ORIGINAL ARTICLE

Distribution of fish species in the upper Po River Basin (NW Italy): a synthesis of 30 years of data

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ABSTRACT

Freshwater fish is the most diverse group of vertebrates but, unfortunately, also one of the most threatened. While some well-known, charismatic species have been subject to important conservation efforts, many others have long been neglected. The aim of this paper is to analyse the distribution over time of seven freshwater fish species and one lamprey in the upper Po River basin (NW Italy), an important biodiversity hotspot. Six of them are native species listed in Annex II of the Habitats Directive (*Lampetra zanandreai*, *Protochondrostoma genei*, *Chondrostoma soetta*, *Telestes muticellus*, *Sabanejewia larvata*, and *Cottus gobio*), while the other two are key invasive species (*Silurus glanis* and *Misgurnus anguillicaudatus*). Data from four regional fish population monitoring campaigns carried out between 1988 and 2019 were analysed. For each species and monitoring campaign, an average Representativeness Index, measuring abundance and population structure, and an Occurrence Frequency were calculated, and then assessed for changes over the time. Of the studied species, *P. genei* declined the most in the last 30 years, while *C. soetta* and *S. larvata* are in a very critical situation, with very few remaining populations. *T. muticellus*, *C. gobio* and *L. zanandreai* seem to have declined only slightly, but their vulnerability should not be underestimated. The two invasive species, on the other hand, show a substantial increase in occurrence as well as range. While some drivers for the decline in the native species vary according to their ecological and biological characteristics, others are more general and linked to the overall degradation of the river environments: habitat alterations, loss of connectivity, excessive water abstraction, pollution, and the presence of invasive alien species. The effects of climate change, such as the rise of inland water temperatures and the alteration of hydrological cycles, must also be taken into account. Diverse and far-reaching conservation efforts are needed to improve the fish habitat and thus also protect the unique biodiversity of this region.

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Key words: freshwater fish; biodiversity conservation; endangered species; aquatic ecosystems; species distribution; conservation measures.

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INTRODUCTION

Freshwater habitats support an impressive biodiversity. Although inland waters only cover 0.8% of the Earth's surface, they support approximately 15,000 fish species, representing half of the global fish diversity (Dudgeon *et al*., 2006). Freshwater fish, considered in a broad and non-holophyletic way, including both Cephalaspidomorphi and Actinopterygii, are the most diverse and species rich group of European vertebrates. The Mediterranean region, in particular, is recognised as a biodiversity hotspot for freshwater fish species with approximately 460 species belonging to 101 different genera and 23 families, including a high number of endemisms (Tierno de Figueroa *et al*., 2013; Zerunian, 2003; Freyhof and Brooks, 2011). Italy is an excellent example of this situation: 53 native species are currently recognised in the freshwater ecosystems of this peninsula, 20 of which are endemic to the Padano-Veneto district (Fortini, 2016).

Unfortunately, freshwater fish is also one of the most threatened animal groups at both continental and national level.

In the the European Red List, 37% of the fish species are considered threatened (Freyhof and Brooks, 2011), and 15 species considered Extinct or Extinct in the wild. In Italy, the situation is even more serious: the 2013 Red List of Italian vertebrates classified 48% of species as Endangered (Rondinini *et al*., 2013), while the latest Red List, published in 2022, indicates a further deterioration, with 18 species moving to a higher threat category than in the previous assessment (Rondinini *et al*., 2022).

The decline of freshwater fish populations has multiple causes, that can act at different scales. At the global scale, climate change disrupts hydrological regimes and raise water temperatures: this leads to an increase in the frequency and intensity of extreme hydrological events, especially droughts, and a decrease in available habitats (Barbarossa *et al*., 2021). At the local scale, several factors interact. Among the most relevant are the regulation and abstraction of water flows, pollution, disruption of longitudinal connectivity, morphological alterations (canalisation, construction of dams, flood prevention works, clogging, and other disturbances of the sediment cycle), overfishing, and the invasion of alien species (Dudgeon, 2019). In Italian freshwaters 47 alien species have been reported, representing 47% of the present fish species (Rondinini *et al*., 2022). The spread of ichthyophagous birds, such as the Great Cormorant (*Phalacrocorax carbo sinensis* Blumenbach, 1798), is also having an important impact on some species (Jepsen *et al.*, 2018; Ovegård *et al*., 2021). Naturally, different stressors interact with each other and risk amplifying the conservation threats.

The need for freshwater conservation efforts is increasingly acknowledged in both research and management (Closs *et al*., 2016). However, both research and conservation efforts are disproportionately focused on emblematic species that are of interest to recreational or professional fishermen, with the bulk of freshwater species being largely neglected (Rytwinski *et al*., 2021; Vøllestad, 2023). This also implies that the lack of ecological knowledge, including movement behaviour, habitat requirements, foraging strategies, and species distribution, is particularly notable for small-sized fish species and species with low direct economic value (Smialek *et al*., 2019; Negro *et al*., 2021).

Here, we aim to increase the biogeographic knowledge of five understudied native fish species and one lamprey, as well as two invasive alien species in the upper Po River basin. We map the distribution and abundance over time of the native Po brook lamprey (*Lampetra zanandreai* Vladykov, 1955), South European nase (*Protochondrostoma genei* Bonaparte, 1839), Italian nase (*Chondrostoma soetta* Bonaparte, 1840), Italian riffle dace (*Telestes muticellus* Bonaparte, 1837), Italian golden loach (*Sabanejewia larvata* De Filippi, 1859), and European bullhead (*Cottus gobio* Linnaeus, 1758). The selected native species are of conservation concern at both European and Italian level. With their different ecological characteristics (some are fossorial and prefer still waters, others are benthic, and others are reophilic and migratory with high swimming abilities), they represent a broad spectrum of the fish community of the upper Po River basin. As a contrast, we also perform the same analysis for two highly invasive species found in the same river system: Wels catfish (*Silurus glanis* Linnaeus, 1758) and Pond loach (*Misgurnus anguillicaudatus* Cantor, 1842).

METHODS

Study area

The Po River originates in the Alps and empties into the Adriatic Sea after 652 km, making it Italy's largest watercourse (watershed surface 74,000 km^2; mean annual flow 1540 m^3/s) (Autorità di Bacino del Fiume Po, 2001). The Upper Po watershed, considered in this study, has a drainage area of about 25.592 km^2, a mean elevation of 957 m asl (min: 98 m asl; max: 4750 m asl and a mean annual discharge of about 447 m^3/s (Regione Piemonte, 2007). The watershed covers a large part of the Piemonte region, from the high mountain streams of the Alpine zone to slow-flowing tributaries of the Po Valley. The fish fauna of the region is very diverse and includes species typical of the upper salmonid zone (*Salmo marmoratus* Cuvier, 1829, *Salmo ghigii* Pomini, 1941 and *Cottus gobio* Linnaeus, 1758), the lower salmonid zone (*Thymallus aeliani* Valenciennes, 1848 and *Barbus caninus* Bonaparte, 1839), the reophilous cyprinid zone (*Romanogobio benacensis* Pollini, 1816, *Squalius squalus* Bonaparte, 1837), and the limnophilous cyprinid zone (*Padogobius bonelli* Bonaparte, 1846, *Cobitis bilineata* Canestrini, 1865, *Alburnus alborella* Bonaparte, 1841) (Regione Piemonte, 2009; Fortini, 2016). In total, 28 native species belonging to 14 families are recognised in Piemonte; in the same area, 17 alien species were recorded in 2009, and 23 alien species in 2019 (Regione Piemonte, 2009; Bovero *et al.*, 2021). This number is is likely to increase further: for example, a new alien spined loach, probably of Danubian origin, has been detected in regional waters in recent years (Delmastro *et al.*, 2021).

Monitoring campaigns

In the years 1988/1989, 2004, 2009 and 2017-2019, four freshwater fish monitoring campaigns were carried out in the upper Po River, Piemonte, NW Italy. The presence, abundance and population structure of the fish species were determined over a network of sites covering the whole river catchment using wading electrofishing. The first survey included 297 stations (1988/1989), the second 202 (2004), the third 428 (2009), and the fourth 209 (2017-2019). In this study, we used data from these four surveys to analyse the population trends for the eight selected species. For each target species and survey, a mean Representativeness Index (RI) and an Occurrence Frequency (OF) were calculated. Bovero *et al*. (2021) used the data collected in the four surveys to obtain a single Representativeness Index (RI) that is comparable over time. This index describes the status of a fish population found at a given sampling station as a function of population structure and size, and results in values from 0.4 to 1 (0.4, 0.5, 0.6, 0.8, 1). An RI value of 0.4 refers to a population that may have lost the ability to self-maintain as it is represented by only a few adult specimens. A value of 1.0 refers to abundant and well-structured populations of many individuals (*e.g.*, 30% of juveniles in the pre-reproductive phase, or 20% of sexually mature adults in the total population). The middle values (0.5, 0.6, 0.8) indicate intermediate situations where the population may be abundant but poorly structured (exclusively or predominantly adults), or well-structured but with few or very few individuals present (Bovero *et al.*, 2021). For the first two surveys (1988/1989 and 2004), qualitative and descriptive data on populations were available, which were converted into numerical RI values by Bovero *et al*. (2021). In the last two surveys (2009 and 2017-2019), populations were assigned an abundance index consisting of a number (1-4) related to population size and a letter (a, b, c) related to population structure; this index was converted into RI with the method described by Bovero *et al*. (2021).

To assess changes in native species populations we used 154 stations, of which 104 were common to all surveys and 50 common to the first (1988/1989), third (2009) and last (2017-2019) surveys. The mean RI per species and survey was calculated using the RI values of the species occurrence stations within the subset of 154 selected stations. OF per species and survey was calculated as a relative frequency, by excluding the stations located in areas where the native species is not expected to occur naturally. Using the information available in the bibliography and the elevation of the occurrence points in the four regional surveys, a maximum elevation for the presence of each native species was chosen and all stations above this value were excluded. OF was then obtained from the ratio between the number of stations where the species was found in the survey and the number of stations suitable for the presence of the species according to the elevation criterion.

Lampetra zanandreai is reported to occur up to 600 m asl by Bianco (1986) and up to 647 m asl by Candiotto *et al*. (2023). In the regional surveys, it occurs up to 653 m asl (in the Stura di Demonte River); 700 m asl was therefore used as a limit. For *Protochondrostoma genei*, a maximum elevation of 550 m asl was defined, since the species is reported in the literature to occur up to about 500 m asl (Delmastro, 1982) and was found in the regional surveys up to 533 m asl (in the Sessera stream). *Chondrostoma soetta* is reported by Fortini (2016) at elevations up to 1000 m asl and occurs in the regional surveys up to 241 m asl. Considering the bibliographical data and its migration to the middle and upper reaches of rivers, a maximum elevation of 1000 m asl was used. *Telestes muticellus* was found up to 746 m asl in the regional surveys (in the Tanaro River). It has also been reported in the literature up to 1500 m asl and over 2000 m asl in some alpine lakes (Tortonese, 1975), but these data are unconfirmed (Delmastro and Balma, 2007) and the species has probably been introduced in these high elevations lentic systems. Therefore, the upper elevation limit for its presence was set to 900 m asl. No bibliographical information on this parameter was found for *Sabanejewia larvata*. The maximum elevation at which it occurs in the regional surveys is 319 m asl. This species is often found in association with the Italian spined loach (*Cobitis bilineata* Canestrini, 1865), which was common up to about 450 m asl and sporadically up to almost 700 m asl. during the surveys. As *C. bilineata* has a broader ecological spectrum than *S. larvata*, a maximum elevation of 500 m asl was used for the latter. *Cottus gobio* is considered to be common up to 800-1200 m asl (Tortonese, 1975; Delmastro, 1982; Gandolfi *et al.*, 1991; Zerunian, 2004), and some authors report it up to 2000 m asl (Fortini, 2016). In the four surveys it was found up to 1428 m asl (in the Toce stream). Taking into account the information in the literature, 2000 m asl was set as the maximum elevation.

For the two alien species, no filter was applied to calculate the mean RI and the OF, as their presence and spread over time are due to human introduction, and the ecological characteristics of their local sites may differ from those of their native range. The mean RI was calculated using the RI values of the species occurrence stations within the whole station network of each survey, and OF was calculated as an absolute frequency, by dividing the number of stations where the alien species was present by the total number of stations examined in each survey.

Presence/absence data (Occurrence frequency) from the four regional surveys are more reliable than RI values, which, especially in 1988/1989 and 2004, may have been influenced by the sampling method and the way in which the biological status of the populations was assessed (Bovero *et al*., 2021). For this reason, Kruskal-Wallis tests were used to assess differences in relative (native species) and absolute (non-native species) OF between years within species, while no statistical tests were performed on RI data. Pairwise comparisons were performed using Dunn's test with Bonferroni correction (significance level was set at $\alpha = 0.05$) to determine specific differences between the four surveys. Calculations and analyses were performed using RStudio version 2023.12.0.369 and QGIS version 3.34.3.

In "Results and Discussion", we provide our commentaries on the OF for each species, while the results of the mean RI are discussed in the section "Considerations on Occurrence Frequency and Representativness Index".

RESULTS AND DISCUSSION

Protochondrostoma genei

Our data show a clear decrease in relative OF of *P. genei* in the study area (Tab. 1, Figs. 1A and 2). A significant difference in the relative OF was found between the first and the second survey (KW: chi-squared=15.95, df=3, p=0.001; Dunn's test: adjusted p=0.01). This result indicates that *P. genei* populations decreased significantly from 1988-1989 to 2004, after which the relative OF remained almost unchanged. *P. genei* is endemic to the Padano-Veneto district, and it used to be one of the most abundant and widespread species in lowland lotic systems of this area together with the Italian chub (*Squalius squalus*) and the Italian riffle dace (Regione Piemonte, 2009). It is typically rheophilous and gregarious and inhabits the middle and upper reaches of lowland rivers and their tributaries, preferring clear, fast-flowing, oxygen-rich waters with a gravelly or pebbly substrate (Gandolfi *et al.*, 1991; Zerunian, 2004; Forneris *et al*., 2012). One of the main reasons for its significant decline is related to the presence of weirs and dams along the river course, which prevent adult fish from migrating upstream to areas suitable for reproduction (Gandolfi *et al.*, 1991; Zerunian, 2004; Forneris *et al.*, 2012). Other important causes of decline are the degradation of water quality, and anthropogenic interventions that modify the morphology of watercourses, particularly the composition of the riverbed which may no longer be suitable for spawning (Gandolfi *et al.*, 1991; Zerunian, 2004; Forneris *et al.*, 2012). These factors have caused the local extinction of many populations, contributing to the significant decline of the species underlined by our results, and consequently to its classification as "Endangered" at the national level by the IUCN (Rondinini *et al.,* 2022). In the fourth national report (2013-2018) of the Habitats Directive, its conservation status is classified as "Unfavourable-Bad" in the Continental bio-ecoregion (Ercole *et al.*, 2021).

Chondrostoma soetta

Our data show a very critical situation for *C. soetta* in the study area. In particular, its relative OF is extremely low in all surveys, reaching zero in the second and third (Tab. 1, Figs. 1A and 2). Although a slightly significant difference in the relative OF of the species was found between the surveys (KW: chi-squared=8.38, df=3, p=0.04), Dunn's test showed no significant differences between any pair of surveys (adjusted p>0.05). *C. soetta*, an endemism of the Padano-Veneto district, is restricted to lowland rivers and, therefore, has a naturally low frequency in Piemonte (Forneris *et al*., 2012). Its rarefaction in regional waters has been known since the 1970s (Tortonese, 1975), and the lack of change over the study period found here should therefore not be interpreted as a lack of concern.

For most of the year, this species inhabits lowland and deeper reaches and potholes of the Po and other high-order rivers, then between the end of April and May it migrates in dense shoals in the tributaries to spawn (Delmastro, 1981; Delmastro, 1982; Gandolfi *et al.*, 1991). The main causes of its decline are similar to those of *P. genei*: the presence of dams and weirs that prevent movement and migration, followed by the artificialisation of watercourses, the alteration of riverbeds, and intense predation by ichthyophagous birds (Zerunian 2004; Forneris *et al*., 2012). In Italy, *C. soetta* is classified as "Critically Endangered" by the IUCN (Rondinini *et al.*, 2022) and its conservation status is classified as "Unfavourable-Bad" in the latest report (2013-2018) of the Habitats Directive in both bioecoregions where it occurs, the Alpine and the Continental (Ercole *et al.*, 2021).

Telestes muticellus

For *T. muticellus*, our data show a general decreasing trend in relative OF (Tab. 1, Figs 1A and 2), but no significant difference was found in the relative OF of the species between the four surveys (KW: chi-squared = 5.24, df = 3, p=0.16).

T. muticellus is endemic to Italy, from the Po River basin to Campania region, where it inhabits low order lotic systems with fast flowing, clear, and oxygenated waters with coarse riverbeds (Zerunian, 2004; Forneris *et al.*, 2012; Fortini, 2016). The non-significance of our result might suggest that *T. muticellus* is not in a particularly worrying situation and that it has experienced a slight decline compared to other species. In fact, at the national level *T. muticellus* is listed as "Least Concern" by the IUCN (Rondinini *et al*., 2022) and its conservation status is classified as "Favourable" in the Alpine and Continental bio-ecoregions, and "Unfavourable-Inadequate" in the Mediterranean bio-ecoregion by the fourth report (2013-2018) of the Habitats Directive (Ercole *et al.*, 2021). However, the conservation concern should not be underestimated, as a population reduction that preceded the first regional survey was already reported in 1991 by Gandolfi *et al.* and subsequently confirmed by other authors (Zerunian, 2004; Forneris *et al*., 2012). Indeed, *T. muticellus* has some vulnerabilities: it is sensitive to low water quality and therefore to the various forms of water pollution, including eutrophication (Delmastro *et al*., 2007a), to habitat alterations such as artificialisation of riverbeds and gravel removal, and, depending on the context, to the loss of longitudinal connectivity (Schiavon *et al*., 2024), as well as excessive water abstraction (Gandolfi *et al*., 1991; Zerunian, 2004), and predation by other fish, especially introduced salmonids (Forneris *et al*., 2012; Fortini, 2016).

Tab 1. Native fish species occurrence in the four monitoring surveys.

	Survey	*P. genei*	*C. soetta*	*T. muticellus*	*S. larvata*	*C. gobio*	*L. zanandreai*
Tot. presence	1988-1989	99	14	199	10	93	26
	2004	47	4	130	1	40	9
	2009	85	8	251	0	99	7
	2017/2019	43	2	147	0	59	19
154 stations presence	1988-1989	58	5	121	3	51	13
	2004	23	0	76	1	25	5
	2009	36	0	105	0	35	3
	2017/2019	32	2	113	0	46	13
Altitudinal range	1988-1989	130	145	145	122	154	142
	2004	92	100	100	87	104	98
	2009	130	145	145	122	154	142
	2017/2019	130	145	145	122	154	142
Mean RI	1988-1989	0.77±0.02	0.64±0.04	0.87±0.01	0.6±0.00	0.75±0.02	0.62±0.02
	2004	0.84±0.04	-	0.96±0.01	1.0±0.00	0.91±0.03	0.88±0.08
	2009	0.69±0.04	-	0.91±0.02	-	0.81±0.04	0.80±0.00
	2017/2019	0.68±0.04	0.40±0.00	0.84±0.02	-	0.66±0.03	0.68±0.06
Relative OF	1988-1989	0.45	0.03	0.83	0.02	0.33	0.09
	2004	0.25	0.00	0.76	0.01	0.24	0.05
	2009	0.28	0.00	0.72	0.00	0.23	0.02
	2017/2019	0.25	0.01	0.78	0.00	0.30	0.09

Tot. presence, number of stations where the species was found in each survey; 154 stations presence, number of stations where the species was found to be present in the network of 154 selected stations; altitudinal range, number of stations suitable for the presence of the species (according to elevation criteria) in the network of 154 selected stations; mean RI: mean Representativeness Index calculated in the species occurrence stations within the network of 154 selected stations; relative OF, relative Occurrence Frequency calculated as 154 stations presence altitudinal range.

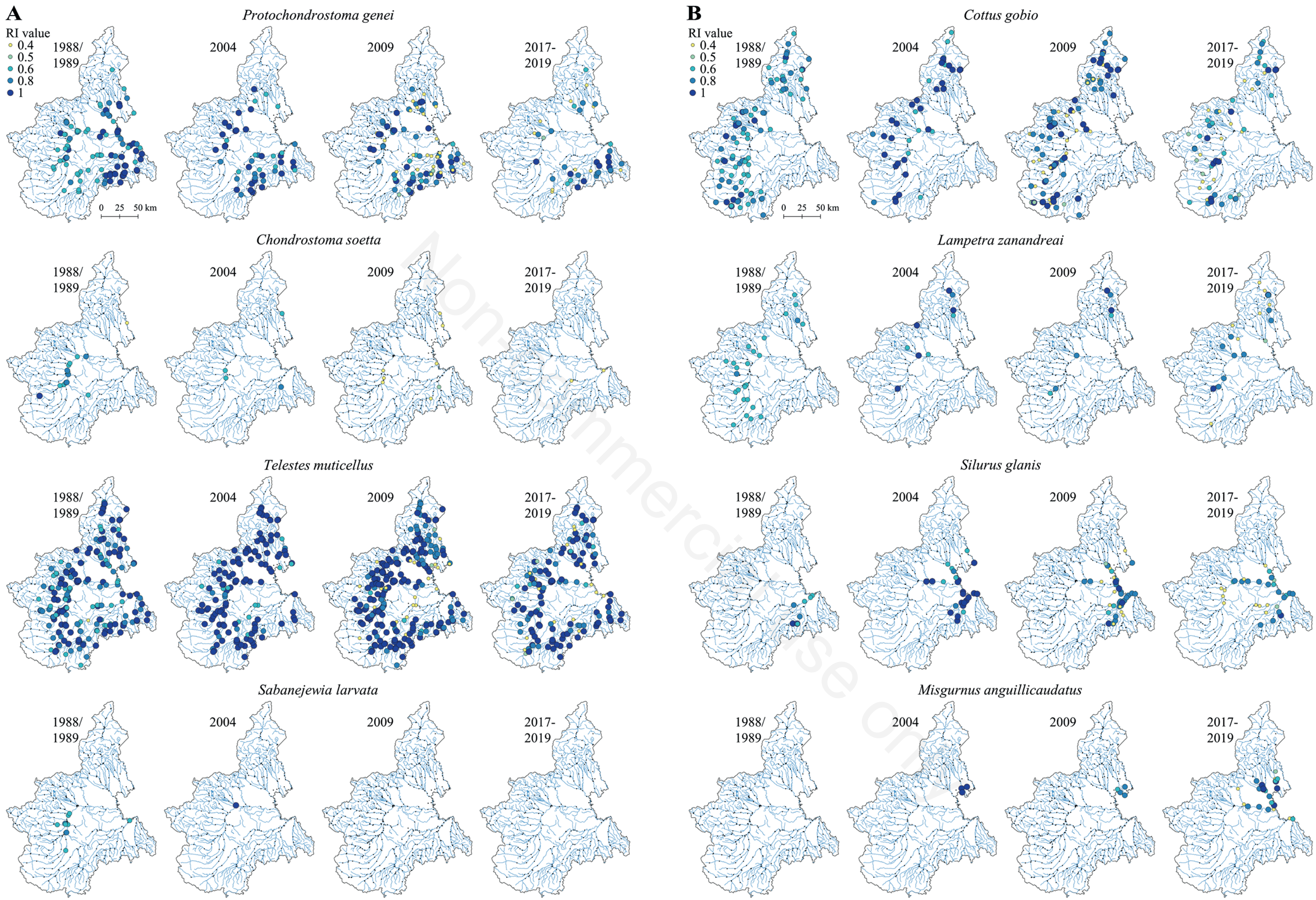

Fig. 1. Points of occurrence of the species in the four monitoring surveys. Black dots represent the stations investigated during the survey. Colored dots are the occurrence points of the species, their size and color varies according to the Representativness Index (RI) value as indicated in the legend.

Sabanejewia larvata

The situation for *S. larvata* is very critical and the species has been absent from the monitoring stations in the regional surveys since 2009 (Tab. 1, Figs. 1A and 2). Its relative OF is very low in all surveys and consequently no significant difference was found between the four surveys for this parameter (KW: chi-squared=5.694, df=3, p=0.13). This is partly due to the fact that *S. larvata* is a naturally rare species (Forneris *et al.*, 2012), restricted to a few areas with specific characteristics. This endemism of the Padano-Veneto district is found exclusively in lowland springs and other watercourses characterised by fine, soft and vegetated bottoms, and moderate or slow currents (Gandolfi *et al.*, 1991; Zerunian, 2004). The biology of this species is poorly understood, but it is likely to be stenoecious and highly demanding in terms of environmental quality (Gandolfi *et al.*, 1991; Zerunian, 2004). In particular, this species seems to be particularly sensitive to the alteration and cementation of the riverbeds, as well as to pollution (Zerunian, 2004; Forneris *et al.*, 2012; Fortini, 2016). For these reasons, the practice of dredging lowland springs has probably contributed to the destruction of one of its ideal environments.

Despite its natural low frequency, in the past *S. larvata* used to be common and abundant in some areas (Delmastro, 1981), contrasting with its absence from the later survey data. At the national level, the species is listed as "Vulnerable" by the IUCN (Rondinini *et al.*, 2022) and its conservation status is "Unfavourable-Bad" in the Alpine and Continental bio-ecoregions, according to the latest report (2013-2018) of Habitats Directive (Ercole *et al.*, 2021).

Cottus gobio

Although *C. gobio* shows a general declining trend in its relative OF (Tab. 1, Figs. 1B and 2), no significant difference was found between the four surveys (KW: chi-squared=2.95, df=3, p=0.39).

From this result, it may seem that *Cottus gobio* is not particularly threatened, but a general decline throughout its range, and at a time scale longer than our study period, was reported in 1991 (Gandolfi *et al*., 1991) and confirmed in subsequent years (Delmastro *et al*., 2007; Forneris *et al*., 2012). This benthic species includes both mountain and lowland springs populations within our study area, with the latter having suffered the greatest decline (Gandolfi *et al*., 1991; Zerunian, 2004). *C. gobio* is present in the Alpine and Continental bio-ecoregions and, according to the fourth report of the Habitats Directive (2013-2018), the populations in the Continental region have a "Unfavourable-Bad" conservation status with a declining trend, while those in the Alpine region have a "Favourable" conservation status (Ercole *et al*., 2021). This difference is probably due to the higher levels of anthropisation and degradation in the Continental region compared to the Alpine region, resulting in poorer environmental and water quality in the former. Indeed, this species is very sensitive in terms of environmental quality and is therefore particularly vulnerable to phenomena such as water pollution, even minor, anthropic actions that modify the bottom of watercourses, excessive water abstraction and also the presence of salmonids that exert predatory pressure (Zerunian, 2004; Forneris *et al*., 2012; Fortini, 2016). Also the increase in temperatures could be responsible of the collapse of a growing num-

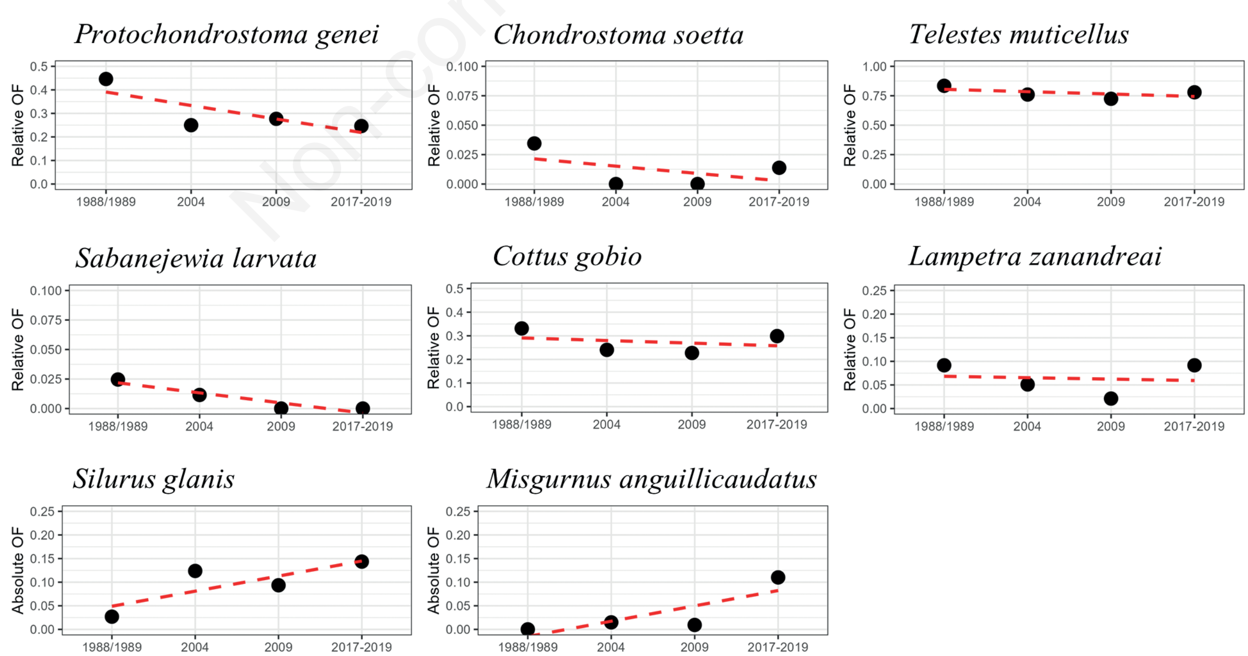

Fig. 2. Relative (native species) and absolute (non-native species) Occurrence Frequency (OF) of the species in the four monitoring surveys. The dashed red line is the population trend line. The OF scale on the y-axis varies between species to better highlight population changes.

ber of populations. In the past, it was subject to intense poaching but at present this species has no angling interest (Fortini, 2016). The IUCN considers this species as "Least Concern" at the national level (Rondinini *et al.*, 2022).

Lampetra zanandreai

A general declining trend is reported for *L. zanandreai* relative OF (Tab. 1, Figs. 1B and 2). Comparing the relative OF of the species between the surveys, a slightly significant difference was found (KW: chi-squared=8.10, df=3, p=0.04), but Dunn's test showed no significant differences between any pair of surveys (adjusted p>0.05).

Although our results are not significant, a marked decline of this species has been reported since the 1980s (Bianco, 1986) and confirmed by more recent authors (Zerunian, 2004; Delmastro *et al.*, 2007a; Delmastro *et al*., 2007b; Fortini, 2016). Some basic aspects of the life cycle of *L. zanandreai* are described in the literature (Zerunian, 2004; Fortini, 2016), but knowledge about its biology, ecology and distribution is still very scarce. A recent work (Candiotto *et al*., 2023) showed that the Po brook lamprey is present in the Belbo and Bormida rivers and in a large part of the Tanaro River, when previous studies hypothesized the absence of this species in the right tributaries of the Po (Bianco, 1986; Fortini, 2016). To be noted, the fact that *L. zanandreai* is a cyclostome, and not a fish, may have unintentionally led to less attentive data collection during the fish monitoring campagins, and hance less accurate occurrence data. Our results for this species are therefore difficult to evaluate, as the data used are likely to be incomplete.

In general, *L. zanandreai* is likely to be present at a low frequency in our waters, as it is associated with environments with specific characteristics. The larval phase, which lasts four to five years, is very sensitive to the quality and characteristics of the water and subsrate (Fortini, 2016; Negro *et al*., 2023). Other vulnerabilities are related to habitat alterations such as the removal of gravel resulting in the disappearance of spawning habitats, the significant increase in surface water temperatures and, in the past, the massive capture of individuals for human consumption (Zerunian, 2004; Fortini, 2016; Candiotto *et al*., 2023). In Italy, *L. zanandreai* is classified as "Vulnerable" by the IUCN (Rondinini *et al*., 2022) and its conservation status is considered "Unfavourable-Inadequate" in the Alpine and Continental bio-ecoregions by the latest (2013-2018) Habitats Directive report (Ercole *et al.*, 2021).

Silurus glanis

During the period studied, this non-native species was characterised by a rapid expansion of its range, which took place mainly at first along the Tanaro river, and then affected the Po and some of its left-hand tributaries (Tab. 2, Figs. 1B and 2). The absolute OF of *S. glanis* was significantly different between the first and the fourth surveys (KW: chi-squared=24.354, df=3, p=0.01; Dunn's test: adjusted p=0.03), but no significant differences were detected between consecutive surveys.

S. glanis is native to central-eastern Europe and north-western Asia and it has been introduced into other countries for recreational fishing. It was first reported in Italy in 1957, in the Adda River (Manfredi, 1957), but it is possible that the species was already present in other areas, such as Lake Como, as early as in the 1930s-40s (Fortini, 2016). For Piemonte, there is a report from 1975 when two juveniles were accidentally released in a small private pond near Valperga (Balma *et al*., 1989). The species seems to have spread from the central-eastern part of the Po basin, where it was acclimatised in the 1970s-80s (Delmastro 1986; Gandolfi *et al*., 1991; Fortini 2016; Fig. 1B). It is well documented that this species has a high invasive capacity, helped by its behavioral plasticity and ability to adapt to different environmental factors, such as rapid variations in oxygen levels and changes in water temperature and pollution (Mancini *et al*., 2022). The species may, however, avoid cold and fast-flowing waters (Nyqvist *et al*., 2022). The impact of *S. glanis* on different ecological groups is now well known, including the fact that it is a generalist predator that can rapidly adapt to new prey species, with devastating effects on native fish communities (Volta *et al*., 2018; Mancini *et al*., 2022).

Misgurnus anguillicaudatus

M. anguillicaudatus was absent during the first survey, extremely localised in the easternmost part of Piedmont during the second survey, whereas subsequent campaigns revealed its rapid spread westwards, along the Po and Sesia rivers (Tab. 2, Figs. 1B and 2). A significant difference in the absolute OF of this

Tab 2. Alien fish species occurrence in the four monitoring surveys.

	Survey	*S. glanis*	*M. anguillicaudatus*
Tot. presence	1988-1989	8	0
	2004	25	3
	2009	40	4
	2017/2019	30	23
Mean RI	1988-1989	0.75±0.06	-
	2004	0.89±0.03	1.00±0.00
	2009	0.69±0.03	0.75±0.05
	2017/2019	0.62±0.04	0.71±0.05
Absolute OF	1988-1989	0.03	-
	2004	0.12	0.01
	2009	0.09	0.01
	2017/2019	0.14	0.11

Tot. presence, number of stations where the species was found in each survey; mean RI, mean Representativeness Index calculated in the species occurrence stations within the whole station network of each survey; absolute OF, absolute Occurrence Frequency of the species calculated as total presence/total stations of each survey.

species was found between the third and the fourth surveys (KW: chi-squared=70.755, df=3, $p=8.99 \times 10^{-7}$; Dunn's test: adjusted $p=1.38 \times 10^{-5}$), indicating an expansion from 2009 to 2017-2019.

The first report of *M. anguillicaudatus* in Italy dates back to 1997, when an individual was found in the irrigation network of Carbonara Ticino (PV, Lombardia, Italy). In the following years numerous adults and juveniles were found in the province of Pavia, confirming the establishment of the species (Razzetti *et al*., 2001). It is likely that it has invaded Piemonte from this area. This is in line with its presence in the hydrographic network of Vercelli in 2004, where in the two most recent surveys it is increasingly widespread and abundant (Fig. 1B).

M. anguillicaudatus is a Cobitidae native to south-east Asia and introduced into various European areas as an ornamental fish. It has benthic habits and can live in hypoxic and even anoxic waters thanks to its ability to extract atmospheric oxygen through the digestive canal (McMahon, 1987). This species can also survive in polluted environments characterised by high levels of ammonia, nitrites and nitrates (NOx), and turbidity (Keller *et al.*, 2007). Due to its high reproductive and dispersal capacity, the Pond loach is highly adaptable and is of great concern because of the potential trophic and habitat competition with other benthic aquatic organisms (including the threatened *S. larvata*) and because of the role it may play in the spread of parasitic diseases (Razzetti *et al*., 2001).

CONSIDERATIONS ON OCCURRENCE FREQUENCY AND REPRESENTATIVENESS INDEX

Changes in OF are a good indicator of population trends, as its decrease indicates the disappearance of the species from certain stations and thus a contranction in its range, whereas its increase indicates its expansion. All native species show a general declining trend in OF while the two invasive species show an expansion of their occurrence. (Figs. 1 and 2). *P. genei* is the species that has suffered the greatest decline in the last 30 years, showing a significant difference in relative OF between the surveys. *C. soetta* and *S. larvata* do not show significant differences in relative OF but both of them have extremely low values of this parameter (reaching zero in some surveys), indicating a very critical situation, close to extinction. The populations of *L. zanandreai*, *T. muticellus* and *C. gobio* show an overall trend in decline over the period studied.

The mean RI (Tab. 1) is useful for understanding the status of populations in terms of abundance and structure, also if it should be evaluated together with the OF data in order not to be misleading. For example, *S. larvata* reached the maximum index in 2004, but the information refers to the single station where the species was found (Tab. 1; Fig. 1A) so that this data indicates a very fragile situation. However, all the species analysed, both native and introduced, show a similar trend for this parameter, with a mean RI increasing from 1988-1989 to 2004, and then decreasing in subsequent surveys (Tabs. 1 and 2). As explained in Materials and Methods, the estimation of this parameter for the first two surveys may be less accurate, and is therefore possible that the data from the first survey (1988-1989) were underestimated. After the 2004 peak, however, the species show a decrease in mean RI as well. For native species, this decrease is consistent with the general decline observed in relative OF: populations have probably declined in abundance and structure to the point of local extinction in some cases. For the two alien species, the decrease in mean RI may be due to the application of containment plans, or, more likely, to the fact that an alien species may have few individuals in an area where it has recently arrived during its expansion.

CONSIDERATIONS ON REGIONAL SURVEYS DATA

The data analysed in this study refer to an extensive monitoring network and provide a reliable general picture of changes in species populations in Piemonte over the last 30 years. However, this network of stations is fixed and predefined, so the data do not always give a complete picture, especially for species with very limited distribution or specific ecological characteristics. For example, *S. larvata* now appears to be extinct in Piemonte, although fortunately a few populations are still known (Fenoglio and Candiotto, 2024). Similarly, it is unlikely that *C. soetta* was extinct in 2004 and 2009, but the few areas where the species was present in those years were not included in the surveys. In addition, and importantly, the decline of these two species had already been reported before the first regional survey, so that the 1988/1989 data represent an already impaired situation.

L. zanandreai is a particular case because the data collected during regional surveys are unlikely to be representative of the real situation of the species, which deserves an independent assessment of its distribution and conservation status, as well as in-depth studies to better understand its biology, ecology and behavior.

For more common and widespread species, such as *P. genei*, *T. muticellus* and *C. gobio*, the data are certainly more representative of the regional situation and allow us to understand the true variations in OF, abundance, and population structure.

THREATS TO NATIVE FISH FAUNA

A wide range of interacting stressors threaten native fish species. Although effects vary according to the species ecological and biological characteristics, many stressors are common to most species. The general degradation of the river environment (Dagnino *et al*., 2013), *i.e.* the diffuse alteration of stream morphology, hydrology and water quality impacts the ecosystem as a whole. Dams and weirs hinder the migratatory spieces, such as *P. genei* and *C. soetta*, but also more stationary fish depend on longitudinally connected rivers for dispersal and short distance migrations in response to environmental change (Jones *et al.*, 2021; De Fries *et al*., 2023; Schiavon *et al*., 2024). Non native species can, for example, increase predation pressure (*e.g.* Wels catfish; Candiotto *et al*., 2011), alter the physio-chemical environment (*e.g. M. anguillicaudatus*; Keller *et al*., 2007), increase the intensity of competion for resources or cause hybridisation, all with potentental effects on fish populations and the ecosystem as a whole (Erarto and Getahun, 2020). Relatedly, Great Cormorant (*Phalacrocorax carbo sinensi*) and other piscivourus birds have greatly expanded their range and numbers in the region in recent decades, with sometimes drastic effects on the local ichtiofauna (Ovegård *et al*., 2021; Bianco and Delmastro, 2011), perhaps especially diurnal, pelagic, medium-sized fish (such as *P. genei* and *C. soetta*; Foneris *et al*., 2012). Climate change impacts hydrological cycle and water temperature, contributing to the list of interacting stressors on local fish populations (Volta and Jeppesen, 2021). The long list of general stressors make it difficult to pinpoint direct causes of fish population declines, but highlight the need for conservation efforts to increase population resilience (Lawrence *et al*., 2014).

CONSERVATION ACTIONS TO REVERSE THE DECLINE OF NATIVE FISH FAUNA

A large number of activities can and need to be implemented in order to conserve native fish species. In our study area, the LIFE Minnow project has planned several types of conservation action to reverse the decline of the six native species considered in this study. One is the re-establishment of longitudinal connectivity in water bodies that are currently fragmented by the presence of barriers, allowing the free movement of species and encouraging the greater exchange between sub-populations. Another is the improvement of habitat quality through the restoration of some springs as well as selective riverbank restoration to increase the availability of suitable areas for the different life stages of the target species. In addition, the spawners of four species (*P. genei*, *C. soetta*, *T. muticellus*, *C. gobio*) will be bred in breeding facilities to release juveniles in the project area. In particular, the remaining small and non-self-sustaining subpopulations will be supplemented, and populations will be reintroduced in areas where the species are extinct. Finally, the control of invasive alien species is planned. In this context, an innovative aspect of the project is the reuse of biomass from the capture of *Silurus glanis* for the production of animal feed through collaboration with a supply chain. These wide-ranging actions will contribute to improve the health of river habitats in the upper Po River basin and the conservation status of the six target species, as well as other native species not directly involved in the project.

CONCLUSIONS

The results of this analysis show how most of the considered native fish species have suffered a great decline in the last thirty years or are in a dramatic situation, with very few remaining populations in Piemonte, while invasive ones are quickly expanding their presence along the upper Po River hydrographical network. This picture represents a general trend and contributes to define conservation priorities at the local level. The present study also highlights the potential importance of grey-literature to inform about understudied species and outline population trends, and emphasizes the value of publicly available local monitoring data. The lack of knowledge for many small sized freshwater fish species, particularly endemisms, underscores the need for research efforts concerning their biology and ecology of small sized fish species. This is urgently needed to inform effective conservation measures.

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