and translocated to a climate room of Leiden University, the Netherlands, between October 26th and 2 November 2020. Another tank with 5 L Milli-Q water was set as a control with low bacterial abundance (possibly from zebrafish larvae), leading to a total of 17 tanks (Figure 2). The temperature setpoint of this climate room was 22°C to obtain a normal living situation for zebrafish larvae, with light between 7 a.m. and 11 p.m. every day. Around 100 zebrafish larvae (~3 mm) of 3 days old were put into each tank to let them release their DNA into the water. The zebrafish larvae originated from a standard zebrafish culturing system in the same building as the climate room and were cultured according to standard protocols (http://ZFIN.org).

TABLE 1 Species-specific primers and probe information, including sequence, length and annealing temperature, that were used for amplification of zebrafish Cytochrome c Oxidase subunit 1 (COI) gene and the bacterial 16S rRNA gene during ddPCR analysis.

Species	Sequences (5′-3′)	Gene	Primer length (bp)	Amplicon length (bp)	Annealing temperature (°C)
Danio rerio	F: GGTGCTTGAGCCGGAATAGT	COI	20	73	55
	R: GTGCTCCTGGTTGGCTAAGT		20		
	FAM- ACCGCATTAAGCCTCTTAATCCGA -BHQ1		24		
All Bacteria	F: TGGCTGTCGTCAGCT	16S rRNA	15	338	60
	R: ACGGGCGGTGTGTAC		15		
	FAM-CAACGAGCGCAACCC-BHQ1		15		

For each tank, the water was screened through a sterilized mesh with pore size around 1 mm to another sterilized tank to remove the zebrafish larvae after about 27 h. No zebrafish larvae died in any of the tanks. Immediately afterwards, all tanks were transferred to another climate room (time point 0) with a lower temperature (18°C) and no light to extend eDNA persistence and to allow for the inclusion of more sampling time points before all eDNA had decayed. The light was kept off during the eDNA monitoring period to avoid its potential impacts on eDNA degradation. Water samples were taken from each tank separately at times 0, 12, 24, 36, 48, 60, 72 and 84 h. At each point in time, 120 mL water was taken after gently mixing the water and sequentially filtered with a plastic syringe (BD Plastipak™) using a polyethersulfone (PES) membrane filter with pore size of 5 µm, followed by filtration of another filter with pore size of 1.2 µm. Our previous study showed that over 80% of zebrafish eDNA is at > 1.2 µm size ranges (Zhao et al., 2021). Therefore, this set-up allowed us to obtain most zebrafish eDNA of extracellular but intra-mitochondrial DNA (1.2–5 µm) and intracellular DNA (>5 µm) origin. Two size ranges at eight sampling times of each for the 17 tanks resulted in 272 eDNA samples for further zebrafish eDNA concentration detection. An additional 60 mL of water was filtered by a plastic syringe (BD Plastipak™) using a 0.2 µm pore size PES membrane providing an additional 136 samples to catch bacterial DNA. After filtering, each filter was immediately put into a 2 mL tube together with 700 µL CTAB Lysis buffer (AppliChem GmbH, DE) and stored at 4°C. Before every sampling round, all injectors, membrane containers, and glass wear had been soaked in 10% bleach over 10 min, subsequently being washed by deionized water and then air-dried on clean paper towels, to decontaminate the reusables of any DNA residues (Goodyear, 2012; Beentjes et al., 2021).

2.2 Abiotic factors

Simultaneous to taking the 5 L water from each of the 16 field locations, four additional tubes of 50 mL water were taken from the same location at the same time using 50 mL sterilized centrifuge tubes from SARSTEDT (https://www.sarstedt.com/). This total of 64 tubes of water was immediately taken to a lab where phosphate and nitrate were quantified. Phosphate concentrations were determined using the MERCK Phosphate Test kit (product No. 114848, Supelco) following the manufacturer's protocol. Likewise, nitrate concentrations were determined using the MERCK Nitrate Test kit (product No. 109713, Supelco) following the manufacturer's protocol. All glass wells and cells were cleaned with acetone first, then with demineralized water before use. DO and pH of each location were determined in the field utilizing HANNA-EDGE instruments with a DO probe (HI764080) and pH probe (HI-11310). For each abiotic factor (phosphate, nitrate, pH and DO), the average value of the four replicate quantifications was used in further analysis.

2.3 Zebrafish eDNA and bacteria DNA extraction and quantification

At the next day following each sampling, DNA was extracted from each filter following a CTAB protocol used in previous eDNA studies (Barnes et al., 2014; Turner et al., 2014) and eluted in 50 µL Tris-EDTA buffer solution (Sigma-Aldrich, US). All extracted DNA samples were quantified using Thermo Scientific™ NanoDrop™ 2000 and then stored at -20°C awaiting further analysis.

Quantification of zebrafish eDNA concentrations was performed using the QX200 ddPCR system (Bio-Rad) through measuring the copy concentration of cytochrome c oxidase I (COI) gene region, utilizing an assay specific to zebrafish designed in one of our previous studies (Zhao et al., 2021). Bacteria concentration was quantified by measuring the copy concentration of the 16S rRNA gene region, utilizing an assay designed by a previous study (Rothrock et al., 2013). Primer sets and probes were ordered from Sigma-Aldrich (https://www.sigmaaldrich.com/), and the sequences of the primers and probes are shown in Table 1.

The ddPCR reaction mix was prepared following the ddPCR protocol (Droplet Digital™ PCR Applications Guide-Bio-Rad, https://www.bio-rad.com/), each 22 µL reaction included 4.4 µL DNA template, 900 nM of each forward and reverse primer, 250 nM of TaqMan probe, 11 µL ddPCR™ Supermix for Probes (No dUTP) and nuclease-free water. Due to the high concentration, each bacteria DNA template except those from the control tank was diluted 10^3 times. 20 µL of each reaction was subsequently transferred to the middle line of a DG8™ Cartridge, and 70 µL of Droplet Generation Oil for Probes was added to every oil hole on the cartridge to generate droplets using the QX200 Droplet Generator after being covered by DG8™ Gaskets. Around 3 min later, 40 µL generated droplets of each sample were transferred to a ddPCR™

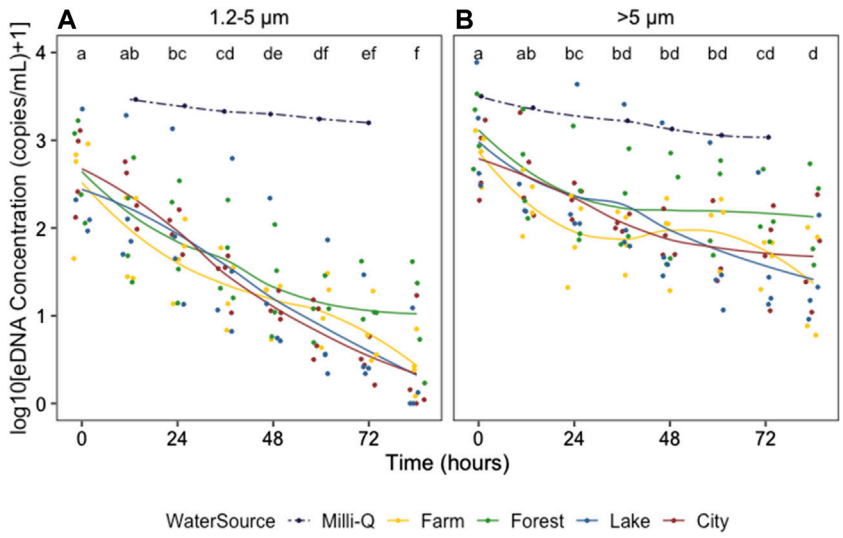

FIGURE 3
Zebrafish eDNA concentration (log10 (X + 1)-transformed) from 0 to 84 h after removing the organisms from field water (solid lines) and Milli-Q water (dotted lines) at 1.2–5 **(A)** and >5 µm size ranges **(B)** Colors indicate the ecosystem type of the water source. Different letters identify significant differences between sampling times according to the Tukey HSD posthoc tests on a one-way ANOVA for all water sources combined (excluding those in Milli-Q water). To indicate the changing trends, smooth lines were added using geom_smooth (method = loess) in R.

96-Well Semi Skirted PCR Plate (Bio-Rad). Each PCR plate was used either for zebrafish eDNA or 16S rRNA gene region quantification and contained two negative controls using Tris-EDTA buffer solution as a template. Plates for zebrafish quantification also contained one positive control of the PCR reaction using zebrafish tissue DNA as a template. Thermal reactions were carried out as follows: 10 min at 95°C, 40 cycles of 30 s at 94°C, and 1 min at the annealing temperature of corresponding primers (Table 1), then 10 min at 98°C before 4°C conservation. Every sample was tested in duplicate, allowing for more accurate quantification of low eDNA concentrations as indicated by previous studies (Jerde et al., 2016; Mauvisseau et al., 2019). The high precision among technical replicates and the nature of the ddPCR analysis make two technical replicates was sufficient, and complies to procedures of other ddPCR studies (Nathan et al., 2014; Doi et al., 2015). Using QX200 Droplet Reader and QuantaSoft (V.1.7.4, Bio-Rad), the fluorescence of each droplet was detected and assigned as positive or negative based on the positive and negative controls following the Quantasoft manual as described before (Zhao et al., 2021). Subsequently, the concentration in copies/µL of each template of both zebrafish eDNA and the 16S rRNA gene region was calculated following the Quantasoft manual through merging the two duplicate measurements, employing the ratio between positive and negative droplets assuming a Poisson distribution (Miotke et al., 2014). The ddPCR results were converted into DNA concentrations (copies/mL) of the water sample based on the various dilutions involved in the analysis process.

2.4 Statistical analysis

Six eDNA samples were excluded as an exceptionally low concentration of extracted DNA (<0.1 ng/µL, NanoDrop results) indicated the failure of the CTAB DNA extraction. All eDNA concentrations including zeros were log10 (X + 1)-transformed before analysis to meet the assumption of normality. To explore the change in eDNA concentration with time, between size ranges and in water sources from different ecosystems (excluding the control treatment) and their interactions, a three-way ANOVA test was performed followed by a Tukey HSD post-hoc test using the multcomp package (V.1.4-17, https://CRAN.R-project.org/package=multcomp) for significant main factors. After that, to better understand the significant interactions between time and size range, a follow-up analysis was run using a one-way ANOVA followed by a Tukey HSD post-hoc test to evaluate differences in the eDNA concentration between sampling times for each size range without consideration of ecosystem type.

To better understand how differential decay of size classes affects size proportions, the particle size percentage was calculated for both size classes. Next, a Kruskal–Wallis test was used to evaluate the PSP changes with eDNA degradation by time and between ecosystems followed by a posthoc Dunn's test using the Holm method to adjust the *p*-values. To further examine the percentage differences between size ranges and between ecosystems, a Wilcoxon signed-rank test was used to evaluate the percentage difference between 1.2– 5 µm and >5 µm size ranges, and between the control tank with Milli-Q water and each of the other tanks.

The concentration of 16S rRNA gene region (copies/mL) was used to represent bacterial abundance in this study. An ANOVA was used to examine how bacterial abundance changed over time for each type of water source. No discernible changes were found ($p \geq 0.05$, Supplementary Figure S2). Therefore, the average bacterial abundance across all time points in each tank was used in further analyses.

To further analyze eDNA degradation under influence of environmental factors, the eDNA decay rate constants of both

TABLE 2 The result of the three-way ANOVA test for the effects of time, eDNA particle size, and ecosystem of water source on eDNA concentration, including degrees of freedom (DF), F-values and *p*-values.

Response	Factor	DF	F-value	*p*-value	
eDNA Concentration	Time	7	36.683	< 2e-16	**
	Size	1	114.59	< 2e-16	**
	Ecosystem	3	4.376	0.005	**
	Time: Size	7	2.88	0.007	**
	Time: Ecosystem	21	0.71	0.82	—
	Size: Ecosystem	3	0.194	0.9	—
	Time: Size: Ecosystem	21	0.16	0.99	—

Asterisks indicate the statistical significance of the factor (**, $p < 0.01$).

size classes were obtained using the easynls package (V.5.0, https://rdrr.io/cran/easynls/) in R software, through fitting eDNA concentration data (N) to an exponential decay model $N(t) = N_0e^{-\lambda t}$. This model has been shown to fit eDNA decay well (Eichmiller et al., 2016; Collins et al., 2018). The decay rate constants λ were log10 (X + 1)-transformed before further analysis to meet the assumption of normality. The impact and interaction of ecosystem type and size range on eDNA decay rate constants were first explored using a two-way ANOVA test, followed by a Tukey HSD post-hoc test. This was followed by an Akaike information criterion (AIC) analysis to find out the best-fit model for eDNA decay rate for both size classes separately, using bacteria abundance, phosphate, nitrate concentration, pH and DO as variables (thus replacing the possible effects of ecosystem types). After that, another two-way ANOVA was used to evaluate the effect and interaction between bacteria abundance and pH on the decay rate constant. The effect of each environmental factor and bacteria abundance on the eDNA decay rate was also tested separately by one-way ANOVAs. R software (V.4.0.2, https://www.r-project.org/) was used for all analyses.

3 Results

3.1 Temporal changes of eDNA concentration

The zebrafish eDNA concentrations from 0 to 84 h after removing the organisms in water from all 16 field locations and the control tank containing Milli-Q water are shown in Figure 3, for the 1.2–5 and >5 μm size ranges separately (Data in Supplementary Table S1). The eDNA concentration showed a significant decrease after removing the zebrafish from the water for all field locations, and was significantly different between 1.2–5 and >5 μm size ranges and between water from different ecosystems, as indicated by the three-way ANOVA (Table 2). The eDNA concentration at 1.2–5 μm size ranges decreased faster than at >5 μm size ranges and the difference in eDNA concentrations increased over time as shown by the significant interaction between time and size range. The post-hoc test following the three-way ANOVA indicated that the eDNA concentration in water from the forest ecosystem was different from the other three ecosystems ($p < 0.05$). It was slightly higher than in other ecosystems after 48 h (Figure 3). eDNA concentrations in the control tank decreased much slower than in tanks with field freshwater, and was barely different between 1.2–5 and >5 μm size ranges.

3.2 eDNA particle size proportion

The eDNA concentration percentage changed strongly with time ($p < 0.01$) for both 1.2-5 and >5 μm size ranges (Figure 4; Supplementary Table S1). The percentage of 1.2–5 μm size range decreased with time with a concomitant increase in the >5 μm size range, and the percentage was significantly different between size ranges ($p < 0.01$). The percentage changes tended to be significant after 36–48 h (Figure 4; Supplementary Table S2). No significant differences in eDNA percentage were found between the field water from different ecosystems ($p = 0.55$) at neither 1.2–5 nor >5 μm size ranges (Supplementary Figure S3). Interestingly, eDNA PSP in the control tank with Milli-Q water was dissimilar from those in all other tanks containing field water (Supplementary Figure S1), with the percentages of 1.2–5 and >5 μm size ranges being similar and barely changing over time.

3.3 eDNA decay rate between size ranges and different ecosystems

The two-way ANOVA indicated that the zebrafish eDNA decay rate constant demonstrated a trend towards being impacted by ecosystem type ($p = 0.07$, F-value = 2.7, DF = 3) which could be mainly appointed to the difference between farm and city as indicated by the post-hoc test (Supplementary Table S3), but not by the eDNA size range ($p = 0.49$, F-value = 0.5, DF = 1), and there was no interaction between these two factors ($p = 0.8$, F-value = 0.3, DF = 3). The eDNA decay rate constant decreased in the order of farm, forest, lake to city. Correspondingly, the estimated retention time showed a slight increase following the same order (Figure 5; Supplementary Table S4). The estimated decay rates of eDNA at both 1.2–5 and >5 μm size ranges in the control tank were extremely low (<0.02), consequently causing longer estimated retention times than in the field water, and was 183 and 291 h for the >5 μm and 1.2–5 μm size range, respectively.

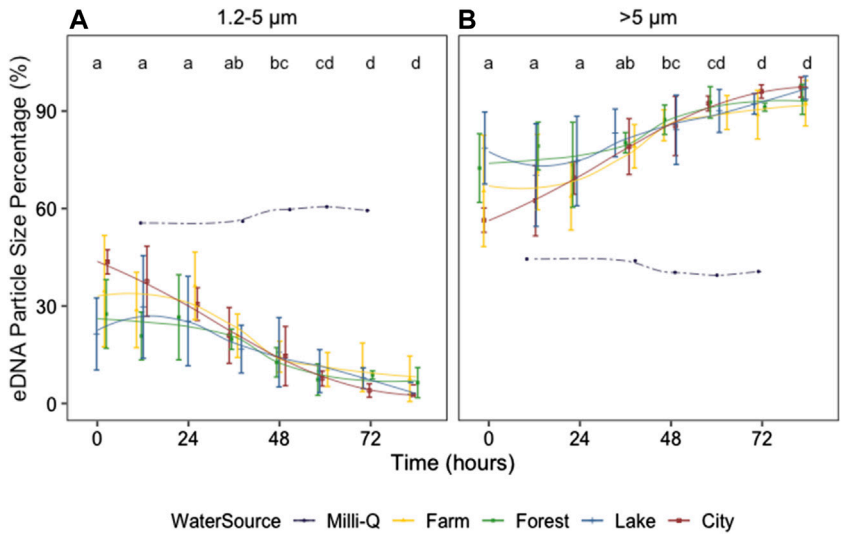

FIGURE 4
Zebrafish eDNA particle size percentages after removing the organisms at 1.2–5 **(A)** and >5 μm size range **(B)** from 0 to 84 h after removing the organisms. Colors indicate the ecosystem type of the water source. Different letters identify significant differences between sampling times according to the Dunn's test on a Kruskal–Wallis test for all ecosystem types combined (excluding those in Milli-Q water). Error bars show the standard errors (SE). To indicate the changing trends, smooth lines were added using geom_smooth (method = loess) in R.

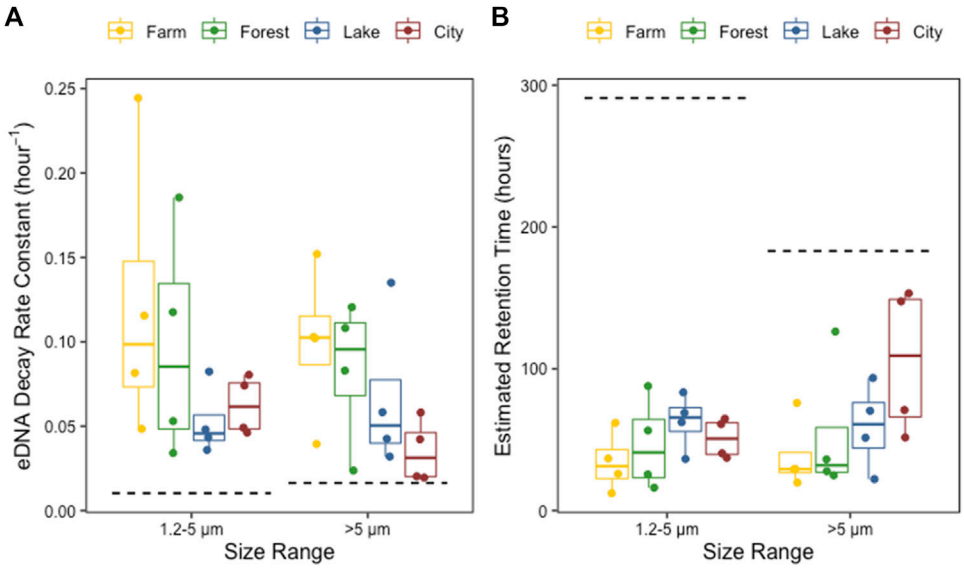

FIGURE 5
The eDNA decay rate constants **(A)** and the estimated retention time of eDNA **(B)** in the field water from farmland ditches (yellow), forest rivers (green), lakes (blue) and city rivers (red) ecosystem types, at 1.2–5 and >5 μm size range separately. The dashed lines indicate the eDNA decay rate constants **(A)** and the retention times of eDNA **(B)** in the control tank containing Milli-Q water.

3.4 eDNA decay rate as affected by environmental factors and bacterial abundance

The values of the four abiotic factors of each tank are shown in Supplementary Table S1. The range of pH, DO, nitrate and phosphate concentration of the field water was 7.62–8.34, 3.86–6.19 mg/L, 0.71–3.05 mg/L and 0–1.83 mg/L. Model selection on the effects of bacterial abundance (copy concentration of the 16S rRNA gene), phosphate, nitrate concentration, pH and DO on the eDNA decay constant showed that only bacterial abundance together with pH influenced eDNA decay rate (Supplementary Tables S4, S5; Figure 6). The two-way ANOVA expressing the best model suggests that, differences in pH ($p < 0.01$, F-value = 13.2, DF = 1) were stronger than that of bacteria concentration ($p = 0.02$, F-value = 5.9, DF = 1) while no

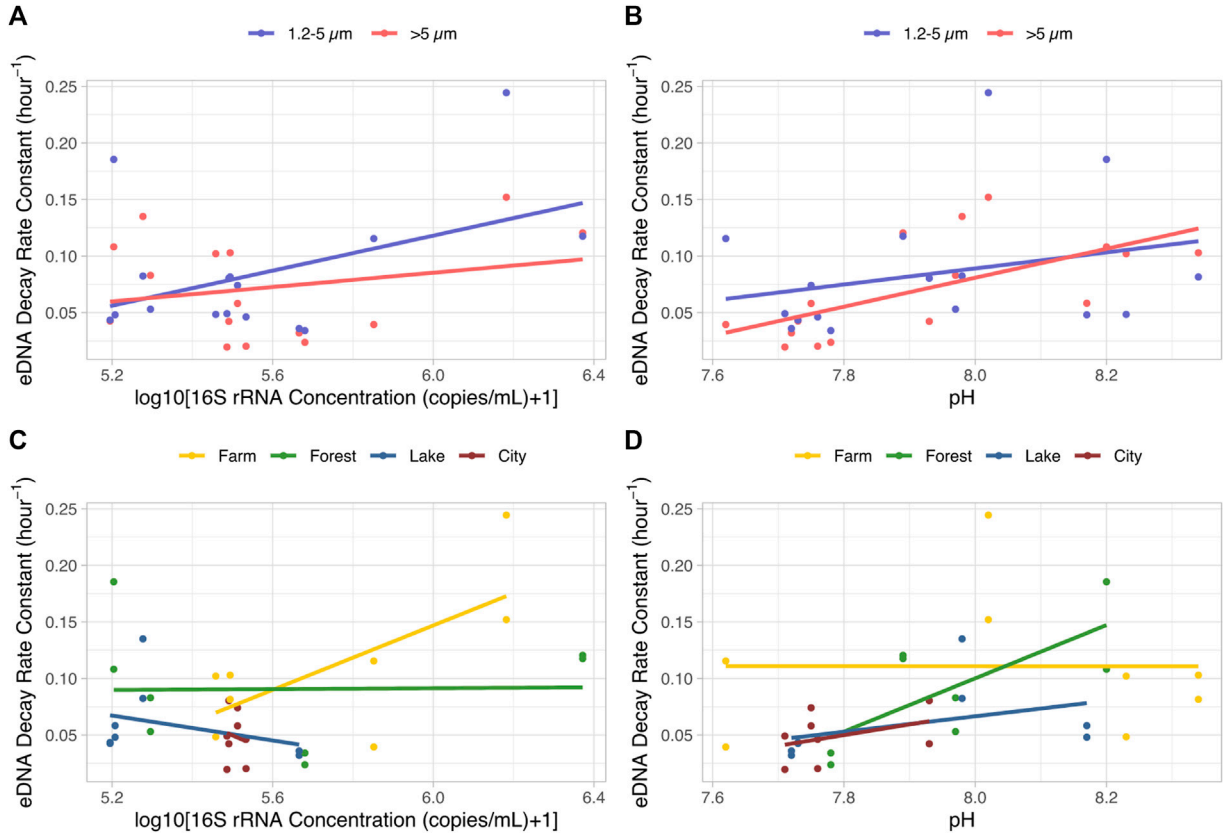

FIGURE 6
eDNA decay rate constants as affected by bacteria abundance (**(A, C)**, log10(16S rRNA gene concentration+1)) and pH **(B, D)**, clustered by eDNA particle size ranges **(A, B)** and the ecosystem types providing the source of water **(C, D)** The lines indicate linear regression lines for each data set.

interactions were found. This was supported by the one-way ANOVA tests of each variable in which only bacteria concentration and pH showed obvious impacts ($p < 0.05$) on the eDNA decay rate constant, rather than phosphate, nitrate concentration, or DO (Supplementary Figure S4).

4 Discussion

While many studies on aquatic eDNA persistence hypothesize that eDNA degradation is strongly affected by the abundance and activity of microorganisms (Strickler et al., 2015; Salter, 2018), there is no direct evidence to support this premise so far for eDNA in natural conditions. To test this hypothesis and improve the knowledge on how bacteria influence eDNA degradation and how abiotic factors are involved in this process, we studied zebrafish eDNA decay rates of two particle size ranges in freshwater from four ecosystem types varying in bacterial abundance, phosphate, nitrate, pH and DO. This setup allowed assessing the main factors that promote eDNA degradation individually and in concert. Our findings indicate that bacterial abundance together with pH enforces aquatic eDNA degradation in water from various ecosystem types, whereas eDNA barely decays in Milli-Q water containing only a few bacteria. eDNA concentration decreases were demonstrated to be different between small and large size ranges. Consequently, the eDNA PSP of the same species was shown to change with eDNA degradation.

4.1 eDNA decay rate and environmental factors

The effect that environmental factors have on eDNA degradation has been demonstrated to strongly impact eDNA applications in an increasing number of studies (Tsuji et al., 2017; Mächler et al., 2018; Jo et al., 2020; Jo and Minamoto, 2021; Mauvisseau et al., 2022). Previous studies indicated that pH is related to eDNA degradation (Strickler et al., 2015; Seymour et al., 2018). In our study, the pH of all tanks was greater than seven, and the eDNA decay rate showed an increase with increasing pH, which corresponds to previous studies (Strickler et al., 2015; Lance et al., 2017). While bacterial abundance was found not to affect eDNA degradation in a previous study (Tsuji et al., 2017), we observed that bacterial abundance strongly boosted the eDNA decay rate. Correspondingly, the decay rate constant in the Milli-Q water containing only a few bacteria was extremely low. It seems that bacteria promoting eDNA degradation need a certain level of nutrients to nurse their activity. Yet phosphate, nitrate, or DO show no impacts on the eDNA decay rate in the field water in this study. This may be due to the limited ranges of physical and

chemical concentrations across the ecosystem types in the present study (with a possible exception for phosphate which showed more variation). Moreover, the relatively high phosphate, nitrate concentrations and relatively stable DO concentrations may have been sufficient to generally support microbial activity, thereby not limiting eDNA degradation. A final potential explanation may be that none of these individual factors may consistently limit bacterial abundance, while the combination of factors does have an effect on bacterial abundance (and through bacterial abundance on the eDNA degradation). In that light, the extremely low decay rate in the Milli-Q water in this study may be explained by the accompanying low, phosphate, nitrate, and DO values in this medium. Consequently, these environmental factors might have to be considered as contributors to a favorable environment for microbial abundance and activity, thereby supporting eDNA degradation as proposed by one previous study (Strickler et al., 2015).

In combination, these findings suggest that there was a direct effect of pH on the microbial activity which may influence the production of enzymes related to eDNA degradation, or directly affect the activity of these enzymes. eDNA present in aquatic ecosystems occurs mostly in the form of organic matters since eDNA is primarily intracellular and in organelles (Turner et al., 2014; Jo et al., 2019a; Zhao et al., 2021). Microorganisms release ecto-hydrolases, e.g., aminopeptidase and alkaline phosphatase, to decompose organic materials into small molecules which in turn can be consumed by microorganisms as nutrition (Grossart and Simon, 1998; Logue et al., 2015; Fabian et al., 2016). It is highly probable the way in which microorganisms cause eDNA degradation as in aquatic ecosystems eDNA is mostly encapsulated by organics such as cell membranes. Additionally, the microbial activity was affected by bacterial abundance (which in turn was possibly affected by the combination of local environmental conditions without one clearly limiting driver). Further studies are encouraged to monitor eDNA degradation as well as bacterial abundance, bacterial activity and where possible the associated enzymes. Once these relationships are established, eDNA persistence may be estimated and quantitatively predicted by bacterial data and measurements of environmental conditions.

4.2 eDNA decay rate and ecosystem type

Ecosystem type and accompanying abiotic factors displayed no significant influence on eDNA degradation and eDNA persistence in the current study. The stronger variation in pH, DO and nutrient levels within ecosystem types than between (with the exception of nitrate concentrations in cities) could explain this general lack of effect of ecosystem type and accompanying abiotic factors. Consequently, no eDNA degradation differences between ecosystem types were found except for a slight difference between farm and city, which may be caused by the limited number of replications. However, the significant differences in eDNA persistence between lentic, lotic and marine ecosystems reported by Harrison et al. (2019) support that ecosystems with different levels and combinations of environmental factors, could differentially affect eDNA degradation and eDNA persistence. A comprehensive understanding of eDNA degradation variation within and between ecosystems will help predict eDNA persistence thereby contributing to reducing the workload of eDNA approaches in biomonitoring.

4.3 eDNA decay rate and particle size

eDNA PSD (or PSP) influences the eDNA degradation, thereby affecting eDNA persistence (Wilcox et al., 2015; Moushomi et al., 2019; Jo and Minamoto, 2021). Therefore, knowledge of potential decay rate differences between size ranges is important to explore in order to understand eDNA persistence. In the current study, both size ranges of eDNA degraded over time, resulting in a significant decrease in eDNA concentration for both size ranges. Importantly, the concentration changes at 1.2–5 μm were faster than those of the >5 μm size range. These findings were further supported by the significant interaction between time and size range. This translated into a reduction of the eDNA percentage at the 1.2–5 μm size range and a concomitant increase for the >5 μm size range. Interestingly, the zebrafish eDNA PSP in the Milli-Q water where eDNA barely decayed was largely different to those in field water, while the zebrafish eDNA PSP in our previous study was somewhere in between (Zhao et al., 2021). This indicates that eDNA PSP (or PSD) of the same species could be different depending on the abiotic and biotic (bacterial abundance) conditions, which in turn impact eDNA degradation.

The change in eDNA PSD (or PSP) with degradation may also be affected by biotic and abiotic conditions as, contrary to the present results, a decrease in the percentage of larger eDNA particle size (>10 μm refers intracellular eDNA) has been reported elsewhere (Jo et al., 2019a; Jo et al., 2019b). In these studies, faster decay rates of smaller sizes (0.8–10 μm refers to extracellular but intra-mitochondrial eDNA) may have been compensated by an influx of these smaller sizes through the degradation of larger particles, maintaining a higher PSP of the smaller sizes (Jo et al., 2019a; Jo et al., 2019b). Environmental factors (both biotic and abiotic) may explain this eDNA degradation differences between size ranges between different studies. Thus, before using eDNA PSD (or PSP) to evaluate the eDNA degradation in field studies, the relationship between eDNA degradation and eDNA PSD (or PSP) has to be established for a range of biotic and abiotic conditions.

In our study, the effects of particle size and environmental factors were independent of each other. Another study, however, showed that temperature had an impact on eDNA decay rate while interacting with eDNA particle sizes (Jo and Minamoto, 2021). In that case, the temperature may not have only impacted the activities of bacteria and enzymes, but also specific enzymes—as related to the degradation of specific size classes—more than others. This leads to the hypothesis that the structure of the eDNA particles determines what enzymes it is sensitive to, and how fast it decays under a certain temperature. Yet, what enzymes digest large or small eDNA particles and how temperature influences its activity in the field remains unknown, and might be quite essential to explore in order to improve the accuracy of eDNA methods for biomonitoring in the field.

4.4 Conclusion

In conclusion, we have demonstrated that bacteria abundance and pH impact aquatic eDNA decay rate in water from different ecosystems. Phosphate, nitrate, and DO did not directly influence the eDNA decay rates, suggesting that none of these factors was consistently limiting bacterial abundance or activity. The concentration of small sized eDNA (1.2–5 μm) decreased faster than of large sizes (>5 μm) in the field water, resulting in a change in eDNA PSP with degradation. This supports the ability of eDNA PSP in assessing the degree of eDNA degradation. Overall, aquatic eDNA persistence relies on the environmental conditions (mostly bacterial activity) and eDNA PSP (or PSD). The present results contribute to understanding aquatic eDNA degradation, thereby help improve the accuracy of eDNA methods by evaluating the mechanisms leading to eDNA persistence, and ultimately lead to few false-positive outcomes of species occurrences in eDNA biomonitoring. In conclusion, this improvement could benefit biomonitoring and tracing migrations, especially for rare species.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

PB, KT, and BZ designed the experiments, analysed the data and wrote the draft of the manuscript. BZ performed the experiments. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2023.1025105/full#supplementary-material

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Phytoplankton community structure and water quality assessment in Xuanwu Lake, China

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Phytoplankton community structure influences the water quality of freshwater lakes and attracts the attention worldwide. The physicochemical parameters including dissolved oxygen (DO), total nitrogen, total phosphorus, NH_4^+-N and COD_{Mn} and biological index such as Chl.a concentrations were measured in water of different sites of Xuanwu Lake, China in varied seasons. The Trophic level index (TLI) was calculated to evaluate the trophic status of the lake based on critical water quality parameters. The phytoplankton community structure and biomass in the water was also identified and detected. Obvious change of physicochemical parameters and biological index was detected in varied sampling time. The results showed that TN, TP, NH_4^+-N, COD_{Mn} and Chl.a had higher concentrations in June and August with higher temperature. Obtained Trophic level index values from key water quality indicators revealed that Xuanwu Lake exhibited a state of mesotrophic level in December 2021, mild eutrophic level in October and February, and remained in a state of moderate eutrophication during other periods having higher temperature. *Chlorophyta* exhibited the highest species diversity and accounted for 48.1% of the entire community. *Cyanobacteria* had higher density compared to other phytoplankton, which suggesting the potential ecological risk in the lake. These findings aligned with the outcomes of the analysis of the phytoplankton community structure, underscoring a certain degree of correlation between the primary water quality indicators and phytoplankton abundance in Xuanwu Lake. That is, higher discharge of contaminant including nutrients potentially dominated the phytoplankton community structure. The continuous monitoring of phytoplankton community and water quality enabled the assessment of its trophic status of urban lake. Appropriate measures such as adjustment of phytoplankton community structure were proposed to mitigate the eutrophication status of lake. The investigation indicated that phytoplankton variation was the important indicator of water quality and supplied direct evidence for the water quality management and ecosystem restoration of urban lakes.

KEYWORDS

water quality assessment, water quality indicators, phytoplankton, eutrophication, Xuanwu Lake

1 Introduction

Lakes are vital for the global environmental landscape, which offer critical habitats to diverse species and serve as integral components of aquatic ecosystems (Brirch et al., 1999). Lakes situated within or in close proximity to urban areas are collectively referred to as "urban lakes," comprising both natural and artificial lake types (Andersson et al., 2014). Urban lakes represent intricate systems that interconnect natural, social, and economic elements, thereby constituting a crucial component of urban ecosystems. Frequently located on the periphery of cities, these lakes playing essential roles in water regulation, tourism, recreational opportunities, and dissemination of urban cultural values (Gkelis et al., 2014). Due to the intensive anthropogenic activities around the lakes, large amount of contaminants such as nutrients was discharged into the lakes and induce the potential eutrophication in water and attract the attention worldwide (Yang et al., 2017; Radosavljevic et al., 2022).

Phytoplankton, with their rapid reproductive rates and short life cycles, serve as valuable indicators of environmental parameters such as water nutrient levels, heavy metal concentrations, pesticides, and other organic pollutants (Bu-Olayan et al., 2001; et al., 2014). Consequently, they are commonly employed to approve and assess the health of aquatic ecosystems (Chen et al., 2003; Chakraborty et al., 2010; Xu et al., 2013; Ligorini et al., 2023). Yuan et al. (2021) proposed that phosphorus could be removed from lake sediments by *Potamogeton crispus*. In addition, nutrients including phosphorus and nitrogen had remarkable effects on phytoplankton distribution in the lake (Bu-Olayan et Çeleklia et al., 2020). Notably, variations in phytoplankton community structure, including shifts in species composition, exhibit meaningful correlations with water quality conditions and developmental trends (Su et al., 2016). However, the long-time response of phytoplankton community structure to water quality change of urban lakes are still limited and need more evidence.

Xuanwu Lake located in Nanjing City, China has long been a research hotspot of urban water management efforts, and is subjected to the degradation of ecological and environmental quality As a typical an urban lake (Xu et al., 2010). After experiencing eutrophication since the late 1980s, cyanobacterial bloom occurred in Xuanwu Lake in July 2005. Subsequent monitoring between 2006 and 2019 indicated a transition from moderate to mild eutrophication, primarily attributed to total nitrogen and total phosphorus as prominent environmental limiting factors (Hu et al., 2011; Chen et al., 2021; Wu et al., 2022). Phytoplankton community was gradually recovered and water quality was improved in this lake over time (Xu et al., 2010). Through comprehensive investigations into the phytoplankton community structure and eutrophication status of lakes, targeted strategies for lake restoration and conservation can be effectively formulated and adjusted. This approach holds promise in strengthening the management of lake's aquatic ecology and realizing sustainable water clarity and ecological equilibrium. Furthermore, it facilitates a comprehensive and precise understanding of the lake's ecological status, thereby promoting the orderly and sustainable development of aquatic environment of lakes.

2 Materials and methods

2.1 Research area and sampling

Xuanwu Lake, an typical urban lake, China, spans an area of approximately 3.68 km^2, with a mean water depth of 1.14 m and a maximum depth of 2.31 m. The lake's surface is segmented into three distinct sections by four small islands, namely, the North Lake, Southeast Lake, and Southwest Lake (Xu et al., 2010). Four sampling sites (Figure 1) were collected within Xuanwu Lake to facilitate bi-monthly sampling at regular intervals, specifically on October, 2021, December, 2021, February, 2022, April, 2022, June, 2022, and August, 2022, respectively.

In addition, *In-situ* measurements were conducted using a multi-parameter water quality monitoring instrument (YSI, USA) to assess various water quality parameters, including water temperature (WT), conductivity (Cond.), potential of hydrogen (pH), turbidity (Tur), and dissolved oxygen (DO). Additionally, transparency (SD) was gauged by employing the Secchi disk method. Water samples were collected from a depth of 0.5 m utilizing organic glass water samplers and preserved in 500 mL polyethylene bottles after acidification to pH < 2 with H_2SO_4 except for common parameters except chlorophyll-a (Chl.a). Subsequent laboratory analyses encompassed a range of parameters, such as total nitrogen (TN), total phosphorus (TP), ammonium nitrogen (NH_4^+-N), suspended solids (SS),

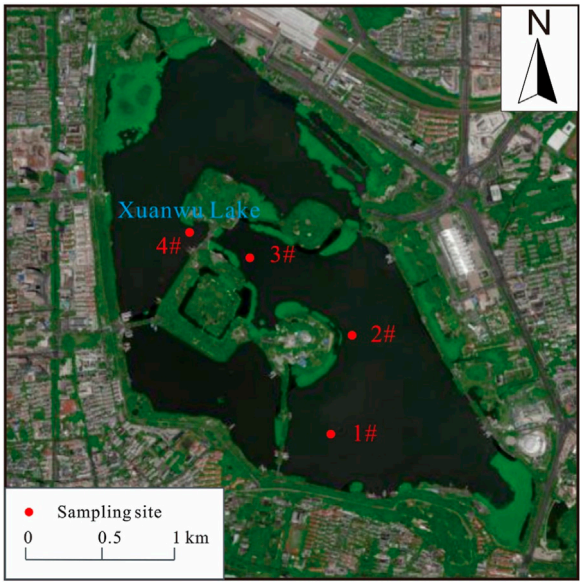

FIGURE 1
Sampling sites in Xuanwu Lake.

TABLE 1 Correlation coefficients (r_{ij}) and their squares (r_{ij}^2) between selected parameters and Chl.a in Chinese lakes and reservoirs.

Parameter	Chl.a	TP	TN	NH_4^+-N	COD_{Mn}
r_{ij}	1	0.84	0.82	−0.83	0.83
r_{ij}^2	1	0.7056	0.6724	0.6889	0.6889

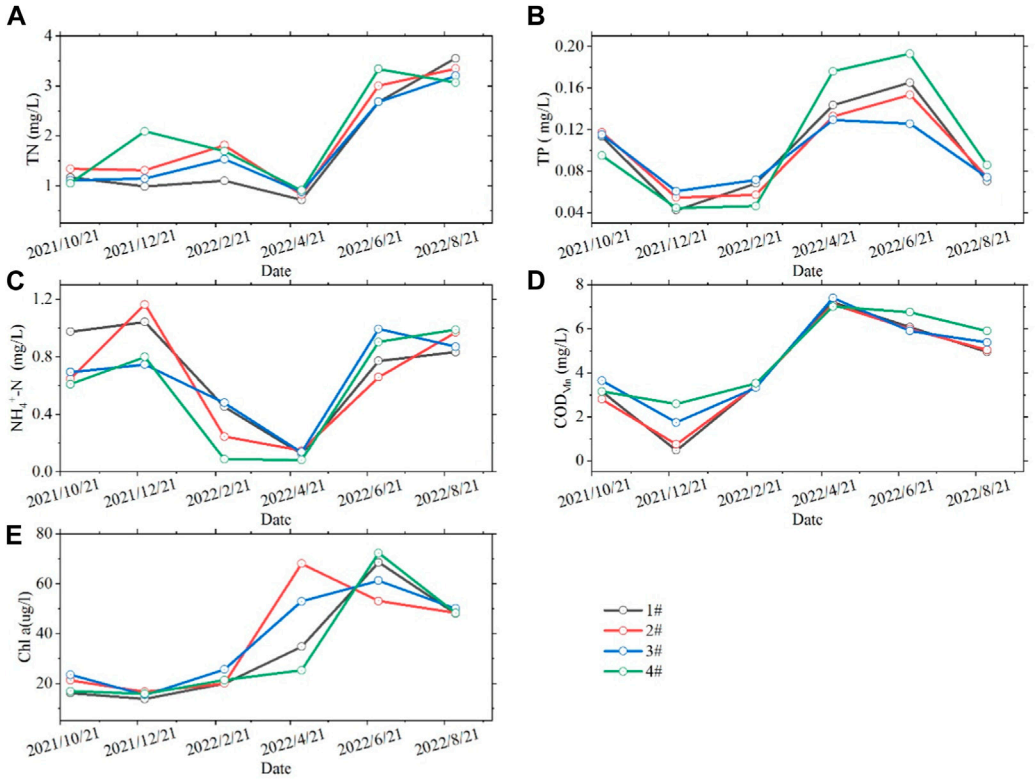

FIGURE 2
Variation trend of environment indexes including TN, TP, NH_4^+-N, COD_{Mn} and Chl a concentrations over time. The lines refer to different sampling sites.

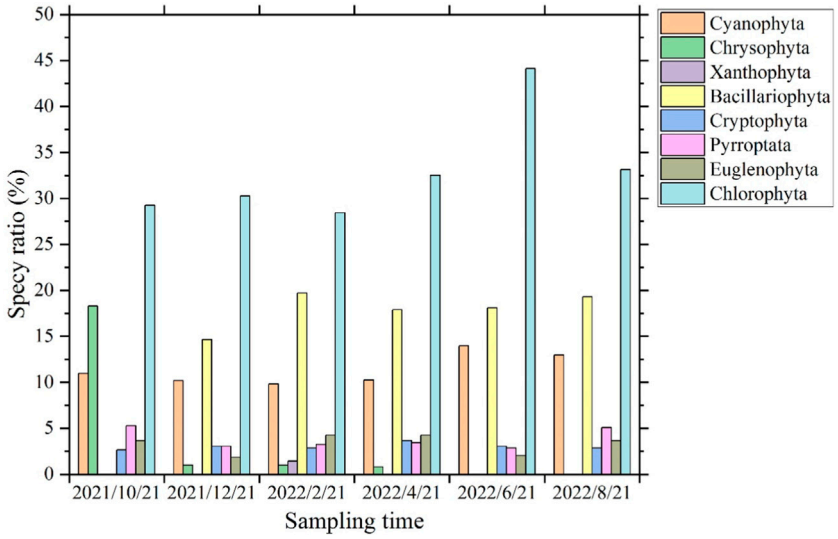

FIGURE 3
Phytoplankton species composition in Xuanwu Lake.

Chl.a, and permanganate index (COD_{Mn}), adhering to the protocols outlined in the *Water and Wastewater Monitoring and Analysis Methods* (4th Edition) (Ministry of Environmental Protection of the People's Republic of China, 2002).

2.2 Phytoplankton collection and processing

Quantitative samples of phytoplankton were collected from the surface water using a 1 L water sampler, poured into sample bottles,

TABLE 2 Dominant phytoplankton species and their dominance values in Xuanwu Lake.

Dominant species	*Cyanobacteria*		*Bacillariophyta*	*Cryptophyta*		*Chlorophyta*	
	Raphidiopsis sinensia	*Pseudanabaena* sp.	*Synedra* sp.	*Chroomonas acuta*	*Cryptomonas erosa*	*Ankistrodesmus* sp.	*Scenedesmus quadricauda*
Dominance Value ($Y \geq 0.02$)	0.09	0.09	0.05	0.04	0.05	0.03	0.03

labeled with unique sample numbers, and immediately treated with Lugol's reagent in an amount of 1%–1.5% of the water sample volume (Chen et al., 2021). The identification and counting process of phytoplankton primarily adhered to the guidelines presented in Freshwater Algae of China: Systematics, Taxonomy, and Ecology (Hu and Wei, 2006). In brief, following a 48 h settling period in the laboratory, the supernatant of the sample was removed via siphoning, and the remaining volume was adjusted to 30 mL with distilled water. Phytoplankton identification and counting were conducted utilizing the counting frame method. In brief, 0.1 mL of 30 mL quantitative sample was rapidly drawn into a 0.1 mL counting frame (20 mm × 20 mm) beneath a cover slip. Subsequently, three to five rows in each field of view were meticulously counted utilizing a high-power microscope. In cases of low phytoplankton abundance, whole-slide counts were performed. Each sample was counted on two slides, and their mean values were recorded. The discrepancy between the counts on the two slides and their mean values were required to remain within ±15%. If this criterion was not met, a third slide counting was conducted, and the process was repeated until the difference between the three slide counts and their mean values did not surpass 15%. The mean values of the two closest values among the three slide counts would then be considered the final result.

Phytoplankton density was calculated as follows (Hu and Wei, 2016):

$$N = \frac{N_0}{N_1} \cdot \frac{V_1}{V_0} \cdot P_n \quad (1)$$

Where N denotes the total number of phytoplankton cells (cells/L); N_0 represents the total area of the counting frame (mm^2); N_1 is the area counted within the frame (mm^2); V_1 is the volume of the water sample after concentration (mL); V_0 represents the volume of the counting frame (mL); P_n denotes the number of phytoplankton counted.

Due to the approximate density of phytoplankton being close to 1, individual biomass could be directly estimated using the volume conversion method. Based on the morphological dimensions, such as length, width, and diameter, of phytoplankton species, a minimum of 50 randomly selected individuals from each species were measured. The volume of each species was calculated based on the most suitable geometric formula, and the mean value was obtained. Multiplying this mean value by the number of individuals of the respective algal species in 1 L of water sample yielded the biomass of that particular phytoplankton species in the sample. Summation of all phytoplankton species' biomasses determined the total phytoplankton biomass in 1 L of water sample, expressed in mg/L.

2.3 Data processing

The Trophic level index (TLI) was employed to evaluate the trophic status of the lake based on critical water quality parameters, including TN, TP, COD_{Mn}, Chl.a, and SD (Wang and Liu, 2002). The TLI(∑) was calculated using the following equation:

$$TLI(\sum) = \sum_{j=1}^{m} Wj \cdot TLI(j) \quad (2)$$

Where TLI(∑) represents the comprehensive trophic status; W_j denotes the relative weight of the trophic state index for the *jth*

TABLE 3 Major water quality indicators and their categories in Xuanwu Lake.

Indicator	DO (mg/L)	TN (mg/L)	TP (mg/L)	NH_4^+-N (mg/L)	COD_{Mn} (mg/L)
Mean value	13.02	1.85	0.10	0.64	4.45
Water category	Class I	Class V	Class IV	Class III	Class IV
Inferior Class V	0%	37.50%	0%	0%	0%
Class V	0%	12.50%	45.83%	0%	0%
Class IV	0%	29.17%	41.67%	8.33%	25.00%
Class III	0%	20.83%	12.50%	58.34%	25.00%
Class II	0%	0%	0%	8.33%	37.50%
Class I	100%	0%	0%	25.00%	12.50%

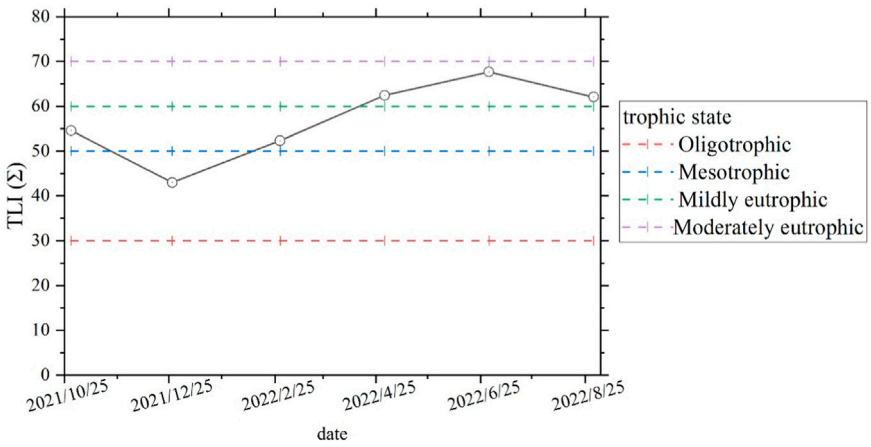

FIGURE 4
Trends in TLI(∑) for Xuanwu Lake.

parameter; *TLI(j)* signifies the trophic state index corresponding to the *jth* parameter. The quality thresholds of TLI index for each parameter is summarized in Table 3.

By selecting Chl.a as the reference parameter, the normalized weight (*Wj*) for each parameter was computed as follows (Wang and Liu, 2002):

$$Wj = \frac{r_{ij}^2}{\sum_{j=1}^{m} r_{ij}^2} \quad (3)$$

Where r_{ij} denotes the correlation coefficient between the *jth* parameter and the reference parameter, Chl.a; *m* indicates the total number of parameters under evaluation. Table 1 presents the correlation coefficients (r_{ij}) and their squares (r_{ij}^2) between selected parameters concerning Chl.a in Chinese lakes and reservoirs.

The TLI for each parameter was calculated as follow (Wang and Liu, 2002)s:

$$TLI(Chl.a) = 10(2.5 + 1.086\ln Chl.a) \quad (4)$$

$$TLI(TP) = 10(9.436 + 1.624\ln TP) \quad (5)$$

$$TLI(TN) = 10(5.453 + 1.694\ln TN) \quad (6)$$

$$TLI(NH_4^+ - N) = 10(5.118 - 1.94\ln SD) \quad (7)$$

$$TLI(COD_{Mn}) = 10(0.109 + 2.661\ln COD_{Mn}) \quad (8)$$

Where Chl.a is expressed in mg/m^3, SD in m, and the other indicators in mg/L.

The determination of dominant phytoplankton species was based on their respective dominance values (*Y*), which were calculated as follows:

$$Y = \frac{N_i}{N} \times f_i \quad (9)$$

Where N_i represents the number of individuals of the *ith* species, *N* denotes the total number of individuals across all species, and f_i signifies the occurrence frequency of the *ith* species. Species with *Y* values greater than or equal to 0.02 were categorized as dominant species (Ministry of Agriculture of the People's Republic of China, 2010).

3 Results and discussion

3.1 Physicochemical parameter variation

The physicochemical parameters including WT, DO, Cond, pH, Tur, SD, TN, TP, NH_4^+-N and COD_{Mn} were plotted in Figure 2. These parameters did not display the remarkable difference in

different sampling sites, indicating the homogeneity of water quality. The WT in Xuanwu Lake exhibited a range of 5.43°C–34.21°C, with mean value of 21.8°C. Notably, it displayed significant seasonal variations, reaching its peak in June 2022 and its nadir in December 2021. DO concentrations showcased fluctuations between 9.94 and 21.1 mg/L, with the highest monthly mean value of 19.55 mg/L recorded in October 2021 and the lowest of 10.72 mg/L in June 2022. The DO concentrations were remarkably higher than Datong Lake and Shijiuhu Lake which are also situated in the Yangtze River, China (Chao et al., 2022; Yuan et al., 2023). Based DO threshold value proposed by GB3838-2002 standards (China), Xuanwu Lake consistently maintained a DO concentration within Class I criteria (National Standard of the People's Republic of China, 2002). The Cond. Values ranged from 221.2 to 342.3 uS/cm, with mean value of 273.52 uS/cm. The pH values exhibited variation between 7.37 (December) and 12.12 (Apri), with mean value of 9.9, indicating a certain alkaline characteristic. Tur demonstrated values within the range of 1.39–23.88 NTU, with the lowest value appearing in December 2021 and the highest in June 2022. Additionally, the SD varied between 0.21 m and 1.94 m, with the lowest value observed in June 2022 and the highest in December 2021. The temporal trends of DO and Tur exhibited pronounced seasonality and were consistent with WT.

The TN content in Xuanwu Lake ranged from 0.72 to 3.55 mg/L, with mean value of 1.85 mg/L. The lowest monthly mean value of 0.83 mg/L was observed in April 2022, followed by a gradual increase, reaching the highest value of 3.29 mg/L in August 2022, which were higher than that in Datong lake with TN value less than 1.5 mg/L (Chao et al., 2022). Furthermore, TN levels remained relatively lower in October 2021, December 2021, and February 2022, with respective values of 1.17 mg/L, 1.38 mg/L, and 1.53 mg/L. *As per* GB3838-2002, the compliance rate of Xuanwu Lake's surface water with Class IV standards (<1.5 mg/L) was 66.7%. However, TN content exceeded Class V standards (>2 mg/L) in June and August 2022, indicating significant N pollution level in this lake.

The TP in the lake water ranged from 0.042 to 0.19 mg/L, with a mean value of 0.10 mg/L. The lowest monthly mean value of 0.051 mg/L was observed in December 2021, followed by a gradual increase, reaching the highest value of 0.16 mg/L in June. Additionally, TP concentrations remained relatively lower in August 2022 and October 2021, with values of 0.076 and 0.11 mg/L, respectively. Throughout the study period, Xuanwu Lake did not meet the Class III standards for TP specified in GB3838-2002. In December 2021, February 2022, and August 2022, it met the Class IV standards, while the remaining months fell under Class V standards. However, the TP concentrations in Xuanwu Lake were lower than that in Datong lake with TP value higher than 0.1 mg/L and higher than that in Shijiuhu Lake with TP value less than 0.05 mg/L, respectively (Chao et al., 2022; Cai et al., 2023).

Ammonium nitrogen (NH_4^+-N) in Xuanwu Lake ranged from 0.001 mg/L to 0.037 mg/L, with mean value of 0.013 mg/L. The highest monthly mean value of 0.019 mg/L was observed in December 2021, followed by a gradual decrease, reaching the lowest value of 0.0021 mg/L in April 2022. NH_4^+-N concentrations remained stable in June and August 2022. Ammonium nitrogen (NH_4^+-N) concentrations were generally lower than that in Datong lake (Chao et al., 2022). Nitrate nitrogen (NO_3^--N) varied from 0.082 to 1.16 mg/L, with mean value of 0.64 mg/L. The highest monthly mean value of 0.94 mg/L was recorded in December 2021, followed by a gradual decrease, reaching the lowest value of 0.12 mg/L in April 2022. Relatively higher values were observed in June 2022, August 2022, and October 2021, with respective values of 0.83 mg/L, 0.91 mg/L, and 0.73 mg/L. The water quality of Xuanwu Lake met Class III standards for NH_4^+-N throughout the study period. In February 2022, it met Class II standards, and in April 2022, it met Class I standards. Generally, N in Xuanwu Lake is at a low level of pollution.

In addition, COD_{Mn} in Xuanwu Lake ranged from 0.47 mg/L to 7.4 mg/L, with mean value of 4.45 mg/L, which was generally lower than that in Datong Lake with COD values higher than 5 mg/L (Chao et al., 2022). The highest monthly mean value of 7.18 mg/L was observed in April 2022, followed by a gradual decrease, reaching the lowest value of 1.39 mg/L in December 2021. In February 2022, COD_{Mn} exhibited a relatively lower value of 3.43 mg/L. According to GB3838-2002 standards, Xuanwu Lake met Class IV standards in April and June 2022, Class III standards in August 2022, Class II standards in October 2021 and February 2022, and Class I standards in December 2021.

Finally, the Chl.a concentration in the lake was also shown in Figure 2. Significant increase of Chl.a was found during the monitoring period. The Chl.a values ranged from 13.71o 72.23 μg/L, with an mean value of 35.69 μg/L. The highest monthly mean value of 63.73 μg/L was observed in June 2022, followed by August 2022 and April 2022, with values of 48.69 and 45.23 μg/L, respectively. In October 2021, December 2021, and February 2022, Chl.a levels were relatively lower, with values of 19.43 μg/L, 15.37 μg/L, and 21.72 μg/L, respectively. In general, remarkable variation of physicochemical and biological parameters were found in Xuanwu Lake during the changed sampling times, which were also detected in other lakes (Tong et al., 2021; Yuan et al., 2023). High pH is concordant with high chl.a values. Photosynthesis consumes inorganic C from the water, which shifts the pH towards high values by loss of the alkaline reserve in the form of phytoplankton (Brettum, 1996). This mechanism was speculated to be responsible for the higher pH values in warm period.

3.2 Phytoplankton community structure

A visual representation of the mean phytoplankton species composition in Xuanwu Lake is depicted in Figure 3. During the period from 2021 to 2022, the phytoplankton community in Xuanwu Lake was composed primarily of *Chlorophyta*, *Cryptophyta*, *Cyanobacteria*, *Chrysophyta*, *Bacillariophyta*, *Pyrrophyta*, *Euglenophyta*, and amounting to a total of 65 species. Among these, *Chlorophyta* exhibited the highest species diversity, encompassing 32 different species, which accounted for 48.1% of the entire community. Similar high abundance of *Chlorophyta* was also found in Xingyu Lake, China (Su et al., 2016). Following closely, *Bacillariophyta* comprised 14 species, representing 24.2% of the community, while *Cyanobacteria* constituted ten species, making up 15.4%. As the biological indicator of eutrophication of lake ecosystem, *Cyanobacteria* contributed 14.8% to the community in this lake (Rönicke et al., 2021). The groups *Pyrrophyta* and *Euglenophyta* were relatively rare, collectively contributing 8.3% to the overall community. Interestingly, *Chrysophyta*, represented solely by the species *Dinobryon* sp., had the lowest representation in the Lake.

A comprehensive overview of the dominant phytoplankton species and their corresponding dominance values can be found in Table 2. The phytoplankton community in Xuanwu Lake is characterized by two dominant species, namely, *Raphidiopsis sinensia* and *Pseudanabaena* sp., both exhibiting a dominance value of 0.09. Dominant phytoplankton species attached to *Cyanobacteria* suggested potential ecological risk in Xuanwu Lake (Gkelis et al., 2014; Xue et al., 2021).

3.3 Phytoplankton density and biomass

The monthly mean phytoplankton density in Xuanwu Lake exhibited a range from 8.26×10^5 to 2.18×10^7 cells/mL, with the lowest value observed in December 2021 and the highest in June 2022. Notably, *Cyanobacteria* dominated the phytoplankton community during October 2021, June 2022, and August 2022, reaching its peak density in June 2022 (1.88×10^7 cells/L) when higher temperature occurred. Similar high density of *Cyanobacteria* were also found in Taihu Lake in warm period (Qin et al., 2018). Conversely, the lowest density of *Cyanobacteria* occurred in February 2022, recording 1.73×10^4 cells/L. In October 2021, *Cyanobacteria* accounted for 80.67% of the total density, while *Cryptophyta* and *Chlorophyta* contributed 8.78% and 7.71%, respectively. However, in December 2021, *Cryptophyta* experienced a substantial increase, becoming the dominant group with a proportion of 75.31%, and *Chlorophyta* also increased to 10.53%. In February 2022, *Cyanobacteria* reached the lowest density, and *Cryptophyta* further increased to 83.47%, with the appearance of *Chrysophyta* noted for the first time. In April 2022, *Bacillariophyta* exhibited a substantial rise, comprising 40.62% of the total density, followed by *Cryptophyta*, *Cyanobacteria*, and *Chlorophyta*, contributing 24.41%, 18.74%, and 15.12%, respectively. Both in June and August 2022, *Cyanobacteria* dominated the community with proportions of 86.14% and 84.1%, respectively, indicating the potential eutrophication in the lake. In general, elevated temperatures facilitated the increase of the dense of phytoplankton communities in the lake (Hao et al., 2018).

The monthly mean biomass in the Lake exhibited a range from 0.91 to 8.45 mg/L, with the highest value observed in April 2022 and the lowest in October 2021. In October 2021, *Cryptophyta* dominated the biomass, contributing 53.86%, while *Bacillariophyta* also made a significant contribution with a proportion of 12.36%. Similar predominance of *Cryptophyta* was also found in Baiyangdian Lake, China (Liu et al., 2010). During December 2021, *Cryptophyta* remained the predominant group, accounting for the highest biomass (63.36%), followed by *Pyrrophyta*, contributing 20.78%. In February 2022, *Cryptophyta* and *Euglenophyta* contributed the majority of the biomass, comprising 56.28% and 31.09%, respectively. In April 2022, *Bacillariophyta* dominated the biomass (52.91%), and *Cryptophyta* constituted the second most abundant group (35.73%). In June 2022, *Bacillariophyta* accounted for the highest biomass (48.74%), followed by *Chlorophyta* and *Cryptophyta*, with proportions of 19.96% and 16.5%, respectively. Finally, in August 2022, *Cryptophyta* represented the highest biomass proportion (45.6%), followed by *Bacillariophyta* and *Chlorophyta*, contributing 24.58% and 13.02%, respectively. In general, *Cyanobacteri* did not show significantly higher biomass than other phytoplankton species, suggesting relative healthy community structure of phytoplankton in Xuanwu Lake.

3.4 Correlation between water quality and phytoplankton

The categorization of major water quality indicators in Xuanwu Lake is also summarized in Table 3. The evaluation of key water quality indicators in this Lake, based on their annual mean values, revealed that the overall water quality fell within Class I for DO, Class V (GB3838-2002) for TN, Class IV for TP, Class III for NH_4^+-N, and Class IV for COD_{Mn}. An analysis of all sample indicators indicated that DO fully met Class I standards at 100%, and TN met inferior Class V standards at a rate of 37.5%. In addition, 12.5% of TP, 91.67% of NH_4^+-N, and 75% of COD_{Mn} complied with Class III or higher surface water standards. The TLI(∑) was calculated using five indicators (TN, TP, Chl.a, COD_{Mn}, and SD), resulting in values ranging from 36.34 to 69.67, with an overall mean of 56.84. Monthly variations in TLI(∑) were observed within the range of 42.76–67.38. The variation trends of TIL values are plotted in Figure 4. The data analysis revealed that approximately 16.67% of the time, Xuanwu Lake experienced mesotrophic conditions, predominantly observed in December 2021. The majority of the remaining months exhibited eutrophic conditions, with October 2021 and February 2022 falling under mild eutrophication, and April, June, and August 2022 indicating moderate eutrophication. Remarkable deterioration of water quality was found during the research period when temperature tended to be higher. Similar variation was also found in Baiyangdian Lake and Shijiuhu Lake (Liu et al., 2010; cai et al., 2023). That is, warm period facilitate the accumulation of pollutant including nutrients and degradation of lake water quality.

Based on the established eutrophication assessment criteria for lakes in China, which categorize phytoplankton density and biomass levels, we defined oligotrophic conditions when the phytoplankton density was below 3×10^5 cells/mL or the biomass was less than 1.5 mg/L. Mesotrophic conditions occurred when the density fell within the range of $(3–10) \times 10^5$ cells/mL or the biomass lay between 1.5 and 5 mg/L. The phytoplankton community structure could be altered t due to their sensitivity to nutrient concentrations (Su et al., 2016). Eutrophic conditions were identified when the density exceeded 10×10^5 cells/mL, or the biomass ranged from 5 to 10 mg/L. Analyzing the phytoplankton density and biomass data in Xuanwu Lake, we found that the lake predominantly experienced eutrophic conditions throughout the observation period, except for December 2021, during which it exhibited mesotrophic characteristics. Liu et al. (2010) proposed that the physico-chemical factors had a positive correlation with phytoplankton community structure. Seasonal variation of phytoplankton was also detected in Taihu Lake and Chaohu Lake, China, indicating the potential nutrient limitation in different seasons in lake water

(Xu et al., 2013; Jiang et al., 2014). Generally, the TLI(∑), derived from relevant water quality indicators, aligned with the assessment based on phytoplankton density and biomass, suggesting a significant correlation between the phytoplankton community structure and relevant water quality indicators in Xuanwu Lake. The improvement measurements of water quality such as the emission reduction of N and P loads from the terrestrial sources can adjust the phytoplankton community structure and biomass of urban lakes.

4 Conclusion

The present study aimed to analyze the temporal variations in environmental factors and assess the trophic status of a freshwater lake, Xuanwu Lake, through an examination of its phytoplankton community. The results demonstrated that:

1) Xuanwu Lake exhibited elevated nutrient levels, with mean TN concentration of 1.85 mg/L and mean TP concentration of 0.1 mg/L over time. Seasonal fluctuations were observed, with TN water quality falling into inferior Class V during June and August 2022 and TP water quality being classified as Class V in October 2021 and April and June 2022.
2) The comprehensive survey identified a total of 65 phytoplankton species, with *Chlorophyta* and *Bacillariophyta* predominating. Species abundance displayed seasonal patterns, reaching its nadir in December and peaking in June. The lowest phytoplankton density was observed in December, while the highest occurred in June. Regarding biomass, the lowest values were recorded in October, while the highest values appeared in April.
3) Water quality deteriorate with the increase of temperature. Warm period facilitate the accumulation of nutrients and degradation of lake water quality. The TLI(∑) aligned with the assessment based on phytoplankton density and biomass suggested a significant correlation between the phytoplankton community structure and relevant water quality indicators in Xuanwu Lake. The investigation supply direct evidence for the water quality management and ecosystem restoration of urban lakes.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

SQ: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing–original draft, Writing–review and editing. JZ: Investigation, Writing–review and editing.

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Conflict of interest

Author JZ was employed by Jiangsu China Tobacco Industry Co., Ltd. Nanjing Cigarette Factory.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Losses in fishery ecosystem services of the Dnipro river Delta and the Kakhovske reservoir area caused by military actions in Ukraine

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We studied the development of commercial and recreational fishing on the Kakhovske Reservoir (aka Kakhovka) and the Dnipro (aka Dnieper) River lowlands in 2020–2023. The fish assemblage of the Kakhovske Reservoir is under consideration for the period 1956–2021. The dynamics of the fish population transformation, species extinction, and the emergence of new invasive species are given. The losses in Kakhovske Reservoir's ecosystem services as a result of the Kakhovska Hydroelectric Power Plant's (HPP) Dam explosion in June 2023 are analyzed. The states and prospects for local recreational and commercial fishing development are assessed. By field research and monitoring observations of the Kakhovske Reservoir and the Dnipro River lowland using the Earth remote sensing data, it was established that 2 months after the accident, the area of the remained reservoir water surface was ~430 km^2 (about 19% of the initial, including the restored Dnipro River bed). The newly formed shallow waterbodies, which do not have a water connection between each other, occupy an area of about 300 km^2. These areas continue to dry out, shrink, and become overgrown with vegetation. The draining of the Kakhovske Reservoir caused an ecological disaster for about 40 species and subspecies of fish. The total monetary losses of commercial fishing are about $5.5 million annually. Losses in fishery from the vanishing of spawning grounds are estimated at 20,000 tons of fish resources (~$40 million). The negative consequences of the loss of the Kakhovske Reservoir aquatic ecosystems will affect the socio-economic development of the entire South of Ukraine for a long time. Among the major ecosystem services lost is the cessation of water supply. Ukraine's priority issues are the post-war rehabilitation of the country, its degraded lands, territories, and water areas, and ensuring water and food security. One of the urgent problems will be the feasibility of reconstruction of the Kakhovska HPP's Dam and restoration of the Reservoir, renovation of water supply, fishery, navigation, energy, and recreation. Biodiversity is a basis for the efficient and sustainable ecosystem functions that provide many ecosystem services, and it should be considered for the post-war recovery and development of Ukraine.

KEYWORDS

ecosystem services, freshwaters, Dnipro reservoirs Cascade, fish resources, food resources, post-war recovery

1 Introduction

Freshwater ecosystems are key elements in the development and functioning of humankind and the environment. Today, they play a key role in achieving the strategic objectives (No.6) of sustainable development (UN-Water, 2016). Unfortunately, the progressive increase in wars and conflicts threatens the functioning of aquatic systems already depleted from anthropogenic pressures. Water conflicts are categorized into three types (Gleick, 2019a): "trigger", "weapon" and "casualty". According to available information (Water Conflict Chronology, 2024), more than 1,600 conflicts are accounted for in human history. The lion's share (almost 90%) occurred in the foreseeable past, between 2000 and the present (Figure 1). Predominantly, they have occurred between countries of the former Soviet Union (Peña-Ramos et al., 2021), the Middle East (Amery, 2002; Gleick, 2019b), and the African continent (Aston, 2008); a somewhat smaller share occurs in other parts of the world (Gleick and Heberger, 2014; Schillinger et al., 2020). Between 2020 and 2023 alone, 543 conflicts were recorded in which 285 times water

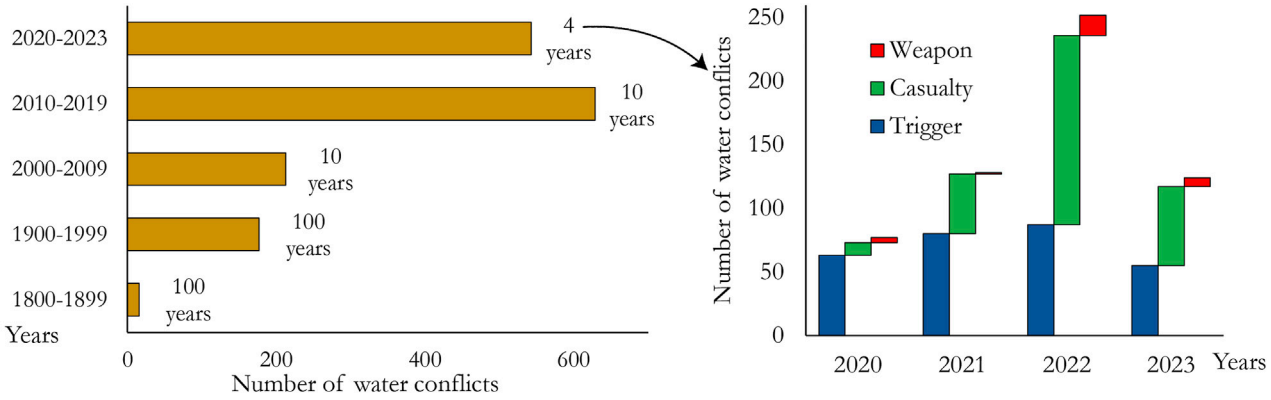

FIGURE 1
World statistics on water conflicts and their forms (according to Water Conflict Chronology, 2024).

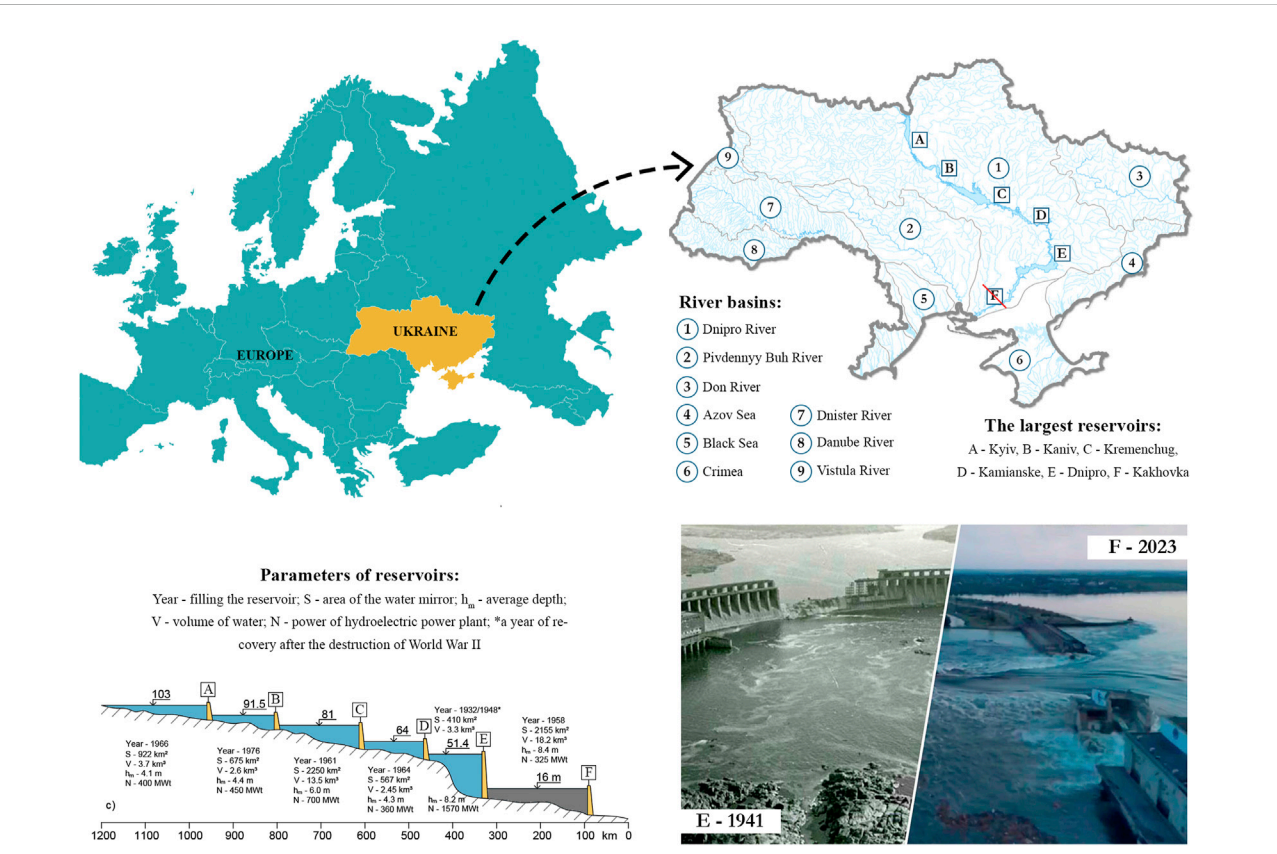

FIGURE 2
An overview map of Ukraine and general characteristics of the Dnieper Reservoir Cascade.

was used as a trigger, 268 times as a weapon, and 28 times as a casualty. The majority of these recorded cases are in the Russian-Ukrainian and Palestinian-Israeli wars.

Since the beginning of February 2022 and throughout the entire period of military operations on Ukrainian territory, critical infrastructure has been subjected to constant missile and artillery strikes. A significant number of hydraulic structures (dams, dikes, canals, water pipelines, pumping stations, etc.) have been destroyed. The total losses of the economy as a result of the war (fall in GDP levels, cessation of investment, labour outflow, additional expenditures on defense and social support, etc.) amount to about $600 billion (Kyiv School of Economics, 2023).

It should be noted that in the history of the Ukrainian state, there has already been a case of destruction of a large hydroelectric facility. To impede the movement of German troops during World War II, the Dnieper Dam was blown up in 1941 (Figure 2). At that time, at the height of the war and during the post-war reconstruction period, the impact of the reservoir's emptying on the loss of ecosystem services and ecological catastrophe for the environment was hardly investigated. The fortunate choice of the location of the hydrosystem site within the canyon-type area did not cause significant flooding in the area compared to other plain-type reservoirs. Industrialisation and rapid post-war development of the country's economy made it possible to rebuild the hydraulic structure and restore the reservoir within a short period of 4 years (1944–1947).

Ukraine is the country with one of the lowest water availabilities in Europe, and rapid climatic changes and anthropogenic pressure on aquatic ecosystems are leading to a decrease in the water volume in rivers and deterioration of water quality there (Hapich et al., 2022a; Chushkina et al., 2024). Today, the total volume and runoff of water resources in the territory of Ukraine is estimated at 55 km^3 per year (Khilchevskyi, 2021). The development and necessity to provide water resources for industries, municipal and household water supply, agriculture, and fishery led to the construction of a large number of reservoirs and ponds in the 20th century. The six largest reservoirs were created on the Dnipro (aka Dnieper) River: Kyivske (aka Kyiv) Reservoir, Kanivske (Kaniv), Kremenchutske (Kremenchuk), Kamianske, Dniprovske (Dnieper) Reservoir, and Kakhovske (Kakhovka) one–the last in the Dnipro Cascade (Vyshnevskyi and Shevchuk, 2021). In June 2023, as a result of the explosive demolition of the Kakhovska Hydroelectric Power Plant (HPP) Dam and hydrodynamic accident, the Kakhovske Reservoir, which provided numerous ecosystem services in the arid territory of southern Ukraine, was completely drained and ~18 km^3 of fresh water was irretrievably lost (Hapich and Onopriienko, 2024). The scale of the disaster as a crime against nature has already been characterized as « ecocide» (Stakhiv and Demydenko, 2023).

The flooding from the Kakhovska Dam washed small islands, areas of floodplain forests and steppes, floodplain meadows, and slopes with all their inhabitants into the Black Sea. The bottom sediments of the Kakhovske Reservoir contain pollutants (Cd, Mn, Fe, and many others) accumulated over decades because of huge industrial emissions (Afanasyev, 2023b; Klitina, 2023). Monitoring stations recorded high levels of heavy metals (Cd, As, and Cu), petroleum by-products, and PCBs in the Black Sea (Stone, 2024). The consequences of sea poisoning will sequentially manifest in the Black Sea region: in Romania, Bulgaria, Turkey, and then in Russia (Klitina, 2023). Bacteriological and chemical pollution has been recorded in both the lower Dnipro River and the northwestern part of the Black Sea (Vyshnevskyi et al., 2023)

As a result of the demolition of the Kakhovska HPP Dam, the near-estuarine zone of the Black Sea has undergone a rapid desalination process. The salinity of water off the Odessa coast was almost 2.7–3.7 times lower than normal for a certain period. Some marine fish and mussels have died, but this amount is not critical to populations (Hubareva, 2023; Stone, 2024). At the same time, the breakthrough of a huge amount of fresh water was disastrous for the few sturgeons living in the Dnipro River, because the beginning of summer is the peak of the breeding season in the spawning grounds downstream of the Kakhovska Dam, where the fish come from the Black Sea. Many Black Sea roaches (Ministry of Agrarian Policy of Ukraine, 2022), the Dnieper barbels (Ministry of Agriculture of Ukraine, 2023), and the Sarmatian bleaks (Brown et al., 2007) appear to have died. The population of estuarine perch (Sander marinus (Havrylenko, 2018)) is highly likely to be extinct (Afanasyev, 2023b; Stone, 2024). Ecological disasters due to the explosion of the Kakhovska HPP Dam may lead to significant changes in the structure of communities not only of fish but also of their parasites. How this may affect the fishery's ecosystem services cannot yet be predicted.

The military actions of the Russian Federation against Ukraine began in March 2014 with the occupation of the Crimean Peninsula and parts of Donetsk and Luhansk regions. During 8 years of the hybrid war, later (24.02.2022) Russian forces invaded the territory of Ukraine. In the 21st century, the Russian-Ukrainian war exceeds all other military conflicts studied in the last 80 years in terms of scale and consequences (Shevchuk et al., 2022). Military actions during war have a significant impact on landscapes and territories, causing diverse long-term negative consequences (Pereira et al., 2022). The war in Ukraine has caused significant damage and worsened the landscapes of more than 16% of natural territories (~104,000 km^2), affected the access of people to quality drinking water, and the loss of many ecosystem services in water bodies (Afanasyev, 2023a). The disruption of many dams as strategic elements of transport connections and structures for the accumulation and retention of water resources has caused significant social and economic losses and caused a certain danger to aquatic ecosystems and their biodiversity (Shevchuk et al., 2022; Novitskyi et al., 2024). In addition to the Kakhovske Reservoir, accidents on the Irpin River (Kyiv Region), the devastation of the Oskolske Reservoir (from the initial water volume of 435 million m^3 only 80 million m^3 remained), damage to the Karachunivske Reservoir, *etc.*, are some other examples of such nature destruction (State Agency, 2023). The volume of commercial fishing in the Dnipro Reservoirs Cascade declined in half, and in the Black and Azov Seas by more than 80%. All this has led to negative consequences in recreational and commercial fishing, and therefore in the food that freshwater ecosystems provide humans as an ecosystem service.

Scientists note that freshwater biodiversity in Europe is declining at an alarming rate: 37% of freshwater fish species are now threatened with extinction (Reid et al., 2018) and fish numbers in existing populations are declining at a particularly rapid rate (Tickner et al., 2020). Habitat loss due to climate change and anthropogenic modification of river hydrology is recognised as a key stressor affecting biodiversity in freshwater ecosystems (Strayer

and Dudgeon, 2010; Ekka et al., 2020; Novitskyi et al., 2023). However, the current negative situation with biodiversity in Europe may get worse due to the emergence of new threats, which are military conflicts and wars (Baumann and Kuemmerle, 2016). During military operations, all environment-oriented, reproduction activities and resource use become more complicated, and ecosystem services are disrupted.

The purpose of our research is to study the consequences of the Kakhovske Reservoir drainage and preliminary assess the loss in fishery and ecosystem services of the Dnipro River Delta and the area of the former reservoir.

2 Materials and methods

In 2020–2023, we studied the development of industrial and recreational fishing in the Kakhovske Reservoir and the lowlands of the Dnipro River. The research was based on theoretical (retrospective review, comparative analysis, mathematical modeling, and forecasting), field, and experimental methods. Official data from the Departments of the State Agency of Land Reclamation and Fisheries of Ukraine (State Agency, 2023) were used.

During the assessment of environmental consequences and economic losses caused to the fishery, the following research methods were used: analytical–a collection of information from official sources of enterprises and institutions that carried out economic activities, control and monitoring observations of the reservoir; hydrobiological–determination of the species composition of fish and other hydrobionts; statistical–qualitative and quantitative assessment of industrially valuable ichthyofauna and the economic losses caused to the fishery; geo-informational–assessment of the modern hydrological regime of the river within the reservoir; to determine the spatial layout, quantitative and qualitative characteristics of water bodies, which were formed after the drainage of the reservoir; predictive–assessment of the potential transformation of the biodiversity in the ecosystems over time under various conditions of further development of the water management industry in Ukraine.

The ichthyological complex of the Kakhovske Reservoir was studied in the period from 1956 to 2021 with an emphasis on the dynamics of the fish population transformation, species extinction, and the emergence of new invasive species. The material was collected according to standard research methods (Arsan et al., 2006; Bonar and Hubert, 2009). Fish protection categories were determined according to the Red List of Ukraine (2021) and the IUCN Red List categories (2023). Systematic names of species are given according to the recent updates (Movchan, 2011; Nelson et al., 2016).

According to the UN document « Millennium Ecosystem Assessment. Ecosystems and Human Wellbeing: Synthesis» (2005) a variety of ecosystem services (ES), that people obtained from the Kakhovske Reservoir and which were lost as a result of its destruction in 2023, were identified.

Research on the state of recreational fishing was conducted in the following directions: a) accounting of recreational fishers (fishing trips); b) collection of information on qualitative and quantitative indicators of catches (Lockwood et al., 1999; Grati et al., 2021; Buzevych et al., 2022). The number of fishing trips was recorded at different times of the day, on weekdays, and on days off in all seasons. The fishers who were fishing simultaneously during the accounting period in the studied area of the reservoir were subject to accounting. Fishers who fish from the shore (bridges, piers, etc.) and watercraft (boats, cutters) were counted separately.

In addition to our observations, the interview method of field survey (Giovos et al., 2018; Novitskyi et al., 2022) was used to collect primary information and study some social aspects of recreational fishing.

The number of fishing trips for a certain period (month, season, year) was calculated according to the average daily indicator for the given period and the number of days in the period Formula (1):

$$\hat{N}_p = \frac{\sum_{j=1}^{k_w} N_{wj}}{k_w} D_w + \frac{\sum_{j=1}^{k_r} N_{rj}}{k_r} D_r = \bar{N}_w D_w + \bar{N}_r D_r, \quad (1)$$

where, $\hat{N}_p$– estimated number of fishing trips for period p (*fishing trips*); N_{wj}–number of fishing trips on a weekday based on the accounting result *j*; N_{rj}–number of fishing trips on days off based on the accounting result *j*; $\bar{N}_w$–the average number of fishing trips on weekdays; $\bar{N}_r$–the average number of fishing trips on days off; k_w–number of accountings carried out on weekdays; k_r–number of accountings carried out on days off; D_w–number of weekdays per period; D_r–number of days off per period.

One completed fishing trip corresponds to one recreational fishing effort applied by one recreational fisher and is evaluated in *fishing hours*. One fishing effort covers the time from arrival at the fishing site to departure. Fishing hours reflect the total amount of time in hours spent on fishing by amateur fishers for a certain period on the reservoir (or its area) (Maksymenko, 2015).

The fishing effort of recreational fishing was calculated according to Formula (2):

$$E = \bar{h}\hat{N}_p, \quad (2)$$

where, *E*–the fishing effort of recreational fishing, calculated for the period p (*fishing hours*); $\bar{h}$–average duration of completed fishing (*hours*).

The assessment of losses in industrial and recreational fishing in the Kakhovske Reservoir due to the complete drainage of littoral areas and the loss of spawning grounds, biotopes for the feeding period of young fish, and feed base for the development of fish farming was carried out according to the following methods. Following the well-described methods (Methodology, 1995), the calculation of spawning productivity for a specific type of fish was made based on the expected industrial return from the ratio:

$$P_{sp} = (B \cdot d \cdot Q \cdot k \cdot r) \Big/ \left(F_{sp} \cdot 10\right), \quad (3)$$

where, P_{sp}–fish productivity of spawning grounds by industrial return, kg/ha; *B*–total industrial stock for 2023, tons; *d*–coefficient that takes into account the share of breeders in the industrial stock; *Q*–average fertility, thousands of eggs; *k*–industrial return from caviar, %; *r*–share of females in the herd, %; F_{sp}–area of spawning grounds, ha.

Losses in monetary value:

$$N = P_{sp} \cdot F_i \cdot W, \quad (4)$$

FIGURE 3
Drainage of the Kakhovske Reservoir near the city of Zaporizhzhia (12 July 2023, 1 month after the destruction of the HEP dam), *photo by M. Maksymenko*.

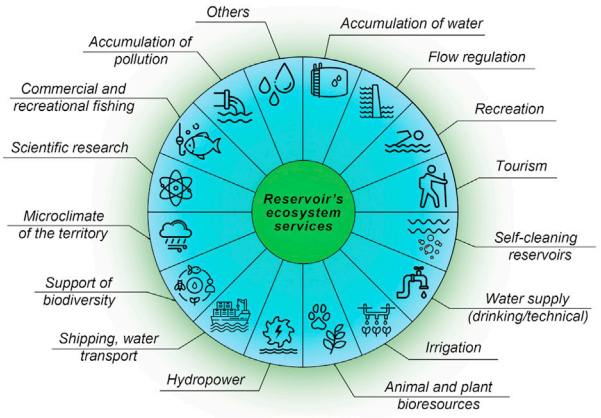

FIGURE 4
Main losses of ecosystem services in the former Kakhovske Reservoir.

where, N–loss in fishery, $; F_i–damage area of spawning grounds, ha; W–cost of 1 kg of fish at market prices in the region in June 2023. The calculation was based on the condition that $F_i = F_{sp}$

Indicators of limits and forecasts of permissible special use of water bioresources of national importance in the Kakhovske Reservoir for 2023 were used as primary fish data for damage calculations (On the approval, 2022). At the same time, it was taken into account that in 2023 the amount of permissible catch was set as 30% of the formed industrial stock. The share of breeders in the commercial herd was determined following the norms of bycatch of immature fish (Rules for commercial fishing, 2023) and was accepted as d = 0.8. Biological indicators for the ichthyofauna in the Kakhovske Reservoir were determined as previously described (Methodology, 2004). Data processing and analysis (Zar, 2010) were made with Statistica 10.0 (Statsoft Inc.).

It should be noted that today the continuation of military activities in the southern and eastern parts of Ukraine, on the left bank of the Dnipro River within Kherson and part of Zaporizhzhia regions and in the Dnipro River's estuary, which are under Russian occupation, is the limiting factor for the field research. It makes having direct access to the entire water area and conducting thorough comprehensive research impossible.

3 Results

3.1 General information about the Kakhovske reservoir

The Kakhovske Reservoir was built and filled with water in 1955–1958. In the Dnipro Reservoirs Cascade, it was the second largest (after Kremenchutske one) with an area of 2,155 km^2 and more than 18 km^3 of fresh water. The throughput capacity of the Kakhovska HPP was 2,600 m^3/s. In the 1960s–1990s, water exchange of the reservoir occurred 2–3 times a year. As a result of current climate change, global warming, and low water in the rivers of Ukraine, the recent water exchange of the Kakhovske Reservoir in some years was less than 1 time per year.

On 6 June 2023, the Kakhovska HPP Dam was destroyed. According to the Ukrainian Hydro-meteorological Institute of the State Emergency Service of Ukraine and the National Academy of Sciences of Ukraine (Kakhovske Reservoir, 2023) on 17 June 2023, the total water surface area of the remained shallow areas in the Kakhovske Reservoir together with the restored watercourse of the Dnipro was 655.9 km^2 (31.8% of its initial area). On 18 June 2023, the complete emptying of the reservoir and the full stop of further intensive discharge of water through the destroyed Kakhovska HPP was recorded.

The use of the *LandViewer* of the *EOSDA* portal allowed us to monitor the water area of the Kakhovske Reservoir and the lowlands of the Dnipro River employing Earth remote sensing data (RSE). It was established that 2 months after the accident, the area of the remained water surface of the reservoir was ~430 km^2 (about 19%) from the initial, including the restored watercourse of the Dnipro River (Figure 3). At the same time, a significant part of it falls on disconnected water bodies of the former reservoir, which occupy an area of about 300 km^2 and continue to decrease, and vegetation is actively developing in the reservoir bed.

3.2 Ecosystem services of the Kakhovske reservoir and their losses

In 2005, the UN published a report « Millennium Ecosystem Assessment. Ecosystems and Human Wellbeing: Synthesis», where the expanded concept of "ecosystem services" (ES) is given. Ecosystem services were recognized as advantages and benefits that people get from ecosystems–obtaining resources, drinking water, food, regulatory services, support services, cultural, and other tangible and intangible benefits. The document outlines four ES categories: supporting, regulating, provisioning, and cultural (Millennium Ecosystem, 2005).

The most important ecosystem service of the Kakhovske Reservoir (as a large technical-natural reservoir) is the accumulation of water for

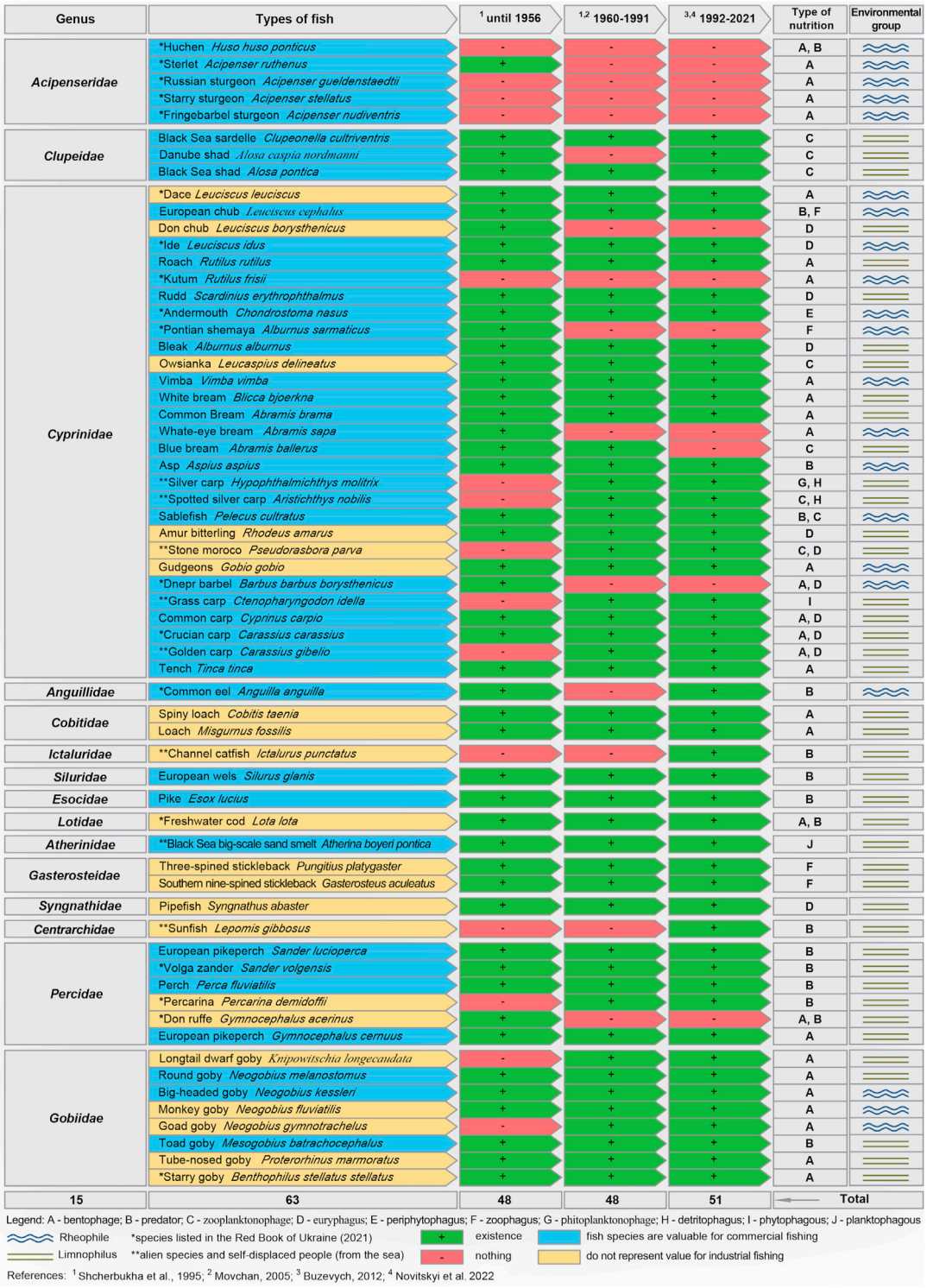

FIGURE 5
Structural and functional characteristics of fish assemblage in the former Kakhovske Reservoir.

various needs. Water supply is the most important service for the wellbeing of people and various sectors of the economy, provided by river (reservoir) ecosystems. The second ES of the reservoir is the ability to regulate the flow in general and flooding in particular. Regulating the flow led to the emergence and operation of another service–the improvement of shipping conditions (transportation conditions). Many other services are important, for example, regulation of water levels and erosion, self-cleaning, and accumulation of pollutants, including radioactive ones (Rudakov et al., 2023). Recreation and ecotourism are the most valuable cultural services.

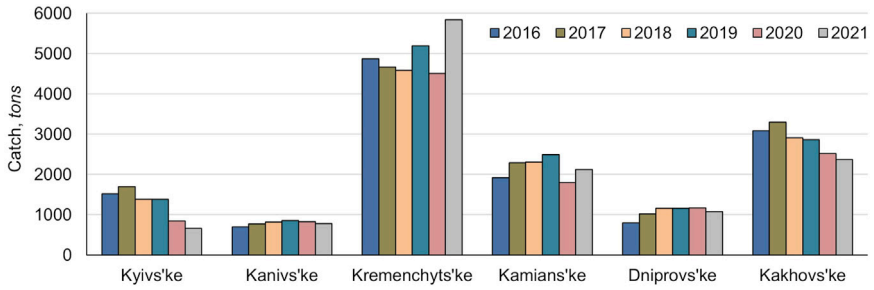

FIGURE 6
Harvest of aquatic biological resources in the Dnipro Reservoir Cascade.

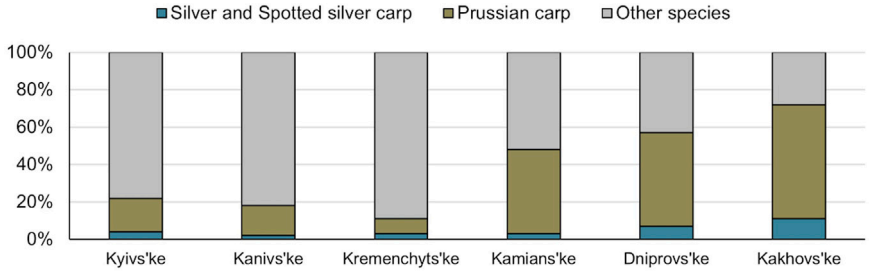

FIGURE 7
Average long-term share of Golden carp and herbivorous fish in the commercial catches at the Dnipro Reservoir Cascade.

According to recent research (Uzunov and Protasov, 2019; Protasov and Uzunov, 2021), Kakhovske Reservoir provided an important ES for delivering drinking and technical water to more than five million people by filling the North Crimean Canal, the Dnipro-Kryvyi Rih Canal, and the Kakhovka and North Rohachynsk irrigation systems with a total area of ~750,000 ha. In addition, the reservoir provided a cooling service for the condensers of Zaporizhzhia NPP (6 GW) and Zaporizhzhia TPP (3.6 GW). For all reservoirs of the Dnipro Cascade, and the Kakhovske in particular, it is possible to note the services of plankton (production and destruction of organic matter, increasing the food base for fish), services of benthos (due to filter mollusks, a significant potential for self-cleaning of reservoirs is achieved), services of periphyton (production of hundreds of thousands of tons of oxygen per season), nekton services (food for fish, biological resources). After the catastrophic drainage, the Kakhovske Reservoir lost almost all the ecosystem services that people obtained from the reservoir as benefits (Figure 4).

In the period from the Dam construction (1952–1956) according to different research, the fish assemblage in the Kakhovske Reservoir had got from 42 (Buzevych, 2012) to 56 species (Shcherbukha et al., 1995). A recent study (Novitskyi et al., 2022) registered 39 species of fish (Figure 5). Fish are known to play an important role in nutrient and energy cycling at different trophic levels by regulating aquatic ecosystem food net dynamics (McManamay et al., 2011). They also contribute to food and livelihoods for millions of people worldwide (Lynch et al., 2016).

The catch and sale of aquatic bioresources from the Kakhovske Reservoir in the pre-war period provided no less than 22% of freshwater fish harvest (Novitskyi and Horchanok, 2022) on the market of Ukraine. In terms of commercial fish catch, the Kakhovske Reservoir took second place in the overall harvest in the Dnipro Reservoirs Cascade (after the Kremenchutske Reservoir) (Figure 6).

Commercial fishing (fishery) was based on 20 industrially valuable fish species, among which the main share of Golden carp and herbivorous fish–Silver carp and Spotted silver carp (Figure 7).

Following the above-mentioned methods of loss calculating in the fishing industry, it was established that the average long-term commercial catch of fish in the Kakhovske Reservoir was in the range of 2,500–3,000 tons per year. Taking into account the current prices for fresh fish in Ukraine in 2023, the total loss in monetary value amounts to about $5.5 million per year. Losses of fisheries from the disappearance of spawning grounds are estimated at 20,000 tons of fish (~$40 million).

Recreational fishing is known to be a type of ecosystem service (Kaemingk et al., 2022; Lennox et al., 2022). Active water recreation (boating, swimming, fishing, etc.) provides many psychosocial advances and generates corresponding economic outcomes, providing important health benefits (Venohr et al., 2018). At the same time, recreational fishing is a powerful factor influencing aquatic ecosystems, fish assemblages, and plant communities (Wortley, 1995). Novitskyi (2015) calculated the taking out of aquatic bioresources from the reservoirs of Ukraine as fishery (commercial fishing) that averaged 30%–35%, recreational fishing–40%–45%, and illegal fishing–at least 25%–30% of the total harvest.

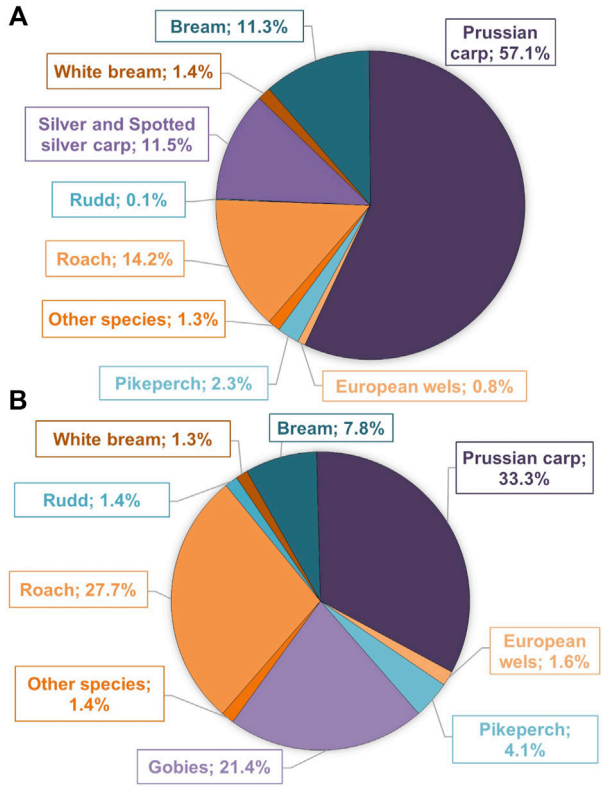

FIGURE 8
Main fish species in the catches at the Kakhovske Reservoir: **(A)** commercial catch, **(B)** recreational fishing.

Thus, recreational fishing is a powerful factor to be considered in nature management, competing with industrial freshwater fishing in Ukraine. Our study notes that recreational fishers caught 34 species in the Kakhovske Reservoir. The ratio of the main commercial fish species in commercial and recreational fish catches is presented in Figure 8. Golden (Prussian) carp was found as dominant in amateur catches.

The total annual catch of fish by recreational fishers at the Kakhovske Reservoir within the boundaries of the Zaporizhzhia region (47.2% of the total water area of the reservoir) was about 586 tons or 6.93 kg/ha of the water area in 2017–2021. Goby species dominated in the total catch in terms of specimen number (Figure 9), and Golden carp (179.6 tons)–in terms of catch weight. The catch volume of the roach was 95.3 tons, and the common bream was 52.9 tons. The total share of predatory fish species (pike, European chub, asp, wels catfish, pikeperch, and perch) was 69.96 tons. The pike perch (43.6 t of the total catch) and wels catfish (21.4 t) are dominant among predators. At the same time, over the entire Kakhovske Reservoir, the total catch was estimated at 747 tons or 3.5 kg/ha (Maksymenko, 2015; Novitskyi et al., 2022).

According to the obtained data on the upper and middle parts of the Kakhovske Reservoir, the largest number of fishing trips per year is concentrated in the 1 section of the reservoir (41%) and floodplains (35% of the total number of trips). The upper part of the Kakhovske Reservoir is up to 28% of the reservoir water surface area, but this part of the reservoir has the largest number of fishing trips–53% of the total accounted number (3.2 fishing trips/ha). The lowest number of trips falls on the lower part of the reservoir–19%, or 1.1 fishing trips/ha.

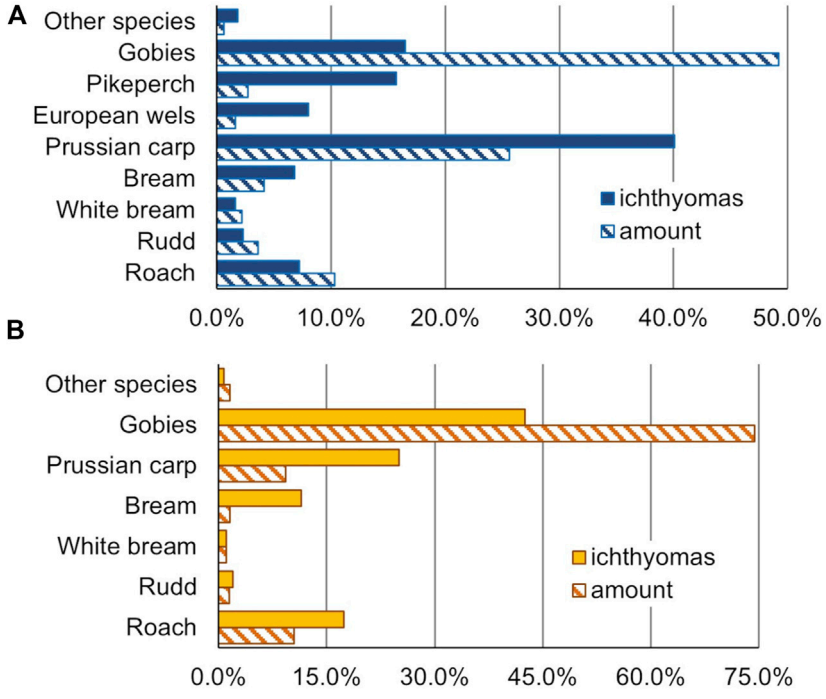

FIGURE 9
Proportion of catch of main fish species in the Kakhovske Reservoir: **(A)** commercial catch, **(B)** recreational fishing.

TABLE 1 Indicators of recreational fishers visits to the Kakhovka Reservoir in 2000–2021.

Indicator	Year			
	2000 (Drobot et al., 2003)	2002–2006 (Maksimenko, 2011)	2013 (Maksimenko and Rudyk-Leuska, 2013)	2021 (Novitskyi et al., 2022)
Area of the water surface **S**, thousand ha	215.5			
Estimated number of fishing trips **N**, thousand	568.3	278.3 ± 46.9	356.7	256.4
The average duration of completed fishing **h**, hours	5.0 ± 0.0	5.8 ± 0.2	5.6 ± 0.0	4.9 ± 0.1
Estimated annual indicator of fishing effort **E**, thousands of fishing hours	2,855.8	1,620.8 ± 425.6	1980.2	1,256.4
Estimated annual indicator of density N_S, fishing trip/ha per day	2.6	1.3 ± 0.2	1.7	1.2
Loading E_S, fishing hour/ha	13.3	7.5 ± 1.3	9.2	5.8

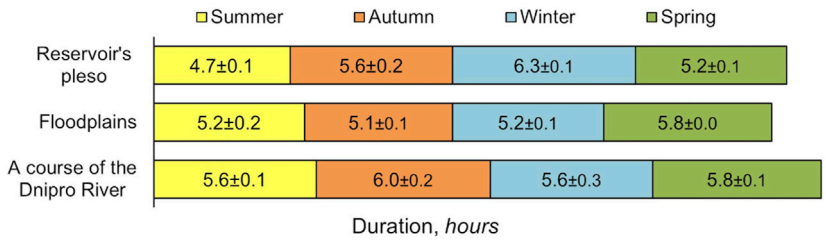

FIGURE 10
The average duration of completed fishing at the Kakhovske Reservoir depending on the season and place.

In Table 1 the averaged data on visits to the Kakhovske Reservoir by recreational fishers in 2000–2021 are presented. The visitation is related directly to the amount of anthropic impact on the shores and water area of the reservoir. The duration of completed fishing indicates directly the prospects of a certain section of the Kakhovske Reservoir for successful fishing. The average duration of completed fishing in the water area of the reservoir, depending on the season and fishing location, varied from 4.7 to 6.3 h (Figure 10).

The destruction of the Kakhovske Reservoir Dam in June 2023 caused an ecological disaster for about 40 species and subspecies of fish. In general, more than 11,000 tons of fish were lost due to the impact of sudden drainage, and estimated losses from disruptions of ecosystem services reach about $270 million (State Agency, 2023).

In Figure 11, data on the ecological losses in the Kakhovske Reservoir from the destruction of the fishery, forage base of fish, and spawning grounds, including in financial terms are presented.

It should be noted that the research covered shallow water areas of 9,313 ha throughout the reservoir, which ensures their high reliability and representativeness. Thus, the obtained results can be extrapolated to the entire water area of the reservoir and partly to the Dnipro River Delta.

4 Discussion

After the Dam destruction, the significant water flow velocity during dewatering resulted in flooding of the lower sections of the reservoir from Kherson, Oleshki, Gola Prystan, and further to the Dnipro estuary. On 9 June 2023, the rate of water level decline in the upper section of the Kakhovske Reservoir allowed many fish to respond to the change in hydrological regime and mostly leave the shallowed areas. However, the majority of limnophilic species groups moved downstream, to the Dnipro estuary, or got to the newly formed floodplains beyond the former shoreline. The fauna of the reservoir, carried away by the flow of water into the floodplains formed below the Kakhovska HPP dam, mostly died with the further lowering of the “flood” wave and washing ashore. Unlike fish, which could move actively and migrate downstream, benthic organisms, primarily mollusks, remained on the drained bottom of the Kakhovske Reservoir. According to the estimates of the Institute of Hydrobiology of the National Academy of Sciences of Ukraine, headed by Professor Serhii Afanasiev, in the first days of the disaster, no less than 500,000 tons of bivalve mollusks were found on land and died (Afanasiev, 2023b). He also claims that the sudden desalination of significant water masses in the Dnipro estuary could contribute to the destruction of rare species populations protected by the Red List of Ukraine and the IUCN, in

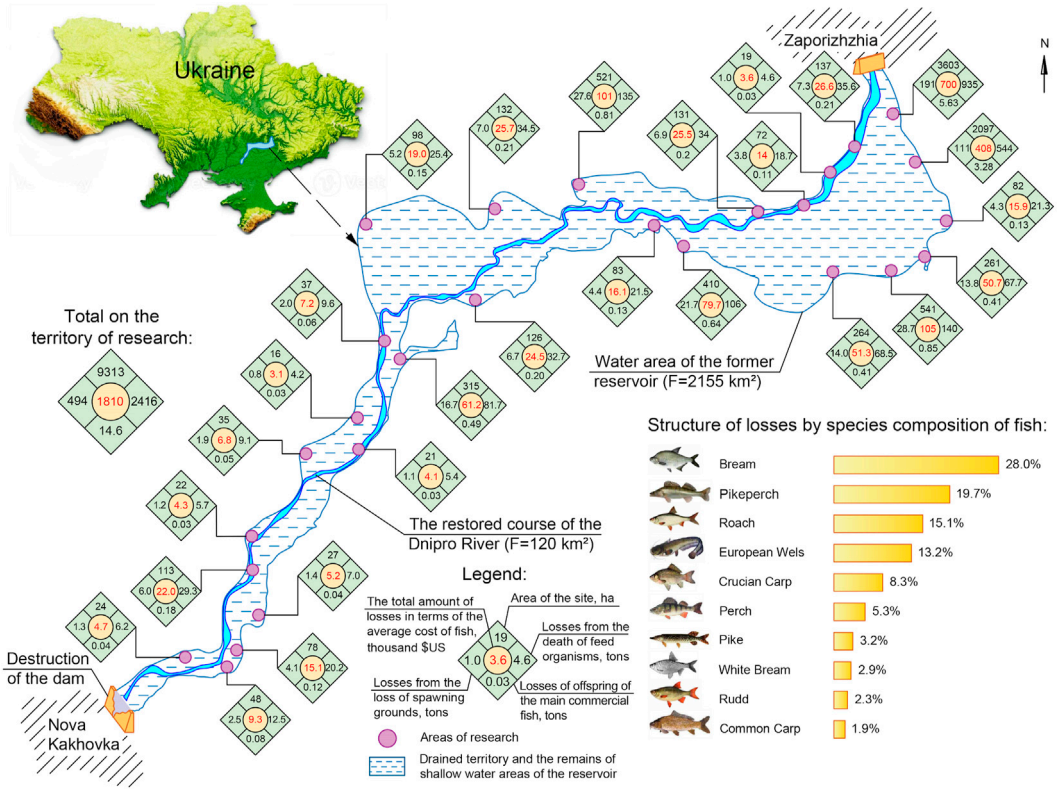

FIGURE 11
Summarized data on ecological losses in the fishing at the Kakhovske Reservoir.

FIGURE 12
Fish mortality in the Kakhovske Reservoir near the village of Lysogirka, Zaporizhzhia district (15 June 2023, on the 10th day after the HPP destruction), *photo by M. Maksymenko*.

particular, the pike *Sander marinus*, which lived there and in the Dnipro-Buh estuary ecosystem in general.

On 18 June 2023, the complete emptying of the reservoir and ending of further intensive evacuation of water through the Kakhovska HPP was recorded. The death of hydrobionts continues in the newly-formed isolated lakes: part of the Balabinsk Bay, lakes in the Khortytsia Island floodplain, parts of the rivers Sukha Moskovka, Mokra Moskovka, Yanchekrak, Konka, Karachekrak and others (Figure 12).

In this study, we did not calculate and assess the vegetation loss in the Kakhovske Reservoir, the reserves of which in the reservoir are significant and amounted to about 50,000 tons in air-dry mass (Spesivyi, 2006). Phytoplankton biomass values according to the seasons made it possible to assign the Kakhovske Reservoir to the category of reservoirs with high nutrition (forage). Due to the production of phytoplankton, it was possible to ensure a potential increase in the ichthyomass of planktonophagous fish in the range of 62–112 thousand tons (Report, 2001), which increases significantly the number of potential losses of fish farming in the Kakhovske Reservoir.

Calculations of feed base losses in the reservoir may also be underestimated because it is impossible to calculate the exact amount of zoobenthos species loss. In 1996–2000, the reservoir was classified as medium or highly nutritious in terms of the level of forage zoobenthos development. The average fish capacity in the Kakhovske Reservoir assessed by zoobenthos was 632.7 kg/ha (Report, 2001).

The catastrophic devastation of the reservoir led to the appearance of a lotic hydroecosystem as it was in the early 1950s. In fact, the Dnipro River from the city of Zaporizhzhia to the Dnipro River estuary (340 km) is no longer regulated. Hydrological changes are taking place in the Dnipro-Buh estuarine region, where the lack of fresh water is gradually being compensated by filling the shelf zone with salt water from the Black Sea. Reducing the flow of the Dnipro and increasing salinity will change significantly the conditions of the existence of hydrobionts,

especially self-displaced and alien species (Semenchenko et al., 2015; Novitskyi et al., 2019; 2023). These species can displace native flora and fauna, leading to a loss of natural local biodiversity. A significant social-economic problem of increasing water salinity in the water system of the Dnipro-Buza estuary region may cause a change in the water management status of the Dnipro outflow.

According to the annual reports of the Department of Ecology and Natural Resources in the Kherson region, on average, about 1,035 million m^3 of Dnipro water is used for drinking, sanitary and hygienic, production, agricultural and other needs (Korzhov and Kucheriava, 2018). Water from the outflow of the Dnipro is ly used for water supply in more than 30 settlements, together with Kherson and Mykolaiv.

Before the draining, the reservoir was characterized by a small percentage of shallow waters with depths of up to 2 m (5%), which was always a significant adverse factor limiting the number of phytophilous fish in the reservoir (Report, 2001). Currently, the Kakhovske Reservoir has lost all spawning grounds, which makes it impossible for the effective reproduction of phytophiles in river conditions in the near future. Increased mortality due to the loss of protected biotopes (locations) is one of the reasons for the decline of mature (adult) fish populations (Arthington et al., 2016; Hermoso et al., 2016). According to forecasts (State Agency, 2023), it will take at least 10–12 years to restore stocks of the main commercial aquatic biological resources to the state that preceded the disaster.

According to the reports of recreation fishers, only 8 species of fish (Golden carp, Common bream, Roach, Common carp, Bleak, White bream, Perch, Round goby) were caught in the Dnipro water course (Zaporizhzhia city) in August-September 2023. The status of predatory piscivorous fish downstream of the Dnipro remains uncertain. It is known that a change in the number and diversity of large predatory fish causes the launch of trophic cascades on the scale of the entire hydroecosystem (Fox et al., 2012), which have detrimental consequences for the further functioning and sustainability of ecosystems, as well as human livelihoods (Dudley, 2008).

In this study, special attention is paid to such an ecosystem service of the Kakhovske Reservoir as recreational fishing, which is a type of active recreation on the water and is also a powerful factor in nature management. The scale of recreational fishing in the water area of the Kakhovske Reservoir and the lowlands of the Dnipro can be evidenced by the following fact: before 24 February 2022, about 55,000 small-sized fleet vehicles (boats and cutters) were registered in the state registration authorities in the Zaporizhzhia region.

Before the explosion of the Kakhovska HPP dam, recreational fishers caught 800–1,100 tons of fish annually in the Kakhovske Reservoir (Novitsky, 2015), which was at least 25%–33% of the commercial fishery. The draining of the Kakhovske Reservoir will make it impossible or reduce drastically recreational fishing, swimming, diving, underwater hunting, and yachting for almost 800,000 fishers in the Zaporizhzhia, Dnipropetrovsk, and Kherson regions. These annual losses of fishery ecosystem services can reach millions of dollars.

The negative consequences of the loss of the reservoir water ecosystem will affect the socioeconomic development of the southern region of Ukraine for a long time. Water and food security in Ukraine are the main components of national security in the context. The loss of water supply is also a key one among ecosystem services (Hapich et al., 2024b). Unfortunately, the qualitative and quantitative indicators of water resources of small and medium-sized rivers in this region are not able to satisfy fully the needs of industry and the population with fresh water (Hapich et al., 2022b). Irrigated agriculture, where water is the limiting factor for development, will also stop (Hapich et al., 2023).

The post-war restoration of Ukraine, its degraded lands, territories, and water areas, and ensuring food security are today's top priority issues. One of the urgent problems will be the reasonability of rebuilding the Kakhovska HPP Dam and restoring the Kakhovske Reservoir, reviving water supply, fishery, energy, recreation, and irrigation (Report, 2001; United Nations, 2023; Hapich et al., 2024a).

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

RN: Conceptualization, Investigation, Methodology, Supervision, Validation, Writing–original draft. HH: Conceptualization, Formal Analysis, Methodology, Validation, Visualization, Writing–original draft. MM: Investigation, Visualization, Writing–original draft. PK: Conceptualization, Investigation, Methodology, Visualization, Writing–review and editing. VG: Conceptualization, Formal Analysis, Methodology, Validation, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Loss of native brown trout diversity in streams of the continental Croatia

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Introduction: The genetic diversity of brown trout in the Western Balkans has been disrupted by the introduction of non-native Atlantic phylogenetic lineages and non-native haplotypes of the Danubian phylogenetic lineage. The Western Balkans is characterized by the greatest phenotypic and genotypic diversity of trout populations, and a large part of the internal territory belongs to the Black Sea basin, where the Danubian Da1 haplotype is native. Artificial propagation of non-native lineages in the Western Balkans has a long history, and these populations are often the only available material for stocking rivers attractive for fishing.

Material and Methods: Fifteen populations in the Danube basin of the continental Croatia were analysed. The analysis of eight microsatellite loci was performed to determine the structure of brown trout populations, as well as the degree of introgression of non-native genetic material into the native.

Results and Disscusion: The results of this study showed significant genetic similarity among brown trout populations, confirming a long history of introduction with non-native genetic material. The main reason was uncontrolled stocking with inadequate material, which is available in fish farms and consists mainly of brown trout of the Atlantic phylogenetic lineage. The results of this study also indicated stocking with brown trout of the non-native haplotypes of the Danubian phylogenetic lineage. The potential breeding origin of brown trout carrying the Danubian Da2 mtDNA haplotype and ways of its introduction into rivers have yet to be investigated. For the survival of the unique gene pool of brown trout in Croatian rivers, it is of fundamental importance to know the structure of wild and farmed populations with the aim of proposing and implementing conservation measures.

KEYWORDS

brown trout, microsatellites, Croatia, stocking, conservation

Introduction

The commercial value of brown trout *Salmo trutta*, along with its significant importance to humans, is reflected in various aspects, particularly its widespread artificial propagation in fish farms globally. Farming is carried out for various purposes, of which stocking notably impacts the survival of native, genetically pure, and geographically unique brown trout populations.

Numerous studies on this species, still considered the *S. trutta* species complex, have shown that the life history, phenotypic plasticity, and high adaptability to diverse environmental conditions have not yet been clarified, more than likely as a consequence of their exceptionally high morphological and genetic variability (Behnke, 1972; Weiss et al., 2001; Sanz, 2018; Škraba Jurlina et al., 2018). The Western Balkans exhibit a high degree of endemism in salmonid genera and species (Behnke, 1986; Oikonomou et al., 2014). Together with the Iberian Peninsula and the Apennines, the Western Balkans is considered the area with the greatest phenotypic and genetic diversity among trout populations (Bernatchez, 2001) with complex evolutionary mechanisms, including the occurrence of secondary contacts between ancestral lineages, as well as local adaptation (Sanz et al., 2002; Snoj et al., 2008; Vera et al., 2010). The Western Balkans is the home range for populations of three phylogenetic lineages of *Salmo* cf. *trutta* which are defined based on the control region (CR) of mitochondrial DNA (mtDNA): Danubian (DA), Adriatic (AD) and *marmoratus* (MA). The DA lineage inhabits the northern, eastern, and central parts of the Balkan Peninsula, i.e., the Black Sea basin, and the AD lineage inhabits the northwestern and southwestern regions, including numerous lotic and some lentic habitats in the continental part of the Adriatic and Ionian Seas' basins (Georgiev, 2003). The MA lineage is found close to the dispersal range of brown trout of the Danubian and Adriatic lineages, in the narrowest area in the Western Balkans, inhabiting the Adriatic drainages in Bosnia and Herzegovina and Montenegro (Snoj et al., 2010; Mrdak eta al., 2012; Škraba Jurlina et al., 2020; Righi et al., 2023). The status of the MA lineage, which is still often identified as marble trout, has not yet been resolved in Croatia (Ćaleta et al., 2019).

The rivers of the Western Balkans within the Black Sea basin drain a considerable territory and are very important in the context of studying the diversity and life history of different phylogenetic lineages (Škraba Jurlina et al., 2018). The DA lineage, native to the Danube basin, consists of numerous haplotypes, with the Da1 haplotype being the most widespread. Still, not all of the DA haplotypes are considered native to the Western Bakans area/region. The presence of non-native DA haplotypes, as well as other phylogenetic lineages, in most of the studied rivers presents a significant threat to the native population's genetic structure (Snoj, 2004; Marić et al., 2006; Jadan et al., 2007; Tošić et al., 2016; Simonović et al., 2017a; Buj et al., 2020; Ivić et al., 2021).

Breeding brown trout in the Balkans is a profitable enterprise which makes it highly popular across all countries in the region. With the aim of intensive artificial propagation, and thus, a greater commercial profit (Simonović et al., 2017b), the initially domesticated native DA lineage (Gridelli, 1936; Kohout et al., 2012) proved to be insufficient. Consequently, breeders introduced breeding technology from Western Europe and the Atlantic lineage of brown trout suitable for this purpose (Taler, 1949; Pofuk et al., 2017; Piria et al., 2020).

Human-induced translocations of organisms and anthropogenic alterations of natural habitats are the most common causes of hybridization and introgression of non-native genotypes into native populations (Allendorf et al., 2001). *Salmo trutta* is characterized by genetic diversity at the intrapopulation level, which is the reason for the existence of genetically differentiated local populations. The loss of these unique populations could lead to a significant reduction in genetic variability within the species (Laikre et al., 1999). Stocking became a common practice to increase and support wild stocks of many fish species, including brown trout (Righi et al., 2023). In the beginning of the 20th century, it was pointed out that supportive breeding programs could also pose a threat to gene-level biodiversity, by releasing cultured individuals into the wild, with the aim of increasing the size of natural populations (Laikre et al., 2008). These activities could lead to manipulation of reproductive rates, causing reduced effective population size, increased rate of genetic drift and loss of genetic variability (Ryman et al., 1995; Wang and Ryman, 2001; Laikre et al., 2008), which is observed in many populations of brown trout in the Balkans (Jadan et al., 2007; Buj et al., 2020; Piria et al., 2020; Škraba Jurlina et al., 2020).

Although the research on genetic variability of brown trout has been extensively conducted to assess the introgression of farmed lineages, gene-level monitoring programs are still notably absent, despite repeated calls for their implementation (Laikre et al., 2008; Tošić et al., 2016; Simonović et al., 2017a; Škraba-Jurlina et al., 2020). To ensure the survival of a population or species, it is necessary to preserve its genetic diversity, and thus its evolutionary potential (Ryman et al., 1995). Unfortunately, the genotyping of brown trout individuals in brood stocks has been limited to a small number of fish farms in the Western Balkans.

In Croatian fish farms, brown trout of the AT lineage are mainly bred, so consequently the rivers are most likely stocked with these individuals due to their availability (Marić et al., 2022). In this way, non-native genetic material enters natural watercourses (Piria et al., 2020). Brown trout of the AT lineage are already recognized as the main cause of the loss of the original genetic diversity of brown trout (Weiss et al., 2001; Simonović et al., 2017b) and show an invasive character (Simonović et al., 2013; 2015; Piria et al., 2020), but their stocking is not regulated by any law in Croatia.

The goal of this research was to determine the genetic structure of brown trout populations in the Croatian part of the Danube basin, and to assess the degree of introgression of non-native genetic material within studied populations, as well as its possible origin.

Material and methods

A total of 141 samples were collected and analyzed from 15 locations: in the Gorski Kotar area (six locations), in Žumberak (three locations) and on the slopes of Mount Papuk (six locations) (Figure 1). The sampling was carried out in the period of April/May during 2017 and 2018.

For the purposes of genetic analyses, the anal fin clips of each individual were taken and stored in 96% ethanol. The extraction of

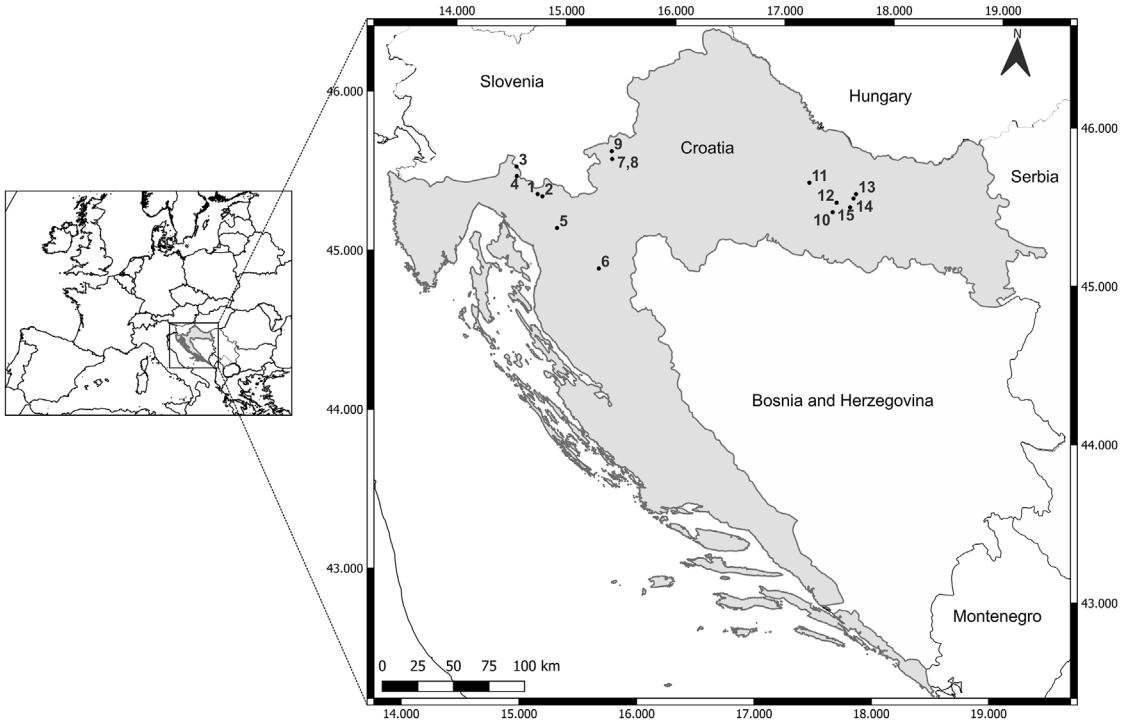

FIGURE 1
Map of sampling sites of brown trout in the Danube River basin of Croatia. Gorski Kotar region: 1—Mala Lešnica, 2—Curak, 3—Čabranka, 4—Bresni Potok, 5—Jasenak, 6—Lička Jesenica; Žumberak region: 7—Kupčina, 8—"Vrabac" fish farm, 9—Slapnica; Mt. Papuk region: 10—Orljava, 11—Toplica, 12—Brzaja, 13—Jankovac Stream, 15—Jankovac Lake, 15—Veličanka.

DNA was conducted according to the protocol of manufacturer of the Quick-gDNA™ MiniPrep (Zymo Research Corporation, United States) extraction kits.

The analysis of the control region of mitochondrial DNA (CR mtDNA) was used to identify the phylogenetic lineages of brown trout and their haplotypes, while restriction analysis of the L-lactate dehydrogenase nuclear locus (LDH-C*) was used to detect if hybrids between AT and DA lineages exist. For a detailed description of the procedures and software analyses refer to Kanjuh et al. (2020).

Population diversity was accessed by analyses of eight microsatellite loci: Str73INRA (Estoup et al., 1993) and Ssa410Uos (Cairney et al., 2000), SsaD190 and SsaD71 (King et al., 2005), Ssa85 (O'Reilly et al., 1996) and SSsp2216 (Paterson et al., 2004), and OMM1064 (Rexroad et al., 2002) and SsoSL438 (Slettan et al., 1995), amplified in four duplex reactions. The total volume of the mixture for all four duplex reactions was 10 µL, and its composition was as follows: forward and reverse primers for a specific locus (with final concentrations: 0.2 µM for SsaD190, SsaD71, SSsp2216 and OMM1064, 0.4 µM for Ssa410Uos and SsoSL438, 0.1 µM for Ssa85 and 0.15 µM for Str73INRA), dH_2O, 1X PCR buffer, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 0.5 U Taq polymerase, and 2 µL DNA sample. The amplification conditions for the first three duplex reactions (Str73INRA and Ssa410Uos, SsaD190 and SsaD71, Ssa85 and SSsp2216) were identical: initial denaturation (94°C, 3 min), 30 cycles of denaturation (94°C, 45 s), primer binding (60°C, 1 min), elongation (72°C, 30 s) and final elongation (72°C, 1 h). An exception in the protocol was the fourth duplex reaction (OMM1064 and SsoSL438), in which the primer binding was set to 57°C, and the number of cycles was 35. Fragment analysis of microsatellite loci was performed in the laboratories of MACROGEN® Europe. For several samples, fragment analysis was performed at the Center for Human Molecular Genetics, Faculty of Biology, University of Belgrade, where the fragment analysis was performed using GeneScan™ 500 LIZ® Size Standard (Applied Biosystems, United States) on an ABI-3130 Genetic Analyzer (Applied Biosystems, United States). Analysis was done using GeneMapper® ID v3.2.1 software (Applied Biosystems, United States).

Statistical analyses of microsatellite loci

Hardy-Weinberg equilibrium (HWE) and *p*-values for F_{IS} within samples, based on 120,000 randomizations, were observed in Fstat 2.9.3.2 software (Goudet, 2002). Observed and expected heterozygosity values, the average number of alleles per locus, allele frequencies, fixation indices, Nei's distances between populations per 1,000 permutations and the Factorial Correspondent Analysis (FCA) were calculated using GENETIX 4.05 software (Belkhir et al., 1996). The software POPULATIONS 1.2.31 (Langella, 2002) was used to calculate shared allele distances (DAS) and to generate a Neighbor-Joining (NJ) tree. The results of Nei's distances were used for CLUSTER analysis using the method of Unweighted Pair-group Average (UPGMA) in STATISTICA 8.0 software (StatSoft, Inc, 2001). Population structure analysis was done using

TABLE 1 Results of control region of mitochondrial DNA showing distribution of the brown trout haplotypes detected in the studied rives in Croatia. n—number of individuals, DA—Danubian lineage, AT—Atlantic lineage (from Kanjuh et al., 2020).

River	Drainage	n	DA			AT
			Da1	Da2	Da22	At-H3 (At1)
Mala Lešnica	Kupa	12	9			3
Curak	Kupa	12	10			2
Čabranka	Kupa	11	9	2		
Jasenak	Kupa	6	4			2
Bresni potok	Kupa	11		2		9
Slapnica	Kupa	10	8	2		
Kupčina	Kupa	14	13			1
"Vrabac" fish farm	Kupa	10				10
Lička Jesenica	Lička Jesenica	9	1	8		
Veličanka	Sava	6	3		1	2
Orljava	Sava	5			5	
Brzaja	Sava	9		1	7	1
Toplica	Drava	13	10	2		1
Jankovac Stream	Drava	5	5			
Jankovac Lake	Drava	8	1	7		
Total		141	73	24	13	31

STRUCTURE 2.3.4 software (Pritchard et al., 2000). The structure analysis for all 141 individuals was conducted with assumed number of groups K = 15 and the test period was set at 100,000 to 200,000, which was repeated seven times for each group K. The STRUCTURE Harvester tool (Earl and vonHoldt, 2012) was used to estimate the most likely value of K according to Evanno et al. (2005). Additional structure analysis was done only for DA individuals, who according to the LDH-C* molecular marker, were homozygous for the LDH-C*100 allele. The assumed number of groups was K = 15 and the test period was set at 100,000 to 200,000, which was again repeated seven times for each value K. The bottleneck effect was evaluated using the BOTTLENECK 1.2.02 software (Cornuet and Luikart, 1996; Piry et al., 1999), within Sign test and Wilcoxon test (Sign-rank test) and two mutation models, two-phase mutation model (TPM) and stepwise mutation model (SMM). As parameters for the assessment of significance for these tests, the proportion of a mutation step in the TPM was set at 95% and the mutation variance for the TPM at 12,000 and 100,000 iterations.

Results

Control region of mitochondrial DNA and L-lactate dehydrogenase nuclear locus

The results of CR mtDNA and LDH-C* locus analyses are presented in detail in Kanjuh et al. (2020). In short, DA and AT phylogenetic lineages were identified in analyzed samples. The presence of non-native brown trout haplotypes was detected in all sampling areas: At1 (At-H3) in eight localities (including the fish farm), Da2 in seven localities and Da22 in three localities (Table 1). The only river where all individuals (five in total) carried the native Da1 haplotype was the Jankovac Stream. Analysis of the LDH-C* locus revealed interbreeding between DA and AT individuals, indicating the presence of hybrids. Even in the Jankovac Stream the crossbreeding between Da1 and At1 individuals was recorded, with one exception that was homozygous for the LDH-C*100 allele. The only genetically pure population was the one from the fish farm, where all the analysed individuals were non-native AT lineage by maternal (CR mtDNA) and paternal (LDH-C*) inheritance. The results obtained with these two molecular markers indicate a strong introgression of non-native genetic material into native, which is most likely a consequence of crosses in the F1 generation, resulting from uncontrolled, long-term stocking with farmed trout. This is supported by the results showing that 21 individuals of the DA lineage were homozygous for the LDH-C*90 allele, which is characteristic trait of the AT lineage, while six individuals of AT haplogroups were homozygous for the LDH-C*100 allele typically found in the DA haplogroup.

Microsatellite loci

The highest expected (Hexp.) and observed (Hobs.) heterozygosities were recorded in the Jasenak River population (Table 2), in which the highest number of alleles, i.e., the highest allelic richness at the loci OMM1064 (16 alleles) and SsoSL438 (14 alleles) was observed (Supplementary Table S1). The lowest

TABLE 2 Average heterozygosity of microsatellite loci in the studied populations. Hexp.—expected heterozygosity, Hn.b.—objective heterozygosity, Hobs.—observed heterozygosity, SD—standard deviation, p—probability, Ān—average number of alleles per locus, LE—Mala Lešnica, CZ—Curak, ČA—Čabranka, JA—Jasenak, BP—Bresni potok, SL—Slapnica, KČ—Kupčina, KČR—"Vrabac" fish farm, LJ—Lička Jesenica, VE—Veličanka, OR—Orljava, TO—Toplica, JP—Jankovac Stream, JJ—Jankovac Lake, BR—Brzaja

	LE	CZ	ČA	JA	BP	SL	KČ	KČR	LJ	VE	OR	TO	JP	JJ	BR
Hexp	0.765	0.659	0.630	0.780	0.764	0.682	0.683	0.663	0.581	0.670	0.542	0.685	0.537	0.653	0.568
SD	0.144	0.200	0.167	0.071	0.090	0.160	0.270	0.121	0.288	0.147	0.269	0.213	0.326	0.144	0.306
Hn.b	0.798	0.687	0.660	0.850	0.800	0.718	0.708	0.698	0.615	0.731	0.603	0.713	0.597	0.697	0.601
SD	0.150	0.209	0.175	0.077	0.095	0.169	0.280	0.128	0.305	0.161	0.298	0.221	0.362	0.153	0.324
Hobs	0.688	0.458	0.511	0.812	0.784	0.525	0.598	0.670	0.542	0.646	0.475	0.615	0.575	0.609	0.514
SD	0.124	0.244	0.266	0.226	0.175	0.287	0.264	0.225	0.281	0.188	0.301	0.240	0.377	0.245	0.302
p (0.95)	1	1	1	1	1	1	1	1	0.875	1	1	1	0.875	1	1
p (0.99)	1	1	1	1	1	1	1	1	0.875	1	1	1	0.875	1	1
Ān	7.625	6.500	4.750	6.250	7.250	6.375	8.875	5.750	5.250	5.250	3.875	6.750	4.375	4.750	5.875

TABLE 3 Genetic distances for populations pairs, based on allele frequencies across the eight microsatellite loci, are shown by fixation index values (F_{ST}) are shown under diagonal, and estimated gene flow values (Nm) are shown above diagonal. VE—Veličanka, OR—Orljava, LE—Mala Lešnica, TO—Toplica, LJ—Lička Jesenica, SL—Slapnica, JA—Jasenak, JP—Jankovac Stream, JJ—Jankovac Lake, BR—Bresni potok, CZ—Curak, ČA—Čabranka, KČ—Kupčina, KČR—"Vrabac" fish farm, BP—Brzaja

Localities	VE	OR	LE	TO	LJ	SL	JA	JP	JJ	BR	CZ	ČA	KČ	KČR	BP
VE		1.84	4.04	1.93	1.23	1	3.02	0.88	1.26	1.39	1.46	1.33	1.38	0.87	1.5
OR	0.12		2.14	1.02	1.42	0.95	1.66	1.39	1.49	5.26	1.64	1.42	1.85	0.84	1.15
LE	0.06	0.10		2.64	2.37	1.46	19.09	2.13	1.82	2.15	4.69	2.21	3.83	1.46	2.41
TO	0.11	0.20	0.09		1.11	1.23	3.25	0.86	1.4	1	1.48	0.97	1.29	1.35	1.73
LJ	0.17	0.15	0.10	0.18		1.06	1.77	0.86	1.04	1.8	2.94	1.05	2.14	0.84	1.15
SL	0.20	0.21	0.15	0.17	0.19		2.1	0.87	1.02	0.93	1.23	0.88	1.6	1.26	1.77
JA	0.08	0.13	0.01	0.07	0.12	0.11		1.26	1.78	1.37	2.58	1.54	2.48	2.33	5.52
JP	0.22	0.15	0.11	0.22	0.23	0.22	0.17		1.46	1.68	1.56	1.2	2.02	0.69	1.1
JJ	0.17	0.14	0.12	0.15	0.19	0.20	0.12	0.15		1.34	1.26	1.54	1.5	1.14	1.72
BR	0.15	0.05	0.1	0.20	0.12	0.21	0.15	0.13	0.16		2.02	1.47	3.07	0.76	0.95
CZ	0.15	0.13	0.05	0.14	0.08	0.17	0.09	0.14	0.17	0.11		1.49	2.84	1.17	1.32
ČA	0.16	0.15	0.1	0.21	0.19	0.22	0.14	0.17	0.14	0.15	0.14		1.36	0.74	1.28
KČ	0.15	0.12	0.06	0.16	0.10	0.13	0.09	0.11	0.14	0.08	0.08	0.15		1.46	1.41
KČR	0.22	0.23	0.15	0.16	0.23	0.17	0.10	0.27	0.18	0.25	0.18	0.25	0.15		2.3
BP	0.14	0.18	0.09	0.13	0.18	0.12	0.04	0.19	0.13	0.21	0.16	0.16	0.15	0.10	

values of Hexp. were observed for populations from Jankovac Stream (0.537) and Orljava River (0.543). The lowest values of Hobs. were recorded for populations from the Orljava River (0.475) and the Curak River (0.458). The lowest average number of alleles was recorded in the Orljava River population (Table 2).

The GENETIX software analysis (Table 3) showed the highest genetic distances (F_{ST}) between "Vrabac" fish farm and the Jankovac Stream (0.27), as well as the smallest gene flow (Nm) between these two populations (0.69). Similarly, Nei's distances (Table 4) showed the highest values between populations from the "Vrabac" fish farm and those from the Veličanka (1.458) and Čabranka rivers (1.408) and Jankovac Stream (1.318). The significant genetic distance between the populations from the "Vrabac" fish farm and the Jankovac Stream coincides with the results of the CR mtDNA analysis which showed that all individuals from the fish farm belong to At1 haplotype, while all individuals from the Jankovac Stream belong to Da1 haplotype (Table 1).

The CLUSTER analysis of Nei's distances and shared allele distances values showed populations' grouping. Four populations from the Gorski Kotar and Žumberak areas were clustered together (SL, JA, BP and KČR) (Figure 2), and the second cluster consisted of the remaining 11 populations. Two populations stood out compared

TABLE 4 Nei's distances values based on the eight microsatellite loci between pairs of populations. VE—Veličanka, OR—Orljava, LE—Mala Lešnica, TO—Toplica, LJ—Lička Jesenica, SL—Slapnica, JA—Jasenak, JP—Jankovac Stream, JJ—Jankovac Lake, BR—Bresni potok, CZ—Curak, ČA—Čabranka, KČ—Kupčina, KČR—"Vrabac" fish farm, BP—Brzaja

Localities	VE	OR	LE	TO	LJ	SL	JA	JP	JJ	BR	CZ	ČA	KČ	KČR	BP
VE	-														
OR	0.537	-													
LE	0.446	0.513	-												
TO	0.593	0.856	0.482	-											
LJ	0.688	0.470	0.400	0.719	-										
SL	1.395	1.006	0.985	0.890	0.789	-									
JA	0.676	0.750	0.352	0.514	0.608	0.832	-								
JP	1.052	0.492	0.493	1.035	0.741	1.086	0.946	-							
JJ	0.910	0.568	0.706	0.709	0.749	1.139	0.881	0.549	-						
BR	0.583	0.212	0.419	0.781	0.345	0.897	0.766	0.383	0.547	-					
CZ	0.770	0.532	0.306	0.647	0.291	0.852	0.609	0.536	0.783	0.376	-				
ČA	0.740	0.540	0.488	0.987	0.670	1.198	0.853	0.609	0.565	0.458	0.570	-			
KČ	0.810	0.465	0.345	0.769	0.363	0.636	0.619	0.418	0.646	0.264	0.347	0.627	-		
KČR	1.458	1.022	0.863	0.714	0.968	0.822	0.629	1.318	0.858	1.078	0.816	1.408	0.642	-	
BP	1.027	0.953	0.721	0.733	0.866	0.770	0.511	0.972	0.736	1.096	0.979	0.885	0.910	0.520	-

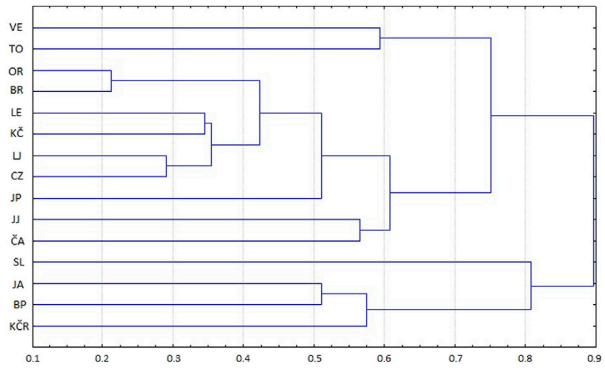

FIGURE 2
CLUSTER analysis of matrix distances of 15 investigated populations according to Nei's distances based on eight microsatellite loci. VE—Veličanka, OR—Orljava, LE—Mala Lešnica, TO—Toplica, LJ—Lička Jesenica, SL—Slapnica, JA—Jasenak, JP—Jankovac Stream, JJ—Jankovac Lake, BR—Bresni potok, CZ—Curak, ČA—Čabranka, KČ—Kupčina, KČR—"Vrabac" fish farm, BP—Brzaja

to the others: the Slapnica River and the Jankovac Stream (Figure 2). The shared allele distances values (DAS) showed small deviations from Nei's distances (Figure 3). Analysis of both DAS and Nei's distances values showed sister relationships between the populations of Čabranka and Jankovac Lake, which follows the low F_{ST} value between these two populations (0.14) (Table 3). In contrast to Nei's distances values, DAS values showed clustering of the population from the Jasenak River with populations from the Toplica River, but also the separation of Veličanka, Mala Lešnica and Kupčina rivers.

The corresponding factorial analysis (CFA) (Figures 4A–C) showed a clear separation of the Slapnica River population (Figure 4A), while a large genetic overlap is shown for the other populations.

The evaluation of the bottleneck effect showed that the He value was higher than the Heq value in the populations from the Orljava River (at five loci according to TPM), Jankovac Stream (at five loci according to TMP and SMM), Brzaja River (at six loci according to TPM and SMM), and Čabranka River (at six loci according to TPM, and at five loci according to SMM) (Table 5). However, no locus had a probability lower than 0.05. Based on both mutation models (TMP and SMM), a significant heterozygosity deficit was recorded in the rivers Brzaja, Curak and the "Vrabac" fish farm, while for the population from the Kupčina River, a heterozygosity deficit was recorded only according to SMM. These results indicate a recent expansion of the above mentioned populations (Table 5).

The STRUCTURE analysis revealed two distinct brown trout genetic clusters (when K = 2, Figure 5A). The structuring of the populations was very much congruent with the mtDNA haplogroup to which the individuals belong (Kanjuh et al., 2020). One group of individuals colored green were those of the DA lineage, while the others colored red were of the AT lineage (Figure 5B). This genetic population structure can best be seen in the brown trout from the Curak River. Twelve individuals of this population were separated during grouping. Ten of them of the DA lineage were grouped with other individuals of the DA lineage (i.e., colored green). Remaining two individuals from the Curak River belonging to AT lineage (one in homozygous state for LDH-C*90, and the other in heterozygous state) were grouped with individuals of the AT lineage (i.e., colored red). The green

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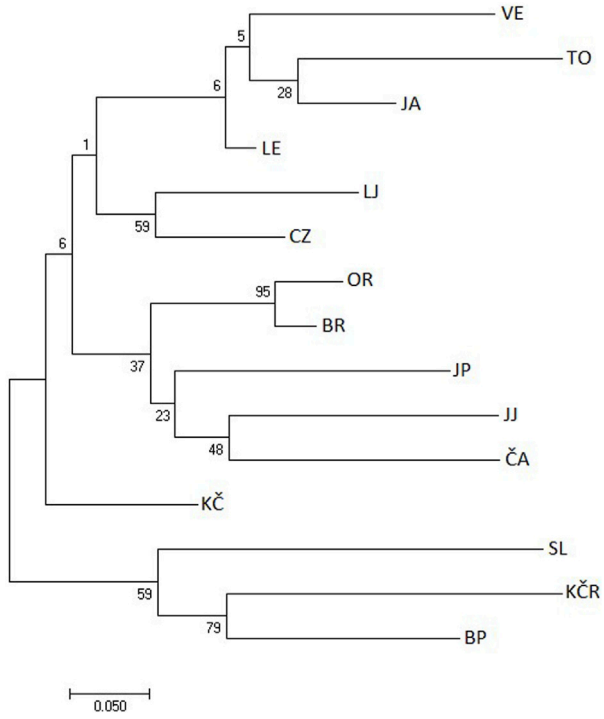

FIGURE 3
Clustering of populations using the Neighbor-Joining rooted bootstrap tree method according to shared allele distances values based on eight microsatellite loci. VE—Veličanka, OR—Orljava, LE—Mala Lešnica, TO—Toplica, LJ—Lička Jesenica, SL—Slapnica, JA—Jasenak, JP—Jankovac Stream, JJ—Jankovac Lake, BR—Bresni potok, CZ—Curak, ČA—Čabranka, KČ—Kupčina, KČR—"Vrabac" fish farm, BP—Brzaja

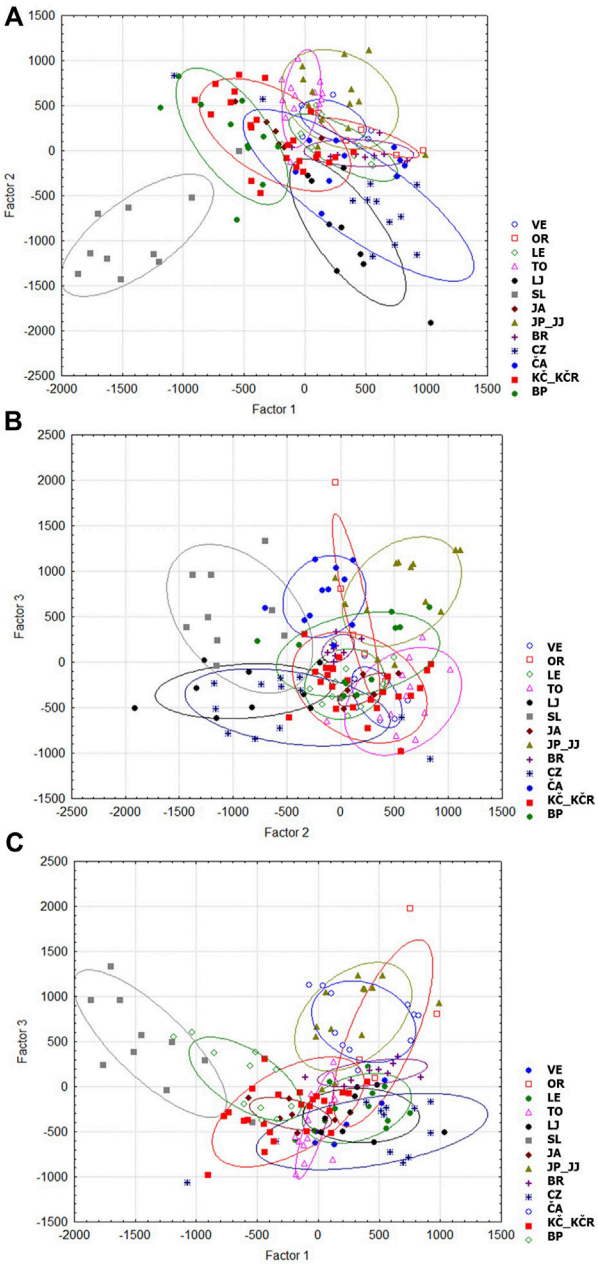

FIGURE 4
Corresponding factor analysis of 15 analysed populations according to factors: **(A)** 1 and 2, **(B)** 2 and 3 and **(C)** 1 and 3. VE—Veličanka, OR—Orljava, LE—Mala Lešnica, TO—Toplica, LJ—Lička Jesenica, SL—Slapnica, JA—Jasenak, JP—Jankovac Stream, JJ—Jankovac Lake, BR—Bresni potok, CZ—Curak, ČA—Čabranka, KČ—Kupčina, KČR—"Vrabac" fish farm, BP—Brzaja

colored group of individuals comprised both DA-AT hybrids and genetically pure DA individuals. Populations from the Čabranka and Lička Jasenica rivers were grouped together (i.e., colored green). All individuals belonged to DA lineage, but among them there were also DA-AT hybrids: two individuals in homozygous state for LDH-C*90 (LJ41 and LJ42), and five individuals in heterozygous state (LJ37, LJ43, ČA5, ČA8 and ČA10) (Figure 5). The grouping with only DA individuals suggested three genetic clusters (K = 3) (Figure 6A). Individuals from the Curak River (CZ) separated into one genetic cluster, while the other two genetic clusters showed overlapping genetic structure (Figure 6B).

Discussion

The results of this study indicated a decrease in genetic variability in the Orljava River and Jankovac Stream. Analysis of CR mtDNA revealed that only one haplotype is present in both populations, non-native Da22 in Orljava River and Da1 in Jankovac Stream (Table 1). Neither Jankovac Stream nor Orljava River population proved to be genetically "pure", considering that DA-AT hybrids were identified there (Kanjuh et al., 2020). In this regard, a bottleneck effect was observed for these two populations. Therefore, to continuously follow the status of these two populations, ongoing monitoring and further investigation are necessary. Evaluating the bottleneck effect in other populations revealed a heterozygosity deficit in the Brzaja, Curak and Kupčina rivers, indicating population expansions, most likely due to the introduction of the non-native farmed At1 haplotype. The same was observed in the "Vrabac" fish farm, but that was to be expected knowing that within the fish farm non-native At1 individuals are farmed.

In comparison with all the analysed populations, it was shown that the Slapnica River population is genetically different. The

TABLE 5 Wilcoxon test results based on two-phase mutation model (TPM) and stepwise mutation model (SMM). Bold values are significantly lower than 0.05. D—heterozygosity deficiency, E—heterozygosity excess, VE—Veličanka, OR—Orljava, LE—Mala Lešnica, TO—Toplica, LJ—Lička Jesenica, SL—Slapnica, JA—Jasenak, JP—Jankovac Stream, JJ—Jankovac_Lake, BR—Brzaja, CZ—Curak, ČA—Čabranka, KČ—Kupčina, KČR—"Vrabac" fish farm, BP—Bresni potok

	TPM		SMM	
VE	0.125D	0.902E	0.098D	0.963E
OR	0.273D	0.770E	0.273D	0.770D
LE	0.578D	0.473E	0.230D	0.808E
TO	0.156D	0.875E	0.125D	0.902E
LJ	0.289D	0.766E	0.289D	0.766E
SL	0.156D	0.875E	0.125D	0.902E
JA	0.808D	0.230E	0.808D	0.230E
JP	0.234D	0.812E	0.234D	0.812E
JJ	0.726D	0.320E	0.726D	0.320E
BR	**0.014D**	0.990E	**0.014D**	0.990E
CZ	**0.020D**	0.986E	**0.010D**	0.994E
ČA	0.680D	0.371E	0.629D	0.422E
KČ	0.125D	0.902E	**0.027D**	0.980E
KČR	**0.010D**	0.994E	**0.006D**	0.996E
BP	0.473D	0.578E	0.422D	0.629E

presence of AT lineage individuals was not recorded there, so this population consists only of individuals of the DA lineage. Nevertheless, the presence of non-native Da2 and DA-AT hybrids in the Slapnica River indicates that its native character was disturbed (Kanjuh et al., 2020). Population from the Slapnica River most likely separates due to the presence of private alleles in hybrid individuals, at microsatellite loci Str73INRA, Ssa410Uos, SsaD190 and OMM1064 (Supplementary Table S1). In all other populations, a large overlap was observed (Figure 2; Figure 3), both within geographically close and among geographically distant ones, as clearly evidenced in populations from the Čabranka River (Gorski Kotar) and Jankovac Lake (Mt. Papuk). This level of overlap indicates a high genetic similarity of the populations, which is a consequence of stocking from hatcheries that import and breed, most likely, the same (AT) phylogenetic lineage of brown trout (Piria et al., 2020). In fish farms throughout Croatia, brown trout carrying the At1 mtDNA haplotype is probably the most farmed and used for stocking of streams attractive for fishing. Minor genetic differences between populations observed in this study, most likely caused by stocking with the same genetic material, were additionally confirmed by the results of the population structure analysis. Separation of only two genetic clusters indicates strong introgression of the non-native genotypes, which is consistent with molecular variance results for CR mtDNA haplotypes (Kanjuh et al., 2020), which show little variation among populations. Structuring of only DA individuals also showed genetic similarity between populations. This result indicated the stocking with not only Atlantic (AT) lineage, but also non-native Danubian (DA) haplotypes of brown trout. Two non-native DA haplotypes were recorded in the analysed rivers of the Danube basin in Croatia (Kanjuh et al., 2020). The Da22 haplotype was limited to three localities (Veličanka River, Orljava River and Brzaja River) at the northern slopes of the Mt. Papuk (Kanjuh et al., 2020). This haplotype is native to the Lohnbach and Daglesbach rivers in Austria (Duftner et al., 2003) and in the Una River drainage in Bosnia and Herzegovina (Škraba et al., 2017). The non-native Da2 haplotype was much more frequent than Da22 haplotype (recorded in seven out of fifteen analysed populations) (Kanjuh et al., 2020). The Da2 haplotype is native to the streams and rivers in southern Germany (Bernatchez, 2001) and streams belonging to the Austrian part of the Danube drainage (Weiss et al., 2001). The stocking of various freshwaters of Europe during the 19th century was carried out with fish material from southern Germany (Kohout et al., 2012). It is most likely that these stockings included the territory of today's Croatia. Precise data on fish farm breeding of non-native Danubian haplotypes of brown trout do not exist, but first data on the presence of the Da2 haplotype in freshwater of Croatia was recorded in the Gacka River, together with the non-native At1 haplotype (Jadan et al., 2007). The separation of DA individuals from the Curak River into a single genetic cluster (Figure 6B) is interesting information from the conservation point-of-view. All DA individuals from the Curak River belong to the native Da haplotype and are homozygous to the native LDH-C*100 allele (Kanjuh et al., 2020). It is possible that the influence of the non-native At1 haplotype was suppressed in this population, considering that out of a total of twelve individuals, only one was genetically pure AT and only one was AT-DA hybrid (Kanjuh et al., 2020).

Genetic research of brown trout and their susceptibility to the presence of the introduced AT lineage in Croatia was until now mainly analysed using cyt*b* and CR mtDNA as molecular markers. Buj et al. (2020) and Ivić et al. (2021) emphasized the negative impact of non-native AT lineage on the native populations of the DA lineage (nominal *Salmo labrax*) and the need for conservation of its genetic character in the Plitvice Lakes National Park and Žumberak-Samobor Hills Nature Park, respectively. The presence of the At1 haplotype has been reported for many streams attractive for fishing throughout the Western Balkans (Snoj, 2004; Marić et al., 2006; 2012; Jadan et al., 2007; Simonović et al., 2015; Tošić et al., 2016; Škraba Jurlina et al., 2018; 2020). In this regard, it is significant that apart from the At1 haplotype, the presence of the rare At17 haplotype was recorded in the upper reaches of the Mrtvica River (Adriatic Basin), undoubtedly indicating a unique event of stocking of this river (Škraba Jurlina et al., 2018). As its presence was not detected in the lower part of the river, the authors assume that the trout spawned only in the upper part of the river, producing hybrids with brown trout of the resident Adriatic lineage. They also pointed out that gene flow between the populations in the upper and lower parts of the river was low (despite the absence of physical and/or reproductive barriers), indicating the impossibility of individuals from the upper reaches of the Mrtvica River to migrate downstream and hybridize with the brown trout of the native Adriatic lineage consisting of both resident (predominantly males) and lake-dwelling, migratory (predominantly females) fish (Škraba Jurlina et al., 2018). The negative influence of the At1 haplotype was also recorded in non-fishing rivers, which are characterized by the presence of specific and narrowly distributed haplotypes. One

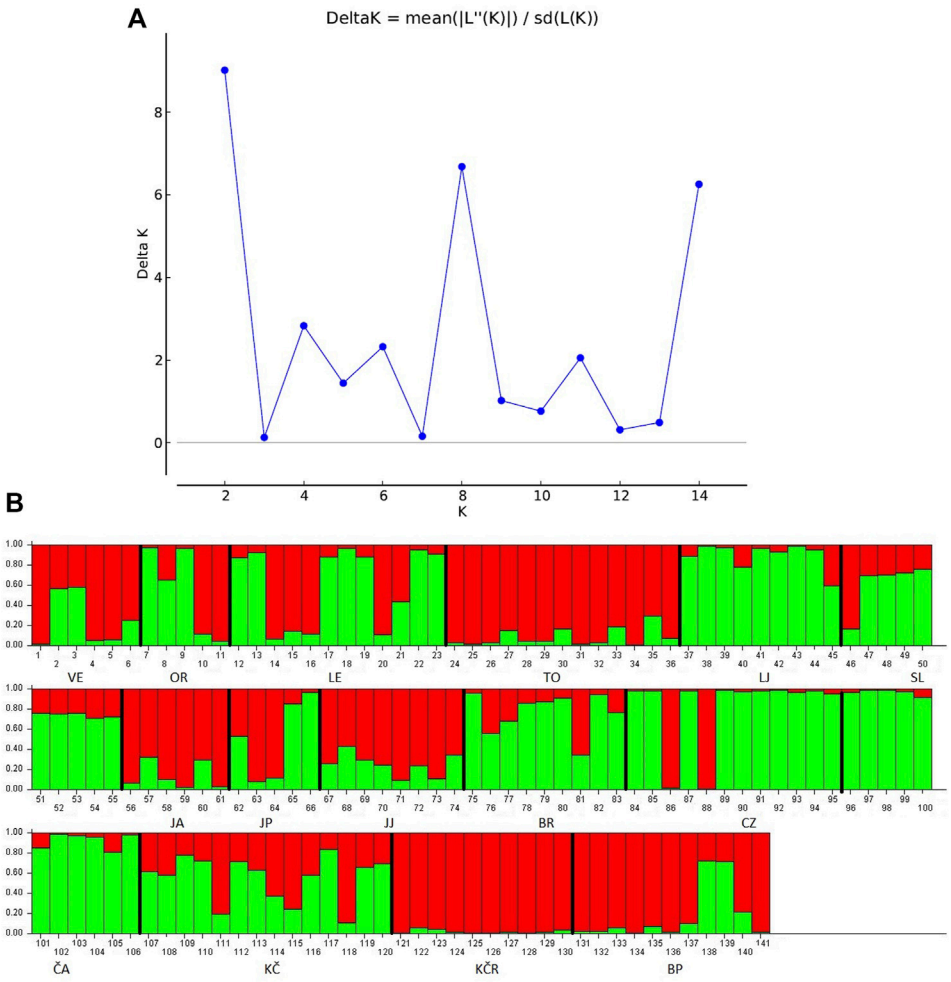

FIGURE 5
(A) Value of ΔK and **(B)** genetic population structuring. VE—Veličanka, OR—Orljava, LE—Mala Lešnica, TO—Toplica, LJ—Lička Jesenica, SL—Slapnica, JA—Jasenak, JP—Jankovac Stream, JJ—Jankovac Lake, BR—Brzaja, CZ—Curak, ČA—Čabranka, KČ—Kupčina, KČR—"Vrabac" fish farm, BP—Bresni potok

such example is the population in the Vratna River, a tributary of the Danube River at the downstream border of the Iron Gate Gorge, where the rare haplotype Da23c occurs, which is currently threatened by the appearance of the stocked brown trout of the At1 haplotype (Tošić et al., 2016; Škraba Jurlina et al., 2020).

In addition to the genetic analyses, the invasive character of the AT lineage has been highlighted in recent research, which showed the rapid adaptation of this lineage to the different conditions in natural watercourses, the consumption of a wide range of available food and competition for food and space, as well as diet overlap with native DA lineage individuals (Piria et al., 2020). Also, in assessment of invasiveness risk in the Danube and Adriatic basins across four Balkan countries, out of 13 existing and four horizon non-native salmonid species, *S. trutta* of the AT lineage showed a very high risk of invasiveness in the current climate conditions (Marić et al., 2022). In addition to At1, the non-native Da2 haplotype has also a significant impact on disrupting the genetic structure of the native Croatian populations of brown trout. Therefore, it would be important for future research to focus on obtaining data on the potential breeding origin and ways of introduction of the Da2 haplotype, but also on assessing the risk of invasiveness to the native genetic pool of the brown trout complex. Apart from the presence of the Da2 haplotype in the rivers of Croatia (Kanjuh et al., 2020), the presence of Da2 haplotype was recorded in the rivers of Serbia (Marić et al., 2006; Simonović et al., 2015; Tošić et al., 2016), Bosnia and Herzegovina (Mrdak et al., 2012; Škraba et al., 2017), and Montenegro (Mrdak et al., 2012).

Everything said above points out to a long history and the current management practice of stocking with the hatchery-reared brown trout. There are clear warnings of the negative impact of the AT hyplotypes on native and wild brown trout populations. Although the detrimental effects of strong introgression in wild brown trout populations are visible, management procedures in many countries are apparently not strict enough and properly applied. Despite proposals for potential management plans, the native gene pool of brown trout remains under serious threat. Studying the wild, native, and locally specific populations of brown trout on the territory of

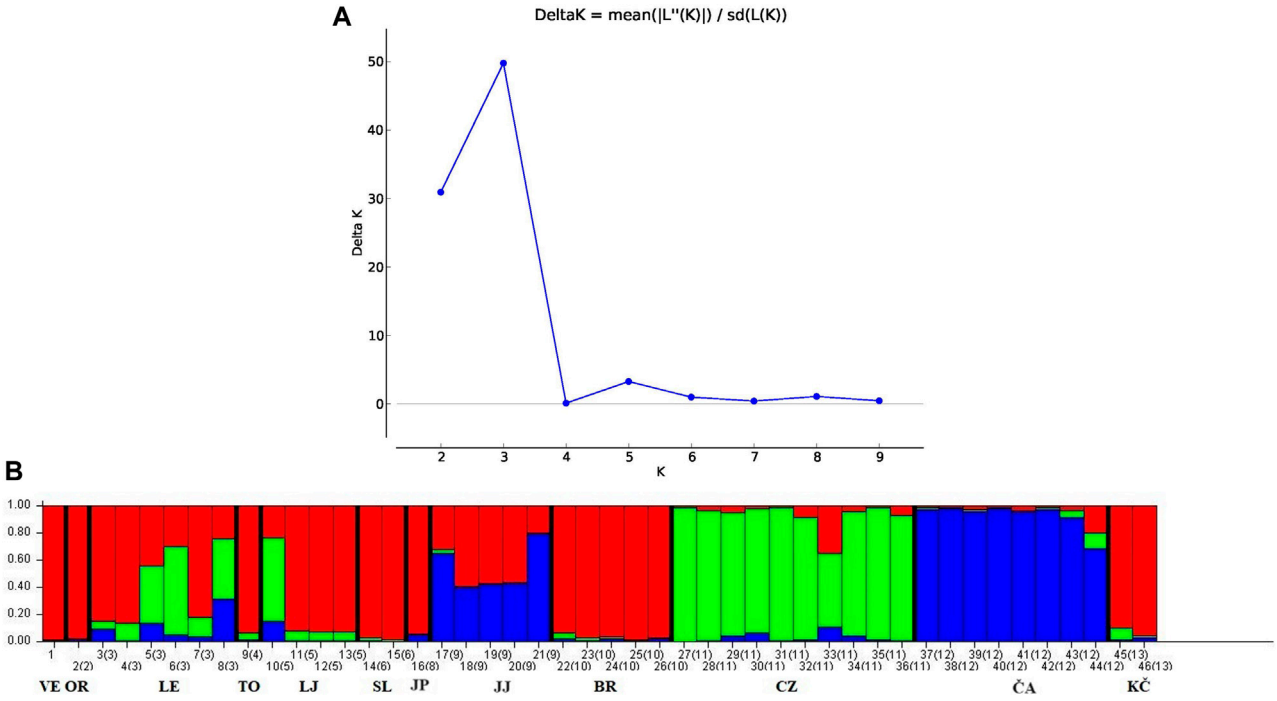

FIGURE 6
(A) Value of ΔK and **(B)** genetic population structuring of the Danubian lineage individuals. VE—Veličanka, OR—Orljava, LE—Mala Lešnica, TO—Toplica, LJ—Lička Jesenica, SL—Slapnica, JP—Jankovac Stream, JJ—Jankovac Lake, BR—Brzaja, CZ—Curak, ČA—Čabranka, KČ—Kupčina.

Croatia, determining of their status and genetic structure should be a priority to maintain and preserve their original genetic stocks. Genotyping the remaining brown trout stock from inland waters is essential. It is to be implemented into the national legislation, in order to set a mandatory genotyping of hatchery brood stocks and to identify and register native brood fish. This practice is currently weakly enforced in Croatia, and in many countries of the Balkan Peninsula. It is important to examine the genetic structure of brown trout grown in fish farms, as well as the stocks used for stocking, which, so far, has not been legally regulated or implemented as a necessary practice in majority of countries in the Western Balkans. Although some authors point out that stocking as a measure of fisheries management should be re-examined (Škraba Jurlina et al., 2018), for the recovery of the degraded status of the brown trout populations, stocking would be desirable. Stocking should imply a controlled, strictly defined, and planned approach, and the stocked material should be bred as a "foundation brood stock". In addition, all of the populations that would be studied and marked as genetically "pure" and/or unique to a certain locality should be protected from the negative influence of fishing pressure. Implementing a mandatory unconditional "catch-and-release" angling regime (Simonović et al., 2018) as a compulsory legal protection measure is proposed as a possible solution that would ensure the self-sustainability of the trout stock without the need for restocking, but also the sustainability of the fishery. Many authors additionally pointed out that long-term stabilization of the density of trout populations is often not possible without environmental improvement strategies, such as habitat restoration, removal of migration barriers, improvement of hydrological regimes, which are generally more effective than stocking (Cowx, 1994; Fjellheim et al., 2003; Oosterhout et al., 2005; Ferguson, 2007; Škraba Jurlina et al., 2018), and the brown trout habitats are certainly special in their characteristics, being very sensitive to all kinds of anthropogenic pressures.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The animal study was approved by Ministry of Agriculture and Ministry of Environmental Protection and Energy. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

TK: Conceptualization, Formal Analysis, Methodology, Resources, Writing–original draft. AM: Formal Analysis, Methodology, Resources, Writing–review and editing. DŠ: Writing–review and editing. PS: Resources, Writing–review and

editing. IS: Formal Analysis, Resources, Writing–review and editing. MP: Resources, Writing–review and editing. IM: Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2024.1379878/full#supplementary-material

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Effects of irrigation dams on riverine biota in mountain streams

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Mountain streams harbor unique biodiversity and provide essential ecosystem services to human societies. Yet, these ecosystems face numerous threats, such as the construction of dams and land use changes, leading to rapid habitat degradation, water pollution, and biodiversity loss. In this study, we assess the effect of irrigation dams on mountain riverine biota using traditional biotic indices and trait-based approaches. We selected diatom and macroinvertebrate communities surveyed between 2015 and 2017 in mountain streams located in different regions in northern Spain (Cantabrian Cordillera, Iberian System, and Pyrenees) under natural and altered flow conditions (i.e., downstream of irrigation dams). Hydrological and biological changes related to the presence of dams, the mountain range, and the interaction between these two factors were identified. Summer flows, frequency of high flow events, and minimum annual flows timing were significantly affected by irrigation dams, independently of the region. Winter flows, the magnitude of high flow extremes, and the number of flow rises and falls varied significantly with the dam-mountain range interaction. The frequency and duration of flow pulses depended on the mountain range only. In the Cantabrian Cordillera, a region with larger reservoirs (>150 hm^3), impacted sites showed a marked inversion of the seasonal flow patterns (i.e., increased summer flows but reduced winter flows). In the other mountain ranges, reservoirs had smaller storage volumes and multiple purposes, causing significant flow change frequency variations. Diatom traits, taxonomic richness, diversity, and IPS score varied with dam presence and mountain ranges, while macroinvertebrate traits and biotic indices responded weakly. These findings suggest that diatom communities might be more sensitive to hydrological alteration, while macroinvertebrates might be more influenced by space-related factors, such as biogeography and dispersal, overriding dam-related impacts. Furthermore, dam-related changes in ecosystems may depend not only on the presence of dams and their characteristics (e.g., reservoir size and operation), but also on local conditions and biogeography. Our findings emphasize that, when using pre-existing biomonitoring datasets, although some dam-related patterns emerge (e.g., with diatoms), other patterns may be constrained by the datasets' low spatio-temporal coverage and taxonomic resolution, highlighting the need of well-structured study designs.

KEYWORDS

freshwater biota, flow regime, irrigation dams, river regulation, diatoms, benthic macroinvertebrates

1 Introduction

Mountain streams provide a unique set of habitat conditions in the river network (i.e., fast water velocities and low temperatures) and harbor an important portion of the global biodiversity (Rahbek et al., 2019). Their high runoff from rainfall and the delayed releases of water stored in the form of snow or lake reserves characterize mountains as "water towers," providing lowlands with relatively constant essential water resources and ecosystem services (Viviroli and Weingartner, 2008; Immerzeel et al., 2020). Population dependence on mountain water runoff is expected to grow steadily, reaching 1.5 billion people critically relying on these water resources by 2050 (Viviroli et al., 2020). At the same time, due to the narrow niches of endemic species, mountain environments are extremely vulnerable to global change. As elevation can amplify global warming effects (Grabherr et al., 2001; Christmann and Oliveras, 2020), even small environmental changes can cause significant ecological impacts, such as the reductions of glaciers and the ice-melt inputs into mountain rivers (Fell et al., 2017). Thus, mountain rivers might experience more rapid climate change effects than their lowland counterparts (Pepin et al., 2015).

An extensive number of mountain rivers have been affected by dams and other water infrastructures, e.g., in the Pyrenees (López-Moreno et al., 2008), in the Alps (Spitale et al., 2015), in the Colorado Front Range (Wohl, 2006), in the Andean Amazon region (Anderson et al., 2018), and in the High Mountain Asia (Li et al., 2022). Apart from the potential gravitational energy, which is extensively exploited for hydropower production, mountain dams store and build up water reserves during seasons of heavy snow melt to assure lowland irrigation and domestic water uses during low flow periods, as well as supply water to mountain lakes used for tourism and fishing (Marnezy, 2008; Consoli et al., 2021). Irrigation dams tend to homogenize flow dynamics (i.e., reducing peak flows and increasing minimum flows; Moyle and Mount, 2007), reduce or increase the downstream water temperature (depending on the residence time, size of reservoir, release depth, and timing of releases; Lessard and Hayes, 2003; Prats et al., 2010), as well as alter the nutrient and sediment fluxes (Olden and Naiman, 2010; Maavara et al., 2020), and limit the habitat connectivity (Grill et al., 2019). It leads to pervasive effects on riverine biodiversity and functioning, e.g., increases in planktonic diatoms (Goldenberg-Vilar et al., 2021), reduction of macroinvertebrate richness (Kennedy and Turner, 2011) or of salmon populations downstream of dams (Moore et al., 2011), among others. Therefore, identifying, monitoring, and managing these impacts on mountain riverine ecosystems while securing human water needs for socioeconomic development has become a major challenge for water resource managers, especially in highly vulnerable regions (e.g., wetlands and mountains; Pang et al., 2018).

Diatoms and macroinvertebrates have been widely used as environmental biomonitoring indicators due to their easy sampling, widespread occurrence, diversity, and reliable indication of anthropogenic impacts (Bonada et al., 2006; Resh, 2008; Pompeu et al., 2023) and are among the biological quality elements recommended to monitor and assess water bodies' ecological state within the European Water Framework Directive (European Commission, 2000). Taxonomy-based biotic indices are extensively employed to measure ecological responses to nutrient enrichment and organic pollution; however, the effects of flow modification have been more rarely addressed (Birk et al., 2012). In addition, the taxonomic-based metrics may be subject to significant biogeographic and evolutionary constraints (Soininen et al., 2016), reducing their broad geographic applicability (Dolédec et al., 1999). Thus, species traits (i.e., organism's biological and ecological characteristics) have been increasingly used in ecological research (e.g., White et al., 2017; Brown et al., 2019; Wu et al., 2019b) to assess stressor effects in lotic ecosystems (Statzner and Bêche, 2010) and to develop mechanistic trait-stressor links valid across broad geographical scales (Culp et al., 2011).

In this context, the data originally collected for water quality assessment (e.g., European Water Framework Directive (WFD) surveys), including biological communities and physico-chemical habitat conditions, could be potentially used to assess other eco-hydromorphological relationships in rivers, such as the ecological effects of dams (Vaughan and Ormerod, 2010; Krajenbrink et al., 2019). To do so, an efficient monitoring network is essential (Arrighi et al., 2021), since the actual effects of hydrological alteration may be underestimated with an inadequate spatial (e.g., lack of gauges immediately downstream of dams; Peñas et al., 2016) or temporal coverage (e.g., biological sampling not covering inter and intra-annual hydrological variability; Munné and Prat, 2011).

In this study, our main objective was to assess the effects of dams primarily used for irrigation (although they might include other minor uses such as water supply and hydropower) on the river flow regime, water quality characteristics, and riverine biota (i.e., diatom and macroinvertebrate communities) of mountain rivers using biological datasets originally collected within the EU WFD. We selected streams under natural and regulated flow regimes (i.e., downstream of irrigation dams) and, in order to minimize the effects of geographical distance on generating differences among riverine communities (Pompeu et al., 2022a), our stream selection was clustered around three mountain ranges in northern Spain: the Cantabrian Cordillera, the Iberian System, and the Pyrenees.

We hypothesized that 1) the presence of dams would be linked to hydrological and water physico-chemical alterations, such as changes in the seasonal flow patterns, homogenization of flow dynamics, and reduction of nutrients downstream of dams. As pointed out by Peñas and Barquín (2019), the altered flow patterns caused by dams in Spanish rivers depend, in part, on local factors, e.g., geographical context and the pre-dam river characteristics). Therefore, we expect that 2) other hydrological and physico-chemical changes, such as changes in flow dynamics associated to snow melt and altitude and water quality parameters caused by different geological characteristics, would be linked to the local conditions present in each mountain range (i.e., the "mountain factor"). 3) Taxonomic biotic indices (e.g., richness, diversity, water quality sensitive indices), which are commonly used in diatom and macroinvertebrate bioassessment worldwide, would also significantly respond to the presence of dams. We expect a decrease in taxonomic richness and diversity downstream of dams, and specific indices, such as the Specific Polluosensitivity Index (IPS) for diatoms, the Iberian Biomonitoring Working Party (IBMWP) and Lotic invertebrate Index for Flow Evaluation (LIFE index) for macroinvertebrates, would likely show negative responses to flow regulation. These trends have been observed previously in Spanish rivers (Ladrera and Prat, 2013; Quevedo et al., 2018; Mellado-Díaz et al., 2019; Pompeu et al., 2022b), even though

these specific indices have not been designed for the assessment of the impacts of dams but to evaluate the effects of organic pollution (e.g., IPS and IBMWP). 4) Trait-based approaches, however, would provide clearer a insight into the effects of dams on biological communities compared to biotic indices based on taxonomy and designed to respond mainly to organic pollution. Unlike taxonomy, traits are consistent across broad spatial scales and provide mechanistic linkages of biotic responses to stressors (Menezes et al., 2010; Culp et al., 2011). Thus, we expect to detect significant changes, such as changes in diatom ecological guilds or macroinvertebrate feeding groups associated to dam-related impacts.

2 Materials and methods

2.1 Study area and site selection

Our study area covered streams located in three mountain ranges in northern Spain: the Cantabrian Cordillera, the Iberian System, and the Pyrenees (Figure 1A). These mountains constitute the natural geographical limits of the Ebro catchment (>85.000 km^2) and present a significant number of structures to attend water and energy demands (e.g., dams and reservoirs for hydroelectric power generation, irrigation, urban water supply, and recreation; López-Moreno et al., 2008; García de Jalón et al., 2019), making them an interesting case study. Most dams in the Pyrenees, for example, were constructed between the 1950s and the 1980s to supply water demands in the Mediterranean region (i.e., irrigation and hydropower production; López-Moreno et al., 2008). In the Cantabrian Cordillera, besides several small hydroelectric power plants, important reservoirs (e.g., Ebro, Aguilar de Campoo, Riaño) supply water for agricultural and urban uses. Reservoirs in the Iberian System, in general, have smaller capacities in relation to the other mountain ranges and provide water for irrigation, urban uses, and hydropower generation.

Our sampling site selection was restricted to sites complying with the following requirements: 1) river reaches classified as "good" or "very good" ecological status according to the River Basin Management Plans 2015-2021 (BOE, 2015) within the European WFD; 2) river reaches with available hydrological and water physico-chemical data; and 3) at least one survey on biological communities (diatoms or macroinvertebrates) between June and October 2015, 2016, or 2017. These criteria allowed us to reduce the environmental variability and the effect of other anthropogenic stressors, such as water pollution and nutrient enrichment associated with human activities (e.g., extensive urban or agricultural land uses), while ensuring a sufficient spatial coverage. In total, we selected 23 sampling sites (Figure 1A; Table 1), from which 11 were located downstream of dams, mostly with multiple uses (e.g., hydroelectric power generation, irrigation, recreation), but primarily used for irrigation (i.e., altered sites, see Supplementary Table S1) and 12 were under minimally disturbed conditions (i.e., control sites).

River gauge stations provided by the Centre for Hydrological Studies (CEDEX, Ministry for Ecological Transition, Spain) located nearby each sampling site recorded the daily river flow discharge. The normalized daily flow series of the three hydrological years (2015–2017), which presented relatively heterogeneous climatic characteristics (2015 marked by extremely warm and dry conditions, 2016 warm and wet, and 2017 warm and very dry; Agencia Estatal de Meteorología, 2023) was then used to generate 35 hydrological indices (HIs; Supplementary Table S2). These HIs characterized the five flow regime components (magnitude, frequency, duration, timing, and rate of change; Olden and Poff, 2003).

2.2 Biological surveys and taxonomic-based biotic indices

Diatom and macroinvertebrate communities were surveyed annually by regional water agencies as part of the water bodies' ecological status assessment within the European WFD. This biological information was then compiled by the Spanish Ministry for the Ecological Transition in the national database NABIA (BOE, 2015). We selected streams surveyed between June and October, during the low flows (when the streams are wadeable), in 2015, 2016, and 2017 to reduce the seasonal flow variation. In some of these sites, surveys occurred for three consecutive years and/or covered both biological communities. In combination with the biological sampling, the NABIA database provided water temperature (T_{WATER}, °C), pH, dissolved oxygen (DO, mg.L^{-1}), electrical conductivity (µS.cm^{-1}), nitrite (NO_2^-; mg N.L^{-1}), nitrate (NO_3^-; mg N.L^{-1}), ammonium (NH_4^+; mg N.L^{-1}), and phosphate (PO_4^{3-}; mg P.L^{-1}) concentrations that were collected and determined using standard protocols (UNE-EN ISO 15681-2:2005 for phosphates, UNE-EN ISO 13395:1997 for nitrite and nitrates, UNE-EN ISO 11732:2005 for ammonium).

Diatom communities were surveyed following UNE-EN13946: 2014 (CEN, 2014b). Five to ten cobbles were randomly selected from the benthos (circa 100 cm^2 of exposed surface area) and the upper part of the substratum was scrubbed with a dishwasher brush. The material was decanted in a sample bottle and preserved using formaldehyde. From each sample, 400 diatom valves were identified in laboratory using a microscope (1000 × magnification) at the lowest feasible taxonomic level, according to the standard procedure UNE-EN 14407:2014 (CEN, 2014a). In total, we gather results from 56 sampling events of diatom communities collected in the 21 sampling sites (see Supplementary Table S1).

Benthic macroinvertebrate surveys followed the standard procedure UNE-EN 16150:2012 (CEN, 2012). The sampling was distributed among the different habitat types present along a 100-m reach. Using a kick hand net (0.1 m^2 and 0.5 mm mesh size), community samples were collected and preserved in 96% ethanol. The identification in the laboratory was mainly at the family level, except for Hydracarina, Nematoda, Ostracoda, Copepoda, and Oligochaeta. In total, we gather results from 51 sampling events of macroinvertebrate communities collected in the 21 sampling sites (see Supplementary Table S1).

We eliminated rare taxa (those representing less than 2% of the total sampled individuals when considering the total number of occurrences; Lavoie et al., 2009). In sites surveyed over multiple years, the abundance of taxa was averaged to obtain a site-specific assemblage structure and to reduce interannual variation, following Paavola et al. (2003), resulting in 21 averaged communities for diatoms and 21 for macroinvertebrates. Even though this approach might lead to a simplification of the

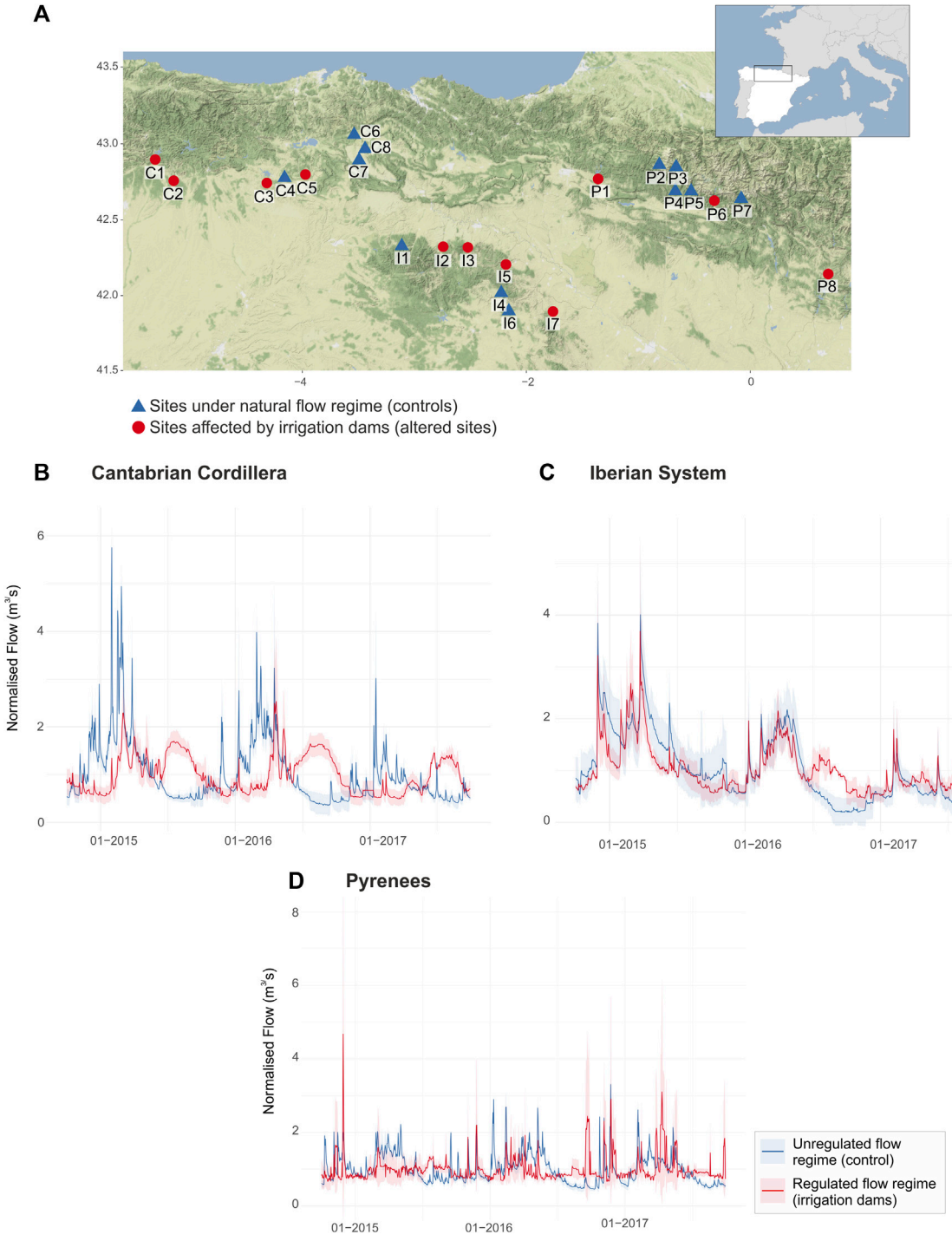

FIGURE 1
(A) Location of the 23 studied sites: controls (n = 12) and sites downstream of irrigation dams (n = 11). Mean normalized daily discharge between 2015 and 2017 in controls and sites influenced by irrigation dams in **(B)** the Cantabrian Cordillera, **(C)** the Iberian System, and **(D)** the Pyrenees. Shaded areas represent 1SD confidence intervals. See also Supplementary Table S1 for information on sampling sites, reservoirs' capacity, and purpose. Map tiles by Stamen Design, under CC BY 3.0 Data by OpenStreetMap, under ODbL.

ecological complexity and the temporal dynamics, as well as the comparison of sites with different sampling efforts (i.e., sampled in less than 3 years), it allowed us to focus on the effect of the presence of dams rather than on the annual climatic and environmental variability. Likewise, for each site-specific assemblage, the average physico-chemical characteristics were calculated.

In each averaged biological community, taxa richness and Shannon-Wiener diversity index were computed using the R package *vegan* (Oksanen et al., 2019). Additionally, for diatom communities, we computed the Specific Polluosensitivity Index (IPS; CEMAGREF, 1982; Ministerio de Agricultura, Alimentación y Medio Ambiente, 2013a). For macroinvertebrates, the Iberian

TABLE 1 Catchment-scale environmental variables in controls and sites affected by dams in each region describing topography, climate, the fractions occupied by land use and land cover, and lithology. Values refer to the catchment mean ± sd upstream of the sampling site.

	Cantabrian Cordillera		Iberian system		Pyrenees	
	Controls (n = 3)	Altered (n = 3)	Controls (n = 3)	Altered (n = 4)	Controls (n = 5)	Altered (n = 3)
Catchment size (km^2)	287.1 ± 166.6	640.4 ± 293.4	76.9 ± 29.6	421 ± 160.1	86.1 ± 81.5	581.5 ± 382.2
Elevation above sea level (m)	642.2 ± 121.3	859.9 ± 132.9	992.1 ± 75	586 ± 62.9	989.9 ± 179.9	609.7 ± 185.1
Slope in segment (0–1)	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Mean distance to dam (km)	-	20.1	-	22.6	-	11.3
Annual mean precipitation (mm)	806.6 ± 71.4	1053.2 ± 241.5	828.3 ± 318.6	738.5 ± 154.1	1538.5 ± 170.6	1378.1 ± 268.8
Annual mean temperature (°C)	10.1 ± 0.8	8.6 ± 1.1	8.9 ± 0.2	9.5 ± 0.4	8.1 ± 1.3	9.8 ± 1.1
Annual mean evapotranspiration (mm)	522 ± 26.1	531.3 ± 34.6	440.7 ± 52	433.4 ± 30.9	426.9 ± 31.6	507.5 ± 59.4
Urbanisation	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Agriculture	0.3 ± 0.1	0 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	0 ± 0	0 ± 0
Pastures	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.4 ± 0.2	0.3 ± 0.1
Broadleaf forests	0.2 ± 0.1	0.2 ± 0	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.2
Coniferous forests	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0
Plantations	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0
Heathlands and shrubs	0.1 ± 0	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0 ± 0	0.2 ± 0.1
Denuded areas	0.1 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0.2 ± 0.1	0.1 ± 0.1
Calcareous rocks	0.9 ± 0.1	0.4 ± 0.3	0.7 ± 0.5	0.7 ± 0.3	0.6 ± 0.3	0.4 ± 0.2
Siliceous rocks	0 ± 0	0.1 ± 0.2	0.3 ± 0.5	0.1 ± 0.2	0 ± 0	0 ± 0
Conglomerate rocks	0.1 ± 0.1	0.4 ± 0.2	0 ± 0	0.1 ± 0.1	0.4 ± 0.3	0.2 ± 0.1
Sandstone	0 ± 0	0 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0.3 ± 0.2
Sedimentary rocks	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Volcanic rocks	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0.1

Average Score per Taxon (IASPT) and the Lotic invertebrate Index for Flow Evaluation (LIFE; Extence et al., 1999) were computed using the R package *biomonitoR* (Laini et al., 2019).

Diatom species were assigned to ecological guilds, life forms, and cell size (biovolume; see Supplementary Table S3), following Passy (2007) and Rimet and Bouchez (2012). In macroinvertebrate samples, taxa were classified into 11 biological traits for European macroinvertebrate genera (Tachet et al., 2010; Schmidt-Kloiber and Hering, 2015). The traits (Supplementary Table S4) described the organisms' life history, morphology, food source, feeding habits, respiration, and reproduction, among other characteristics using a fuzzy coding approach. Since most of the taxa were identified at the family level but traits are reported at the genus level, each trait affinity was averaged and re-scaled to this lower taxonomic resolution using affinities of all genera computed within a family. Although the use of family-level identification can result in a loss of ecological information, previous studies (Dolédec et al., 2000; Gayraud et al., 2003; García-Roger et al., 2014) reported minor impacts on the functional description of macroinvertebrate communities.

2.3 Data analysis

We used a two-way analysis of variance (ANOVA; factors: dam/no dam and mountain range) to test (a) the general effects of the presence of irrigation dams (control vs. altered sites), (b) the effect of the mountain range (Cantabrian Cordillera, Iberian System, and Pyrenees), i.e., the influence of local factors, and (c) the interactions between the presence of dams and the mountain range to assess the effects of irrigation dams within each mountain range. Independent analyses were done for the HIs encompassing the three hydrological years (2015, 2016, and 2017), water physicochemical characteristics, and biotic indices. The tests were calculated with type III sums of squares due to the unequal sample sizes in control and altered sites among mountain ranges (Quinn and Keough, 2002). The homogeneity of variances was checked using Levene's tests and variables were log-transformed when needed. Following the significant two-way ANOVA tests, *post hoc* pairwise t-tests with Bonferroni correction were used to identify significant differences ($p < 0.05$) among factors.

Spearman's rank correlations were also used to identify statistically significant relationships ($p < 0.05$) among diatom and

macroinvertebrate biotic indices, HIs, and physico-chemical characteristics. In line with the two-way ANOVA analyses, the HIs used in the Spearman's rank correlations were also computed based on the 2015–2017 hydrological years.

The relationships between organisms' traits and environmental descriptors were investigated with the RLQ and fourth-corner analyses using the R packages *ade4* (Dray and Dufour, 2007) and *adespatial* (Dray et al., 2020). The RLQ analysis (Dolédec et al., 1996), an exploratory ordination method, combines three tables: table R ($n \times m$) containing the measurement of m environmental variables (i.e., HIs encompassing the three hydrological years and physico-chemical variables) at n sites; table L ($n \times p$) with the abundance of p taxa (i.e., diatom and macroinvertebrate taxa) at n sites; and table Q ($p \times s$) describing s traits (i.e., diatom and macroinvertebrate traits) related to the same p taxa. The method generates a fourth matrix representing the cross-variance structure explained by environmental variables and functional traits, providing a broad overview of how they are associated (Brown et al., 2014; Thioulouse et al., 2018). The overall significance of the trait-environment relationship was then assessed with 9999 permutations using a two-step procedure (Dray et al., 2014), which simultaneously test the permutation of sites (testing the null hypothesis that the species distribution is randomly attributed to sites, irrespective of the site characteristics) and species (testing the null hypothesis that species are distributed irrespective of their traits) while controlling the type I error (Ter Braak et al., 2012). Further, the fourth-corner analysis was used to identify significant individual trait-environment relationships. This method computes a matrix ($s \times m$) with one-to-one trait-environment correlations (Dray et al., 2014).

Prior to the analyses, physico-chemical variables were standardized (zero mean and unit standard deviation) to reduce the effects of different measurement units and to improve normality (Quinn and Keough, 2002). The statistical significance level for the ANOVA tests, Spearman's rank correlation, and RLQ and fourth corner analyses was set to $p \leq 0.05$. All the analyses were performed in the R environment (R Core Team, 2020).

3 Results

3.1 Dam effects on HIs and physico-chemical variables

The two-way ANOVA tests associated with the presence of dams only (D factor in Figure 2) identified significant changes uniquely due to this factor in the summer (+M7,8,9), winter (-M12), and spring flows (-M3), the frequency of high flow events (-FRE3), and the timing of minimum flows (-JMin). These findings indicated that irrigation dams produced seasonal flow inversions (i.e., increased summer flows but reduced spring and winter flows) and less frequent high flow events independently of the mountain range, i.e., the geographical region.

In turn, when considering the mountain factor alone (M factor in Figure 2), the number of high and low flow pulses within a year (nPulsesHigh, nPulsesLow) and their duration (MeanDPHigh, MeanDPLow) showed significant changes. In the Pyrenees, the *post hoc* tests showed that the high and low flow pulses were significantly more frequent than in the Iberian System ($p < 0.04$), but shorter in duration in comparison to other regions ($p < 0.001$ for MeanDPHigh in all cases; $p = 0.006$ for MeanDPLow in relation to the Iberian System). These findings indicated that the frequency and duration of flow pulses varied among mountain ranges independently of the presence of dams.

The interaction term that allowed us to analyze the effect of irrigation dams based on the comparison of control and altered sites within each mountain range (D × M in Figure 2), showed significant differences in the winter (M1,2) and early summer flows (M6), the magnitude of short-term high flow extremes (1, 3, 7HF), and the number of days with increasing and decreasing flows (nPos, nNeg). The *post hoc* tests revealed that most of the significant differences were found between control and altered sites in the Cantabrian Cordillera (p at least <0.015 for M1, M2, M6) and in the Pyrenees (p at least <0.045 for nPos and nNeg).

In contrast to hydrology, physico-chemical characteristics did not seem to be significantly influenced by the presence of dams or their geographical location (Figure 3). Nitrate was the only variable that presented a marginally significant reduction downstream of dams ($p = 0.052$), independently of the mountain range (D factor in Figure 3).

3.2 Biological communities and the effects of dams on biotic indices

We retained 55 diatom species for the analyses (see Supplementary Table S5 for the taxa list). The most ubiquitous species were *Achnanthidium pyrenaicum* and *Achnanthidium minutissimum*. Control sites also showed higher mean abundances of *Gomphonema angustivalva* and *Achnanthidium subatomus*, while altered sites presented higher abundances of *Achnanthidium lineare* and *Cocconeis euglypta*.

Taxonomic-based biological indices, in contrast to hydrological indices, did not show significant differences linked to dam or mountain range effects alone. However, the dam-mountain range interaction seemed to significantly influence the diatom taxonomic richness, Shannon-Wiener diversity index, and the IPS score (Figure 4A). The effect of dams on biological communities varied among regions, with streams in the Iberian System (IS in Figure 4A) showing clearer changes than the other two mountain ranges. In this regard, diatom richness and diversity increased, while the IPS score decreased in altered sites in comparison to controls; despite this, the *post hoc* pairwise t-tests with Bonferroni correction did not detect significant differences. The Spearman's rank correlations (Supplementary Figure S2A) indicated that, in general, richness and Shannon-Wiener diversity were significantly linked to summer flows and water temperature.

In macroinvertebrate communities, a total of 43 taxa were retained for the analyses (Supplementary Table S6). Baetidae, Chironomidae, and Gammaridae were the most ubiquitous families. Control sites showed higher mean abundances of Chironomidae, Heptageniidae, and Leuctridae, while Simuliidae, Hydrobiidae, and Elmidae were more abundant in impacted sites.

The LIFE score (Figure 4B) was the only macroinvertebrate biotic index that presented significant changes associated with dams and the dam-mountain range interaction, although the *post hoc* pairwise t-tests with Bonferroni correction did not detect significant differences between groups. On average, altered sites in the Cantabrian

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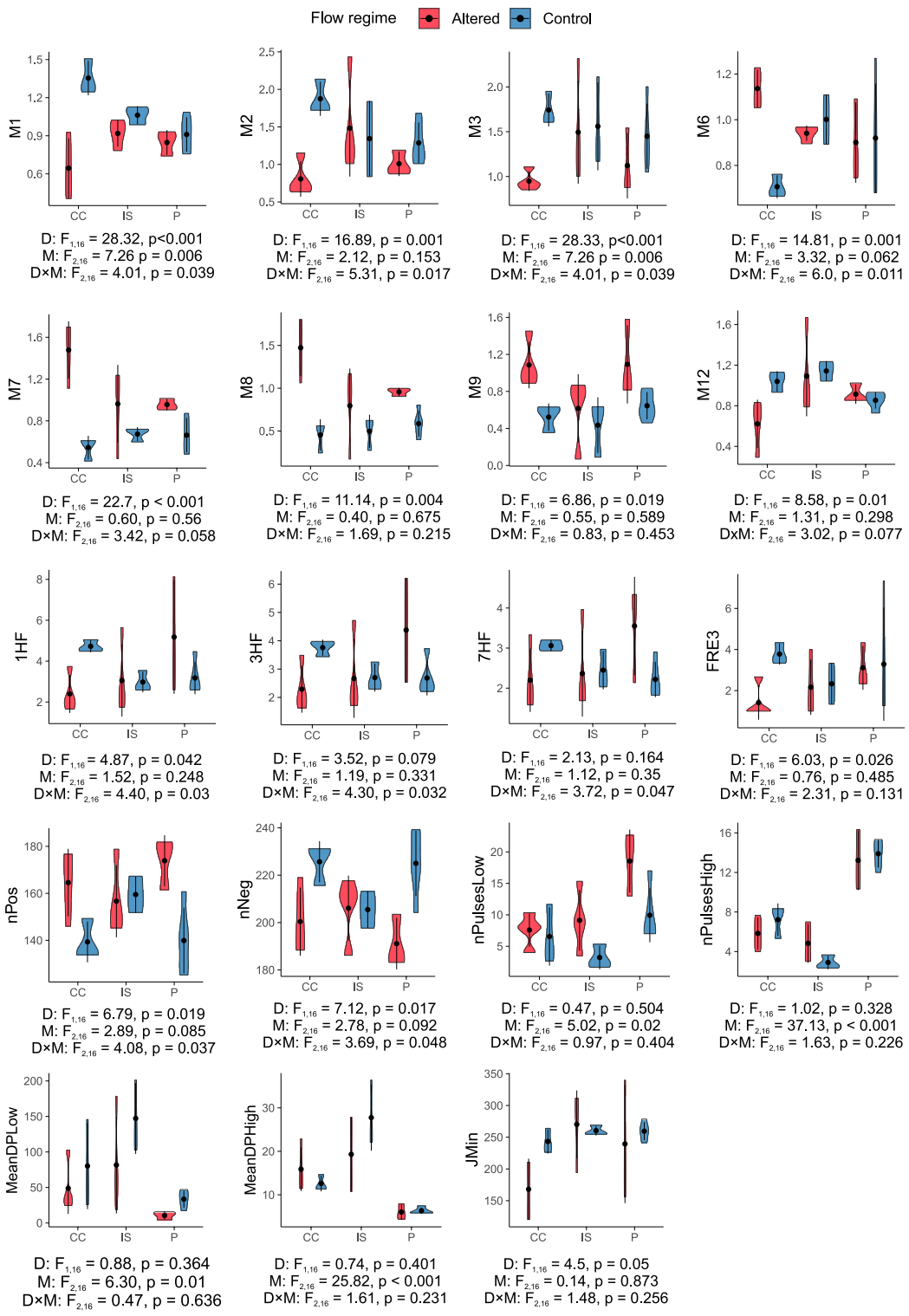

FIGURE 2
Violin plots representing the distribution of hydrological indices in the Cantabrian Cordillera (CC), Iberian System (IS), and Pyrenees (P) among control (blue) and altered (red) sites. The two-way ANOVA tests are shown: "D" refers to the ANOVA test with flow regime (dam presence) as a factor, "M" to mountain range as a factor, and "D × M" as the interaction between these factors. Only hydrological indices with significant differences are shown (see all variables in Supplementary Figure S1). Vertical bars represent the standard deviation.

Cordillera scored higher than control sites, while in the other mountain ranges, scores in control and altered sites were similar. The Spearman's rank correlation (Supplementary Figure S2B) indicated that the LIFE score was positively correlated to summer flows and the magnitude of low flow extremes but negatively correlated to winter flows and the frequency of high flow events.

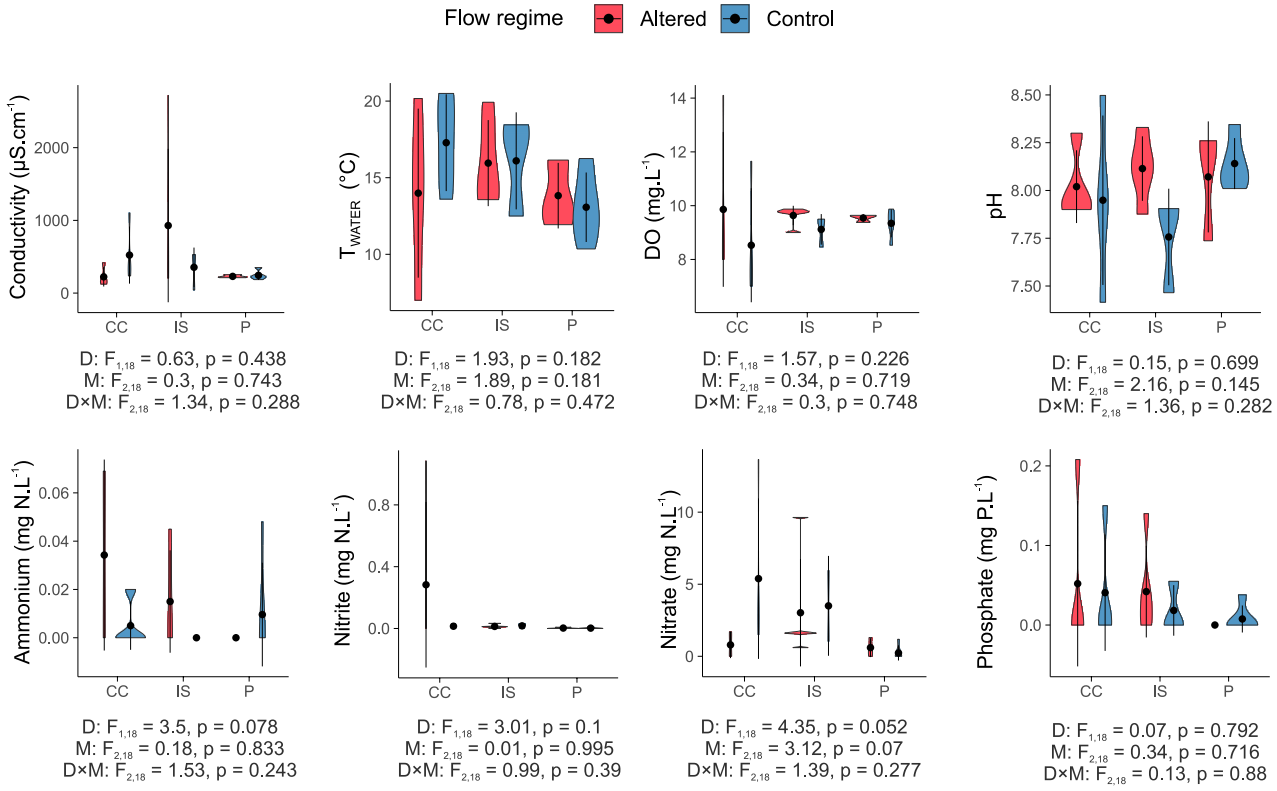

FIGURE 3
Violin plots representing the distribution of physico-chemical variables in the Cantabrian Cordillera (CC), Iberian System (IS), and Pyrenees (P) among control (blue) and altered (red) sites. The two-way ANOVA tests are shown: "D" refers to the ANOVA test with flow regime (dam presence) as a factor, "M" to mountain range as a factor, and "D × M" as the interaction between these factors. Vertical bars represent the standard deviation.

3.3 Trait-based responses to dams in diatom communities

The overall RLQ permutation test on diatom communities showed a marginally significant trait-environment relationship (permutation across sites $p = 0.05$, across species $p = 0.064$). The first two axes of the RLQ analysis explained 85.7% of the cross-variance between diatom traits and HIs and physico-chemical conditions (Figure 5).

Along the first axis (67.7% of the total variance, Figure 5), the Cantabrian Cordillera and Iberian System controls were concentrated on the negative side (left). Sites I5 and P8, which were affected by the multipurpose reservoirs Enciso and Escales, respectively (see Supplementary Table S1), were also clustered with controls. They were linked to more frequent and stronger high flow events (+FRE3,7, +1-90HF), higher February (+M2) and March (+M3) flows, and higher water conductivity. These conditions favored high profile and colonial diatoms while reducing the low profile guild. In contrast, altered sites in the Cantabrian Cordillera and most of the altered sites in the Iberian System were linked to higher mean magnitudes of spring (+M5) and autumn (+M10) flows and long-term low flow extremes (+7,30,90LF), favoring pioneer diatoms and reducing the high profile guild.

A further 17.9% of the total variance was explained by the second RLQ axis (Figure 5). The higher phosphate and ammonium concentrations, as well as the stronger rates of flow change (+meanPos, -meanNeg), observed especially in the C2 site, favored adnate diatoms. In turn, pedunculate diatoms were linked to less strong flow change rates (+meanNeg) and lower nutrient concentrations.

3.4 Trait-based responses to dams in macroinvertebrate communities

The overall RLQ permutation test revealed a non-significant macroinvertebrate trait-environment relationship (permutation across sites $p = 0.318$, across species $p = 0.47$). The first two RLQ axes accounted for 70.3% of the total variance (Figure 6).

Along a gradient of flow regulation, control sites were mainly concentrated in the bottom part of the ordination, except for site I5, which was affected by the reservoir Enciso. The Cantabrian Cordillera and Iberian System controls were mostly clustered in the bottom-left quadrant. These sites were associated with increased frequency (+FRE7) and magnitude of high flow events (+30HF), winter (+M1,2) and spring (+M3,4) flows, later timing of minimum and maximum annual extremes (+JMin, +JMax), and higher water temperatures ($+T_{WATER}$). These conditions favored macroinvertebrates with small body sizes (<1cm, Small, e.g., Hydroptilidae), more than one reproductive cycle per year (Multivoltine, e.g., Sphaeriidae), deposit feeders, and fine detritus (FineDetritus, e.g., Ostracoda, Caenidae) and animal detritus (AnimalDetritus; e.g., Lymnaeidae) as food sources.

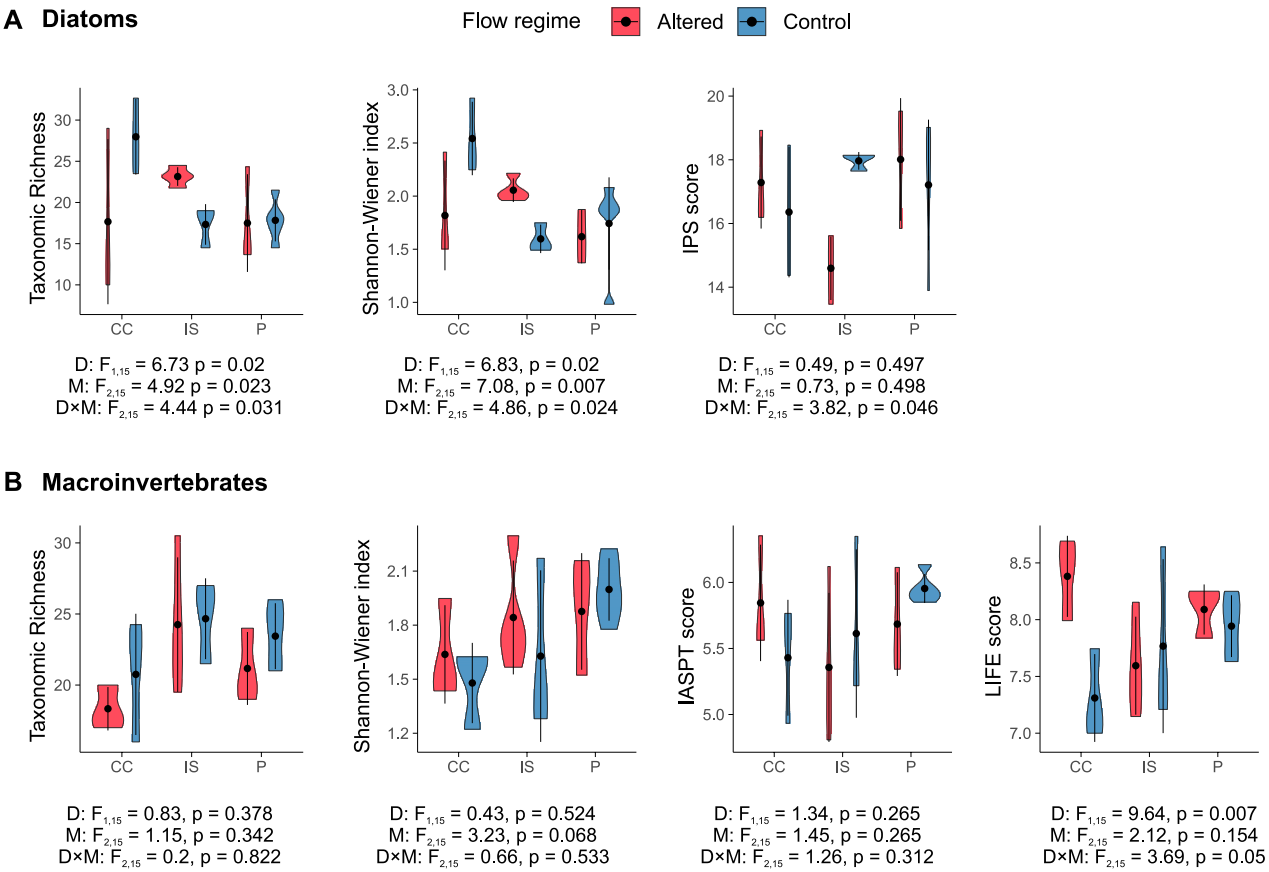

FIGURE 4
Violin plots representing the distribution of taxonomic biotic indices in **(A)** diatom and **(B)** macroinvertebrate communities in the Cantabrian Cordillera (CC), Iberian System (IS), and Pyrenees (P) among control (blue) and altered (red) sites. The two-way ANOVA tests are shown: "D" refers to the ANOVA test with flow regime (dam presence) as a factor, "M" to mountain range as a factor, and "D × M" as the interaction between these factors. Vertical bars represent the standard deviation.

In turn, altered sites were mostly concentrated in the top-right quadrant (except for control sites P5 and P7). They were associated with increased spring (+M5), summer (+M6,7), and autumn (+M10) flows and increased magnitude of short-term low flow extremes (+1,3LF). These conditions favored macroinvertebrates with larger body sizes (>4 cm, Large, e.g., Pediciidae), one potential reproductive cycle per year (Univoltine), larval aquatic stages (Larva, e.g., Perlidae), aquatic (AquActive, e.g., Ephemerellidae) and aerial active (AerialActive, e.g., Heptageniidae) dispersions.

Macroinvertebrate communities in the Cantabrian Cordillera sites showed a more pronounced difference among control and altered sites than the other mountain ranges. Along the second axis (23.1% of the total variance, Figure 6), the Cantabrian Cordillera impacted sites, which were linked to higher summer flows (+M6,7), showed to favor taxa with larger body sizes and larval aquatic stage. In contrast, control sites were associated with increased winter flows (+M1,2) and higher water temperatures, and favored small-sized macroinvertebrates and detritus feeders.

Nutrient concentration and flow change gradients were observed along the first axis (47.2% of the total variance, Figure 6). Iberian System sites were more concentrated on the left side with increased nitrate concentration and longer low flow pulses, favoring macroinvertebrates with medium to large body sizes (MedLarge, e.g., Rhyacophilidae) and adult aquatic stage (Adult, e.g., Hydrobiidae, Gammaridae). In contrast, lower nitrate concentrations and more frequent and shorter flow pulses (+nPulsesHigh, -MeanDPLow) were more associated with Pyrenees' sites, favoring macroinvertebrates with medium body sizes (Medium, e.g., Heptageniidae), aquatic larvae, and aerial active dispersion.

4 Discussion

In this study, we assessed the effects of irrigation dams on the riverine flow regime, water quality, and diatom and macroinvertebrate communities of mountain streams. Summer flows (e.g., magnitude of July, August, and September flows), frequency of high flow events, and the timing of minimum annual flows showed to be significantly affected by the presence of dams, independently of the local environmental conditions, as hypothesized. Water physico-chemical variables, however, did not show to be significantly influenced by the presence of dams. Our second hypothesis was also partially supported. Flow pulse patterns showed to be significantly dependent on the mountain conditions (i.e., mountain range factor), while the magnitude of winter flows

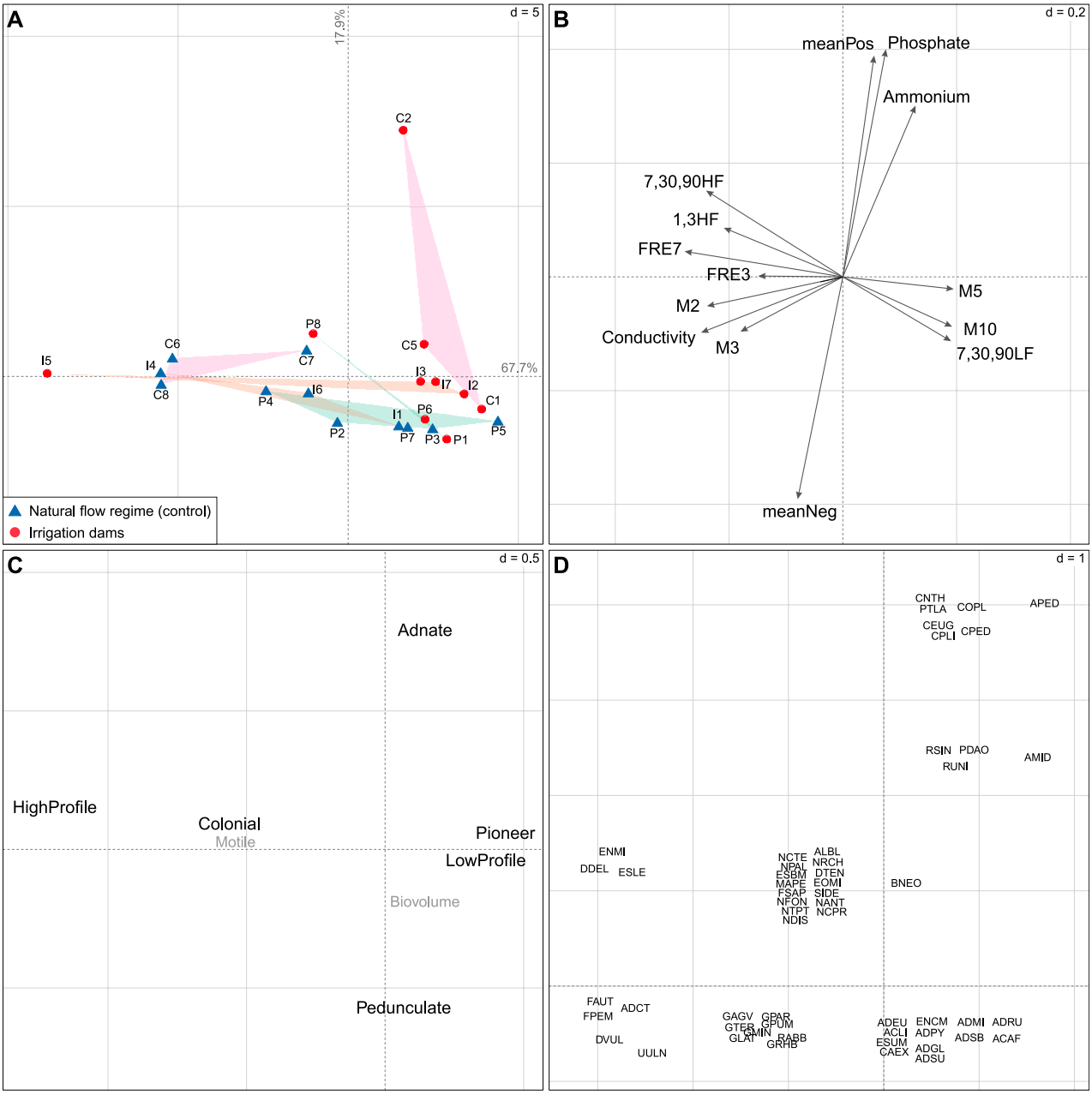

FIGURE 5
Ordinations showing the first two RLQ axes on diatom communities. **(A)** Sampling site scores classified according to the presence (red circles) or absence (blue triangles) of dams and geographical regions: Cantabrian Mountain Range (C; in pink), Iberian System (I; orange), and Pyrenees (P; green). **(B)** Coefficients for HIs (see Supplementary Table S2 for codes) and physico-chemical variables with strong links to traits (see fourth corner analysis in Supplementary Figure S3). **(C)** Coefficients for traits (see Supplementary Table S3 for codes). Traits with no significant relationships (see fourth-corner analysis, Supplementary Figure S3) are represented in grey. **(D)** Coefficients for species (see Supplementary Table S5 for codes). The "d" values give the grid size for scale comparison across the graphs.

and high flow extremes and the rate of flow change were influenced by both factors. Water physico-chemical variables did not exhibit significant changes in response to the mountain conditions.

Diatom communities presented significant responses to dam impacts in terms of biotic indices and traits across the three mountain ranges, partially supporting Hypothesis 3. Macroinvertebrate communities, in contrast, showed less evident responses to geographical and dam-induced changes when analyzing both biotic indices and functional traits. In general, traits showed to be useful in understanding dam-related effects on biological communities, in contrast to biotic indices, as expected in Hypothesis 4.

4.1 Abiotic changes associated with dams

In our study, the magnitude of seasonal flows, the frequency of high flow events, and the timing of minimum annual flows, in

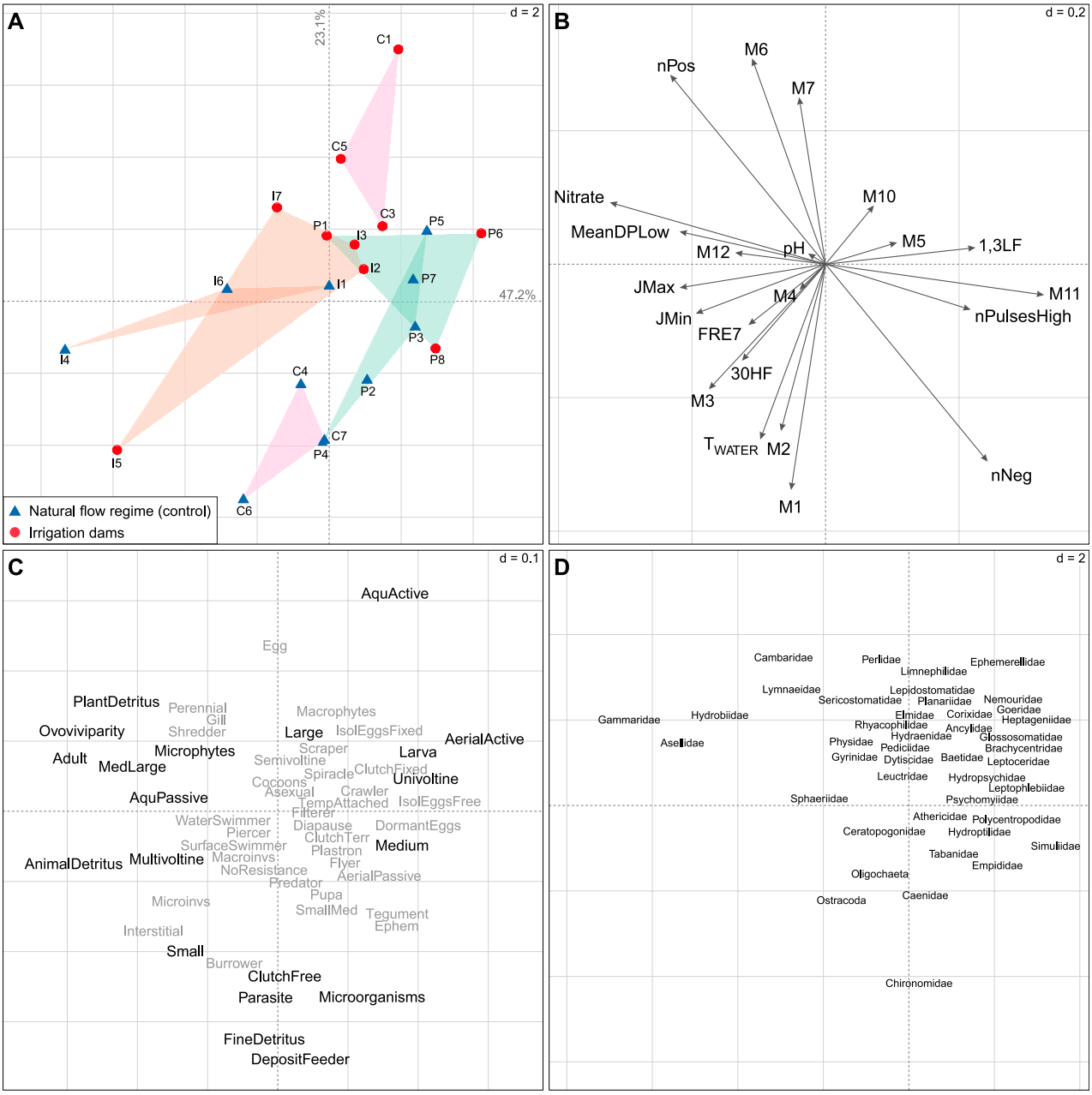

FIGURE 6
Ordinations showing the first two RLQ axes on macroinvertebrate communities. **(A)** Sampling site scores classified according to the presence (red circles) or absence (blue triangles) of dams and geographical regions: Cantabrian Mountain Range (C; in pink), Iberian System (I; orange), and Pyrenees (P; green). **(B)** Coefficients for HIs (see Supplementary Table S2 for codes) and physico-chemical variables with strong links to traits (see fourth corner analysis in Supplementary Figure S4). **(C)** Coefficients for traits (see Supplementary Table S7 for codes). Traits with no significant relationships (see fourth-corner analysis, Supplementary Figure S4) are represented in grey. **(D)** Coefficients for species (see Supplementary Table S9). The "d" values give the grid size for scale comparison across the graphs.

general, varied significantly with the presence of dams. Although the hydrological period analyzed was relatively short (3 years), we observed significant alterations across the three mountain ranges. The magnitude of the alteration (and in some cases the direction, e.g., M6) varied between the three regions, with particularly relevant hydrological alterations in the Cantabrian Cordillera. Winter flows, high flow extremes, and the number of flow rises and falls were shown to be significantly affected by the interaction between flow regulation and mountain range factors. Moreover, changes in other HIs such as flow pulse changes were related exclusively to the mountain range factor (i.e., affected by local conditions only). These results indicate that there is an influence of the regional characteristics (e.g., climate, LULC, geology) besides dam-induced changes. This also agrees with Peñas and Barquín (2019), who observed a geographical dependence of the patterns of altered river flow regimes on the river's pre-dam characteristics.

As it has been briefly discussed above, dams located in the Cantabrian Cordillera displayed the most pronounced inversion of the seasonal flow regime compared to the other mountain ranges. This inversion results from the retention of winter flow peaks and the release of impounded water during summer to address seasonal water demands, as also reported in previous studies (Belmar et al., 2013; García de Jalón et al., 2019). The relatively large capacity of these reservoirs (e.g., Riaño, Ebro, Juan Benet, >150 hm^3 of storage volume; see Supplementary Table S1) contributed to the pronounced seasonal flow inversions. Conversely, in the Pyrenees and the Iberian System, no significant changes in HIs associated to seasonal patterns were detected. The substantially lower storage volumes of these reservoirs may not be the only responsible for these results. Other dam uses (e.g., Bubal, Lanuza, and Escales are also used for flood control and hydropower production; López Moreno et al., 2016) might also contribute to the observed differences. Accordingly, we found that HIs associated with hydropower, such as nPos and nNeg, were significantly altered in the Pyrenees, in agreement with previous studies (Almeida et al., 2020; Chalise et al., 2021; Pompeu et al., 2022b).

Independently of the dam presence, the streams in the Pyrenees exhibited shorter and more frequent high and low flow pulses in comparison to the Iberian System. This can be associated to the higher altitudes, slope, and increased precipitation found in Pyrenean streams. In addition, altered sites in both Iberian System and the Pyrenees showed a slight reduction in the spring flows (see Supplementary Figure S1), which could be related to the retained spring snowmelt flows occurring in these mountain areas (Dott et al., 2022). These results highlight the importance of the regional mountain conditions and the dam characteristics in the hydrological responses to flow regulation.

The physico-chemical variables did not show significant changes associated to irrigation dams or the mountain range. Although water temperature and nutrient concentration have been reported to be strongly impacted by dams (Olden and Naiman, 2010; Chandesris et al., 2019; Maavara et al., 2020), in our case, the available physico-chemical data (spot measurements collected along with the annual biological surveys) might be insufficient to show these effects. For instance, attending to our criteria for sampling site selection (i.e., sites in "good" ecological status), we limited the range of nutrient concentrations, which certainly contributed to the low ability to detect significant differences in nutrient-related variables. Moreover, the long distances between sampling sites and the dams (>20 km downstream of dams in some cases), might buffer the dam-related effects on the temperature. A study conducted by Zaidel et al. (2021) reported persistent changes in water temperature for an average of only 1.3 km downstream of dams. Therefore, a continuous monitoring strategy in closer proximity to dams would be needed to better understand the effects of irrigation dams on physico-chemical variables (Vilmin et al., 2018).

4.2 Changes in taxonomic biotic indices associated with dams and mountain ranges

The diatom biotic indices showed significant changes to the flow regulation-mountain range interaction, suggesting that the dam-induced effects on diatom communities may be dependent on the mountain range. In the Iberian System, slightly higher taxonomic richness and diversity were detected in regulated sites, in agreement with previous studies (Smolar-Žvanut and Mikoš, 2014; Krajenbrink et al., 2019). It suggests that diatom communities might benefit from more stable flows in regulated sites, as these conditions favor the development of biofilm algae (Biggs et al., 1998; Biggs and Smith, 2002). The IPS score, which was originally developed to target organic pollution (CEMAGREF, 1982) but also widely used in riverine bioassessment in Spain within the WFD (BOE, 2015), showed a reduction in flow-regulated sites in the Iberian System only. Since the index did not respond significantly to HIs in our study, it could be linked to local environmental factors (e.g., higher altitudes and higher siliceous geology in controls in comparison to altered sites in the Iberian System) or other water quality characteristics not covered in our study. These findings can be linked to the influence of local habitat characteristics and spatial-related factors (e.g., dispersal and biogeography) on the ecosystems besides the presence of dams. Previous studies reported this interactive effect of river hydrological variation, local, and spatial factors on the diatom and macroinvertebrate communities (Karaouzas et al., 2019; Laini et al., 2019; Pompeu et al., 2022a; Krajenbrink et al., 2022).

The macroinvertebrates' taxonomic richness and the Shannon-Wiener diversity did not show significant responses to flow regulation. Although numerous studies indicate that dam construction decrease macroinvertebrate richness (Wu et al., 2019a), these biotic indices could be weakly correlated to hydromorphological alterations, as observed in our study (see Spearman's rank correlation in Supplementary Figure S2) and elsewhere (Feld et al., 2014). Thus, taxonomic replacement associated with hydrological alteration may not cause important changes in biotic indices based on the whole community (i.e., richness and diversity), as different species can replace those lost due to hydrological impacts (Feld et al., 2014). Both IBMWP and IASPT macroinvertebrate scores, which are commonly used in the WFD river health assessment, did not show significant responses to flow regulation, contradicting previous research conducted on Spanish rivers (Quevedo et al., 2018; Mellado-Díaz et al., 2019). Click or tap here to enter text.These findings could be attributed to the fact that IBMWP and IASPT are based on macroinvertebrate families' sensitivity to organic pollution and were originally designed to target water quality rather than other stressors (Friberg et al., 2011). Hence, our findings support the argument that these indices should only be used to assess the impacts for which they were designed while they are prone to fail when assessing the effects of other stressors (e.g., flow regulation by dams).

Contrary to expected, the LIFE index, a UK-based index designed to evaluate hydromorphological stress (Extence et al., 1999), failed to detect impacts downstream of irrigation dams. Moreover, altered sites in the Cantabrian Cordillera scored higher than controls, in contrast to the findings reported by White et al. (2017). This divergence could be attributed to the study design and the distances between the dam and the sampling location. Mellado-Díaz et al. (2019) reported an increase in the LIFE score immediately downstream dams (between 0 and 4 km downstream), then they showing a recovery across the downstream gradient (4–20 km). Pompeu et al. (2022b),

following an optimized and statistically robust control-impact design, highlighted clear biological and ecological responses downstream irrigation dams. Local factors other than hydrology (e.g., water quality, channel morphology, and anthropogenic alteration of riverine habitats), which were not fully addressed in our study might also have a significant impact on the macroinvertebrate community structure and thus, on the recorded LIFE scores (Worrall et al., 2014; Laini et al., 2018; Laini et al., 2022).Therefore, long-term biological sampling schemes following optimal control-impact designs are critical to develop biological indices able detect changes in macroinvertebrate communities associated with natural gradients and specific stressors, such as irrigation dams (Vaughan and Ormerod, 2012).

4.3 Changes in trait-based communities associated with dams and mountain ranges

As hypothesized, the trait-based biological responses varied with the different flow regime alteration patterns and the regional control. In the Cantabrian Cordillera, communities showed a clear segregation between control and impacted streams, which can be linked to the marked hydrological alteration identified in this region (i.e., seasonal flow inversion, with increased summer flows and lower winter flows) and the larger size and regulation capacity of these reservoirs. In contrast, in the Iberian System and the Pyrenees, the community shifts associated to seasonal flow inversions were less evident. This was especially obvious in some specific altered sites (e.g., I5 and P8), where impacted communities were not different from controls. In these sites, we argue that the reduced influence of dams on the biological communities might be associated to the smaller reservoir sizes, and the significant sampling site-dam distances (exceeding 20 km, with tributaries joining the main stem; see Supplementary Table S1) would act as a buffer, mitigating dam-related hydrological and biological impacts. Moreover, the multiple-purpose character of these dams (used for irrigation, urban water supply, and hydropower generation) may cause a less clear-cut hydrological alteration (e.g., changes in the flow rise frequency). These findings highlight the importance of thoroughly planning appropriate biomonitoring study designs when investigating the effects on riverine communities. Factors such as the distances between the dams (or other stressors) and the sampling sites, the similarities between impacted and unimpacted sites, the geographical location or the sampling period and consistency along large periods, among others, must be carefully considered.

The diatom trait-based analysis indicated that control sites, in general, favored the high profile guild and colonial life forms, which are both traits linked to low flow disturbances (Passy, 2007). Dam-impacted sites, in turn, favored pioneer and low profile diatoms, i.e., traits resistant to flow disturbances (Passy, 2007; Tornés et al., 2015). Our results contrast with Goldenberg-Vilar et al. (2021), who reported that resistant traits (e.g., small-sized, non-colonial diatoms) in unregulated streams were replaced by colonial life forms, large-sized, and loosely attached diatoms in sites affected by dams in northern Spain. These divergences can be attributed to the different sampling periods and the effect of environmental variability present in our dataset. The study conducted by Goldenberg-Vilar et al. (2021) sampled biological communities in 2017 using a more straightforward study design, which was specifically planned to investigate the effects of dams on diatom communities.

The increased flow rates of change may also have contributed to the significant diatom community shifts observed in our study, which favored adnate life forms and reduced pedunculate ones. Adnate life forms adhere to the substrate and are highly resistant to repeated scour disturbances (Tornés et al., 2015). However, we highlight that diatom responses to flow regime components may be confounded by the interactions between dam-related effects and environmental variability (Wagenhoff et al., 2017; Krajenbrink et al., 2019), e.g., differences in the altitude, land use and land cover, and geology among mountain ranges. Therefore, reducing the environmental variability would contribute to better assess dam-related impacts.

In contrast to diatoms, macroinvertebrates did not show a globally significant relationship between trait composition and the hydrological and physico-chemical variables, although the impacted sites in the Cantabrian Cordillera streams were clearly segregated from controls. This result highlights the potential use of diatom traits to assess the dam-related impacts over relatively broad spatial scales. Nevertheless, the individual significant trait-hydrology relationships identified in the macroinvertebrate analyses might be useful to understand the mechanistic effects of dam-related impacts. Traits better adapted to the dynamic flow conditions (e.g., small body sizes, multiple reproductive cycles per year) in unregulated mountain streams, especially in the Cantabrian Cordillera, were replaced by univoltine taxa, larger body sizes, larval aquatic stages, and active dispersion, as also reported in previous studies (Bonada and Dolédec, 2018; Piano et al., 2020).

4.4 Study limitations

This study relies on data originally collected for the water bodies' ecological status assessment within the European WFD. Post hoc analyses of these existing datasets may support hydroecological research and help identifying some general patterns at large spatial scales (e.g., Krajenbrink et al., 2019; Feeley et al., 2020; Kelly-Quinn et al., 2020); however, several inherent challenges arise when using data surveyed for other purposes (Vaughan and Ormerod, 2010).

One key issue potentially obscuring the emergence of more clear patterns is the high degree of data heterogeneity in WFD datasets. In this regard, adhering to rigorous criteria for sites selection allowed us to obtain statistically meaningful results and conclusions, but also revealed several barriers establishing a robust study design. These barriers include, among others, the difficulty in finding comparable rivers within and between different regions (i.e., mountain ranges), the distance to the dam, the temporal extent (e.g., surveys carried out in years with different hydrological and environmental legacies), the limited spatial coverage due to the lack of essential data in many sites (e.g., relatively few sampling sites paired to flow gauges), the variations in sampling efforts among sites, and the simplification of the ecological complexity when averaging the biological communities sampled in different years.

Additionally, the lack of relevant variables measured in the field (e.g., hydromorphological conditions, sediments or longer-term water quality) and longer river flow discharge series (>10 years) to characterize long-term hydrological patterns may also have contributed to the uncertainties observed in this study. All these

issues should be specifically addressed in the design of future works aiming at improving our understanding on the effects of different types of dams on riverine biological communities. In this sense, previous studies have demonstrated the suitability of WFD datasets to understand the main environmental factors controlling biological communities at large spatial scales (Feeley et al., 2020; Sturbois et al., 2021; Pompeu et al., 2022a; Heß et al., 2023; Pompeu et al., 2023). In contrast, their suitability to test hypotheses dealing with the effect of specific stressors (i.e., dams) in more localized areas (i.e., local and regional studies) could be more uncertain and controversial, as highlighted in this study. In this case, to discern between the effect of the dams and the natural variability of the riverine ecosystem, more explicit and meticulous study designs are necessary (England et al., 2021; Pompeu et al., 2022b). Long-term monitoring strategies should be also considered to capture the temporal dynamics of biotic communities and overcome the ecological complexity under variable environmental conditions (Jackson and Füreder, 2006; England et al., 2021).

In addition, as required by the lotic bioassessment protocols in Spain (e.g., IBMWP index; Alba-Tercedor et al., 2002; Ministerio de Agricultura, Alimentación y Medio Ambiente, 2013b), macroinvertebrate communities were mainly identified at the family level. Although family-level identification is reported to be cost-effective and suitable for bioassessment (Gayraud et al., 2003; Heino and Soininen, 2007), the use of finer taxonomic resolution may be necessary to detect subtle impacts in macroinvertebrate communities (Chessman et al., 2007). Likewise, taxonomic resolution may cause significant differences in LIFE scores (Monk et al., 2012) and in the detection of the key environmental variables controlling community patterns (Jiang et al., 2013).

We also point out that the RLQ permutation tests failed to indicate a significant overall trait-environment association in macroinvertebrate communities. These findings indicate that macroinvertebrate taxa distribution was independent of the environmental characteristics (i.e., hydrology and physico-chemistry) or functional traits. Even though all selected sites were located in mountain areas, the environmental and hydrological heterogeneity among regions (e.g., variations in altitude, climate, geology, and hydrological characteristics) may produce substantial trait-based differences among communities at the regional scale (Bonada et al., 2007; Pompeu et al., 2022a; 2022b) and substantially increase the noise in the analyses. Previous research has also reported that controlled settings produced more significant outcomes since the effect of confounding variables is reduced and a direct investigation of the trait-environment response is allowed (Brown et al., 2019). Thus, future research should focus on the reduction of hydrological and environmental variability. Methods such as control-impact study design, grouping dams with similar characteristics (McManamay et al., 2016), and hydrological classification (Arthington et al., 2006) would reduce the noise arising from the inherent environment variability and, therefore, enable a more precise analysis of dam-related impacts (Pompeu et al., 2022b).

4.5 Implications for biomonitoring and conservation efforts

In contrast to taxonomic biotic indices, trait-based analyses allowed establishing mechanistic linkages of biotic functional responses to hydrological and physico-chemical conditions (Culp et al., 2011), such as changes in the diatom life forms and biovolume along the dam impact gradient, which has been reported elsewhere (Murphy et al., 2017; Troia and McManamay, 2019). In combination with HIs, traits can provide a template to create and test large-scale hypotheses regarding flow-ecology responses (Poff and Allan, 1995; McManamay et al., 2015). Although research in this field is growing (Wu et al., 2019b; Goldenberg-Vilar et al., 2021; Larsen et al., 2021; Pompeu et al., 2022b), due to the lack of long-term discharge data and paired biological samples, linkages between the flow regime and biological communities are usually not fully explored. Therefore, setting biomonitoring networks in which communities are sampled more frequently (e.g., covering seasonal variability), over a long and meaningful time frame (e.g., at least 10 years), and with adequate spatial coverage (e.g., relatively close to dams and with paired river gauging) would be beneficial for monitoring and tackling dam impacts on river ecosystems, as well as for identifying future research priorities.

Furthermore, exploring the responses of different organisms to dam-related impacts is essential to select meaningful biomonitoring metrics (Poff and Zimmerman, 2010). For instance, we demonstrated that diatom communities showed significant responses to dam-related changes, whereas the influence on macroinvertebrate communities was not so clear. The differences between diatoms and macroinvertebrates in terms of body size, life-history strategies, and dispersal capacity may have contributed to their responses to local environmental characteristics and stressors (Grenouillet et al., 2008; Shurin et al., 2009; De Bie et al., 2012). Diatoms have good dispersal abilities and fast population growth rates and, therefore, are considered good bioindicators to track environmental change over large spatial scales (Bennett et al., 2010). Macroinvertebrates, in contrast, are weaker dispersers in comparison to diatoms, thus spatial-related processes may have a greater influence on their community structure than environmental control (Heino, 2013; Padial et al., 2014).

In conclusion, our findings highlighted the main hydrological alterations associated to irrigation dams in mountain rivers and their effects on riverine biota, which varied among biotic groups, mountain ranges, and dams' operational rules and impoundment capacity. Trait-based analyses allowed us to capture more complex changes in the communities (e.g., life form, body sizes, life history strategies) along the dam impact gradient than the taxonomic approaches. Diatoms were shown to better respond to the presence of dams than macroinvertebrates, which could be linked to their lower spatial dependency. These results contribute to the development of efficient biomonitoring designs and strategies (e.g., selection of suitable biotic groups, location of control and altered sites at adequate spatial scales) and the definition of flow-ecology relationships (e.g., ecological responses to key HIs) as part of riverine conservation strategies (e.g., ELOHA environmental flow framework; Poff et al., 2010).

Data availability statement

The data that support the findings of this study are available from the Spanish Ministry for the Ecological Transition and were used under license for this study. The datasets are available upon reasonable request with the permission of the national authority.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

CRP: Data curation, Formal Analysis, Investigation, Methodology, Software, Visualization, Writing–original draft, Writing–review and editing. FP: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing–review and editing. JB: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2024.1332268/full#supplementary-material

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Effects of phytoplankton diversity on resource use efficiency in a eutrophic urban river of Northern China

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Freshwater biodiversity has been declining in urban areas, which may threaten ecosystem functions. Although many studies have demonstrated a positive correlation between biodiversity and ecosystem functioning (BEF) in terrestrial and marine ecosystems, little is known about the BEF relationship in freshwater environments, especially in highly urbanized regions where water pollution is a major concern. Eutrophication in urban water bodies may trigger algae blooms, decreasing the evenness or functional divergence (FDiv) of phytoplankton communities, thus negatively affecting ecosystem functioning. Through an annual field investigation, we clarified the relationship between phytoplankton diversity and ecosystem functioning, represented as resource use efficiency (RUE), in an urban river in northern China. Results indicated that evenness in the phytoplankton community contributes most to driving ecosystem functioning compared to environmental factors. The relative abundance of dominant Bacillariophyta was positively correlated with the resource use efficiency of phytoplankton (RUEpp) but negatively correlated with the resource use efficiency of zooplankton (RUEzp). Both phytoplankton evenness and functional divergence were negatively linked to RUEpp but positively to RUEzp. Our findings suggest that the reduction of phytoplankton evenness and functional divergence may seriously threaten resource use efficiency (RUE), and its potential mechanism can provide a crucial reference for water quality protection and sustainable water resource utilization in the basin.

KEYWORDS

ecosystem functioning, evenness, functional traits, algae blooms, functional divergence

1 Introduction

Environmental pressures resulting from intensified human activities severely threaten biodiversity and ecosystem structure, with freshwater ecosystems being particularly affected (Yi et al., 2014; Newbold et al., 2015; Zhao et al., 2019; Wang J. et al., 2021; Moi et al., 2022; Polazzo et al., 2022). One major threat is eutrophication (Rosset et al., 2014), which disrupts the natural balance of nutrients crucial for the stability of aquatic ecosystems (Huisman et al., 2018). Excessive nutrient inputs can trigger algal bloom outbreaks, reduce planktonic biodiversity (Wang et al., 2022), alter community structure (McQuatters-Gollop et al., 2009; Bužančić et al., 2016; Wang H. et al., 2021), reduce ecological niche differentiation, and intensify interspecific competition, thereby affecting ecosystem functions (Amorim and Moura, 2021). These blooms, often dominated by species such as Cyanobacteria,

Chlorophyta, and Bacillariophyta, increase water turbidity and pH, resulting in anoxia and organism mortality (Visser et al., 2016). Furthermore, the intensity of the bloom also increases phytoplankton nutrient utilization but blocks the energy transfer from primary producers to zooplankton (Grey et al., 2000), which is not conducive to the acquisition of nutrients by zooplankton and negatively affects ecosystem functioning (Tian et al., 2017).

The relationship between biodiversity and ecosystem functioning (BEF) has long been a central theme in ecology. Considerable evidence suggests that biodiversity is critical for maintaining ecosystem functioning, and a loss of biodiversity reduces the capacity of ecosystems to provide multiple services (Cardinale et al., 2012; Naeem et al., 2012; Hagan et al., 2021; Mitchell et al., 2024), i.e., biodiversity positively influences ecosystem functioning (Cardinale et al., 2011; Tilman et al., 2014; Oliver et al., 2015; Soliveres et al., 2016; Slade et al., 2017). However, this relationship may also exhibit a non-linear pattern influenced by interspecific interactions and environmental heterogeneity (Cardinale et al., 2011; Thompson et al., 2017). Most of the early research on BEF relationships has focused on terrestrial ecosystems (Soliveres et al., 2016; Schuldt et al., 2018), particularly terrestrial plants, while aquatic ecosystems have received less attention (Daam et al., 2019). Globally, the biodiversity of freshwater systems is declining faster than that of terrestrial and marine systems (Vaughn, 2010; Zhang et al., 2019), and its loss will seriously threaten ecosystem stability and productivity.

The BEF relationship of phytoplankton was first proposed by Ptacnik et al. (2008), who used resource use efficiency (RUE) to indicate ecosystem functioning. RUE quantifies ecosystem functioning by measuring the ratio of resources acquired by organisms to the amount of biomass they transform and serves as a better indicator of the level of resource use by organisms (Hodapp et al., 2019). As one of the most important primary producers in river ecosystems (Varol, 2019), phytoplankton communities reflect their fundamental and critical role in material cycling, pollutant degradation, water self-purification, and ecosystem stability (Yang et al., 2022). Due to their tiny unicellular size and extreme sensitivity to environmental changes (Feio et al., 2009), phytoplankton are susceptible to both abiotic factors, such as nutrient salinity and water temperature, as well as biotic factors, such as grazing and interspecific competition. Consequently, they are considered ideal aquatic ecological indicator organisms (Wojciechowski et al., 2017). In the context of China's rapid urbanization and industrialization, changes in phytoplankton diversity significantly reflect the response and adaptation of river ecosystems to environmental stress. In particular, increased nutrients from industrial wastewater and urban runoff cause eutrophication of water bodies, affecting phytoplankton community structure and function, reducing water quality, and threatening the overall aquatic biodiversity (Mustapha et al., 2013; Barakat et al., 2016). Therefore, monitoring and analyzing changes in phytoplankton diversity not only assesses the health of ecosystems but also provides a critical scientific basis for developing effective environmental management strategies.

While numerous studies have confirmed the overall positive correlation between BEF, most have focused solely on taxonomic diversity metrics such as species richness (Duffy et al., 2017; Amorim and Moura, 2021). In contrast, evenness metrics, which reflect the uniformity of species distribution within communities, and functional diversity metrics, which describe the functional traits of species, have received limited attention. Recent studies have concluded that the relationship between phytoplankton evenness and ecosystem functioning (measured by resource use efficiency) generally shows a negative effect in highly disturbed ecosystems, i.e., dominant species with superior resource utilization capabilities outcompete others (Filstrup et al., 2019; Otero et al., 2020). Furthermore, evenness is considered a sensitive indicator of changes in biodiversity, often reflecting the impacts of anthropogenic activities or environmental changes better than species richness and eliciting rapid responses in ecosystem functioning (Chapin III et al., 2000; Wilsey and Potvin, 2000; Filstrup et al., 2014; Hodapp et al., 2015). In contrast, species richness ignores the effects of differences in relative abundance among species on interspecific interactions and overemphasizes the importance of rare species (Zhang et al., 2012; Filstrup et al., 2014), which does not adequately represent the diversity associated with ecosystem functioning. In particular, changes in evenness often occur with little or no change in species richness, i.e., species tend to decline in number before going extinct, and evenness can capture such species declines (Wilsey and Potvin, 2000; Hillebrand et al., 2008). Consequently, the effects of evenness on ecosystem functioning warrant further in-depth investigation (Wilsey and Potvin, 2000).

Field and experimental studies have demonstrated that functional diversity is a highly effective predictor of ecosystem functioning, making it a critical aspect of contemporary biodiversity research (Griffin et al., 2009; Abonyi et al., 2017). In this context, the functional diversity indices proposed by Villéger et al. (2008), which comprehensively assess functional traits such as growth forms, habitat preferences, and resource use strategies, have received widespread recognition in the international scientific community (Eisenhauer et al., 2019). Compared to traditional research focused on taxonomic diversity, this species trait-based method of analysis more accurately reflects the actual functional roles of species within ecosystems. This approach highlights the functional differences among species and addresses the problem of traditional biodiversity indices that treat the contributions of each species equally (Cadotte et al., 2011), making it a promising tool for biological monitoring and ecosystem management. In particular, in the study of phytoplankton, by analyzing the relationship between their functional diversity and ecosystem functions, we can better understand the critical contributions of these microorganisms to primary productivity and nutrient cycling in water bodies. This will further elucidate the complex response mechanisms of aquatic ecosystems to environmental change, providing a solid foundation for the formulation of scientific ecological conservation and management strategies.

Currently, studies of aquatic biodiversity tend to focus on biodiversity effects within individual trophic levels, with less attention paid to vertical biodiversity effects across multiple trophic levels (Duffy et al., 2007). This approach may overlook potential trophic connectivity effects during urbanization, resulting in a lack of understanding of the role of individual taxa in food webs and their impact on ecosystem functioning (Eisenhauer et al., 2013; Li et al., 2021; Moi et al., 2021). Organisms drive ecosystem

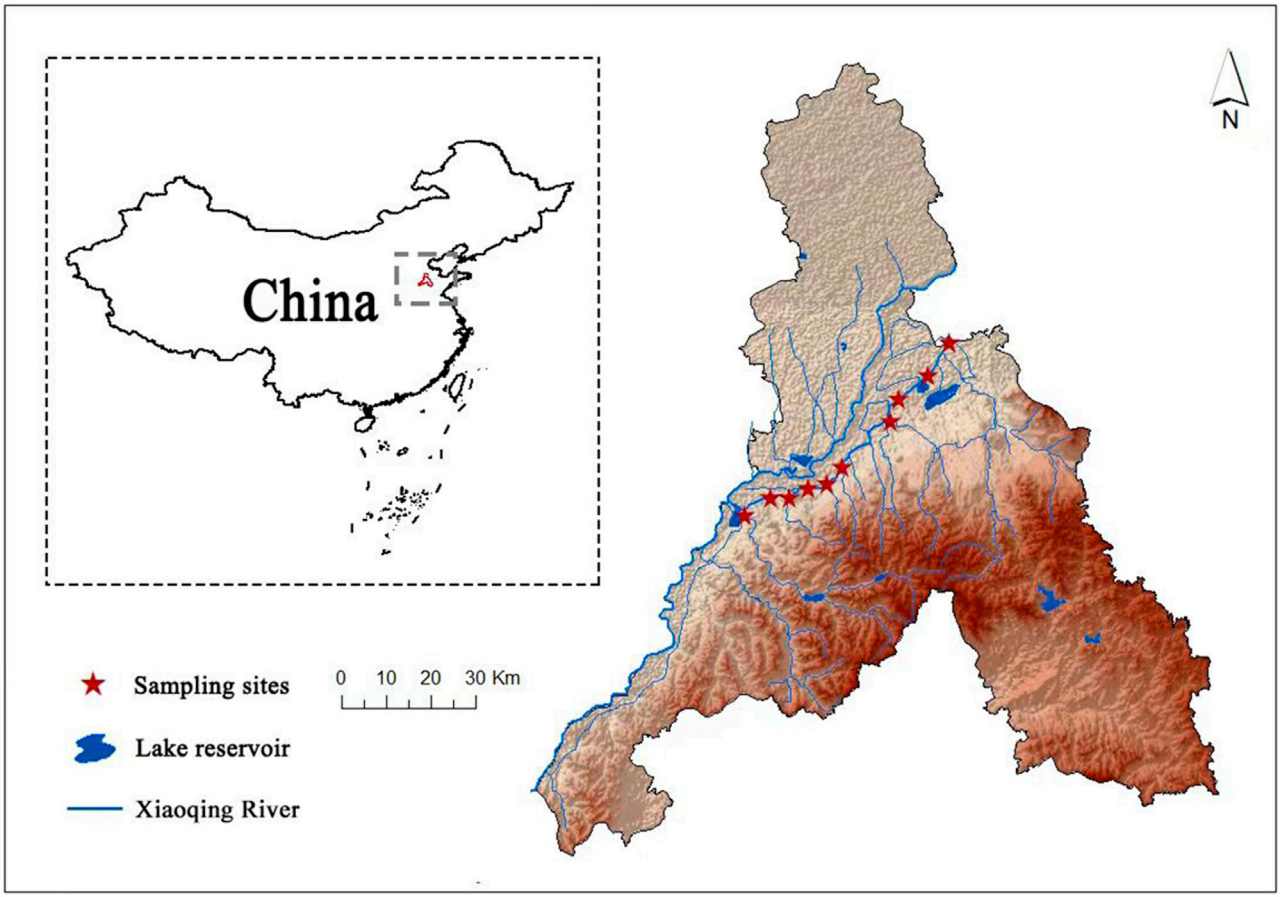


FIGURE 1
Sampling locations of the Xiaoqing River in northern China.

functioning at multiple trophic levels, and considering trophic complexity is critical to understanding the role of biodiversity at different trophic levels (Li et al., 2020). Primary producers can have different effects on ecosystem functioning within and across trophic levels (Filstrup et al., 2014). Results that consider only a single trophic group are likely to underestimate the importance of biodiversity and fail to effectively reflect the complexity of river and lake ecosystems (Weijters et al., 2009; Soliveres et al., 2016; Li et al., 2020). Therefore, it is necessary to integrate data from multiple trophic levels and horizontally explore the ecosystem functions of communities at different trophic levels to improve our understanding of the ecological health of rivers.

The Xiaoqing River, situated in northern China, is a highly urbanized area where intensive human activities have led to eutrophication of the water and the outbreaks of algal blooms. This makes it an ideal ecosystem to empirically test the relationship between biodiversity and river ecosystem functioning (Wang and Yin, 2023). We used phytoplankton taxonomic diversity (evenness) and functional diversity (functional divergence) as indicators of biodiversity, and resource use efficiency of phytoplankton (RUEpp) and zooplankton (RUEzp) communities as measures of ecosystem functioning. As one of the most important primary producers in river ecosystems, the diversity change of phytoplankton can reflect the response of rivers to environmental stress, while RUE can comprehensively reflect the level of resource utilization by biomes, and the correlation analysis between the two can help to elucidate the intrinsic mechanism underlying the biodiversity-ecosystem functioning relationship. We tested the following research hypotheses: (1) Phytoplankton evenness would be negatively correlated with RUEpp but positively correlated with RUEzp. (2) Functional divergence (FDiv) of the phytoplankton community would be negatively correlated with RUEpp but positively correlated with RUEzp. (3) The increase of dominant species caused by algal blooms would lead to an increase in RUEpp and a decrease in RUEzp.

2 Materials and methods

2.1 Study area

The Xiaoqing River, with a length of approximately 240 km, originates in Jinan City (He et al., 2018), Shandong Province, northern China (Figure 1). It spans five cities in Shandong Province (Jinan, Binzhou, Zibo, Dongying, and Weifang) (Jiang et al., 2017), with a watershed area of approximately 10,336 km^2 (Zhang et al., 2020). This area has a temperate monsoon climate, with an average annual mean temperature of 14.7°C and an average

annual precipitation of 1,043.4 mm. The Xiaoqing River is mainly used for flood control, irrigation, and navigation (He et al., 2020). However, with the rapid economic and social development of Jinan City, the land use in the basin has changed significantly (Liu et al., 2019), leading to the worsening of eutrophication in the Xiaoqing River and severe damage to the ecosystem (Zhang et al., 2023). In the present study, we monitored ten fixed sites along the Jinan section of the Xiaoqing River on a monthly basis from April 2020 to March 2021.

2.2 Collection and measurement of environment parameters

A portable multi-parameter water quality monitor (AZ86031) was used in the field to measure water temperature (WT), water depth (D), pH, dissolved oxygen (DO), transparency (Trans), turbidity (Tur), suspended solids (SS) and conductivity (EC) at each sampling site (Jiang et al., 2017). We collected 2 L of water samples at a depth of 0.5 m below the surface. The water samples were stored in a cold, dark environment, transported to the laboratory for further analysis, and then divided into two subsamples: one for water chemistry analysis and the other for quantitative phytoplankton analysis. The following indicators were determined according to the national environmental protection standards issued by the Ministry of Environmental Protection: ammonia nitrogen (NH_3^--N), nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N), and phosphate (PO_4^{3-}-P) were determined by ultraviolet spectrophotometry, the permanganate index (COD_{Mn}) was determined by the permanganate method (GB11892-89), total nitrogen (TN) was determined by UV spectrophotometry using alkaline potassium persulphate digestion, and total phosphorus (TP) was determined by the ammonium molybdate spectrophotometric method (Zhou et al., 2022). Chlorophyll-a content was determined by the hot ethanol method: the samples were extracted with 90% ethanol solution after filtration, and the supernatant was taken after a water bath, shaking, centrifugation, and the chlorophyll-a concentration was determined spectrophotometrically (Chen and Gao, 2000).

2.3 Plankton sampling and analysis

The collected phytoplankton quantitative samples were placed in 1 L plastic bottles, labeled, and fixed in the field by adding 1%–1.5% (v/v) of Lugol's reagent. After 24 h of sedimentation, the samples were concentrated to 100 mL (Zhang et al., 2021). Quantitative samples of zooplankton were collected with a water sampler at different water levels in each section, with 50 L of mixed water samples filtered through a 25# plankton mesh (64 μm mesh diameter) (Lu et al., 2021). The filtered samples were then preserved in 100 mL sample bottles with 4% formaldehyde solution.

Referring to the relevant diagrams (Wang, 1961; Jiang, 1979; Zhu and Chen, 2000; Hu and Wei, 2006; Wang, 2020), phytoplankton and zooplankton samples were identified and analyzed to the lowest possible taxonomic level (i.e., species level when possible) using the field-of-view method under a 400x light microscope (SOPTOP, EX20). Density and biomass of phytoplankton and zooplankton in quantitative samples were calculated based on the identification results (Supplementary Appendix S1, S2), where biomass was derived from density multiplied by the average individual weight (biomass = density x weight).

The density of phytoplankton (i.e., the number of phytoplankton per liter of water, N_p) was calculated as follows:

$$N_p = \frac{C_s}{F_s F_n} \times \frac{V}{v} \times P_n$$

Where:
N_p is the number of phytoplankton per liter of water;
C_s is the area of the counting frame (mm^2);
F_s is the area of one field of view;
F_n is the number of fields of view counted;
V is the volume of 1 L of water sample after sedimentation and concentration (100 mL);
v is the volume of the counting frame (0.1 mL);
P_n is the number of individual phytoplankton counted in Fn fields of view.

The density of zooplankton (i.e., the number of zooplankton per liter of water, N_z) was calculated as follows:

$$N_z = \frac{V_s \times n}{V \times V_a}$$

Where:
N_z is the number of zooplankton per liter of water;
V_s is the volume after sedimentation (100 mL);
N is the number of individuals obtained;
V is the sampled volume (50L);
V_a is the volume of the counting frame (1 mL).

The Technical Specification for Classification and Monitoring of Algal Blooms, issued by Guangdong Province in December 2020, indicates that the common phylum of water blooms, including Cyanobacteria and Bacillariophyta, can be classified into five categories. The results of the phytoplankton density calculations conducted in this study indicate that the present study area can be defined as Class III Bacillariophyta bloom (density $>5 \times 10^6$cells/L).

2.4 Phytoplankton taxonomic and functional diversity

In this study, taxonomic diversity and functional diversity were used to investigate BEF relationships in the Xiaoqing River across different seasons. Measures of phytoplankton taxonomic diversity included species richness and evenness. Species richness was quantified as the number of species in a community, while evenness was a measure of the uniformity in the distribution of individual species abundances within a community. A higher value of evenness indicates a more balanced distribution of individuals among species and a more stable community. Evenness is calculated as $J' = H'/\ln S$, where H' is the Shannon-Wiener diversity index and S is the number of species in the sample.

TABLE 1 Trait classification of phytoplankton.

Traits	Categories
Algae size	<20um,20-200um,>200um
Algae morphology	Unicellular, chain or filamentous, colonial
Motability	Yes/no
Silica	Yes/no
Mode of reproduction	Vegetative reproduction, sexual propagation, asexual propagation
Ability to fix nitrogen	Yes/no
Accessory pigment composition	Chlorophyll-b, Chlorophyll-c, phycobilin
Habitat	PNL and MNL, ENL, mixed aquifer, hydrostatic, high current
Tolerances	Solarization, Low light, and low nutrition, nutrient stratification, stir, cleanse, high BOD
Sensitivities	Mixing, stratification, cleanse, pH, nutritional deficiency, predation, high light

TABLE 2 Multiple regression analysis models based on predictor variables (numerous environmental factors and biodiversity indexes) and response variable RUEpp. The "Contribution" column in the table quantifies the contribution of a single explanatory variable to the multiple linear regression model of the dependent variables RUEpp.

Variable	P	VIF values	Contribution (%)	Estimate	Std. Error
WT	0.401	1.517	0.401	0.089	0.105
D	0.920	1.284	0.005	0.010	0.097
Trans	0.116	1.782	1.666	0.180	0.114
DO	0.197	1.697	1.070	−0.144	0.111
NO_2^--N	0.201	1.561	0.963	0.137	0.107
NO_3^--N	0.011	1.47	3.632	0.26	0.103
TP	<0.001	1.889	8.664	−0.411	0.117
COD_{Mn}	0.003	1.598	5.436	0.326	0.108
TSS	0.860	1.411	0.016	0.018	0.100
Evenness	<0.001	1.307	66.933	−1.143	0.097
FDiv	<0.001	1.354	7.591	−0.385	0.101
FEve	0.007	1.333	3.900	−0.276	0.100
FRic	0.621	1.322	0.124	0.049	0.099

Table Notes: WT: water temperature; D: water depth; Trans: transparency; DO: dissolved oxygen; NO_2^--N: nitrite nitrogen; NO_3^--N: nitrate nitrogen; TP: total phosphorus; COD_{Mn}: permanganate index; TSS: suspended solids; Evenness: evenness; FDiv: functional divergence; FEve: functional evenness; FRic: functional richness.

With reference to previous studies (Litchman and Klausmeier, 2008; Otero et al., 2020; Zhou et al., 2022), we selected ten categories of traits related to phytoplankton morphology, growth and reproduction, and habitat characteristics, including algal size, morphology, motility, reproduction, nitrogen-fixing capacity, and habitat suitability traits. These traits are closely related to phytoplankton resource acquisition, predator avoidance, and reproduction (Table 1). Phytoplankton trait data and density data were then used to calculate functional richness (FRic), functional evenness (FEve), and functional divergence (FDiv) of the phytoplankton community in the "FD" package of the R language. Among the indices, FRic refers to the volume of multidimensional space occupied by all species in the functional space of the community. It reflects the degree of utilization of ecological space, with a larger index indicating a higher degree of utilization. (2) FEve is defined as the uniformity of the distribution of functional traits of organisms in a community in an ecological space. The larger the index, the more comprehensive the utilization of effective resources, and the higher the efficiency of resource utilization. (3) FDiv reflects changes in biological characteristics within a community, as well as the degree of ecological niche differentiation and the degree of competition for resources. A higher FDiv indicates a higher degree of complementarity among the organisms within the community, accompanied by a weaker degree of competition.

TABLE 3 Multiple regression analysis models based on predictor variables (numerous environmental factors and biodiversity indexes) and response variable RUEzp. The "Contribution" column in the table quantifies the contribution of a single explanatory variable to the multiple linear regression model of the dependent variables RUEzp.

Variable	P	VIF values	Contribution (%)	Estimate	Std. Error
WT	0.098	1.517	3.892	0.278	0.166
D	0.267	1.284	1.473	−0.171	0.153
Trans	0.460	1.782	0.903	−0.134	0.180
DO	0.190	1.697	2.770	−0.234	0.178
NO_2^--N	0.821	1.561	0.074	0.038	0.169
NO_3^--N	<0.001	1.471	19.483	−0.622	0.164
TP	0.826	1.889	0.084	−0.041	0.186
COD_{Mn}	0.753	1.598	0.148	0.054	0.172
TSS	0.625	1.411	0.311	0.079	0.160
Evenness	<0.001	1.307	71.535	1.191	0.159
FDiv	0.188	1.354	2.295	0.213	0.161
FEve	0.778	1.333	0.101	−0.045	0.159
FRic	0.418	1.322	0.823	0.128	0.157

Table Notes: WT: water temperature; D: water depth; Trans: transparency; DO: dissolved oxygen; NO_2^--N: nitrite nitrogen; NO_3^--N: nitrate nitrogen; TP: total phosphorus; COD_{Mn}: permanganate index; TSS: suspended solids; Evenness: evenness; FDiv: functional divergence; FEve: functional evenness; FRic: functional richness.

2.5 Resource use efficiency

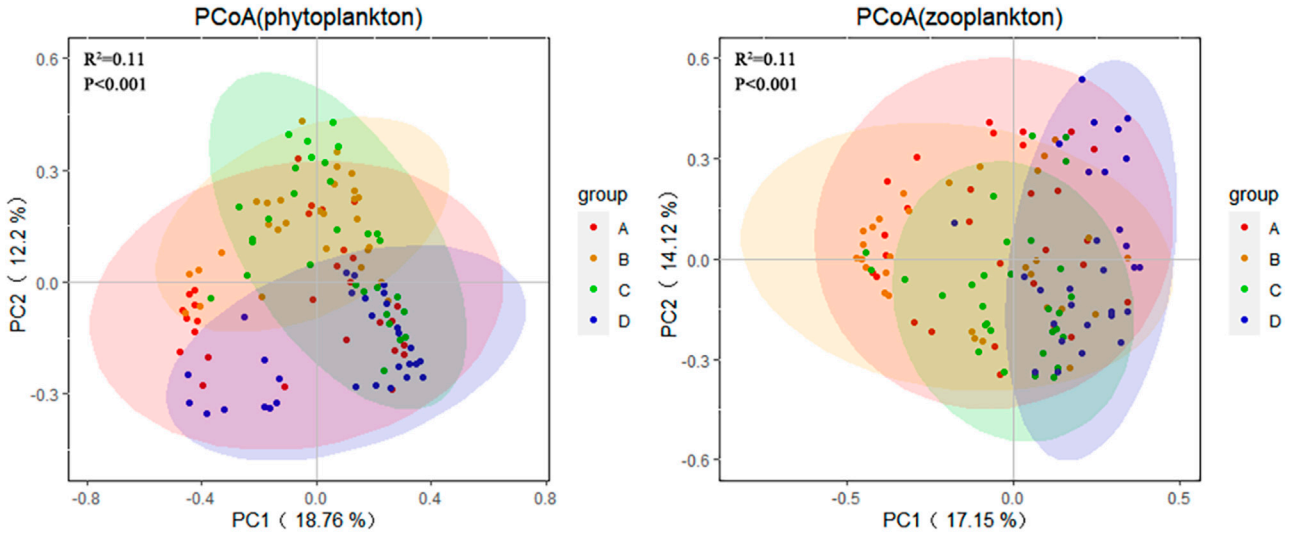
We used resource use efficiency (RUE) to quantify the ratio of realized resources to potential productivity as a metric for river ecosystem functioning (Hodapp et al., 2019). Total phosphorus (TP) was used as a proxy for potential phytoplankton productivity, while phytoplankton biomass represented the realized resources. Phytoplankton resource use efficiency (RUEpp) was expressed as the ratio of phytoplankton biomass to TP concentration. On the other hand, zooplankton resource use efficiency (RUEzp) was calculated as the ratio of zooplankton biomass (realized resource) to phytoplankton biomass (potential productivity) (Ptacnik et al., 2008; Filstrup et al., 2014; 2019). The specific equations are as follows:

$$\text{RUEpp} = \text{phytoplankton biomass}/\text{TP}$$
$$\text{RUEzp} = \text{zooplankton biomass}/\text{phytoplankton biomass}$$

2.6 Statistical analyses

Two multiple linear regression models were constructed using the "lm" function in R to assess the impact of the predictor variables—environmental factors and biodiversity—on the response variables, specifically RUEpp and RUEzp. Sixteen environmental variables and five biodiversity indicators were included in the models: water temperature (WT), water depth (D), transparency (Trans), turbidity (Tur), pH, electrical conductivity (EC), dissolved oxygen (DO), ammonia nitrogen (NH_3^--N), nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), total nitrogen (TN), total phosphorus (TP), phosphate (PO_4^{3-}-P), permanganate index (COD_{Mn}), suspended solids (TSS), chlorophyll-a (Chl-a), species richness, evenness, functional divergence (FDiv), functional evenness (FEve), and functional richness (FRic). The analysis quantified the overall explanatory power of these variables on RUE and determined the relative contribution of each predictor, calculated as the square of its regression coefficient divided by the sum of the squares of all regression coefficients (Johnson and Lebreton, 2004). The Shapiro-Wilk normality test of normality indicated a non-normal distribution of RUEpp and RUEzp, which prompted a logarithmic transformation. All variables were standardized using Z-scores prior to regression analysis. The vif function of the "car" package assessed multicollinearity through variance inflation factor (VIF) values, and Pearson correlation coefficients were calculated to assess relationships between variables, facilitating the identification and resolution of multicollinearity.

Based on the Bray-Curtis distance calculated for plankton community composition, we used Principal Coordinate Analysis (PCoA) and PERMANOVA in the "vegan" package in R to test whether there were significant multivariate differences in phytoplankton and zooplankton community structure and assembly between seasons. The *post hoc* function pairwiseAdonis was used for multiple comparisons between seasons in the PERMANOVA analysis (Sun et al., 2023). One-way ANOVA was used to assess variability in RUE between seasons, while the Kruskal-Wallis test was used to assess differences in relative phytoplankton abundance between seasons. Results were presented as box plots. Watershed boundaries and stream systems were depicted using digital elevation modeling in ArcGIS version 10.8. The calculation of phytoplankton evenness and species richness was conducted in the R language "vegan" package, and the phytoplankton functional richness (FRic), functional evenness (FEve), and functional divergence (FDiv) were implemented in the package "FD". We used Pearson's correlation analyses to



pairs	R²	p.adjusted
A vs B	0.06848961	0.0012
A vs C	0.06068111	0.0012
A vs D	0.07233302	0.0012
B vs C	0.04214869	0.0020
B vs D	0.11870265	0.0012
C vs D	0.10252026	0.0012

pairs	R²	p.adjusted
A vs B	0.03455683	0.0230
A vs C	0.06657738	0.0012
A vs D	0.09670409	0.0012
B vs C	0.07075866	0.0012
B vs D	0.11444848	0.0012
C vs D	0.10009566	0.0012

FIGURE 2
Principal coordinate analysis (PCoA) and PERMANOVA tests for phytoplankton and zooplankton communities in four seasons based on Bray-Curtis distance. Different graph colors represent different seasons: A is spring, B is summer, C is autumn, and D is winter, and $p < 0.05$ is considered significant. The horizontal and vertical axes represent the first and second principal coordinates and their contribution to community differences.

determine the relationship between dominant algae and RUE, and the direction and strength of the BEF relationship, with predictor variables standardized for Z-scores before analysis. Pearson's correlation analyses, one-way ANOVA, Kruskal-Wallis tests, correlation heatmaps, and box plots were implemented in the Origin 2023 software, with significance levels set at $p < 0.05$, and the rest of the analyses were performed in R language (4.3.1).

3 Results

3.1 Contribution of environmental factors and biodiversity

After calculating the VIF values and the correlation coefficients between the variables, we found that the VIF values of all 21 variables were less than 10. Therefore, we addressed multicollinearity based on the correlation coefficients by discarding one of the variables with correlation coefficients higher than 0.5 to ensure the model's robustness (Appendix S3). The removed variables included turbidity (Tur), pH, electrical conductivity (EC), ammonia nitrogen (NH_3^--N), total nitrogen (TN), chlorophyll-a (Chl-a), and species richness (Richness). The results of the multiple linear regression showed that the adjusted R^2 of the RUEpp and RUEzp models were 0.658 and 0.499, respectively, indicating that the models were able to explain a considerable portion of the variation in the data. The higher the contribution, the more the independent variable explains the variation in the dependent variable. In the models, evenness had the most significant explanatory effect on both RUEpp and RUEzp, with contributions as high as 66.933% and 71.535%, respectively, which were statistically highly significant ($p < 0.001$). Among the functional diversity indicators, functional divergence made the largest contribution, with the degree of explanation for RUEpp being particularly important. Additionally, TP contributed the most to RUEpp among environmental factors, while NO_3^--N contributed the most to RUEzp (Tables 2, 3).

3.2 Differences in plankton community composition and RUE across seasons

The plankton community composition collected from the Xiaoqing River during the four seasons tended to cluster separately. The phytoplankton and zooplankton community compositions differed significantly among seasons (R^2 = 0.113,

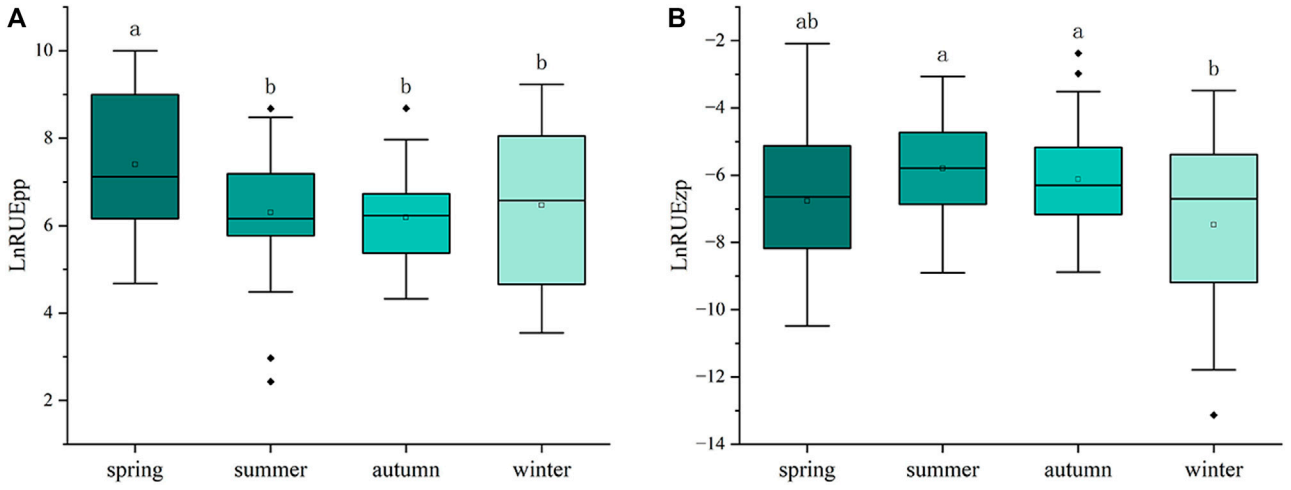


FIGURE 3
Box plots depicting the Resource Use Efficiency (RUE) of phytoplankton **(A)** and zooplankton **(B)** across spring, summer, autumn, and winter. One-way ANOVA was employed to assess the seasonal differences in RUE. To further determine specific differences between seasons, the Least Significant Difference (LSD) post-hoc test was performed. Different lowercase letters above each season indicate statistically significant differences ($p < 0.05$).

$p < 0.001$; $R^2 = 0.117$, $p < 0.001$), and the pairwise differences in phytoplankton community composition among all four seasons were also significant ($p < 0.05$), indicating that structure of phytoplankton and zooplankton assemblages was significantly affected by seasonal changes (Figure 2).

Significant differences were observed in RUEpp and RUEzp across seasons (Figure 3), with RUEpp being significantly higher in spring than in the other seasons. However, RUEzp was higher in summer and autumn compared to winter. In addition, the relative abundance of Bacillariophyta was significantly higher than other phyla in all four seasons, and they dominated the phytoplankton community (Figure 4), followed by the Chlorophyta and Cyanobacteria, whereas the species of Chrysophyta, Xanthophyta, Cryptophyta, Euglenophyta, and Dinophyceae accounted for a smaller total proportion. As the relative abundance of Bacillariophyta increased, RUE showed an increasing trend ($R^2 = 0.111$, $p < 0.001$), while RUEzp was negatively correlated ($R^2 = 0.164$, $p < 0.001$) (Figure 5). This finding indicates that the increase in the Bacillariophyta population significantly contributed to RUEpp, while it significantly reduced RUEzp.

3.3 Effect of taxonomic diversity on resource use efficiency

RUEpp showed a decreasing trend with increasing evenness in all seasons except autumn (spring: $R^2 = 0.672$, $p < 0.001$; summer: $R^2 = 0.372$, $p < 0.001$; winter: $R^2 = 0.671$, $p < 0.001$). In contrast, RUEzp exhibited an increasing trend with increasing evenness in spring, summer, and winter (spring: $R^2 = 0.488$, $p < 0.001$; summer: $R^2 = 0.208$, $p = 0.006$; winter: $R^2 = 0.557$, $p < 0.001$) (Figure 6). These results suggest that the dominant phytoplankton species (e.g., Bacillariophyta) exhibit specific physiological characteristics and thus utilize resources more efficiently, but the acquired resources were not effectively transferred to the next trophic level.

3.4 Effect of functional diversity on resource use efficiency

Except in winter, RUEpp showed a significant negative correlation with FDiv (spring: $R^2 = 0.258$, $p = 0.002$; summer: $R^2 = 0.216$, $p = 0.005$; autumn: $R^2 = 0.167$, $p = 0.014$), whereas RUEzp was significantly positively correlated (spring: $R^2 = 0.183$, $p = 0.010$; summer: $R^2 = 0.167$, $p = 0.014$; autumn: $R^2 = 0.221$, $p = 0.005$) (Figure 7). These results suggest that the higher the degree of divergence in phytoplankton functional traits, the lower the RUEpp, whereas zooplankton feeding on a more functionally diverse phytoplankton assemblage exhibited a higher RUE.

4 Discussion

Although most BEF-related studies have focused on single sampling and single taxa, our study investigated the BEF relationship across trophic levels under different seasons. Our results demonstrated that the increase in the relative abundance of dominant species caused by Bacillariophyta blooms significantly impacted the RUEpp and RUEzp. Meanwhile, phytoplankton evenness negatively affected RUEpp but positively affected RUEzp. Similar correlation trends were also reflected in the functional divergence index. We evaluated the roles of environmental factors and biodiversity in explaining variations in RUE, among which evenness emerged as the most significant contributor.

The results of the multiple linear regression modeling showed that evenness had a significant effect in both RUEpp and RUEzp models and was the main factor explaining the variation. This is in agreement with Hodapp et al. (2015) who found that evenness was a key driver of phytoplankton productivity and RUE compared to environmental factors such as temperature and light. In communities with lower evenness, a few dominant species have a competitive advantage and are able to use resources more efficiently

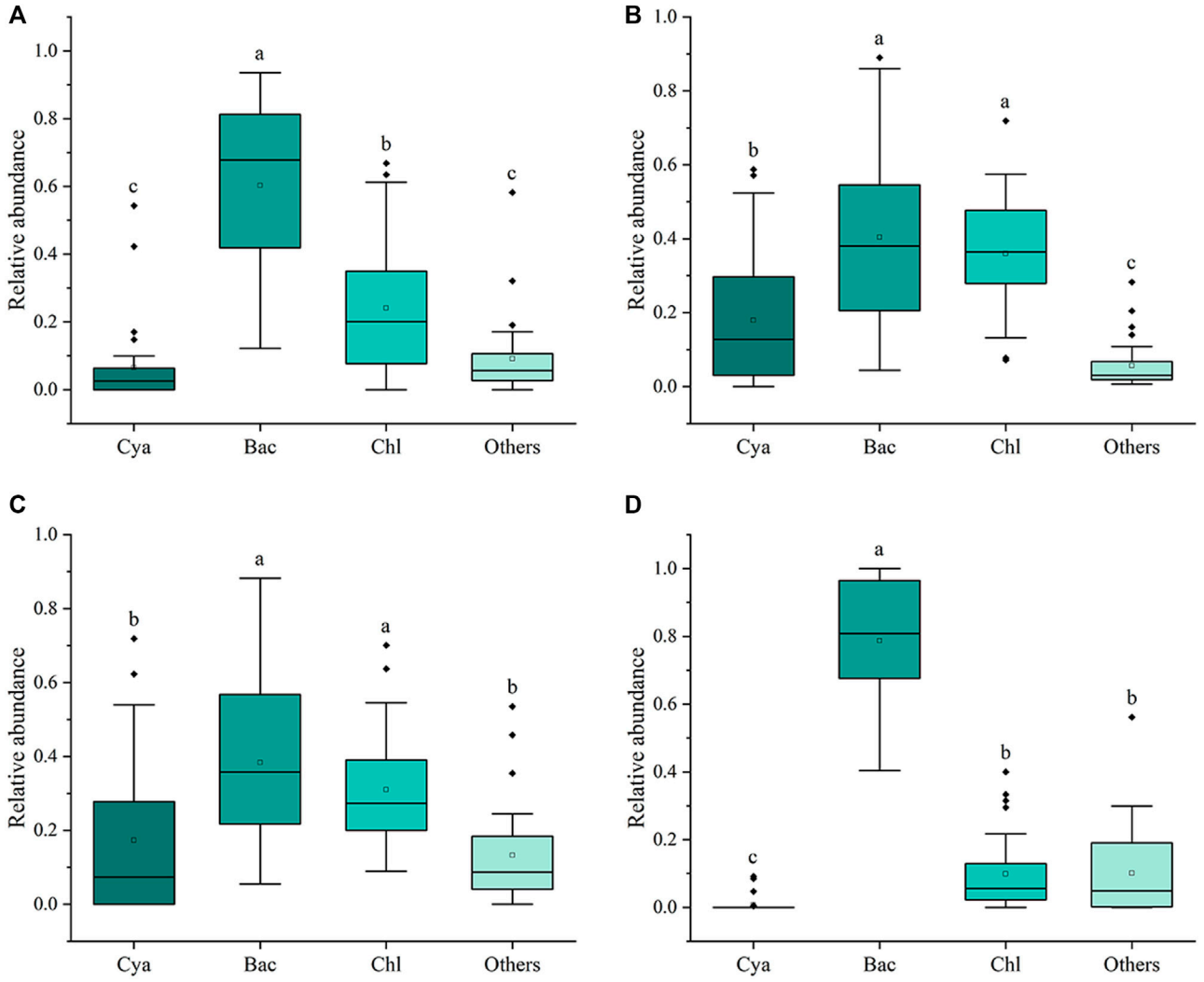


FIGURE 4
Box plot showing the relative abundance of phytoplankton phyla under four seasons: spring **(A)**, summer **(B)**, autumn **(C)**, and winter **(D)**, where Cya represents the Cyanobacteria, Bac means the Bacillariophyta, Chl means the Chlorophyta, and Others means the total relative abundance of the Chrysophyta, Xanthophyta, Cryptophyta, Euglenophyta, and Dinophyceae. Significant differences are indicated by different lowercase letters ($p < 0.05$).

due to their higher physiological or chemical adaptations (Hodapp et al., 2015). In contrast, higher phytoplankton evenness may mean that zooplankton have a more diverse food source, which not only provides richer nutrients, but also improves zooplankton growth and reproductive efficiency (Wang et al., 2024).

In the RUEpp model, NO_3^--N and COD_{Mn} had positive effects on resource use efficiency, while total phosphorus (TP), evenness, functional divergence and functional evenness all had negative effects. High phosphorus levels in eutrophic waters can make nitrate a limiting factor, and as nitrate levels increase, resource use efficiency can be improved by supporting more phytoplankton growth (He et al., 2021). Meanwhile, higher COD_{Mn} values indicate the presence of more biodegradable substances in the water body, which may release additional nutrients (e.g., nitrogen and phosphorus) during degradation that can be used by phytoplankton to further improve resource use efficiency (Reinl et al., 2022). The possible reason for the negative correlation of functional divergence is that under eutrophication conditions, functional convergence of algae reduces ecological niche occupancy and ecosystem resources cannot be fully utilized (Zhang et al., 2019; Dunck et al., 2019). In contrast, in the RUEzp model, NO_3^--N had a significant negative effect on RUEzp, except for phytoplankton evenness, which had a significant positive effect. We hypothesize that the possible reason is that elevated nitrate-nitrogen concentrations usually contribute to the occurrence of algal blooms, in which some harmful algal species bloom and may not be consumed by zooplankton. In addition, algal blooms can cause rapid changes in water quality (e.g., oxygen depletion and toxin release) that stress zooplankton populations and reduce their growth and reproductive success (He et al., 2021).

Principal coordinate analysis revealed significant seasonal variations in the composition of phytoplankton and zooplankton communities. This finding indicates that the structure of riverine organisms is largely influenced by seasonal differences, such as temperature and flow. The results of the one-way ANOVA

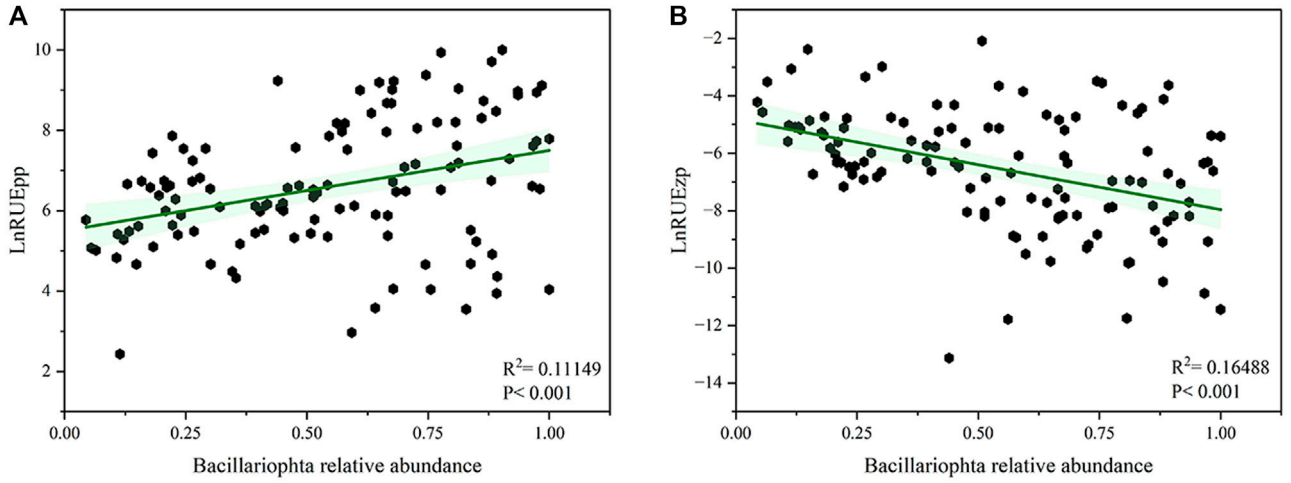


FIGURE 5
Relative abundance of dominant algae about phytoplankton resource use efficiency [RUEpp; **(A)**] and zooplankton resource use efficiency [RUEzp; **(B)**]. The predictor variable was the relative abundance of Bacillariophyta, and the response variable was transformed to a natural logarithm before analysis, and $p < 0.05$ was considered a significant difference.

showed that RUEpp and RUEzp also exhibited seasonal differences. Among the variables, RUEpp exhibited a significantly higher value in spring than in other seasons. This indicates that phytoplankton growth activity was higher in the spring, probably due to increased light and temperature, which significantly increased resource use efficiency. Furthermore, the higher RUEzp observed in summer may be attributed to the availability of phytoplankton as a food source, which in turn facilitates the growth and reproduction of zooplankton. For the study of algal bloom conditions in the Xiaoqing River, Bacillariophyta dominated the phytoplankton community during the sampling period and formed high-density algal blooms (density $>5 \times 10^6$ cells/L) several times. The strongest bloom was found in spring, which could reach up to class Ⅳ, followed by class Ⅲ in summer, and the weakest degree of bloom was found in autumn and winter. Compared with Chlorophyta and other higher aquatic plants, Bacillariophyta have a more efficient photosynthetic rate, faster exponential growth rate, and more robust salinity tolerance, which allows them to fully utilize water resources. In contrast, zooplankton feeding strategies favor algae such as Chlorophyta, which are more palatable, and avoid Bacillariophyta (Vincent and Bowler, 2020). Possible mechanisms include the release of substances by Bacillariophyta that can inhibit zooplankton predation (Malej and Harris, 1993), their hard siliceous shells, and the production of toxic aldehydes, which are also important predation avoidance mechanisms (Miralto et al., 1999). Furthermore, studies of eutrophic lakes have found that when blooms occur, RUEpp often increases while RUEzp decreases (Filstrup et al., 2014; Gao et al., 2022). If bloom algae with higher RUE and competitive advantages are well adapted to environmental conditions, they may produce greater RUEpp; simultaneously, zooplankton may reduce predation on these dominant species with lower nutrient quality (Hassett et al., 1997), thereby decreasing RUEzp.

The positive correlation between phytoplankton species richness and RUE has been widely demonstrated in the assessment of ecosystem functioning (Ptacnik et al., 2008; Striebel et al., 2009). However, evenness tends to have a better effect on ecosystem functioning than species richness, because it captures changes in the abundance of each species rather than just the loss and gain of species (Filstrup et al., 2019). Meanwhile, under dramatic environmental change, evenness can better reflect the rapid changes in the external environment, and higher evenness means that the abundance of species in the community tends to be more balanced and the whole tends to be more stable (Otero et al., 2020). Most current studies have focused more on the relationship between primary producer diversity and RUE and less on the RUE of herbivores and even higher trophic levels. Therefore, a comprehensive understanding of the effects of biodiversity requires the integration of both horizontal and vertical dimensions of biodiversity, especially when specific taxa with unique traits dominate primary producers (Duffy et al., 2007). Our results consistently showed that phytoplankton evenness is significantly correlated with RUEpp and RUEzp across seasons (although the strength of the correlation may be differ) and exhibits opposite trends, which is consistent with the findings of Filstrup et al. (2014) in eutrophic lakes. These findings can also be explained by ecological stoichiometry, i.e., when RUEpp is high, RUEzp decreases due to reduced trophic quality (Hassett et al., 1997). Notably, in contrast to other seasons, we found that the relationship between phytoplankton evenness and RUE was not significant in the autumn, speculating that this finding may be related to the large amount of tree litter input to the river from the riparian zone. As an essential source of carbon, nitrogen, and phosphorus, leaf litter can significantly increase phytoplankton biomass and chlorophyll content and promote photosynthesis (Zhang et al., 2018). However, large amounts of leaf litter can also form a cover on the water surface that reduces light transmittance, diminishes the amount of light available to phytoplankton in the water column, and reduces photosynthetic efficiency (Domingues et al., 2014). Some light-loving taxa, such as Microcystis and Scenedesmus, are reduced, while shade-tolerant species, such as

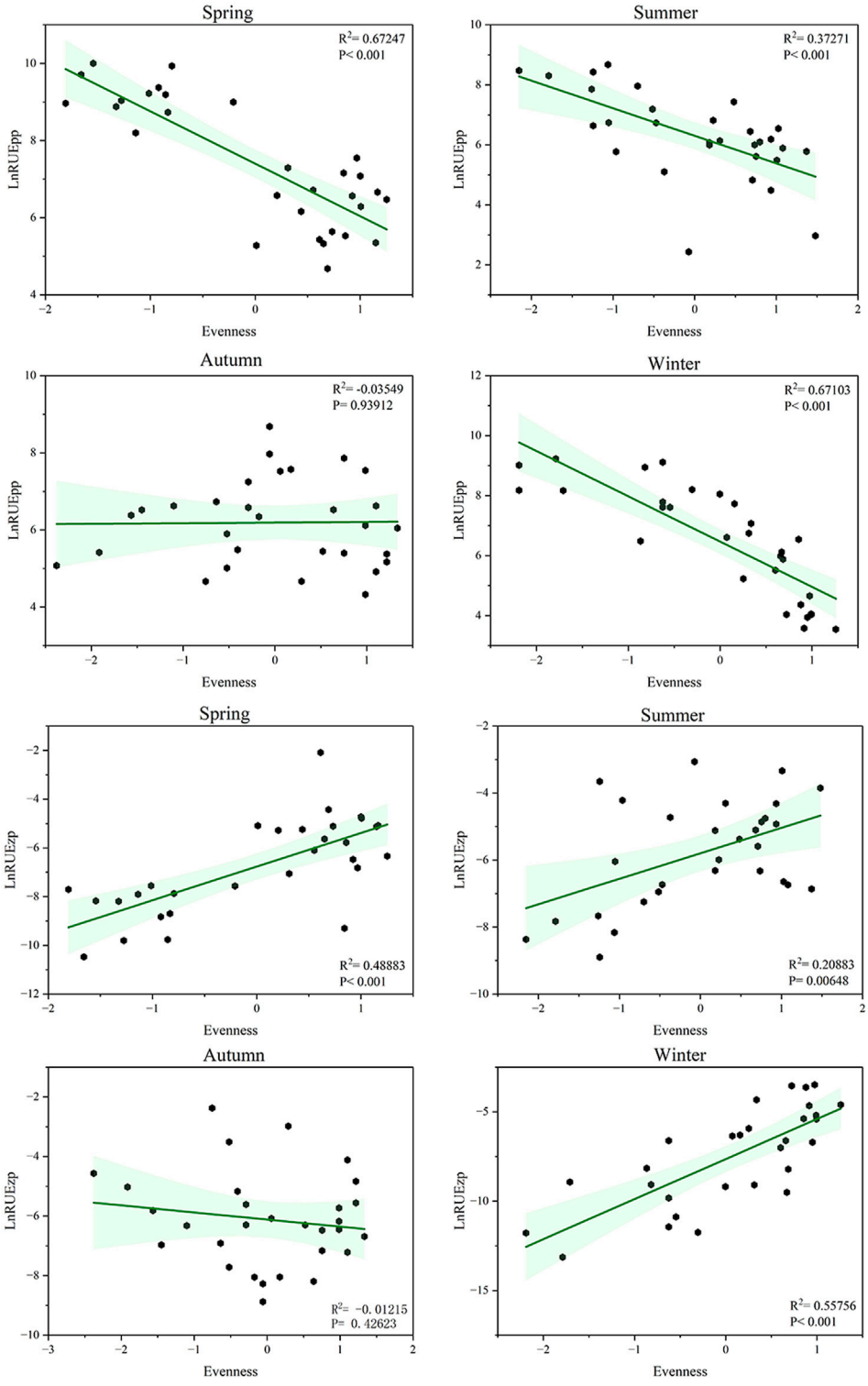


FIGURE 6
Relationship between taxonomic diversity and ecosystem function in Xiaoqing River in each season. The response variables were phytoplankton resource use efficiency (RUEpp) and zooplankton resource use efficiency (RUEzp), and the explanatory variable was phytoplankton evenness. Green shading represents 95% confidence intervals, and the response variables were transformed to natural logarithms before analysis.

Cryptomonas and Euglena, are benefitted (Reynolds et al., 2002; Padisák et al., 2009). Furthermore, leaf litter accumulation may lead to localized stagnation of the water column, affecting mobility, nutrient transport, and light distribution, disrupting vertical migration of phytoplankton, and ultimately limiting their distribution and growth (Li et al., 2013).

Individual ecology-based classification of phytoplankton functional groups can more accurately characterize habitats,

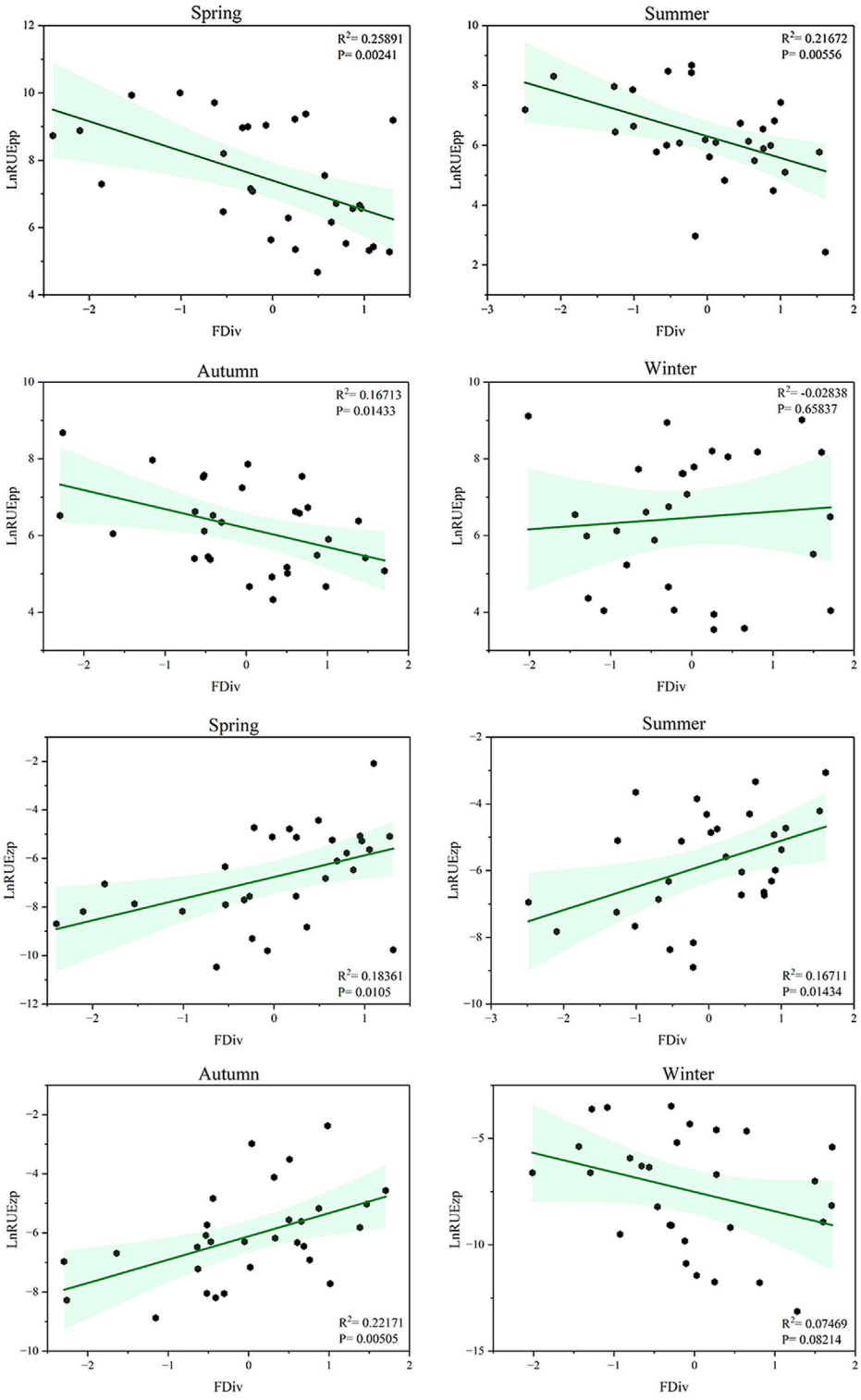


FIGURE 7
Relationship between functional diversity and ecosystem function in Xiaoqing River in each season. The response variables were phytoplankton resource use efficiency (RUEpp) and zooplankton resource use efficiency (RUEzp), and the explanatory variable was phytoplankton functional divergence index. Green shading represents 95% confidence intervals, and the response variables were transformed to natural logarithms before analysis.

reveal dynamic relationships between phytoplankton functional groups and the aquatic environment, and predict changes in ecosystem function (Abrantes et al., 2006; Borics et al., 2021). Our results are consistent with the hypothesis that FDiv has some explanatory power in predicting ecosystem functioning. Its response pattern is consistent with taxonomic diversity (evenness), i.e., FDiv has a negative correlation effect on RUEpp and a positive correlation on RUEzp, indicating that FDiv well reflects the

distribution of species in the functional trait space and their effects on RUE. The higher the FDiv of phytoplankton, the weaker the competition for resources among species, the more fully utilized the ecological niche space, and the higher the primary productivity generated, so that RUEzp exhibited an upward trend (Ptacnik et al., 2010). Several studies have demonstrated that functional diversity can be a better predictor of ecosystem properties because adding or subtracting species with similar functions has little effect on ecosystem functioning (Carmona et al., 2016; Abonyi et al., 2017; Gross et al., 2017; Ye et al., 2019). Due to the heterogeneity of ecosystem habitats and resources, communities with similar functions cannot achieve maximum resource utilization. In contrast, communities with greater functional differences can improve RUE and enhance ecosystem functioning (Cadotte et al., 2011). Therefore, the functional characterization approach is considered a more promising tool in stream ecosystem biomonitoring and management (Abonyi et al., 2017; Breton et al., 2017; Leruste et al., 2018). The present study demonstrated that both taxonomic and functional diversity were significantly correlated with ecosystem functioning. However, the model fit for taxonomic diversity was slightly better than that for functional diversity. This observation contradicts the results of previous studies, and this discrepancy may stem from the fact that our ability to quantify traits remains limited, and the trait profiles covered need to adequately capture slight differences in species at specific ecological niches. For instance, differences in light requirements, which may be a key trait in determining species' efficient use of heterogeneous resources within the environment, may need to be better captured, thus reducing the percentage of variance explained by functional diversity in RUE. Furthermore, trait-mediated effects due to interactions among taxa must be considered, which may limit the applicability of currently used traits (Klais et al., 2017).

Our study supports a bottom-up ecosystem regulatory mechanism in which resource use drives competition among species at the primary producer level, with more competitive populations gaining a resource advantage and dominating the community, i.e., selection effects. At the herbivore level, resource heterogeneity and differences in food selectivity drive changes in trophic transfer efficiency (Filstrup et al., 2019), and such interactions across trophic levels form a more complex and rich BEF relationship. In this study, only two groups of organisms were involved, and we should consider expanding the scope of the study based on field observations to investigate the BEF mechanism of organisms at higher trophic levels. Considering the advantages of studying BEF relationships based on functional traits, our results improve the quantitative description of phytoplankton functional traits in subsequent studies. This study contributes to a more comprehensive identification of various ecologically relevant traits for a more comprehensive assessment of river ecosystem functioning (Abonyi et al., 2017; Kremer et al., 2017). Furthermore, the findings of this study will provide theoretical guidance for the protection and management of eutrophic waters. Specifically, we propose the implementation of measures to control nutrient inputs, maintain the structural stability of phytoplankton communities, and strengthen ecosystem monitoring and assessment. These measures aim to slow down the eutrophication process, maintain the functional balance and diversity of primary producer communities, and thus improve the resource use efficiency and ecological service function of the whole ecosystem. These protection strategies should be further refined and a comprehensive eutrophication water management plan should be formulated and implemented with the ultimate goal of curbing eutrophication and realizing the improvement and restoration of the aquatic ecosystem.

5 Conclusion

In summary, this study has established the patterns and mechanisms between biodiversity and ecosystem function in natural phytoplankton communities under eutrophic conditions. The results verified our preliminary hypothesis that both phytoplankton evenness and FDiv significantly affect RUE and have opposite patterns at the different trophic levels. Due to the increase of dominant species caused by algal blooms, the abundance of each species tends to be unbalanced, the occupation of the trophic ecological niche decreases, and it becomes difficult to effectively transfer and utilize resources to the upper trophic level. When the dominant harmful algal blooms declined, a relative equilibrium state was reached among the species, the whole ecological niche was gradually utilized more comprehensively, and the ecosystem function was enhanced. Therefore, in order to effectively manage and protect the health of water bodies, we suggest controlling the input of nutrients and slowing down the eutrophication process; taking measures to restore and maintain an appropriate phytoplankton community structure and avoiding the absolute dominance of a single species; and strengthening the monitoring and assessment of the structural and functional indicators of phytoplankton communities and formulating corresponding protection management and strategies. This study provides important insights into the study of ecosystem functioning across trophic scales. It supports the view that increased ecological niche utilization can improve resource use efficiency, providing an important reference for subsequent studies of BEF mechanisms at higher trophic levels.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

MM: Conceptualization, Formal Analysis, Methodology, Software, Visualization, Writing–original draft, Writing–review and editing. JL: Data curation, Methodology, Software, Writing–review and editing. AL: Data curation, Methodology, Software, Writing–review and editing. PZ: Investigation, Visualization, Writing–review and editing. XY: Funding acquisition, Project administration, Supervision, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2024.1389220/full#supplementary-material

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Contaminated freshwater as a Harbinger of tropical disease spread in Europe

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Human-induced environmental changes, including climate change and pollution, significantly affect host-parasite interactions, potentially altering the geographical spread and severity of various parasitic diseases. These changes may particularly influence the dynamics of diseases like schistosomiasis, posing significant public health concerns. This review explores how pollutants such as organophosphate pesticides, antibiotics, heavy metals, cities' landfills, and microplastics can affect the development and transmission dynamics of parasites, especially *Schistosoma* spp. Our researches highlight that pesticides promote parasitic disease development, while pharmaceuticals have mixed effects on the life cycles of these parasites. Similarly, heavy metals found in water systems disrupt host-pathogen interactions, and microplastics are linked to significant changes in snail stressor genes, a critical intermediate host for several parasites. With the rising impacts of anthropogenic activity on the environment, there is an urgent need to reassess and adjust regulatory policies to minimize these threats. By studying the implications of pollution on host-parasite interactions, we can develop better strategies for disease control and improve the preservation of our ecosystem's health.

KEYWORDS

water quality, aquatic ecosystems, environmental parasitology, *Schistosoma* spp., chemical pollutants

1 Introduction

Freshwater ecosystems, including lakes, rivers, streams, wetlands, and ponds play a crucial role in regulating biodiversity, water quality, nutrient cycling, and mitigating the impacts of droughts providing crucial ecosystem services to humans (Gray et al., 2022). These ecosystems are worldwide distributed and figure among the most diverse habitats on the planet while dealing with faster degradation than terrestrial ones (Turak et al., 2017). Freshwater species populations have experienced a staggering decline of 84% since 1970 (McRae et al., 2020), mainly as a consequence of water contamination by human activities (Aryal et al., 2020; Albert et al., 2021). Four main sources of chemical contamination can be identified: Firstly, agricultural contamination via the use of pesticides to control pests, the use of which is regulated, however, these substances can persist and run-off, contaminating the environment for long periods (Syafrudin et al., 2021). Secondly, industrial chemicals, including micro- and nano-plastics, heavy metals, and organic compounds used on production lines. Thirdly, chemicals used in the health sector, such as antibiotics, antidepressants, and endocrine disruptors, among others, are found in trace amounts in

the environment. Finally, the full range of substances that can be detected in cities, including organic contaminants, microplastics, or products contained in cigarette butts. Depending on the conditions and the substance in question, they can contaminate several links in the trophic chain and their persistence in the environment depends on physico-chemicals factors (Chevalier et al., 2016; Tudi et al., 2021).

The disruption of ecosystem balance as a consequence of the exacerbation of climate crisis intensify the situation further. Rising temperatures associated to unbalance water cycle have serious consequences, not only for natural ecosystems but also for agriculture, industry and health sector, as they lead to more frequent, severe, and prolonged extreme events (VijayaVenkataRaman et al., 2012). Stratification, water levels, and flow regimes are all significantly impacted with broad-ranging impacts on biodiversity (Birk et al., 2020; Sures et al., 2023).

The emergence of freshwater zoonotic diseases, infectious maladies transmissible between animals and humans, has witnessed a notable increase. Approximately 60% of established infectious diseases and up to 75% of novel or emerging infectious diseases are estimated to have zoonotic origins, either transmitted or not transmitted by freshwater (Salyer et al., 2017). The dynamic interactions among humans, animals, and pathogens cohabiting the same environment align with a "One Health" approach, emphasizing interconnections between human, animal, and environmental health—a pivotal aspect of disease control and prevention strategies (Kahn, 2011). This concept is based on the sophisticated principle that protecting human health involves protecting the health of animals and their interactions with the environment, and is driven by three main objectives; vigilance against infectious diseases, combat resistance to anti-infectives, and recognize the disruptions in our environment that are likely to encourage the emergence of new diseases (Destoumieux-Garzón et al., 2018; Parodi, 2021). Freshwater-snail-borne parasites, such as those causing schistosomiasis, play instrumental roles in species biodiversity contributing to the dynamics of natural ecosystems (Sures et al., 2023). It strives to comprehend how environmental conditions, freshwater pollution between them, impact parasite life cycles, and their evolution, including their effects on host behaviour, physiology, and population dynamics, as well as their influence on food webs and ecosystem functioning (Nachev and Sures, 2016; Sures et al., 2017a). By unravelling the environmental factors that influence parasites spreading, it is possible to develop strategies for disease prevention, control, and management. However, new approaches are needed to counter the progression of freshwater diseases, which are on the rise in Europe (Levy et al., 2018; Semenza et al., 2022). Although some studies have examined the impact of pollution on vector biology, further research is required to understand its effect on the pathogens themselves. Parasites, such as schistosomes, pose an enduring threat to organisms across various phyla, with their virulence defined as the impact on their host's fitness, being shaped by host-parasite combinations and environmental conditions. In this intricate interplay, biological and chemical features act as drivers emerging as a potential factor affecting parasite virulence, a still neglected aspect of water pollution effect (Augusto et al., 2019). Systems biology approaches, combining functional genomics, offer promising methods to address challenges by elucidating the molecular mechanisms underlying host-pathogen interactions in changing environmental scenarios. A key area of research in functional genomics is the use of CRISPR-based technology to edit vector host or pathogen genomes, potentially leading to more effective treatment and prevention strategies for infectious diseases. Despite the difficulties and uncertainties associated with using CRISPR-based methods in mosquito-borne malaria models, it is recognized that there is a need for further technological advancements in vector hosts or their associated parasites in the context of freshwater-related diseases (Tajudeen et al., 2023).

This review elucidates the influence of freshwater quality and its potential ramifications on the spread of waterborne diseases in Europe. Concentrating on Schistosomiasis, a parasitic ailment traditionally confined to Africa, Asia, and Central/South America, but recently observed in France (Corsica), we explored the repercussions of freshwater pollution on the snail-parasite relationship that potentially impacts disease dissemination within Europe.

2 Study topic: *Schistosoma* and pollutants

2.1 Schistosomiasis (importance, consequences, and distribution)

Schistosomiasis or bilharziasis is the world's second most common parasitic disease. This parasitic anthropozoonosis disease is endemic to sub-Saharan Africa, Central and South America, and Asia which recently spread to countries in temperate zones as a result of migratory movements, globalisation, and tourism (Tchouanguem et al., 2016). Currently, this disease is progressing in Europe and has already been endemic in Corsica for several years, responsible for multiple contaminations every year (Berry et al., 2016; Kincaid-Smith et al., 2017; Gabrielli and Garba Djirmay 2023). Several species of parasites are responsible for this disease, two of which are the majority in terms of infection, but they differ in their intermediate hosts. First of all, *Schistosoma mansoni*, (Silva, 1908), transmitted by the snail *Biomphalaria* spp., (Say, 1818) is mainly studied because it is widespread in Africa and present in Latin America. Secondly, *Schistosoma haematobium* (Bilharz, 1852), having the snail *Bulinus* spp. (Müller OF Geschichte der Perlen-Blasen) as a vector, which is mainly present in sub-Saharan Africa and that tends much more to migrate towards Europe, already present in Corsica, Spain, Portugal, Greece, and Italy (Gabrielli and Garba Djirmay 2023). These two parasites mainly have humans as definitive hosts but can also affect other mammals which may also contribute to human infections (McManus et al., 2018; Liang et al., 2022). In humans, Schistosomiasis can cause numerous symptoms, infecting the intestine, liver, kidneys, and genitals, causing inflammation in both men and women and even death (McManus et al., 2018). According to the World Health Organization (WHO), in 2021, at least 251.4 million people needed preventive treatment against Schistosomiasis, but only a fifth received it, demonstrating that new measures need to be put in place to counter the advance of this disease and reduce the number of deaths (Kokaliaris et al., 2022). Currently, the fight against schistosomiasis aims to reduce the number of sufferers through the development of new treatments and improved sanitary conditions, which only serve to reduce transmission (Kokaliaris et al., 2022).

This conjuncture will only intensify in the years to come, with the amplification of climate change which promotes the transmission and

spatio-temporal distribution of parasites by affecting their reproduction, their duration of maturation, and the incubation period of which they need to become their preferred vector (Semenza et al., 2022). The increase in migratory flows contribute also to extending the geographical distribution of schistosomiasis (Aagaard-Hansen et al., 2010) underlining the need to find solutions to curb this parasitic disease. Moreover, warmer climates are also associated with faster disease transmission as well as the persistence of more virulent parasitic strains (Altizer et al., 2013; Cable et al., 2017). In addition, genetic mixtures between species of schistosomiasis can also take place, producing hybrids like those found in Corsica (Noël et al., 2018). These hybridization phenomena promote contamination or extend the range of hosts that can be affected, which further complicates the determination of sources and the methods of action to fight against epidemics (Gabrielli and Garba Djirmay 2023). All of these factors make this disease, no longer tropical but global, a major public and veterinary health problem (Berger et al., 2022).

The situation is all the more alarming from the point of view of aquatic ecosystems, which represent important poles of biodiversity (Gray et al., 2022). As the life cycle of these parasites is closely linked to aquatic environments, their state under the effect of climate change is central to understanding the evolution of this disease and finding solutions. By their very nature, freshwater ecosystems are subject to many fluctuations in physico-chemical parameters (water availability, temperature, salinity, and pH), which affect both the parasite and its host population dynamics (Woodward et al., 2010). Anthropogenic modifications to these ecosystems add a new level of complexity, affecting these populations in deleterious or healthy ways, mainly through the expansion of agriculture, which leads to the contamination of these environments (Prajapati et al., 2022). Indeed, under the effect of higher temperatures, the parasitic life cycle could be affected at different layers, the populations of snails or cercariae can thus increase in numbers but the lifespan of the snails can be found reduced, also reducing the period of release of the cercariae. In addition, the presence of fertilisers and pesticides facilitates the development of food resources, such as algae (periphyton) as well as the development of intermediate hosts (Douchet et al., 2023) and could also increase parasites' virulence (Mennerat et al., 2010). Other factors are more deleterious in the overall dynamics of transmission, such as predation or competition (Douchet et al., 2023). Faced with all these factors, the host's metabolism and immune system are decisive in the response to infection: tolerance, resistance, or susceptibility (Downs et al., 2019). Despite these numerous findings, it is necessary to consider the net effects on the entire life cycle of the parasite as well as the entire ecosystem to glimpse the links between climate change and the development of parasitic diseases within aquatic ecosystems (Marcogliese et al., 2015).

2.2 Effects of the different pollutants on host-parasite interactions

Numerous studies have shown that the presence of chemical compounds in the environment can also have synergistic or antagonistic effects on parasite development (Table 1). Organophosphate pesticides have an impact on the development of parasitic diseases, either by promoting or hindering their development cycle, as in the case of schistosomiasis (Sures et al., 2017b). Indeed, it has been shown that Esfenvalerate, authorised in Europe, can promote the disease by reducing snail predators, thus favouring their biomass, or can negatively impact snail-susceptible competitors such as Macrobrachium shrimp species, thus increasing their abundance and the rate of trematode transmission (Haggerty et al., 2023). There is also evidence that Glyphosate, also authorised in Europe, reduces transmission by affecting snail reproduction (Hoover et al., 2020). On the other hand, the pesticides Atrazine, Chlorpyrifos and Profenofos, present in ecosystems even though they are not authorised in Europe, have demonstrated an impact on the dynamics of algae, affected by these substances, thus favouring snail populations and amplifying transmission. At the same time, predator mortality is increased, which also favours transmission (Hoover et al., 2020). Snail hosts have also been shown to have a high tolerance to even high concentrations of pesticides, which, combined with the effects of these substances on predators and the food reservoir of this intermediate host, further increases the risk of disease transmission (Ganatra et al., 2023).

These effects are likely to be amplified by the eutrophication of environments due to the migration of nutrients from crops to water (increase in population densities, land occupation), which is constantly growing and leading to increased exposure to trematode cercariae (larval forms that infect the definitive host). However, these observations remain highly context-dependent (type and concentration of pesticide, distance from agriculture, population status, predation, climatic factors, etc.). Besides the effect of pesticides on the host-parasite relationship, there are other chemical contaminants due to anthropogenic activity, such as pollution from health facilities, cities and industry that could also impact it. Pollution related to human health is commonly linked to pharmaceuticals and personal care products (PPCPs), hormones, and endocrine disruptors released into the environment. One of the components of PPCPs easily found in freshwater is antibiotic residues (Danner et al., 2019). A recent study showed the influence of antibiotics on parasite dynamics. This study explored the effect of tetracycline, a common broad-spectrum antibiotic, on the host-parasite life cycle of the parasite *S. mansoni* and its intermediate host, *Bi. glabrata*. They studied the impact of an ecologically relevant low-dose concentration on the host and parasite. Hosts affected by tetracycline contamination showed an increase in parasite production as the infection developed. Subsequently, the presence of antibiotics increased egg-laying in snails, potentially making new hosts for the parasite (Melchiorre et al., 2023). Another study showed the impact of polyhexamethylene biguanide hydrochloride (PHMB) used as a disinfectant and antiseptic, capable of inhibiting the embryonic hatching of *Bi. glabrata* (de Oliveira Melo et al., 2019; de Oliveira Melo et al., 2019). Another study demonstrates the harmful effects of antiviral drugs that are often released into the environment. Lamivudine and stavudine were found to have a stimulatory effect on *Bu. tropicus* embryonic development, producing mean embryo lengths greater than controls. In contrast, efavirenz and nevirapine showed an overall inhibitory effect on embryonic growth. Antiretroviral exposure to *Bu. tropicus* affected embryo length, hatching and mortality. The influence of these antiretrovirals on the development and growth of the snail vector confirms the need for further studies into the ecological impact of these pharmaceutical compounds (Bouwman et al., 2020).

Meanwhile, cities represent a substantial part of global pollution with residential and industrial processes. First, polycyclic aromatic hydrocarbons (PAHs) from organic material combustion or

TABLE 1 Summary of the main chemical substances from four main areas.

Source of the pollutant	Chemicals	Type of pollutant	Biological impact on host-parasite interaction	References	Legislation in Europe
Agriculture	Glyphosate	Herbicides (Glycine derivatives)	Affect snail reproduction	Hoover et al. (2020)	Authorised
	Atrazine	Herbicides (Triazine)	Affect the algal community		Unauthorised
	Chlorpyrifos, profenofos	Insecticides (Organophosphorus - OPPs)			
	Esfenvalerate	Insecticides (Pyrethroid)	Reduce snail predators and Negative effect on *Macrobrachium* shrimp species (predators)	Haggerty et al. (2023)	Authorised
	Λ-cyhalothrin, permethrin				Unauthorised
	Chlorpyrifos, terbufos	Insecticides (OPPs)			Unauthorised
	Malathion				Authorised
Industries	Cadmium	Metal ions	Increasing *Biomphalaria alexandrina* snails infection rate by *Schistosoma mansoni*	El-Din et al. (2010)	Unauthorised
	Lead	Metal ions	Reduction in *Schistosoma* worm burden, tissue egg load and ova excretion/Increase oxidative stress of *Schistosoma* infected host	El-Gohary, Yassin, *et* Shalabya (2003)	Unauthorised
Health	Tetracycline	Antibiotic	Increase, *Schistosoma* parasite production and increased *B. glabrata* egg-laying in snails	(Melchiorre et al., 2023)	Authorised
	Polyhexamethylene biguanide hydrochloride	disinfectant and antiseptic	Inhibit the embryonic hatching of *B. glabrata*	de Oliveira Melo et al. (2019)	Authorised
	Lamivudine and stavudin	antiviral drugs	Increases the average size of embryos of *B.tropicus*	Bouwman et al. (2020)	Authorised
Cities, Industries	Microplastics, macroplastics, Nanoplastics	Cigarette butts, bottles, food packagings	Alteration in the expression stress response genes in *B. glabarata*. (HSP70, CYP450, and MIF)	Merrill (2022)	Authorised
Cities, Industries, Agriculture	Polycyclic aromatic hydrocarbons (PAHs)	Cigarette butts, wood combustion, vehicular emissions, gas flaring	Increase in ROS production, protein carbonylation and DNA damage of land snails, presence detected in *B. bulinus*	Ediagbonya et al. (2022) Dobaradaran et al. (2020) Itziou et al. (2011); Kaloyianni et al (2011); Dimitriadis et al. (2011)	Authorised

Agriculture, industry, health and cities - authorised in Europe, which may have a biological impact on *Schistosoma* spp. or its vector. Pesticides use authorizations determined by the European Commission's, and European Union's official website.

discarded cigarette butts have been detected in elevated levels in snails, including *Bu. globosus* (Dobaradaran et al., 2020; Ediagbonya et al., 2022). In addition, terrestrial snails *Eobania vermiculata* (Müller OF 1774) demonstrated the profound effects of high concentrations of pollutants such as PAHs; their findings revealed a statistically significant rise in reactive oxygen species (ROS) production, protein carbonylation, and DNA damage (Itziou et al., 2011). However, no studies about the impact of these contaminants have been performed on aquatic snails such as *Bi. glabrata* or *Bu. truncatus* so far.

Moreover, microplastics, found in an average abundance of 28.13 ± 4.18 particles across all snail populations (An et al., 2022), have been linked to notable changes in the behaviour of the aquatic snail, *Potamopyrgus antipodarum* (Gray, 1843) (Romero-Blanco et al., 2021). Furthermore, studies have demonstrated that the build-up of nanoplastics within *Bi. glabrata* can trigger changes in the expression of numerous stress response genes. These include heat shock protein-70 (HSP70), cytochrome P450 (CYP450), and macrophage migration inhibitory factor (MIF) (Merrill, 2022). These potentially hazardous compounds, by influencing snails physiology, may also adversely affect the virulence of *Schistosoma*, thereby posing further ecological risks, further research is needed.

Finally, heavy metals found in polluted water such as cadmium, lead, and mercury are responsible of disbalanced in host-pathogens interaction. It has been shown that cadmium concentration in *Bi. alexandrina* snails is positively correlated to their infection rate by *S. mansoni* (El-Gohary et al., 2003; El-Din et al., 2010). Table 1 have also shown that chronic lead exposure of *S. mansoni* and *S. haematobium* infected hamsters showed significant reductions in worm burden, tissue egg load, and ova excretion in stool, liver, and intestine while increasing the oxidative stress compared to the non-infected group.

However, the effect of freshwater contamination on parasites has not been well studied. Previous work has revealed that the use of pesticides could not just affect the snail host but also exerts a long-lasting impact on parasite virulence (Mello-Silva et al., 2011; Augusto et al., 2017; 2021). Therefore, the intricate

relationship of the abovementioned pollutants on host and parasite outcomes contributes to the need to study the effects of schistosomiasis in different environmental conditions, such as in Europe. At the same time, however, it is imperative to note that in real life, these contaminants do not normally present themselves as pure, as evidenced by laboratory experiments. In natural settings, an acute or latent concentration of a dangerous substance mixture is present in the environment; however, freshwater snails and parasites are also present. Therefore, more research is needed to determine the impact of environmental pollution on the prevalence of schistosomiasis. This type of crosscutting may contain crucial data and optimal solutions for parasitic illnesses due to pollution.

3 Conclusion and future perspectives of concerns and improvement

Here, we reviewed how freshwater pollution modifies the host-parasite relationship, significantly impacting snail hosts and the transmission risk of schistosomiasis. However, an often overlooked aspect is the potential effect of pollution on parasite virulence. The virulence of metazoan parasites isn't solely determined by their genetics; environmental factors play a crucial role in controlling virulence gene expression. Specifically, pollution may enhance the expression of these genes, thus influencing the spread of schistosomiasis in Europe. Additionally, studies in realistic environmental settings associated to functional genomic approach are essential to better understand and control the spread of freshwater-borne diseases like schistosomiasis.

Author contributions

AC: Conceptualization, Data curation, Investigation, Writing–original draft, Writing–review and editing. HG: Conceptualization, Data curation, Investigation, Writing–original draft, Writing–review and editing. AS: Conceptualization, Data curation, Investigation, Writing–original draft, Writing–review and editing. MT: Conceptualization, Data curation, Investigation, Writing–original draft, Writing–review and editing. RA: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing–original draft, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Water filter: a rapid water environmental DNA collector in the field

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Biological monitoring using environmental DNA (eDNA) technology has expanded from micro- to macro-organisms. In aquatic eDNA studies, large volumes of water need to be filtered rapidly in the field, which requires development of effective eDNA collection devices. In this study, we introduce a novel portable eDNA collection system containing a GM dual-channel water filter and a DNA extraction kit adapted to large filter membranes (ø 100 mm). The water filter is powered by a high-capacity lithium battery (9,000 mA), which operates two peristaltic pumps and maintains a continuous filtration rate of up to 1 L/min for 5 h in outdoor settings. For sample collection, the optimum conditions are still water and turbidity below 8 nephelometric turbidity units. This allows for the filtration of 10 L of water within 10 min by use of a 0.22-μm filter. Metagenomic and 12S metabarcoding sequencing showed that the DNA extraction quality and species annotation accuracy of our custom DNA extraction kit, which was tailored for this system, rivaled the performance of established kits. The GM water filter's enrichment mode gave consistent results with vacuum filtration, which greatly reduced the filtration time for large water samples, while accurately reproducing species annotations. This innovation streamlines the eDNA collection and annotation process and offers substantial benefits for biodiversity monitoring and conservation efforts.

KEYWORDS

aquatic ecosystem, eDNA sample collection, metabarcoding, metagenome, quick filtering

1 Introduction

Environmental DNA (eDNA) is DNA released by an organism into its surroundings. This DNA can be detected in various matrices, including air, water, and soil (Thomsen and Willerslev, 2015). Initially used to investigate microbial communities in marine sediments (Ogram et al., 1987), eDNA sampling has more recently revolutionized the study of multicellular organisms (Pawlowski et al., 2020). This includes the detection of various organisms such as diatoms, macrozoobenthos, fish, and earthworms (Nagler et al., 2022). An outstanding advantage of eDNA sampling lies in its ability to detect organisms that are rare or challenging to sample, including invasive non-native species (Dejean et al., 2011; James et al., 2024) and native species of conservational concern (Wilcox et al., 2013; Mauvisseau et al., 2020).

To date, three principal detection strategies have been used in eDNA research: single-species eDNA detection by quantitative PCR (Ratsch et al., 2020) and digital PCR (Capo et al., 2020), multispecies community composition analysis through metabarcoding (West et al., 2020), and shotgun sequencing of the entire metagenome (Pinfield et al., 2019; Roesma et al., 2021). The latter two approaches necessitate high-throughput sequencing, which enables the simultaneous annotation of numerous species within a single experiment. The evolution of high-throughput sequencing technology over the past decade has facilitated the application of eDNA analyses across a diverse array of aquatic ecosystems (Beng and Corlett, 2020; Takahashi et al., 2023), including subterranean environments (Saccò et al., 2022), Antarctic geothermal sites (Fraser et al., 2018), coral reefs (Madduppa et al., 2021), and deep oceans (McClenaghan et al., 2020). Research on aquatic eDNA generally requires enrichment procedures because of the low concentration of DNA (organismal or extra-organismal) (Minamoto, 2022). Advances in eDNA sampling methodologies, particularly for large-volume sampling, are ongoing (Thomas et al., 2018). Existing protocols often necessitate the transportation of water samples back to the laboratory, which limits the sample volume and introduces the possibility of DNA degradation before filtration or preservation can be performed (Goldberg et al., 2016). Development of portable eDNA filtration systems, particularly for large-volume sampling, can improve the efficiency, sterility, and replicability of aquatic eDNA sampling for field users. Thomas et al. designed a novel backpack eDNA filtration system with a flow rate threshold of 1.0 L/min. It greatly improved the sampling speed and replicability and minimized the risk of contamination. Nevertheless, this system was adapted to two mixed cellulose ester filter pore sizes (1 and 5 μm) rather than the small pore-sized filters (0.45–1.0 μm) used in a large number of eDNA studies (Rees et al., 2014). Another portable aquatic eDNA filtration system developed by DeHart et al. incorporated a field-portable pump to filter large volumes of water conveniently with 0.22-μm or 0.45-μm Sterivex filters with an unobstructed filtration rate of 150.05 ± 7.01 mL/min and 151.70 ± 6.72 mL/min, respectively (DeHart et al., 2023). Undoubtedly, this rate is still time-consuming for field filtration of large-volume water samples. Therefore, the development of a portable eDNA sampling system that supports a high flow rate (e.g. 1.0 L/min) and is compatible with commonly used filter membrane pore sizes (e.g. 0.22 μm and 0.45 μm) could greatly streamline eDNA pre-processing by enabling the filters to be easily carried and reducing DNA degradation. In the laboratory setting, vacuum filtration remains the predominant method for eDNA enrichment, with the standard filter membrane diameter being 47 mm (Schweiss et al., 2020; Wong et al., 2020). For eDNA extraction from these filters, the Qiagen DNeasy PowerWater Kit and MP Biomedicals MagBeads FastDNA Kit for Soil are frequently used (Lear et al., 2018; Schweiss et al., 2020; WANG et al., 2022). However, these extraction kits are specifically designed for 47-mm filter membranes and may not be compatible with larger filters that could be used in portable field systems.

In this study, we developed an innovative portable eDNA collector system using a GM dual-channel water filter (developed by GeneMind Biosciences Co., Ltd, Shenzhen, China) with a Water Filter DNA Extraction Kit specifically tailored for large filter membranes (ø 100 mm). This system was engineered to enhance the efficiency, sterility, and replicability of aquatic eDNA sampling in the field. We provide a comprehensive account of the system's design, including its stress testing, and comparative experiments against conventional extraction kits and vacuum filtration methods.

2 Methods

2.1 System design

It is well-established that the flow rate is positively correlated with pressure differences when the flow resistance remains constant. In suction filtration, the maximum achievable pressure difference is capped at 1.0 atm, whereas gravity filtration exhibits a minor pressure difference, which is dictated by the water column's height. A positive pressure filtration system enhances the pressure difference across the filter membrane surface beyond the 1.0 atm threshold, which surpasses the limitation of gravity filtration. When considering the driving force, a peristaltic pump outperforms centrifugal, diaphragm, and plunger pumps in terms of self-priming capacity, flow capacity, and pump liquid efficiency. Consequently, we opted for a positive pressure filtration system paired with a peristaltic pump. To monitor clogging during the filtration process, we incorporated a pressure monitoring device, which allowed for the cessation of filtration by setting the upper and lower pressure thresholds. Additionally, we implemented two timers to assist the operator with tracking the filtration duration. The system was designed with a lightweight structure and was powered by a high-capacity lithium battery (9,000 mA), which makes it suitable for the collection of water samples in the field. For more turbid water bodies, we have designed a bucket-shaped filter (120 mesh) and a butterfly filter (pore size of 100 μm), which can be connected sequentially in front of the inlet pipe for pre-filtration. The GM dual-channel water filter contains one battery meter, two pressure gages, and two timers (Figure 1).

2.2 Water turbidity stress testing

We evaluated the system's performance using 12 freshwater samples sourced from groundwater, streams, lakes, and ponds in Shenzhen, China (Table 1). Water turbidity was quantified by using a suspended matter turbidity detector (LH-XZ03, Lohand Biotechnology Co., Ltd., Hangzhou, China), with measurements of four replicates. Four replicate filter samples were collected using mixed cellulose ester membranes with a pore size of 0.22 μm (XYMCE0065, Shanghai Hai Xin Ya Purification Equipment Co., Ltd). The filtration time required to process 10 L of water through the dual channel system was recorded.

2.3 Pressure testing

To ascertain the maximum pressure tolerance during the filtration process and validate the system's capability to filter 10 L water within 10 min, we conducted an experiment using water samples from Gankeng Reservoir with an original turbidity

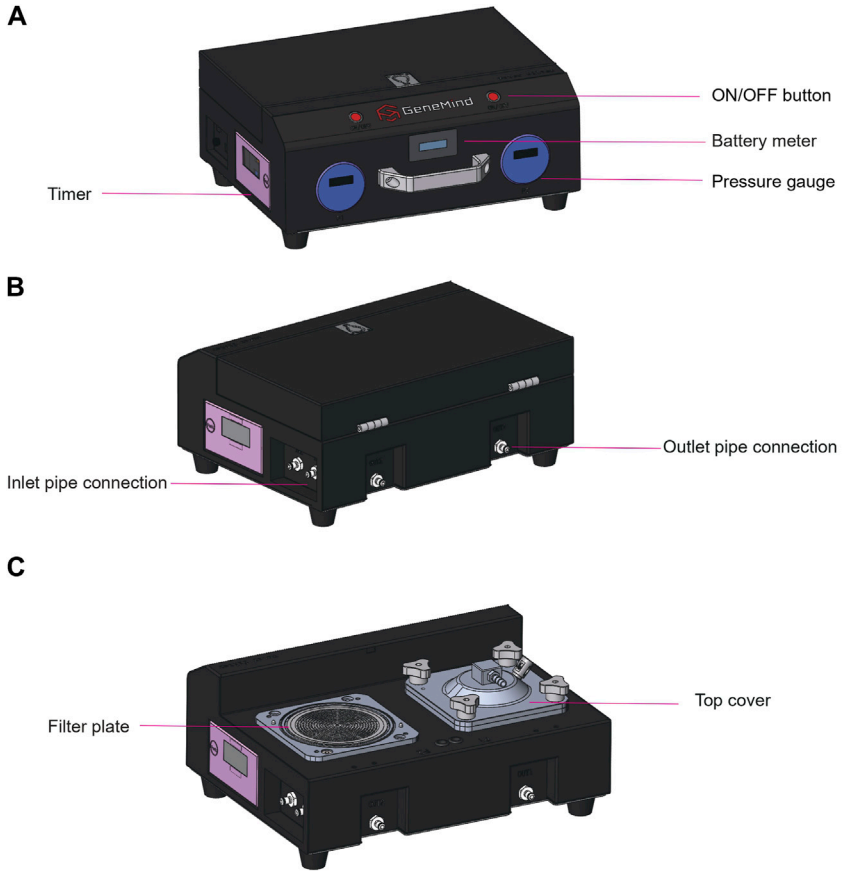

FIGURE 1
GM dual-channel water filter. **(A)** Front view showing the window for the battery meter, two pressure gages, and one timer on the left side. **(B)** Rear view showing two outlet and inlet pipe connections. **(C)** Rear view showing two filter plates, two top covers, and one timer on the left side.

of 8.81 ± 0.71 nephelometric turbidity units (NTU). We supplemented the samples with pure water to facilitate pressure testing and to prevent rapid clogging of the filter membrane. Finally, a total volume of 200 L and a turbidity of 2.61 NTU water were used to ascertain the pressure limit during the filtration process. The flow rate was calculated from the volume of the water filtered per minute, and dynamic pressure changes were monitored. Experiments were conducted separately for each channel, with four trials for both channels in total, and the filtration endpoint was set at 15 min.

2.4 Comparison with different DNA extraction kits

Following the pressure testing, we performed three consecutive filtration rounds with the above water (2.61 NTU), which resulted in the collection of six filter membranes. The volume of water filtered by each filter membrane was approximately 5.5–6.2 L. Two membranes were divided into four and subjected to eDNA extraction using a MagBeads FastDNA Kit for Soil (MP Biomedicals, Santa Ana, CA) and a DNeasy PowerWater Kit (Qiagen, Dusseldorf, Germany). The remaining four membranes were processed using our in-house Water Filter DNA Extraction Kit (A000026, GeneMind Co., Ltd, Shenzhen, China).

The process involved the addition of 7.5 mL of lysis solution and grinding beads, followed by vigorous vortex mixing at 2,500 rpm for 10 min and centrifugation at 4,000 × g for 1 min. The supernatant (6.5 mL) was then transferred to a clean collection tube, and 100 µL of proteinase K solution was added. The mixture was vortex-mixed briefly to ensure thorough mixing and then incubated at 65°C for 20 min. Subsequently, 3 mL of isopropyl alcohol and 80 µL of magnetic beads (50 mg/mL) were added, the tube was vortexed or inverted to mix, and then placed on a shaker for 9 min to facilitate binding. The tube was then set on a magnetic rack for 5 min to allow the magnetic beads to settle, after which the supernatant was discarded. Add 1 mL of wash solution I to re-suspend the magnetic beads by vortexing, and then deposit the tube on the magnetic rack for 2 min to ensure that the beads aggregate along the tube wall. Thereafter, the supernatant was carefully discarded without disturbing the bead pellet. Following this, a second wash was performed with 1.2 mL of wash solution II, followed by a third wash with an additional 700 µL of wash solution II. After the final wash, the remaining solution was carefully aspirated, and the tube was returned to the magnetic rack for drying at room temperature. The drying process was ceased when all of the visible moisture on the magnetic nanoparticles had evaporated. Then, 100 µL of elution buffer was added to the dried beads. The beads were re-suspended and incubated at 55°C for 5 min. After that, the tube was placed on

TABLE 1 Filtration time of 10 L water with different turbidities via 0.22-μm filter membranes.

Sample ID	Turbidity (NTU)	Time for 10 L water	Information about sampling point
G1-GK	1.70 ± 0.05	8′16″±42″	Groundwater 1—Gankeng base
G2-GK	1.23 ± 0.04	8′8″±37″	Groundwater 2—Gankeng base
S1-GK	27.9 ± 0.73	14′15″±1′25″	Stream—Gankeng base
IL-ELP	12.9 ± 0.39	95′±7′34″	Inner Lake—East Lake Park
S1-ELP	10.73 ± 0.73	83′±6′8″	Stream—East Lake Park
P1-SUST	18.6 ± 1.06	62′±3′52″	Pond 1—SUST
LS-SUST	9.85 ± 0.32	42′±4′43″	Lower stream—SUST
US-SUST	11.18 ± 0.15	45′±6′7″	Upper stream—SUST
P2-SUST	9.44 ± 0.29	65′±5′19″	Pond 2—SUST
P3A-SUST	7.91 ± 0.36	8′7″±25″	Pond 3—SUST
P3B-SUST	8.67 ± 0.56	13′5″±2′23″	Pond 3—SUST
P3C-SUST	6.91 ± 0.26	9′37″±56″	Pond 3—SUST

Note: SUST, Southern University of Science and Technology; the value in turbidity or time for 10 L of water is the average and standard deviation for fourth tests.

the magnetic rack for 5 min to allow the magnetic beads to settle. Finally, 98 μL of the supernatant (eluted DNA) was transferred to a clean 1.5-mL micro-centrifuge tube. The extracted DNA could be stored at −20°C for extended periods. All devices and extraction kits came with operation manuals.

The concentration and purity of the extracted eDNA were quantified by using a fluorometer (Qubit 4, Thermo Fisher Scientific Inc, Waltham, Mass) and a spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific Inc, Waltham, Mass), respectively. The integrity of the eDNA was assessed by agarose gel electrophoresis (gel concentration: 1%; voltage: 5 V/cm; electrophoresis time: 20 min) and by using a NanaLyzer (GeneMind Co., Ltd, Shenzhen, China). Subsequently, 12 metagenomic libraries were constructed from the 12 DNA samples. Sequencing was conducted via the FASTASeq 300 platform (GeneMind Co., Ltd, Shenzhen, China) using the PE150 mode. Species annotation was performed using our previously published method (Feng et al., 2023). Briefly, Kraken 2 was used for taxonomic assignment of metagenomics sequencing reads through exact *k*-mer matches (https://ccb.jhu.edu/software/kraken2/). The Kraken2_standard_16G_20230314 database was chosen for aligning. Using the internal *k*-mer (default parameters) to the LCA (lowest common ancestor) mapping algorithm, Kraken 2 assigns a taxonomic label (confidence: 0.9). The relative abundance was calculated based on the proportion of fragments assigned to the taxa by Kraken 2.

2.5 Comparison with vacuum filtration

Vacuum filtration is a prevalent technique in eDNA laboratory research. The standard filtered water sample volume for this technique is 500 mL. In this part, we compared the metagenomic and 12S sequencing outcomes for filter membranes obtained after processing 2 L of stream water from the Gankeng base (turbidity: 25.98 ± 0.56 NTU). For vacuum filtration, a 47-mm filter membrane (pore size: 0.22 μm) was used for every 500 mL of water, and the four resulting membranes were pooled for eDNA extraction. The GM water filter system processed 2 L of water by using a single filter membrane (ø 100 mm, pore size: 0.22 μm). Four biological replicates were conducted for each filtration method, and a Water Filter DNA Extraction Kit (A000026, GeneMind Co., Ltd, Shenzhen, China) was used for eDNA extraction. Each of the eight DNA samples was divided into two aliquots. One aliquot was subjected to metagenomic sequencing by the FASTASeq 300 platform using the PE150 mode, while the other was sent to Magigene Co., Ltd (Guangzhou, China) for 12S metabarcoding sequencing using a NovaSeq 6000 in the PE150 mode. The subsequent data analysis followed the methods outlined in a previous study (Zhang et al., 2024). Briefly, Fastp (v0.12.4) was utilized to perform quality control on the raw sequencing data. Subsequently, the cutadapt software was employed to eliminate primer sequences, thereby generating clean reads. These clean reads were then processed using usearch-fastq_mergepairs version (v11.0.667), which required at least a 16-bp overlap between reads derived from the opposite ends of the same DNA fragment, with a maximum of 5-bp mismatches permitted within the overlap region. The resulting sequences were clustered into operational taxonomic units (OTUs) using the UPARSE software with a 97% sequence similarity threshold. These OTUs with reads <10 were removed. The remaining OTUs were compared against the reference databases MitoFish and GenBank to facilitate species annotation. For alpha diversity, Chao and Shannon indices were computed, and beta diversity was assessed through non-metric multidimensional scaling (NMDS) analysis based on the Bray–Curtis dissimilarity matrices.

3 Results

3.1 System implementation

The GM Water Filter is versatile and can accommodate various filter membrane pore sizes (0.22 and 0.45 μm) and materials, including mixed cellulose ester, organic nylon, and polyether sulfone, all with a

FIGURE 2
Typical GM dual-channel water filter workflow for environmental DNA (eDNA) sampling: (1) the equipment, (2) connecting the inlet and outlet tubes, (3) tightening the screws and closing the upper covers, (4) placing the inlet tubes into the alcohol and turning on the rinse, (5) loading the filter membrane with the edge of the filter membrane aligned with the supporting filter, (6) loading the two filter membranes, (7) turning on the power and setting the filtering time, (8) setting the pressure switch, and (9) pressing the on–off button and starting the filter.

standard diameter of 100 mm. The setup of the GM water filter and the standard sampling procedure are depicted in Figure 2. Operators must always wear sterile gloves to ensure aseptic conditions during the experimental procedure. Before initiating the filtration process, 20 mL of 75% alcohol was aspirated into the water inlet pipe to effectively sanitize the internal conduit. Subsequently, the water sample designated for filtration was introduced into the apparatus to ensure that any residual alcohol was thoroughly rinsed from the pipe by the incoming water sample. After assessing the flow rate, we used a mixed cellulose ester membrane. Post-filtration, the filter membrane was carefully collected and stored in a sterile 50-mL centrifuge tube, which was then transported to the laboratory at low-temperature conditions (4°C). The pipeline system was thoroughly cleaned using 75% ethanol and pure water. Additionally, the panel components are disinfected by applying 75% alcohol, followed by wiping with a clean cloth to ensure the removal of any residual alcohol and contaminants.

3.2 Determination of the optimal turbidity

We assessed 12 freshwater sites to establish the recommended turbidity for achieving filtration of 10 L of water within 10 min. The turbidity distribution was 1.23–27.90 NTU (Table 1). A filtration rate of 10 L in 10 min was feasible when the turbidity was below 8 NTU. Even for relatively pristine groundwater (1.23–1.70 NTU), filtration of 10 L of water took approximately 8 min. Although there was a positive correlation between the filtration time and turbidity, the relationship was not strictly linear (Supplementary Figure S1).

3.3 Pressure threshold

During the filtration process, channel A exhibited two distinct plateaus in the relationship between the flow rate and pressure within 15 min, while channel B demonstrated a negative correlation between these parameters (Figure 3). At the end of filtration, the pressures of channels A and B stabilized at 174.75 ± 1.5 and 155.55 ± 3.70 kPa, respectively. The corresponding flow rates were 0.110 ± 0.008 and 0.178 ± 0.005 L/min, respectively. The volumes of water filtered through the channels were 3.81 ± 0.14 L (A) and 3.17 ± 0.03 L (B) at 5 min, 5.64 ± 0.41 L (A) and 5.06 ± 0.05 L (B) at 10 min, and 6.34 ± 0.44 L (A) and 6.09 ± 0.09 L (B) at 15 min. The results for the four replicates confirmed that

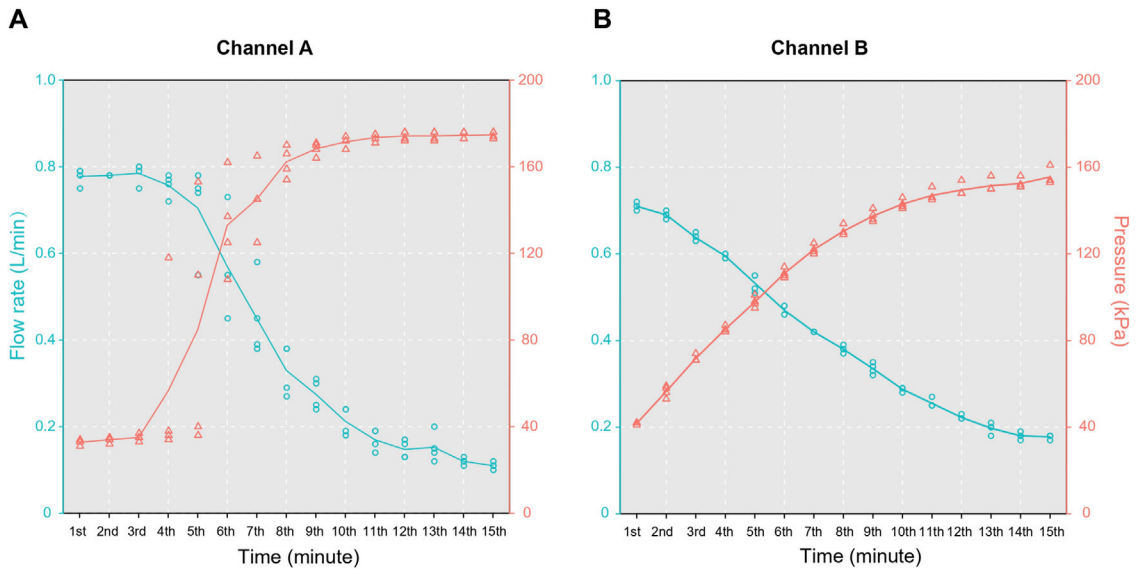

FIGURE 3
Results of the pressure tests for the two channels in the water filter: **(A)** channel A and **(B)** channel B.

TABLE 2 Comparison of DNA quality obtained by the eDNA extraction reagent.

Sample	A260/A280	A260/A230	Qubit (ng/μL)	Volume (μL)	DNA (μg)
GM-1	1.83	1.98	112.0	80	8.96
GM-2	1.84	1.55	114.0	100	11.40
GM-3	1.81	1.56	100.0	80	8.00
GM-4	1.87	1.64	116.0	85	9.86
MP-5-1	1.94	1.38	24.8	91	2.26
MP-5-2	1.89	1.48	20.2	85	1.72
MP-5-3	1.86	1.56	23.6	80	1.89
MP-5-4	1.82	1.54	23.4	77	1.80
Qiagen-6-1	1.90	0.36	50.2	100	5.02
Qiagen-6-2	1.91	0.28	42.0	100	4.20
Qiagen-6-3	1.92	0.65	64.0	100	6.40
Qiagen-6-4	1.90	0.90	38.6	100	3.86

Note: GM-1, GM-2, GM-3, and GM-4 mean membrane 1#, 2#, 3#, and 4#, respectively. MP-5-1 means quarter of membrane 5#. Qiagen-6-1 means quarter of membrane 6#. GM means Water filter DNA Extraction Kit, MP means MagBeads FastDNA™ Kit, and Qiagen means DNeasy® PowerWater® Kit.

the dual-channel system was capable of filtering at least 10 L of water within 10 min.

3.4 Performance of the GM extraction kit

Benchmarking results indicated that DNA integrity with the GM extraction kit was comparable to that with the MP and Qiagen kits (Table 2), while the DNA concentration and total yield were evidently lower with the MP kit than with the other kits (Supplementary Figure S2). Specifically, the total DNA yields from a single filter using the GM, MP, and Qiagen kits were 9.56, 7.66, and 19.48 μg, respectively (Table 2). The efficiency of the GM extraction kit was comparable to that the MP kit, but was less efficient than the Qiagen kit when considering DNA quality and quantity.

Metagenomic sequencing and species annotation were performed on the 12 DNA samples. The clean data ranged from 22.6 to 26.9 million reads, with Q20 > 94% (Supplementary Table S1). Figure 4 shows the relative abundances at the phylum and genus levels. The three kits presented highly consistent species annotation at the phylum level, with only minor variations observed at the genus level. Generally, the inter-group differences were more pronounced than the intra-group differences. The more distinct difference

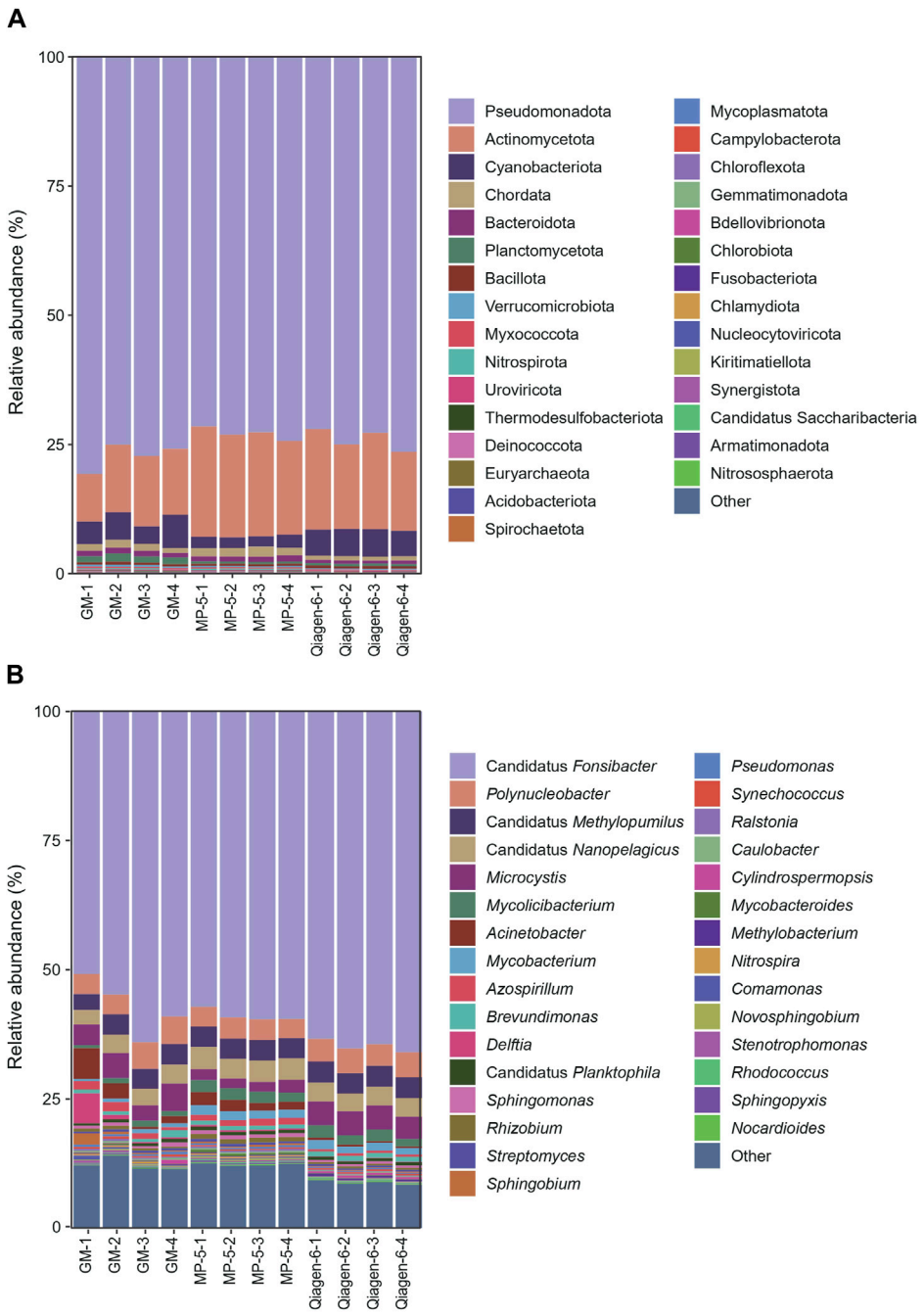

FIGURE 4
Relative abundances at the phylum **(A)** and genus **(B)** levels of the 12 DNA samples from three kits. The 30 most abundant species are shown. "Other" indicates species other than the top 30 across all samples. Abbreviations: MP, MagBeads FastDNA Kit for Soil; Qiagen, DNeasy PowerWater Kit; and GM, water filter DNA extraction kit.

observed within the GM group could be attributed to the separate extraction of the four filter membranes. This was in contrast to the MP and Qiagen kits, where four aliquots were extracted from the same filter membrane. It seems like the water was not fully mixed during the filtration, and although we agitated the water bucket to ensure homogeneity and started the filtration, it remained undisturbed. Nevertheless, these differences are considered acceptable and are consistent with the variations observed in biological replicates.

3.5 Impact of filtering methods in eDNA sequencing

To filter 2 L of water, the vacuum filtration system took 644.02 ± 39.74 min and the GM water filter system took 27.61 ± 2.67 min. Metagenomic sequencing was conducted on eight samples, which were divided into two groups (labeled VF for vacuum filtration and GM for the GM water filter system). The data volume ranged from 15.5 to 16.6 million reads (Supplementary Table S2). The heatmap

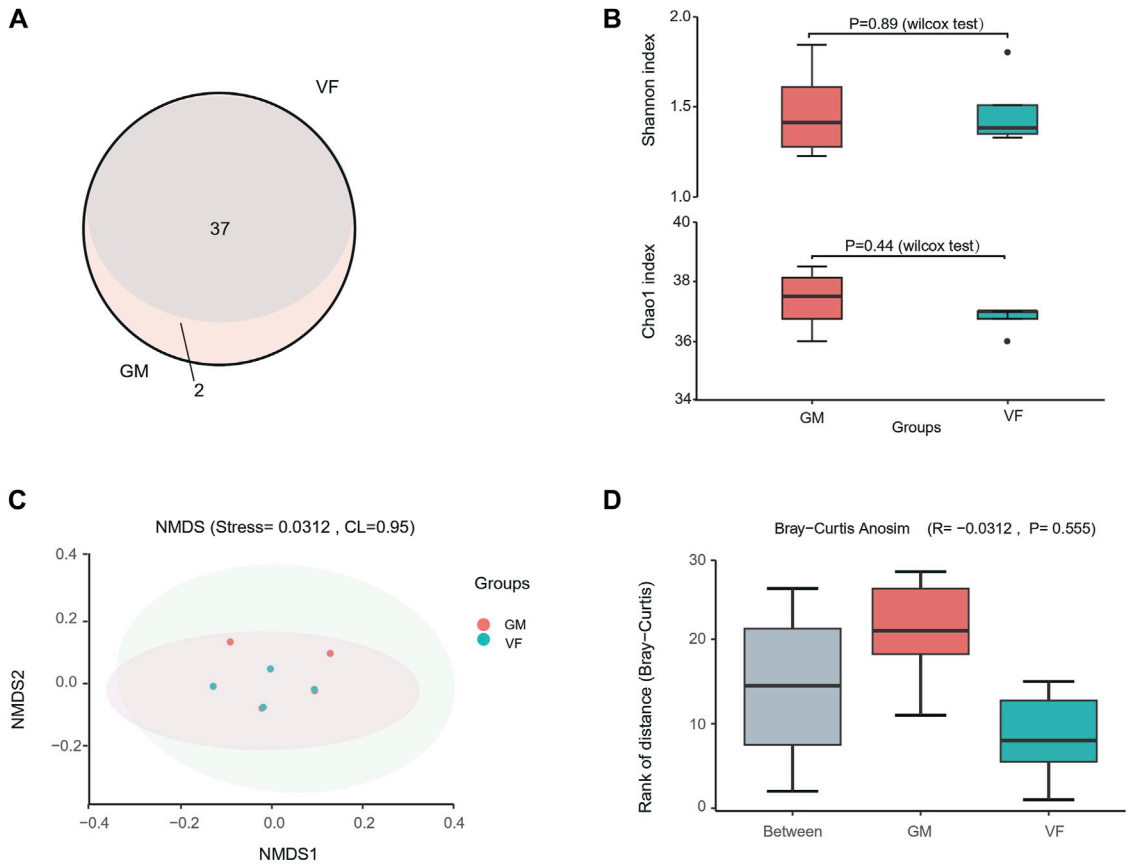

FIGURE 5
12S metabarcoding sequencing of sample filters by vacuum filtration and the GM water filter system. **(A)** Venn diagram of the OTU annotations for the GM water filter system (GM) and vacuum filtration (VF). **(B)** Alpha diversity of the two groups. **(C)** Non-metric multidimensional scaling (NMDS) scatter plot for the Bray–Curtis distance of the two groups. The shaded areas represent the confidence intervals (0.95). The stress value reflects the quality of the NMDS analysis results. When stress <0.05, the analytical result has excellent representativeness. **(D)** Box plot of significant differences between two groups obtained using analysis of similarity (AnoSim) for the Bray–Curtis dissimilarity. R < 0 indicates that the difference between the groups is smaller than the difference within the group. $P < 0.05$ indicates statistical significance.

representing the relative abundances of the top 20 genus and species taxa demonstrated a high degree of concordance among the four biological replicates within each group, with these replicates clustering together in a single clade (Supplementary Figure S3). Inter-group comparisons also revealed high consistency in the relative abundance profiles. In both VF and GM groups, the genera *Cylindrospermopsis*, Candidatus *Fonsibacter*, *Polynucleobacter*, and *Synechococcus* were predominant at the genus level. When examining the species level, the four most abundant were *Cylindrospermopsis raciborskii*, Candidatus *Fonsibacter ubiquis, Polynucleobacter* sp. MWH-UH24A, and *Cylindrospermopsis curvispora*. The consistent distribution of highly abundant genera and species between the two groups indicated that both the filtering methods had a minimal influence on species annotation via metagenomics sequencing.

The 12S metabarcoding sequencing data for the eight samples ranged from 329,000 to 401,000 reads, with an average of 360,000 reads (Supplementary Table S3). The VF and GM groups yielded 281,000–320,000 tags and 231,000–311,000 tags, respectively, which culminated in the identification of a total of 271 OTUs. Thirty-seven OTUs were annotated in the genus or species levels for the VF group, and 39 OTUs were annotated for the GM group. The GM group included all 37 OTUs from the VF group (Figure 5A). The alpha diversity indices, Shannon and Chao1, indicated that there were no significant differences between the two filter models (Figure 5B). Furthermore, the NMDS analysis from Bray–Curtis dissimilarity matrixes showed a highly consistent ordination pattern for both groups, with a stress value of <0.05 (Figure 5C). Supplementary beta diversity analysis (analysis of similarity) showed that the inter-group differences were less pronounced than the intra-group differences (R < 0); however, it was not statistically significant ($P > 0.05$) (Figure 5D). Collectively, the metabarcoding sequencing (12S) results suggested that there were no significant differences between the species information obtained by the two eDNA enrichment methods when both alpha and beta diversity were considered.

4 Discussion

We engineered a portable water filtration device using a GM dual-channel water filter for eDNA collection. This innovative system markedly expedites the filtration process for large-volume water samples (10 L) and reduces the time from several hours

required by traditional vacuum filtration methods to only 10 min. When we evaluated commercially available eDNA extraction kits, we observed that kits tailored for smaller filter membranes (ø 47 mm) dominated (Schweiss et al., 2020; Wang et al., 2022), which also makes extraction of DNA from environmental water samples tedious and expensive. Therefore, to complement the GM water filter, we designed a compatible eDNA extraction kit, which was optimized for the large filter membranes (ø 100 mm) used by our system. Pilot studies with the GM dual-channel water filter and the Water Filter DNA Extraction Kit showed high agreement in terms of species annotation.

When sampling eDNA from aquatic environments, the choice of the filter material, the pore size of the filter membrane, and the volume of the water filtered are critical factors that significantly affect the capture efficiency of eDNA (Majaneva et al., 2018). In this study, the mixed cellulose ester filter membrane was selected for its superior eDNA yield when compared with polyethene sulfone, polyvinylidene fluoride, and polycarbonate filters (Liang and Keeley, 2013; Majaneva et al., 2018). The pore size determines the retention of eDNA-laden particles. Small pore-size filters [0.20 μm (Liang and Keeley, 2013)] have been shown to enrich the eDNA yield, while they may lead to faster clogging of the filter, especially in algal blooming or turbid waters. In such conditions, pre-filtration seems to be a good strategy to intercept larger particles, such as sediment, organic debris, and other macroscopic materials. Our developed GM dual-channel water filter integrated a bucket-shaped filter (120 mesh) at the water inlet to perform a pre-filtration step to enhance the efficiency of the subsequent finer filter membrane designed for eDNA capture. Employing a larger pore-size filter membrane can significantly reduce the filtration time required for turbid water samples. However, this efficiency gain was accompanied by a notable reduction in eDNA recovery, presumably due to the increased pore size, allowing a portion of the eDNA-containing particles to pass through the filter (Turner et al., 2014). Alternatively, augmenting the volume of water may compensate for the diminished capture rate; however, it necessitates an extension of the filtration time. In summary, a subtle equilibrium must be struck, given the interplay between the membrane pore size, the volume of the water sample, the duration of filtration, and the efficiency of eDNA capture. The GM dual-channel water filter employed a pre-filtration (120 mesh) step followed by fine filtration (0.22 μm or 0.45 μm) to allow the filtering of 10 L of the water sample within 10 min, thereby ensuring a high efficiency of eDNA enrichment.

For still water samples with turbidity levels below 8 NTUs, the filtration process takes approximately 10 min, and interestingly, relatively pristine groundwater with turbidity ranging from 1.23 to 1.70 NTUs also requires a similar duration. This suggests that the filtration time does not scale linearly with decreasing turbidity. The association between turbidity and flow rate appears to be more complex than a straightforward negative linear correlation (Supplementary Figure S1). Moreover, turbidity is not the sole factor governing the filtration rate. The free flowing rate (no-loading filter membrane) of our peristaltic pump is 0.7 L/min, and it rapidly increases until the sample turbidity is above 8, at which point filtration slows down drastically. Consequently, for optimal filtration efficiency, we recommend targeting still water bodies with turbidity levels below 8 NTUs. If the water body has relatively high turbidity (>8 NTU), pre-filtration should be applied.

We acknowledge the limitation inherent in the validation experiment of this study. Our benchmarking comparison with vacuum filtration was predominantly centered on the 12S rRNA metabarcoding sequencing (Majaneva et al., 2018), a method commonly applied for the identification of eukaryotic phytoplankton and fish. Its focus did not extend to other established sequencing methods, including 16S rRNA (Harrison et al., 2021), 18S rRNA (Minerovic et al., 2020), ITS (Fahner et al., 2016), and CO1 (Tagliabue et al., 2023) sequencing for bacteria, fungi and protists, fungi, and metazoan species identification, respectively. These methodologies were also extensively utilized in the profiling of the eDNA metabarcoding study and should be performed in subsequent studies to fully assess the strength and weakness of our equipment in the application of metabarcoding.

Our eDNA collection system has been widely disseminated among numerous collaborators conducting field studies in diverse aquatic ecosystems, spanning from urban reservoirs to lakes within national reserves. The invaluable feedbacks received have driven the ongoing enhancement of our system, focusing on simplifying its operation, reducing its weight, and creating extraction kits that are compatible with larger filter membranes, all while minimizing costs. By embracing an open-hardware philosophy, forthcoming iterations of this portable filtration system are poised to incorporate advancements such as higher-flow pumps, integrated large-capacity batteries, and materials that contribute to less overall weight, thereby enhancing field usability. At present, our systems are being utilized in the Shenzhen Academy of Environmental Sciences, the Key Laboratory of Poyang Lake Hydrology and Ecology Monitoring and Research, and the Eco-Environmental Monitoring Center of Dongting Lake in Hunan Province research programs. Collaborative input has been instrumental in guiding the design evolution, allowing us to refine the system's field reliability and processing capacity.

5 Conclusion

We have successfully developed a portable water filtration system, the GM dual-channel water filter, complemented by a water filter DNA extraction kit that is specifically designed for the collection of environmental DNA (eDNA) from large-volume water samples quickly, such as 10 L volume in 10 min. This innovative system is particularly user-friendly, even for individuals with minimal prior experience in eDNA sampling, thanks to the provided operation manual. It holds great promise for field applications, as it could significantly simplify the process of water eDNA collection. By streamlining the filtration process and facilitating rapid species identification, this system is instrumental in enhancing biodiversity monitoring and conservation initiatives.

Data availability statement

The datasets presented in this study can be found in online repositories. CNGB Sequence Archive (https://db.cngb.org/cnsa/) under project accession number CNP0005350.

Author contributions

PW: conceptualization and writing–original draft. JF: writing–original draft. MJ: methodology and writing–original draft. SW: conceptualization and writing–review and editing. WH: methodology, validation, and writing–review and editing. MW: formal analysis, visualization, and writing–original draft. JnL: methodology and writing–review and editing. LfZ: conceptualization and writing–review and editing. YG: conceptualization, methodology, and writing–review and editing. JZ: data curation, resources, and writing–review and editing. MjL: data curation, resources, and writing–review and editing. HG: data curation, resources, and writing–review and editing. LdZ: methodology, visualization, writing–review and editing. JaL: writing–review and editing. MzL: methodology, supervision, and writing–review and editing. QY: project administration and writing–review and editing. LS: project administration and writing–review and editing. YL: writing–original draft, writing–review and editing, and project administration.

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Conflict of interest

Authors PW, MJ, WH, MW, LfZ, YG, JZ, MjL, HG, LdZ, JaL, MzL, QY, LS, and YL were employed by GeneMind Biosciences Company Limited.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2024.1415338/full#supplementary-material

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Spawning grounds model for neotropical potamodromous fishes: conservation and management implications

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Introduction: Freshwater fish migrations are an important natural process. All main river basins in South America have potamodromous fish that migrate upstream to spawn. Therefore, these species withstand fisheries and are socially, economically, and ecologically important. Hydropower dams cause one of the main threats to these fish's survival. Hydropower is the main source of low-carbon electricity in South America, where the most diverse and endemic riverine fish fauna inhabit. However, hydropower development rarely considers spawning areas or cumulative impacts in fish migratory routes at a macro-basin scale in their environmental impact assessment (EIA) studies. In the present case study conducted in the Magdalena basin in Colombia, a distribution model of potential spawning areas of migratory fish species was developed. The objective of the current research is to demonstrate the potential use of early planning tools at the macro-basin scale to ensure that freshwater ecosystems remain functional in supporting fish migrations.

Methods: Potential spawning areas for 15 migratory fish species were determined using ichthyoplankton sampling records, embryonic and larval time development, water velocity, and average flow time estimations. Spawning distribution grounds, analyzed for species diversity and richness, were overlaid with the national hydropower projects portfolio to examine the potential loss of reproduction areas due to hydropower dam development.

Results and discussion: Our basin-wide model calculated spawning areas for all of the identified species in available ichthyoplankton samples, using available data on the duration for larval and embryonic development. The proposed model estimated the potential impacts of projected hydropower development in the Magdalena basin and revealed spawning grounds encompassing 11,370 km of rivers, spanning Strahler orders three to eight, which represented 11.2% of the entire river network. These areas overlapped with 80 hydropower projects (56.7% of the total), with a projected 45.0% loss experienced in reproduction areas for potamodromous species.

Conclusion: Management measures to promote freshwater fish species conservation must avoid river fragmentation and critical habitat loss, while

promoting habitat connectivity. This model provides a solution to analyze fragmentation impacts from hydropower dam development in data-limited basins. It supports science-based decision-making for choosing dam location arrangements that minimize impacts (connectivity and reproductive habitat loss), while ensuring that rivers continue to support migratory fish for better conservation and food security outcomes.

KEYWORDS

development by design, early planning, environmental impact assessment, freshwater migratory fish, hydrological modeling, mitigation hierarchy, species spatial modeling

1 Introduction

Inland aquatic ecosystems and their biodiversity provide irreplaceable services to both nature and people (Lynch et al., 2023). Despite being very important, the wetlands are disappearing globally, three times faster than forests, and rate of decline of populations of inland aquatic vertebrates is more than twice than that of terrestrial or marine vertebrates (Albert et al., 2021). Over the past 50 years, 30% of inland aquatic ecosystems and 83% of their species have disappeared, thereby posing a severe threat to people who depend on rivers, lakes, and tributaries for water, food, and their economic well-being (Albert et al., 2021; Almond et al., 2022; Deinet et al., 2024). This global accelerated biodiversity loss has been called by scientists as the freshwater biodiversity crisis (Albert et al., 2021).

Studies have established threats to freshwater biodiversity (Dudgeon et al., 2006), with loss of connectivity being one of the main threats (Grill et al., 2019). Furthermore, dams and other types of infrastructure have been particularly damaging in fragmenting freshwater ecosystems and disrupting movements of water, species, sediments, and nutrients (Opperman et al., 2017; Brink et al., 2018; Grill et al., 2019; Tickner et al., 2020; Angarita et al., 2021; Deinet et al., 2024). Water resource planning is not accorded prime importance in the maintenance of natural ecosystems and their constituent species in a relatively intact state (Flitcroft et al., 2019). To address this challenge, Tickner et al., 2020 developed an emergency recovery plan to reverse the loss of freshwater biodiversity. This plan proposed safeguard measures to prevent further loss and to restore river connectivity as one of its six priority actions.

Hydropower provides approximately 17% of electricity worldwide (IEA, 2021). In several countries in Latin America, hydropower provides more than 50% of the total electricity supply and remains a key source of low-carbon energy and is likely to be largest renewable source across the region in future (IHA, 2022). Though hydropower is important for achieving sustainable development and economic goals, the creation and operation of hydropower dams can cause considerable social and environmental harm (Opperman et al., 2015). These impacts include the isolation of spawning grounds from feeding and growth habitats of migratory fish species; such isolation leads to a decline in the population of these species (Asmal et al., 2000; Agostinho et al., 2007; Grill et al., 2019), as well as a reduction in freshwater ecosystem services that impoverish local fishers (Hoeinghaus et al., 2009).

Potamodromy, the predominant migration type in stream fishes (Flecker et al., 2010), is crucial for nutrient energy flows and sustaining artisanal fisheries, especially in tropical regions, where it accounts for more than 60% of fish catches (Welcomme, 1985; Welcomme et al., 2015; Zhao et al., 2015; Barletta et al., 2016; Ainsworth et al., 2023; Deinet et al., 2024). The highest riverine fish biodiversity and the highest number of endemic species are found in South Africa (Oberdorff et al., 2011; Tedesco et al., 2017; Jézéquel et al., 2020), with at least 20% of these fish species being potamodromous (Carolsfeld et al., 2003). However, in this region, Andean rivers are the target for hydropower project development (Tognelli et al., 2016; Anderson et al., 2018). However, Environmental Impacts Assessment (EIA) studies of hydropower projects often overlook fish migratory routes and spawning grounds. Additionally, these assessments are typically conducted on a project-by-project basis rather than considering impacts at a macro-basin scale, which would take into account cumulative impacts, including those on wide-ranging migratory fish. Lack of data on the spatial distribution of migratory fish and their habitat use is one of the challenges in making EIA studies. The difficulties of observing freshwater fish significantly hinder the ability to develop an accurate understanding of these resources and to provide users with the feedback needed for effective management in the wild (Zhang et al., 2020). Identifying spawning grounds is essential for this management; however, a few studies have been documented for tropical potamodromous species (Godinho et al., 2017; Miranda-Chumacero et al., 2020; Moreno-Arias et al., 2021). To overcome this challenge, different models on species distribution are employed to predict the population responses of these species or species groups under different scenarios and identify an accompanying management strategy (Langhans et al., 2019).

Because of the economic and social importance of potamodromous fishes, the present study aims to develop and test a framework for constructing a distribution model to identify potential spawning areas for migratory fish species. We intend to use the findings of this approach to highlight the value of early planning tools in achieving a balance between hydropower dam development and the preservation of functional freshwater ecosystems that sustain migratory fish. This might minimize conflicts between fishing communities and hydropower projects while assessing fragmentation and potential critical habitat loss for various species of conservation and economic importance.

The proposed model is an innovative combination of straightforward mathematical hydraulic analysis and field-collected ichthyological data. It offers a practical solution for environmental agencies and consultants worldwide conducting EIAs in basins with limited fish distribution data. This model can be used by various stakeholders to evaluate the potential impacts of

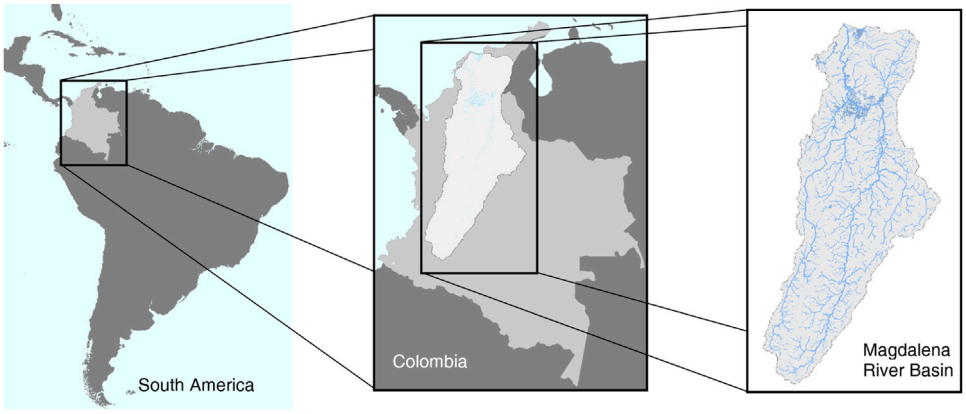

FIGURE 1
Location of the Magdalena basin (Colombia).

dam-induced habitat fragmentation and critical habitat loss by dams on freshwater migratory ichthyofauna more effectively.

2 Methods

2.1 Study area

The Magdalena River basin is located in the northwestern region of South America. It exhibits a bimodal hydrological cycle, which has two rainy and two dry seasons, annually. The basin has two primary drainage areas: the Magdalena River and the Cauca River (Figure 1). The Magdalena River flows 1,500 km from its source in the Andes mountains to the Caribbean Sea and spans approximately 273,000 km^2 basin area. The basin covers nearly a quarter of Colombia's land area, with a mean annual flow of 7,300 cubic meters per second, making it the fifth-largest river in South America.

The basin is densely populated, containing approximately 75% of the Colombian population (or 36 million people; Opperman et al., 2017). Due to its hydrography and proximity to existing transmission infrastructure and key water demand centers, this basin has been the target of several hydropower dam projects. These dams represent 84% of Colombia's reservoirs, with 35 operational hydropower dams, most of which exceed 15 m in height (Opperman et al., 2017). These dams generate approximately 70% of the Columbia's power (UPME, 2018) and over 100 new dams could be installed in the future to fulfill the country's needs (DNP, 1979).

The Magdalena basin is home to a diverse range of fish species, with 237 recorded so far (DoNascimiento et al., 2024). Of these species, 23 are identified as migratory fish species (Usma et al., 2009; Zapata and Usma, 2013; López-Casas and Jiménez-Segura, 2015; López-Casas et al., 2016; Jiménez-Segura et al., 2020), which support artisanal fisheries and account for half of the 40 to 45 commercial species consumed in the basin (Lasso et al., 2011; The Nature Conservancy, Fundación Alma, Fundación Humedales, & AUNAP, 2016). These migratory species undertake two annual upstream migrations from their feeding and growing habitats in the floodplains of the basin to their reproductive habitats in the upper river stretches, up to 1,200 m a.s.l., in the Cauca sub-basin (Mojica et al., 2012) and approximately 1,000 m a.s.l. in the Magdalena basin (Jiménez-Segura et al., 2016). Their catches represent approximately 50% of Colombia's inland fisheries harvest. The fishing industry in this basin supports approximately 61,000 fishers directly, without considering their families, of which 84.7% get their food from fishing (AUNAP and PNUD, 2021).

2.2 Data sets

To build the species model, different ichthyoplankton data sets were compiled and systematized in a database that contained information on the date recorded, sampling point name and coordinates, taxonomic identification, and individual development phases: early embryos and larvae classification according to their embryonic and larval stage of development. Ichthyoplankton sampling data were obtained from fieldwork conducted by The Nature Conservancy (TNC) and the University of Antioquia (UA), with data gathered from reports of the National Authority of Environmental Licensing (or Autoridad Nacional de Licencias Ambientales (ANLA) in Spanish) and ichthyoplankton monitoring of the El Quimbo hydropower plant, which was facilitated by the ENEL-EMGESA Environmental Department (Table 1).

Data were collected from different projects and by different researchers, and all followed standardized ichthyoplankton sampling methods for the basin; data sampling was done daily over 15 consecutive days during at least two different reproductive seasons (Jiménez-Segura, 2007). All larvae were classified by development phases and taxonomically or genetically identified by experts in the Universidad de Antioquia, Universidad Surcolombiana, or Centro de Investigación Piscícola de la Universidad de Córdoba. Genetic identification was used in the TNC-UA data set, which allowed for the classification of species that are difficult to identify in their first stages of life, such as two species from the genus *Pimelodus* (*Pimelodus grosskopfii* and *Pimelodus yuma*).

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TABLE 1 Name and location of the ichthyoplankton sample points and origin of the data sets.

ID	Sampling point	Locality (latitude; longitude)	Data origin
1	Magdalena River Main Channel I	Upstream La Miel River (5°45′37.63″N; 74°39′41.77″W)	Field work
2	Samaná Sur River	San Miguel (5°42′8.12″N; 74°44′29.66″W)	Field work
3	Magdalena River Main Channel II	Upstream Nare River (6°10′51.49″N; 74°35′2.31″W)	Field work
4	Nare River	Puerto Nare (6°12′42.56″N; 74°36′36.03″W)	Field work
5	Magdalena River Main Channel III	Puerto Berrío (6°29′18.27″N; 74°23′53.06″W)	Field work
7	Carare River	Puerto Parra (6°46′5.46″N; 74° 6′25.14″W)	Field work
9	Opon River	Yondó (6°56′48.17″N; 73°53′17.89″W)	Field work
10	Magdalena River Main Channel IV	Barrancabermeja (7°11′14.25″N; 73°56′6.05″W)	Field work
11	Sogamoso River	Barrancabermeja (7°11′57.95″N; 73°54′39.71″W)	Field work
13	Boque River	Simití (7°53′2.31″N; 73°55′53.78″W)	Field work
14	Cesar River	Puente Canoa (9°39′1.54″N; 73°38′45.19″W)	Field work
16	San Andrés River	Ituango (7° 7′52.43″N; 75°39′55.70″W)	Field work
17	Espíritu Santo River	Espíritu Santo (7°14′58.85″N; 75°26′20.01″W)	Field work
18	Cauca River Main Channel I	Valdivia (7°15′4.04″N; 75°26′30.48″W)	Field work
19	Cauca River Main Channel II	Caucasia (7°57′36.61″N; 75°11′57.85″W)	Field work
20	Nechí River	Nechí (8° 5′36.85″N; 74°45′7.75″W	Field work
21	San Jorge River	San Jorge River at bridge autopista Caucasia – Planeta Rica (8° 4′4.36″N; 75°21′30.10″W)	Field work
22	Cauca River Main Channel III	Pinillos (8°54′49.39″N; 74°28′49.47″W)	ANLA reports
23	Cauca River Main Channel IV	Cáceres (7°34′44.75″N; 75°21′23.14″W)	ANLA reports
24	Magdalena River Main Channel V	Puerto Seco (2°29′42.82″N; 75°32′36.02″W)	ANLA reports
25	Magdalena River Main Channel VI	RM (2°20′6.82″N; 75°36′56.87″W)	ANLA reports
26	Cauca River Main Channel V	Buga (3°55′3.37″N; 76°19′46.79″W)	ANLA reports
27	Cauca River Main Channel VI	La Virginia (4°53′11.03″N; 75°52′18.64″W)	ANLA reports
28	Cauca River Main Channel VII	La Pintada (5°44′44.58″N; 75°36′25.71″W)	ANLA reports
29	Cauca River Main Channel VIII	Santafe de Antioquia (6°33′31.84″N; 75°48′4.37″W)	ANLA reports
30	Cauca River Main Channel IX	La Ilusión (8° 1′0.97″N; 75° 4′57.92″W)	ANLA reports
31	Magdalena River Main Channel VIII	Bengala (2°21′18.3″N 75°35′55.6″W)	Emgesa Field work
32	Magdalena River Main Channel IX	Peña Alta (2°10′7.70″N 75°41′23.73″W)	Emgesa Field work
33	Magdalena River Main Channel X	Puerto Seco (2°29′14.2″N 75°34′05.8″W)	Emgesa Field work
34	Suaza River	Upstream from the Magdalena (2°10′22.8″N 75°40′11.5″W)	Emgesa Field work
35	Páez River	Upstream from the Magdalena (2°26′51.8″N 75°34′39.3″W)	Emgesa Field work
36	Magdalena River Main Channel XI	Bilú (2°34′56.15″N 75°29′39.10″W)	Emgesa Field work

Samples came from 36 localities across the basin. Nevertheless, the lower basin of both the Magdalena and Cauca rivers, as well as the upper Cauca River, were under-represented because a majority of the data were collected from the TNC-UA data sets, which was focused on the middle Magdalena basin, while ANLA environmental licensing reports contained data about hydropower generators, excluding significant parts of the basin.

A literature review was conducted to set up the post-fertilization time (in hours) of each development stage for each of the species reported in the data sets. During the study review period, each collected individual for a single fish species takes to reach the development phase in which it was collected. The review searched for information on the early development of migratory fish from the Magdalena Basin, or congeneric and related species of

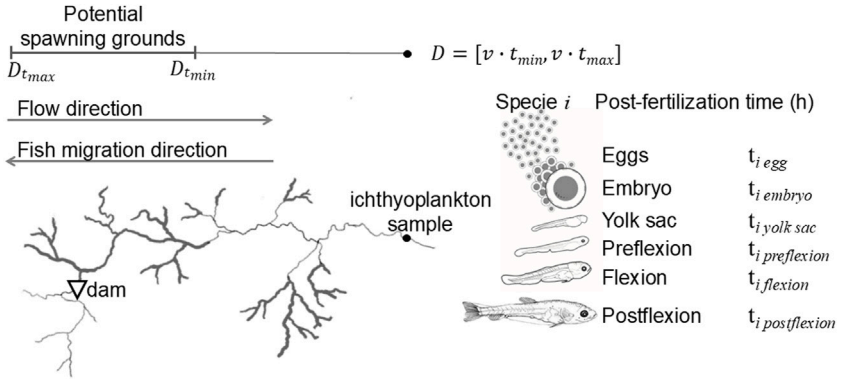

FIGURE 2
Schematic model of the spawning grounds spatial distribution model. The black dot represents ichthyoplankton sampling points, the triangle represents a dam, and the dark gray lines represent the stretches of river where spawning occurred and from where ichthyoplankton were drifting, considering water velocity in each section and the development time of each individual, as determined from the ichthyoplankton sampling.

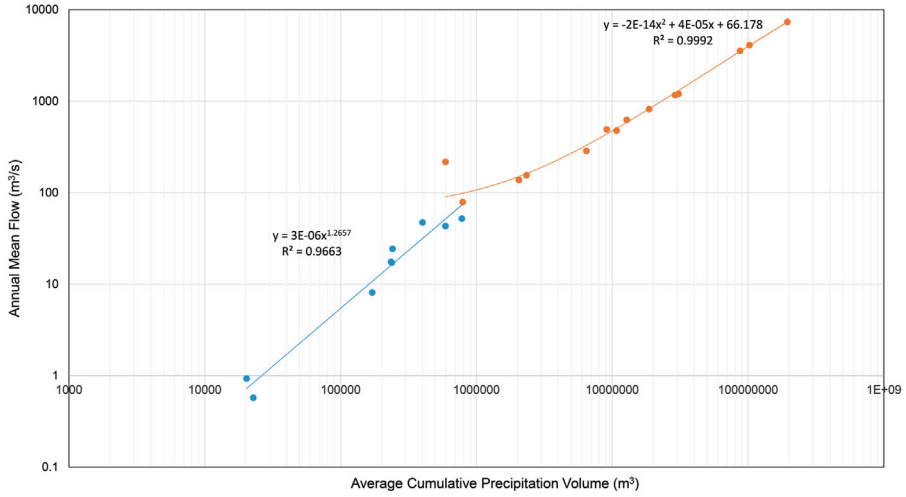

FIGURE 3
Relation between cumulative precipitation and annual mean flow for the Magdalena basin.

the Magdalena or other neotropical basins, for those species with unknown development time. Most of the reports corresponded to initial development under controlled conditions at water temperature of 26°C to 28°C (Contreras and Contreras, 1989; Atencio, 2001; Nakatani et al., 2001; Aristizábal-Regino et al., 2004; Novoa and Cataño, 2005; Arias-Gallo et al., 2010; Valbuena-Villareal et al., 2012b; Valbuena-Villarreal et al., 2012a; Stevanato, 2016; Montes-Petro et al., 2019; Arashiro et al., 2020). This time of initial development was used to determine downstream drifting time from a spawning ground.

2.3 Modeling and data analyses

The tier 1 complementary tool is a combined method using a hydraulic approximation to create the average flow velocity (flow time) and ichthyological records from embryonic and larval sampling.

First, a topological fluvial network for the Magdalena basin was created using a digital elevation model (SRTM, 90 m) and a conventional GIS procedure described by Baumbach et al. (2015). This resulted in a topological network with 34,046 river stretches with a Strahler order ranging from 1 to 8. Data from the Institute of Hydrology and Meteorology of Colombia (IDEAM, 2023) were used to determine annual mean flow for each reach and to correlate flow and cumulative precipitation.

Using aerial photographs and satellite images, and considering wide rectangular channels, the association between hydraulic radius and mean annual flow was estimated. To estimate the velocity (U) for each reach in the drainage network, the Manning equation was used:

$$U = \frac{1}{n} R_h^{2/3} S^{1/2},$$

where S denotes the slope of the reach and was calculated from the digital elevation model. R_h is the hydraulic radius and was deduced

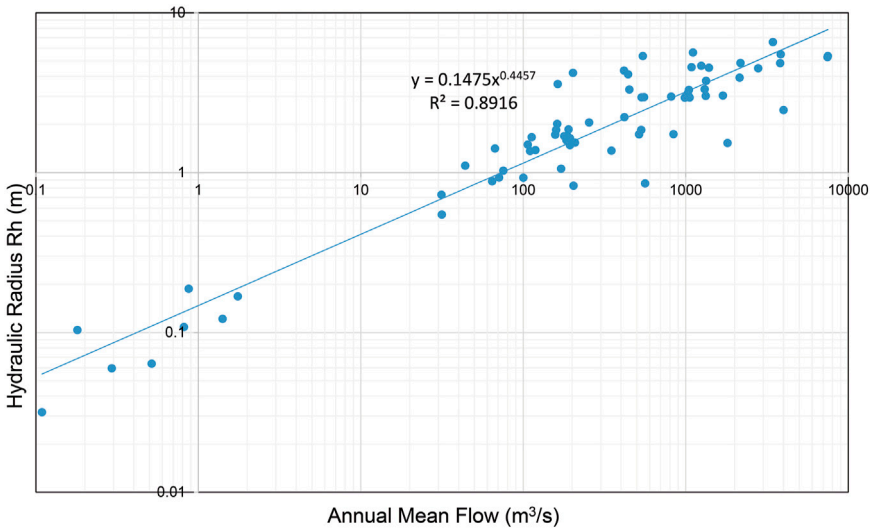

FIGURE 4
Relation between annual mean flow and hydraulic radius for the Magdalena basin.

using the relation between cumulative precipitation and annual mean flow for the Magdalena basin (Figure 3), and n represents roughness, which was estimated according to the values recommended by Bathurst (1997) based on channel slope (Manning, 1891). Using these data as a tier 1 approximation, velocity could be calculated for each reach of the system.

After determining the velocities, the flow time was calculated as

$$t = \frac{L,}{U}$$

where t is the flowing time and L denotes the reach length.

After estimating the flow time for each reach, an efficient algorithm in MATLAB (MathWorks, 2017) was developed to analyze the topological fluvial network. In this code, the user must define the location of the ichthyological sample including information on the development time (embryonic or larval stage) for each collected species, i.e., the time from spawning. To delimit a river stretch, it was necessary to set up a maximum and a minimum time for each species, otherwise spawning ground would be marked as a dot. An elevation and a Strahler order limit were set to delimit the accumulation of river stretches. The algorithm accumulates the flow time through the river network from the arc (river stretch) where the collection of the sample was indicated. Based on simple time rules of embryonic or larval time development obtained from literature, the potential stretches where spawning occurred were identified for each of the analyzed fish species (Figure 2). The algorithm was also used to locate the barriers (e.g., hydropower projects) to consider the effects of infrastructure in the topological network. The hydropower project sites were used from the 1979 master plan formulated by the Colombian government with support from the German Cooperation Agency, which generated approximately 100 points on the Magdalena River main stem, as well as several of its tributaries (DNP, 1979).

In Colombia, dams are generally located in Andean regions, upstream of key feeding and growth habitats in the floodplains. As Colombian dams are typically big (>15 m in height) and lack fish passage facilities, they act as barriers to fish reaching these critical spawning areas. Moreover, in addition to the impacts of habitat loss and isolation, even when trapped individuals might spawn in the reservoir upstream of the dam, reservoirs located between spawning grounds and floodplains can entirely block the downstream drift of eggs and larvae, thereby preventing them from reaching their critical feeding and growing habitats (Pelicice and Agostinho, 2008). Consequently, all upstream river stretches of a dam, identified in the baseline as potential spawning grounds were considered lost. In our algorithm, this type of disconnection meant that spawning drift was interrupted downstream by these barriers. To simulate this, in the special arcs where a barrier is located, the cumulative flow time is set to zero. It implies that spawning drift is completely interrupted in these arcs and travel time is reset in the arc downstream of the barrier, assuming total interruption of connectivity.

Additionally, to highlight the importance of some river basins or river sections, the richness of potential spawners in each river stretch was plotted by accumulating the number of species that potentially spawn in it.

3 Results

The ichthyoplankton samples were abundant and representative of modeling concerns. We obtained 102,303 individuals (embryos and larvae) registered in samples collected by the TNC-UA, comprising 19,748 individuals in mid-2013 and 82,555 individuals in 2014. Additionally, 2,932 larval individuals from 11 potamodromous fish species were extracted from 15 reports submitted to ANLA between 2013 and 2018. Fifteen individuals were obtained from data sets provided by ENEL-EMGESA, collected between 2014 and 2017. A final data set of 105,250 individuals and 15 fish species was used in the analysis.

In the proposed model, the river basin topological network developed consisted of 101,110 km of rivers represented in 34,046 river stretches, and mean annual flow and cumulative

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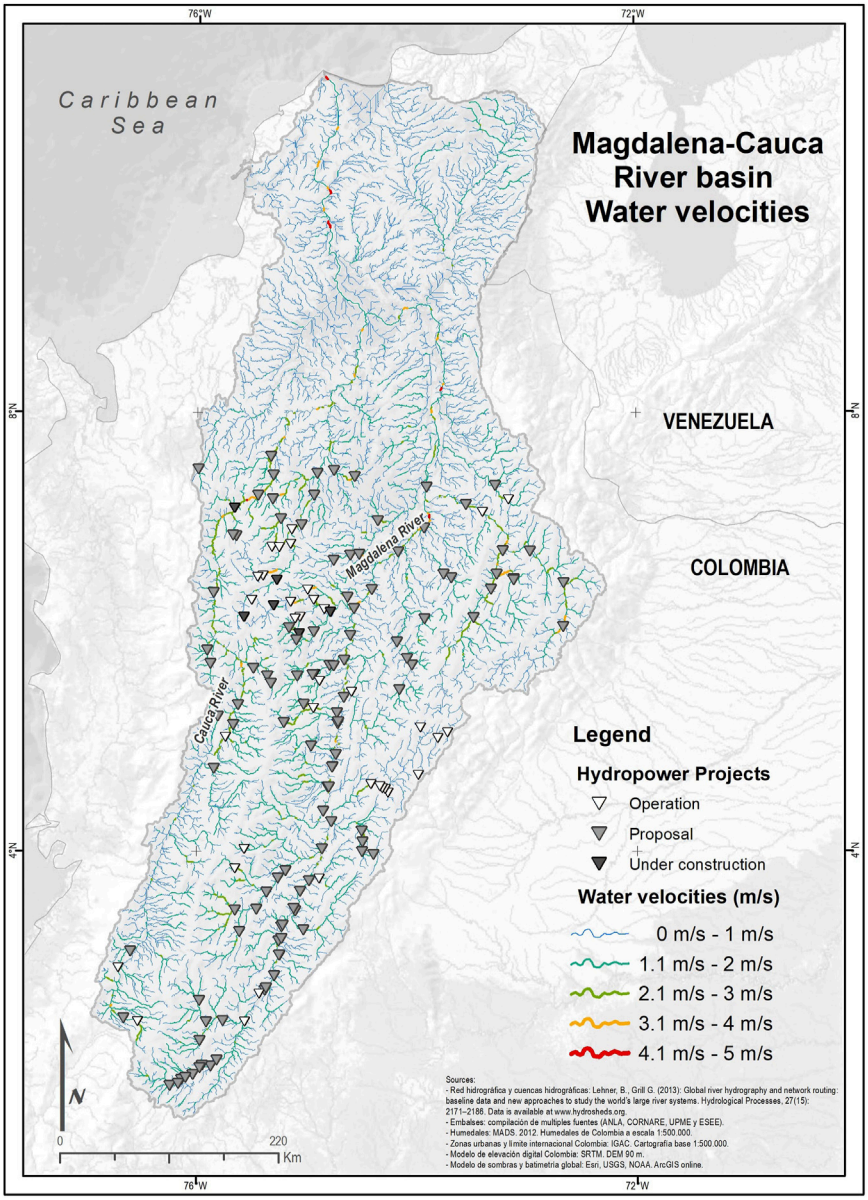

FIGURE 5
Water velocities estimated for the river network of the Magdalena basin.

precipitation in this network were both positively and significantly correlated with hydraulic radius and mean annual flow (Figures 3, 4). The relation between hydraulic radius and mean annual flow can be represented by the following:

$$Rh = 0.145Q^{0.4457}.$$

The velocities obtained from the modeling process for the entire basin ranged between 0.01 m/s and 4.89 m/s (Figure 5), whereas the velocities obtained using the flowmeter for days when eggs and larvae occurred in the samples ranged between 0.20 m/s and 4.86 m/s.

Through a simple algorithm, potential spawning areas in the Magdalena–Cauca basin were delimited considering the effects of the barriers. With an elevation limit of 1,000 m a.s.l. for the accumulation of spawning areas, the potential spawning grounds for the 15 processed species in the current (baseline) scenario accounted for 11,370 km of rivers, including Strahler order from two to eight (Figure 6A), corresponding to 11.2% of the 101,110 km of the total network) or 3656 river stretches of the basin topological network. The spawning grounds overlapped with 80 hydropower projects (56.7% of the total). Under the scenario for full development of the hydropower portfolio, spawning areas were predicted to be reduced by 45.0% for river kilometers and 45.8% for river stretches (Figure 6B; Table 2).

Total spawning area length differed by species and between the baseline and full development scenarios. Species like *Pseudoplatystoma magdaleniatum*, *M. muyscorum,* and *Prochilodus magdalenae* had a larger number of potential rivers available for spawning, ranging from 7.9% to 9.9% of the Magdalena basin river network, while other species like *S. affinis* and from the

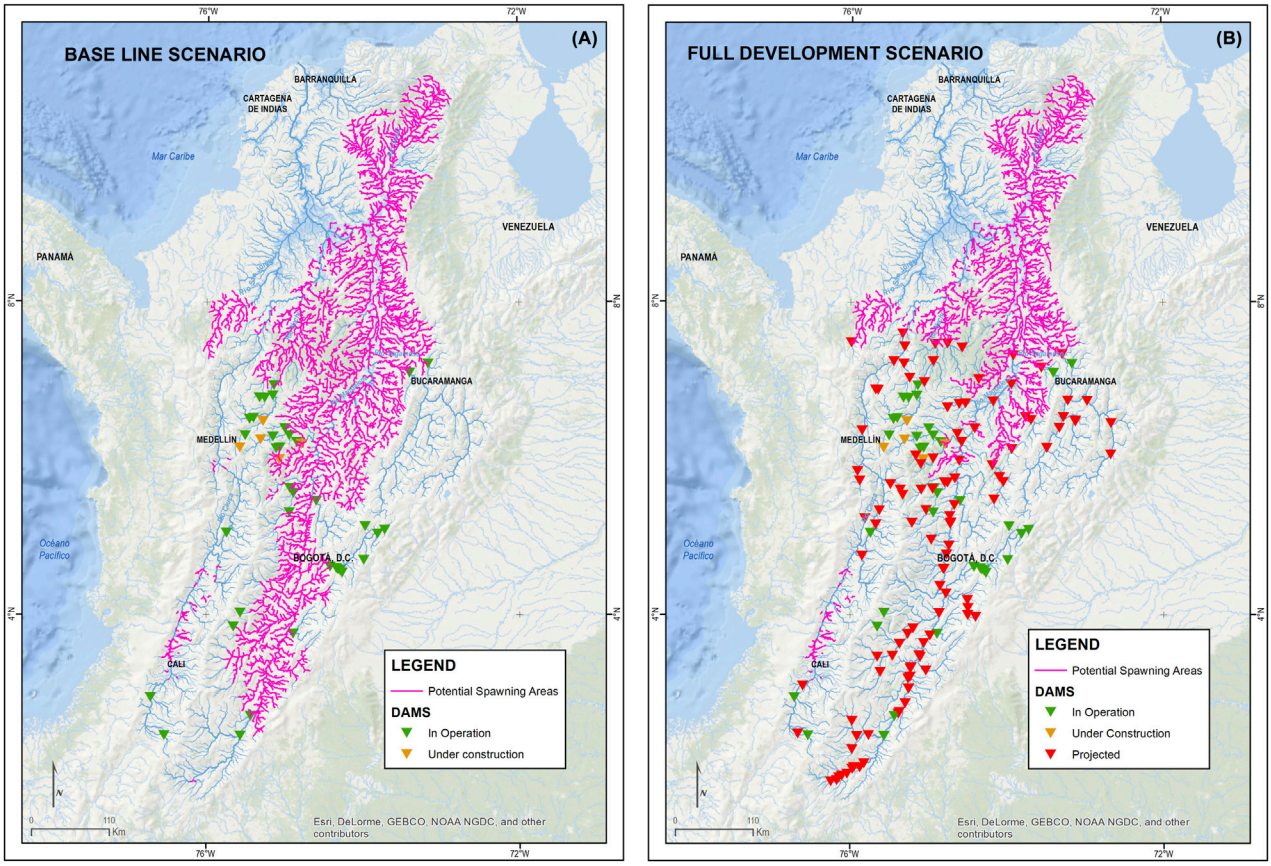

FIGURE 6
Potential spawning grounds distribution for 15 potamodromous fish species of the Magdalena–Cauca basin in **(A)** baseline scenario and **(B)** full development scenario.

genus *Brycon* had fewer and more restricted spawning areas, respectively, comprising 1.2% and ~2%, of the river network. Species that were difficult to identify as larvae, which may have been underestimated in samples, such as those from the genera *Pimelodus*, *Pseudopimelodus,* and *Astyanax*, had smaller areas, ranging from 0.1% to 5.7% of the river network (Table 1). Samples collected for *Pseudopimelodus atricaudus* species in the Cauca River did not have spawning areas available under the baseline scenario due to recent dam construction.

Potential habitat loss for each species differed among the analyzed fish species and was independent of the total length of respective spawning areas. Species with restricted spawning grounds and those with widely distributed spawning grounds were predicted to have significant habitat loss under the full hydropower project portfolio development scenario. Species with restricted reproductive areas, like those from the genera *Pseudopimelodus* and *Brycon*, were predicted to be worse affected, with respective losses of 73% and 65% of reproductive habitats, while species like *S. affinis* and those of the genera *Astyanax* and *Pimelodus* were potentially the least affected. Fish species with wide spawning ground distributions, like *P. magdalenae* and *M. muyscorum*, were predicted to lose approximately half (55.9% and 50.3%, respectively) of their reproductive areas (Table 1).

Spawning areas are not homogeneously distributed in the basin, and some river stretches showed higher richness of spawners species than others. In both the baseline and full development scenario, there are some river stretches in which up to 10 species spawn, whereas in other spawning areas, one species was found (Figures 7, 8). In both hydropower scenarios, spawner species richness mode was five species (Figure 8) in the largest number of river sections. Greater habitat loss was experienced on river sections with three, nine, and ten fish spawners species (60.2%, 74.7%, and 56.2% of the cumulative spawning area, respectively), while river stretches with one or two species loss were less than 20% of its area (Figure 8). Dam projects on rivers stretch with a higher Strahler order and located in a central position of the basin, demonstrated a greater number of predicted impacts, as shown by spawning grounds loss, than those located at the headwaters of the river network (Figure 7). Due to limited data available from the upper and middle Cauca River and the lower Magdalena River basins, their potential spawning grounds could not be precisely determined for those river stretches.

4 Discussion

Species distribution models are quantitative tools that combine species occurrence data with environmental estimates, thereby offering valuable insights into ecology and evolution while predicting distributions across landscapes (Elith and Leathwick,

TABLE 2 Potential spawning grounds length (km of rivers or number of rivers stretches) for each of the analyzed fish species in the baseline and full hydropower development scenarios and potential habitat loss between the two scenarios. Knowing the migratory fish behavior, the model was restricted to river stretches between 3 and 8 Strahler order and below 1,000 m a.s.l.

Species	Spawning grounds length				Habitat loss	
	Baseline scenario		Full development scenario		(km)	(%)
	(km)	(% of total network)	(km)	(% of total network)		
Astyanax spp	3941.1	3.9	2493.1	2.5	1448.0	36.7
Brycon spp	1985.9	2.0	683.1	0.7	1302.8	65.6
Curimata mivartii	5624.3	5.6	3091.0	3.1	2533.3	45.0
Megaleporinus muyscorum	8758.1	8.7	4352.8	4.3	4405.3	50.3
Pimelodus spp	4223.4	4.2	2741.4	2.7	1482.1	35.1
Pimelodus grosskopfii	121.4	0.1	61.3	0.1	60.1	49.5
Pimelodus yuma	121.4	0.1	61.3	0.1	60.1	49.5
Prochilodus magdalenae	8011.9	7.9	3536.8	3.5	4475.1	55.9
Pseudopimelodus spp	5800.0	5.7	1570.6	1.6	4229.4	72.9
Pseudopimelodus atricaudus	300.7	0.3	280.8	0.3	19.9	6.6
Pseudopimelodus magnus	0.0	0.0	0.0	0.0	0.0	
Pseudoplatystoma magdaleniatum	9970.4	9.9	5166.0	5.1	4804.4	48.2
Salminus affinis	1562.0	1.5	1221.1	1.2	340.9	21.8
Sorubim cuspicaudus	5585.1	5.5	3356.8	3.3	2228.3	39.9
Triportheus magdalenae	4294.0	4.2	2221.7	2.2	2072.3	48.3
Total km of rivers	11,370.2	11.2	6257.8	6.2	5112.4	45.0
River stretches (n)	3656		1982		1674.0	45.8
% of stretches of the total network	10.7		5.8			

2009). The proposed model, with its simple assumptions and calculations, and without rigid limits or data extrapolations, serves as a Tier 1 tool for rapid assessments and early planning, aiming to minimize environmental impacts. Its reliance on ichthyoplankton samples and current knowledge of embryonic and larval development enables efficient mapping of potential spawning areas at the macro-basin scale, despite its spatial and temporal limitations. Furthermore, it provides a foundation for ecosystem-based management planning (Langhans et al., 2019). Moreover, the model allows for easy improvement with the incorporation of new data.

This study has some limitations, such as gaps in sampling coverage and incomplete knowledge of developmental stages for some species, although this is the first time that a map of spawning areas has been obtained for Colombia. Notably, spawning grounds were likely underestimated due to limited data from several key sections of the river, while some species could not be modeled because of a lack of early developmental data, even among congeneric species. Similarly, inadequate sampling frequency and imprecise taxonomic identification could dramatically affect the determination of the major spawning rivers and the detection of spawning events of some migratory species (Pompeu et al., 2023). Thus, some migratory species (*Cynopotamus magdalenae*, *Cyphocharax magdalenae*, *Ichthyoelephas longirostris,* and *Pimelodus cripticus*) recognized as migratory in the basin (López-Casas et al., 2016; Jiménez-Segura et al., 2020), were found absent in our samples, highlighting the need for broader temporal and spatial data collection. Taxonomic challenges in identifying species of taxonomically challenging groups of species (*Astyanax* spp, *Brycon* spp, *Pimelodus* spp, and *Pseudopimelodus* spp) at early life stages also require genetic tools for improved accuracy.

Notably, data sets were compiled from different years. Specifically, data on *Pseudopimelodus atricaudus* were collected in the Cauca River before the construction of the Hidroituango dam. Currently, the river is dammed, and the model recognizes the dam as a barrier to fish migration, resulting in no identified spawning areas in the baseline scenario. Still, to improve the accuracy of quantifying available spawning kilometers, we explored the possibility of constraining the network using the river's Strahler order and altitude. A deeper understanding of spawning ground requirements—incorporating geomorphological and hydraulic factors—could further refine this approach. Furthermore, integrating hydrodynamic models could significantly improve the spatial and temporal resolution of our analysis, offering a more comprehensive and precise understanding of spawning habitats.

Overlaying spawning grounds and hydropower projects provides a useful approach for prioritizing hydropower dam planning. With a portfolio of more than 100 planned projects in

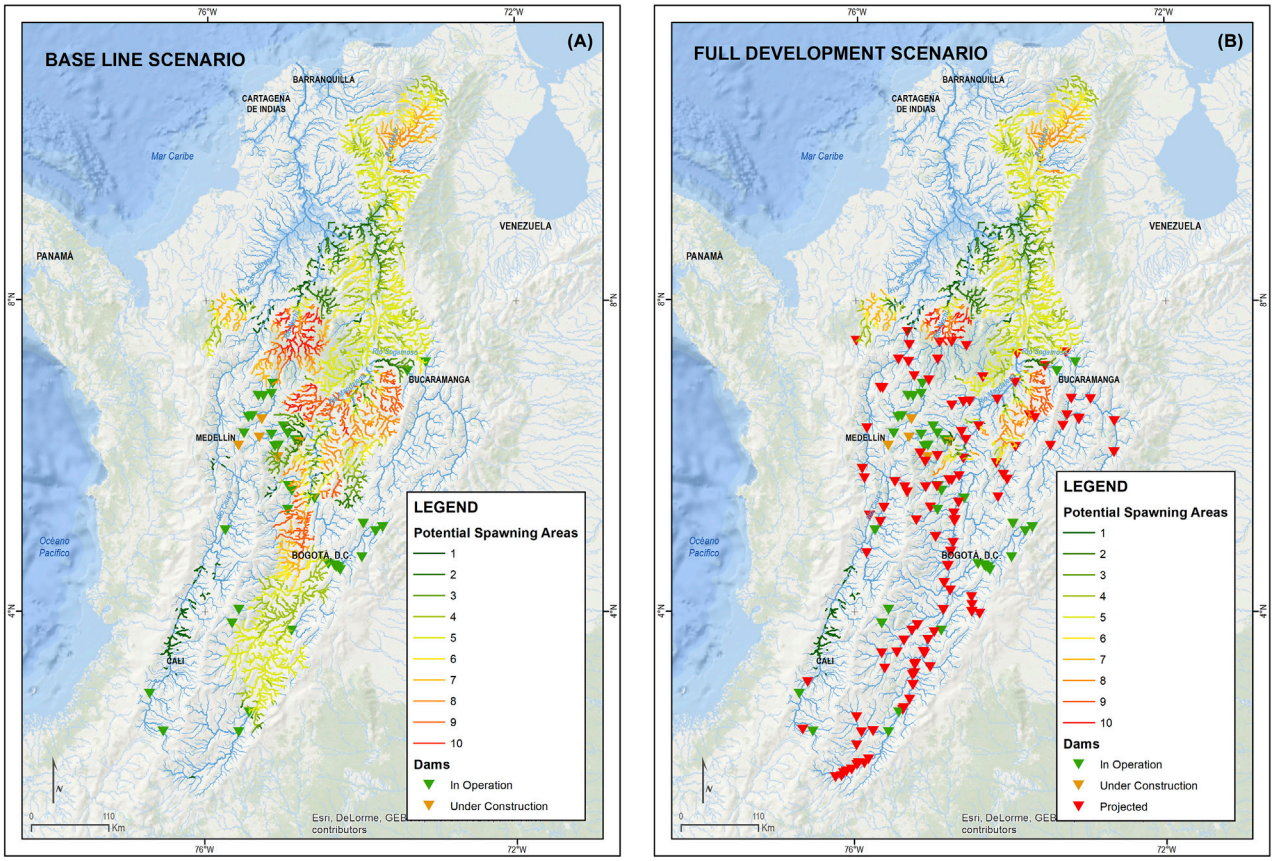

FIGURE 7
Spawners species richness for 15 potamodromous fish species of the Magdalena basin in **(A)** baseline scenario and **(B)** full development scenario.

the basin (DNP, 1979), the absence of suitable policies that address river fragmentation and conservation of potamodromous fish species underscores the importance of the current analysis. By prioritizing projects based on basin-wide planning, policymakers can better balance hydropower development with freshwater biodiversity conservation. This information can be considered in the early planning stages of a project at the macro-basin scale to eliminate or minimize environmental impacts and find optimal.

Our findings emphasize the need for integrated, basin-wide cumulative impact assessments rather than the traditional isolated project evaluations. Typically, EIAs consider the potential habitat impacts and loss associated with an individual project only. Therefore, a single project may be approved for construction, despite causing minimal habitat loss (measured in river kilometers of spawning grounds) for one or a few species. Yet, when viewed at the macro-basin scale, these river kilometers may represent critical habitats for species with limited spawning grounds (such as *Brycon* spp. and *Pseudopimelodus* spp.). Therefore, this seemingly minor loss can have far-reaching consequences, compromising the ecosystems' ability to sustain these species and their fisheries.

Prioritizing regional and basin-wide planning for dam placement is crucial for striking a balance between conflicting energy and biodiversity interests in the energy sector while ensuring that freshwater ecosystems remain functional to support migratory fish populations and the services these species and ecosystems provide, as suggested to invigorate freshwater conservation (Flitcroft et al., 2019). Nevertheless, it is crucial not only to preserve the spawning grounds but also to feed and grow habitats (the floodplain systems) and the river stretches that connect them. This integrated approach is vital for preserving essential global freshwater ecosystem services and effectively addressing the current freshwater biodiversity crisis (Albert et al., 2021).

Our results revealed that certain river stretches and basins are more critical as spawning habitats for potamodromous fish than others, indicating that not all rivers are of equal importance for the conservation and maintenance of these species. Though it may seem intuitive to prioritize spawning areas with high species richness, such as those supporting 11 species, conservation efforts should instead focus on river stretches that encompass the entire distribution of each species. This approach aligns with the principles of systematic conservation planning. This stresses the need for comprehensive landscape management strategies that balance production and protection (Margules and Pressey, 2000), following comprehensiveness, adequacy, representativeness, and complementarity criteria.

Dam projects occupying a central position in the network have greater impacts on habitat loss for the reproduction and maintenance of potamodromous fish species. Although we could not conduct a comprehensive analysis of the Magdalena hydropower project portfolio, the maps indicated that projects

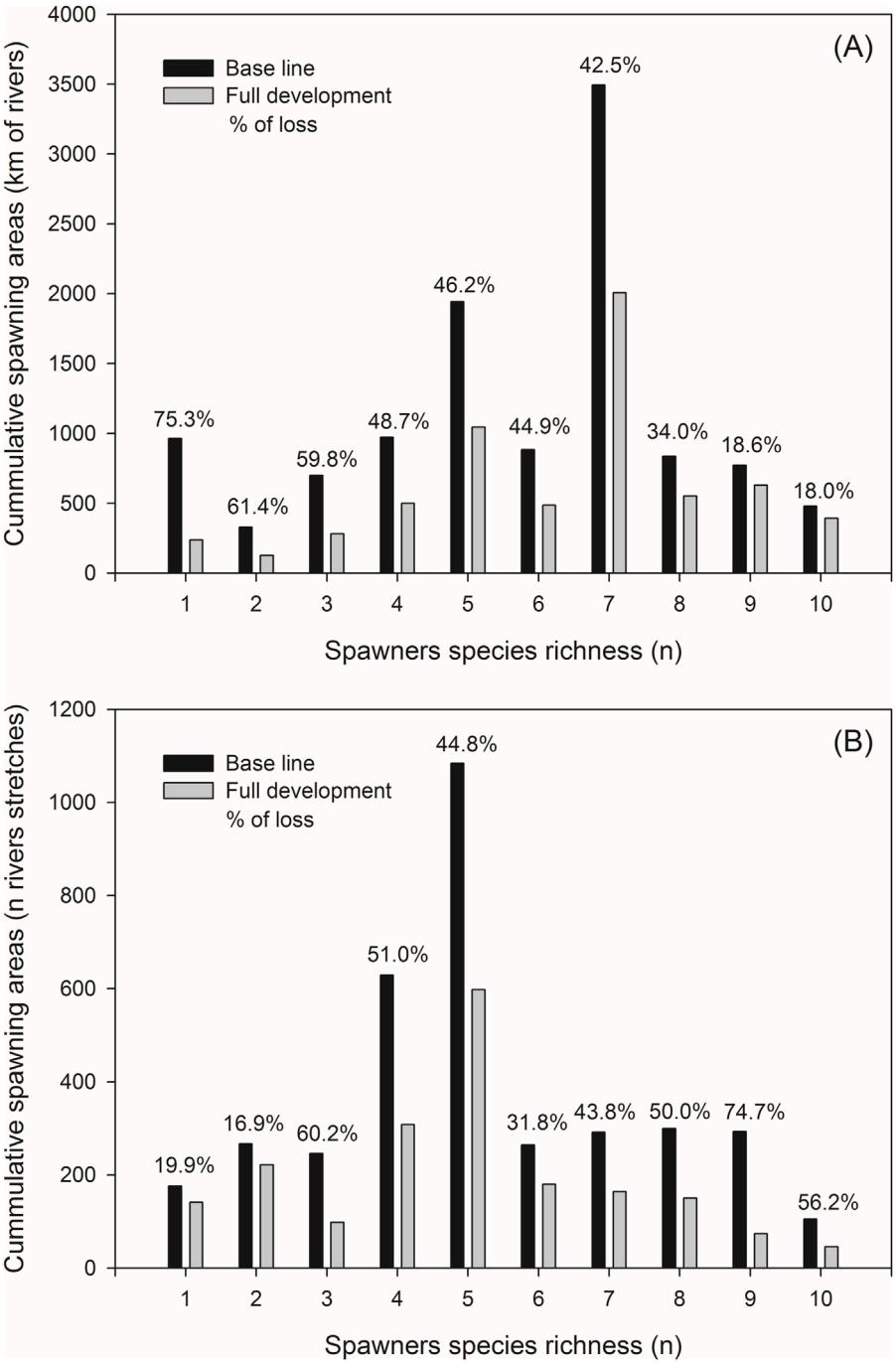

FIGURE 8
Cumulative spawning areas by species richness values calculated for 15 potamodromous fish species of the Magdalena basin in baseline scenario (dark gray) and full development scenario (light gray). **(A)** Length. **(B)** Number of river stretches.

located on major rivers (Strahler orders 8 and 7, such as the Cauca and Magdalena Rivers) tended to have more impacts and would disproportionately affect critical spawning habitats for commercially and ecologically significant potamodromous fish. Notably, even a single project can lead to the loss of critical habitats for multiple species, resulting in the elimination of spawning areas with both high and low species richness. This risk stresses the importance of maintaining connectivity within dendritic river systems for fish and fisheries conservation (Koning et al., 2020) as documented in the emergency recovery plan to bend the curve of global freshwater biodiversity loss (Tickner et al., 2020).

5 Conclusion

The present study demonstrates the application and potential benefits of the proposed model, in minimizing habitat loss through a quantitative case study of hydropower development in the

Magdalena River basin. The results elucidate how the proposed model can help minimize environmental impacts for projects, particularly those related to the loss of critical spawning areas for potamodromous fish species and disruptions to fish migration patterns that affect fisheries, considering that projects are part of a larger system. Furthermore, we can also conclude that the model can also help identify solutions that balance economic benefits with biodiversity conservation, resulting in lower environmental and social impacts and greater economic benefits.

Data availability statement

The code associated with this paper is openly available at https://github.com/N4W-Facility/Spawning_Ground_Model.

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because we used datasets of ichthyoplankton samples from different projects, so we did not handle or use live animals.

Author contributions

SL-C: conceptualization, data curation, formal analysis, methodology, supervision, visualization, writing–original draft, and writing–review and editing. CR-P: conceptualization, formal analysis, methodology, software, validation, visualization, writing–original draft, and writing–review and editing. VA-G: data curation, formal analysis, writing–original draft, and writing–review and editing. CM-Á: data curation, writing–original draft, and writing–review and editing. DA: data curation, writing–original draft, and writing–review and editing. KR-C: data curation, writing–original draft, and writing–review and editing. LJ-S: data curation, writing–original draft, and writing–review and editing.

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Conflict of interest

Author DA was employed by Integral S.A.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2025.1425804/full#supplementary-material

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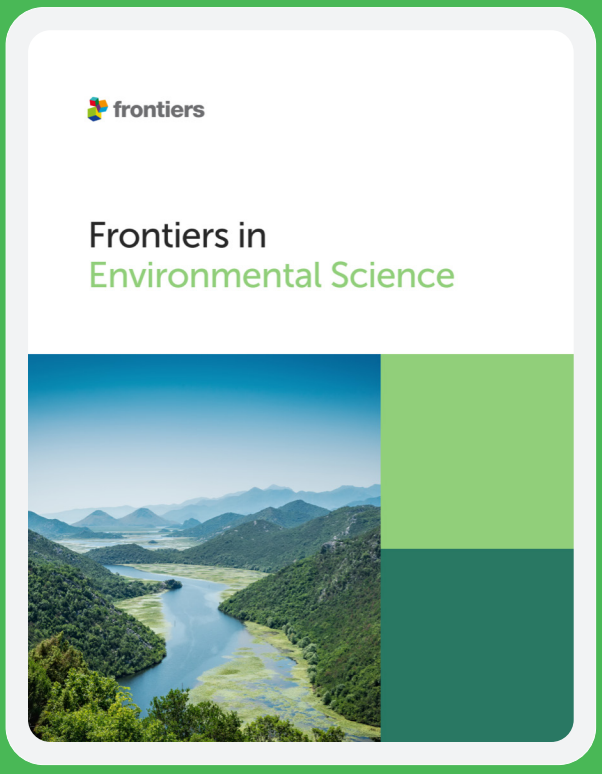

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