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CICLE XXXII

Coordinator Prof. Barbujani Guido

*THE ROLE OF EXOTIC SPECIES
AND ENVIRONMENTAL STRESSORS
IN THE DECLINE OF ITALIAN
FRESHWATER FISH*

Ph.D. candidate

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Abstract

The importance of biodiversity for ecosystem functioning and resilience is widely acknowledged. Nevertheless, biodiversity constantly declines worldwide. Among different ecosystems, freshwaters appear more vulnerable to biodiversity loss due to several anthropic disturbances, to cite some, habitat degradation, pollution and overexploitation of water resources, introduction of exotic species, overfishing and climate change. The scope and magnitude of these pressures has been investigated in many studies but some uncertainties still remain on the interplay between different pressures and especially on the effects of exotic species.

This thesis investigates the decline of native fish community in Italian rivers and canals, analyzing the effects and the interactions of environmental pressures and exotic fish species.

Official data from provincial and regional fish inventories were collected and digitized in homogenous datasets which, at following steps, extended from the whole Emilia-Romagna Region, to the Padano-Veneto District, and to the whole Italian territory, from North to South, including main islands, for a total of 4126 fish sampling sites along the Italian watercourses.

Data analysis is organized in three main parts: 1) the assessment of the fish communities composition and the identification of the most important factors of disturbance, 2) the analysis of alpha and beta diversity of native and exotic components of the fish communities, and 3) the effects of exotic fish species on functional diversity of fish communities.

The results have highlighted that 1) exotic species are widespread and in many cases the invasion degree was severe. Environmental features generally resulted less detrimental to the native fish community than exotic species. This trend reached an apparent paradox in the highly altered Oglio River, where the two dams, unpassable to fish, present in the middle course, acted as a defense, rather than an obstacle, for the surviving native community, as prevent exotic species upstream migration from the highly invaded Po River. Other evidences have further supported this observation, with exotic species pushing the native ones on the edge of local extinction in their optimal native range and displaced some residual populations on the edge of their natural altitudinal distribution.

Similar results were found also in term of diversity 2) with alpha and beta native diversity higher in the upland rivers, whereas exotic species resulted dominate large rivers and canals in the lowlands. The results suggest also a homogenization process in fish communities due to few exotic species, widespread and dominant in most of lowland waterways.

Inside invasion dynamic, the results 3) suggest that not all generalist traits are equally important for a successful invasion. Temperature and low oxygen tolerant species, with some eurytopic traits resulted more advantaged than native ones, and dominant in the exotic communities.

On the scale of the whole Italy, a high invasion degree corresponded to lower functional diversity. Furthermore exotic species showed a lower functional diversity than native species and had more negative effects than translocated species on the diversity of fish communities.

Overall, results of this thesis have also provided an updated picture on the state of the fish communities in Italy, calling for urgent need of conservation and management actions.

Keywords: Biodiversity, fish, abiotic pressures, water quality, dam, exotic species, species invasion

List of publications

This thesis is based on the following publications:

[1] **Gavioli, A.**, Mancini, M., Milardi, M., Aschonitis, V., Racchetti, E., Viaroli, P., Castaldelli, G. (2018). Exotic species, rather than low flow, negatively affect native fish in the Oglio River, Northern Italy. *River Research and Applications*, (December 2017), 1–11. <https://doi.org/10.1002/rra.3324>

Highlights

- Exotic fish species affected native ones more than water quality and hydromorphological variables.
- Native species were found abundant in low flow and shallow depth waters, where exotics were not widespread.
- The presence of dams in the middle reach block exotic species migrating upstream preserving native populations.

[2] Milardi, M., Aschonitis, V., **Gavioli, A.**, Lanzoni, M., Fano, E. A., Castaldelli, G. (2018). Run to the hills: exotic fish invasions and water quality degradation drive native fish to higher altitudes. *Science of The Total Environment*, 624, 1325–1335. <https://doi.org/10.1016/j.scitotenv.2017.12.237>

Highlights

- Exotic species were found to be a major factor in native species abundance distribution in freshwater habitats of Northern Italy.
- The combined feedback of exotic species and eutrophication drove most native fish species on the edge of their natural distribution and towards extinction.
- Site- and species-specific conservation actions could benefit from a carefully considered prioritization provided in our work.

[3] Aschonitis, V. G., **Gavioli, A.**, Lanzoni, M., Fano, E. A., Feld, C., Castaldelli, G. (2018). Proposing priorities of intervention for the recovery of native fish populations using hierarchical ranking of environmental and exotic species impact. *Journal of Environmental Management*, 210, 36–50. <https://doi.org/10.1016/j.jenvman.2018.01.006>

Highlights

- Need of combined and well-designed interventions to restore environmental quality and restricting exotics invasion.
- A new method was proposed here to design spatially explicit priorities of intervention.
- The method showed that the northwestern lowland areas of Emilia-Romagna region presented the higher priority for intervention to preserve/restore native communities.

[4] **Gavioli, A.**, Milardi, M., Lanzoni, M., Mantovani, S., Aschonitis, V., Soana, E., Fano E.A, Castaldelli, G. (2018). Managing the environment in a pinch: red swamp crayfish tells a cautionary tale of ecosystem based management in northeastern Italy. *Ecological Engineering*, 120(July), 546–553. <https://doi.org/10.1016/j.ecoleng.2018.07.013>

Highlights

- Red swamp crayfish was less established in large, deeper and fast flowing waterways, especially when these are unvegetated.
- Fish predation was also found a significant factor in limiting red swamp crayfish abundance.
- A different hydraulic management, which can also permit a major predation, could slow down or even reverse the invasion process.

[5] **Gavioli, A.**, Milardi, M., Soininen, J., Fano E.A., Castaldelli, G. (2019). Diversity patterns of native and exotic fish species suggest homogenization processes, but partly fail to highlight extinction threats, (January), 1–12. <https://doi.org/10.1111/ddi.12904>

Highlights

- In lowlands, exotic species richness was higher than native species one, especially in large rivers and drainage canals.
- Large rivers in the uplands are important in maintaining native fish diversity.
- Rare native species can show low contribution to beta diversity, although they required conservation actions.

[6] Milardi, M., **Gavioli, A.***, Castaldelli, G., Soininen, J. (2020). Partial decoupling between exotic fish and habitat constraints remains evident in late invasion stages. *Aquatic Sciences* 82,14

Highlights

- Generalist traits were widespread in nearly all areas where exotic species occurred with the dominance of temperature tolerants, low oxygen tolerants and eurytopic traits
- Invasion dynamics could partly decouple environment and biotic communities
- Exotic species ecofunctional diversity hotspots were located in human disturbed areas

[7] Milardi, M., **Gavioli, A.***, Soininen, J., Castaldelli, G. (2019). Exotic species invasions undermine regional functional diversity of freshwater fish. *Scientific Reports* 9:17921 <https://doi.org/10.1038/s41598-019-54210-1>

Highlights

- Lower diversity of ecological traits in communities corresponded to a high invasion degree
- Exotic component of communities showed less diverse ecological traits than the native one
- Exotic species invasions could reduce the functional diversity of native communities

[8] Milardi, M., **Gavioli, A.**, Soana, E., Lanzoni, M., Fano, E. A., Castaldelli, G. (2020). The role of species introduction in modifying the functional diversity of native communities, *Science of the Total Environment* Volume 699, 2020, 134364. <https://doi.org/10.1016/j.scitotenv.2019.134364>

Highlights

- Exotic and translocated fish species had different geographical distribution patterns.
- Invasion can diminish the ecofunctional diversity of host fish communities.
- Exotic species might have worse effects than translocated ones.

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Henceforth, the publications are referred by their number between square brackets.

Other works not included in the main thesis' project:

Milardi, M., Lanzoni, M., **Gavioli, A.**, Fano, E. A., Castaldelli, G. (2018). Long-term fish monitoring underlines a rising tide of temperature tolerant, rheophilic, benthivore and generalist exotics, irrespective of hydrological conditions. *Journal of Limnology*. <https://doi.org/10.1002/cphc.201601380>

Highlights

- Exotic fish species invasion progressed with time irrespective of mean annual discharge and temperature.
- The total number of species fluctuated without a clear trend with a progressive substitution of native species with exotic ones.
- Native species nested more strongly at the beginning of the period and progressively declined over time.

Milardi, M., Lanzoni, M., **Gavioli, A.**, Fano, E. A., Castaldelli, G. (2018). Tides and moon drive fish movements in a brackish lagoon. *Estuarine, Coastal and Shelf Science*, 215(September), 207–214. <https://doi.org/10.1016/j.ecss.2018.09.016>

Highlights

- Fish movements between habitats were influenced by moon and tide factors
- European flounder catches were positively correlated with disk illumination, while sand and black goby were influenced by the moon phase
- Water temperature, salinity and the presence of invertebrate prey had significant but contrasting effects on some of the fish species

Milardi, M., **Gavioli, A.**, Lanzoni, M., Fano, E.A., Castaldelli, G. Meteorological factors influence marine and resident fish movements in a brackish lagoon (2019) *Aquatic Ecology* <https://doi.org/10.1007/s10452-019-09686-4>

Highlights

- Prevalent wind direction and rain on the day before sampling were the most effective meteorological variables in enhancing fish movement
- Movement of lagoon resident species seemed to be enhanced by wind factors (both direction and wind speed)
- Marine species movement was enhanced by rain factors, but also by dissolved oxygen linked to prevailing wind direction

Soana, E., **Gavioli, A.**, Tamburini, E., Fano, E. A., Castaldelli, G. (2018). To mow or not to mow: reed biofilms as denitrification hotspots in drainage canals. *Ecological Engineering*, 113, 1–10. <https://doi.org/10.1016/j.ecoleng.2017.12.029>

Highlights

- Nitrogen removal in biofilms on dead reed was investigated in relation to water velocity.
- Biofilms maintain high denitrification potential in winter.
- Water velocity (0–6 cm s⁻¹) promotes denitrification.

Soana, E., **Gavioli, A.**, Vincenzi, F., Fano, E. A., Castaldelli, G. (2019). Nitrate availability affects denitrification in *Phragmites australis* sediments. *Journal of Environmental Quality* 16608653, <https://doi.org/10.1002/jeq2.20000>

Highlights

- Plant-mediated denitrification was the dominant NO₃⁻ sink in summer
- Denitrification was lower in winter but still stimulated by *P. australis* biofilms
- Plant-mediated denitrification showed a near-linear response up to 5.0 mg N L⁻¹

1. INTRODUCTION

Understanding how biodiversity (i.e. the variety of organisms considered at all levels, including the variety of ecosystems; Wilson, 1992) is distributed has important implications, for example in conservation and management plans, but also in studies which aim at quantifying the species' niche, to assess impacts (e.g. climate change, land use), and to assess of species invasion dynamics (see Guisan and Thuiller, 2005).

Biodiversity is not equally distributed on the Earth, but shows strong geographical variation. For examples, biodiversity changes across latitudinal gradient, with many species close to the equator than the poles, and across elevation with a decrease of species from low to high altitudes (Gaston K.J., 2000), although some exceptions are present (e.g. Teittinen *et al.*, 2016). Furthermore, there are areas featured by high richness of species, rare species and endemic species defined as hotspot of biodiversity (Fig. 1; Myers *et al.*, 2000), in which Mediterranean basin is included.

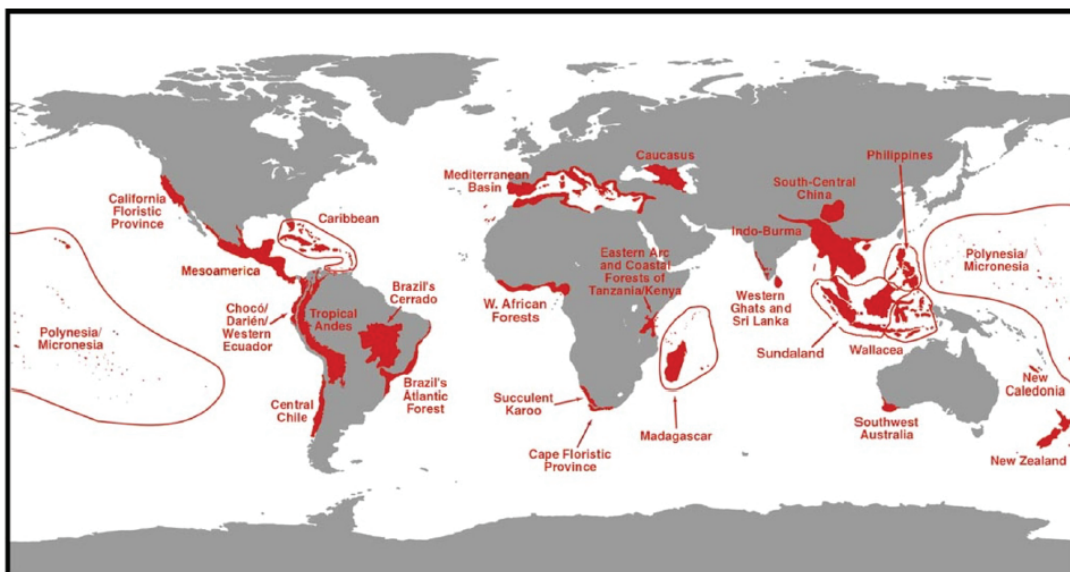


Figure 1. The 25 hotspot of biodiversity identified from Myers *et al.*, 2000.

Despite the importance of biodiversity, its decline is a worldwide trend which affects almost all taxa and ecosystems (Butchart *et al.*, 2010; Dirzo *et al.*, 2014; Ceballos *et al.*, 2015). For example, from 8 million of total estimated number of animal and plant species on Earth (including 5.5 million insect species), up to 1 million of species are threatened with extinction, with a rate of extinction higher than the rate to average over the last 10 million years, and the rate is accelerating (IPBES Report 2019).

The loss of biodiversity causes ecosystems change through the alteration of ecosystem functioning and ecosystem services that in turn even affect human health (Díaz *et al.*, 2006; Cardinale *et al.*, 2012).

Freshwater ecosystems appear more vulnerable to biodiversity loss due to many and heterogeneous pressures (Vörösmarty *et al.*, 2010; Carpenter, Stanley and Vander Zanden, 2011), to the high number of endemic and rare species and limited spatial extension (<1% of the Earth's surface) are intrinsically vulnerable (Gleick, 1998; Balian *et al.*, 2008; Collen *et al.*, 2014). Five main threats to freshwater biodiversity were identified by Dudgeon *et al.*, (2006): overexploitation, water pollution, habitat degradation, flow modification and exotic species introductions (Allan *et al.*, 2005; Strayer, 2010; Wen, Schoups and Van De Giesen, 2017; Bierschenk *et al.*, 2019; Boddy *et al.*, 2019). Furthermore, also climate change has contributed more and more, in last years, to biodiversity loss (e.g. Pecl *et al.*, 2017; Dudgeon, 2019). To complicate this scenario, an important role in biodiversity

loss could be played by the interactions between these threats (e.g. Johnson, Olden and Vander Zanden, 2008) making even more difficult to investigate the loss of biodiversity.

How to detect biodiversity?

The most common way to investigate biodiversity trends and changes is the study of variations in taxonomical species diversity (Colwell and Coddington, 1994), largely correlated with other levels of diversity, such as genetic diversity or functional diversity (e.g. Meynard *et al.*, 2011).

In 1960, Whittaker proposed the taxonomical diversity as the result of three components: alpha (local diversity), beta (variation of community composition among sites) and gamma diversity (regional diversity; Whittaker, 1960, 1972). Many index are proposed to investigate diversity in each component as, for example, the common ecological Shannon-Wiener index and the species richness (see e.g. Magurran, 2004), the turnover and nestedness component of beta diversity proposed by Baselga, 2010, or the Local Contribute To Beta Diversity proposed by Legendre and De Cáceres, 2013.

In the last decades, more attention is focusing also on functional diversity in order to take into account the role of species in the ecosystems, and also because it is generally considered reflecting more closely the interaction between the environment and biotic communities in space and time (see e.g. Baskin, 1994) and could be more informative than taxonomical diversity to ecosystems change (e.g. Vandewalle *et al.*, 2010). Changes in functional diversity can be interpreted through the analysis of the ecological-traits composition of living communities, at different spatial scales. However, similarly to taxonomical diversity, many measures were proposed to estimate functional diversity (see e.g. Petchey and Gaston, 2002; Villéger, Mason and Mouillot, 2008; Laliberte and Legendre, 2010) but see also the review of Schmera *et al.*, 2017.

Why study freshwater fish?

Freshwater fish account significantly to the overall biodiversity with 15.750 described species and accounting with approximately 25% of vertebrate diversity (Closs, Krkosek and Olden, 2015). Furthermore, freshwater fish supply ecosystem services for humans and play ecological role in the ecosystems functioning. For examples, freshwater fish are important for human nutrition, with an increase of human consumption from 1961 (UNEP, 2010; FAO, 2018), for recreational activities (Arlinghaus and Cooke, 2009) and for the economic value (e.g. Butler *et al.*, 2009). Freshwater fish play also a key role in driving ecosystems, as the case of apex predators in the food web (e.g. Vejřík *et al.*, 2017).

The main impacts on freshwater fish

Freshwater fish are more sensible to anthropogenic impacts on freshwater ecosystems, as results more than 1500 species assessed by IUCN are threatened with extinction, and in the Mediterranean basin fish are the most threatened taxon among the vertebrates (IUCN, 2017). Fish are affected by all threats impacting on freshwater ecosystems and especially habitat degradation (including flow modification), water pollution, overexploitation, and exotic (i.e. non-native) species (Closs, Krkosek and Olden, 2015; Dudgeon, 2019).

Changes on natural flow regimes of rivers can affect freshwater habitats and fish communities (Bunn and Arthington, 2002). A most common way through which humans alter flow regime is damming. Dams stop movements of materials and species (e.g. Jansson, Nilsson and Malmqvist, 2007; Lehner, Liermann, Revenga, Vörösmarty, *et al.*, 2011) with negative effect on fish communities and especially on migratory fish (Liermann *et al.*, 2012; Göthe *et al.*, 2019). Also water abstractions for irrigation and other needs decreasing downstream water availability for fish (e.g. Boddy *et al.*, 2019).

Water pollution can include many categories of contaminants, from nutrients load to pharmaceuticals products, which reflect human activities and consequent pressures on fish community. For example, the use of pesticides in agriculture were found impact on chinook salmon population altering natural behavior with negative consequences for survival and reproductive success (Scholz *et al.*, 2000). Also chemical compounds similar to hormones can cause endocrine alteration in fish such as barbell in Italian Po River (Viganò *et al.*, 2001) and roach in British rivers (Jobling *et al.*, 1998). Nutrients enrichment due to human activities and land use (e.g. agriculture, farms, urbanization) are common in most of the rivers in the world affecting aquatic communities, and fish too (Chalar *et al.*, 2013; Alexander, Vonlanthen and Seehausen, 2017). However, also freshwater fish can affect water quality by increasing eutrophication and turbidity through sediment resuspension (Richardson, Whoriskey and Roy, 1995; Chumchal, Nowlin and Drenner, 2005).

Overexploitation due to commercial fisheries seem took place mainly in marine ecosystems and overfishing in freshwaters is largely unrecognized and seems to be a primary pressure only when concur with other impacts (Arlinghaus, Mehner and Cowx, 2002; Allan *et al.*, 2005). However, evidences that overfishing can directly affect fish stocks have been found for species with a long and complex life cycle, such as sturgeons in the Mississippi River (Koch *et al.*, 2009) and eels in Europe (Aschonitis *et al.*, 2016). For example, the impossibility to perform artificial reproduction of eels resulted in a strong fishing pressure on juveniles, mainly illegal, which in turn resulted in a drop of recruitment down to less than 1% of the maximum historic record (Dekker and Casselman, 2014). Freshwater fish are also affected by recreational fishing, which concur to shifts of community structure (e.g. removal of big fish) and non-native species translocation and introduction (Lewin, Arlinghaus and Mehner, 2006; Pierce, 2010; Arlinghaus *et al.*, 2019).

Fish species are one of the most introduced taxa worldwide, included Europe, (Gozlan *et al.*, 2010) with different introduction routes such as sport fishing, aquaculture, ornamental and aquarium hobby (e.g. Elvira and Almodóvar, 2001; Maceda-Veiga *et al.*, 2013). However, despite the large number of introduced species, only a subset of these species can establish the new environment (Jeschke and Strayer, 2005) and spread and increase in abundance becoming invasive (Colautti and MacIsaac, 2004). Fish introductions are one of the major drivers of native fish species contraction in Mediterranean region (Crivelli, 1995) and cause of homogenization in fish community (Leprieur *et al.*, 2008; Villéger *et al.*, 2011). For examples, abundance of invasive fish species was found as the best single predictor of native species decline in Iberian streams (Hermoso *et al.*, 2011) but also play a central role in native fish decline also in Italian rivers (Castaldelli *et al.*, 2013). Although some authors remained sceptic about the extent and intensity of exotic species effects on native communities (e.g. Gurevitch and Padilla, 2004; Gozlan, 2008) the main mechanisms through which exotic species can affect native ones have been identified (e.g. Vitule, Freire and Simberloff, 2009; Ribeiro and Leunda, 2012). The most intuitive pressure from exotic species to native ones is predation, in fact piscivorous species (e.g. wels catfish or pikeperch) were found catch mainly native fish especially in natural rivers (Alp *et al.*, 2008; Carol *et al.*, 2009). Exotic species can also compete for food, space, shelter or mates reducing their availability for native species (e.g. Nasmith *et al.*, 2010) and can decrease genetic heterogeneity through hybridization, most common for example in cyprinids or trout (e.g. Meldgaard *et al.*, 2007). Furthermore, exotic species can change habitat as for example the crucian carp that promote water turbidity (Richardson, Whoriskey and Roy, 1995) and the grass carp that remove water vegetation (Milardi *et al.*, 2019).

However, some uncertainties still remain on the interplay between different pressures, as often they overlap in space and time and their effects on the ecosystem are not easy to disentangle. Nevertheless, their cumulative impacts are more than disruptive for native species and ecosystems (Dudgeon, 2019).

2. QUESTIONS OF THE RESEARCH

The aim of this thesis is to investigate the causes of native fish community decline using Italian waterways as case study, focusing on the northern part of the Italian peninsula as hot spot of disturbance. The specific purpose is to identify and study the role of the most important environmental stressors and exotic species in affecting native fish community.

To do so, I collected and organized an extensive amount of data (nationwide, both official fish inventories and institutional monitoring of abiotic parameters) into datasets. I also participated in some sampling campaigns on fish and water parameters, in the Po River and its delta. Through this field work I had the main goal to acquire the sensitivity needed to evaluate and harmonize the data already collected by others.

The main research questions investigated are:

1. How do multiple pressures affect fish communities?

Through this question, the main pressures (i.e. damming, water availability, water quality and exotic species) on fish community were taken into account in order to identify the main threats for native community. This question is assessed in papers [1-4].

2. How do alpha and beta diversity change in native and exotic communities?

The hypothesis under this question is that native and exotic community showed different diversity patterns due to different response to the pressures that in turn determine a different spatial distribution among native and exotic species. This point is addressed in paper [5].

3. How do exotic fish species affect the functional diversity?

This is one of the most pending questions in literature. Some authors found an increase of functional diversity in community after invasions (e.g. Toussaint *et al.*, 2018), some others found a decrease (e.g. Matsuzaki, Sasaki and Akasaka, 2016). What happens in Italian fish community after a late invasion stage is assessed in papers [6-8].

These results are useful to understand spatial and ecological dynamics acting in fish community that, in turn, can be useful to improve management and conservation actions in freshwaters.

3. MATERIALS AND METHODS

3.1. Study area

The study area embraces freshwater waterways from north to south of Italian peninsula, including the rivers in the main islands (Fig 2a). Typically, Italian rivers are divided in three districts: the Padano-Veneto district (PDV), the Tosco-Laziale district (TL) and the islands district (ISL). The PDV district includes the largest river basin in Italy, the Po River basin (71,000 km²) within the Po River plain, limited in the north by the Alps and in the south by the Apennines. All rivers in this district flow ultimately in the Adriatic Sea. The TL district is characterized by the highest longitudinal extension; with Apennines separate the rivers that flow in the Tyrrhenian Sea, in the west Italian coast, from rivers that flow in the Adriatic Sea and Ionian Sea. The ISL district encompasses the two major Italian islands, completely isolated from the continental lands by sea stretches.

Due to the extension of this work, heterogeneous waterways are taken into account: canals, rivers and streams (Fig 2b, c, d). Canals, located in the lowlands, are strongly human regulated for drainage and irrigation of agricultural lands. Rivers are mainly located from lowlands to foothills and hills area, they are natural waterways, although a wide gradient of anthropic alterations is present (e.g. dams). Streams are natural ecosystem located in the hills and in the mountains.

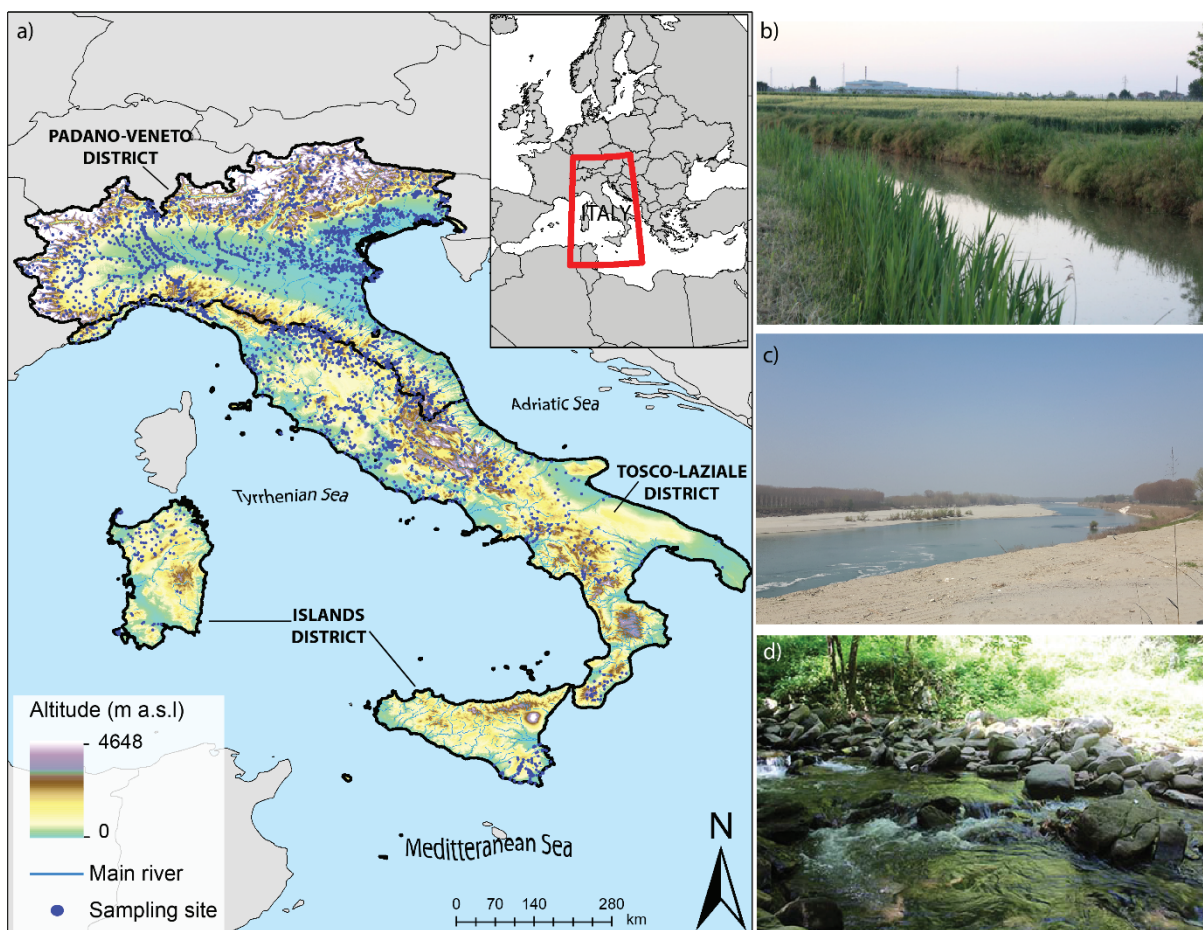


Figure 2. a) Map of the studied area with sampling sites, the main Italian rivers and the altitude profile, the solid black lines shows the border of the Padano-Veneto (PDV), Tosco-Laziale (TL) and Islands (ISL) districts. A typical view of canals b), rivers c) and streams d) are shown.

The article [1] focused on the Oglio River in northern Italy, the articles [2] and [3] on the main waterways of the Emilia-Romagna region and the article [4] on the specificity of the canals and rivers

of the Po River Delta. In the articles [5], [6] and [7], the analysis was extended to the whole Po River basin and to the Brenta River, as reference in Veneto region and in the article [8], to the whole Italian territory.

3.2. Data collection

Fish fauna

Fish dataset [1-8] was compiled from monitoring data within official monitoring programs (Fish Inventories) run by provincial or regional administrations according to regional or national laws. References are detailed in Supplementary table 1. We collated and digitized data from 4126 sampling events, but we discarded all sites without fish or those sampled more than once, narrowing our dataset to 3734 sampled sites (Fig.2a).

Fish sampling was performed by electrofishing, adapting the standard national monitoring guidelines to the particular conditions of each watercourse, and using direct current at 400–600 V and 4–5 A (Reynolds, 1996). River stretches were sampled once, during daylight, in an upstream zigzag direction, ensuring that the range of present macrohabitats of each site was fully surveyed. Electrofishing is considered the best quantitative method for fish sampling in shallow waters, up to a maximum of 1 m (Zalewski and Cowx, 1990) but its efficacy may be low in deeper waters, with big and mobile specimens, or with high conductivity. Such special conditions occurred in almost all the lower stretches of rivers and in the canals of the lowlands. For this reason, at these sites, electrofishing was immediately followed up with a standard set of trammel nets (with variable mesh size from 90 to 5 mm), with the support of professional fishermen (Backiel and Welcomme, 1980). In order to allow comparisons between sites (i.e. river stretches), the sampling effort at each sampling site was standardized according to the national fish monitoring guidelines (APAT 2007).

Fish species were classified according to Kottelat and Freyhof (2007), taking into account recent taxonomic determinations and common names as listed in FishBase (Froese and D. Pauly, 2019). Following Leprieur *et al.*, 2008, fish species were also identified as native (i.e. originally from a geographic area, e.g. Italy), exotic (i.e. originally from a distant geographic area, e.g. outside Italy) and translocated (i.e. originally from a nearby geographic area, e.g. outside a geographical district).

Fish functional traits

To investigate ecological changes in community composition we have taken into account the functional composition of fish community (Milardi and Castaldelli, 2018). The main ecological functions examined are feeding, reproduction, migration, tolerance and habitat use. Among each ecological function fish species are classified in guilds that describe a group of species that exploit the environmental resources in a similar way (Milardi and Castaldelli, 2018). Fish species were divided into functional guilds using the information from continuously updated online databases such as FishBase (Froese and D. Pauly, 2019) or through peer-review papers, when available. When no information was available expert knowledge was used to fill the gaps, usually assuming that the species would share functional characteristics with the closest related species for which information was available (Table 1; Supplementary table 2).

As result, in the feeding ecological function, based on the prevalent diet of adult individuals, fish were assigned to the following guilds: planktivores, herbivores, benthivores, invertivores, piscivores, parasites and generalists. With respect to reproduction, fish were assigned to the following guilds: lithophils, phytophils, phytolithophils, psammophils, ostracophils, pelagophils or live breeding and polyphils. With respect to migration, guilds were based on the range of movement reported in literature for the species, including short, medium and long ranges of movement. In the tolerance

ecological function, fish were divided into two mutually exclusive guilds of tolerance/intolerance to low oxygen (indicatively below 3 ppm) and to high temperature (indicatively above 20 °C). In the habitat ecological function, fish species were divided into two broad guilds based on current speed (rheophils, limnophils or eurytopic) and water transparency (fish adapted to clear water, turbid waters or adaptable to a wide range of water turbidity).

Table 1 – Ecological functions and guilds overall considered for each species (from Milardi and Castaldelli, 2018).

Ecological function	Guild	Description
Feeding	Planktivores	Plankton feeders
	Herbivores	Vegetation feeders
	Benthivores	Bottom feeders
	Invertivores	Invertebrate feeders
	Piscivores	Fish feeders
	Parasite	Ematophages
	Generalists	Unspecialized feeding
Reproduction	Lithophils	Spawning on stones or gravel
	Phytophils	Spawning on submerged vegetation
	Phytolithophils	Spawning on stones or vegetation
	Psammophils	Spawning on sand or mud
	Ostracophils	Spawning in molluscs
	Pelagophils or live breeding	Pelagic spawners or live spawners
	Polyphils	Generalist spawners
Sea spawning	Saltwater spawners	
Migration	Short	Within or close to the site
	Intermediate	Up and downstream or into flooded areas
	Long	Anadromous and catadromous species
Tolerance	Low oxygen tolerants	Tolerance/intolerance to low oxygen
	Low oxygen intolerants	(indicatively below 3 ppm)
	High temperature tolerants	Tolerance/intolerance to high temperature
	High temperature intolerants	(indicatively above 20 °C)
Habitat use	Rheophiles	Preferring fast flowing water
	Limnophiles	Preferring slow or no current
	Eurytopics	Having no preference on current velocity
	Clear water	Clear water adapted
	Turbid water	Turbid water adapted
	Wide range of conditions	Adapted to a wide range of water turbidity

Crayfish sampling

The invasive crayfish *Prokambarus clarkii* was taken into account in paper [4]. The sampling of red swamp crayfish was conducted between May and June 2009, in the waterways in the lower stretch of the Po River. In each waterway, crayfish presence was investigated in stretches with homogenous morphology, hydrology and with no tributaries or discharges using plastic traps baited with a can of fish-flavored cat food (see e.g. Lappalainen and Pursiainen, 1995). The traps (40×25×25 cm, 0.3 cm mesh size) had two openings at opposite ends, plus a central opening for extraction (Fjälling, 1995). A set of 15 traps was used at each sampling event, placed along the waterway banks at depths between 0.5 and 1 m, and left overnight (12 h, from 19.00 to 7.00). Three replicates of the

sampling were performed at each site, and catches were expressed as average CPUE, defined as the mean number of crayfish caught per trap, per sampling event.

Water chemistry and physical data

Water physicochemical sampling was performed by Regional Environmental Protection Agencies (ARPA) for Po River, Brenta River and Emilia-Romagna rivers and by Oglio River Water Authority and University of Parma in the Oglio River [1-7]. Sampling stations belong to the regional monitoring networks and generally are overlapping the fish monitoring stations. When not, the closest station for water monitoring was associated to the one for the fish monitoring, after an accurate evaluation of the representativeness of the fish sampling site features and never farther than 3 km on the same river reach. Eight water physicochemical variables were included as follows: water temperature ($^{\circ}\text{C}$), electrical conductivity ($\mu\text{S cm}^{-1}$), chemical oxygen demand (COD [$\text{O}_2 \text{ mg l}^{-1}$]), biological oxygen demand (BOD5 [$\text{O}_2 \text{ mg l}^{-1}$]), total suspended solids (mg l^{-1}), total phosphorus (P mg l^{-1}), ammonia (N mg l^{-1}) and nitrate nitrogen (N mg l^{-1}).

In the study [4], the Oxygen concentration ($\text{O}_2 \text{ mg l}^{-1}$) was measured with a multiparametric probe (Ocean Seven, 316) and levels of chlorophyll a (Chl-a, $\mu\text{g l}^{-1}$), total suspended solids (mg l^{-1}), and of Biochemical Oxygen Demand (BOD5 [$\text{O}_2 \text{ mg l}^{-1}$]) were sampled in the crayfish sampling sites and determined according to APHA (2005).

Spatial data

The stream order of sampling sites [5-7] was calculated from Digital Elevation Model (DEM) data (ISPRA, Italian Institute for Environmental Protection and Research) through the ArcGIS 10.1 software. Using the Hydrology Spatial Analyst Tool, the flow direction and the flow accumulation based on DEM layer were calculated. Then, for the entire river network generated by flow accumulation, the stream order with the Strahler method (Strahler, 1957) was calculated. This procedure resulted reliable for upland streams, whilst in the lowland, it was less accurate possibly due to the fact that in the lowlands, the flow direction and magnitude have been modified by humans. The Strahler stream order was thus manually checked and revised when necessary in lowland rivers and streams.

In order to balance the number of rivers sampled in each Strahler stream order, rivers were grouped into four classes based on stream order: class 1 – rivers with 1 and 2 Strahler stream order, class 2 – rivers with 3 and 4 stream order, class 3 – rivers with 5 and 6 stream order, and class 4 – rivers with Strahler stream order higher than 6. As the drainage and irrigation canals located in lowlands could not be assigned into any natural class, they were assigned into a separate class.

Land cover data were acquired from the CORINE database (2012, <https://www.eea.europa.eu/data-and-maps/data/copernicus-land-monitoring-service-corine>) [6-7]. In the lowlands, where estimation of watershed areas is more difficult due to low slopes and human-regulated flow, the land cover of the whole river basin or of the administrative province was used. CORINE land cover classes were merged in five categories based on the main land use in order to better describe the study area: urban use, agricultural use, forest, other natural area, freshwater and brackish water. Land cover was expressed as the share of each of these categories in the watershed of each site.

3.3. Data analysis

Fish diversity measures

The alpha diversity was considered as richness, calculated as number of species in each sites [5-8]. To study the uniqueness of fish community composition across sites [5-7], the Local Contribution to Beta Diversity (LCBD) was calculated for each sampling based on Legendre and De Cáceres (2013).

This method calculates the Total Beta Diversity (BD_{Total}) from the total variance of a site by species community table. The LCBD was derived by partitioning the BD_{Total} into the local contributions, and the sum of the LCBDs for all sites is equal to 1. For this metric, higher values of LCBD of a site indicate an unusual species composition compared with the average community composition in the whole set of stations examined. From an ecological point of view, the LCBD values represent the degree of uniqueness of the sampling units in terms of community composition (Legendre and De Cáceres, 2013). We measured also the relative importance of each species in affecting beta diversity as the Species Contribution to Beta Diversity (SCBD) that shows the degree of variation of a species across the considered area (Legendre and De Cáceres, 2013).

In order to investigate the degree to which there is community structural variation within a stream order class, a test of homogeneity of dispersion (PERMDISP) was used (Anderson, 2006; Anderson, Ellingsen and McArdle, 2006). Through the average dissimilarity from individual observations to their group centroid, this test calculates the degree of dispersion, that is beta diversity (when based on presence-absence data) and the community structural variation (when based on abundance data) (Anderson, Ellingsen and McArdle, 2006; Heino *et al.*, 2013).

The functional diversity was investigated through the functional dispersion metric (FDis; Laliberte and Legendre, 2010), which is a metric based on community-specific fish functional traits. This metric was chosen because it can be calculated on all communities (including the species-poor ones), should not be overly affected by species richness, can be computed from any distance or dissimilarity measures and takes into account any number and type of traits (also qualitative traits, as in this study), is not strongly influenced by outliers, and accounts for species abundances (Laliberte and Legendre, 2010). Large values of FDis showed communities more diverse in species traits combinations.

Analysis of fish community and multiple pressures

Multivariate analysis [1-8] were applied to investigate the complex interactions between fish community and controlling factors. At first, the Detrended Correspondence Analysis (DCA) was performed in order to select the most appropriate response model (between linear or unimodal) for gradient analysis (ter Braak and Šmilauer, 2002; Lepš and Šmilauer, 2003). Based on the dominant gradient length value in DCA, the Canonical Correspondence Analysis (CCA) as unimodal method or the Redundancy Analysis (RDA) as linear method were used in the studies to investigate species and environmental drivers relationships.

Communities changes along environmental gradients were analyze using the Threshold Indicator Taxa ANalysis (TITAN, Baker and King, 2010; Baker, King and Kahle, 2015) [1-2]. TITAN uses indicator taxon scores (IndVal) to integrate occurrence, abundance and directionality of taxon responses along environmental gradients. The method identifies the environmental threshold (the optimum value of a continuous variable) that partitions sampling units and distinguishes negative (=losses: z-) and positive (=gains: z+) taxon responses. Thus, TITAN helps to identify taxon-specific change points along an environmental gradient at which the decline/increase in a given taxon's frequency and abundance is most prominent.

To assess how a response variables (e.g. species richness) was influenced by environmental features a machine learning method, Boosted Regression Trees analysis (BRT) (Elith, Leathwick and Hastie, 2008) was applied [5-6]. The BRT is an efficient method to describe non-linear relationships between variables (e.g. thresholds) and it automatically incorporates interactions between variables. The effect of predictors is showed through the fitted functions that provide a useful basis for interpretation, although they are not perfect representation in case of strong interactions between predictors (Elith *et al.*, 2008).

To evaluate how much of the explained variation of a dependent variable can be independently and jointly attributed to environmental variables the hierarchical partitioning was performed (Chevan and Sutherland, 1991; Mac Nally and Walsh, 2004) [4]. This approach allows to break up the variation explained by a set of independent variables into independent components (I), which reflect the relative importance of individual variables, and joint contributions (J), which are cumulative effects of each variable with all other variables.

The spatial distribution of interested variables (e.g. the invasion degrees), for all the areas covered by sampling points, was performed by ordinary kriging in ArcGIS software version 10.1 (ESRI, 2011).

The DCA, CCA and RDA analysis were performed in CAONOCO 4.5 for Windows (Lepš and Šmilauer, 2003). The other ones were performed in R software (R Core Team, 2017) through 'vegan' (Oksanen *et al.*, 2017), 'adespatial' (Dray *et al.*, 2018), 'gbm' (Ridgeway & Southworth, 2017), 'hier.part' (Walsh and Nally, 2013), 'TITAN' (Baker and King, 2010) R package.

4. MAIN RESULTS AND DISCUSSION

Along with this research project, 3734 sampled sites in Italian waterways were taken into account. Of them 1940 sites (52%) showed some degree of invasion, and introduced species constituted on average 42.5% of the community in invaded sites, reaching maximums of 100% (i.e. the native community was completely lost in 130 sites, 3.5% of all sites examined). The most relevant invasion hotspot was located along the Po River, but other highly invaded areas resulted in the central and southern part of the Italian peninsula, as well as in the islands (Fig. 3). A total of 99 fish species were sampled in Italian waterways (Supplementary table 2), 37 species of them were exotic species (i.e. originally from outside of the Italian geographical barriers) and 62 were native to Italy.

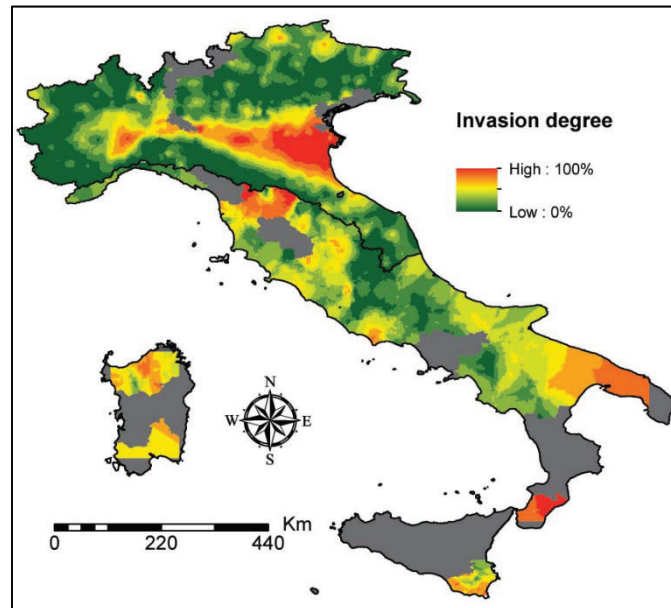


Figure 3. Invasion degree in fish communities of Italian inland waters based on spatial interpolation of 3734 sampling sites. The solid black line dividing the northern part of the peninsula represents the boundary between the Padano-Veneto district (to the north) and the Tosco-Laziale district (to the south). Grey shaded areas represent zones for which data data were not made available by the authorities competent on the territory.

4.1 How do multiple pressures affect fish communities?

4.1.1. The role of flow and damming

Dams resulted to strongly affect freshwater habitats and fish species, in agreement with the international studies on the topic (see e.g. Bunn and Arthington, 2002 for a review) as also rivers biodiversity (Vörösmarty, P B McIntyre, *et al.*, 2010; Hermoso *et al.*, 2018). One of the main consequence of dams presences is the interruption of longitudinal connectivity which, together with the alteration of fish life cycles, alter the whole river functioning (Poff *et al.*, 1997; Prenda *et al.*, 2006). Another consequence is also the alteration of natural flows, since water is, in most of the cases, taken out from the river to hydroelectrical plans and/or irrigation supplies. River flows and water availability are not term of minor relevance, since hydrological conditions can play a central role in fish community composition and also in affecting the intensity of invasion by exotic species, for example by providing flow conditions that are more suitable to exotics than natives (Bunn and Arthington, 2002). However, in an opposite dynamic, altered hydrological conditions can also disadvantage invasive species, as for example in New Zealand, where the hydrological disturbance associated with human activities blocked invasion effects of brown trout, supporting the native fish population (Leprieur *et al.*, 2006). As for the

flow, also the loss of connectivity due to barriers can play contrasting and in some cases opposite effects, where an invasion by exotic species is ongoing (e.g. Fausch *et al.*, 2009).

In the case of the Oglio River [1], flow conditions were found to have less impact on native fish than degraded water quality and the abundance of exotic species. We studied the reach of the Oglio River originating from the subalpine Iseo Lake (185 m a.s.l.) and flowing into the Po River (16 m.a.s.l.), with a total length of 156 km. Human alterations of hydraulics date back to 19th century, when the abstraction of water aimed at two primary uses: agriculture and hydropower production. More recently, in 20th century, further uses have become important, as the distribution of drinking water and the discharge of effluents from sewage treatment plants. Two main interruptions of longitudinal connectivity are present in the middle and lower reaches of the river, around 70 and 110 km downstream of the Iseo Lake, near the towns of Pontevico and Isola Dovarese, respectively. The present composition of native fish community reflects these disturbances and physical alterations. In the upper reaches, rheophilic and clear water native species were still present, despite severe water abstraction determining a drastic reduction of flow and depth (Fig. 4). However, native species abundances decreased rapidly in the lower reach of the river, where flow and depth increased. Contrarily to native species distribution, exotic species were dominant in the lower reach of the Oglio, corresponding to high flow values and high depth. This peculiar distribution could be explained by the presence of dams in the middle reach, which hindered exotic species to migrate upstream from the highly invaded Po River (Castaldelli *et al.*, 2013). Moreover, the lower flow and depth in the upper reaches determined a very low habitat suitability for invasive exotic species potentially invading from the Iseo lake (Fig. 4a,b).

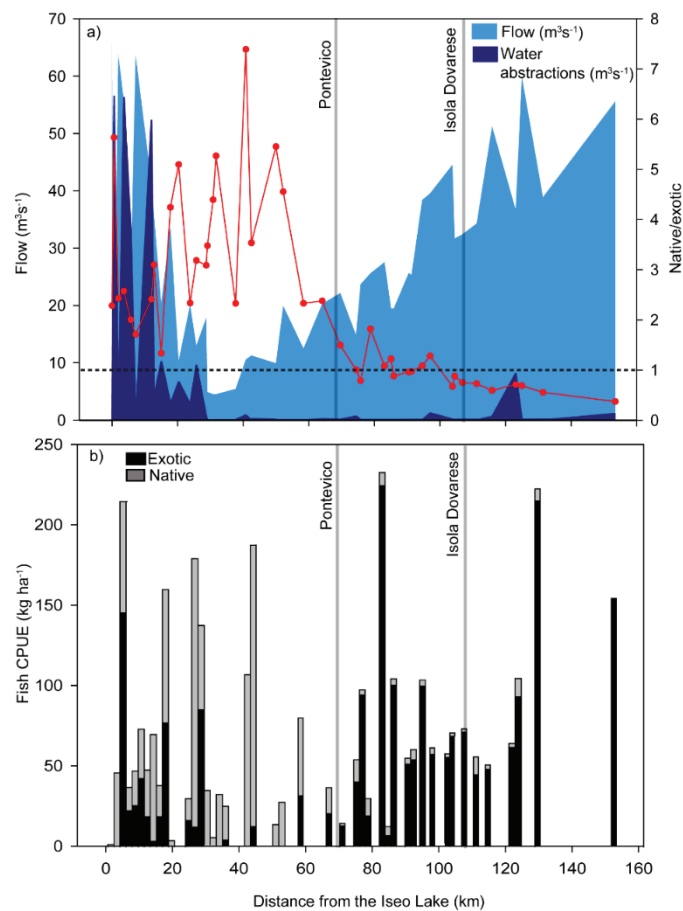


Figure 4. Water flow, water abstraction, and the ratio between native and exotic CPUEs; values of the ratio below one (dashed line) indicate the prevalence of exotic fish (a). Sampled fish CPUEs, with a

representation of the share of exotics and natives at each sampling sites (b). The most important longitudinal connectivity interruptions are shown as vertical grey lines.

These results suggest that the barriers removal and the increase of flow values for the conservation of the native fish community could be less than effective, if not even misleading for native species, when exotic species are present. For example, the increase of flow, as the new flow conditions might alter wetted habitats and potentially result in lower habitat suitability for some species (Bradford, Higgins, Korman, & Snee, 2011). Although only the relationship between environmental parameters and fish communities through ordination methods was explored, similar results were also reported in field studies as for examples Marks *et al.*, 2010 that found a greater recovery of native fish species in response to exotic species removal than to flow restoration due to dam removal.

4.1.2. The role of water quality

Hydrological alteration and damming are not the only disturbance to fish fauna in rivers. Water quality degradation (i.e. degradation of chemical, physical and biological features) and eutrophication (i.e. surplus of nutrients) can affect fish communities (Alexander, Vonlanthen and Seehausen, 2017), but the impacts of these factors on native species are difficult to identify and isolate from other pressures, especially when exotic invasions have occurred or are ongoing (Leprieur *et al.*, 2008).

In the second paper [2], this difficulty was addressed using ordination methods and variance partitioning. The analysis was performed on the low reach of rivers in Emilia-Romagna region (from -1.00 to 389.00 m a.s.l), in sites where both native and exotic species were present. In these selected areas, it was evident that water quality had less effect on native species than exotic species presence (Fig. 5a).

It was also clear that in lowland areas fish communities were dominated by exotic species, whereas native species resulted mostly located at higher altitudes where exotic species presence were not widespread (Fig. 5b). This result suggested that many native species were pushed to higher altitudes, with cooler, less eutrophic water and less invaded sites, where exotic fish dominated lowland sites

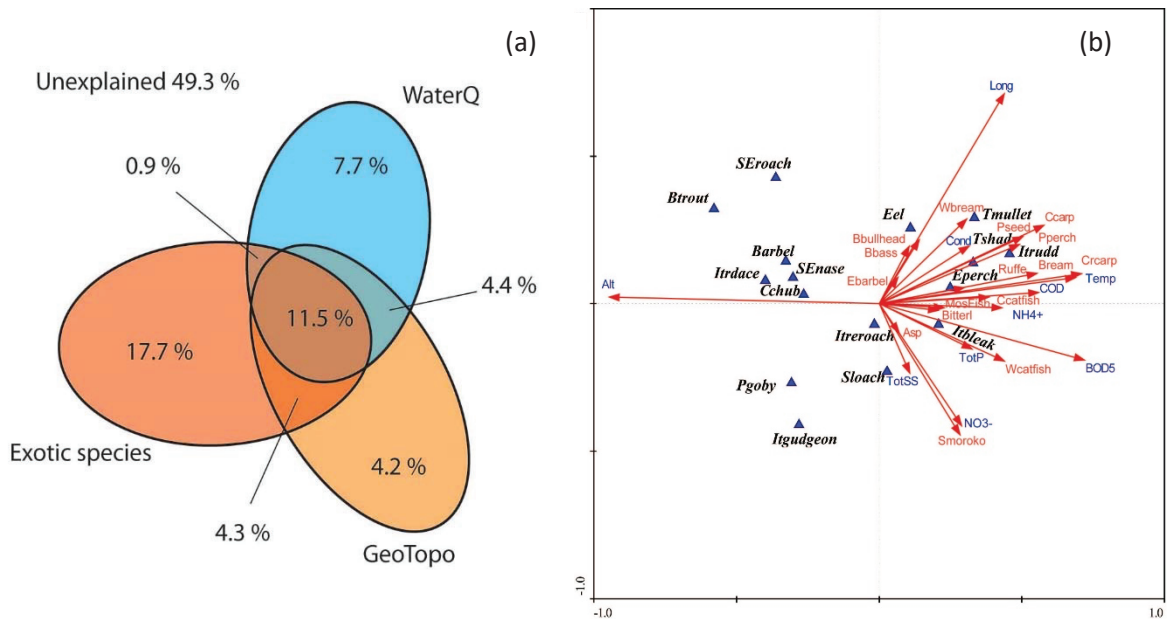


Figure 5. (a) Variance partitioning with the unique and joint effects of geographical (GeoTopo), water quality (WaterQ) and exotic species on native species distribution and abundance. The numbers indicated the variance explained by each component. (b) Triplots of CCA ordination results showing the direction of combined effects of environmental variables (in blue) and exotic species on native

species (in italics). Species are identified with codenames derived from contractions of their common names.

As a matter of fact, Fish communities in lowland sites occasionally include native North-American species, but are mostly composed by species native of the Danube River drainage (e.g. wels catfish). The few native species in the zone of overlap with exotic species are clearly the most resilient to interactions with the exotics (e.g. Italian rudd *Scardinius hesperidicus* and Italian bleak *Alburnus alburnella*). This fish community status, with the loss of peculiar species, highlighted a homogenization process also emerged also in [6-8].

In order to design spatially priorities of intervention for the recovery of native communities of these fish community, in work [3] a new method was proposed to rank monitoring sites of Emilia-Romagna Rivers according to the threats on native species and thus the need of strong management interventions. The results showed that the native communities in the lowland of the Po River delta (Fig. 6) are strongly compromised, requiring the most effective policy (and probably not economically sustainable) for management and restoration plans.

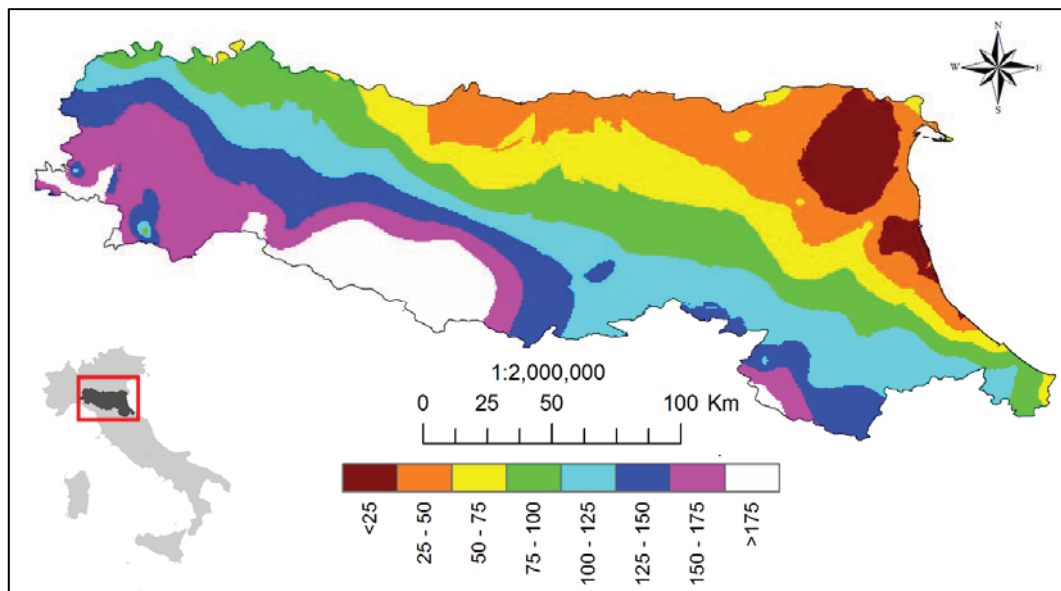


Figure 6. Spatial interpolation of priority ranking of sites. From high priority of interventions (low values) due to deprecated sites conditions (both for water quality and exotic species) to low priority of intervention (high values).

In these priority areas, native fish species are strongly affected by exotic species that dominate fish community. There are many ways in which exotic species can affect native species, directly, with predation, for example, by the invasive wels catfish (*Silurus glanis*), or indirectly as, for example, causing the decline of aquatic vegetation and the increase of water turbidity, with a general habitat deterioration (e.g. the common carp *Cyprinus carpio* and crucian carp *Carassius* spp.). Furthermore, in addition to the disturbance exerted by the exotics, also the most important water quality stressors (e.g. COD, BOD and temperature, all connected to oxygen depletion) were concomitantly present in the same rivers and canals, suggesting a double pressure on native species.

4.1.3. Not only fish as invaders: the top invader red swamp crayfish (*Procambarus clarkii*)

Studies on the dynamics between exotic and native fish species rarely take into account other components which may be important. As a matter of fact, a rich web of interactions between fish and crayfish species have been highlighted in freshwater ecosystems. This interaction include both top-

down of fish on crayfish (e.g. change in crayfish behavior; Wood, Kelley and Moore, 2018) and bottom-up effects of crayfish on fish (e.g. predation of fish eggs; Ilhéu, Bernardo and Fernandes, 2007), as also interaction between exotic fish and exotic crayfish species (e.g. Elvira, Nicola and Almodovar, 1996).

The article [4] focused on the direct and indirect interactions between environment, fish community and red swamp crayfish (*Procambarus clarkii*), taken as case study the lower Po river plain. In this area, a complex network of canals of more than 4000 km in linear extension was built for irrigation and drain supplies, although natural rivers are also present. The red swamp crayfish was first reported in this area in 1996, but the invasion probably started in the mid '80s (Gherardi *et al.*, 1999). This is one of the most invaded area of the country where also fish species being introduced around 1970 from Asia or East Europe (Lanzoni *et al.*, 2018).

Results showed that the red swamp crayfish were most abundant in less likely to establish in large, deeper and fast flowing waterways, especially when these are deprived of vegetation. Among environmental drivers, aquatic vegetation was one of the most important ones in favoring red swamp crayfish presence. This is not surprising, in fact, vegetation can provide habitat and shelter from fish (Carol *et al.*, 2009; Musseau *et al.*, 2015) and water birds (Huner, 2000). Negative effect of the invasive crayfish on fish, evidenced in other environments as predation on eggs, larvae and juveniles (e.g. Souty-Grosset *et al.*, 2016), were not evidenced by the analysis. What appeared more clearly is a negative effect on crayfish by fish predators, most of them invasive (e.g. common carp, wels catfish).

These results are representative, not only for the hydrological network assessed, but indirectly also for at least the canal network of the low Po River plain, accounting for 15.000 linear km, invaded as well by the same fish species and by the red swamp crayfish (Ficetola *et al.*, 2012; Lanzoni *et al.*, 2018). The study's outcomes have highlighted how complex can be the interactions at play in an ecosystem invaded by exotic fish species and by the red swamp crayfish (Filipe, Filomena Magalhães and Collares-Pereira, 2010).

4.2. How do alpha and beta diversity change in native and exotic communities?

What had emerged from the previous articles [1-4] is the critical status of native fish community, especially in the lowland reaches of the rivers in the Padano-Veneto District. Although different pressures contributed to the native fish decline (e.g. dams construction, water pollution, etc...), exotic species seem to play a central role in driving this decline.

Following the results of the previous paragraphs, the study of the fish community was deepened in the article [5] by exploring metrics of fish diversity (i.e. alpha and beta diversity). Alpha diversity was investigated as number of species in each site (i.e. species richness) and beta diversity was calculated as Local Contribute to Beta Diversity (LCBD, Legendre and De Cáceres, 2013). The dataset used account sampling sites from the waterways of the Emilia-Romagna Region, the whole Po River course, one Po left tributary, the Oglio, and one important river on the hydrological left of the Po, the Brenta River. Although the rivers of Emilia-Romagna region mainly contributed to the dataset, the area as a whole is representative of the Padano-Veneto District, where the Veneto territory is partly covered by the dataset accounting for hydrologically and ecologically relevant River Brenta.

Although it was already evidenced that headwater streams may promote fish diversity (e.g. Matthews, 1986), this study highlighted the importance of also large rivers in the uplands (>100 m a.s.l.) in maintaining native fish diversity (both alpha and beta diversity). These ecosystems provide unique habitats and less human altered conditions, as well as a lower pressure by exotic invasive species. Exotic species richness increased with stream order classes, with the highest exotic richness in the largest rivers and drainage canals network (Fig. 7a). Conversely, native richness showed an

increase across stream order only in the uplands (Fig. 7b), whereas in the lowlands, native species richness decreased in large rivers and drainage canals network.

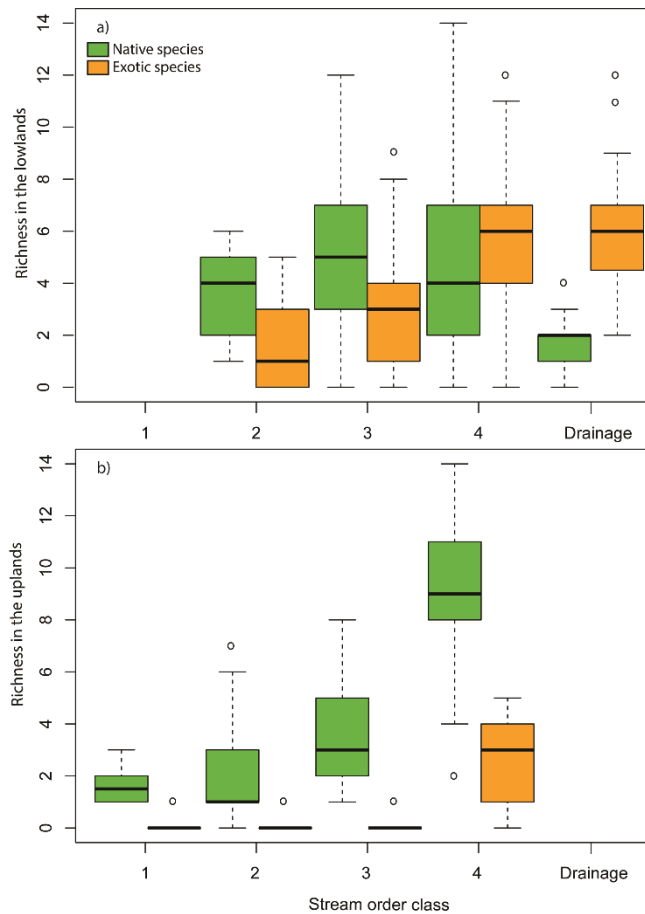


Figure 7. Boxplots representing the values of exotic (orange) and native (green) fish species richness in the lowlands (a) and uplands (b) along stream order classes. The horizontal bars in the boxes represent the median, the boxes' hinges represent the first and third quartile, and the notches represent the 95% confidence interval of the median.

In the lowlands, fish communities showed similar Local Contribution to Beta Diversity (LCBD) among stream order classes, indicating a homogenous species composition across lowland sites, probably driven by the most widespread exotic ones such as common carp and crucian carp. That these two species of cyprinids, are able to promote homogenization in fish community, especially in Palearctic regions has been previously suggested by Toussaint et al., 2016. Homogenization, which commonly refers to an increase in the similarity of communities, usually suggest a loss of unique species and their replacement by more common species with a negative effect of biodiversity (e.g. McKinney and Lockwood, 1999). The findings that upland sites hold the highest native beta diversity has practical feedbacks, calling the attention to the protection of these last semi-preserved native communities of the Padano-Veneto District.

4.3. How do exotic fish species affect the functional diversity?

All the evidence gathered up to this point shows that, in the environmental context of northern Italy, the main determinant of the recent disappearance of native fish biodiversity is the introduction, spread and establishment of some exotic fish species. The understanding of these dynamics therefore passes not only through the study of spatial patterns of diversity but required to consider also the effects on functional traits of resident fish community. In order to investigate the functional diversity

of fish community, the FDis metric proposed by Laliberte and Legendre, 2010 was chosen due to its applicability on qualitative traits.

Exotic and native functional diversity showed different spatial patterns and different responses to environmental constraints [6-8].

Mountain streams showed a low functional trait diversity [6], probably related to lower habitat complexity and lower resource availability, but higher habitat quality than lowlands rivers (Bouska, 2018). This trend could also be linked to a lower species richness in these areas [5], as species number is known to affect diversity measures (Legendre, 2014).

Generalist traits of feeding, reproduction, tolerance to environmental stressors and habitat use resulted widespread in exotic communities but differences in magnitude were present across each trait (Fig. 8). Some generalist traits related to broad tolerance and flexible habitat use (e.g. adaptation to a wide range of turbidity) resulted dominant among exotic communities but other generalist traits (e.g. polyphils) were dominant in native communities suggesting that not all generalist traits are equally important to determine invasion success.

Functional diversity of native and exotic species [7], responded similarly to most environmental constraints (Fig. 9), except for altitude. Altitude appeared to be one of the main factors of divergence for native and exotic functional diversity, not only for the link to other factors (e.g. habitat quality and human disturbance) but also for the massive exotic presence in the lower reach of all rivers [1-3,5-6,8].

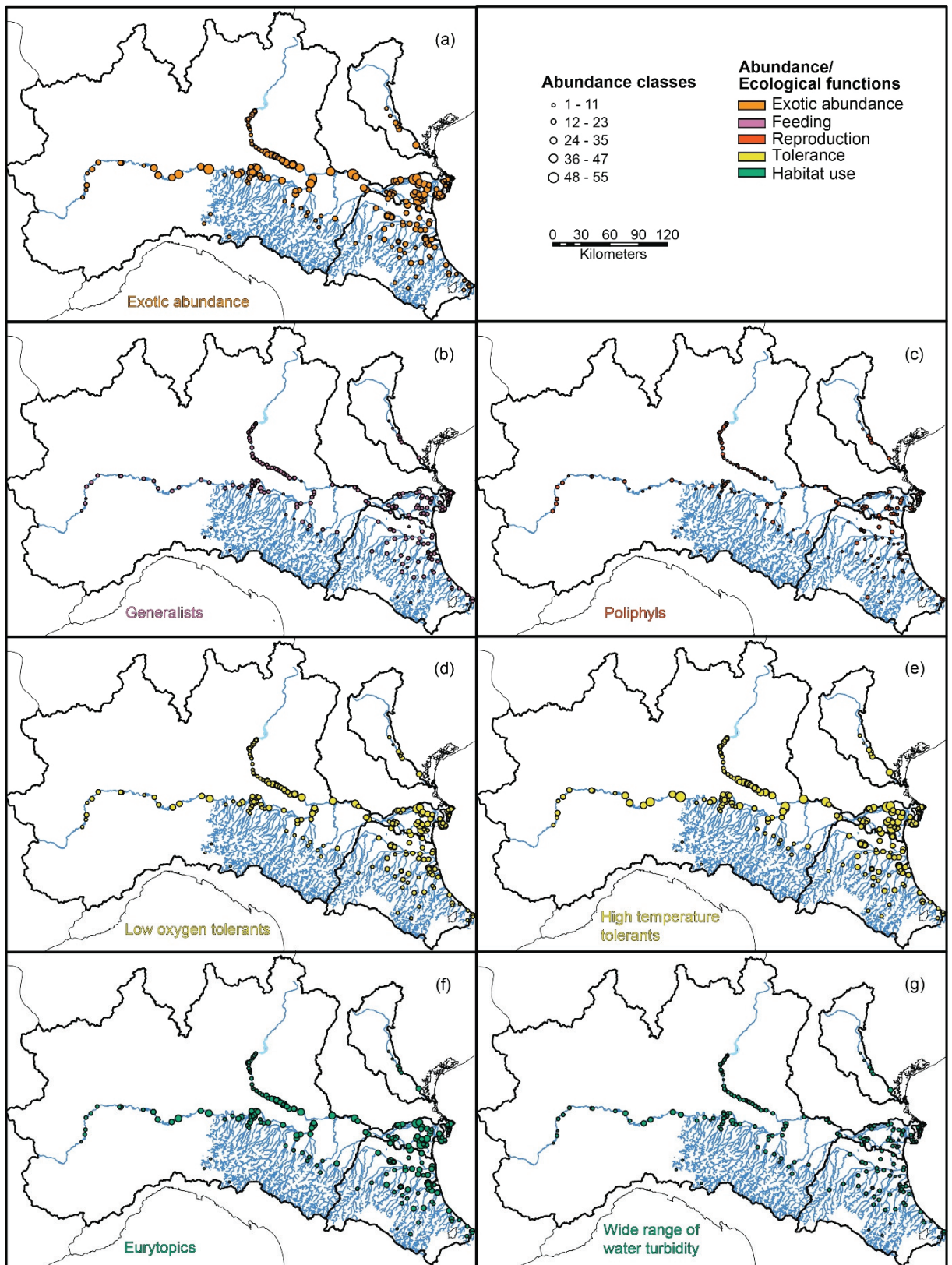


Figure 8. Spatial distribution of exotic abundances in the study area (a) and respective abundances of generalist traits within different ecological functions. Feeding – Generalists (b), Reproduction – Polyphils (c), Tolerance – Low oxygen tolerants (d) and High temperature tolerants (e), Habitat use – Eurytopics (f) and Wide range of turbidity conditions (g).

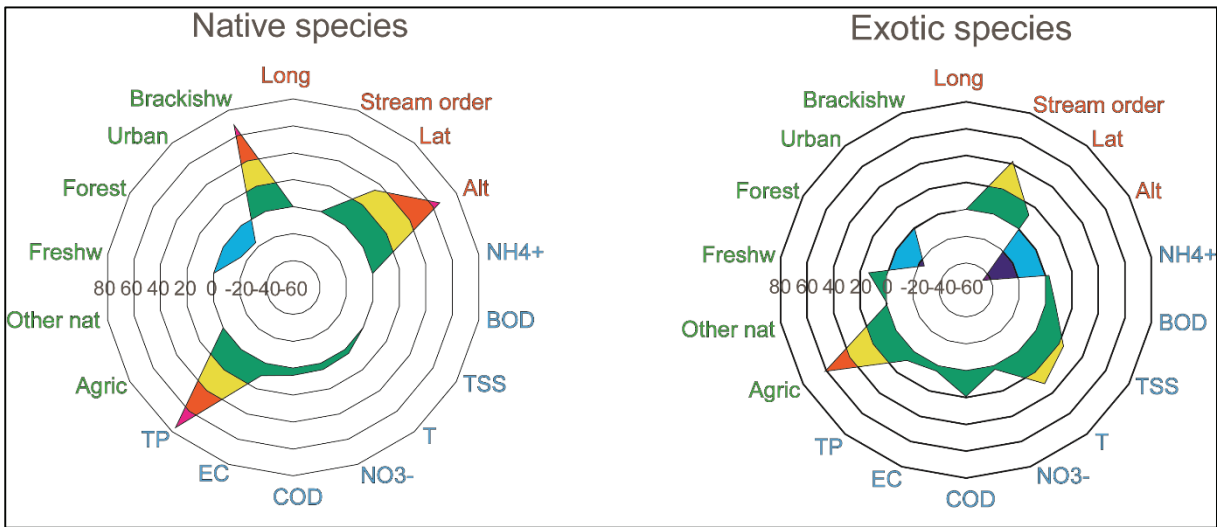


Figure 9. Boosted Regression Tree (BRT) summary showing the relative influence of geographical variables (in orange), water physico-chemical variables (in blue) and land use (in green) on freshwater fish functional diversity (calculated through the functional dispersion, FDis, metric) for native (left panel) and exotic (right panel) species. The variable abbreviations stand for: *Long* – longitude, *Lat* – latitude, *Alt* – altitude, NH_4^+ – ammonia, *BOD* – biological oxygen demand, *TSS* – total suspended solids, *T* – water temperature, NO_3^- – nitrate nitrogen, *COD* – chemical oxygen demand, *EC* – electrical conductivity, *TP* – total phosphorus, *Agric* – agricultural, *Other nat* – other natural area, *Freshw* – freshwater, *Forest* – forest, *Urban* – urban and *Brackishw* – brackish water.

Lower functional diversity was found in communities with high invasion degree, not only in the Padano-Veneto district, but also considering in the analysis all sampling station available across Italy (Fig. 10; $R^2 = 0.23$, P -value<0.05). This evidence suggested a detrimental effects of exotic invasion on functional diversity, found also by Shuai *et al.*, 2018, in the Pearl River (Southern China) and highlighted the sensitivity of functional diversity to exotic invasions.

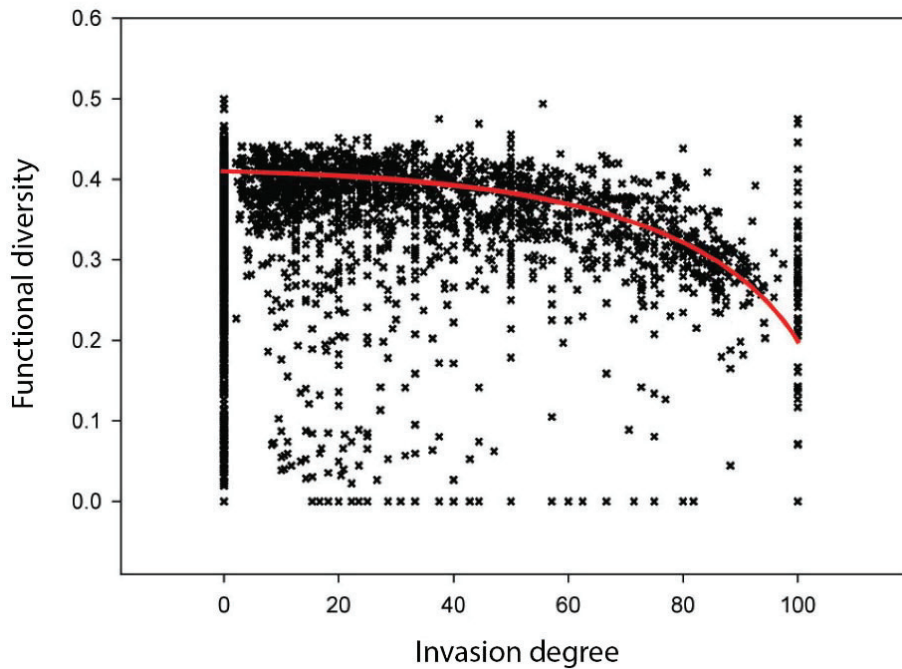


Figure 10. Italian freshwater fish communities' functional diversity (calculated through the FDis metric applied to the ecological traits of species), along the invasion gradient. The red line represents the result of the best-fitting regression analysis.

The process of invasion seems diminishing the functional diversity of fish communities, however a different role is played by exotic species (i.e. originally outside Italy) and translocated ones (i.e. originally from a nearby geographical district) [8]. Translocated species showed a light effect in affecting native functional diversity than exotic species (Fig. 11). This result is explained, at least in PDV and TL districts, by the higher functional similarity of native species to the translocated ones, than the exotic species, whose traits have evolved in markedly different ecosystems.

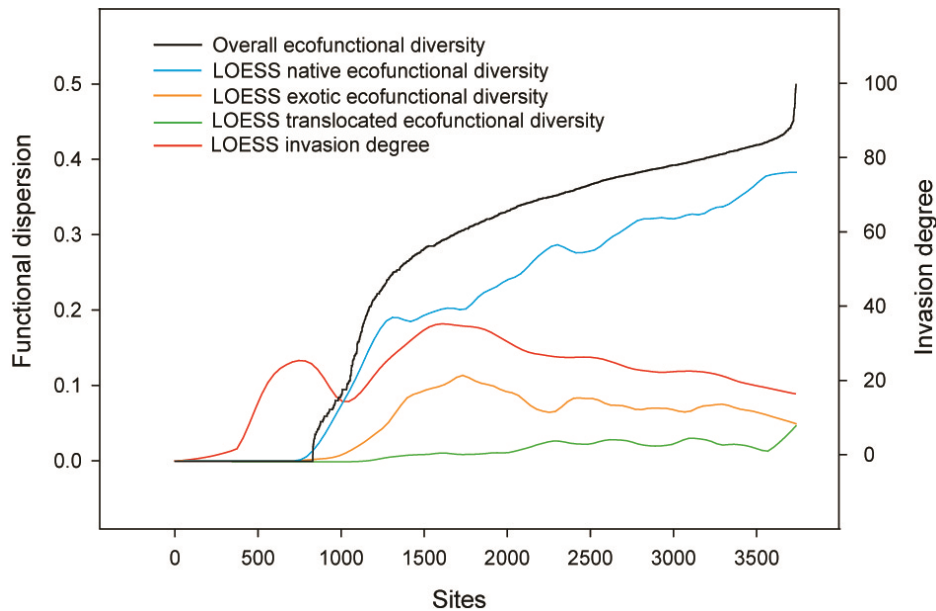


Figure 11. Native (blue), exotic (orange) and translocated (green) species contribution to the overall functional diversity, measured through the functional dispersion metric (black line), in fish communities of Italian inland waters. Sites were sorted in ascending order of functional diversity and the distributions of native, exotic and translocated species contributions were LOESS-smoothed to reduce noise. The red line indicates the LOESS-smoothed invasion degree of each site, scaled on the right vertical axis.

A greater impact of exotic species than translocated ones on functional diversity was found also by Matsuzaki, Sasaki and Akasaka, 2013, although with a contrary tendency. These authors found an increase of functional diversity in invaded fish community, due to the functional uniqueness of exotic species compared with native ones.

Discrepancy of outcomes between other studies (e.g. Toussaint *et al.*, 2018) could be led by different approach applied, by the use of species abundance measures and by the finer spatial scale considered.

5. CONCLUSIONS

On the wider scale of the whole Italy, native fish communities resulted strongly altered, with many species threatened to local extinction in lowland and middle course of waterways and less affected native communities present at higher altitudes.

This research provided clear evidences that in the present state of disturbance, exotic species invasions, almost in the Padano-Veneto District, are more detrimental to native fish community than water quality, land use, dams and flow alteration. One of the most counterintuitive results was found in presence of dams whose presence, in the Oglio River, has prevented the invasion and spread of exotic species and thus protected remaining native species.

It has been evidenced the strong role of exotic species in determining the spatial distribution of residual native species, limited to higher altitudes whereas almost absent in the large rivers and canals of the Emilia-Romagna region, dominated by exotic species. At the foothill, native species showed also the highest beta diversity. This decreased taxonomic diversity, highlighted the homogenization process occurred in the lowland communities, which are dominated by few and widespread exotic species.

Exotic species resulted detrimental not only for the taxonomic diversity but also for the functional diversity of fish community, especially when community showed more than 50% of invasion degree. This result seems to be driven by some generalist traits, particularly eurytopic, as temperature and low oxygen tolerance, widespread in all invaded community. These aspects add new dimension to the effects of exotic invasions which should be of great interest to conservation science and management.

In a management perspective, plans for these areas should require strong actions to reduce exotics' presence, not only for the direct impacts on native fish diversity, but also for their ability as ecosystem engineers, in altering the habitat. Furthermore, prevention actions and educational programs should also be promoted to avoid further introductions into rivers.

In order to contrast exotic species, the European Union recently adopted the EU regulation No 1143/2014 on the prevention and management of the introduction and spread of invasive alien species. Based on risk assessment (i.e. the description of the species and the assessment of the potential pathways of introduction, establishment and spread), the EU regulation addressed the member states to adopt prevention measures to avoid exotic species presence, to early detect their presence, and to carry out eradication measures in an early stage on invasiveness. At last, management measures should be applied in each member state when the exotic species are widespread and the eradication measures should have already resulted ineffective and/or too expensive (Britton, Gozlan and Copp, 2011).

However, despite the EU regulation, some Mediterranean countries do not strictly apply national and international legislation aimed at limiting the presence and spread of exotic invasive species, or even allow the introduction of exotic species when they can bring economic benefits (Anastácio *et al.*, 2019). This work, which took into account, for the first time, the available information on the fish community in the whole Italian territory, highlighted the critical state of native fish communities and, more in general, put in evidence the detrimental effects of exotic species on the native ones. Starting from these results, future research on invasion dynamics should include a wider scale of Mediterranean region and complementary process occurring in invasion dynamics, such as the effects of climate change.

Last, but not least, updated information of species presence are fundamental for conservation and management actions which, in turn, highlights the importance to ensure funding for field monitoring campaigns and studies for the protection of native fish fauna.

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Supplementary table 1 – Sources of stream fish communities' data used in this study. The first column reports the main provider of data/publication as reference, but we do acknowledge the work of people that conducted the surveys in the field and compiled the data for the publication. The second column acknowledges people that have provided unpublished data or facilitated the retrieval of published data.

	Data source	Acknowledgments
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2	Indipendent Region of Valle d'Aosta, Individuazione, salvaguardia e riabilitazione delle popolazioni di trote autoctone in Valle d'Aosta e in Alta Savoia, 2006	Dr. Daniele Stellin (Consorzio Regionale Pesca)
3	Province of Varese, Carta delle Vocazioni ittiche della Provincia di Varese, 2001	Dr. Fabrizio Stefani (National Research Council)
4	Province of Como, Carta ittica, unpublished data	Dr. Carlo Romanò (Province of Como)
5	Province of Milano, Carta Provinciale delle Vocazioni ittiche, 2007	Dr. Fabrizio Stefani (National Research Council)
6	Province of Bergamo, Carta delle Vocazioni ittiche, 2001	Dr. Fabrizio Stefani (National Research Council)
7	Province of Brescia, Carta ittica della Provincia di Brescia, 2004	Dr. Fabrizio Stefani (National Research Council)
8	Province of Pavia, Carta Ittica Provinciale, 2010	Dr. Fabrizio Stefani (National Research Council)
9	Province of Cremona, Revisione della carta provinciale delle vocazioni ittiche della Provincia di Cremona, 2000	Dr. Fabrizio Stefani (National Research Council)
10	Province of Mantova, Carta delle Vocazioni Ittiche, 2006	Dr. Fabrizio Stefani (National Research Council)
11	Province of Bolzano, Provincia Autonoma di Bolzano - Servizio Faunistico, Carta Ittica, unpublished data, 2014	Dr. Luigi Spagnolli and Dr. Nicola Merlo (Province of Bolzano)
12	Province of Trento, Provincia Autonoma di Trento - Servizio Faunistico, Carta Ittica del Trentino, 2006	Dr. Leonardo Pontalti (Province of Trento)
13	Province of Verona, Carta Ittica, 2008	
14	Province of Vicenza, Monitoraggio della Carta Ittica della Provincia di Vicenza: zona della bassa pianura, 2011	
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17	National Park of Dolomiti Bellunesi, Monitoraggio dei popolamenti ittici negli ambienti acquatici del Parco Nazionale delle Dolomiti Bellunesi, 2000	
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19	Province of Padova, Aggiornamento dello stato delle conoscenze sui popolamenti ittici della Provincia di Padova, 2010	
20	Province of Rovigo, Carta ittica della Provincia di Rovigo - Acque Dolci Interne, 2010	
21	Friuli Venezia Giulia Region - Ente Tutela Pesca del Friuli Venezia Giulia, Aggiornamento parziale della Carta Ittica 1992	Dr. Mirvana Feletti (Liguria Region)
22	Province of Imperia, Carta Ittica della Provincia di Imperia, 2004	Dr. Mirvana Feletti (Liguria Region)
23	Province of Savona, Terza Carta Ittica della Provincia di Savona, 2009	Dr. Mirvana Feletti (Liguria Region)
24	Province of Genova, Carta Ittica della Provincia di Genova - Indagine d'aggiornamento 1999-2003, 2005	Dr. Mirvana Feletti (Liguria Region)
25	Province of La Spezia, Carta ittica della Provincia della Spezia, 2007	Dr. Mirvana Feletti (Liguria Region)
26	Emilia-Romagna Region, Carta Ittica dell'Emilia-Romagna - Zone C, 2005	
27	Emilia-Romagna Region, Carta Ittica dell'Emilia-Romagna - Zone A e B, 2008	
28	Emilia-Romagna Region, Carta Ittica dell'Emilia-Romagna - Zone D, 2002	
29	Province of Pistoia, Carta Ittica della Provincia di Pistoia, 2002	
30	Province of Firenze, Carta ittica della Provincia di Firenze, 2012	
31	Province of Livorno, Carta ittica delle acque interne della Provincia di Livorno, 2009	
32	Province of Pisa, Carta Ittica della Provincia di Pisa, 2010	
33	Province of Arezzo, Relazione sullo status delle popolazioni delle specie ittiche autoctone italiane presenti nelle acque interne della Provincia di Arezzo, 2016	Dr. Stefano Porcellotti
34	Province of Grosseto, Carta delle Vocazioni Ittiofaunistiche della Provincia di Grosseto, 2004	Dr. Simona Piccini (UTR Grosseto)
35	Province of Prato, Carta Ittica della Provincia di Prato, 2008	Dr. Lorena Di Iulio Chiacchia (UTR Prato)
36	Umbria Region, Aggiornamento della Carta ittica del bacino del Chiascio e Topino, 2009	
37	Umbria Region, Aggiornamento della Carta ittica del bacino del Nera, 2010	
38	Umbria Region, Aggiornamento della Carta ittica del bacino del Paglia-Chiani e del Nestore, 2012	
39	Umbria Region, Aggiornamento della Carta ittica del bacino residuo del Tevere, 2015	
40	Marche Region, La Carta Ittica delle Marche, 2012	
41	Lazio Region, Carta della Biodiversità ittica delle acque correnti del Lazio, 2012	Dr. Stefano Sarrocco (DIREZIONE REGIONALE CAPITALE NATURALE, PARCHI E AREE PROTETTE)
42	Province of Aquila, Carta Ittica, 2009, unpublished data	Dr. Lino Ruggeri (Ecogest)
43	Province of Teramo, Carta Ittica, 2005	Dr. Lino Ruggeri (Ecogest)
44	Province of Pescara, Carta ittica della Provincia di Pescara, 2007	

	Data source	Acknowledgments
45	Province of Chieti, Progetto di cooperazione Pianificazione della salvaguardia delle specie ittiche nel territorio della Provincia di Chieti, 2008	
46	Molise Region, Carta ittica della regione Molise, 2005	
47	Province of Avellino, Carta ittica della Provincia di Avellino 2001.-2003, 2004	
48	Campania region, Alberto Gentile, unpublished data	Dr. Alberto Gentile (FIPSAS federation council)
49	Regional office for the prevention and protection of the environment - ARPA Puglia, unpublished data	Dr. Erminia Sgaramea (ARPA Puglia)
50	Basilicata Region, Carta Ittica Regionale, 2008	
51	Province of Reggio Calabria, I corsi d'acqua della Provincia di Reggio Calabria, 2005	Dr. Giuseppe Postorino (Province of Reggio Calabria) and Prof. Andrea Donato (University of Reggio Calabria)
52	Province of Ragusa, Carta ittica della provincia di Ragusa, 2008	Dr. Salvatore Buonmestieri (Province of Ragusa)
53	Province of Siracusa, La carta ittica della Provincia di Siracusa, 2005	
54	Province of Sassari, Carta ittica di I livello della Provincia di Sassari: indagine conoscitiva sull'ittiofauna presente nelle acque scorrenti, 2012	Dr. Antonio Zara and Eng. Salvatore Circoستا (Province of Sassari)
55	Province of Cagliari, Carta ittica di primo livello dei principali bacini idrografici della Provincia di Cagliari, 2007	Dr. Nicola Carboni and Dr. Maria Giuseppina Liggi (Province of Cagliari)
56	Province of Olbia-Tempio, Carta Ittica della Provincia di Olbia-Tempio, 2012	
57	Authority of the Po River Basin, Monitoraggio dell'ittiofauna e redazione della Carta ittica del Fiume Po, 2009	
58	Oglio River Water Authority, Evaluation of the minimum vital flow of the OglioRiver (2009–2015)''	Eng. Massimo Buizza (Oglio River Water Authority Director) and Dr. Marco Mancini

Supplementary table 2 - Full species matrix, including ecological functions (feeding, reproduction, migration, tolerance and habitat preferences) and guilds, as well as body-size classes (1: <10g, 2: 10-50g, 3: >50g), for each fish species included in this thesis.

Species	Feeding										Reproduction										Migration										Tolerance										Habitat									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
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PAPERS

4.1 How do multiple pressures affect fish communities?

4.1.1. The role of flow and damming

[1] Gavioli, A., Mancini, M., Milardi, M., Aschonitis, V., Racchetti, E., Viaroli, P., Castaldelli, G. (2018). Exotic species, rather than low flow, negatively affect native fish in the Oglio River, Northern Italy. *River Research and Applications*, (December 2017), 1–11. <https://doi.org/10.1002/rra.3324>

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RESEARCH ARTICLE

WILEY

Exotic species, rather than low flow, negatively affect native fish in the Oglio River, Northern Italy

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Abstract

Rivers worldwide are impacted by human activities such as habitat degradation, habitat fragmentation, waterway flow regulation, and introduction of exotic species, which are responsible for the reduction or the disappearance of native species in many parts of the world. The Oglio River, a tributary of the Po River in Northern Italy, is a good example of a river with a long history of human alteration and where exotic invasions are present. We used data on water parameters and fish communities along the watercourse to investigate whether low flow conditions, degraded water quality, abundant exotic species, and the presence of migration barriers could be a disadvantage for native species. We used ordination methods (redundancy analysis), variance partitioning analysis, and the threshold indicator taxa analysis to explore changes in community composition and ecofunctional traits along an altitude gradient. We found that exotic species affected native ones more than water quality and hydromorphological parameters. Native species were most abundant in the upper reach of the Oglio River, despite low flow and shallow depth. Moreover, rheophilic and clear water native fish decreased rapidly in the lower reach of the river, where exotic species increased. This distribution could be explained by the presence of barriers in the middle reach, which block exotic species migrating upstream from the highly invaded Po River, and by a lower suitability of the upper reach for some exotic species. Our results provide a general description of the fish fauna of a strongly regulated river and can contribute to develop more effective fish and water management practices.

KEYWORDS

dam, discharge, habitat fragmentation, hydromorphological alterations, non-native species, water abstraction

1 | INTRODUCTION

Rivers are some of the most human-altered ecosystems worldwide (Dudgeon et al., 2006). Anthropogenic pressures such as impoundment (Haas, Blum, & Heins, 2010), habitat degradation (Meador, Brown, & Short, 2003), longitudinal interruptions and lateral connectivity, riverbed, and flow modifications (Bunn & Arthington, 2002;

Nilsson, Reidy, Dynesius, & Revenga, 2005), and exotic species introduction (Ribeiro & Leunda, 2012) are common in all medium and large rivers worldwide (Tharme, 2003; Tockner, Stanford, Tockner, & Stanford, 2002). Although there is evidence that exploitation of rivers increased exponentially in the 20th century (Rosenberg, McCully, & Pringle, 2000), human impact on river ecosystems is an age-old phenomenon in some regions. Large lowland rivers in Europe were

influenced by landscape changes in their catchment area because the Stone Age and main alterations on flow regime began already in the Middle Ages (Müller, 1995).

European rivers thus offer good examples of habitats impacted by hydrological and morphological stressors, which, together with exotic species invasions, are the major drivers of native fish loss in this region (Corbacho & Sánchez, 2001; Crivelli, 1995; Hermoso, Clavero, Blanco-Garrido, & Prenda, 2011; Maceda-veiga, Mac, & De Sostoa, 2017).

Despite the undeniable importance and necessity of maintaining a flow suitable for aquatic life (i.e., “minimum flow”; Bunn & Arthington, 2002), humans abstract water for agriculture and power production causing severe disturbance to biotic communities (Gehrke, Gilligan, & Barwick, 2002; Nilsson et al., 2005; Poff & Zimmerman, 2009). However, a unique definition, terminology, or parametrization of minimum flow is still lacking (Tharme, 2003). This is due to the high variability of riverine ecosystems worldwide, the differences in estimation methods, and the fact that minimum flow values could vary for different target species (Murchie et al., 2008).

Traditional studies attempting to investigate minimum flow on target populations included instream habitat models, used to estimate the impact of flow changes (King, Brown, & Sabet, 2003; Lamouroux, Doutriaux, Terrier, & Zylberblat, 1999), or before–after studies on the effects of flow restoration (Lamouroux et al., 2006; Schmutz et al., 2016). Unfortunately, the response of biotic communities to variations in flow resulted largely unpredictable or not assessable, leading to contrasting results (Dudgeon et al., 2006; Poff & Zimmerman, 2009). Furthermore, even if some evidence that flow regulation might favour exotic fish species exists (Bunn & Arthington, 2002), studies on minimum flow that distinguish between native and exotic communities are generally lacking (with few exceptions, see e.g. Marks, Haden, O'Neill, & Pace, 2010; Caiola, Ibáñez, Verdú, & Munné, 2014).

In this study, we used the Oglio River to analyse the interplay between the fish community and environmental factors, such as flow. The Oglio River is a strongly regulated river, which can be representative of medium-sized European rivers with a long history of anthropic modification, where the discussion on minimum flow is currently still ongoing. The water quality (Bartoli et al., 2012; Soana, Racchetti, Laini, Bartoli, & Viaroli, 2011), macroinvertebrate, and plant communities (Bolpagni & Piotti, 2015; Guareschi et al., 2014) of the Oglio River are well known, but no studies have been carried out on the fish fauna and its relations with environmental descriptors, so far. The aim of this study was to investigate how flow conditions, water quality, and the presence of dams affect the balance between native and exotic fish species. We hypothesized that low flow conditions would be detrimental to the native fish community. We also hypothesized that degraded water quality and abundant exotic species would also be, to a lesser extent, disadvantageous for native species. Finally, we hypothesized that migration barriers could disrupt the longitudinal connectivity, further negatively impacting native fishes. We used threshold gradient analysis, ordination methods, variance partitioning, and nonparametric correlations to test which of these factors could play a central role in affecting the native fish community status, among multiple disturbances. Using these results, we also considered whether minimum flow regulations could be effective towards native fish conservation in the currently altered river conditions.

2 | MATERIALS AND METHODS

2.1 | Study area

The study was carried out on the Oglio River, a watercourse of 156 km originating from the subalpine Iseo Lake (185 m a.s.l.) and flowing into the Po River (16 m.a.s.l.), with a watershed total surface of 3,800 km² in the Lombardy Region, Northern Italy. The Mella, Strone, and Chiese Rivers are the three major tributaries of the Oglio River, with an extended drainage network dispersed in the agricultural lands. Agricultural lands cover the 58% Oglio River watershed, with intensive maize cultivation and livestock farming practices. Urbanized lands covered around the 12% of the watershed area. The Po River basin is characterized by a Mediterranean continental climate, an annual average precipitation of 1,036 mm, a mean temperature of ~12°C, a variable flow regime, and seasonal drought periods (Montanari, 2012; Turco, Vezzoli, Da Ronco, & Mercogliano, 2013). The Po River is also known as a hot spot of exotic species invasion (Lanzoni, Milardi, Aschonitis, Fano, & Castaldelli, 2018; Milardi, Aschonitis, et al., 2018), and the Po River tributaries, such as the Oglio River, could be good models to study the interactions between exotic and native fish species in the framework of a long-term history of hydraulic interventions.

In the second half of the 19th century, water abstraction was aimed at two primary uses: agriculture and hydropower production. In the 20th century, other uses have gained importance, namely, the distribution of drinking water and the discharge of polluted water from sewage treatment plants and from different industrial activities. Presently, the Oglio River can be divided into three distinct sections based on patterns of abiotic features (river flow and depth) and water abstractions. In the upper reach, from the Iseo Lake to 25 km downstream, there are six hydropower plants and the largest water abstraction for agriculture. The middle reach, between 25 and 60 km, is characterized by low flow and low depth. The lower reach, from 60 km to the confluence with the Po River, is characterized by the constant increase of flow due to the contribution of the primary tributaries (the Strone River, the Chiese River, and the Mella River) and of the aquifer, seeping directly into the river.

Two main longitudinal connectivity interruptions are also present on the middle and lower reaches of the river, around 70 and 110 km downstream of the Iseo Lake, near the towns of Pontevecchio and Isola Dovarese, respectively (Figure 1).

2.2 | Surveys and data collection

Water quality and fish data were taken from 44 sampling stations in the Oglio River course. Sampling was conducted during the warm season (from April to September) of the period 2009–2010 as a part of the project “Evaluation of the minimum vital flow of the Oglio River (2009–2015)” supported by the Oglio River Water Authority (in Italian, *Consorzio dell'Oglio, Regione Lombardia*).

Fish sampling was performed by electrofishing along both shorelines of each sampling station, for a variable length according to river width (five times the maximum width of the river at that location, to ensure that all relevant macrohabitats were sampled). Sampling was

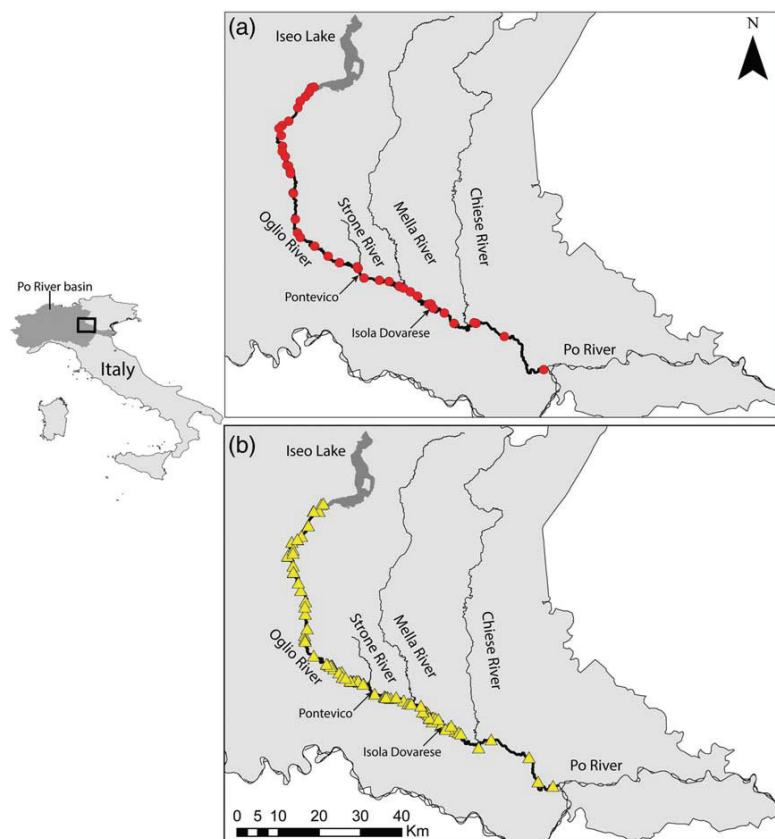


FIGURE 1 Map of Italy and the Po River basin (in darker grey, on the left) and of the study area (right panels) with locations of the fish and abiotic parameters sampling sites (red dots, a) and water abstraction sites (yellow triangles, b) [Colour figure can be viewed at wileyonlinelibrary.com]

conducted by wading, where depth was less than 1 m, and from a boat in deeper waters, keeping operators and gear constant. Captured fish species were classified according to Kottelat and Freyhof (2007), taking into account recent taxonomic determinations and common names listed in FishBase (<http://www.fishbase.org>; see Table 1). Fish data were expressed as CPUEs (unit of sampled mass [kg] per unit of sampled water surface [ha]).

A total of eight water quality parameters were measured at each sampling site. Water temperature and saturation of oxygen (%) were measured in situ using a handheld sonde (Yellow Spring Instruments Inc.). The ammonia (NH_4^+) and nitrate (NO_3^-) nitrogen, BOD_5 at 20°C , total phosphorus, chlorophyll *a*, and total suspended solids (TSS) were measured in the laboratory according to American Public Health Association (2005) standards. Shading, mean depth of the river, water velocity, and flow were also recorded for each sampling site (see Table 2). Water velocity and flow were also measured daily by the Oglio River Water Authority; for this study, we considered only values corresponding to our sampling dates. Water abstraction data (mean water abstraction for derivations and power plants) was provided by the Oglio River Water Authority.

2.3 | Fish ecofunctional traits

To investigate ecological changes in community composition along altitude and distance from Iseo Lake gradients, we took into account the ecofunctional traits of fish species. The habitat ecological function was chosen due to its power to inform on habitat choice; within this

ecological function, there are two broad guilds based on current speed and water transparency preference. The first one identified the flow preference of species: rheophilic species that prefer fast flowing water, limnophilic species that prefer slow or no current, and eurytopic species that no have flow preference. The second guild identified water preference: species preferring clear water, species preferring turbid water, and species with a broad range of conditions (Table 1). Every species was assigned to a single category within each of the two ecofunctional guilds: ecological functions, guilds, and traits of each species follow the work of Milardi and Castaldelli (2018).

2.4 | Statistical analysis

To assess the relationship between different variables and fish species, the data were used to form four groups of variables: native fish species (Native species), exotic fish species (Exotic species), morphological descriptors (Morpho), and water quality parameters (WQ; Tables 1 and 2). Fish CPUE were log transformed, whereas abiotic parameters were log and arcsin transformed (see Table 2). The data transformation was made in order to reduce normality departures for achieving simpler and more linear responses and for reducing the influence of descriptors' extreme values and the influence of highly variable abundances of fish species as suggested by Lepš and Šmilauer (2003) and Baker and King (2010).

Detrended correspondence analysis was initially performed in order to select the most appropriate response model, between linear or unimodal, for gradient analysis (Lepš & Šmilauer, 2003). Taking into

TABLE 1 Fish species sampled in the 44 sampling sites of the Oglio River

Family	Species	Common name	S ^a	NP ^b	Mean (g ha ⁻¹) ^c	Abbrev	Flow preference guild	Water clarity preference guild
Anguillidae	<i>Anguilla</i> (Linnaeus, 1758)	European eel	N	19	2025.08	Aang	R	W
Cyprinidae	<i>Leucos aula</i> (Bonaparte, 1841)	Italian red-eye roach	N	32	410.87	Laul	L	W
	<i>Squalius squalus</i> (Bonaparte, 1837)	Cavedano chub	N	44	10487.13	Ssqu	E	W
	<i>Telestes muticellus</i> (Bonaparte, 1837)	Italian riffle dace	N	27	1167.36	Tmut	E	W
	<i>Phoxinus phoxinus</i> (Linnaeus, 1758)	Eurasian minnow	N	15	119.17	Ppho	E	C
	<i>Tinca tinca</i> (Linnaeus, 1758)	Tench	N	15	1371.16	Ttin	L	C
	<i>Scardinius hesperidicus</i> Bonaparte, 1845	Italian Rudd	N	33	1078.64	Shes	E	W
	<i>Alburnus arborella</i> (Bonaparte, 1841)	Italian bleak	N	42	385.73	Aaal	E	W
	<i>Chondrostoma soetta</i> Bonaparte, 1840	Italian nase	N	11	13.22	Csoe	L	C
	<i>Protochondrostoma genei</i> (Bonaparte, 1839)	South European Nase	N	11	75.53	Pgen	R	C
	<i>Gobio benacensis</i> (Pollini, 1816)	Italian gudgeon	N	19	20.25	Gben	R	C
	<i>Barbus plebejus</i> Bonaparte, 1839	Italian barbel	N	41	7270.77	Bple	R	C
	<i>Carassius</i> spp.	Goldfish/Crucian carp	E	25	1903.69	Ccar	E	T
	<i>Cyprinus carpio</i> Linnaeus, 1758	Common carp	E	22	20836.67	Ccarp	E	T
	<i>Abramis brama</i> (Linnaeus, 1758)	Common bream	E	6	754.92	Abra	E	T
	<i>Blicca bjoerkna</i> (Linnaeus, 1758)	White bream	E	1	42.95	Bbjo ^d	E	T
	<i>Rutilus rutilus</i> Linnaeus, 1758	Roach	E	5	35.37	Rrut	E	W
	<i>Rutilus pigus</i> (Lacépède, 1803)	Pigo	N	3	249.80	Rpig	E	W
<i>Rhodeus sericeus</i> (Pallas, 1776)	Bitterling	E	31	143.13	Rser	L	W	
<i>Pseudorasbora parva</i> (Temminck and Schlegel, 1846)	Stone moroko	E	23	28.27	Ppar	E	W	
<i>Leuciscus aspius</i> (Linnaeus, 1758)	Asp	E	7	469.08	Lasp	R	T	
Cobitidae	<i>Misgurnus anguillicaudatus</i> (Cantor, 1846)	Pond loach	E	6	11.33	Mang	R	W
	<i>Cobitis bilineata</i> Canestrini, 1856	Italian spined loach	N	17	29.95	Cbi	R	C
Siluridae	<i>Silurus glanis</i> Linnaeus, 1758	Wels catfish	E	32	23399.16	Sgla	E	T
Ictaluridae	<i>Ameiurus melas</i> (Rafinesque, 1820)	Black bullhead	E	1	10.98	Amel ^d	L	T
Esocidae	<i>Esox cisalpinus</i> (Bianco & Delmastro, 2011)	Southern pike	N	10	1370.47	Ecis	L	C
Poeciliidae	<i>Gambusia holbrooki</i> Girard, 1859	Eastern mosquitofish	E	2	0.40	Ghol ^d	E	T
Cottidae	<i>Cottus gobio</i> Linnaeus, 1758	Bullhead	N	1	2.60	Cgob ^d	R	C
Centrarchidae	<i>Micropterus salmoides</i> (Lacépède, 1803)	Largemouth black bass	E	4	23.98	Msal	L	C
	<i>Lepomis gibbosus</i> (Linnaeus, 1758)	Pumpkinseed	E	12	110.98	Lgib	L	C
Percidae	<i>Perca fluviatilis</i> Linnaeus, 1758	European perch	N	21	532.19	Pflu	E	C
	<i>Sander lucioperca</i> (Linnaeus, 1758)	Zander or Pike-perch	E	6	514.58	Sluc	L	T
Mugilidae	<i>Mugil cephalus</i> Linnaeus, 1758	Flathead grey mullet	N	5	456.49	Mcep	E	W
Gobiidae	<i>Padogobius bonelli</i> (Bonaparte, 1846)	Padanian goby	N	39	134.00	Pbon	R	C

Note. Abbrev: fish name abbreviation. Flow preference guild: rheophilic species (R), limnophilic species (L), and eurytopic species (E). Water clarity preference guild: clear water species (C), turbid water species (T), and species in wide range of water clarity conditions (W).

^aS: status where N is for native and E is for exotic species.

^bNP: number of sampling sites where the species is present.

^cMean (g ha⁻¹): Mean CPUE of each species collected in 44 sampling sites.

^dRare species occurring in less than three sampling sites was removed.

account the results of detrended correspondence analysis, the linear gradient method (redundancy analysis [RDA]) was ultimately selected (Lepš & Šmilauer, 2003). Full and partial RDAs were performed using native species as dependent variables and the remaining groups as descriptor variables, similarly as Aschonitis et al. (2016). Species sampled in less than three sites were considered rare and excluded from the RDA (Table 1; Aschonitis et al., 2016); this affected one native species (*Cottus gobio*) and three exotic species (*Blicca bjoerkna*, *Gambusia holbrooki*, and *Ameiurus melas*). Collinear variables, with a variance inflation factor higher than 8 were excluded before the RDA (Zuur, Ieno, & Smith, 2007).

A variance partitioning scheme (Borcard, Legendre, & Drapeau, 1992) was applied for each group of variables on the basis of the overall variance explained by the partial RDAs. This procedure allowed the distinction between unique effects (i.e., the variance explained by a single group of variables), joint effects (i.e., the variance jointly

explained by variables of two or three groups), and unexplained variance. Variance partitioning was also run with all variables to identify the marginal effects (λ -1) and the conditional effects (λ -A) of each descriptor variable.

To investigate the changes in the fish community along the river and to assess possible correlations between water abstractions, flow, depth, TSS, and fish assemblages, a Spearman rank correlation was performed using untransformed data, except for fish assemblages. For this step, fish assemblages were expressed as the ratio between the total native CPUE and the total exotic CPUE, both log transformed, for each sampling site (Nat/Exo).

The threshold indicator taxa analysis (TITAN; Baker & King, 2010) was used to investigate changes in the fish community composition in terms of ecofunctional guilds, along the correlated gradients of altitude and distance from the Iseo Lake. This analysis identifies at which point of the environmental gradients the decline/increase in CPUEs of

TABLE 2 Average and range of abiotic parameters in the 44 sampling sites

Parameter	Abbrev.	Unit	Transformation	Minimum	Maximum	Average	SD	Group ^a
Longitude	Long	Dec. degrees	$\log(x + 1)$	9.84	10.65	10.06	0.22	-
Latitude	Lat	Dec. degrees	$\log(x + 1)$	45.04	45.66	45.36	0.18	-
Altitude	Alt	m a.s.l.	$\log(x + 1)$	17	183	80	54.59	-
Shading	Shading	%	$\arcsin(x/100)^{0.5}$	0.00	45.00	12.50	13.32	Morpho
Depth	Depth	m	$\log(x + 1)$	0.40	2.90	0.61	1.46	Morpho
Water velocity	WaterV	m s^{-1}	$\log(x + 1)$	0.11	1.16	0.40	0.22	-
Flow	Flow	$\text{m}^3 \text{s}^{-1}$	$\log(x + 1)$	4.54	65.80	28.23	17.55	Morpho
Ammonia nitrogen	N_NH4	N mg l^{-1}	$\log(x + 1)$	0.02	0.15	0.06	0.03	WQ
Nitrate nitrogen	N_NO3	N mg l^{-1}	$\log(x + 1)$	0.17	7.72	4.07	2.65	WQ
BOD ₅	BOD5	$\text{O}_2 \text{ mg l}^{-1}$	$\log(x + 1)$	0.40	4.45	1.84	1.00	WQ
Total phosphorus	TP	P mg l^{-1}	$\log(x + 1)$	15.50	222.50	55.91	43.58	-
Chlorophyll <i>a</i>	Chla	$\mu\text{g l}^{-1}$	$\log(x + 1)$	0.50	33.25	3.46	4.76	WQ
Total suspended solids	TSS	mg l^{-1}	$\log(x + 1)$	0.80	103.35	11.93	16.40	WQ
Saturation of oxygen	O%	%	$\log(x + 1)$	88.50	114.00	101.52	6.79	WQ
Water temperature	WT	°C	$\log(x + 1)$	20.2	25.8	23.0	1.3	-

Note. TSS: total suspended solids; RDA: redundancy analysis.

^aVariable coded “-” not used for RDA due to collinearity (variance inflation factor > 8).

fish belonging to each guild was most prominent. TITAN was performed with 500 replicates for the bootstrap resampling on “TITAN2” R package (Matthew, Baker, & King, 2015).

3 | RESULTS

The Oglio River fish fauna consisted of 34 species, 19 native, and 15 exotic, belonging to 12 families. The *Cyprinidae* was the most represented family, accounting for almost 60% of the total number of species (Table 1).

Among native species, the most abundant was *Squalius squalus* (mean density: $10487.13 \text{ g ha}^{-1}$), which was also the only ubiquitous species. The least represented native species sampled was *Cottus gobio*, present only in one site and with a low density (2.60 g ha^{-1}). Among exotic species, *Cyprinus carpio* and *Silurus glanis* had the highest average densities (20836.67 and $23399.16 \text{ g ha}^{-1}$, respectively). The least represented were *A. melas*, *G. holbrooki*, and *B. bjoerkna*, sampled in only few sites.

Among abiotic parameters, temperature showed relatively constant values over the sampling period with a mean of $23.0 \pm 1.3^\circ\text{C}$ (Table 2). Along the river, hypoxic conditions were never found and the oxygen values ranged from 88.50% to 114.00%. Both chlorophyll *a* and TSS were generally low, with mean values of $3.46 \mu\text{g l}^{-1}$ and 11.93 mg l^{-1} , respectively. The most downstream sampling site, however, had the highest values ($33.25 \mu\text{g l}^{-1}$ of chlorophyll *a* and 103.35 mg l^{-1} of TSS). Ammonia, BOD₅, and total phosphorus showed a large variability that reflected the heterogeneity of the sampling sites and the diverse contributions of the tributaries. Nitrates ranged from 0.17 to 7.72 mg l^{-1} . The percentage of shading due to riparian vegetation was generally low, with a mean value of less than 13% and a maximum of 45% (recorded only in two sampling stations).

Mean river depth showed a high variability in the first 30 km ($0.40\text{--}2.90 \text{ m}$; Figure 2a). Further downstream depth and flow

increased until the Isola Dovarese dam, after which flow increased and depth decreased, due to a substantial widening of the river bed in the last stretch before the confluence with the Po River. TSS were low in the first 50 km and increased progressively downstream until the confluence with the Po River (Figure 2b).

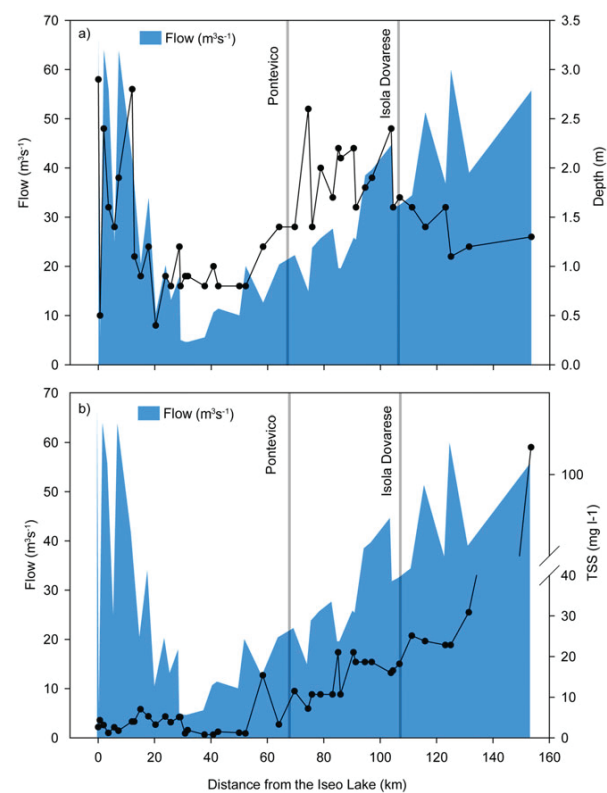


FIGURE 2 (a) Depth and (b) total suspended solids along the Oglio River watercourse. The most important longitudinal connectivity interruptions are indicated with vertical grey lines [Colour figure can be viewed at wileyonlinelibrary.com]

3.1 | Weights of native species descriptors in RDA

Longitude, latitude, altitude, distance from the Iseo Lake, water velocity, total phosphorus, water temperature, *Pseudorasbora parva*, and *Abramis brama* were all excluded from the RDA due to their high collinearity (variance inflation factor > 8).

The proportion of native species variance explained by exotic species group was higher (16.60%) than the variances explained by morphological (5.70%) and water quality (12.90%) groups (Figure 3a,b). The partial joint effects were 10.50%, 26.20%, and 27.90% for morphological, water quality, and exotic species groups, respectively (Figure 3a). Overall, the total joint effect was 28.70% (Figure 3b). Among abiotic parameters, the marginal ($\lambda-1$) and conditional ($\lambda-A$) effects of each covariate (Figure 3c) showed a high contribution of TSS, nitrogen nitrates, flow, and depth in affecting the native fish community. Among exotic species, the most important were *Lepomis gibbosus*, *Leuciscus aspius*, *C. carpio*, and *S. glanis*.

The direction and magnitude of the descriptors effects on the native fish community is shown in Figure 4. High TSS negatively affected native species, such as *S. squalus* and *Protochondrostoma genei*, but showed a positive effect on exotic species such as *L. aspius* and *C. carpio* and the native *Mugil cephalus*. High values of flow and depth were positively related to *S. glanis*; conversely, many native species were negatively affected by these factors. Among native species, only *M. cephalus* showed a positive relationship with the exotics *L. aspius*, *C. carpio*, and *S. glanis* (Figure 4).

3.2 | Relationship between river conditions and fish species

Flow, depth, and TSS were positively correlated among each other but negatively correlated with the ratio between native and exotic species

(Table 3). Water abstraction was not correlated with any of the other parameters. Water flow ranged from 4.54 to 65.8 m³ s⁻¹, with a mean of 28.23 m³ s⁻¹. The highest flow was measured in the upper reach, 20 km from the Iseo Lake (Figure 5a,b).

Native species were most abundant in the middle reach of the river, from 30 to 50 km downstream of the Iseo Lake (Figure 5a). In this reach, the mean native species CPUE was 58.96 kg ha⁻¹, with a maximum of 174.69 kg ha⁻¹ and a minimum of 5.59 kg ha⁻¹. Contrarily, exotic species had the lowest CPUEs (mean of 2.85 kg ha⁻¹). Exotic CPUEs increased in the lower reach of the river, especially downstream of the Isola Dovarese dam up to the confluence with the Po River, where the fish community was composed by almost entirely by exotic species (Figure 5a,b). A corresponding change in the fish community ecofunctional composition was evident 60 to 80 km downstream of the Iseo Lake, at an altitude of 45–40 m a.s.l., corresponding with the location of the Pontevecchio dam (Figures 5 and 6). Upper- and middle-reach communities of the Oglio River were generally dominated by rheophilic and clear water species, whereas lower-reach communities were dominated by turbid water species. Species adapted to a wide range of flow and water clarity conditions were distributed rather evenly across the river course, whereas limnophilic species did not have a distribution predictable according to the gradients analysed (Figure 6).

4 | DISCUSSION

Contrarily to our initial hypothesis, in the Oglio River, low flow conditions did not seem to be detrimental to the native fish community. Conversely, according to our initial hypothesis, degraded water quality and the abundance of exotic species had a clear negative effect on native fish, with a greater influence than flow conditions. Migration

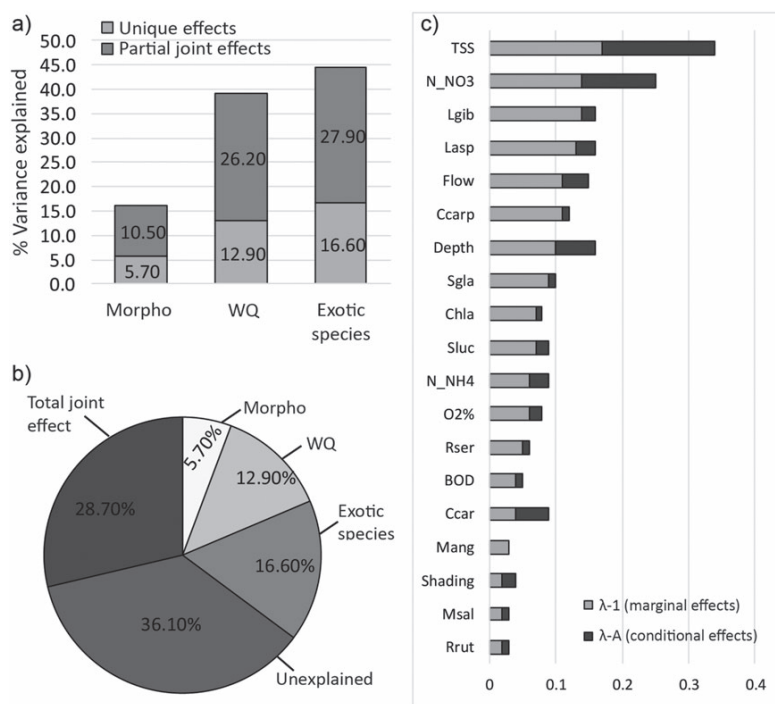


FIGURE 3 Unique and partial joint effects for each one of the three groups of variables (a), unique and total joint effects (b), marginal $\lambda-1$ and conditional $\lambda-A$ effects of each parameter affecting native species from the full redundancy analysis (c)

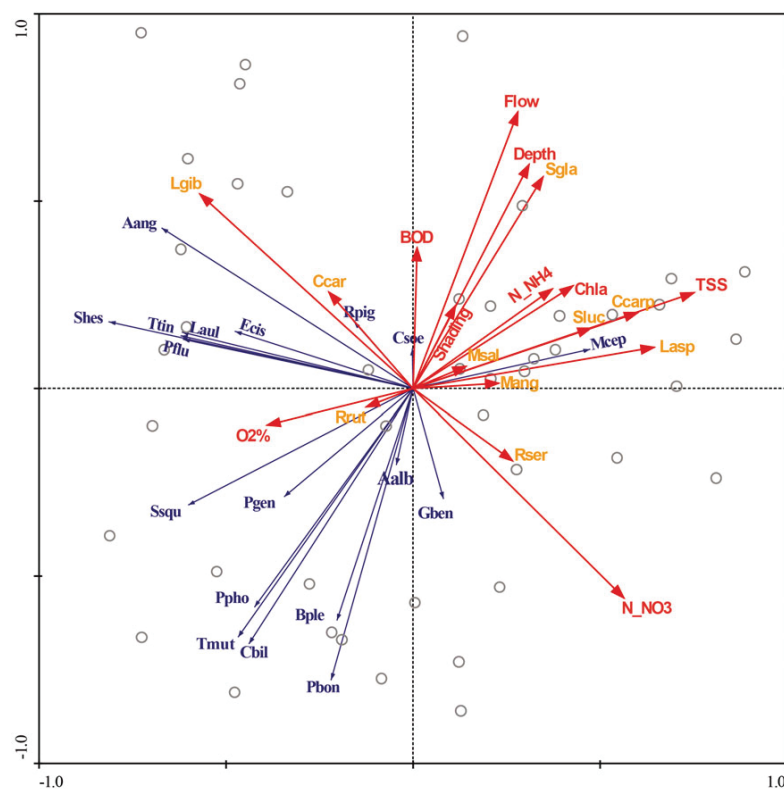


FIGURE 4 Triplot of redundancy analysis of native species as dependent variables (blue arrows) and morphological and water quality descriptors (red label) and exotic species (orange label) as descriptor variables (red arrows) in the 44 sampling sites (grey circles). Abbreviations are given in Tables 1 and 2 [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Spearman rank correlations for abstraction, flow, depth, and TSS and the ratio between native and exotic species

	Nat/Exo	Abstraction ($\text{m}^3 \text{s}^{-1}$)	Flow ($\text{m}^3 \text{s}^{-1}$)	Depth (m)	TSS (mg l^{-1})
Nat/Exo	1				
Abstraction ($\text{m}^3 \text{s}^{-1}$)	0.2201	1			
Flow ($\text{m}^3 \text{s}^{-1}$)	-0.5828**	-0.0237	1		
Depth (m)	-0.5489**	-0.1755	0.6294**	1	
TSS (mg l^{-1})	-0.8316**	-0.1212	0.4259*	0.3848*	1

Note. TSS: total suspended solids.

** $p < 0.001$. * $p < 0.05$.

barriers limited the upstream migration of exotic species from the highly invaded Po River and thus offered a counterintuitive protection to native fish. Ordination methods showed that the effect of exotic fish species was stronger than all other environmental variables together and that, among abiotic parameters, flow was not the main variable affecting native fish. In light of these results, the concept of minimum flow for the conservation of the native fish community appears to be less than effective, if not even misleading. Minimum flow should therefore be reconsidered in a more integrated way, accounting for multiple disturbances affecting the native communities, including hydromorphological disruptions.

Perhaps counterintuitively, flow was not retained by RDA as a strong descriptor of the native community and higher flow values seem to have favoured exotic species over native ones (Figures 4 and 5). An increase in flow does not always result in a positive change for native fish, as the new flow conditions might alter wetted habitats and potentially result in lower habitat suitability for some species

(Bradford, Higgins, Korman, & Snee, 2011). Indeed, our results indicated that native fish were caught more frequently in the upper and middle stretches of the Oglio River, characterized by highest water abstraction and low flow. Large exotic predators such as *S. glanis* were abundant in deep sections with high flow, whereas small native species such as *A. arborella* and *P. bonelli* were more abundant in the middle reach, in shallow sections with low flow. The peculiar hydromorphological conditions of the middle reach, with low flow and low depth, are unsuitable for big predators such as *S. glanis* (which was never caught in this area) and thus effectively provided “refuge areas” for the residual native community. Despite substantial water abstraction, water was never completely absent due to the continuous supply from the aquifer and the numerous tributaries. Furthermore, contrarily to other rivers characterized by higher slopes, the Oglio River was never steep enough to cause the complete absence of water downstream of the abstraction. Further downstream, water from the tributaries and the aquifer progressively increases the flow again until

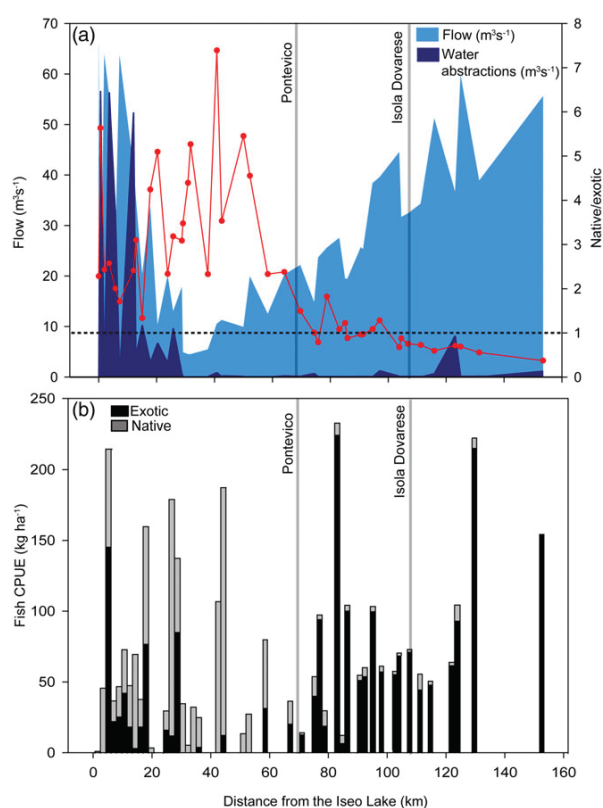


FIGURE 5 Water flow, water abstraction, and the ratio between native and exotic CPUEs; values of the ratio below one (dashed line) indicate the prevalence of exotic fish (a). Sampled fish CPUEs, with a representation of the share of exotics and natives at each sampling sites (b). The most important longitudinal connectivity interruptions are indicated with vertical grey lines [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

values prior to abstraction are reached and even surpassed; this is the area where fish communities were mostly composed by rheophilic species. However, this increase in flow did not correspond to an increase in native species abundance but rather to a steep decrease. Although we investigated the relationship between environmental parameters and fish communities through ordination methods, which can only explore the relationships between variables rather than explain them, similar results were reported in field studies that

artificially manipulated the flow or the fish community (Arthington, Naiman, McClain, & Nilsson, 2010; Coggins, Yard, & Pine, 2011; Marks et al., 2010). Previous studies have underlined how flow and other environmental parameters could potentially influence the effectiveness of fish sampling (i.e., the catchability of fish) (Gwinn, Beesley, Close, Gawne, & Davies, 2016). However, the information currently available based on other species and other environments (see, e.g., Speas, Walters, Ward, & Rogers, 2004 or Lyon et al., 2014) is insufficient to infer that this could be a relevant issue in our data, and we recommend that future studies investigate this aspect specifically. Although there is mounting evidence that minimum flow restoration could be ineffective on the native community status if water quality improvement and exotic species control are not fully addressed, we advise that future work should further explore the key factors identified in our study to assess their explanatory potential experimentally.

Among exotic species potentially affecting native ones, *L. gibbosus* had the highest conditional and marginal effects and was positively related with the density of *Anguilla anguilla*. This is likely due to the preference of *L. gibbosus* for similar habitat and water quality of the native species, that is, low turbidity, low depth, low flow, and mesotrophic conditions with a higher availability of diversified microhabitats (Top, Tarkan, Vilizzi, & Karakuş, 2016). Conversely, the high ranking of *L. aspius* in the ordination graph is likely driven by its capture in only seven sites of the lower reach, where the native community was not significantly represented and other exotic species were also present. *S. glanis* and *C. carpio*, two species with a high rank in the ordination graph, are known to severely affect the native community. *S. glanis* is a generalist predator and a successful invader thanks to adaptability to a wide range of prey and habitat conditions (Copp et al., 2009). Adverse effects on native fish populations in artificial or highly modified environments have been reported in the literature (Castaldelli et al., 2013), although these do not necessarily extend to all waters (Guillerault et al., 2015). It is widely documented that *C. carpio* can alter habitat conditions, increasing turbidity and reducing aquatic vegetation, and thus indirectly affecting native fish communities (Crivelli, 1995).

Among water quality parameters, TSS and nitrates were the two most important factors negatively affecting native fish distribution. Phytoplankton blooms due to nitrates availability were not at the origin of turbidity, as chlorophyll *a* was not retained in the analysis as a strong descriptor. Most likely, a combined effect of the inorganic

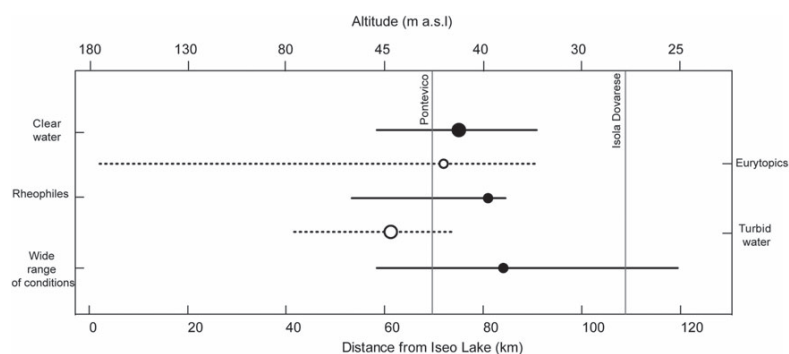


FIGURE 6 Significant thresholds for different ecofunctional guilds related to habitat choice along gradients of altitude and distance from the Iseo Lake (horizontal axes). Lines represent guilds distribution across the gradients, with circles identifying the distribution peak. Solid lines and circles represent guilds that decline along the gradient (grouped on the left), whereas dashed lines and empty circles represent species, which distribution increases along the gradient (grouped on the right). The most important longitudinal connectivity interruptions are indicated with vertical grey lines

fraction of the TSS and phytoplankton blooms is at the root of this effect (Bartoli et al., 2012; Delconte et al., 2014; Soana et al., 2011). Watershed uses, in particular agriculture, are the likely causes of the increase of nitrates and TSS, particularly in the middle reach of the Oglio River. This area was affected by extensive manure spreading and irrigation by submersion, a traditional practice still made possible by high water availability. Through this practice, surface run-off brings inorganic fine materials to the river, which increase TSS (Bartoli et al., 2012). Furthermore, due to the coarse texture and consequent high permeability, water percolates from fields directly to aquifer and then back to the river, transporting a significant nitrates load. This is not uncommon in Mediterranean rivers where TSS and nutrient load typically increase towards the lower areas due to a more intense agricultural land use. The high turbidity could also be partly due to the resuspension of fine sediments operated by *C. carpio* and *Carassius* spp., which dominated the turbid-adapted lower-reach fish communities. Both species are known to thrive in turbid, eutrophic waters and to positively feedback eutrophication by resuspension of sediments of sedimentary phosphates through their feeding (Huser, Bajer, Chizinski, & Sorensen, 2016). In turn, the high turbidity in the lower reach may have further favoured exotic species accustomed to it and disadvantaged native ones, adapted to clearer water conditions and more abundant in the upper and middle reaches.

Ultimately, our results downplay the significance of minimum flow for native fish conservation and highlight the importance of other parameters such as the degree of interruption of longitudinal connectivity and the effects of exotic species. Although longitudinal connectivity is an undeniable necessity for the fish community (Bunn & Arthington, 2002), its interruption can have counterintuitive effects. In the Oglio River, interrupted longitudinal connectivity paradoxically favoured some native species by blocking the invasion of exotics migrating upstream from the highly invaded Po River (Castaldelli et al., 2013). This is most evident in Figure 4, where the exotic community overtakes the native one downstream of the Isola Dovarese dam (the first impassable barrier, the second being a few km upstream, in Pontevico). Our results also suggested that low flow in the Oglio River may have favoured some native species over exotic ones by altering depth, velocity, and sediment features. Consequently, the native community was counterintuitively more nested in the upper and middle reaches of the river, where water abstraction was most intense. In the future, because of climate change, flow in the Po River basin is expected to be further diminished due to a substantial decrease of seasonal precipitation and an accelerated snow melt in spring (Ravazzani, Barbero, Salandin, Senatore, & Mancini, 2014) but net losses and gains of habitats suitable for native species have not yet been modelled. Furthermore, higher temperatures could favour exotic species invasions (Vitousek, D'Antonio, Loope, Rejmánek, & Westbrooks, 1997) even though this might not be a relevant factor in this area (Milardi, Lanzoni, Gavioli, Fano, & Castaldelli, 2018).

5 | CONCLUSIONS

Our study analysed for the first time the interplay between the fish community and environmental factors in the Oglio River. Our results

underlined how anthropogenic impacts on river morphology and flow, occurred centuries to decades ago, affected the fish community, favouring some native rheophilic species despite reductions in flow, and blocking the invasion of exotics from invaded downstream areas. Although modified flow regimes in regulated rivers can negatively affect fish communities, there is a wider range of community responses to flow alterations (Murchie et al., 2008). Therefore, we suggest that additional attention should be given to restoring natural hydromorphology when exotic species invasions are ongoing. Management and conservation plans should model the changes in habitat suitability and consider the effect of exotic species before further altering the river hydromorphology.

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4.1 How do multiple pressures affect fish communities?

4.1.1. The role of water quality

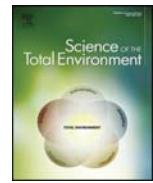
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Run to the hills: exotic fish invasions and water quality degradation drive native fish to higher altitudes

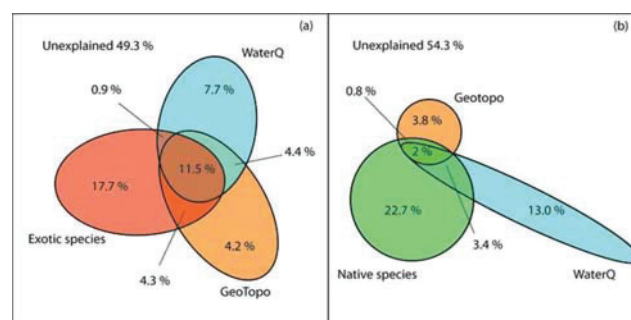
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HIGHLIGHTS

- Exotic species were found to be a major factor in native species abundance distribution in freshwater habitats of Northern Italy.
- Exotics effects were evident three decades after the initial invasion and comparable in magnitude to eutrophication.
- The combined feedback of exotic species and eutrophication drove most native fish species on the edge of their natural distribution and towards extinction.
- Site- and species-specific conservation actions could benefit from a carefully considered prioritization provided in our work.

GRAPHICAL ABSTRACT



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ABSTRACT

While the significance of anthropogenic pressures in shaping species distributions and abundances is undeniable, some ambiguity still remains on their relative magnitude and interplay with natural environmental factors. In our study, we examined 91 late-invasion-stage river locations in Northern Italy using ordination methods and variance partitioning (partial-CCA), as well as an assessment of environmental thresholds (TITAN), to attempt to disentangle the effects of eutrophication and exotic species on native species. We found that exotic species, jointly with water quality (primarily eutrophication) and geomorphology, are the main drivers of the distribution of native species and that native species suffer more joint effects than exotic species. We also found that water temperature clearly separates species distributions and that some native species, like Italian bleak (*Alburnus alburnella*) and Italian rudd (*Scardinius hesperidicus*), seem to be the most resilient to exotic fish species. We also analyzed the dataset for nestedness (BINMATNEST) to identify priority targets of conservation. As a result, we confirmed that altitude correlated negatively with eutrophication and nestedness of exotic species and positively with native species. Overall, our analysis was able to detect the effects of species invasions even at a late invasion stage, although reciprocal effects seemed comparable at this stage. Exotic species have pushed most native species on the edge of local extinction in several sites and displaced most of them on the rim of their natural distribution. Any potential site- and species-specific conservation action aimed at improving this situation could benefit from a carefully considered prioritization to yield the highest results-per-effort and success rate. However, we encourage future research to update the information available before singling out specific sites for conservation or outlining conservation actions.

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1. Introduction

The significance of anthropogenic pressures in shaping terrestrial and aquatic ecosystems at the global scale is undeniable (Foley et al., 2005; Halpern et al., 2008; Syvitski et al., 2005). The scope and magnitude of these pressures has been investigated in several studies which attempted to find the thresholds of changes that lead to new dynamic states (Samhouri et al., 2010). From the worldwide effects of anthropogenic-driven climate warming (e.g. Behrenfeld et al., 2006) down to the local scale consequences of water abstraction or infrastructure construction (Jeppesen et al., 2015), some uncertainties still remain on the interplay between different pressures, as often they overlap in space and time and their effects on the ecosystem are not easy to disentangle.

A new factor that has been introduced in the equation of anthropogenic pressures is the introduction of exotic species, which enhances the complexity and the uncertainties of interpreting the individual role of specific stressors (Leprieur et al., 2008; Pyšek et al., 2010). The assessment of invasion impacts could be accomplished in controlled experimental conditions (e.g. Johnson et al., 2009) or, where feasible, backtracking the effects in the sedimentary record (e.g. Milardi et al., 2016). Several studies have recently attempted to address the role of human-mediated species introductions in the loss of socio-economic values (e.g. Pimentel et al., 2005) or biodiversity (Wilcove et al., 1998). It is generally recognized that exotic species constitute a relevant pressure on the environment at different scales (Meyerson and Mooney, 2007). However, while several authors pointed out that exotic species invasions could be a major driver of native species extinction and homogenization of ecosystems (Wilcove et al., 1998), others remained sceptic about the extent and intensity of their effects (e.g. Gurevitch and Padilla, 2004). This controversy could also be partially due to the fact that exotic species invasions are not a flash process: invasion impacts could be highest in the initial stages, but changes in native species abundance and distribution could take a longer time to complete. Furthermore, some studies might have failed to grasp the full effects of species invasions because overlapping factors (e.g. eutrophication levels) might jointly drive the interactions between native and exotic species, thus confounding the final outcome of invasions (Leprieur et al., 2008).

While there might remain a certain amount of uncertainty on their exact rank among pressures or their combined effects, it is undeniable that human-mediated species introductions played a role in homogenizing the worldwide biota (Clavel et al., 2011; Rahel, 2000). This homogenization is particularly evident in the case of the fish fauna (Rahel, 2000), where a few species that are highly sought after for aquaculture or fisheries (e.g. the common carp, *Cyprinus carpio* or the rainbow trout, *Oncorhynchus mykiss*) have been introduced so widely that they can be now found in all continents. Most successful invaders are also generalist species which thrive in different environmental conditions, and could be favored by environmental degradation, even though patterns of invasion success might differ among taxa (Crawley et al., 1986). However, the environment plays a major role in regulating the distribution of species: therefore the success of species invasions might be mostly due to a synergic effect between the changing environmental conditions and the exotic species, regardless of the native biota (Moyle and Light, 1996). However, the reciprocal effect of native species on exotic ones has not yet been thoroughly investigated. Furthermore, albeit there is no agreement as to how long an invasion takes to reach a “late stage”, areas where exotic and native species interactions have had decades to play out could be most interesting to explore this issue.

The Mediterranean region has been the focus of many studies dealing with biodiversity loss due to species invasions (e.g. Didham et al., 2007; Lloret et al., 2005). In this region, it has been argued that fish introductions constitute one of the major drivers of extinction, at least for fish species (Crivelli, 1995). However, the Mediterranean is one of the regions where anthropic modification of the environment has

been undertaken at least since the Roman Empire, thus potentially creating degraded habitat conditions that could further favor the establishment of exotic species. However, more than a few challenges persist in disentangling and prioritizing biodiversity loss causes as well as in optimizing conservation efforts, due to the lack of a truly multidisciplinary approach and of adaptive management (Pooley et al., 2014). Methods of investigation that would be able to address these challenges could be very useful to.

To investigate the impact of exotic species invasions, and its relation to environmental conditions, we selected an area at a late stage (over 30 years) of exotic fish species invasion in the Mediterranean region, where native fish species were still present. We hypothesized that exotic species would be a major driver of native species distribution and that the reciprocal effects would be much smaller. We also hypothesized that water quality (primarily eutrophication) and geomorphological factors could play a role in both native and exotic fish distributions, but would favor the latter. We used ordination and variance partitioning methods to quantify the relative contribution of environmental gradients and exotic species in shaping the occurrence, distribution and abundance of native species. We also tested the application of a method to rank sites based on species population nestedness and rank species based on their nesting capacity, which can be used to identify potential priority targets of site-specific and species-specific future conservation measures.

2. Materials and methods

2.1. Study area, surveys and data collection

The study area is located in Northern Italy and it is defined by the administrative boundaries of Emilia-Romagna Region with a total coverage of 22,446 km² (Fig. 1). It is naturally bound north and south by the Po River and the Apennines Mountains, respectively, and has a Mediterranean continental climate. In this area, exotic fish species introduction date as far back as the XVII century (common carp), with few North American species (rainbow trout, brown bullhead, *Ictalurus melas*, and some Centrarchidae) arriving in the early part of the last century and most species being introduced around 1970 from Asia or East Europe (e.g. grass carp, *Ctenopharyngodon idella*, or wels catfish, *Silurus glanis*).

We selected 91 sampling sites within the study area, where both exotic and native fish species were present. The selected sites were mostly located in the lowlands, including both natural and artificial water bodies, over an altitudinal gradient of ~120 m (full range – 1–389 m a.s.l.). Sites with fully native (at high altitudes) or fully exotic (at low altitudes) fish communities were excluded from the analysis, because the focus was to specifically investigate the area where native and exotic species distributions overlap. Further descriptions of sites, species and communities excluded from this analysis can be found in Lanzoni et al. (2017, accepted for publication).

The sampling sites were located in waterways with section width ranging from 8 to 350 m (the maximum value corresponds to the Po River) (Fig. 1). The sampling was performed in both natural (e.g. Po, Trebbia, Taro, Secchia, Panaro, Reno, Lamone, Fiumi Uniti, Bevano, Marecchia etc) and artificial (irrigation canals, mainly located in the lowlands, e.g. Po di Volano, Po di Primaro, Canal Bianco, Canale Circondariale) riverine habitats during the warm season (from April to September) of the period 1998–2005 as part of the latest institutional regional monitoring program for the compilation of the official Fish Inventories of the Emilia-Romagna Region (Regione Emilia Romagna, 2008). Unfortunately, due to the amount of funding required for such work, no more recent data was available as this was the latest survey performed at the regional scale. The dataset included information on each site location, altitude and main water quality parameters (e.g. nitrogen and phosphorus concentration, Biochemical Oxygen Demand – BOD₅, Chemical Oxygen Demand – COD, temperature and pH). Yearly LIM (*Livello di Inquinamento da Macroscrittitori*, Pollution Level from

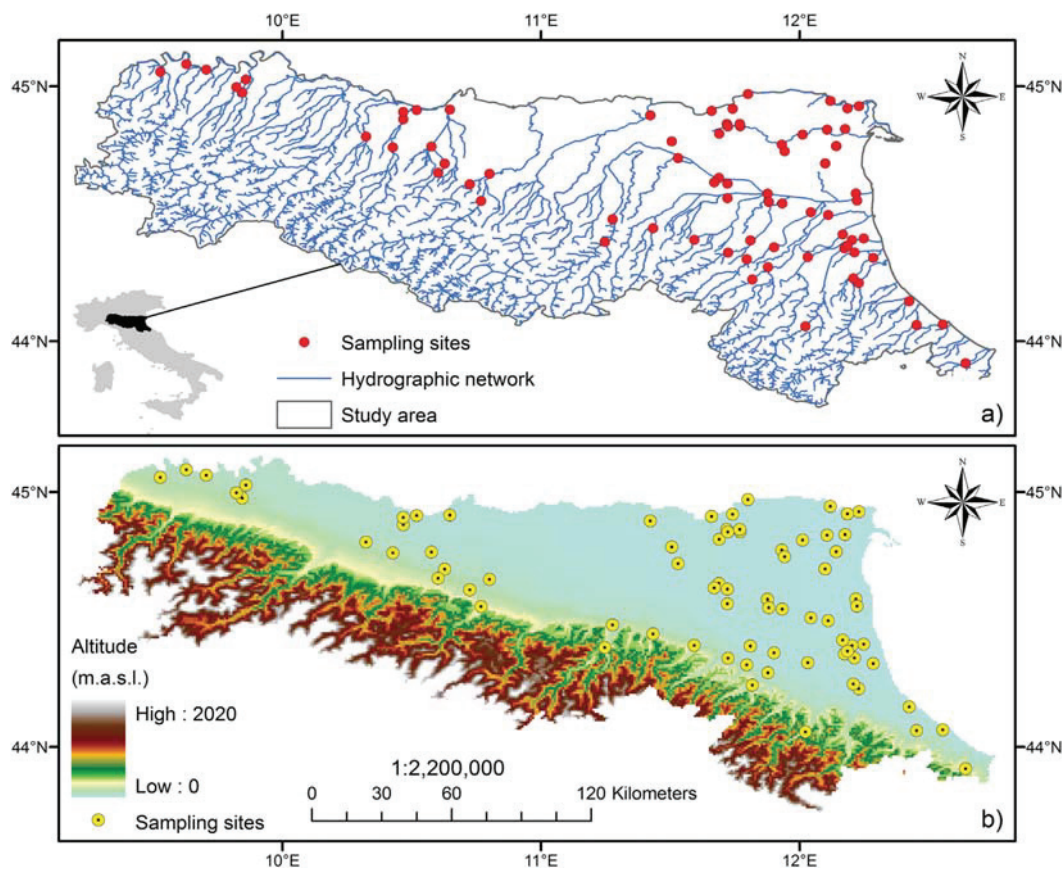


Fig. 1. Location of the 91 sampling sites based on a) major rivers, streams and canals (source: <http://www.eea.europa.eu/data-and-maps/data/european-river-catchments-1>), and b) altitude profile of the area (source: <https://lta.cr.usgs.gov/GTOPO30>).

Macro-descriptors, in English) scores were measured in the same period as fish sampling (1998–2005); LIM measures the environmental status based on the concentration of 7 different parameters representative of the chemical status of the water, sampled at monthly intervals. These parameters are dissolved oxygen, BOD, COD, *Escherichia coli*, phosphorus and nitrogen dissolved compounds, therefore LIM does not strictly measure chemical pollution, but rather provides a measure of the eutrophication level (the higher the value, the lower eutrophication). Fish sampling was performed by electrofishing, adapting the standard guidelines to the particular conditions of waterway typologies, using direct current at 400–600 V and 4–5 A (Backiel and Welcomme, 1980; Reynolds, 1996). Sites were sampled once, during daylight, in an upstream zigzag direction by wading, when depth was <1 m, and by boat in deeper waters. The transect lengths were equivalent to 10 times the river width ensuring that the range of present macrohabitats of each site was fully surveyed (Hankin and Reeves, 1988). The duration of sampling was therefore quite variable ranging from half an hour to more than 2 h, as in the case of the Po River. Electrofishing is considered the best quantitative method for fish sampling in shallow waters, up to a maximum of 1 m (Zalewski and Cowx, 1990) but its efficacy may be low in deeper waters, with big and mobile specimens, or with high conductivity. Such special conditions occurred in almost all the lower stretches of rivers and in the canals of the lowlands. For this reason, electrofishing in these sites was immediately followed by sampling with a standard set of trammel nets (with variable mesh size from 90 to 5 mm), with the support of professional fishermen (Backiel and Welcomme, 1980). Fish species were classified according to Kottelat and Freyhof (2007), taking into account recent taxonomical determinations and common names as listed in FishBase (Froese and Pauly, 2017). Site-specific fish

abundances were expressed in Moyle classes (Moyle and Nichols, 1973), ranging from 1 (low abundance, 1–2 individuals per site) to 5 (high abundance, >50 individuals per site).

2.2. Ordination methods and variance partitioning

The data were used to form four groups of variables: Group 1 (native fish species - Ns), Group 2 (exotic fish species - Es), Group 3 (geographical variables - GeoTopo) and Group 4 (water quality parameters - WaterQ) (the latter two groups are summarized in Table 1). The data of abiotic environmental parameters (Table 1) were log-transformed before analysis to reduce normality departures (Aschonitis et al., 2016; Feld and Hering, 2007).

Detrended Correspondence Analysis (DCA) was initially performed in order to select the most appropriate response model (between linear or unimodal) for gradient analysis (Lepš and Šmilauer, 2003; Ter Braak and Šmilauer, 2002) and Canonical Correspondence Analysis (CCA) (unimodal method) was finally used in all the studied cases instead of a linear method (e.g. Redundancy Analysis - RDA) because the dominant gradient length in DCA was always >4 (Lepš and Šmilauer, 2003) (results not given). CCAs were performed either using Es group or Ns group or both groups as dependent variables versus the remaining groups of each case as descriptor variables. Each CCA was performed targeting either all the remaining groups (case of full-CCA) or one group after partialing out the effects of the parameters of the remaining ones, which were used as co-variables (case of partial-CCA). CCA was performed for each possible combination of targeted descriptor and co-variables using CANOCO 4.5, based on species correlations, and standardized species scores (Ter Braak and Šmilauer, 2002). Significant

Table 1
Geographical and water quality parameters and their grouping.

Parameter	Abbreviation	Unit	Transformation	Minimum	Maximum	Average	Standard deviation	Group
Latitude (WGS84 ellipsoid)	Lat	Dec. degrees	log(x + 1)	43.91	45.09	44.62	0.28	GeoTopo
Longitude (WGS84 ellipsoid)	Long	Dec. degrees	log(x + 1)	9.53	12.64	11.62	0.73	GeoTopo
Altitude	Alt	m a.s.l	log(x + 5)	−1.00	389.00	28.80	54.69	GeoTopo
Ammonia nitrogen	NH4 +	N mg/L	log(x + 1)	0.02	6.35	0.60	1.02	WaterQ
Nitrate nitrogen	NO3-	N mg/L	log(x + 1)	0.17	9.87	2.07	1.48	WaterQ
BOD ₅	BOD5	O2 mg/L	log(x + 1)	0.63	10.50	4.44	2.10	WaterQ
COD	COD	O2 mg/L	log(x + 1)	4.33	52.25	17.10	8.54	WaterQ
Electrical conductivity	EC	µS/cm	log(x + 1)	334.50	3660.67	810.82	408.00	WaterQ
Total phosphorus	TP	P mg/L	log(x + 1)	0.01	1.68	0.25	0.28	WaterQ
Total suspended solids	TotSS	mg/L	log(x + 1)	0.50	297.83	52.51	49.26	WaterQ
Water temperature	Temp	°C	log(x + 1)	12.75	24.57	20.24	2.41	WaterQ

descriptors for each group were identified using CANOCO's forward selection procedure and Monte Carlo permutation test (499 permutations) (Aschonitis et al., 2016; Feld and Hering, 2007). Collinear variables with a variance inflation factor VIF > 8 were excluded before the CCA analysis (Zuur et al., 2007). Rare fish species (present in ≤1% of sites) were excluded from ordination analysis (Aschonitis et al., 2016). These species were southern pike (*Esox cisalpinus*), tench (*Tinca tinca*), flathead grey mullet (*Liza ramada*), European flounder (*Platichthys flesus*), brown trout (*Salmo trutta*), roach (*Rutilus rutilus*), grass carp and pond loach (*Misgurnus anguillicaudatus*).

A variance partitioning scheme (Borcard et al., 1992; Liu, 1997) was applied for each group of variables based on the overall variance explained by the partial CCAs. This procedure allowed the distinction between unique effects (i.e. the variance explained by a single group of variables), joint effects (i.e. the variance jointly explained by variables of two or three groups), and unexplained variance. The proportion of variance explained by different groups of variables are expressed as the sum of all canonical eigenvalues of partial-CCA (or CCA) divided by the total inertia (Feld and Hering, 2007). Variance partitioning was also run with all variables to identify the marginal effects ($\lambda-1$) and the conditional effects ($\lambda-A$) of each descriptor variable. The marginal effect of a descriptor variable is equal to the eigenvalue of a partial CCA if the corresponding variable was the only environmental variable (additionally to the variance explained by covariables). The conditional effect of an environmental variable is equal to the additional amount of variance in species assemblages explained by the corresponding variable at the time it was included into the model during a selection procedure (additionally to the variance explained by covariables). Such effects were also examined to assess the relative contributions of environmental variables for predicting the community composition (Ter Braak and Smilauer, 2002).

2.3. Assessment of environmental thresholds – TITAN analysis

The main gradients identified through ordination methods were used to analyze the losses and gains of taxa along these gradients using the Threshold Indicator Taxa Analysis (TITAN, Baker and King, 2010). Altitude (Group 3) and temperature (Group 4) were selected based on their ranking within variable groups for both native and exotic species. Additionally, crucian carp abundance (Group 2) was selected to identify thresholds solely for native species. TITAN uses indicator taxon scores (IndVal) to integrate occurrence, abundance and directionality of taxon responses along environmental gradients. The method identifies the environmental threshold (the optimum value of a continuous variable) that partitions sampling units and distinguishes negative (= losses; z−) and positive (= gains; z+) taxon responses. Thus, TITAN helps to identify taxon-specific change points along an environmental gradient at which the decline/increase in a given taxon's frequency and abundance is most prominent. Bootstrapping (500 repetitions) is used to estimate two important diagnostic indices (reliability and purity) as well as uncertainty around the location of individual taxa and

community change points (Baker and King, 2010). Indicator purity is the proportion of change-point response directions (positive or negative) among bootstrap replicates that agree with the observed response. Pure indicators (e.g., purity ≥ 0.95) are consistently assigned the same response direction, regardless of abundance and frequency distributions generated by resampling the original data. If bootstrap resampling substantially alters the probability of obtaining an equal or larger IndVal based on random permutations of the data, then that particular taxon is not a reliable indicator. Indicator reliability is estimated by the proportion of bootstrap change points whose IndVal scores consistently result in P-values below one or more user-determined probability levels (e.g., P ≤ 0.05). Reliable indicators (e.g., P ≥ 0.95) of the bootstrap replicates achieving P ≤ 0.05 are those with repeatable and consistently large IndVal maxima (Baker and King, 2010). Similarly as for ordination analysis, the same species which were found in <3 sites were excluded also from TITAN analysis.

2.4. Nestedness analysis

The binary matrix nestedness temperature calculator ("BINMATNEST"; Rodríguez-Gironés and Santamaría, 2006) was used to quantify the level of nestedness in native and exotic species distributions. The calculator's algorithm permutes rows (fish species) and columns (sampling sites) in such a way that matrix nestedness is maximized and a temperature T ranging from 0° (complete order) to 100° (complete disorder) is calculated. In an ordered dataset, every site contains a proper subset of the species at all of the sites above it. To determine the statistical significance of the observed T value, BINMATNEST provides three probability values, associated with different null models. The BINMATNEST is used in this study not for estimating nestedness temperatures of the populations but rather because it additionally provides two types of rankings through the final packed matrix: a) a ranking of species based on their nesting capacity and b) a ranking of sites based on species population nestedness.

We initially used BINMATNEST to test its use for ranking of native, exotic and all species grouped to identify priorities for species-specific conservation actions. Species at the top of the rank should be the most nested and widespread, thus at the lowest risk of extinction, while species at the bottom of the rank should be the most vulnerable and at the highest risk of extinction. Furthermore we produced also site rankings to test potential prioritization of site-specific conservation actions. For each site, two different nestedness rankings were calculated based on native and exotic species, which were then combined according to the following formula:

$$S_{nest_i} = Inv(NR_{Ei}) + NR_{Ni} \quad (2)$$

where NR_E and NR_N are the nestedness rankings of sites from the packed matrix of "BINMATNEST" based only on exotic species or native species, respectively, and i is the sampling site number. Both NR_E and NR_N get values from 1 (highest nesting) to x (lower nesting), where x is the

number of sampling sites. $Inv(NR_E)$ inverts the NR_E ranking values from $1, 2, \dots, x$ to $x, \dots, 2, 1$. Thus, if a site i has $Inv(NR_E) = x$ and $NR_N = x$ means that it has the most nested exotic and the least nested native populations. Thus, higher values of $Snest_i$ indicate a both higher nested exotic and low nested native population in site i and is used to describe the highest potential threat of widespread exotic species on less common native species, for the purposes of species-specific conservation. Lower values of $Snest_i$, on the other hand, identify sites where least nested exotic species coexist with some of the most nested native species. Therefore, such sites are primary targets of site-specific conservation measures. Using the outcomes of ordination analysis, we tested correlations between ranks and $Snest_i$ values with the main environmental gradient (i.e. altitude) using generalized linear models. Trying to account for some of the general conditions of the sites prior to sampling, which could have contributed in shaping the current species distribution, we also used average LIM values to test dependencies between eutrophication level and nestedness rank, through a Spearman Rank correlation.

As the purpose of nestedness analysis was to test its use to identify priority species and sites for conservation measures, all species records were used including those of rare species.

3. Results

3.1. Ordination methods and variance partitioning

Native species distribution presented substantially higher joint effects associated to geographical parameters and exotic species than vice-versa (Fig. 2a). Exotic species abundance and distribution, on the other hand, was almost twice as uniquely affected by water quality parameters (i.e. temperature) (Fig. 2b). Overall, native species distribution and abundance were more affected by joint effects of other variable groups (21.1%), when compared to exotic species (6.2%).

Among geographical factors, CCA showed that altitude was a strong explanatory variable of both native and exotic species distribution (Fig. 3). Marginal and conditional effects of exotic species on native species were still about one-third of the magnitude of altitude effects. Among water quality parameters, temperature was one of the strongest explanatory variables but other variables linked to eutrophication (COD and BOD_5) were also relevant. Ultimately, exotic species like crucian carp (*Carassius carassius*), common carp and common bream (*Abramis brama*) had the greatest negative effect on native species (see also Fig. 4). Conversely, effects of native species were less directional but

indicated that Italian rudd (*Scardinius hesperidicus*) and Italian bleak (*Alburnus alburnella*) distribution and abundance had a positive effect on exotic species, whereas Italian chub (*Squalius squalus*) and Italian nase (*Protochondrostoma genei*) had a negative effect (Figs. 3, 4).

3.2. TITAN

TITAN analysis revealed that several native species such as Italian bleak and Italian nase were distributed across the full range of altitudes in this sub-area (Fig. 5a), in sympatry with equally widespread exotic species such as stone moroko (*Pseudorasbora parva*) and, to a lesser extent, other exotics (e.g. crucian carp, common carp, common bream, wels catfish). A temperature threshold was found close to 20 °C, with native species decreasing before the threshold and exotic species (with the exception of Italian rudd) preferring warmer waters, likely at lower altitudes (Fig. 5b). Ultimately, TITAN analysis showed that only Italian bleak and Italian rudd coexisted with all densities of crucian carp, while a group of native species such as South European roach (*Sarmarutilus rubilio*), Padanian goby (*Padogobius bonelli*), Italian barbel (*Barbus plebejus*) and chub clearly decreased in presence of higher densities of crucian carp (Fig. 5c).

3.3. Nestedness analysis

Overall, all-inclusive nestedness analysis revealed that Italian bleak, crucian carp, common carp, stone moroko and wels catfish were the most widespread species and coexisted in some sites (Table 2). The native species Italian bleak was the least affected by exotic species presence. Lowest ranking species were the least widespread, including occasional saltwater species (e.g. European flounder, *Platichthys flesus*), threatened native species (e.g. tench, *Tinca tinca*), least successful exotics (e.g. roach, *Rutilus rutilus*).

Among native species, Italian bleak, Italian rudd and chub were the most widespread and clustered together, while brown trout, thinlip grey mullet and European flounder were the least widespread and found only occasionally and never together. However, some of the most threatened native species (e.g. tench or southern pike) did not rank at the lowest places. Among exotic species, stone moroko, crucian carp and common carp were the most widespread and formed the backbone of exotic assemblages in several sites. At the other end of the scale, roach, white bream (*Blicca bjoerkna*) and grass carp (*Ctenopharyngodon idella*) were the least widespread.

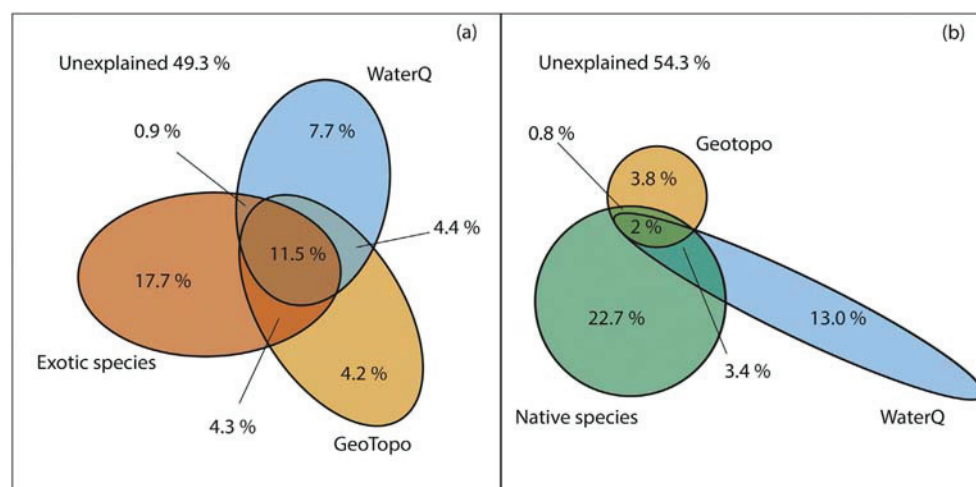


Fig. 2. Euler-Venn diagram of unique and joint effects of geographical (GeoTopo), water quality (WaterQ) and exotic species on native species distribution and abundance (a) and the reciprocal representation of the same effects for exotic species, using native species as explanatory variables (b). The numbers indicated the variance explained by each component.

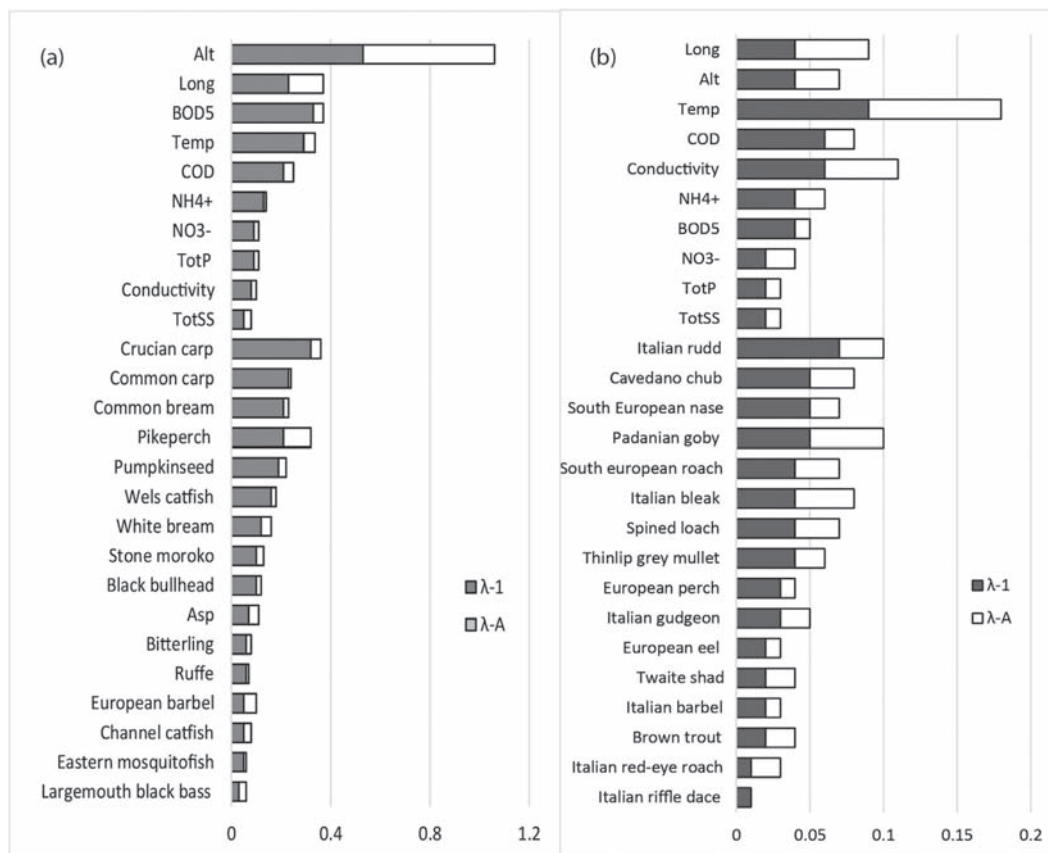


Fig. 3. Marginal ($\lambda-1$) and conditional ($\lambda-A$) effects of variables within variable groups that affect native (a) and exotic (b) species distribution and abundance. Bars are arranged according to a decreasing order of magnitude of marginal effects, by variable group (GeoTopo, WaterQ and exotic or native species).

Sites with highest nestedness in terms of native species hosted communities formed by the most common and clustered species (Italian bleak, Italian rudd and chub) whereas the lowest ranking sites hosted only one native species, albeit common (i.e. Italian bleak). Sites on the top of the exotic species rank hosted the most widespread exotic

species, whereas those at the bottom of the rank were those least invaded.

The results of site nestedness analysis based on native and exotic species correlated significantly with altitude, showing a positive correlation for native species (Correlation Coefficient = 0.605, P-value <

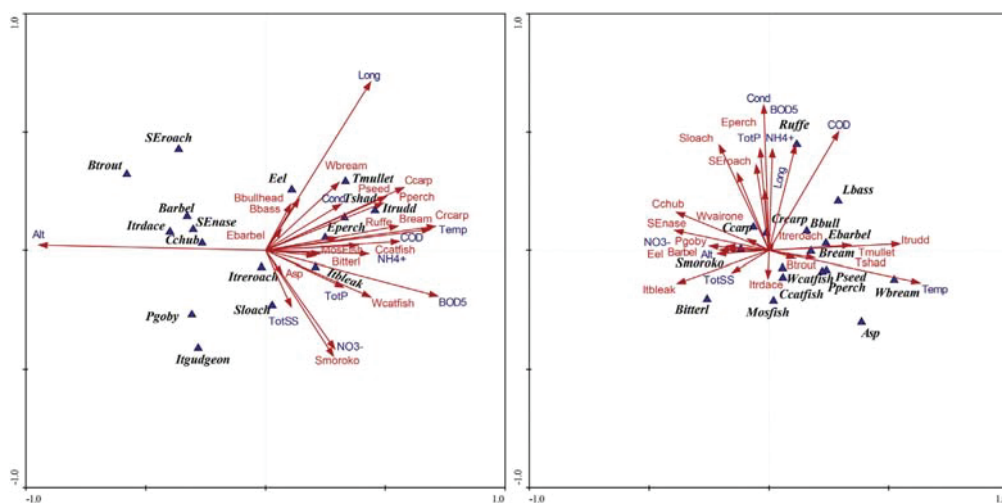


Fig. 4. Triplots of CCA ordination results showing the direction of combined effects of environmental variables (in blue) and exotic species on native species (in italics), on the left panel. The combined effects of environmental variables (in blue) and native species on exotic species (in italics) is shown on the right. Species are identified with codenames derived from contractions of their common names (see also Table 2). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

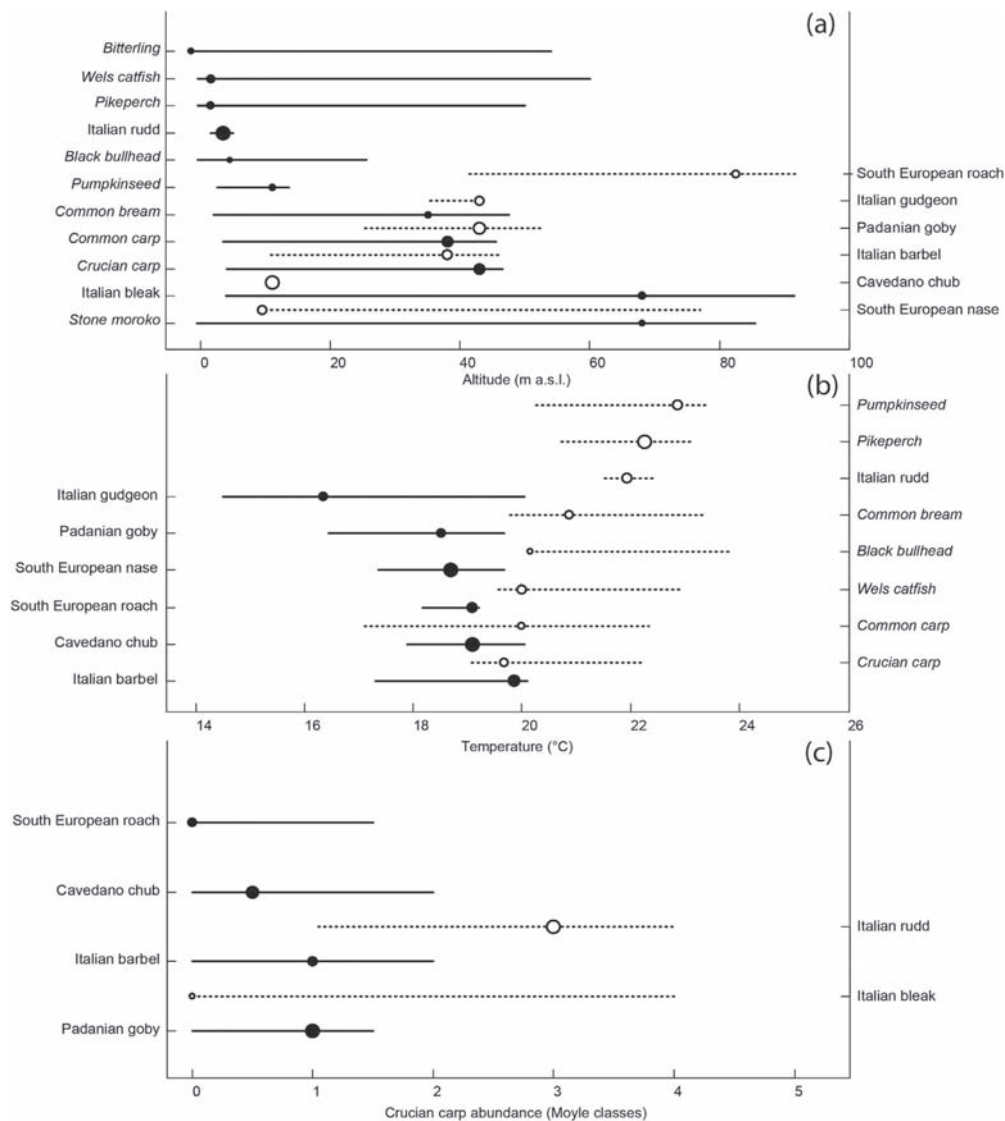


Fig. 5. Losses and gains of both exotic (in italics) and native species along altitude (a), and temperature (b), as well as losses and gains of native species along distribution/abundance of crucian carp (c), according to the TITAN analysis. Lines represent species distribution across the gradient, with circles identifying the distribution peak. Solid lines and circles represent species that decline along the gradient, while dashed lines and empty circles represent species which distribution increases along the gradient. Species not shown in these figures have a distribution not predictable according to the environmental gradients examined.

0.05) and a negative one for exotic species (Correlation Coefficient = -0.457 , P -value < 0.05) (Fig. 6a). The sites with lowest S_{nest_t} values had the highest native rank and the lowest exotic species rank; the S_{nest_t} values correlated negatively with altitude of the site (Fig. 6b, Correlation Coefficient = -0.686 , P -value < 0.05). Despite the relatively small altitude gradient, there was a significant negative correlation between eutrophication levels expressed through average LIM and altitude (Fig. 6c, Correlation Coefficient = 0.665 , P -value < 0.05).

The results of site nestedness analysis based on all species did not correlate with average eutrophication index LIM (P -value > 0.05). However, ranking of sites based on exotic species alone had a slight positive but not significant correlation with average LIM (Spearman rank corr. = 0.15 , P -value > 0.05) whereas ranking of sites based on native species alone had a significant negative correlation with average LIM (Spearman rank corr. = -0.31 , P -value < 0.05).

4. Discussion

Up to 2005, both native and exotic species coexisted at lower altitudes, but assemblages were dominated by exotic species, revealing a marked altitudinal effect despite the relatively small gradient (~ 120 m) while native fish species in the study area were mostly located at higher altitudes. Confirming our initial hypothesis, ordination methods revealed that exotic species still had strong effects on native species abundance and distribution after ca 30 years from the latest species introductions but, perhaps counterintuitively, underlined a similar size of unique and joint effects of exotics on natives. This is likely because the very few native species which were still present in this area were those least affected by exotic species and eutrophication, which was confirmed by all analyses. Our tests confirmed that nestedness analysis could be useful in identifying sites as priority targets of

Table 2

Family, scientific name and common name of fish species in the sub-area where native and exotics overlap. The table includes their native or exotic status (N/E) and the number of sites within the sub-area where each species was present (#S). RankN represents the rank calculated for native species alone, RankE the rank calculated for exotic species alone and the OverallR value represents the global rank (considering all species). Bold values underline the first and last 5 species of each ranking.

Family	Species	Common name	N/E	#S	RankN	RankE	OverallR
Anguillidae	<i>Anguilla anguilla</i> (Linnaeus, 1758)	European eel	N	9	8		17
Clupeidae	<i>Alosa fallax</i> (Lacépède, 1803)	Twaite shad	N	8	9		26
Cyprinidae	<i>Sarmarutilus rubilio</i> (Bonaparte, 1837)	South European roach	N	8	11		22
	<i>Leucos aula</i> (Bonaparte, 1841)	Italian red-eye roach	N	1	12		21
	<i>Squalius squalus</i> (Bonaparte, 1841)	Cavedano chub	N	32	3		6
	<i>Squalius lucumonis</i> (Bianco, 1982)	Toscana stream chub	N	2	17		31
	<i>Telestes muticellus</i> (Bonaparte, 1837)	Italian riffle dace	N	3	18		27
	<i>Tinca tinca</i> (Linnaeus, 1758)	Tench	N	1	16		39
	<i>Scardinius hesperidicus</i> Bonaparte, 1841	Italian rudd	N	30	2		8
	<i>Alburnus arborella</i> (Bonaparte, 1841)	Italian bleak	N	74	1		1
	<i>Protochondrostoma genei</i> (Bonaparte, 1841)	South European nase	N	20	4		13
	<i>Romanogobio benacensis</i> (Pollini, 1816)	Italian gudgeon	N	9	10		19
	<i>Barbus plebejus</i> Bonaparte, 1839	Italian barbel	N	21	6		14
	<i>Barbus barbus</i> Linnaeus, 1758	European barbel	E	3		16	30
	<i>Carassius spp.</i> (Linnaeus, 1758)	Crucian carp	E	67		3	4
	<i>Cyprinus carpio</i> Linnaeus, 1758	Common carp	E	76		2	2
	<i>Abramis brama</i> (Linnaeus, 1758)	Common bream	E	30		6	10
	<i>Blicca bjoerkna</i> (Linnaeus, 1758)	White bream	E	4		17	29
	<i>Rutilus rutilus</i> Linnaeus, 1758	Roach	E	1		19	40
	<i>Rhodeus sericeus</i> (Pallas, 1776)	Bitterling	E	26		5	7
	<i>Pseudorasbora parva</i> (Temminck & Schlegel, 1846)	Stone moroko	E	70		1	3
	<i>Ctenopharyngodon idella</i> (Valenciennes, 1844)	Grass carp	E	2		18	33
	<i>Leuciscus aspius</i> (Linnaeus, 1758)	Asp	E	6		14	28
Cobitidae	<i>Misgurnus anguillicaudatus</i> (Cantor, 1842)	Pond loach	E	1		15	36
	<i>Cobitis bilineata</i> Canestrini, 1865	Italian spined loach	N	14	5		15
Siluridae	<i>Silurus glanis</i> Linnaeus, 1758	Wels catfish	E	43		4	5
Ictaluridae	<i>Ameiurus melas</i> (Rafinesque, 1820)	Black bullhead	E	18		9	12
	<i>Ictalurus punctatus</i> (Rafinesque, 1820)	Channel catfish	E	4		13	24
Esocidae	<i>Esox cisalpinus</i> Bianco & Delmastro, 2011	Southern pike	N	1	15		34
	<i>Salmo trutta</i> complex	Brown trout	N	2	19		32
Poeciliidae	<i>Gambusia holbrooki</i> (Girard, 1859)	Eastern mosquitofish	E	5		12	20
Centrarchidae	<i>Micropterus salmoides</i> (Lacépède, 1803)	Largemouth black bass	E	9		11	23
	<i>Lepomis gibbosus</i> (Linnaeus, 1758)	Pumpkinseed	E	23		7	11
Percidae	<i>Perca fluviatilis</i> Linnaeus, 1758	European perch	N	3	13		25
	<i>Gymnocephalus cernua</i> (Linnaeus, 1758)	Ruffe	E	9		10	18
	<i>Sander lucioperca</i> (Linnaeus, 1758)	Zander or Pike-perch	E	27		8	9
Mugilidae	<i>Liza ramada</i> (Risso, 1827)	Thinlip grey mullet	N	3	20		38
Gobiidae	<i>Padogobius bonelli</i> (Bonaparte, 1846)	Padanian goby	N	16	7		16
Pleuronectidae	<i>Platichthys flesus</i> (Linnaeus, 1758)	European flounder	N	1	21		37

conservation measures, but was not as useful when applied to species. Ultimately, careful consideration needs to be given to the outputs of this analysis prior to the elaboration of conservation plans. Unfortunately, even if our analysis drew on the most updated data available, this was probably already outdated and no concrete suggestions for prospective conservation could be drawn from it. Before invasion, numerous native species were present in the area, including the lowlands (Castaldelli et al., 2013), but were pushed to higher altitudes, with cooler and less eutrophic water, by a combination of factors where exotic species likely played a major role.

4.1. Ordination methods and variance partitioning

In 2005, native species abundance and distribution seemed to be largely driven by the presence of exotic species jointly with water quality and geographical factors after ~30 years from the latest species introductions. The large joint effect of geographical factors (over 20% versus a 4.2% of unique effects) is very likely a product of exotic species pressure and water quality pressure that pushed several natives' distribution areas towards the upstream part of the rivers. However, our analysis focused on a late invasion stage, thus not detecting the full effects of exotic species at the invasion peak, but rather the product of those effects. Therefore, the results of our analysis underline the outcome of past interactions, which have driven down and away native species unable to cope with the presence of exotic ones, combined with other environmental factors. The few native species in this zone of overlap with exotic species are clearly the most resilient to interactions with the exotics,

which likely explains the similar size of unique and joint effects of natives on exotics (and vice-versa).

Water quality is a good example of the complex interactions having a strong unique effect on exotic species distribution. The most abundant and widespread exotic species thrive in eutrophic waters and some of these are known to affect water quality by increasing e.g. eutrophication and turbidity through sediment resuspension (Bonneau and Scarnecchia, 2015; Richardson et al., 1995) thus creating a positive feedback cycle. That different exotic species could mutually facilitate each other in the invasion is not a new hypothesis (Simberloff and Von Holle, 1999), but it remains somewhat controversial (Simberloff, 2006), and it could well be that exotic species would have complex interactions, both positive and negative, with native species and other exotics (Goodenough, 2010). In most invaded sites, exotic fish communities include predators such as pikeperch and wels catfish, and their prey: crucian and common carp, common bream and few other smaller bodied cyprinids. Pikeperch and wels catfish are adapted for predation in turbid waters (Ali et al., 1977; Bruton, 1996) while common bream, crucian and common carp are ecosystem engineering species that increase turbidity and eutrophication and are likely to have co-evolved specific predator avoidance mechanisms (Bonneau and Scarnecchia, 2015; Castaldelli et al., 2013; Richardson et al., 1995). Ultimately, the outcome after almost 30 years of invasion is a homogenization of the fish fauna, with communities that occasionally include native North-American species (introduced much earlier), but are mostly composed by species native of the Danube River drainage, similar to what underlined by Castaldelli et al. (2013) in a smaller section of this area.

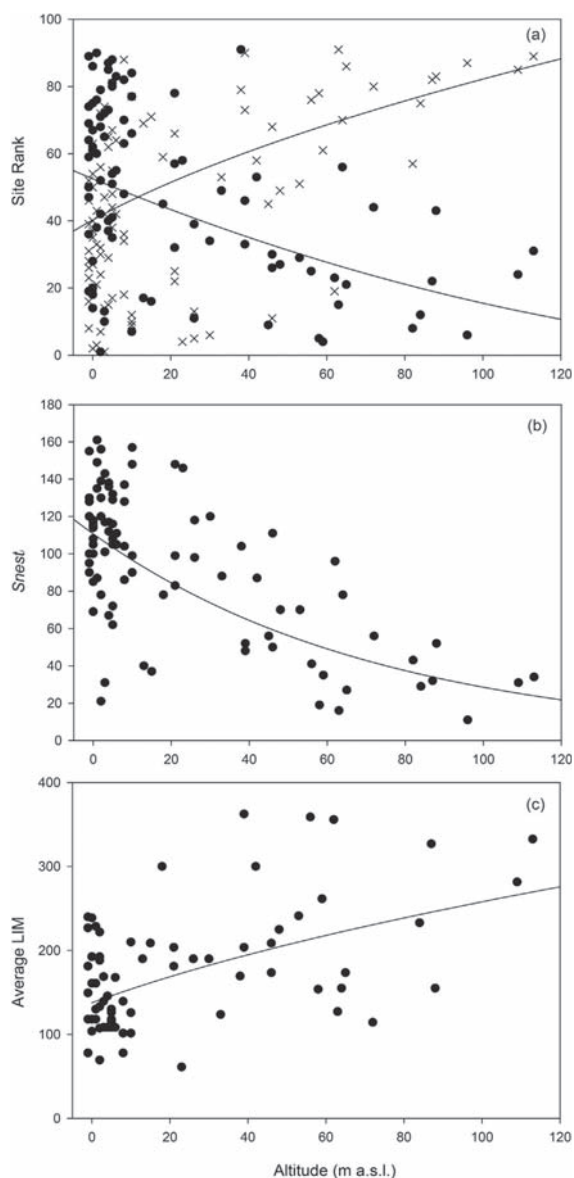


Fig. 6. Relations between altitude and (a) rank of sites based on native (full circles) and exotic species (crosses), (b) S_{nesti} values, and (c) average LIM values. Solid lines represent significant regression curves.

The “Danubification” of the Po River could be a good example of faunal homogenization and invasional meltdown in fish communities of which, so far, very few examples are known (e.g. the opposite effect found in Britton et al., 2010).

4.2. Assessment of environmental thresholds – TITAN analysis

The results of ordination analysis cannot be interpreted without considering other factors, but can help direct further investigations. Considering a combination of the results of ordination methods and TITAN it was clear that altitude itself could not be the sole limit for the expansion of exotic species, but temperature should be also accounted for. Water temperature generally correlates negatively with altitude and, to a limited extent, this was true even over the limited altitude gradient present among our sampling sites (see e.g. Fig. 3a). However, it must be noted that native species distribution in the lowlands was not limited by

temperature, before the exotic invasion (Castaldelli et al., 2013). Temperature might still play a relevant role, as some exotic species could lose part of their competitive edge at cooler temperatures (e.g. crucian carp, Roberts, 1966; Vornanen et al., 2009). Other factors, not accounted for in the analysis, such as the presence of migration barriers (i.e. weirs or dams), could further explain the distribution of alien and native species and likely contribute to the distribution pattern emerging from our data (Rolls, 2011). Furthermore, it could be worth to consider that our analysis worked on numerical abundances rather than biomass. Biomass is usually considered to give a stronger representation than numerical abundance, when analyzing ecological patterns in animal and plant communities, as it reflects more directly the allocation of energy among the species (Abrahamson and Caswell, 1982; Sprules and Munawar, 1986; Strayer, 1986), especially given the large size span of most fish communities. As an example, the native species coexisting with exotic in invaded sites were small bodied cyprinids, such as Italian bleak, which were not numerically abundant and therefore likely to constitute an even smaller part of the fish communities’ biomass. Overall, this further strengthens the loss of native biota (i.e. in terms of native biomass as well as number of species) revealed by our results.

Exotic species clearly distributed in sites with a wide range of eutrophication levels (summarized by the LIM index), showing that these species that were not significantly affected by eutrophication. This indirectly confirmed that the most widespread exotic species, if anything, could thrive in eutrophic habitats. While it is possible that native species were negatively affected by eutrophication, as some of them were poorly distributed in sites at higher eutrophication levels, it is also true that eutrophication levels correlated with altitude (Fig. 6c). Therefore, it might not be possible to fully disentangle the interplay of these two factors. However, it is interesting to note that altitude had a stronger effect on native species distribution than any single parameter of water quality related to eutrophication (Fig. 3a), which strongly suggests that eutrophication could be a lesser factor than exotic species in regulating native species distribution. This strengthens and broadens the conclusions drawn in Castaldelli et al. (2013), where water quality did not seem to play a strong role in native species decline. The notion that exotic species play a minor role in threatening biodiversity (e.g. Davis, 2003), seems to be inapplicable to our study area.

4.3. Nestedness analysis

The few native species still present in eutrophic sites were widely distributed and might not deserve species-specific conservation efforts. Conversely, species at the bottom of the native species ranking might be more plausible species-specific conservation targets, as these species were found only in few sites. However, to further implement a management strategy, further checks need to be performed on adjoining sites, both to verify whether these species could be surviving in adjoin areas and to identify surviving populations to use in artificial breeding and restocking. In our case, unfortunately, native species such as tench or southern pike could be found only in one site and are thus were at the highest risk of regional extinction. However, they were not ranked among the lowest scoring species because brown trout (a species typical of higher altitudes, likely at the edge of its distribution area), thinlip grey mullet and European flounder (two species typical of transitional waters, and thus rare in freshwater) scored even lower. This signals that this analysis outputs could not be immediately used, but need further careful consideration as to the geographical range and number of species included. In fact, locally endangered native species (i.e. those typical of the area and present in only few sites, such as tench or southern pike) ranked in the middle range when considering solely native species, because they co-occurred with very common native species (e.g. Italian bleak). At this spatial level, the worldwide conservation status of species defined by IUCN is usually less relevant than the status of local populations: species relatively common at larger spatial scales still face local/regional extinction, thus increasing population

fragmentation. A more appropriate ranking of these species for conservation purposes was obtained when considering exotic and native species together in the analysis.

In principle, nestedness analysis based on native species distribution should have identified sites where the least widespread and clustered native species could be found. Theoretically, if native species were also not abundant at these sites these could be the most likely locations of local extinctions. However, as mentioned above, since the rarest native species (i.e. tench, southern pike, Toscana stream chub and Western vairone) often occurred together with some of the most widespread species (i.e. Italian bleak, Italian rudd or chub) these sites were not ranked at the bottom. Perhaps counterintuitively, the least ranked sites contained the most widespread and clustered species (i.e. Italian bleak). Yet these sites were correctly identified as some of the primary targets of site-specific conservation, as they will be easily lost to a full exotic community if, for any cause, that single native species is lost. Therefore, while the single native species is of no particular conservation value in itself, as it is in no danger of extinction on a large scale, the site probably deserves some attention because it is at higher risk of local total extinction of native species. This is what has already occurred in some of those sites since the last sampling in 2005 (Lanzoni, unpublished data). However, further consideration needs to be given also to this ranking, as there could be discrepancies and valuable lessons learned from a more holistic perspective.

In a world where conservation resources are limited and need to be optimally allocated to obtain the most significant results per effort, we further tested the use of $Snest_i$ as a measure of balancing conservation priorities taking into account both native and exotic species distribution. According to this method, sites with lowest $Snest_i$ could be identified as primary conservation targets, as they host robust native communities (highest native ranks) and weak exotic communities (lowest exotic ranks). In our analysis, these sites did not fully correspond to those identified using either native or exotic species alone and could be considered as an optimal sub-group. Site-specific conservation actions could be most effective and yield the highest success rate or results-per-effort, if applied to this kind of sites. However, given that the most updated data available was still several years old, we could not single out specific sites for future conservation or outline potential conservation actions. We encourage future research in this field to update the information available before any reasonable concrete conclusion could be drawn. Furthermore, this is a method to devise a-posteriori actions, which face the struggle to reverse a situation that is already compromised and therefore might not be considered acceptable by natural resources managers and the public at large. We advise that precautionary action is preferable to restoration actions, bearing in mind the challenges of managing exotic species include also a time lag (Crooks, 2009). Furthermore, ecosystem services evaluations might help to underline that the gap between socio-economic and biodiversity conservation goals could be narrower than commonly thought (Nelson et al., 2009).

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4.1 How do multiple pressures affect fish communities?

4.1.2. The role of water quality

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Research article

Proposing priorities of intervention for the recovery of native fish populations using hierarchical ranking of environmental and exotic species impact

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ABSTRACT

The freshwater populations of native fish species (Ns) have reached critical levels in many parts of the world due to combined habitat deterioration by human interventions and exotic fish species (Es) invasions. These alarming conditions require combined and well-designed interventions for restoring environmental quality and restricting Es invasion. The aim of the study is to propose a method to design spatially explicit priorities of intervention for the recovery of Ns populations in highly impacted freshwater systems by exotic multi-species invasion and water quality (WQ) degradation. WQ and Es are used as Ns descriptors, which require intervention. The method uses gradient analysis (ordination method of Canonical Correspondence Analysis) for assessing the weights of Ns descriptors' effects, which are further used to develop weighted severity indices; the severity index of WQ (Swq) and Es invasion (Se), respectively. Swq and Se are further merged to one combined total severity index St . The proposed method provides a) a ranking of the sites, based on the values of S_p , which denotes the priority for combined intervention in space and can be visualized in maps, b) a ranking of the most important Ns descriptors for each site to perform site-specific interventions, and c) Es rankings based on their potential threat on Ns for species-specific interventions. WQ, Es and Ns data from 208 sampling sites located in the Emilia-Romagna Region (Northern Italy) were used as a case study for the presentation of the proposed method. The application of the method showed that the north and northwestern lowland areas of Emilia-Romagna region presented the higher priority for intervention since the Ns of these areas are the most impacted from combined Es invasions and WQ degradation. Specific Es belonging to cyprinids, which are mostly responsible for the decline of aquatic vegetation and the increase of water turbidity, and a top Es predator (Wels catfish) were mostly present in these areas. Additionally, the most important WQ stressors of Ns were found to be COD, BOD and temperature that are all connected to oxygen depletion. The aforementioned conditions in the areas described by high priority for intervention can be used as a basis for the development of specific Ns conservation practices targeting the containment of the most harmful Es, the restoration of aquatic vegetation and the improvement of oxygen conditions.

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1. Introduction

The decline in native fish species (Ns) populations of freshwater systems have reached critical levels globally (IUCN, 2016). The most important causes are habitat degradation (flow modification, pollution) (Gehrke et al., 1995; Lee et al., 2016), invasions of exotic

fish species (Es) (Strayer, 2010; Trumpickas et al., 2011; Castaldelli et al., 2013a), overfishing (Allan et al., 2005; Aschonitis et al., 2017) and the interaction of these causes (Dudgeon et al., 2006; Light and Marchetti, 2007).

Habitat degradation due to pollution (Carpenter et al., 1998; Khun et al., 2012), destruction of aquatic vegetation, damming, river bed and flow modification (Nilsson et al., 2005; Elosegi and Sabater, 2013) have led to reduction or complete loss of both lateral and longitudinal connectivity of streams (Jansson et al., 2007). These conditions have also led to a consequent reduction

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Abbreviations*	
A1	Group of sites with no fish presence due to severe human impact
A2	Group of sites with no fish presence due to severe natural conditions
B	Group of sites only with exotic fish species present
C	Group of sites with both native and exotic fish species present
CCA	Canonical Correspondence Analysis
D	Group of sites only with native fish species present
DCA	Detrended Correspondence Analysis
EQD	Environmental Quality Degradation
Es	Exotic species
IDW	Inverse Distance Weighted interpolation method
Kr	Kriging interpolation method
<i>N</i>	Total number of sampling sites
Ns	Native species
<i>Nwq</i>	Number of WQ variables used in Eq. (1)
<i>Ne</i>	Number of Es variables used in Eq. (1)
RDA	Redundancy Analysis
<i>Pd</i>	Priority ranking of all Ns descriptors (i.e. both Es and WQ variables) for intervention in a specific site (integer ordinal values 1,2,3...) (the lower values denote higher priorities)
<i>Ps</i>	Priority ranking of Es variables for intervention considering all sites (integer ordinal values 1,2,3...) (the lower values denote higher priorities)
<i>Pt</i>	Priority ranking of sites for combined interventions on both Es and WQ variables (integer ordinal values 1,2,3...) (the lower values denote higher priorities)
SC	Severity class
<i>Se</i>	Severity index of sites based only on Es impact (higher values denote higher severity)
<i>Sd</i>	Severity index of an Ns descriptor for a specific site (higher values denote higher severity)
<i>Ss</i>	Severity index of an Es variable considering all sites (higher values denote higher severity)
<i>St</i>	Total or combined severity index of sites based on both Es and WQ impact (higher values denote higher severity)
<i>Swq</i>	Severity index of sites based only on WQ impact (higher values denote higher severity)
<i>W</i>	Weight of Ns descriptors (equivalent to $\lambda-1$ from the CCA analysis)
WQ	Water Quality
<i>i</i>	Sequence number of sampling sites
<i>j</i>	Sequence number of WQ variables
<i>k</i>	Sequence number of Es variables
<i>n</i>	Sequence number of all Ns descriptors (i.e. both Es and WQ variables)
$\lambda-A$	Conditional effects of Ns descriptors obtained by an ordination method (e.g. CCA or RDA)
$\lambda-1$	Marginal effects of Ns descriptors obtained by an ordination method (e.g. CCA or RDA)

of habitat availability for growth and reproduction of Ns (Castaldelli et al., 2013a). Conversely, it seems that these conditions may promote the success of many invasive Es (Bunn and Arthington, 2002; Didham et al., 2005; Leprieur et al., 2008; Alexander et al., 2014).

The increase of Es invasions is alarming (Leprieur et al., 2008; Marr et al., 2013) creating an important threat for freshwater ecosystems. Es can promote a) habitat deterioration/alteration through consumption of aquatic vegetation, b) increase of turbidity and nutrients release due to sediment resuspension that promote phytoplankton blooms and eutrophication, c) genetic alterations within populations, d) spreading of pathogens and parasites, e) competition with and replacement of native species (Crivelli, 1983, 1995; Crivelli and Maitland, 1995; Dibble and Kovalenko, 2009; Leprieur et al., 2009; Leunda, 2010; Badiou et al., 2011; Ribeiro and Leunda, 2012; Ilhéu et al., 2014).

Although, both environmental and biotic factors (e.g. exotic fishes) regulate Ns river communities (Godinho and Ferreira, 1998, 2000), the majority of survey studies consider only a group of explanatory variables (biotic or abiotic) or even a single factor for analyzing Ns responses (Meffe, 1984; Meffe and Sheldon, 1988; Harvey, 1987; Osborne and Wiley, 1992; Poff and Allan, 1995; Woodford et al., 2005; Pelicice and Agostinho, 2009; Alexandre et al., 2010; Pavlova and Rabadjiev, 2014). On the other hand, very few studies have explicitly related the response of Ns communities to both Es and environmental variables (e.g. water quality, aquatic vegetation, hydromorphological conditions, climate, topography, land uses of the surrounding environment) (Godinho and Ferreira, 1998, 2000; Moyle et al., 2003; Giannetto et al., 2012; Ilhéu et al., 2014; Carosi et al., 2015) analyzing the relative weight of Ns

descriptors using ordination methods such as Canonical Correspondence Analysis (CCA) or Generalized Linear Models (GLMs).

The interactions among Ns and Es in combination with the effects of abiotic or other biotic environmental variables are extremely complicated and their description face several problems due to high covariation of anthropogenic and natural gradients, existence of complex scale-dependent mechanisms, non-linear responses, difficulty in separating present-day from past influences (e.g. past hydrological changes due to human intervention) (Rinne and Stefferud, 1999; Leprieur et al., 2008). These problems lead to significant limitations for planning management measures for the recovery of Ns populations, which require a) as a first step, the identification of abiotic (e.g. water quality) and biotic (e.g. Es) variables related to Ns community degradation and the assessment of their relative weight as Ns descriptors, and b) as a second step, the development of hierarchical ranking of priorities for interventions for improving the conditions of native fish species. Until now, there are only generalized proposals (Thom et al., 2016; Nguyen et al., 2016), either site-specific, event-specific or species-specific proposals (Rieman et al., 2003; Cooke et al., 2009; King et al., 2010; Woodford et al., 2011) without providing a method for prioritizing interventions based on a decision support protocol for designing management plans for Ns recovery. It is indicative that an integrated method for decision support purposes that can assist Ns recovery plans for large scale applications considering both multi-species invasion and human impact is still missing (Didham et al., 2007; Britton et al., 2011).

The aim of the study is to propose an integrated method that provides priority rankings for intervention facilitating management plans for Ns recovery in highly impacted freshwater systems by Es invasions and human activities. The method is based on gradient analysis for assessing the weight of Ns descriptors' effects,

* Abbreviations in italics denote mathematical/statistical variables.

weighted severity indices related to environmental degradation and Es invasion, and classification of sites based on presence/absence of Es and Ns. The proposed method provides a) a ranking of the sites, which denotes the priority for combined intervention in space and can be visualized in maps, b) a ranking of the most important Ns descriptors for each site to perform site-specific interventions (site-specific priorities), and c) Es rankings based on their potential threat on Ns for species-specific interventions (species-specific priorities for the whole study area). The proposed method is applied in this study using as a case study the Emilia-Romagna Region (Northern Italy), which is described by extremely high rates of multi-species invasion and human interventions.

2. Materials and methods

2.1. Study area

The study area is located in Northern Italy and it is defined by the administrative boundaries of Emilia-Romagna Region with total coverage of 22,446 km² (Fig. 1a). It is naturally bounded north and south by the Po river and the Apennines Mountains (Fig. 1b), respectively, while the general direction of water flow in natural rivers and streams is towards north (Po river) and north-east to the Adriatic Sea (Fig. 1a). The study area is the south-eastern part of the large watershed of Po river.

Agricultural lands cover 55% of the territory, 26.3% is covered by forests with oaks (*Quercus* spp.) being the dominant species, urban areas cover 4.7% and mosaic grasslands/shrublands cover the rest 14% (Fig. 1c). Point source pollution at upland sites is limited to organic wastes originated from small urban settlements and livestock farms. The lowlands are characterized by a high degree of urbanization and intensive agriculture, with a dense network of artificial ditches regulating the drainage and flow conditions. In the lowlands, the intensive cropping of maize and wheat has led to fertilizer leaching, particularly nitrate to ground and superficial waters (Castaldelli et al., 2013b).

2.2. Field survey

Fish and water quality data from 208 river monitoring sites were collected in this study (Fig. 1a) covering a wide range of freshwater habitats at different altitudinal zones, different forms of land use and different eco-hydrological conditions (Fig. 1a–c). The samplings were performed in natural rivers (e.g. Po, Trebbia, Taro, Secchia, Panaro, Reno, Lamone, Fiumi Uniti, Bevano, Marecchia etc) and in large artificial drainage canals, which are mainly located in the lowlands (e.g. Po di Volano, Po di Primaro, Canal Bianco, Canale Circondariale etc). Details for each sampling position are given in Table S.1 of the supplementary material).

The sites were homogeneously positioned in 64 waterways of the region (Fig. 1a) with their section width ranging from 8 to 350 m (the maximum value corresponds to Po river). The samplings were conducted during the warm season (from April to September) of the period 1998–2004 as part of the institutional regional monitoring program for the compilation of the official Fish Inventory of the Emilia-Romagna Region (Gandolfi and Piccinini, 2002; AA.VV., 2005; Castaldelli and Rossi, 2008).

2.2.1. Fish samplings

Fish sampling was performed by electrofishing adapting the standard guidelines to the particular conditions of waterway typologies (Backiel and Welcomme, 1980; Reynolds, 1983). Electro-fishing was performed with a D.C. at 400–600 V and 4–5 A (Reynolds, 1983; Godinho and Ferreira, 2000) once during daylight,

in an upstream zigzag direction by wading, when depth was less than 1 m, and by boat in deeper waters. The transect lengths were equivalent to 10 times the river width ensuring that the range of present macrohabitats of each site was fully surveyed (Hankin and Reeves, 1988; Godinho and Ferreira, 2000). The duration of sampling was therefore quite variable ranging from half an hour to more than two hours, as in the case of the Po river. Electrofishing is considered the best quantitative method for fish sampling in shallow waters up to a maximum of 1 m (Zalewski and Cowx, 1990) but its efficacy may be low in deeper waters, with high conductivity, or in the presence of big and mobile specimens. Such special conditions occurred in almost all the lower stretches of rivers and in the canals of the lowlands. For this reason, electrofishing in these sites was followed by sampling using a standard set of nets, with the support of professional fishermen working in the same area. The obtained results were used to confirm or to complement the ones achieved using electrofishing (Backiel and Welcomme, 1980). Fish species were classified according to Berg (1932), Sterba (1962), Gandolfi et al. (1991) and Kottelat and Freyhof (2007). Overall, the fish fauna, which was identified in the hydrological network, consisted of 45 species; 25 native and 20 exotic; belonging to 12 families (Table 1). Common and scientific names used in this paper are from FishBase (<http://www.fishbase.org>). The number of native and exotic species per site are given in Table S.1 of the supplementary material. The abundance ranking of the species was expressed using the abundance index of Moyle and Nichols (1973) in accordance with Turin et al. (1999). This approach provides a class of abundance from 1 to 5 (0 value indicates absence) for each species in each sampling site (Table 1).

From the 25 observed native species, 17 are included in the Italian Red List of Threatened Species, 9 are included in the protected species list of Annex II of H.D.92/43/EEC, 2 in the respective list of Annex V of H.D.92/43/EEC and 7 in the respective list of Annex III of Berne Convention 1982 (Table 1). Additionally, 4 species (Italian red-eye roach, Italian bleak, Italian nase and Spined loach) are endemic species of the Northern Mediterranean region (Zerunian, 2003). Three species that existed in the past, the sea lamprey (*Petromyzon marinus* Linnaeus, 1758), the beluga sturgeon (*Huso huso* Linnaeus, 1758) and the common sturgeon (*Acipenser sturio* Linnaeus, 1758) are already considered extinct (Bronzi et al., 2011), while the endemic Adriatic sturgeon (*Acipenser naccarii* Bonaparte, 1836) was never caught during the sampling surveys of this study but it is probably present with few specimens, as reported sporadically by anglers. The generally critical conditions of Ns populations in the study area were highlighted quantitatively by the previous study of Castaldelli et al. (2013a), where comparative analysis for the period 1991–2009 showed that 10 native fish species faced local extinction while many exotic ones showed a population explosion in the lowland province of Ferrara (north-eastern part of the study area).

2.2.2. Water quality samplings

Monitoring of hydro-chemical data was performed from the Regional Environmental Protection Agency of Emilia Romagna (ARPA) at the same sites of fish samplings. 8 water quality parameters were derived for each sampling site (Table 2). Electrical conductivity, dissolved oxygen and water temperature were measured *in situ* using a handheld instrument Y.S.I. (Yellow Spring Instruments Inc.). The COD (Dichromate Reflux Method), BOD₅ at 20 °C, suspended solids, total phosphorus, ammonia and nitrate nitrogen were measured according to APHA (2005) (Table 2). Latitude, longitude and altitude were also recorded by GPS device for each sampling site (Table S.1).

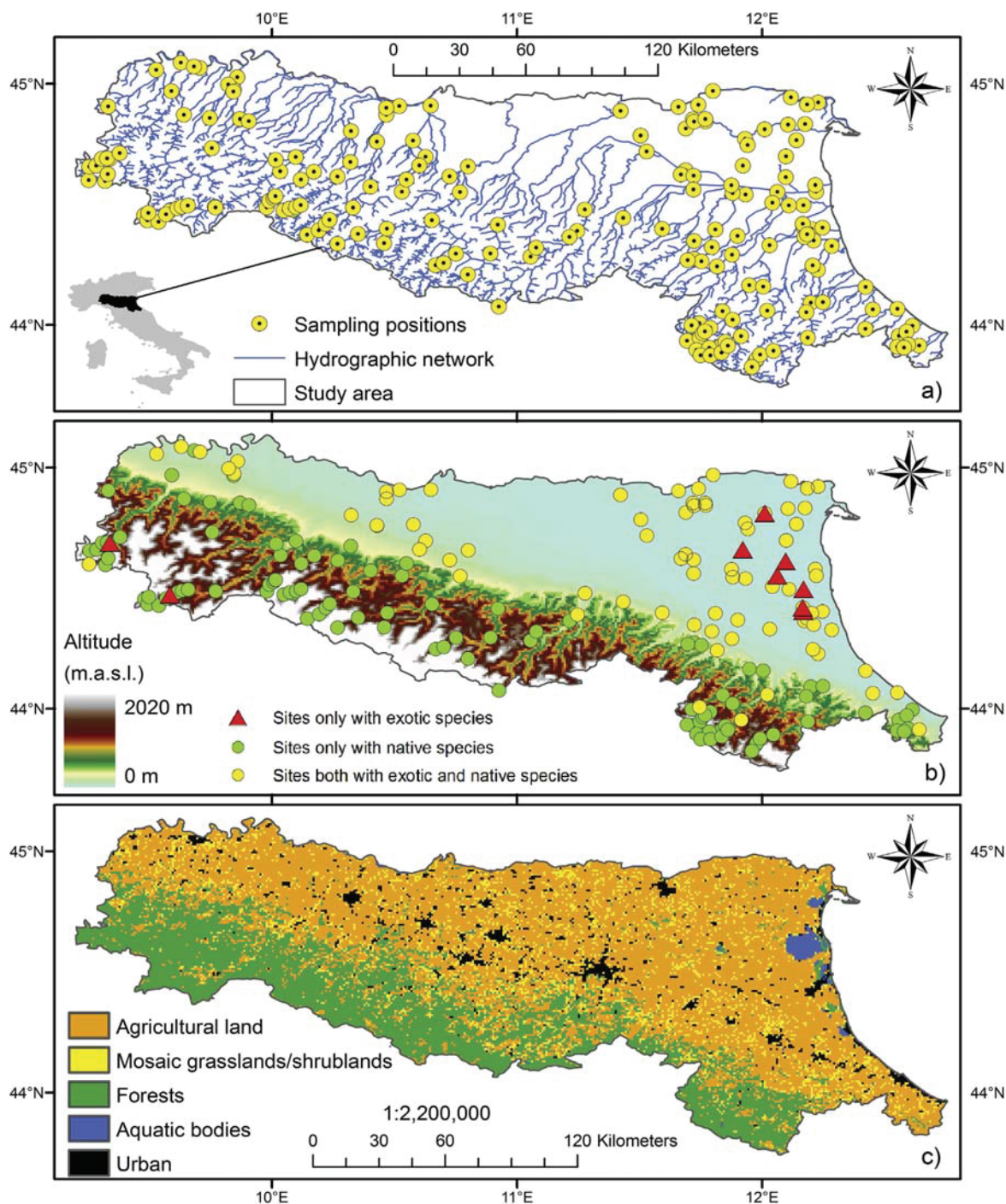


Fig. 1. a) Study area (source: <http://gadm.org>), hydrographic network of main rivers and streams (source: <http://www.eea.europa.eu/data-and-maps/data/european-river-catchments-1>) and sampling sites, b) altitude (source: <https://lta.cr.usgs.gov/GTOPO30>) and separation of sites based on the existence of native and exotic species and c) Main land uses (source: http://due.esrin.esa.int/page_globcover.php) of the study area.

2.3. Methodology for proposing priorities for intervention between sites for the recovery of native fish species

The method for proposing priorities of intervention between sites for the recovery of Ns takes into account the impact of water

quality degradation and exotic species invasion. The basic assumption of the method is that the increase of Es invasions and the increase of environmental quality degradation (EQD) have a combined negative impact on the whole Ns community. This assumption considers that the past attributes of Ns communities

Table 1
Observed fish species in the 208 freshwater sampling sites of Emilia Romagna region.

Family	Species	Common Name	S ^a	NP ^b	Max. Ab. Rank. ^c	IUCN ^d	H.D. 92/43/EEC ^e		Berne C. 1982 ^f
							An. II	An. V	An. III
Anguillidae	<i>Anguilla anguilla</i> (Linnaeus, 1758)	European eel	Ns	17	2	CR			
Clupeidae	<i>Alosa fallax</i> (Lacépède, 1803)	Twaite shad	Ns	8	3	VU	✓	✓	✓
Cyprinidae	<i>Sarmarutilus rubilio</i> (Bonaparte, 1837)	South European roach	Ns	23	5	NT	✓		✓
	<i>Leucis aul</i> (Bonaparte, 1841)	Italian red-eye roach	Ns	6	3	LC			
	<i>Squalius cephalus</i> (Linnaeus, 1758)	Chub	Ns	85	5				
	<i>Squalius lucumonis</i> (Bianco, 1982)	Toscana stream chub	Ns	2	3	CR	✓		
	<i>Telestes souffia</i> (Risso, 1827)	Vairone	Ns	55	5	LC	✓		✓
	<i>Phoxinus phoxinus</i> (Linnaeus, 1758)	Eurasian minnow	Ns	4	1	LC			
	<i>Tinca tinca</i> (Linnaeus, 1758)	Tench	Ns	1	1	LC			
	<i>Scardinius hesperidicus</i> (Linnaeus, 1758)	Italian Rudd	Ns	30	4	LC			
	<i>Alburnus arborella</i> (Bonaparte, 1841)	Italian bleak	Ns	81	5	NT			
	<i>Chondrostoma soetta</i> (Bonaparte, 1840)	Italian nase	Ns	2	1	EN	✓		✓
	<i>Protochondrostoma genei</i> (Linnaeus, 1758)	South European nase	Ns	47	5	EN	✓		✓
	<i>Gobio gobio</i> (Linnaeus, 1758)	Gudgeon	Ns	20	5	LC			
	<i>Barbus plebejus</i> (Bonaparte, 1839)	Barbel	Ns	76	5	VU	✓	✓	✓
	<i>Barbus tiberinus</i> (Bonaparte, 1839)	Tiber barbel	Ns	9	4	VU			
	<i>Barbus barbus</i> (Linnaeus, 1758)	European barbel	Es	3	1				
	<i>Carassius auratus/carassius</i> (Linnaeus, 1758)	Goldfish/Crucian carp	Es	71	5				
	<i>Cyprinus carpio</i> (Linnaeus, 1758)	Common carp	Es	82	4				
	<i>Abramis brama</i> (Linnaeus, 1758)	Common bream	Es	31	4				
	<i>Blicca bjoerkna</i> (Linnaeus, 1758)	White bream	Es	5	2				
	<i>Rutilus rutilus</i> (Linnaeus, 1758)	Roach	Es	1	1				
	<i>Rhodeus sericeus</i> (Pallas, 1776)	Bitterling	Es	27	5				
	<i>Pseudorasbora parva</i> (Temminck and Schlegel, 1846)	Stone moroko	Es	74	5				
	<i>Ctenopharyngodon idella</i> (Valenciennes, 1844)	Grass carp	Es	3	2				
	<i>Aspius aspius</i> (Linnaeus, 1758)	Asp	Es	6	2				
Cobitidae	<i>Misgurnus anguillicaudatus</i> (Cantor, 1842)	Pond loach	Es	1	1				
	<i>Cobitis taenia</i> (Linnaeus, 1758)	Spined loach	Ns	27	2		✓		✓
Siluridae	<i>Silurus glanis</i> (Linnaeus, 1758)	Wels catfish	Es	48	4				
Ictaluridae	<i>Ameiurus melas</i> (Rafinesque, 1820)	Black bullhead	Es	20	4				
	<i>Ictalurus punctatus</i> (Rafinesque, 1820)	Channel catfish	Es	4	3				
Esocidae	<i>Esox cisalpinus</i> (Bianco & Delmastro, 2011)	Southern pike	Ns	1	1				
Salmonidae	<i>Salmo (trutta) trutta</i> (Linnaeus, 1758)	Brown trout	Ns	70	5				
	<i>Oncorhynchus mykiss</i> (Walbaum, 1792)	Rainbow trout	Es	3	5				
Poeciliidae	<i>Gambusia holbrooki</i> (Girard, 1859)	Eastern mosquitofish	Es	6	2				
Cottidae	<i>Cottus gobio</i> (Linnaeus, 1758)	Bullhead	Ns	1	2	LC	✓		
Centrarchidae	<i>Micropterus salmoides</i> (Lacépède, 1803)	Largemouth black bass	Es	9	3				
	<i>Lepomis gibbosus</i> (Linnaeus, 1758)	Pumpkinseed	Es	26	4				
Percidae	<i>Perca fluviatilis</i> (Linnaeus, 1758)	European perch	Ns	3	1				
	<i>Gymnocephalus cernuus</i> (Linnaeus, 1758)	Ruffe	Es	10	3				
	<i>Sander lucioperca</i> (Linnaeus, 1758)	Zander or Pike-perch	Es	29	4				
Mugilidae	<i>Mugil cephalus</i> (Linnaeus, 1758)	Flathead grey mullet	Ns	1	1				
	<i>Liza ramada</i> (Risso, 1827)	Thinlip grey mullet	Ns	3	1				
Gobiidae	<i>Padogobius bonelli</i> (Bonaparte, 1846)	Padanian goby	Ns	43	5	LC			
Pleuronectidae	<i>Platichthys flesus</i> (Linnaeus, 1758)	European flounder	Ns	1	1				

^a S: Status where Ns is for native and Es is for exotic species.

^b NP: number of sampling sites where the species is present.

^c Max.Ab.Rank: Maximum observed abundance ranking based on Moyle Index.

^d IUCN: Species condition at national scale based on the Italian Red List of Threatened Species (<http://www.nationalredlist.org/search2/species-search>) where CR: critically endangered, EN: endangered, NT: near threatened, VU: vulnerable, LC: least concerned.

^e H.D.92/43/EEC: protected species included in Annex II and V of Habitats Directive 92/43/EEC.

^f Berne C. 1982: protected species included in Annex III of Berne Convention 1982.

Table 2
Abbreviations, units and statistics of environmental parameters considering all 208 sampling sites.

Parameter	Abbrev.	Unit	Transformation	Minimum	Maximum	Average	St.deviation	Group
Latitude (WGS84 ellipsoid)	Lat	Dec. degrees	log(x+1)	43.82	45.09	44.47	0.33	Geo
Longitude (WGS84 ellipsoid)	Long	Dec. degrees	log(x+1)	9.25	12.64	11.16	1.02	Geo
Altitude	Alt	m a.s.l	log(x+5)	−4	1137	245.4	281.2	Geo
Ammonia Nitrogen	NH4	N mg L ⁻¹	log(x+1)	0.01	6.35	0.35	0.77	WQ
Nitrate Nitrogen	NO3	N mg L ⁻¹	log(x+1)	0.06	9.87	1.29	1.3	WQ
BOD ₅	BOD ₅	O ₂ mg L ⁻¹	log(x+1)	0.5	18	3.13	2.46	WQ
COD	COD	O ₂ mg L ⁻¹	log(x+1)	1.5	56.33	12.27	9.56	WQ
Electrical conductivity	EC	dS m ⁻¹	log(x+1)	0.242	3.661	0.659	0.418	WQ
Total phosphorus	TP	P mg L ⁻¹	log(x+1)	0.01	1.68	0.16	0.24	WQ
Total suspended solids	TotSS	mg L ⁻¹	log(x+1)	0.5	785.17	36.83	67.45	WQ
Water temperature	Temp	°C	log(x+1)	12.5	24.73	18.06	3.25	WQ

were set based on a dynamic equilibrium among Ns that was regulated by the past local environmental conditions, when Es and EQD were almost null. Thus, any disturbance associated to Es invasions and/or EQD alters the equilibrium among Ns. It is worth mentioning that some Ns may show a positive correlation with Es presence and/or EQD. Even in this case, such positive effects on some Ns can have negative impact on the whole Ns community since an increase of a native predator or a native competitor can lead to reduction or complete loss of other Ns.

The steps of the proposed methodology are described in the following subsections and in Fig. 2.

2.3.1. Step 1: Multiple gradient analysis, ordination and variance partitioning – Canoco analysis

The data were used to form four groups of variables as follows: Group 1 (native fish species - Ns), Group 2 (exotic fish species – Es), Group 3 (geographical variables – Geo) and Group 4 (water quality parameters - WQ) (Tables 1 and 2). The data of abiotic environmental parameters (Table 2) were log-transformed before analysis to reduce normality departures (Feld and Hering, 2007; Aschonitis et al., 2016).

Based on the aforementioned groups of variables, the following procedures were performed using CANOCO 4.5 software (ter Braak and Smilauer, 2002):

- Detrended Correspondence Analysis (DCA) using only Ns data was initially performed in order to select the most appropriate response model (between linear or unimodal) for gradient analysis (ter Braak and Smilauer, 2002; Lepš and Šmilauer, 2003). According to Lepš and Šmilauer (2003) when the dominant gradient length in DCA is greater than 4 then a unimodal gradient method (Canonical Correspondence Analysis - CCA) while when is <3 a linear gradient method (Redundancy Analysis - RDA) should be selected. Based on this criterion, CCA (unimodal method) was finally selected to be used in this study.
- Two cases of CCA were performed using Ns group as dependent variables versus (a) all the descriptors of the remaining groups (Es, WQ, Geo) as independent variables (Full-CCA) and (b) versus one group of descriptors after partialling out the effects of the remaining ones, which were used as co-variables (Partial-CCA). CCA was performed for each possible combination of

targeted descriptors and co-variables based on species correlations and standardized species scores (ter Braak and Smilauer, 2002). Significant descriptors were identified using CANOCO's forward selection procedure and Monte Carlo permutation test (499 permutations) (Feld and Hering, 2007; Aschonitis et al., 2016). Collinear variables with a variance inflation factor $VIF > 8$ were excluded before the CCA analysis (Zuur et al., 2007). CCAs were performed using data only from sampling sites where Ns were present (CCA excluded automatically the data of 9 sites where all Ns variables had 0 values).

- A variance partitioning scheme (Borcard et al., 1992; Liu, 1997) was applied for each group of variables based on the overall variance explained by the Partial-CCA. This procedure allowed the distinction between unique effects (i.e. the variance explained by a single group of variables), joint effects (i.e. the variance jointly explained by variables of two or three groups), and unexplained variance. The proportion of variance explained by different groups of variables is expressed as the sum of all canonical eigenvalues of partial-CCA (or CCA) divided by the total inertia (Feld and Hering, 2007). Variance partitioning was also run with all variables to identify the marginal effects ($\lambda-1$) and the conditional effects ($\lambda-A$) of each descriptor variable. The marginal effect of a descriptor variable is equal to the eigenvalue of a partial CCA if the corresponding variable was the only environmental variable (additionally to the variance explained by co-variables). The conditional effect of an environmental variable is equal to the additional amount of variance in species assemblages explained by the corresponding variable at the time it was included into the model during a selection procedure (additionally to the variance explained by co-variables) (ter Braak and Smilauer, 2002; Titeux et al., 2004).

The Full-CCA and Partial-CCA analysis provide the following information:

- Full-CCA provides a list of significant Ns descriptors through the forward selection procedure but also their weighted effect (W), which corresponds to the estimated value of marginal effect $\lambda-1$. The significant descriptors and their weights are used in the next steps.

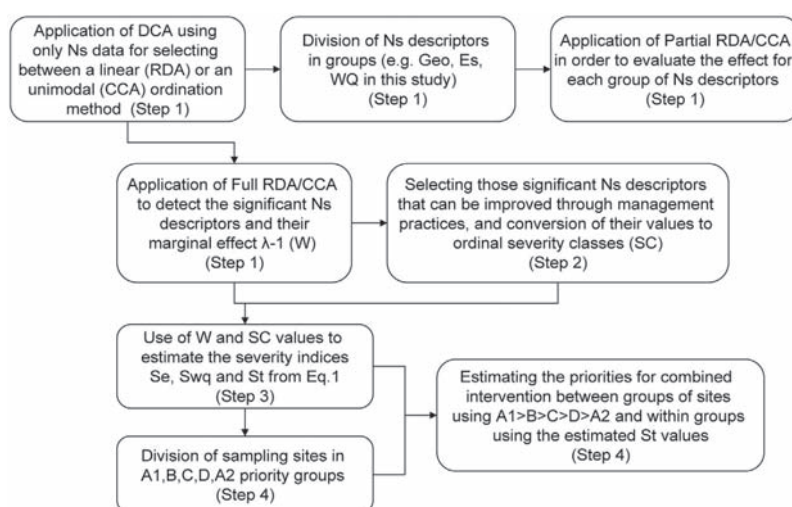


Fig. 2. General diagram of the methodological steps for assessing priorities for combined intervention.

- Partial CCA provides information about the conditional and total joint effects for each group of descriptors (Es, WQ and Geo). The results of this analysis are only used to evaluate the significance of each group and to assist the development of generalized priorities for intervention without considering the next steps. For example, if Es group of variables shows higher explanatory power from WQ group, indicates that interventions on Es is of higher priority.

2.3.2. Step 2: Development of severity classes for Ns descriptors

From the list of significant Ns descriptors indicated by the Full-CCA, are selected those which are associated to environmental-biological quality (e.g. descriptors belonging to WQ and Es) and can be restored/modified by conservation practices. For example, geographic parameters like altitude, latitude or longitude may be significant descriptors but they cannot be modified and for this reason are not used in step 2, while they are used in CCA (step 1) because they can provide more robust and higher purity weights (W) for the other descriptor variables. The data values of the final selected descriptors are classified in severity classes. Five severity classes with values SC ranging from 0 to 4 were used in this study for the selected Ns descriptors belonging to Es and WQ groups (value 0 corresponds to lower severity and 4 to higher severity). For the descriptors belonging to WQ group, SCs were formed based on water quality thresholds obtained by the literature (Rhoades et al., 1992; WHO, 2003; EAP Task Force/OECD, 2007). EAP Task Force/OECD (2007) (Table 14 in the cited report) is an extremely valuable source of severity classification for a wide range of freshwater pollutants including common pollutants such as those belonging to WQ group (Table 2) but also heavy/trace metals, bacteriological parameters, pesticides, insecticides, industrial, urban and agricultural wastes. For the descriptors belonging to Es group, SCs were formed based on the abundance ranking categories of Moyle.

2.3.3. Step 3: Assessing severity indices

The following severity indices Swq , Se and St are estimated taking into account the weight W values from step 1 and the SC values from step 2 of the final selected parameters:

$$St_i = Swq_i + Se_i = \sum_{j=1}^{Nwq} (W_j \cdot SC_{j,i}) + \sum_{k=1}^{Ne} (W_k \cdot SC_{k,i}) \quad (1)$$

where St : is the total severity index estimated by the final selected WQ and Es descriptor parameters, Swq : is the severity index based only on WQ parameters, Se : is the severity index based only on Es, Nwq : is the total number of selected WQ parameters, Ne : is the total number of selected Es, i : is the sequence number of sampling site, j : is the sequence number of WQ descriptor variable and k : is the sequence number of Es descriptor variable. The higher values of St , Swq and Se indicate higher severities. The St provides an integrated aspect for the combined severity related to water quality and exotic species while Swq and Se can be used for assessing the respective aforementioned two severities, separately.

To support the discussion of the results, spearman correlations were also used to evaluate the relationship between Swq , Se , St or other factors (e.g. altitude) that do not participate in the calculation of Eq. (1).

2.3.4. Step 4: Assessing the final priorities for intervention

In many cases, the gradient analysis may neglect significant existing information. For example, the statistical validity of CCA/RDA depends on the removal of many collinear parameters from the descriptors' group. Additionally, some Ns may also be neglected

due to their rare presence while sites, which have only Es, are also excluded from the gradient analysis. This is a very important loss of information especially in the case when there are sites where Ns presence is null due to extreme Es invasion. These sites may be erroneously described by lower values of severity indices compared to sites that host both Ns and Es. Additionally, some sites may present total absence of fish species (neither Ns nor Es) because one water quality parameter or an undetermined environmental parameter is of extreme severity. These latter sites do not participate in gradient analysis and would also present low values of severity indices, which is not valid from a management point of view.

In order to avoid the aforementioned problems, the complete set of sites is classified in the following main groups: sites where no fish species are present (group A), sites where only Es are present (group B), sites where both Es and Ns are present (group C) and sites where only Ns are present (group D). The complete absence of fish species in group A may be due to the high severity of environmental parameters related to human or Es impact, or due to extreme natural conditions (e.g. non reachable or very cold upstream areas of high altitude etc). For this reason group A is further divided in two subgroups A1 (absence of fishes due to human or Es impact) and A2 (absence of fishes due to natural factors) (this division can be performed through expert judgment if data to support the division do not exist).

Taking into account the aforementioned major groups of sites, the priorities for combined intervention Pt are adjusted according to the following priority order $A1 > B > C > D > A2$ where the respective ranking of sites within each group is adjusted after comparing the already determined St severity index from the higher to the lower value. All sampling sites are ordered in sequence ranking using integer values ($Pt = 1, 2, \dots, n$) denoting the priority of sites for combined intervention on both WQ and Es (1 is of the higher priority).

2.3.5. Mapping Swq , Se , St and Pt

The values of Swq , Se , St and Pt can be mapped using Geographic Information Systems. The aforementioned parameters were elaborated in ESRI Arc-GIS environment using two different spatial interpolation techniques, Kriging (Kr) and Inverse Distance Weighted (IDW). The Kr method reduces the local effect of a site in order to provide a more generalized zonation of severity and priority classes based on the combined effects with its neighbor sites while the IDW method increases the local effect of each site. Both interpolation techniques distort at some degree the absolute values of Swq , Se , St and Pt at the sampling sites but they can provide significant information about territories with no observations. Thus, the interventions can be site-specific only for the 208 sites of the case study based on the absolute values Swq , Se , St and Pt , or zone-specific based on the produced maps.

3. Results

3.1. Step 1: CCA analysis

Full-CCA and partial CCA analysis were performed using Ns as dependent variables and Geo, WQ and Es parameters as descriptor variables for the dataset of 199 sites where Ns were present (the 9 sites where Ns were absent were automatically excluded by Canoco).

Full-CCA showed that the overall proportion of variance explained by all Ns descriptors was 42.7%. The marginal ($\lambda-1$) and conditional ($\lambda-A$) effects of Ns descriptors are given in Fig. 3a. The $\lambda-1$ marginal effects of selected Ns descriptors (Fig. 3a) are going to be used as weights (W) in Eq. (1) (analysis of Step 3).

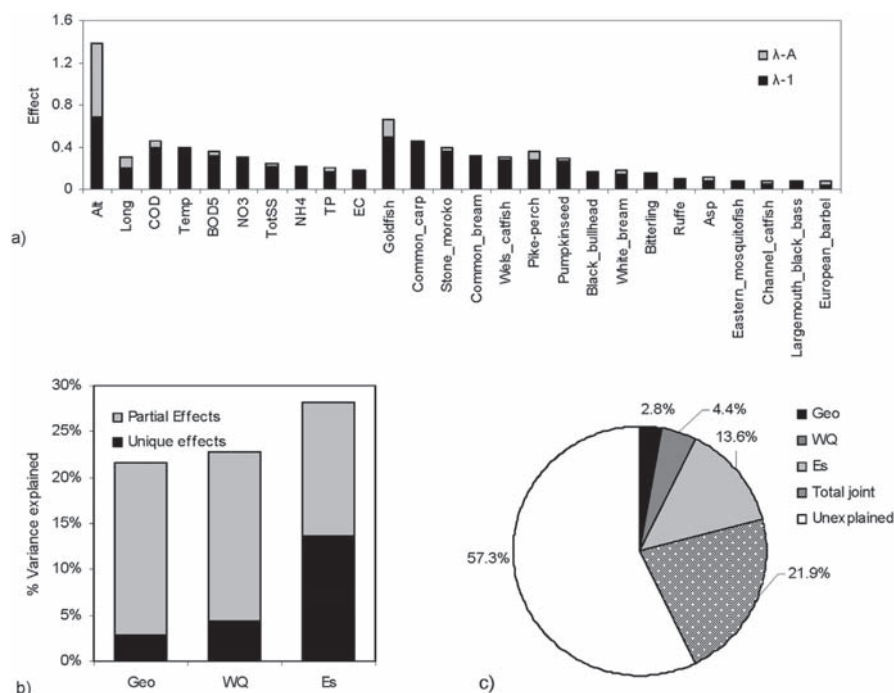


Fig. 3. a) Marginal ($\lambda-1$) and conditional ($\lambda-A$) effects of each Ns descriptor from the full CCA of Ns versus Geo, WQ and Es variables, b) Unique and partial joint effects for each one of the three groups of variables (Geo, WQ and Es) after partitioning Ns species variance, and c) Unique and total joint effects of the three groups of variables (Geo, WQ and Es) based on partitioning Ns species variance.

The use of partial-CCA showed that the unique effect of the three groups of Ns descriptors followed the order Es > WQ > Geo (Fig. 3b and c).

3.2. Step 2: Creating severity classes of the final selected Ns descriptors

After the application of Full-CCA and the estimation of $\lambda-1$ weights (W) (Fig. 3a), the most significant Ns descriptors, which can be improved through management practices, were selected for analyzing the severity indices using Eq. (1). The 8 water quality parameters and the 16 exotic species, which appear in Fig. 3a, were

selected (Altitude and longitude parameters were excluded). The 24 Ns descriptors belonging to WQ and Es groups were classified in five severity classes SC based on the classes given in Table 3. The five severity classes for COD, BOD₅, NO₃, NH₄, TP and Temp were based on the classes provided by EAP Task Force/OECD (2007). The EC severity classes were based on Rhoades et al. (1992) while the respective classes for TotSS on WHO (2003). The severity classes of exotic species were created after re-adjusting the abundance ranking categories of Moyle. The five severity classes of the final selected Ns descriptors get values SC from 0 to 4 (Table 3).

3.3. Step 3: Assessing the Swq, Se and St severity indices

The Swq, Se and St (Eq. (1)) for each sampling site was estimated taking into account the weights W (equivalent to $\lambda-1$) (step 1, Fig. 3a) and the SC values (step 2, Table 3) of the 24 Ns descriptors. The values of severity indices for each site are given in Table S.2a of the supplementary material.

The spatial interpolation of Swq, Se and St severity indices using the IDW and Kr techniques are given in Fig. 4. The interpolated values of Swq (Fig. 4a), Se (Fig. 4c) and St (Fig. 4e) using IDW were classified in 8 classes using quantile ranges. The same ranges were used for the respective classification of interpolated values using the Kr technique (Fig. 4b,d,f) because the IDW quantile ranges represent better the Swq, Se and St values of the sampling sites. Additionally, using the same ranges in the classification of IDW and Kr results better highlight the spatial differences of the two techniques. The visual comparison between Figs. 4 and 1b showed that the severity indices present similar spatial patterns with altitude (altitude was the most significant descriptor in CCA but it did not participate in the calculation of Eq. (1)). For this reason, Spearman

Table 3
Severity classes of the final selected descriptor variables of Ns.

Parameter	Unit	Severity class (SC)				
		I	II	III	IV	V
^a COD	O ₂ mg L ⁻¹	<3	3–7	7–15	15–20	≥20
^b BOD ₅	O ₂ mg L ⁻¹	<3	3–5	5–6	6–7	≥7
^c NO ₃	N mg L ⁻¹	<1	1–3	3–5.6	5.6–11.3	≥11.3
^d NH ₄	N mg L ⁻¹	<0.2	0.2–0.4	0.4–0.8	0.8–3.1	≥3.1
^e TP	P mg L ⁻¹	<0.1	0.1–0.2	0.2–0.4	0.4–1	≥1
^f Temp	°C	<18	18–20	20–22	22–24	≥24
^g TotSS	mg L ⁻¹	<300	300–600	600–900	900–1200	≥1200
^h EC	dS m ⁻¹	<0.7	0.7–2	2–10	10–25	≥25
ⁱ Exotic sp.	Moyle	0	1	2	3	≥4
Value given to each SC		0	1	2	3	4

^a EAP Task Force/OECD (2007) <http://www.oecd.org/env/outreach/38120922.pdf>.

^b Rhoades et al. (1992) <http://www.fao.org/docrep/t0667e/t0667e05.htm>.

^c WHO (2003) http://www.who.int/water_sanitation_health/dwq/chemicals/tds.pdf.

^d Effective for all exotic species.

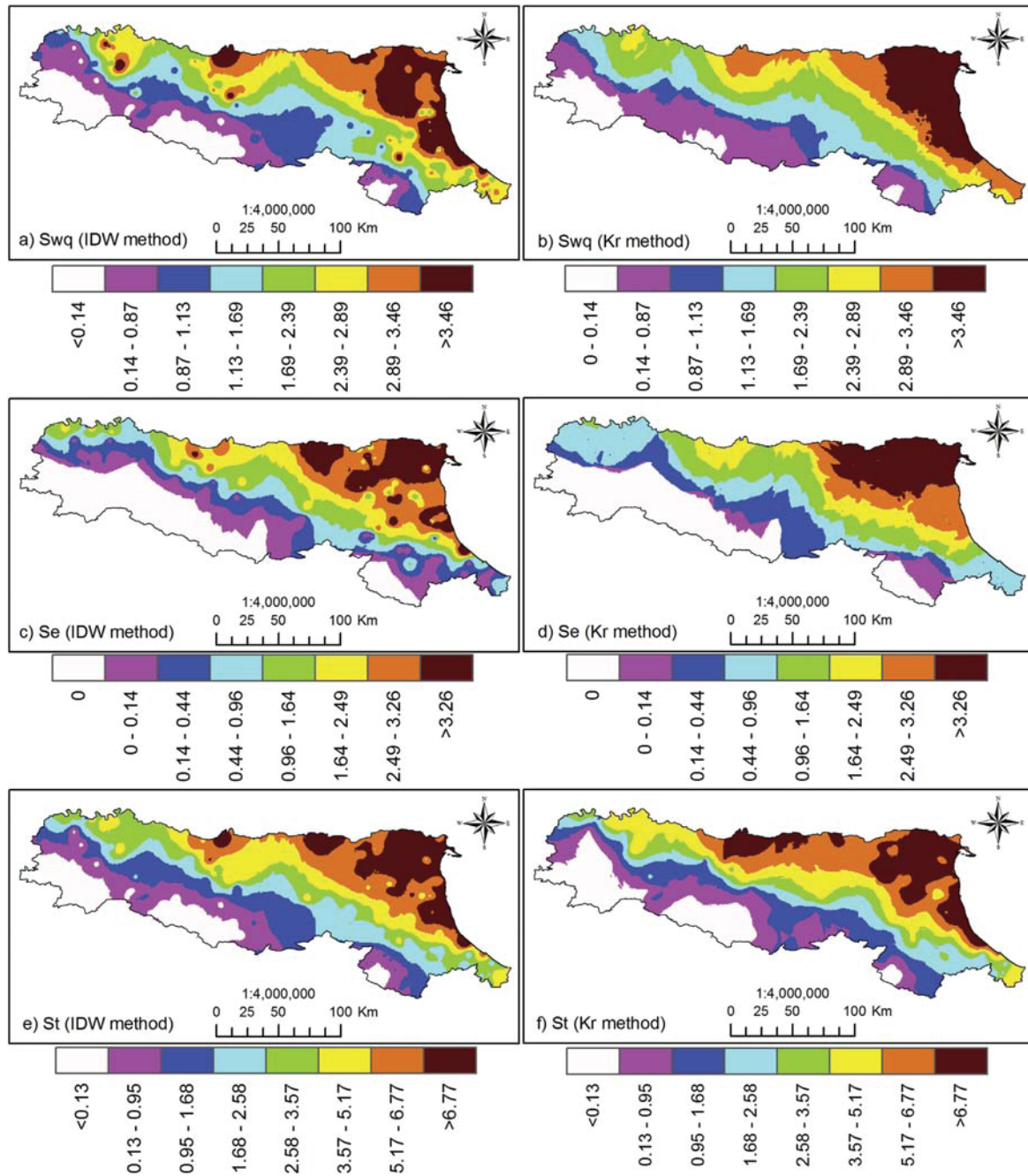


Fig. 4. Spatial interpolation of a) Swq using IDW technique, b) Swq using Kr technique, c) Se using IDW technique, d) Se using Kr technique, e) St using IDW technique, f) St using Kr technique.

Table 4
Spearman correlations Swq, Se, St and altitude values of the sampling sites.

	Swq	Se	St	Alt
Swq	1			
Se	0.750**	1		
St	0.951**	0.889**	1	
Alt	-0.852**	-0.854**	-0.904**	1

**p < .01.

correlations between Swq, Se, St and altitude were also performed and they are given in Table 4.

Taking into account the results of Table 4 and Fig. 4a–f, the following were observed:

- The patterns of Swq, Se and St show a negative correlation with the altitudinal gradient, suggesting that altitude is a surrogate of human and Es impact.

- The fact that *Swq* and *Se* are significantly correlated, indicates that the priorities for intervention on water quality and exotic species should be followed in parallel since their severities follow the same spatial distribution.
- The most impacted zones in terms of water quality and exotic species are those of north and especially the north-east territories (Ferrara province) that present the higher interconnection between the Po river (upper boundary of the study area), tributary rivers, irrigation and drainage channels. The Po river is considered among the most impacted European water bodies by exotic species, which acts as a pool for exotic species (Castaldelli et al., 2013a).
- The comparison between the IDW and Kr showed that the first one provided a better description of the severity indices on the sampling sites while the second one provided a more clear territorial zonation of severities that are attributed to specific local properties. For example, the *Se* severity map obtained by Kr technique (Fig. 4d), shows that the highest severity class (brown color in Fig. 4d) is mostly present inside the Ferrara province boundaries, which are defined by the Po and Reno rivers at the north and south, respectively. The Reno river, which is at the south boundaries of the highest severity *Se* class, may act as obstacle for intensive exotic species invasion in the most south-eastern areas. Of course, Reno river indirectly receives water from Po river through some channels, which are activated to preserve water flow when Reno flow drops below critical limits and for this reason invasions in these territories are inevitable but at lower rates.

Even though the spatial distribution of severity indices is generally satisfactory from an expert judgment point of view, some inconsistencies were observed. For example:

- Sites with no Ns presence and many Es belonging to B group had lower values in severity indices from many sites that had both Ns and Es (Table S.2a).
- Two sampling sites located at the south-west part of the study area at ~597 and ~365 m altitude (Fig. 1b, No. sites 173 and 201 in Table S.1 and S.2a) with no Ns presence and only one exotic (rainbow trout) showed *Se* = 0 (Table S.2a) because the rainbow trout was not a significant descriptor in CCA but also because these two sites were not included in the CCA due to Ns absence.

The aforementioned inconsistencies of severity indices are solved after adjusting the priorities using the major priority groups (Step 4).

3.4. Step 4: Assessing sites' priorities for intervention based on severity indices and major priority groups

The division of sampling sites based on the presence/absence of Ns and Es showed that there are 9 sites only with Es (B group), 92 sites with both Ns and Es (C group) and 107 sites only with Ns presence (D group) (Fig. 1b). In this study, sites with no fish species in the impacted lowlands (case of A1 group) were not observed while respective sites of extreme natural conditions in the upland regions (case of A2 group) were not included in the surveys.

Taking into account the aforementioned major groups of sites, the priorities for combined intervention on both WQ and Es are adjusted according to the following priority order $B > C > D$ where the respective priority ranking of sites within each group was adjusted by the *St* severity index. For sites of the same priority group with the same *St* values, the sites with the higher *Se* values are of higher priority (due to the higher significance of Es compared to WQ descriptors, Fig. 3b and c). Respectively, for sites with the same *Se*, *Swq* and *St*, the priority is higher for the sites of the lower

altitude since altitude is an indirect indicator of severities (altitude shows significant correlations with the *Se*, *Swq* and *St*, Table 4). The final priority rankings for combined intervention *Pt* are given in Table S.2b of the supplementary material.

In order to visualize the priorities for intervention in space, the *Pt* values were interpolated using the IDW and Kr techniques and classified using equal intervals (Fig. 5a and b). As it can be seen from Fig. 5a and b, the patterns of *Pt* values are slightly different from the patterns of *St* severity index (Fig. 4e and f), especially at the southwest locations, since the corrected *Pt* based on the priority groups B,C,D reduced the effects of CCA omissions related to a) sites with no Ns and b) Es that were not included in the CCA.

4. Discussion

4.1. Combined versus one-type interventions

The proposed methodology for assessing priorities for intervention targets more on the promotion of combined interventions on multiple stressors of native species (e.g. both WQ and Es stressors) rather than one-type interventions (e.g. only on WQ or only on Es), because there are serious indications of the potential failure of the latter ones. It is important to note that any planned intervention to achieve Ns recovery should give special attention to habitat restoration since a poor medium "water" flowing in a good matrix is probably an insufficient precondition for recovery (Feld et al., 2011). Conversely, improving only WQ parameters may lead to a further proliferation of Es. On the other hand, targeting only at Es, the recovery of Ns may be undermined by insufficient WQ conditions. Examples, which highlight that good WQ conditions are not enough to support the preservation of Ns are the sites No.173 and 201 (Table S.2a, their *Swq* is extremely low) where a top predator fish (rainbow trout) has completely dominated without other species present (only this species was captured in these sites). It has to be noted that the presence of rainbow trout in these territories is supported by continuous introduction by local fishermen (this is not the case for the other species of the study area). Failure of one-type interventions can also occur due to inconsistent evaluation of descriptors effects due to the fact that intensive invasions of Es in freshwaters increase the statistical uncertainty in purity of other environmental gradients related to human impact and vice versa. This is verified by the extremely high partial effects and the extremely high total joint effect of the descriptor groups given in Fig. 3b and c, respectively. A similar observation was also made by Leprieur et al. (2008), who found that human activity indicators accounted for most of the variation in richness of non-native species at global scale. This indicates that descriptors of human impact and Es go together and thus combined interventions are required. Another finding, which indicates that Es invasions manipulate other environmental gradients effect, is that altitude was the most important descriptor of Ns in the CCA with extremely high both marginal and conditional effects (Fig. 3a). The manipulation of altitude gradient by Es can also be verified by historical records (Bianco, 1995, 1998; Lanzoni et al., 2010; Castaldelli et al., 2013a) where many Ns, which were very abundant in the lower altitude areas of Ferrara province (north-eastern territory of the study area), declined dramatically or completely disappeared, while now they are only present in upstream territories (Castaldelli et al., 2013a).

4.2. Use of the results to determine site-specific and species-specific priorities for intervention

A basic advantage of the proposed method is that it provides additional information to support priority rankings for site-specific or species-specific interventions.

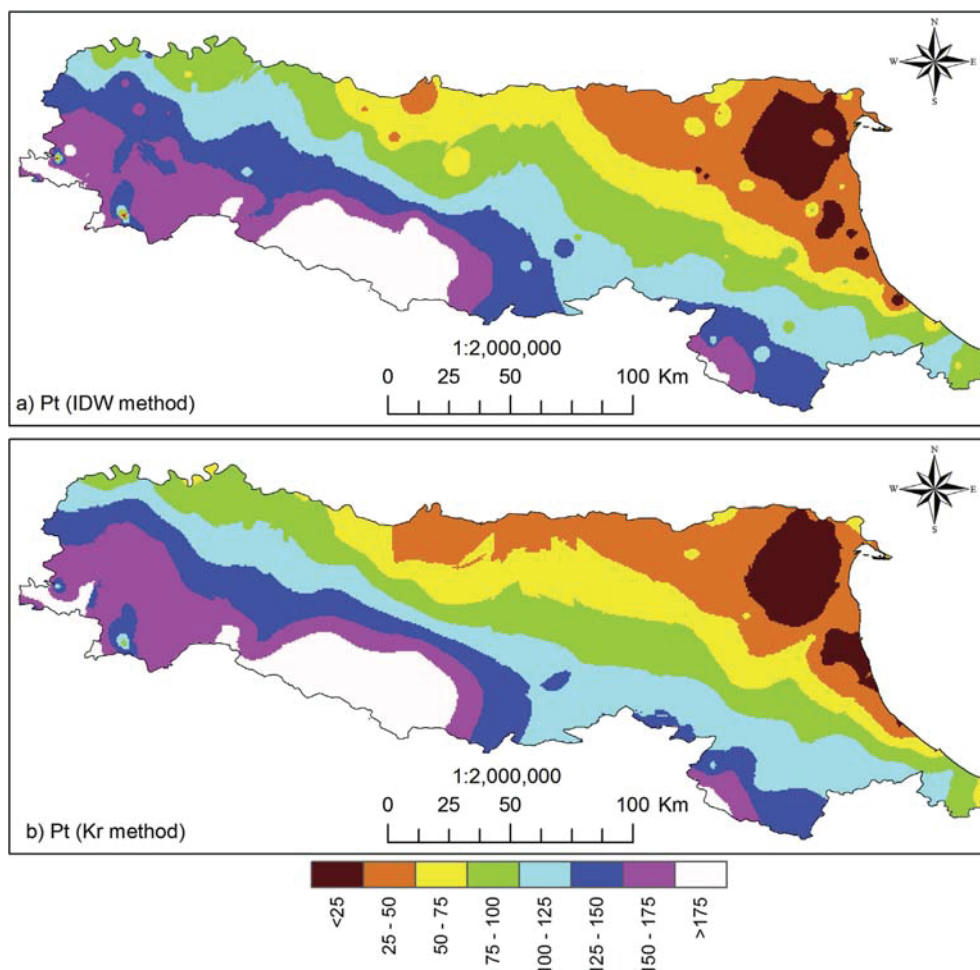


Fig. 5. Spatial interpolation of a) Pt using IDW technique, b) Pt using Kr technique.

For site-specific interventions, the associated severities (SC) and respective weights (W) of each Ns descriptor belonging either to Es or WQ group can provide an internal priority ranking of the descriptors for a specific site according to the following equation:

$$Sd_{i,n} = W_n \cdot SC_{i,n} \quad (2)$$

where $Sd_{i,n}$: is the local severity of a Ns descriptor n for the site i . For example, the larger the value $Sd_{i,n}$ of a descriptor n is in comparison to other descriptors of the site i , the higher is the ranking priority for intervention on this descriptor (Pd). An example of site-specific descriptor priorities Pd is given in Table S.3 of the Supplementary material for the site No.56, which was ranked first according to Pt (Table S.2b, supplementary material). In the case of No.56 site, the site-specific priorities for intervention followed the order: Goldfish > COD > Stone Morocco > BOD₅ > Temp > Common Carp > NH₄ > TP > NO₃ > EC > TotSS.

Similarly, ranking priorities for species-specific interventions on Es can also be performed for each k exotic fish considering all N sites according to the following:

$$Ss_k = \sum_{i=1}^N (W_k \cdot SC_{i,k}) \quad (3)$$

where Ss_k : is the total severity of an Es descriptor considering all N sites. Thus the higher the value of Ss_k of an Es is compared to others, the higher is the priority Ps for interventions on this exotic fish in the whole study area. In the case where there are sites only with Es species and sites with both Es and Ns (existence of both B and C type groups of sites), it is recommended to rank first the Es that were present in the B group and then the remaining Es . Es that were not included in the CCA and hence do not have a weight $\lambda-1$ value, they are ranked last depending on the group of sites (i.e. B,C,D) at which they were present. An example of species-specific priorities Ps based on all sites of this study is given in Table S.4 (supplementary material), where the Ps for intervention on Es showed the following order: Goldfish > Common Carp > Stone Morocco > Wels catfish > Common bream > Pumpkinseed > Zander > Bitterling > Black bullhead > Ruffe > White bream > Eastern mosquitofish > Rainbow trout > Grass carp > Largemouth black bass > Asp > Channel catfish > European barbel > Roach \approx Pond loach.

4.3. Adaptation of the proposed method for including other types of severity or other types of analysis

Another significant issue for discussion is the flexibility of the proposed method to be adapted in different environments or different datasets. For example, the method allows the inclusion of other abiotic descriptors (apart from WQ) affecting Ns such as hydromorphology/hydraulic conditions. For example, damming and other hydraulic parameters (e.g. flow, substrate conditions etc) can be included as an additional descriptor group to denote hydromorphological severity in Eq. (1).

The proposed method can also support the implementation of Water Framework Directive that requires actions not only for the improvement of freshwater quality but also of fish communities (as part of the biological quality elements). Towards this purpose, the proposed method can be further adapted combining sensitive aquatic organisms to pollution (e.g. specific fish, benthic, plant species etc) as dependent variables in order to assess the weight of water pollution indicators and to build new and more integrated indices for assessing the biological quality of surface waters. It is worth mentioning that despite the efforts of many scientists, does not still exist a standard procedure of general use for assessing biological quality of surface waters due to the high uncertainties introduced by the high variability in environmental conditions and biological quality elements (http://ec.europa.eu/environment/water/water-framework/info/intro_en.htm) (Pauls et al., 2002; Hering et al., 2010; Arle et al., 2016).

4.4. Limitations of the proposed method

The basic limitations of the proposed method are the following:

- The method requires extremely high sampling effort (data for many biotic and abiotic parameters from many sites) in order to provide robust weights of the parameters through CCA analysis.
- The weights of the parameters captured by the CCA describe the specific conditions (effects of WQ and Es on Ns) of the sampling period. It is well known that Es alter the evolutionary pathway of Ns by competitive exclusion, niche displacement, hybridization, introgression, predation, and ultimately extinction. Es evolve in response to their interactions with Ns, as well as in response to the new abiotic environment (Mooney and Cleland, 2001). Thus, any future assessment of priorities for intervention requires repetition of samplings for re-assessing the Es and Ns populations and water quality conditions.
- The proposed method cannot be generalized using data from sampling sites belonging to aquatic systems of different regions with no interconnection of fish populations. Sampling sites of different regions may show significant differences of Ns and Es populations but also different relationships between biotic and abiotic factors in general.

4.5. Use of priorities for intervention to support management plans in the study area

The results of the study provided significant information about the expansion of Es in the study area. It is indicative that some Es have reached high spatial distribution (e.g. Goldfish, Common carp, Stone Moroco, Wels catfish) (Table 1) several years after their introduction (an example case is the Wels catfish that was first reported in the middle course of Po during 1972) (Castaldelli et al., 2013a).

It is essential that management measures should be taken to stimulate the recovery of Ns populations. Measures for eradicating

Es in other regions have been put into practice with success in lakes and ponds (Britton et al., 2008; Tsunoda et al., 2010). However, in this study, the Es invasion is extremely expanded over a wide geographic area with large overall biomass, which is continuously supported by recolonization from the Po river. For this reason, eradication is unfeasible. For such conditions, Britton et al. (2011) suggested that containment measures could be the most promising strategy to manage invasions. In the study area, a plan for containment of Wels catfish through fishing began several years ago with the application of legislative Acts (Regional Law N. 11 of 22 February 1993, Directive N. 1574 of the Regional Council of 3 July 1996). This measure was insufficient and the main deficiency appeared to be the spatial and temporal discontinuity of intervention in the extensive canals network and the large stock of Wels catfish, which was always recovering rapidly (Castaldelli et al., 2008). Additionally, the increase of fishing effort on such a large scale is economically unsustainable for public institutions. The failure of local measures was also verified by Castaldelli et al. (2013a) who provided additional data from the year 2009 for Ferrara province (upper right most impacted area according to Figs. 4 and 5 based on the data of 1998–2004), where it was observed that the conditions of Ns further worsened during 2004–2009.

An alternative and more economically sustainable strategy would be a controlled program of commercial and recreational fishing with the support of both local fishermen and public institutions taking into account the priorities for intervention provided by this study. The overall concept can be based on the following: a) use of *Pt* (Fig. 5) to organize intensive professional or recreational fishing activities on *Pt* hotspots controlled by public administrations targeting only on Es, b) indirect or partial compensation (e.g. tax reliefs of professional fishermen, or economical prizes supported by environmental programs or private funds) considering the weight of catches and the captured species, providing higher compensations for the species of higher priority (priority order of species-specific interventions provided in section 4.2 and Table S.4), c) direct compensation of fishermen through the supply of the captured fishes to the local fish-market, which has already established trading routes to countries of eastern Europe where such species have a higher cultural and economic value in the local tradition and cuisine (this strategy has already been activated and has recently been reinforced by the increase in wels catfish demand and wholesale price on the eastern European markets; Castaldelli et al., 2013a), d) parallel actions of habitat restoration together with fishing activities taking into account as a guide the priorities of site-specific interventions such as those given for the example case of No.56 (section 4.2 and Table S.3). It has to be mentioned that the successful implementation of such proposed programs require the involvement of fishermen that are aware about the threats of Es and are fully collaborative with the guidelines provided by the public institutions. An example of failure due to unsuccessful collaboration is the program of commercial fishing, which started in the region in 2014. This program was difficult to be performed on a regular basis due to the violent actions of some local anglers that defend Es, particularly carp and wels catfish, because they are fanatic supporters of the “catch and release” discipline. This unexpected occurrence forced the institutions to stop the program.

As regards habitat restoration, the most effective short-term action is the restoration of aquatic vegetation, which can be achieved through the containment of grass carp, common carp and goldfish. In the longer term, modification of canals morphology to decrease the embankment slope would allow further recovery of emergent vegetation. It is also recommended the maintenance of a constant water level in all branches of the canal network by supplying water through Po river, especially during the months of non

irrigation season (from mid-September to mid-March), when the water depth in the canals is low and the water supply for irrigation or other purposes from the Po river is of low demand. Canals present suitable conditions for Es containment since their upstream and downstream stretches are confined between two hydraulic infrastructures that regulate the flow and act as obstacles to immediate re-invasion. A case of containment and habitat restoration in the study region was performed during 2011–2012 in the stretch of the Fossa Lavezzola close to Berra city centre (Ferrara province), which led to a complete recovery of aquatic vegetation and substantial turbidity reduction (Castaldelli et al., 2012).

Finally, it is crucial to promote the awareness of the public about the ecological problems associated to Es invasions through seminars and public discussions. Apart from the obvious cases of urban pollution, citizens, NGOs and environmental administrations consider that agricultural activities is the main factor for the low water quality (including also the biological water quality) of the nearby water bodies. For this reason, high cost management actions are usually planned targeting agricultural activities. Such plans may not reach the expected target since they do not consider the underlying ecological problems associated to specific Es. The best example is the case of the common carp, which is the Es with the highest NP (number of sampling sites where the species is present) in the study area (Table 1). This species may be almost fully responsible for the loss of aquatic vegetation, low nutrient retention, increase of turbidity and biodiversity reduction in the sites where it presents high abundance (Hicks et al., 2012). Goldfish, grass carp and other Es of the study area also present similar behavior. Diverting the public discussions in order to stress more the effects of Es on water quality (without neglecting their effects on native fish populations), the proposals of management plans for Es may receive higher acceptance and funding because water quality issues are more attractive to public attention and to environmental/political authorities. Such actions may also help to overcome problems associated to groups who defend Es.

5. Conclusions

This study provided a new integrated method to develop priority rankings for combined interventions for the recovery of Ns communities in freshwater habitats, which are threaten by human impact and Es invasions. The method is able to provide spatially explicit priorities in order to target specific locations for restoration but it can also provide site-specific priorities (priorities among Ns drivers per site) and species-specific priorities (priorities among Es). These attributes allow the method to be used as a valuable decision support tool for the designation of management and restoration practices optimizing their efficacy at site and basin scale.

The application of the proposed method for Emilia-Romagna region showed that the Ns populations of the north and north-western lowland areas are the most impacted from combined Es invasions and degradation of water quality. These areas present the higher impact from human activities and the higher interconnection with the Po river, which acts as a pool for Es. Specific Es belonging to cyprinids, which are mostly responsible for the decline of aquatic vegetation and the increase of water turbidity, and a top Es predator (Wels catfish) were mostly present in these areas. Additionally, the most important WQ stressors of Ns were found to be COD, BOD and temperature that are all connected to oxygen depletion. The aforementioned conditions in the areas described by high priority for intervention can be used as a basis for the development of specific Ns conservation practices targeting the containment of the most harmful Es, the restoration of aquatic

vegetation and the improvement of oxygen conditions.

The experience gained from the monitoring surveys, the application of the proposed method and the results of some related management practices already performed in the study area showed that:

- the robust application of the proposed method requires extremely large datasets of high cost, high working time, high requirements of specialized equipment and personnel from different disciplines (biology and ichthyology, hydrochemistry, hydrology, environmental modeling, fishermen etc).
- the successful implementation of actions and conservation measures which are based on the proposed priorities derived by the method require full support and especially high financial support by the authorities but also full consent by the local society.

Recommendations for future studies could be the following:

- the application of the proposed method for other study areas with similar characteristics of Es invasions and water quality degradation in order to detect any possible similarities or differences in the response of Ns. Possible observed similarities could justify possible “global effects” of Es and water quality degradation on Ns while possible differences could be used to detect differences in the influence of Ns stressors.
- the inclusion of additional parameters in the proposed method. For example in the case of WQ group, it was used a number of very common parameters that are mostly associated to urban organic and agricultural pollution. The WQ group could be expanded including heavy metals, industrial pollutants, radioactive pollutants etc depending on the conditions of each study area.
- In the case where Es invasions are insignificant, the Es group can be neglected from the analysis. Thus, the analysis of Ns using WQ descriptors can be used to assess the quality of freshwaters to support aquatic organisms. Considering the above, the proposed method can support the implementation of Water Framework Directive that requires actions not only for the improvement of biochemical water parameters but also of fish communities, which are among the most significant descriptors of biological quality of freshwaters.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jenvman.2018.01.006>.

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4.1 How do multiple pressures affect fish communities?

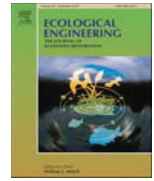
4.1.3. Not only fish as invaders: the top invader red swamp crayfish (*Procambarus clarkii*)

[4] Gavioli, A., Milardi, M., Lanzoni, M., Mantovani, S., Aschonitis, V., Soana, E., Fano E.A, Castaldelli, G. (2018). Managing the environment in a pinch: red swamp crayfish tells a cautionary tale of ecosystem based management in northeastern Italy. *Ecological Engineering*, 120(July), 546–553. <https://doi.org/10.1016/j.ecoleng.2018.07.013>

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Managing the environment in a pinch: red swamp crayfish tells a cautionary tale of ecosystem based management in northeastern Italy

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ABSTRACT

Farmlands are globally widespread and their management should consider both human and environmental needs. In fact, these man-made ecosystems provide subsistence to the human population but are also habitats for plant and animal communities. The worldwide increase of exotic species has affected native communities, but also human activities or health. We used an exploited farmland in northern Italy, where many exotics are present, as a test case for identifying restoration measures based on an ecosystem approach. In particular, we focused on red swamp crayfish for its ecosystem engineering capabilities, and examined the factors affecting its invasion success in order to attempt the definition of management strategies. We used multivariate and regression analysis to evaluate the relationships between the red swamp crayfish, water quality, macrophytes abundance, watercourse hydraulics and the fish community. All analyses indicated that red swamp crayfish was less likely to establish in large, deeper and fast flowing waterways, especially when these are deprived of vegetation and less eutrophicated. Based on our results, fish predation was also a significant factor in limiting red swamp crayfish abundance. We thus concluded that a different hydraulic management, which leaves more water in irrigation canals throughout the winter, could be possibly used to slow down or even reverse the invasion process.

1. Introduction

The sustainable management of human activities has become one of the most imperative goals for the majority of developed countries (UN General Assembly, 2015). A major challenge to achieve this goal has been the divergence between environment conservation and activity development targets (Margules and Pressey, 2000), creating an exacting management gap (Griggs et al., 2013). The concept of ecosystem approach has been developed trying to bridge this gap: it consists of an integrated management of human activities based on the best available knowledge of ecosystems and their dynamics, in order to identify and solve primary causes of ecosystem degradation (UN General Assembly, 1992; Secretariat of the Convention on Biological Diversity, 2004). The product of the ecosystem approach, ecosystem based management, should therefore ensure that development can occur without preventing the ecosystem to provide its services. Being a relatively new concept, its application is not overly widespread and has been perceived to be overly complex and limited in scope to some human activities or

particular environments (e.g. such as with fisheries management, Garcia et al., 2003). Furthermore, several other challenges persist on the development and application of ecosystem based management; namely the difficulties in integrating knowledge from different fields and the incredibly complicated interactions between factors that are at play in human-impacted ecosystems (Long, Charles and Stephenson, 2015; Slocombe, 1998).

The field of agriculture management is a prime example of such difficulties, as it involves complex interactions between soil and water ecology (Altieri, 1995; Kramer, 1969). Farmlands often have a long history and are globally widespread; their modified environment providing sustenance to human populations and, perhaps surprisingly, habitats suitable to some species (Clavero et al., 2015; Freemark et al., 2002; Katayama et al., 2013). Often overlooked as a biodiversity-capable environment, irrigation canals are habitat-simplified watercourses that are connected to natural rivers and constitute an interconnected network within farmlands. Managing irrigation canal environments involves governance at different levels (e.g. on fisheries) but perhaps

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the most relevant is hydraulic regulation (Ricart and Gandolfi, 2017). Hydraulic regulation in canals presents challenges common to other human-impacted watercourses, where conservation and production goals often diverge and a major knowledge gap has been underlined as to the effect of variations in water flow on the freshwater plant and animal communities (Bunn and Arthington, 2002; Poff and Zimmerman, 2009).

Similarly, the fauna living in canals can be influenced by pressures much similar to those of natural aquatic environments; primarily eutrophication derived from field fertilization (Castaldelli et al., 2013a; Huang et al., 2017), habitat degradation (Dudgeon et al., 2006) and the introduction of exotic species (Strayer, 2010).

Exotic species are widely recognized as one of the “big five” causes of biodiversity loss (Sala et al., 2000) and can also become invasive, with adverse consequences extending to human activities as well. Among the most invasive exotic species introduced in temperate freshwaters, crayfishes have recently become increasingly common and have had direct effects on native communities (Gherardi and Lazzara, 2006; Rodríguez-Pérez et al., 2016). One of the most prominent and invasive crayfishes, the red swamp crayfish (*Procambarus clarkii*, Girard, 1852), has been found to affect aquatic macrophytes (Carreira et al., 2014) and even damage watercourse banks due to its burrowing habits (Barbaresi et al., 2004). This species thus presents a veritable threat to ecosystem functioning and hydraulic stability, but also a potential impact on crop production (Anastácio et al., 2000) and restoration practices (Rodrigo et al., 2013) posing a serious question to both conservation and agricultural management.

In the lowest portion of the Po River basin, by far the largest and one of the most exploited farmlands in Italy, the presence of red swamp crayfish was reported in 1996 (for a general view, see Gherardi et al., 1999) but its effects or its potential management have been scarcely investigated. This area is also heavily invaded by exotic fish species, which are crayfish predators (e.g. wels catfish *Silurus glanis* L. (Carol et al., 2009) or common carp *Cyprinus carpio* L. (Britton et al., 2007)), but in turn also pose a question of exotic species management. To examine the factors affecting the invasion success of red swamp crayfish and to attempt the definition of management strategies, we selected both natural and artificial waterways and worked under the hypothesis that low water levels outside of the growing season could favor crayfish invasion by preventing effective fish predation. We used multivariate analysis to evaluate the relationships between the red swamp crayfish, water quality, macrophytes, watercourse hydraulics and the fish community. Using our results, we identify factors that could be accounted for in hydraulic management, which could be possibly used to slow down or even reverse the invasion process. This test case study could be used as a stepping stone to reduce complexity, achieve sustainable management and be transferred to other regulated watercourses where multiple environmental stressors are present.

2. Materials and methods

2.1. Study area

The waterways investigated in this study are located in the lower stretch of the Po River, within the administrative boundaries of the Province of Ferrara (Emilia-Romagna Region, northeastern Italy). In this area, the natural swamp of the delta has been reclaimed over nearly two centuries and was turned into an heavily exploited farmland, extending over 2200 km², half of which is below sea level. To provide water for irrigation, a capillary network of canals was built, which accounts for more than 4000 km in linear extension in the Province of Ferrara. Other waterways include the Po River, a constricted river with a relatively natural flow regime, and the Reno River, a constricted and flow-regulated river.

A total of 34 sampling sites, along 27 different waterways, were sampled to assess presence and abundance of both crayfish and fish

(Fig. 1). Moreover, hydrological and environmental variables were also measured. These waterways had variable widths (2–100 m), depths (0.5–4 m) and water current (0–0.3 m s⁻¹; but peak values up to 1 m s⁻¹ were registered in the Po and Reno rivers). All water courses presented a muddy-silty layer of variable thickness, covering the clayey sediment of the canal bed, except the Po River, where a prevalence of sandy sediment could be found in the sites sampled. These waterways are also affected by severe microhabitat simplification due to management practices, such as frequent vegetation mowing (Castaldelli et al., 2013a). Irrigation canals are flooded during the growing season (typically April to October) to provide water for irrigation; but water levels are significantly lower outside of this time, further limiting the habitat available to aquatic species.

In the lowest portion of the Po River basin, native white-clawed crayfish *Austropotamobius italicus* (Lereboullet, 1858), belonging to the *A. pallipes* complex, was historically present (Morpurgo et al., 2010), but its abundance and distribution sharply declined in the ‘70s, with numerous local extinctions in rivers and canals. Afterwards, two exotic crayfish species accidentally escaped from aquaculture ponds, took advantage of the empty niche: the red swamp crayfish was first reported in 1996, and the spinycheek crayfish, *Orconectes limosus* (Rafinesque, 1817), was reported in 2006 about 60 km downstream of the waterways sampled in this study (see Gherardi et al., 1999 for a general overview). It is unclear whether exotic crayfish presence in this area is due to local introductions or dispersal from nearby invaded areas.

In this area, several species of native fish were historically present but exotic fish species introductions date as far back as the XVII century, with most species being introduced around 1970 from Asia or East Europe (Lanzoni et al., 2018; Milardi et al., 2018a). This is one of the most heavily invaded areas in the country, where severe impacts on the native fish communities have been detected (Castaldelli et al., 2013b; Milardi et al., 2018a) and sites with fully exotic fish communities have been found (Lanzoni et al., 2018).

2.2. Crayfish and fish sampling

Sampling of crayfish was conducted between May and June 2009, a period when crayfish were active and environmental stressors, such as flow variations, were not present. In each waterway, crayfish presence was investigated in stretches with homogenous morphology, hydrology and with no tributaries or discharges using plastic traps baited with a can of fish-flavored cat food (see e.g. Lappalainen and Pursiainen, 1995). No professional or sport fishing targeted the crayfish populations in these waterways.

Plastic traps had proved to be the most reliable survey measure in preliminary tests and previous sampling campaigns in the area (2004–2006), due to the high turbidity and the presence of emergent vegetation along the bank (Lanzoni, unpublished data). The traps (40 × 25 × 25 cm, 0.3 cm mesh size) had two openings at opposite ends, plus a central opening for extraction (Fjälling, 1995). A set of 15 traps was used at each sampling event, placed along the waterway banks at depths between 0.5 and 1 m, and left overnight (12 h, from 19.00 to 7.00). 3 replicate sampling events were performed at each site, and catches were expressed as average CPUE, defined as the mean number of crayfish caught per trap, per sampling event. Carapace and total length of all crayfish were measured to the nearest 0.1 mm using calipers. Crayfish wet weight was measured in the field, after removing excess water, to the nearest 0.1 g using an electronic scale.

A temporally and spatially overlapping sampling of the fish fauna was also performed using a combination of electrofishing and netting, adapting the national standard guidelines to the unique conditions of these waterways (Lanzoni et al., 2018). Fish were identified to the species level and individuals of each species counted. Species abundances were expressed in Moyle classes (Moyle and Nichols, 1973), which range from 1 (low abundance, 1–2 individuals per site) to 5 (high abundance, > 50 individuals per site).

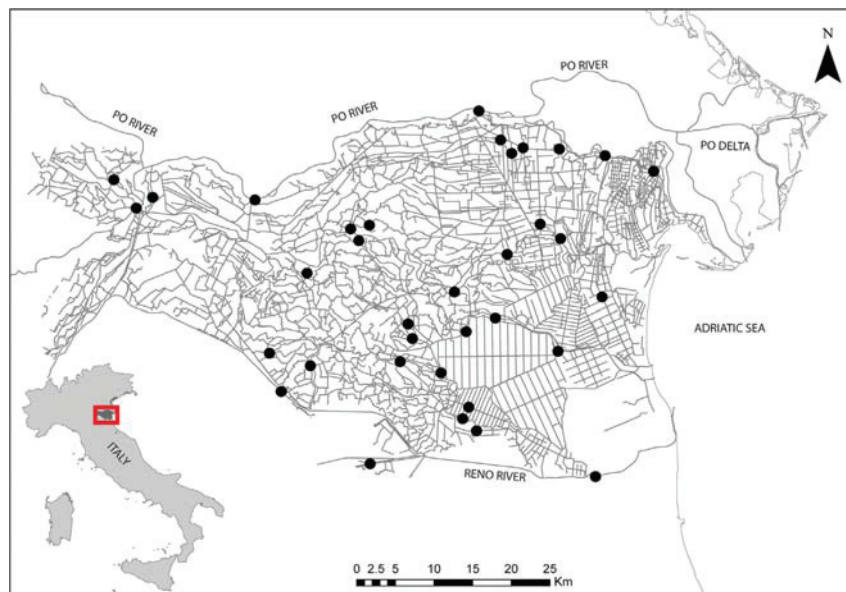


Fig. 1. Map of the study area: the lower Po River plain and the complex hydrological network of the Province of Ferrara (Italy). The 34 black dots represent the location of sampling sites, distributed along 27 different waterways, used in this study.

2.3. Environmental variables

At each of the 34 sampling stations, a number of different environmental variables were measured. Over the span of a year, maximum current velocity (V_{max}) and minimum depth (Depth) were measured respectively during flood events by means of a current meter (Open Stream CurrentMeter 2100) and during drought using a dipping metric tape or, in the largest canals and rivers, a probe (Ocean Seven 316, Idronaut, Brugherio, MI, Italy). The waterway width (Width) was measured with a binocular laser rangefinder (Leica 10x42 Geovid BRF) with metric resolution or with a metric tape, depending on the site characteristics. The span of aquatic vegetation (mostly reeds) along the waterway banks (Veg) was measured with a floating metric pole with centimetric resolution. Oxygen concentration (Oxy) was measured with a multiparametric probe (Ocean Seven, 316). Levels of chlorophyll *a* (Chl-*a*, indicative of phytoplankton biomass and eutrophication levels), total suspended solids (TSS, indicative of turbidity), and of Biochemical Oxygen Demand (BOD_{5s} , indicative of nutrient load) were determined according to APHA (2005).

2.4. Statistical analysis

In order to explore the relationship between crayfish, fish and environmental parameters multivariate statistics were employed. A Detrended Correspondence Analysis (DCA) was initially performed to select the most appropriate response model for gradient analysis (Lepš and Šmilauer, 2003). The dominant gradient length in DCA was always lower than 3 so the Redundancy Analysis (RDA) was finally chosen (Lepš and Šmilauer, 2003). RDA is a linear gradient analysis that allows to quantify the variation of a multivariate data set explained by independent variables (ter Braak and Šmilauer, 2002). The environmental variables were considered as independent parameters, whereas the faunal community (red swamp crayfish and fish species) was considered as dependent. Fish species sampled in less than 3 sites, were excluded from the RDA analysis (Aschonitis et al., 2015; Feld and Hering, 2007; Godinho and Ferreira, 2000). Variables with a Variance Inflation Factor (VIF) higher than 8 should be excluded before the RDA

analysis in order to exclude collinear (redundant) ones (Zuur et al., 2007), but all variables were below the limit.

The relationship between the environmental parameters and the abundance of red swamp crayfish was assessed using a multiple regression analysis. Spearman's correlation coefficient ($|r| > 0.7$) was used to remove redundant variables in environmental data (Dormann et al., 2013). A forward stepwise selection of variables based on an F-to-enter test was performed: at each step, the algorithm adds to the multiple regression the variable that will be the most statistically significant if entered ($F\text{-value} > 4.0$). Variance analysis was performed to test the statistical significance of the fitted model and to check the relationship between crayfish abundance and independent variables.

A hierarchical partitioning was performed to evaluate how much of the explained variation of crayfish abundance can be independently and jointly attributed to each environmental variable (Chevan and Sutherland, 1991; Mac Nally and Walsh, 2004). This approach allows to break up the variation explained by a set of independent variables into independent components (I), which reflect the relative importance of individual variables, and joint contributions (J), which are cumulative effects of each variable with all other variables. The distribution of joint effects shows the relative contribution of each variable to shared variability in the full model. Negative joint effects are also possible for variables that act as suppressors of other variables (Chevan and Sutherland, 1991). To avoid minor rounding errors, only the five most important factors previously identified with forward selection procedures were chosen for hierarchical partitioning (Mac Nally and Walsh, 2004; Walsh and Nally, 2013). The significance of the hierarchical model was assessed using a randomization test with 100 permutations (Walsh and Nally, 2013).

Environmental and crayfish abundance data, were log-transformed with $X' = \log_{10}(X + 1)$ to reduce the departure from normality. The DCA and RDA analyses were performed using CAONOCO 4.5 for Windows (Lepš and Šmilauer, 2003). The multiple regression analysis was performed using StatGraphics Centurion XV software (Statpoint Technologies, Warrenton, VA, USA) and the hierarchical partitioning was performed using R software (R Core Team, 2017) and the "hier-part" package 1.0–4 (Walsh and Nally, 2013).

Table 1

Observed fish species in the 34 sampling sites, status of species (N is for native and E is for exotic species) and number of sampling sites where each species was present (S). Rare species “*” occurring in less than 3 sampling sites were excluded from statistical analyses.

Family	Species	Common Name	Status	S
Anguillidae	<i>Anguilla anguilla</i> (Linnaeus, 1758)	European eel	N	5
Clupeidae	<i>Alosa fallax</i> (Lacépède, 1803)	Twaite shad	N	5
Cyprinidae	<i>Squalius squalus</i> (Bonaparte, 1837)	Italian Chub	N	2*
	<i>Scardinius hesperidicus</i> Bonaparte, 1845	Italian rudd	N	10
	<i>Alburnus arborella</i> (Bonaparte, 1841)	Italian bleak	N	26
	<i>Chondrostoma soetta</i> Bonaparte, 1840	Italian nase	N	1*
	<i>Barbus plebejus</i> Bonaparte, 1839	Barbel	N	1*
	<i>Barbus barbatus</i> (Linnaeus, 1758)	European barbel	E	1*
	<i>Carassius</i> spp.	Crucian carp/Goldfish	E	31
	<i>Cyprinus carpio</i> Linnaeus, 1758	Common carp	E	33
	<i>Abramis brama</i> (Linnaeus, 1758)	Common bream	E	16
	<i>Blitca bjoerkna</i> (Linnaeus, 1758)	White bream	E	1*
	<i>Rhodeus sericeus</i> (Pallas, 1776)	Bitterling	E	14
	<i>Pseudorasbora parva</i> (Temminck & Schlegel, 1846)	Stone moroko	E	25
	<i>Ctenopharyngodon idella</i> (Valenciennes, 1844)	Grass carp	E	5
	<i>Leuciscus aspius</i> (Linnaeus, 1758)	Asp	E	6
	Siluridae	<i>Silurus glanis</i> Linnaeus, 1758	Wels catfish	E
Ictaluridae	<i>Ameiurus melas</i> (Rafinesque, 1820)	Black bullhead	E	10
	<i>Ictalurus punctatus</i> (Rafinesque, 1820)	Channel catfish	E	2*
Poeciliidae	<i>Gambusia holbrooki</i> (Girard, 1859)	Eastern mosquito fish	E	4
Centrarchidae	<i>Micropterus salmoides</i> (Lacépède, 1803)	Largemouth black bass	E	1*
	<i>Lepomis gibbosus</i> (Linnaeus, 1758)	Pumpkinseed	E	13
Percidae	<i>Perca fluviatilis</i> Linnaeus, 1758	European perch	N	1*
	<i>Gymnocephalus cernuus</i> (Linnaeus, 1758)	Ruffe	E	1*
	<i>Sander lucioperca</i> (Linnaeus, 1758)	Pike-perch	E	20
Mugilidae	<i>Liza ramada</i> (Risso, 1827)	Thinlip grey mullet	N	2*

3. Results

3.1. Crayfish and fish

Neither the native white-clawed crayfish nor the exotic spinycheek crayfish were found in traps, so the red swamp crayfish was the only crayfish captured. In six sampling sites, no crayfish were found.

A total of 830 red swamp crayfish specimens were caught and measured. Their average density was 1.6 CPUE (± 2.1 SD); with a maximum average density of 8.4 CPUE (± 4.8 SD). Average total length, carapace length and weight were respectively 57.1 mm (± 35.4 SD), 27.0 mm (± 16.0 SD) and 12.6 g (± 8.7 SD).

The fish fauna consisted of 26 species, 17 exotic and 9 native, belonging to 9 families. Cyprinids were the most represented family, accounting for almost 50% of the total number of species. Gradient analysis was performed on 16 fish species (12 exotic and 4 native) since species present in less than 3 sites were not included in the analysis (Table 1). In almost all waterways, the most abundant species were exotic.

3.2. Environmental variables

Environmental parameters (Table 2) were highly variable among sites. Maximum values of water velocity were measured in a canalized river (130 cm s^{-1}), whereas minimum values were sampled in drainage canals. Minimum water depth ranged from 0.3 m, measured in a small drainage canal, to 3 m measured in a canal used both for irrigation and drainage. Emergent vegetation was mainly represented by *Phragmites australis*, *Typha latifolia* and *Glyceria maxima* and its extension was generally limited (mean 2.3 ± 3.7 SD m). Oxygen showed a high variability from hypoxic condition (2.4 mg l^{-1}) to high concentrations (17.2 mg l^{-1}). Also chlorophyll *a* showed a wide range of values, from 3.0 to $92.6 \mu\text{g l}^{-1}$, but with generally high levels (mean 31.2 ± 26.7 SD $\mu\text{g l}^{-1}$).

3.3. Relationship between crayfish, fish and environmental variables

Red swamp crayfish thrived in smaller waterways rather than larger ones, as defined by Depth and Vmax and Width, and its abundance was

Table 2

Average and range of environmental variables measured at the 34 sampling sites during May/June 2009.

Parameter	Abbrev.	Unit	Minimum	Maximum	Average	SD
Maximum water velocity	Vmax	(cm s^{-1})	5.0	130.0	33.1	32.4
Minimum depth	Depth	(m)	0.3	3.0	1.4	0.7
Width	Width	(m)	4.0	329.0	34.2	60.9
Emergent vegetation	Veg	(m)	0.2	20.0	2.3	3.7
Oxygen concentration	Oxy	($\text{O}_2 \text{ mg l}^{-1}$)	2.4	17.2	7.0	2.9
BOD ₅	BOD ₅	($\text{mg O}_2 \text{ l}^{-1}$)	1.2	11.2	4.5	2.6
Chlorophyll <i>a</i>	Chl- <i>a</i>	($\mu\text{g l}^{-1}$)	3.0	92.6	31.2	26.7
Total suspended solids	TSS	(mg l^{-1})	28.7	205.0	75.3	41.2

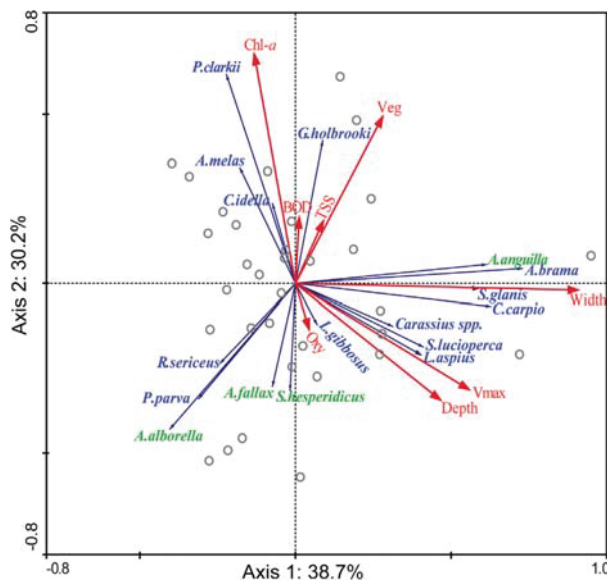


Fig. 2. Redundancy analysis (RDA) triplot showing the relationships between red swamp crayfish, fish fauna (blue arrows, native species with green labels, exotic ones with blue ones) and environmental parameters (red arrows). Empty dots represent sampling sites. Fish species abbreviations are given in Table 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3

Regression analysis results; standard error, *t*-test and *p* values are reported for each retained variable.

Variable	Estimate coefficient	Standard Error	T Statistic	P-Value
Intercept	0.922	0.083	11.051	< 0.001
Vmax	-0.260	0.035	-7.518	< 0.001
Depth	-0.730	0.080	-9.090	< 0.001
Chl-a	0.216	0.037	5.808	< 0.001
Veg	0.185	0.022	8.411	< 0.001
Oxy	-0.340	0.064	-5.343	< 0.001

also positively influenced by high values of Chl-a and Veg (Fig. 2). Crayfish predators such as wels catfish, European eel (*Anguilla anguilla*, L.), common carp and other fish species were also found in large waterways and negatively influenced red swamp crayfish abundance (Fig. 2). BOD, TSS and Oxy did not appear to be as strong descriptors of red swamp crayfish distribution (Fig. 2).

The Spearman rank test showed a high correlation between Width and Depth ($P < 0.01$, Correlation Coefficient = 0.7439). In order to avoid redundant variables, only Depth, as better descriptor of water-course hydraulics, was retained in the multiple regression analysis. The forward selection procedure included Vmax, Depth, Chl-a, Veg and Oxy as significant for red swamp crayfish abundance (Table 3). The fitted multiple regression model explained 41.25% (adjusted R-squared) of the variability in crayfish abundance. Analysis of variance of this model showed a statistically significant relationship between environmental variables and crayfish abundance ($F_{5,579} = 83.01$; $P < 0.001$). Chl-a and Veg positively influenced red swamp crayfish abundance, while Depth, Vmax and Oxy negatively affected it.

The hierarchical partitioning showed that Vmax explained the highest percentage of total variance (29.79%) of red swamp crayfish abundance (Fig. 3a and b), with an independent contribution of 0.13

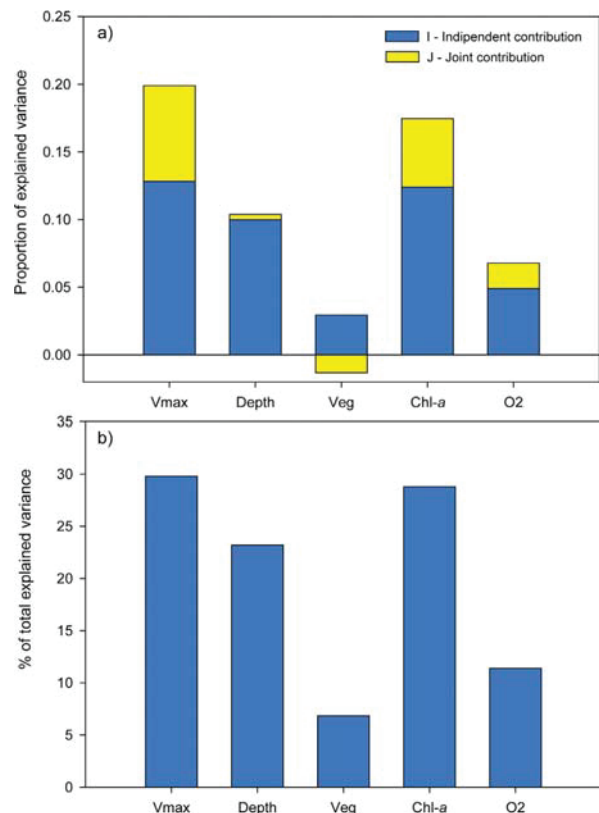


Fig. 3. Hierarchical partitioning of the environmental variables retained in the multiple regression analysis ($P < 0.001$). Proportion of red swamp crayfish abundance variance explained independently and jointly by the variables (a) and percentage of total red swamp crayfish abundance variance explained by each variable (b).

closely followed by Chl-a (23.19% of total variance). Veg and Oxy had the lowest values: 6.84% and 11.28% of total explained variance, respectively (Fig. 3b). Veg was the only variable with a negative joint effect (-0.01). The randomization test retained all variable included in hierarchical partitioning as significant.

4. Discussion

Our results show, for the first time, that the abundance of red swamp crayfish in the lower Po plain is affected by complex interactions between the environment and the fish community, with predation playing a central role. All analyses indicated that red swamp crayfish was less likely to establish in large, deeper and fast flowing waterways, especially when these are deprived of vegetation and less eutrophicated. Larger waterways also host more fish species that prey upon red swamp crayfish, adding to that effect. Unfortunately, it was not possible to clearly disentangle the effect of these factors, but it was clear from all analyses that managing the invasion would require to take into account multiple and often contrasting factors.

Although some authors suggest that hypoxia conditions can affect abundance of red swamp crayfish (Bonvillain et al., 2015), dissolved oxygen did not have a strong influence on red swamp crayfish in our study. Although retained in the forward selection procedure and in the multiple linear regression, dissolved oxygen had the lowest independent contribution to explain the crayfish abundance, probably due to the low variation among sites. However, an indirect effect of low

dissolved oxygen values cannot be excluded; an anoxic zone could limit the presence of fish and consequently decrease predation pressure on the red swamp crayfish. High flow rates and highly variable flow regimes have been previously shown to negatively affect the dispersion of red swamp crayfish (Kerby et al., 2005). An increase of mortality in high flow conditions was also shown in other crayfish species such as the signal crayfish (*Pacifastacus leniusculus*, Dana, 1852) (Light, 2003) and the white-clawed crayfish (Robinson et al., 2000). However, in the studied area, water velocity possibly affected red swamp crayfish density indirectly, acting through other environmental factors. In fact, while maximum water velocity was higher in the rivers of the Province of Ferrara, it was generally low in the canals and presumably even lower among the vegetation of the banks, where red swamp crayfish were found. Water velocity could have a significant effect on the sedimentation of fine detritus: higher flows would cause lower sedimentation rates. Fine detritus is mostly derived from phytoplankton, which is the dominant primary producer in this system (Mantovani et al., 2004). Lower sedimentation could mean less food resources available to the crayfish as phytoplanktonic detritus is a primary food source for the red swamp crayfish in this area (Lanzoni, unpublished data), contrarily to what previously found by Gherardi and Lazzara (2006). Therefore, this could also explain the positive influence of Chl-*a* levels on red swamp crayfish abundance underlined by our data. In turn, the bioturbation activity of red swamp crayfish can release nutrients trapped in sediments and thus favor phytoplankton blooming (Angeler et al., 2001; Rodríguez et al., 2003). Thus, there could be a complex positive feedback mechanism between red swamp crayfish and eutrophication, which could be further reinforced by other species.

Mechanisms of potential invasional meltdown have been previously hypothesized to be at play in the area, favoring the invasion of exotic fish (Lanzoni et al., 2018) through e.g. an increase of eutrophication effects. These mechanisms could further magnify the action of red swamp crayfish, creating a positive synergy among habitat engineering species. For example, bighead and silver carp (*Hypophthalmichthys* spp., L.) have been introduced in this area and found to be able to naturally recruit (Milardi et al., 2017). These fish are plankton feeders, with bighead carp feeding mainly on zooplankton (Dong and Li, 1994), and thus are potentially able to decrease zooplankton grazing pressure and increase phytoplankton blooms, ultimately favoring the red swamp crayfish. However, several exotic fish species are also active crayfish predators, so that the net outcome of these interaction is less than predictable. Common carp was one of the most abundant and widespread fish species in the canals (Castaldelli et al., 2013b; Milardi et al., 2017, 2018b; Lanzoni et al., 2018) and is well-known for its ability to resuspend nutrients from the sediment while feeding (Badiou and Goldsborough, 2015) but can also prey on smaller red swamp crayfish (Britton et al 2007, Lanzoni, unpublished data). Our data seems to suggest that the latter mechanism is predominant, as the presence in deeper watercourses of fish that prey upon crayfish (such as wels catfish or common carp) seemed to correlate negatively with crayfish abundance.

Besides mechanical mowing, also fish presence can influence the presence of aquatic vegetation. Resuspension of sediments by benthivorous fish can increase water turbidity, limiting the amount of light available to submerged vegetation (de Backer et al., 2010). Moreover, some fish can feed directly on aquatic macrophytes: grass carp (*Ctenopharyngodon idella*, Valenciennes 1844), an herbivorous fish capable of overgrazing aquatic plants (Cudmore and Mandrak, 2004), has been massively introduced in the area and found to naturally recruit (Milardi et al., 2015). In all waterways of the study area, submerged vegetation was indeed absent, while emergent vegetation was dominantly represented by reed *Phragmites australis* (Cav. Trin. ex Steud). Except for the early vegetative phase (in this area from early to mid-May), reed is a poor food item for fish or crayfish. Thus, vegetation positively influences red swamp crayfish density by providing habitat and shelter from fish (Carol et al., 2009; Musseau et al., 2015) and water birds (Huner,

2000) predation, rather than a food resource. In northern Italy, the grey and white herons and the little egret have become very abundant in the last decades, and are active predators of red swamp crayfish (Fasola et al., 2010; Fasola and Cardarelli, 2015). In the western portion of the Po River basin, red swamp crayfish has become an increasingly predominant food item in heron diet (Fasola and Cardarelli, 2015). Predation by water birds could be increased by the total absence of submerged vegetation, but higher water depths in winter should in turn decrease predation, by making crayfish less accessible to birds. However, our results show that minimum water depth seemed to favour, rather than limit, red swamp crayfish presence. Perhaps this indicates that fish predation is a stronger pressure than bird predation on the red swamp crayfish population.

5. Conclusions

Given the tight interplay between invasive red swamp crayfish, other exotic fish species, native bird predators and the environmental management of irrigation canals, it is clear that managing the crayfish invasion is a complex task. The invasion of red swamp crayfish is widespread in all Continental Europe, measures of containment have been proposed, but ultimately eradication appears unfeasible (Gherardi et al., 2011). Solving the red swamp crayfish conundrum would require extensive conservation measures, which are sadly not affordable due to current management budget constraints. Similarly, the use of pyrethroid substances to control the invasive crayfish population would have too high social and environmental costs. Thus finding alternative and low-cost management alternatives is of primary importance. However, the understanding of factors favoring the invasion may help to promote dedicated management to control the population in invaded areas and limit the colonization of new areas.

Our results clearly indicate that, in irrigation canals, hydraulic features and hydraulic management play a central role in favouring the presence of red swamp crayfish. If the canals could be excavated to increase depth and width, hydraulic transport would be enhanced and red swamp crayfish presence could be limited. Furthermore, agricultural management practices (i.e. hydraulic management) could also directly affect red swamp crayfish abundance. Fish predation could be enhanced by keeping higher water levels through the winter season, allowing a cost-effective control of red swamp crayfish density. However, fish predation pressure is now mostly given by exotic fish and managing exotic fish has been a generally troublesome undertaking, where several management and conservation issues have arisen (Milardi et al., 2018a,b).

Limiting the presence of some of the exotic ecosystem engineering fish species (e.g. common and grass carp) could favour native fish species conservation by increasing aquatic macrophytes that are used as a spawning substrate (Milardi and Castaldelli, 2018). Unfortunately this could also increase red swamp crayfish predation shelter, so that the ultimate outcome of such management is uncertain and would need to be field-tested.

Ultimately, a reasonable management for red swamp crayfish invasion would have to be ecosystem-based, taking into account all components of the aquatic and terrestrial environment and the socio-economic factors to delineate an optimal strategy.

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4.2 How do alpha and beta diversity change in native and exotic communities?

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Diversity patterns of native and exotic fish species suggest homogenization processes, but partly fail to highlight extinction threats

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Abstract

Aim: Exotic species are a major threat to biodiversity and have modified native communities worldwide. Invasion processes have been extensively studied, but studies on species richness and beta diversity patterns of exotic and native species are rare. We investigate such patterns among exotic and native fish communities in upland and lowland rivers to explore their relationship with environmental drivers.

Location: Northern Italy.

Methods: Exotic and native fish beta diversity patterns were investigated separately in lowland and upland sites using Local Contribution to Beta Diversity (LCBD) and Species Contribution to Beta Diversity (SCBD) analyses. To examine the main environmental variables affecting the LCBD, a Boosted Regression Trees (BRT) method was used. Community dispersion among and within stream orders was investigated with the PERMDISP test.

Results: In lowland sites, exotic species richness was higher than native species richness, especially in large rivers and drainage canals. An opposite trend was found in upland sites, where native species richness was higher than exotic species richness, especially in large rivers. No clear LCBD patterns were found along stream orders in the lowland, whereas higher stream orders in the upland showed the highest LCBD. Its patterns in upland and lowland sites were related to a number of factors, such as total suspended solids and total phosphorus. Community dispersion among stream orders did not show a relationship with environmental heterogeneity. SCBD values were positively correlated with species occupancy in the study area, and native species showed higher SCBD values than exotic species only in the uplands.

Main conclusions: Large rivers in the uplands are important in maintaining native fish diversity and should be protected against invasive fish. In contrast, most lowland rivers have suffered from biological homogenization. Some rare native species can show low contribution to beta diversity, but still need conservation actions due to their risk of local extinction.

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KEYWORDS

Alien species, beta diversity, biodiversity conservation, freshwater, invasions, non-native species, species diversity, species richness

1 | INTRODUCTION

The importance of biodiversity for ecosystem functioning and resilience, as well as for humans through the supply of ecosystem services (e.g., food, pest control, fisheries), is widely acknowledged (Cardinale et al., 2012; Hooper et al., 2005; Worm et al., 2006). Nevertheless, biodiversity constantly declines worldwide (Butchart et al., 2010) and to define management plans that can halt this decline it is necessary to understand biodiversity trends in space and time (Richardson & Whittaker, 2010). A common approach to detect these biodiversity trends is to measure variations in taxonomical diversity (Chiarucci, Bacaro, & Scheiner, 2011; Colwell & Coddington, 1994). In 1960, Whittaker proposed the taxonomical diversity could be defined as the result of three components: alpha (local diversity), beta (variation of community composition among sites) and gamma diversity (regional diversity; Whittaker, 1960, 1972). In recent years, more attention has been focused on beta diversity (Anderson et al., 2011) due to its ability to identify human impacts on diversity (e.g., agriculture, species invasion and climate change) at multiple scales (Socolar, Gilroy, Kunin, & Edwards, 2016). Different measures of beta diversity have been proposed (e.g., Baselga, 2010; Tuomisto, 2010), and recently, Legendre and De Cáceres (2013) proposed a method that not only estimates the overall beta diversity, but also quantifies the Local Contribution to Beta Diversity (LCBD) by single sites and the Species Contribution to Beta Diversity (SCBD) by individual species. Both LCBD and SCBD can also be considered as measures of the uniqueness of sites and species for a region and have been used to investigate species distribution shifts in fish communities (Kuczynski, Legendre, & Grenouillet, 2017) and other taxa such as diatom communities (Jyrkänkallio-Mikkola, Siljander, Heikinheimo, Pellikka, & Soinen, 2018) and stream invertebrates (Heino & Grönroos, 2017; Sor, Legendre, & Lek, 2018; Tonkin, Heino, Sundermann, Haase, & Jähni, 2016).

Despite the importance of diversity measures in explaining taxonomical biodiversity, the main shortcoming of these measures is that all species are typically considered equally, without taking into account evolutionary or ecological differences between species (Chiarucci et al., 2011). For example, taking into account the native or exotic status of a species has important implications in terms of management and conservation, also considering that the invasion sensitivity of the community could be related to diversity measures such as species richness (Hooper et al., 2005). Invasions of exotic species can often cause a native species' decline through predation, hybridization, competition and indirect effects (Blackburn et al., 2014; Simberloff et al., 2013).

Freshwaters are particularly susceptible to exotic species invasions, and in such ecosystems, exotic species are considered one of the main causes of biodiversity loss (Dudgeon et al., 2006). For

instance, in fish communities, exotic species constitute one of the major drivers of extinction in the Mediterranean region (Crivelli, 1995) and can cause taxonomic homogenization (i.e., taxonomic similarity across communities), particularly in the Nearctic and Palearctic regions (Villéger, Blanchet, Beauchard, Oberdorff, & Brosse, 2011, 2015). There is also evidence that only few introduced exotic species (e.g., common carp, *Cyprinus carpio* L.) drive this trend (Toussaint, Beauchard, Oberdorff, Brosse, & Villéger, 2016). There are many studies focusing on the effects of exotic species on native ones (e.g., Milardi et al., 2018); however, large-scale diversity patterns in native and exotic species communities are still understudied, especially in freshwaters (some exceptions: Kuczynski et al., 2017; Leprieur, Olden, Lek, & Brosse, 2009; Maceda-Veiga et al., 2017).

To investigate these patterns, we focused on fish biodiversity in rivers and streams in Northern Italy, one of the most heavily invaded areas in the country. In some stretches of these rivers, the invasion of exotic fish, and a corresponding decline of native species, occurred nearly twenty years ago (Castaldelli, Pluchinotta et al., 2013). Here, we (a) investigated how species richness (i.e., alpha diversity) and the uniqueness of community composition (i.e., beta diversity, LCBD) vary among exotic and native fish species from headwaters to lowland rivers, that is, across stream orders. Secondly, we (b) investigated the relative influence of main water physico-chemical variables on the uniqueness of the community composition at sites (i.e., LCBD). We also (c) examined the variation in exotic and native community within stream orders and studied whether we could relate within stream order variation in communities to the degree of water physico-chemical heterogeneity. Finally, we (d) analysed the species contribution to beta diversity (i.e., SCBD) under the hypothesis that native species might contribute more to beta diversity than exotic ones, which tend to homogenize communities. We also examined if a relationship between species occupancy and species contribution to beta diversity existed.

Our results can help to understand spatial clines in native and exotic species diversity and how these clines respond to different water physico-chemical variables. Such information would in turn be useful to improve management and conservation actions in freshwaters.

2 | METHODS

2.1 | Study area

The study area is located in Northern Italy and includes the largest river basin in Italy, the Po River basin (71,000 km²). The area hosts more than 17 million of inhabitants and is impacted by agricultural activities and livestock farming. The study region has a

Mediterranean continental climate, with an annual average precipitation of 1,036 mm and a mean air temperature of 12°C. The rivers network considered include the Po River in all its course, the Oglio River, one of the most important left tributaries of the Po River, and the right tributaries in the Emilia-Romagna region. As a reference external to the Po Basin, we included the Brenta River, located on the north-east of the Po Basin, and torrents and rivers south of the Po Basin, until the southernmost border of the Emilia-Romagna region.

In the upland rivers, organic material originating from villages and small towns and livestock farms is the main source of pollution. Conversely, a high degree of urbanization and intensive agriculture characterize the lowland rivers, where high nutrient loads have led to eutrophication (Castaldelli, Soana et al., 2013; Soana, Racchetti, Laini, Bartoli, & Viaroli, 2011). To support agricultural activities, a complex network of drainage canals has been established in the lowlands. This system is completely human-regulated with hydrological management directed to drainage or irrigation supply (Castaldelli, Pluchinotta et al., 2013; Milardi, Chapman, Lanzoni, Long, & Castaldelli, 2017). Overall, a total of 337 sampling sites in 105 watercourses were sampled between 1999 and 2010 and included in this study, covering a wide range of freshwater habitats, different

altitudinal zones and environmental conditions (Figure 1). We considered that community turnover would not be a relevant factor in our study, due to the fact that fish communities are typically more temporally stable than other aquatic communities (Korhonen, Soinen, & Hillebrand, 2010). Furthermore, the study area was already in a late invasion stage (Milardi et al., 2018), since loss of native species and exotic invasion occurred mainly prior to 1997 (Castaldelli, Soana et al., 2013), that is, before the data analysed here were collected.

2.2 | Stream surveys

Fish data were collected within a monitoring programme for the compilation of the official Fish Inventories of the Emilia-Romagna region (Emilia-Romagna Region, 2002, 2005, 2008), the Padova province (Padova Province, 2010), the Po River (Po River Water Authority, 2008) and the Oglio River (Oglio River Water Authority, 2016). Fish sampling was performed typically from April to September by electrofishing. In sites of higher water depth and conductivity (e.g., lower stretches of the rivers), electrofishing was combined with the use of nets. For more details on fish sampling methods, see Aschonitis et al. (2018), Gavioli et al. (2018), Milardi et al. (2018).

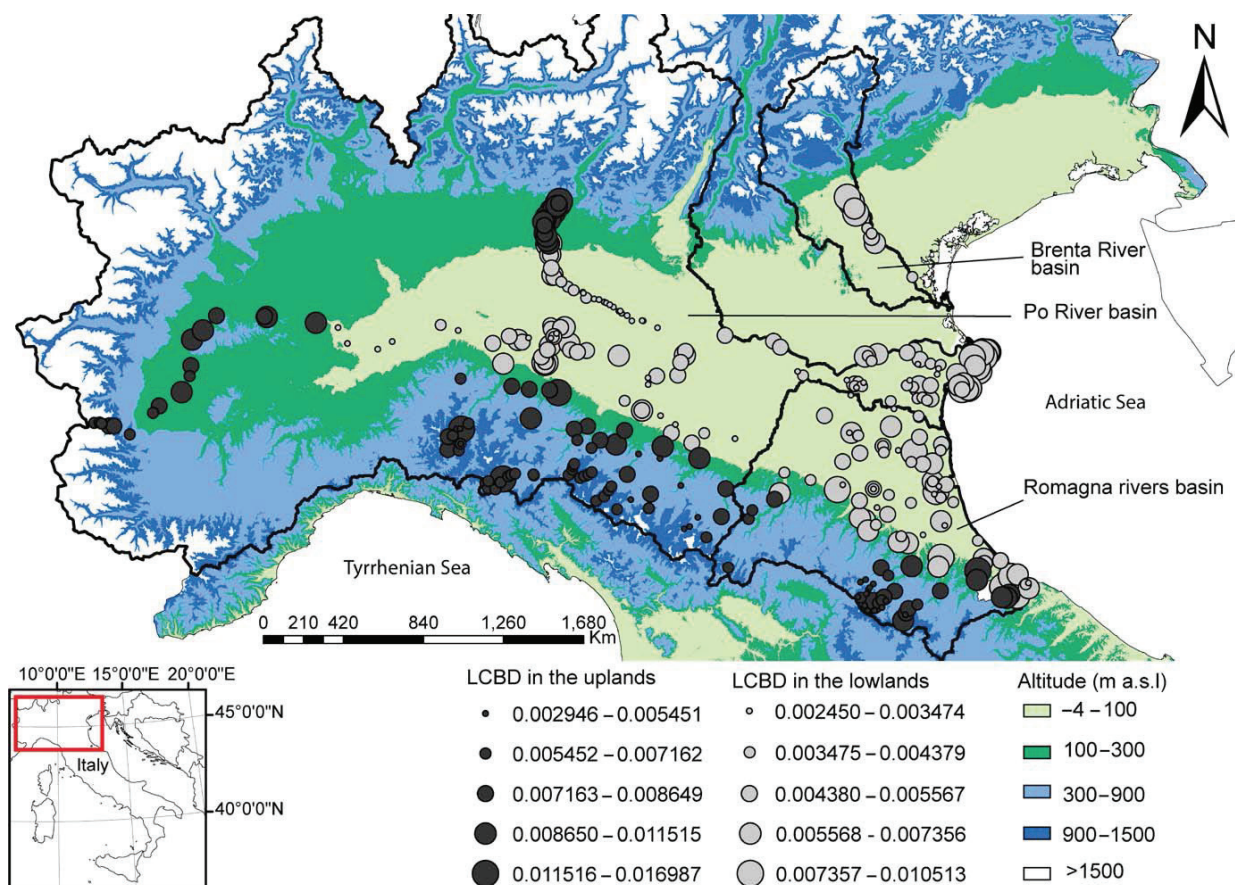


FIGURE 1 Map of sampling sites in the Northern Italy, altitudinal gradient and Local Contribution to Beta Diversity for upland (dark grey circles) and lowland sites (light grey circles) calculated for the total fish community. Po River basin, Brenta River basin and Romagna rivers basin are shown

Fish species were classified according to Kottelat and Freyhof (2007), taking into account recent taxonomic determinations and common names as listed in FishBase (Froese & Pauly, 2017). Each species was categorized as native or exotic: a species was considered as native when naturally present in Italian watercourses, and as exotic when introduced by humans, irrespective of the time elapsed since the introduction. Fish species abundance was expressed using Moyle classes (Moyle & Nichols, 1973) ranging from 1 (lower abundance, 1–2 individuals per site) to 5 (higher abundance, more than 50 individuals per site). Hybrid specimens or uncertain species were excluded from this study in order to avoid taxonomic asymmetries.

Typically, in European rivers, fish communities change from Salmonidae to Cyprinidae dominated, along an altitude gradient, from headwaters to large rivers at low elevation (Aarts & Nienhuis, 2003). Taking into account such community shifts, study sites were divided into two groups: lowland sites (sites below 100 m above sea level) and upland sites (sites above 100 m above sea level). This limit is not absolute and it is not a strong physical barrier for fish species, but it was chosen based on earlier studies in the region (Aschonitis et al., 2018; Milardi et al., 2018) and separate typical lowland impacted environments from the less impacted ones, located in the uplands.

Water physico-chemical sampling was performed with standard methods in proximity to the fish sampling sites by Regional Environmental Protection Agency (ARPA) for Po River, Brenta River and Emilia-Romagna rivers and by Oglio River Water Authority for the Oglio River. Eight water physico-chemical variables were included as follows: water temperature ($^{\circ}\text{C}$), electrical conductivity ($\mu\text{S}/\text{cm}$), chemical oxygen demand (COD [O_2 mg L^{-1}]), biological oxygen demand (BOD_5 [O_2 mg L^{-1}]), total suspended solids (mg/L), total phosphorus (P mg L^{-1}), ammonia (N mg L^{-1}) and nitrate nitrogen (N mg L^{-1}).

2.3 | Stream order analysis

The stream order of each sampling site was calculated from Digital Elevation Model (DEM) data (ISPRA, Italian Institute for Environmental Protection and Research) through the ArcGIS 10.1 software. In order to harmonize the elevation model, the DEM layer was first resampled into 10 m pixel size. Then, using the Hydrology Spatial Analyst Tool, the flow direction and the flow accumulation based on DEM layer were calculated. Finally, for the entire river network generated by flow accumulation, the stream order with the Strahler method (Strahler, 1957) was calculated. This procedure resulted reliable for upland streams, while in the lowland, it was less accurate possibly due to the fact that in the lowlands, the flow direction and magnitude have been modified by humans. The Strahler stream order was thus manually checked and revised when necessary in lowland rivers and streams.

In order to balance the number of rivers sampled in each Strahler stream order, rivers were grouped into four classes based on stream order: class 1—rivers with 1 and 2 Strahler stream order, class 2—rivers with 3 and 4 stream order, class 3—rivers with 5 and 6 stream order and class 4—rivers with Strahler stream order higher than 6. As the drainage and irrigation canals located in lowlands could not

be assigned into any natural class, they were assigned into a separate class called "Drainage."

Overall, in the uplands, six sampling sites were included in stream order class 1, 41 in stream order class 2, 55 in stream order class 3, 6 in stream order class 4 and no sites were sampled in drainage canals. In the lowlands, no sampling sites were included in the first stream order class, 17 were included in stream order class 2, 53 in stream order class 3, 94 in the stream order class 4 and 40 in the drainage canals.

2.4 | Statistical analysis

All statistical analyses were performed for lowland (204 sampling sites below 100 m of altitude) and upland (133 sampling sites above 100 m of altitude) sites separately, taking also into account the distinction between exotic and native fish species.

2.4.1 | Species richness and local contribution to beta diversity in exotic and native fish species

To study the uniqueness of fish community composition across sites, the Local Contribution to Beta Diversity (LCBD) was calculated for each sampling site using the *beta.div* function in "adespatial" R package (Dray et al., 2018) based on Legendre and De Cáceres (2013). This method calculates the Total Beta Diversity (BD_{Total}) from the total variance of a site by species community table. The LCBD was derived by partitioning the BD_{Total} into the local contributions, and the sum of the LCBDs for all sites is equal to 1. For this metric, higher values of LCBD of a site indicate an unusual species composition compared with the average community in the data. From an ecological point of view, the LCBD values represent the degree of uniqueness of the sampling units in terms of community composition (Legendre & De Cáceres, 2013).

To investigate how LCBD and richness varies across stream order classes, the Kruskal–Wallis (KW) test (R function *kruskal.test*) was applied. The choice of Kruskal–Wallis test was due to the fact that data did not meet all assumptions of ANOVA, tested with *ad.test* function in "nortest" package (Gross & Ligges, 2015).

2.4.2 | Relative influence of main water physico-chemical variables on the local contribution to beta diversity

A machine learning method, Boosted Regression Trees analysis (BRT; Elith, Leathwick, & Hastie, 2008), was used to investigate how LCBD was influenced by water physico-chemical variables. BRT has been considered to be an efficient method to describe any nonlinear relationships between variables (e.g., thresholds) and it automatically incorporates interactions between variables. This approach differs from traditional regression methods as BRT analysis combines together a large number of simple tree models using the boosting technique to improve the predictive performance. BRT analysis further calculates the relative influence of predictors on response variable. The effect of predictors is showed through the fitted functions

that provide a useful basis for interpretation, although they are not perfect representation in case of strong interactions between predictors (Elith et al., 2008). BRT was performed with Gaussian distribution, bag fraction of 0.75 and shrinkage of 0.001 in the R software package "gbm" (Ridgeway & Southworth, 2017).

2.4.3 | Variation in exotic and native community dispersion among and within stream orders

In order to investigate the degree to which there is community structural variation within a stream order class, a test of homogeneity of dispersion (PERMDISP) was used (Anderson, 2006; Anderson, Ellingsen, & McArdle, 2006) with a function *betadisper* in the "vegan" R package (Oksanen et al., 2017). Through the average dissimilarity from individual observations to their group centroid, this test calculates the degree of dispersion, that is beta diversity (when based on presence-absence data) and the community structural variation (when based on abundance data; Anderson et al., 2006; Heino et al., 2013) within stream order and test if it differs among stream orders. The PERMDISP analysis was run using Gower dissimilarities on fish abundance data and Sørensen dissimilarity on presence/absence data. Moreover, we also investigated the degree of water physico-chemical dispersion within stream order classes using Euclidian distances. A permutation test with 999 permutations (*permutest* function) was used to compare the degree of within group dispersions among groups. A linear regression analysis was used to test the null hypothesis of no relationship between the distance of centroid based on abundance data (i.e., communities structural variation) and the distance to centroid of water physico-chemical variables (i.e., water physico-chemical heterogeneity) across sites (Heino et al., 2013).

2.4.4 | Differences in species contribution to beta diversity between native and exotic species and the relationship with species occupancy

We calculated the Species Contribution to Beta Diversity (SCBD) that shows the degree of variation of a species across the considered area (Legendre & De Cáceres, 2013). It can be considered as a measure of the relative importance of each species in affecting beta diversity (Heino & Grönroos, 2017). Linear regression was used to investigate the relationship between the SCBD values and the number of sites occupied for each species and the Kruskal-Wallis test was used to investigate difference in SCBD values between the lowlands and uplands.

All statistical analysis was performed in R software, version 3.4.3 (R Core Team, 2017).

3 | RESULTS

A total of 60 fish species were observed in the study area, with 38 native and 22 exotic species. In the upland sites, fish community

was composed of 24 native species and 11 exotic species, whereas in the lowland sites, 38 native and 22 exotic species were found (Supporting information Table S1).

Minimum, maximum, averages and standard deviations of water physico-chemical variables and altitude for lowland and upland sites are reported in Supporting information Appendix S1: Appendix A. Variation of water physico-chemical variables along stream order classes are shown in Supporting information Appendix S1: Appendix B. In summary, lowland sites showed the highest anthropogenic pollution, with the highest values of ammonia and nitrate nitrogen, chemical oxygen demand (COD), biological oxygen demand (BOD₅) and total phosphorus. Also, electrical conductivity, mainly due to brackish waters, and total suspended solids were higher in the lowland sites than upland sites. Due to the altitudinal gradient, the lowest water temperatures were detected in the upland sites.

3.1 | Species richness and local contribution to beta diversity in exotic and native fish species

Exotic fish species richness was higher in lowland sites than upland sites, where only few exotic species were recorded (Figure 2). The exotic species richness showed significant differences among stream order classes in the lowlands (KW $\chi^2 = 53.7$, $df = 3$, $p < 0.001$) and in the uplands (KW $\chi^2 = 71.2$, $df = 3$, $p < 0.001$) with a positive trend towards higher stream orders (Figure 2a, b). Native species showed significant differences among stream order classes in both lowlands (KW $\chi^2 = 54.0$, $df = 3$, $p < 0.001$) and uplands (KW $\chi^2 = 71.2$, $df = 3$, $p < 0.001$). In the lowlands, native richness peaked in stream order class 3 and was lowest in drainage canals, whereas in the uplands richness was highest in stream order class 4 and lowest in class 1 (Figure 2a, b).

Considering all fish species, BD_{total} for lowland and upland sites were 0.631 and 0.607, respectively. The distribution of LCBD values considering all species is shown in Figure 1. The highest values of LCBD in the lowland sites occurred in the Po River Delta and in South-East area of Emilia-Romagna region. In upland sites, LCBDs showed a high spatial variability across the studied area. According to the Kruskal-Wallis test, LCBD values did not show significant differences among stream order classes in the lowlands considering native species (Figure 3a; KW $\chi^2 = 1.7$, $df = 3$, $p > 0.05$). Whereas considering exotic species, LCBD values showed a significant difference among stream order classes (KW $\chi^2 = 9.0$, $df = 3$, $p < 0.05$) more evident between the stream order class 3 and drainage canals class (Figure 3a). In the uplands, LCBD values showed significant differences considering both native (Figure 3b; KW $\chi^2 = 24.7$, $df = 3$, $p < 0.001$) and exotic species (Figure 3b; KW $\chi^2 = 65.6$, $df = 3$, $p < 0.001$) along stream order classes, reaching the highest values in large rivers.

3.2 | Relative influence of main water physico-chemical variables on the local contribution to beta diversity

According to BRT analysis, the total suspended solids and the total phosphorus were retained as the most important factor affecting

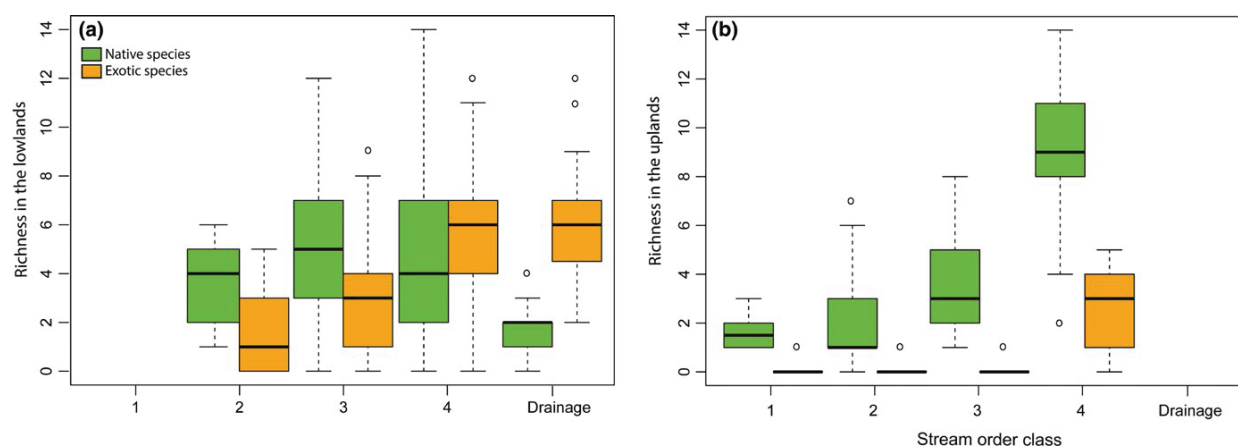


FIGURE 2 Boxplots representing the values of exotic (orange) and native (green) fish species richness in the lowlands (a) and uplands (b) along stream order classes

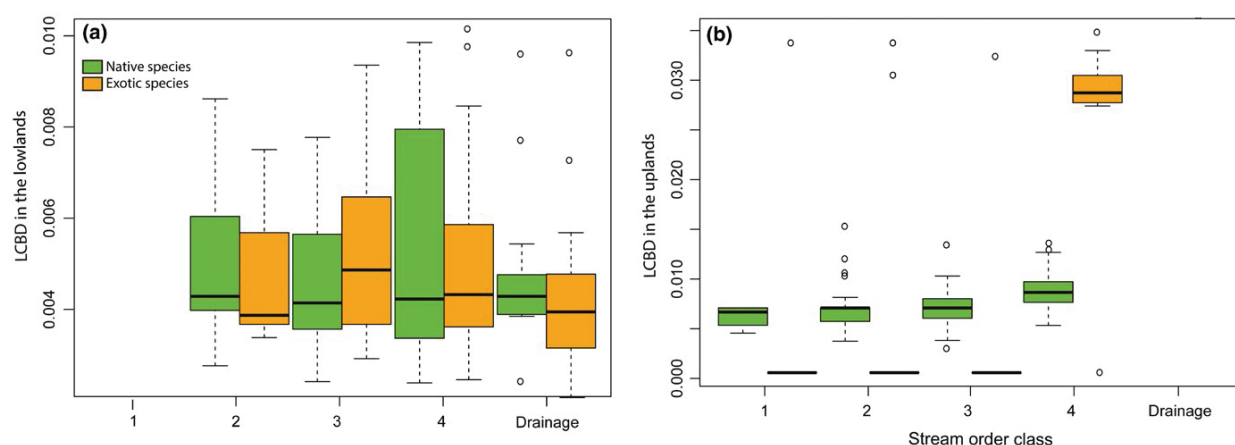


FIGURE 3 Boxplots representing Local Contribution to Beta Diversity (LCBD) values for exotic (orange) and native (green) fish species along stream order classes in the lowlands (a) and uplands (b)

LCBD values for both exotic and native species in the lowlands and in the uplands, respectively (Figure 4). As evident in the fitted functions, these predictors showed negative relationships with respective LCBD. The chemical oxygen demand (COD) was the second most important predictor among native species, for both lowland and upland sites, and it showed a positive relationship with LCBD. Among exotic species, a second important factor was total phosphorus in lowland sites, having a negative influence on LCBD at low phosphorus levels. In upland sites, nitrate nitrogen had the highest influence being positively related with LCBD, having a clear threshold above which LCBD notably rises.

3.3 | Variation in exotic and native community dispersion among and within stream orders

According to PERMDISP analyses, within stream order dispersion varied significantly among stream order classes (Figure 5)

for exotic species both in lowlands ($F_{(3, 200)} = 26.8, p < 0.01$), and uplands ($F_{(3, 129)} = 119.3, p < 0.01$), but also for native species in the lowlands ($F_{(3, 200)} = 20.2, p < 0.01$) and uplands ($F_{(3, 129)} = 61.4, p < 0.014$). Based on the pairwise comparisons, within stream order dispersion differed significantly in larger rivers regardless to altitudinal zones and in drainage canals network. Stream orders did not differ in their water physico-chemical heterogeneity (Supporting information Appendix S1: Appendix C) either in the lowlands ($F_{(3, 200)} = 0.4, p > 0.05$) or in the uplands ($F_{(3, 129)} = 0.5, p > 0.05$). According to linear regression analysis, within stream water physico-chemical heterogeneity had no significant relationship with community dispersion either for native and exotic species in the uplands ($R^2 = 0.004, p > 0.05$; $R^2 = 0.014, p > 0.05$, respectively) or for exotic ones in the lowlands ($R^2 = 0.0001, p > 0.05$). However, a weak but significant relationship was found for native species in the lowlands ($R^2 = 0.024, p < 0.05$).

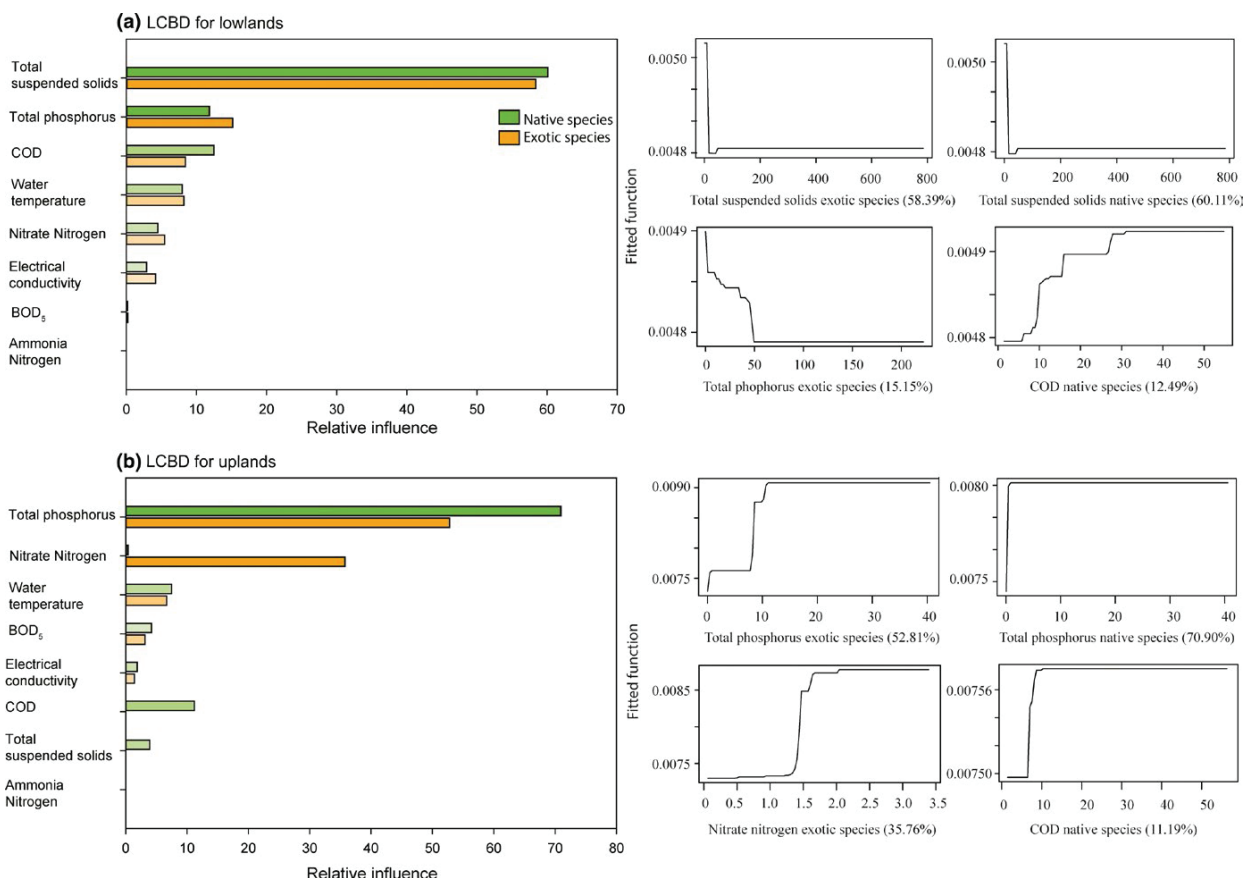


FIGURE 4 Boosted Regression Tree summary showing the relative influence of water physico-chemical variables on Local Contribution to Beta Diversity (LCBD) values for lowland (a) and upland (b) sites. The curves of fitted function for the most important variables are also shown in the panels on the right. COD: chemical oxygen demand

3.4 | Differences in species contribution to beta diversity between native and exotic species and the relationship with species occupancy

SCBD showed a linear positive relationship with the number of sites occupied for each species (Figure 6, both for exotic ($R^2 = 0.91$, $p < 0.001$) and native species ($R^2 = 0.90$, $p < 0.001$) in the lowland sites (Figure 6a) and in the upland sites (Figure 6b; $R^2 = 0.74$, $p < 0.001$ for native species; $R^2 = 0.31$, $p < 0.001$ for exotic species). SCBD values and species occupancy for each species are given in Supporting information Appendix S1: Appendix D. According to the Kruskal–Wallis test, no differences were found in SCBD values between exotic and native communities in the lowlands sites ($KW \chi^2 = 2.4$, $df = 1$, $p > 0.05$), whereas in the upland sites SCBD values were higher for native species than exotic ones ($KW \chi^2 = 8.3$, $df = 1$, $p < 0.01$).

4 | DISCUSSION

Large-scale diversity studies focusing simultaneously on exotic and native species diversity in freshwater ecosystems are still relatively

rare, although exotic species may play a strong role in native species diversity loss. This study investigated diversity patterns and their drivers among exotic and native stream fish species.

4.1 | Species richness and local contribution to beta diversity in exotic and native fish species

An increase of species richness from headwaters to lowland rivers was previously found not only in fish (Beecher, Dott, & Fernau, 1988; Chea, Lek, Ngor, & Grenouillet, 2017) but also in other taxa such as macroinvertebrates and diatoms (Finn, Bonada, Múrria, & Hughes, 2011; Stenger-Kovács, Tóth, Tóth, Hajnal, & Padisák, 2014) suggesting a general diversity pattern. Different mechanisms have been proposed to drive this pattern, including water temperature, river morphology (e.g., depth and width) and habitat diversity (Allan & Castillo, 2007). In our study, only exotic species richness increased with stream order classes with the highest exotic richness in the largest rivers and drainage canals network. In contrast, native richness showed an increase across stream order only in the uplands, whereas in the lowlands, native species richness decreased in large rivers and drainage

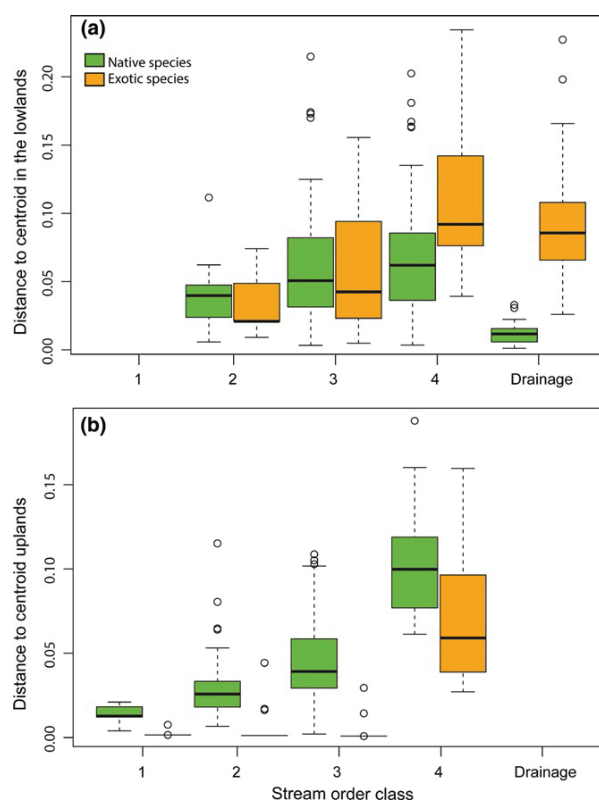


FIGURE 5 Boxplots showing mean distance to centroids along stream order classes, based on Gower dissimilarities of native (green) and exotic (orange) fish species in the lowlands (a) and in the uplands (b)

canals network. Anthropogenic disturbance (e.g., pollution, river modifications and flow regulation) could partly explain low native species richness in lowland rivers, and particularly in the artificial drainage network, but also past exotic species invasions could have played a central role in shaping this distribution. In fact, exotic species have pushed most lowlands native species on the edge of local extinction in several sites and displaced most of them on the boundary of their natural distribution to the highest reach of the rivers (Milardi et al., 2018). However, upstream rivers cannot provide suitable habitats for all such native species and cannot completely compensate the loss of native species of the lowlands.

The decline in native species richness was more evident in drainage canals network where direct effects (e.g., predation and competition) and indirect effects (e.g., changes in water quality) of some successful exotic invaders (e.g., *Silurus glanis* and *Cyprinus carpio*) were amplified due to the lower habitat complexity (Castaldelli, Pluchinotta et al., 2013).

Conversely to richness patterns, LCBD did not show clear differences among stream order classes in the lowland sites for either native or exotic species, suggesting that fish communities in different stream orders had typically similar degree of uniqueness. This result indicated a similar community

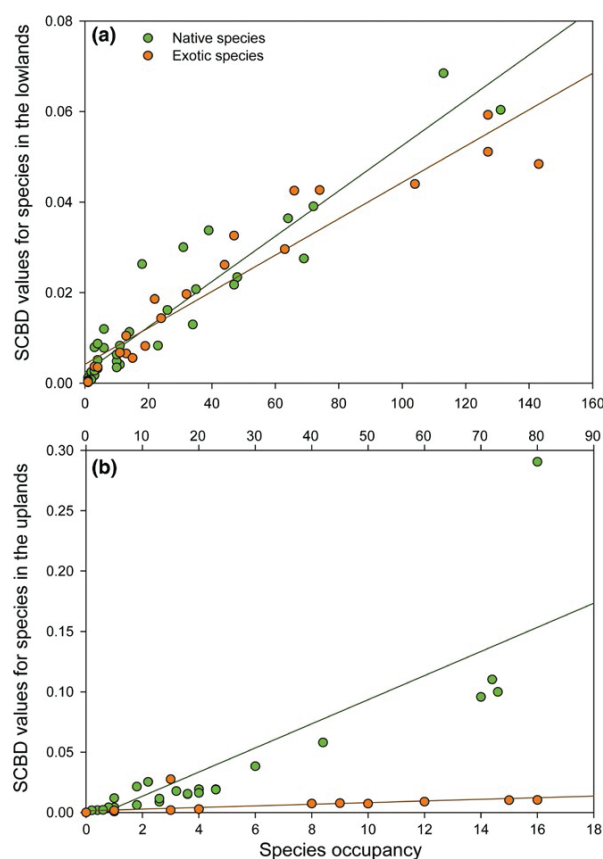


FIGURE 6 Relationship between Species Contribution to Beta Diversity (SCBD) and fish species occupancy (number of sites) for exotic (orange points) and native (green points) species, in the lowland (a) and the upland (b) sites. Please note that scales in occupancy for native and exotic species in panel b) are different for exotic (down) and native (up) species

composition across sites in the lowlands, probably driven by the most widespread exotic species such as the common carp or the crucian carp (*Carassius* spp.). These two species can also promote homogenization in communities especially in Palearctic regions (Toussaint et al., 2016; Villéger, Blanchet, Beauchard, Oberdorff, & Brosse, 2011). Upland sites (high stream order class) contributed strongly to beta diversity of exotic and native species, suggesting that large rivers at higher elevations could provide regionally unique habitats and conditions. Interestingly, high exotic species LCBD values in large upland rivers can be the result of an early invasion process from widespread exotic communities in the lowlands (Milardi et al., 2018) and thus underline a need for conservation and possibly restoration of such sites (Legendre & De Cáceres, 2013). These results suggest that not only headwater streams require conservation attention for native fish species, as suggested in other studies (Matthews, 1986; Paller, 1994), but that large rivers in the uplands can also contribute to regional diversity by harbouring unique native species communities.

4.2 | Relative influence of main water physico-chemical variables on the local contribution to beta diversity

Different water physico-chemical variables were proven important for LCBD in lowland versus upland sites when considering both exotic and native species. The large importance of total suspended solids in explaining LCBD (with negative relationship) reflects not only anthropogenic effects but also the effects of exotic ecosystem-engineering species such as crucian or common carp. In fact, these species can increase water turbidity through the resuspension of sediments while feeding, in turn causing a phytoplankton biomass increase and loss of submerged vegetation, while being able to tolerate high turbidity themselves (Crivelli, 1995). As a consequence, fish community tend to change reflecting this environmental shift, with for example a loss of clear water species with the water turbidity rise.

In upland sites, LCBD was mainly driven by total phosphorus, suggesting a strong role of nutrients on beta diversity patterns. Nutrients can affect beta diversity promoting the presence of highly tolerant species and negatively affecting the most sensitive species. Similar results were found also in Finnish lakes, where species richness of eutrophication-tolerant species increased towards higher nutrient loads (Olin et al., 2002).

Other authors suggested a strong influence of morphological factors (e.g., water depth, width, flow conditions or substratum typology) on diversity patterns, such as substrate features on diatoms (Jyrkänkallio-Mikkola, Heino, & Soininen, 2016), macroinvertebrates (Heino et al., 2013) and fish (D'Ambrosio, Williams, Witter, & Ward, 2009). Unfortunately, in our dataset, data on morphological features were not available, and the investigation of their role in affecting LCBD was not possible. However, supporting the importance of water chemistry, also Maceda-Veiga et al. (2017) recently found that salinization and nutrient pollution (such as nitrate, nitrite, phosphate supply) constitute one of the major threats to native fish, in addition to hydrological features.

4.3 | Variation in exotic and native community dispersion among and within stream orders

The degree of water physico-chemical heterogeneity did not vary among stream order classes either in uplands or in the lowlands, suggesting that water conditions do not differ among stream order classes. We also did not find a relationship between community dispersion and heterogeneity in water physico-chemical variables (except for native communities in the lowlands). The absence of such relationships was also found by Heino et al. (2013) in stream macroinvertebrate communities and by Jyrkänkallio-Mikkola et al. (2016) for diatoms. Different explanations already proposed for such a pattern could also be applicable here: (a) fish species distributions may not have been related only to water conditions but also to dispersal processes, (b) patterns are difficult to see at the community level due to the species-specific responses and

(c) the lack of important habitat descriptors such as river morphology (Heino et al., 2013). Although only the water physico-chemical descriptors were considered in this study, we expect that water physico-chemical patterns might reflect also other, more general, stream alterations due for example to agriculture or farm animals (Allan & Castillo, 2007). Thus, we conclude that the degree of community dispersions does not strongly depend on the level of water physico-chemical heterogeneity within stream order classes. The only exception was native species communities in the lowlands, which showed a weak relationship with water physico-chemical heterogeneity, perhaps indicating their somewhat higher sensitivity to water quality variations due to anthropogenic pressures or to exotic fish species presence as discussed above.

4.4 | Differences in species contribution to beta diversity between native and exotic species and the relationship with species occupancy

As we hypothesized, native species had higher SCBD values than exotic species, but only in the uplands. The fact that SCBD values did not often differ between native and exotic species in the lowlands could be the result of simplified native communities, composed by the few native species most resilient to the invasion process, as previously suggested by other studies in the same area (Lanzoni, Milardi, Aschonitis, Fano, & Castaldelli, 2018; Milardi et al., 2018). It may also indicate that exotic species communities are spatially structured, with different species dominating communities across sites (Clavero & García-Berthou, 2006). One more reason could be the positive relationship between SCBD and the species occupancy, suggesting that the most widespread fish species (which are often exotic species, too) can strongly affect beta diversity. For example, competition and predation mechanisms as well as the fact that exotic species are able to change environmental conditions can concur to exclude native species from a fish community. However, the expected positive relationship between abundance based SCBD values and species occupancy was previously found also in stream insects by Heino and Grönroos (2017) suggesting that species with high SCBD values are expected to have relatively high local abundance and high sites occupancy. Our results also confirm the predictability of SCBD values from species occupancy and abundance (Heino & Grönroos, 2017; da Silva, 2018).

However, perhaps counterintuitively, it is not always true that species with high SCBD (i.e., high contribution to beta diversity) are important to preserve diversity when exotic or native status was considered. For example, in lowland sites, the most widespread exotic species such as the stone moroko (*Pseudorasbora parva*), the crucian, carp and the common carp showed high SCBD but, due to the homogenization effect (Toussaint et al., 2016) and their ability to modify the environment (Breukelaar, Lammens, Breteler, & Tatrai, 1994; Chumchal, Nowlin, & Drenner, 2005; Alain J. Crivelli, 1983), they can negatively affect native species diversity. Of consequences, the high SCBD values can help to

identify the most abundant and widespread exotic species which could have negative effect on native communities. Likewise, high SCBD values in native species can identify species that not need conservation measures due to the high abundance and wide distribution, such as the chub (*Squalius squalus*) or the Italian bleak (*Alburnus albolella*). Contrarily, low SCBD values can identify rare native species, that for the low abundance and restricted distribution that require major conservation measures such as the Italian nase (*Chondrostoma soetta*), and the South European nase (*Protochondrostoma genei*), classified as endangered by IUCN and included in the Habitat Directive (Annex II). It is also possible that some low SCBD values were due to the low sites occupancy of species at the edge of their distribution such as the rainbow trout, *Oncorhynchus mykiss*, in the lowlands or the Eastern mosquitofish, *Gambusia holbrooki*, in the uplands.

Taking into account these aspects, these analysis outputs require accurate consideration as to the geographical range and the exotic or native species status. We encourage future research in this field to update the information available and to better understand the major drives of it.

5 | CONCLUSIONS

Due to the loss of native freshwater biodiversity worldwide (Strayer & Dudgeon, 2010), the need for the identification of priority areas for conservation (Hermoso, Clavero, & Kennard, 2012) and the limited conservation resources available; there are three main implications for future conservation strategies found in this paper: (a) not only headwaters require conservation measures but also large upland rivers are important in contributing to native fish diversity. These systems resulted in a low exotic species presence, promoting zones with high native diversity. (b) In upland sites, native species showed the highest contribution to beta diversity, but this pattern was not found in lowland sites, which shows the importance of protecting native communities in upland sites, while suggesting a general homogenization process in the lowland communities. (c) Some rare native species that are restricted to few sites can show low contribution to beta diversity, but such species may still need conservation actions due to their risk of local extinctions. This suggests to interpret the results of SCBD carefully, because the abundance of rare species is typically underestimated.

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DATA ACCESSIBILITY

Fish data used in this study are shown in Supporting information Table S1. Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.83g8j8f>

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BIOSKETCH

The authors have a background in species diversity distributions (J.S.), fish and invasion ecology (A.G., G.C., M.M.) as well as general ecology (E.A.F.). These different research lines were joined to investigate the spatial distribution of fish species and its drivers, considering the exotic/native species status.

Author contributions: J.S. and G.C. conceived the idea, A.G. collected the dataset and analysed the data, A.G. and J.S. led manuscript writing, M.M., G.C. and E.A.F. provided major input on the manuscript.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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4.3 How do exotic fish species affect the functional diversity?

[6] Milardi, M., Gavioli, A. *, Castaldelli, G., Soininen, J. (2020). Partial decoupling between exotic fish and habitat constraints remains evident in late invasion stages. *Aquatic Sciences* 82, 14.
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Partial decoupling between exotic fish and habitat constraints remains evident in late invasion stages

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ABSTRACT

We investigated the relationships between exotic freshwater fish invasions, environmental factors and ecofunctional diversity in streams. We used data from 335 stream sites, belonging to 105 watersheds and 3 basins in Italy, to test whether the exotic species invasion was dominated by species with generalist traits and whether the environment-ecofunctional trait relationships of exotic and native species would differ from each other. We also tested the hypothesis that ecofunctional uniqueness patterns between exotic and native species would be substantially different. We found that generalist traits were widespread in nearly all areas where exotic species occurred, but not all generalist traits were equally abundant in exotic communities. Only temperature tolerants, low oxygen tolerants and eurytopic traits were typically more dominant in exotic communities than native ones, suggesting that not all generalist traits are equally important in the invasion process and that more complex mechanisms of trait selection could take place. Environment-ecofunctional trait relationships of exotic and native species partly differed both in direction and magnitude, suggesting that invasion dynamics could partly decouple environment and biotic communities, but also that this decoupling might decrease at later invasion stages. Finally, site and trait ecofunctional uniqueness differed between exotic and native species. Exotic species ecofunctional diversity hotspots were located in human-disturbed areas, suggesting that human disturbance might play a strong role in invasion patterns. We advocate for a wider use of ecofunctional approaches in conservation studies in the future, as they could be a key to understand complex ecological processes such as exotic invasions.

Keywords: biodiversity conservation, alien species, species diversity, functional diversity, ecofunctional uniqueness, native species

INTRODUCTION

The study of geographical distribution of species and spatial variation of biodiversity on Earth has a long history. Recently, hundreds of studies have underlined the importance of biodiversity for ecosystem functioning and resilience to different pressures (e.g. Hooper et al. 2005), as well as its general worldwide decline (Wiens 2016). Much effort has been devoted to investigate the dispersal and evolutionary processes driving taxonomical biodiversity in the past (see e.g. Herrera 1995). However, the need for a shift from taxonomical to trait-based approaches in biodiversity research has been acknowledged for over two decades, because of the close linkage between environment and ecological traits, as well as their fast response to environmental changes (see e.g. Poff et al. 2006; Violle et al. 2014). As a consequence, recent works shifted their attention towards functional diversity, exploring its spatial patterns and dynamics.

Functional diversity quantifies the distinctive combination of morphological, biochemical, physiological, structural, phenological or behavioral traits that characterizes living communities and has been advocated to measure ecosystem functioning more reliably than taxonomical diversity (Cadotte et al. 2011). Ecofunctional diversity, a subset of functional diversity, identifies the combination of ecological traits in communities, selected by environmental conditions and habitat availability (Poff and Ward 1990). Habitat diversity, geography, land use, soil or water chemistry might all play a role in determining which ecofunctional traits occur in a community (Comte et al. 2016). In this perspective, the habitat selects which traits prevail in a given environment, ultimately linking habitat diversity with species ecofunctional diversity. This linkage has been explored to infer ecological quality from ecofunctional diversity of biotic communities (Milardi and Castaldelli 2018; Mouillot et al. 2013; Pont et al. 2006; Schmutz et al. 2007), but exotic species invasions have been recognized as a potential confounding factor for this linkage.

Exotic species introduction is a global phenomenon, and one of the main causes of biodiversity loss (Leprieur et al. 2008; Sala et al. 2000) and community homogenization (Rahel 2000). A lot of attention has been devoted to investigate the consequences of exotic invasions on taxonomical diversity at different geographical scales (Sax and

Gaines 2003). A common notion in invasion biology suggests that successful invaders in environmentally degraded areas could be favored by their tolerance to a broad range of environmental conditions and their generalist adaptations (Lurgi et al. 2014; Marvier et al. 2004; Milardi et al. 2018b). If most invaders are generalists, invasion dynamics would override environmental drivers behind species and trait distributions and cause a peculiar ecofunctional diversity distribution for exotic communities, compared to that of native ones which had a longer environment-selection time and thus clear environment-trait relationships. However, such difference between exotic and native communities could decrease with time, as exotic species become more and more selected by environmental constraints after the initial invasion stage.

The overall ecofunctional structure and distribution of fish has been poorly studied, so far, and similarly little research exists on the impact of exotic invasions on the ecofunctional structure of Mediterranean freshwater fish (Colin et al. 2018; Villéger et al. 2013). As in many other geographical areas, exotic invasions are one of the main factors causing biodiversity loss, but this is particularly true in the Mediterranean region and its freshwater ecosystems (Crivelli 1995; Cuttelod et al. 2009; Médail and Quézel 1999). Only recently, an ecofunctional characterization of native and exotic fish species has been defined for some areas (Milardi and Castaldelli 2018), enabling further research on the linkages between biological invasions, ecofunctional diversity and the environment.

In this study, we focused on the northern Italian peninsula, previously highlighted as a good area to investigate the outcomes of freshwater fish invasions (Castaldelli et al. 2013; Gavioli et al. 2018; Gavioli et al. 2019; Lanzoni et al. 2018; Milardi et al. 2018a). We used a spatially-comprehensive dataset, including several river basins, to explore the spatial distribution of riverine freshwater fish ecofunctional traits and investigate the relationships between environmental factors and ecofunctional diversity of native and exotic species. We tested the hypothesis (H_1) that the invasion process would be largely led by less-specialized species, able to adapt to a wide range of environmental conditions (i.e. generalist species).

Under this hypothesis, we expected that generalist ecofunctional traits would be widespread and dominant in exotic species communities of invaded areas, as generalist species are usually leading the invasion process (e.g. Evangelista et al. 2008). However, we expected this dominance to decrease at late-invasion stages. We also tested the hypothesis (H₂) that the environment-ecofunctional traits relationships of exotic and native species would differ. In particular, we expected that the relationships would be overall weaker for exotic species, as native species traits have been selected from the regional

species pool by environmental factors over a longer time than the residence time of exotic species. We ultimately investigated the hypothesis (H₃) that ecofunctional traits of exotic and native species would have different spatial patterns (i.e. between upland and lowland streams), because lowland areas were highlighted as an invasion hotspot (Lanzoni et al. 2018; Milardi et al. 2018a). Finally, our results would help understanding the factors shaping the ecofunctional diversity of fish communities under exotic invasions, one of the major threats to native fish conservation worldwide.

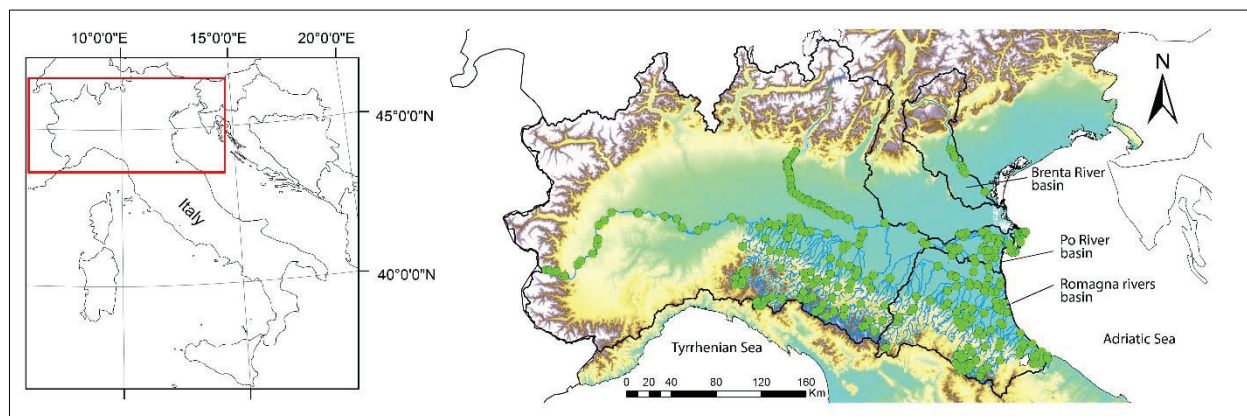


Figure 1 – Distribution of the 335 sampling sites used in this study (green dots), the watercourses and the three main basins considered in northern Italy.

MATERIALS & METHODS

Study area

We focused our investigation on northern Italy (Fig. 1), an area hosting more than 17 million inhabitants and impacted by agricultural activities and livestock farming. The study region has a Mediterranean continental climate, with an annual average precipitation of 1036 mm and a mean temperature of 12° C. Within this area lies the largest river basin in Italy, the Po River basin (71,000 km²), of which we included the Po River itself (in all its course), the Oglio River (one of the most important left tributaries of the Po River) and the right tributaries in the Emilia-Romagna region. We also included two additional groups of rivers outside of this basin: the Brenta River (north-east of the Po River basin) and watercourses of the Emilia-Romagna (south of the Po River basin). Overall, a total of 335 sampling sites (i.e. 335 stretches of 105 watercourses) were included in this study, covering heterogeneous habitats within river ecosystem (e.g. riffles, pools), and different altitudinal zones and environmental conditions (Fig. 1).

In the uplands, organic material originating from villages, small towns and livestock farms is the main

source of river pollution. Conversely, a high degree of urbanization and intensive agriculture characterize the lowland rivers, where high nutrient loads have led to eutrophication (Castaldelli et al. 2013). To support agricultural irrigation, a complex network of drainage canals has been established in the lowlands; this system is completely human regulated with hydrological management directed to both irrigation supply and drainage (Castaldelli et al. 2013; Milardi et al. 2017).

Data collection

Fish data were collected within monitoring programs of the Emilia-Romagna region (Regione Emilia Romagna 2008), the Padova Province (Provincia di Padova 2010), the Po River (Autorita' di bacino del Po 2008) and the Oglio River (Consorzio dell'Oglio 2016). The monitoring programs were carried out separately, in different years, over a relatively long-term period (1999–2010).

Fish sampling was performed by electrofishing, adapting the standard national monitoring guidelines to the particular conditions of each watercourse, and using direct current at 400–600 V and 4–5 A (Backiel

and Welcomme 1980; Reynolds 1996). River stretches were sampled once, during daylight, in an upstream zigzag direction, ensuring that the range of present macrohabitats of each site was fully surveyed (Hankin and Reeves 1988). Electrofishing is considered the best quantitative method for fish sampling in shallow waters, up to a maximum of 1 m (Zalewski and Cowx 1990) but its efficacy may be low in deeper waters, with big and mobile specimens, or with high conductivity. Such special conditions occurred in

almost all the lower stretches of rivers and in the canals of the lowlands. For this reason, at these sites, electrofishing was immediately followed up with a standard set of trammel nets (with variable mesh size from 90 to 5 mm), with the support of professional fishermen (Backiel and Welcomme 1980). In order to allow comparisons between sites (i.e. river stretches), the sampling effort at each sampling site was standardized based on area sampled, according to the national fish monitoring guidelines (APAT 2007).

Table 1 – Ecological functions and guilds (and their abbreviations) considered for each species in this study. Edited from Milardi and Castaldelli (2018).

Ecological function	Guild	Abbreviation	Description
Feeding	Planktivores	P	Plankton feeders
	Herbivores	H	Vegetation feeders
	Benthivores	B	Bottom feeders
	Invertivores	I	Invertebrate feeders
	Piscivores	Pi	Fish feeders
	Parasite	Pa	Ematophages
	Generalists	G	Unspecialized feeding
Reproduction	Lithophils	Lp	Spawning on stones or gravel
	Phytophils	Pp	Spawning on submerged vegetation
	Phytolithophils	PL	Spawning on stones or vegetation
	Psammophils	PS	Spawning on sand or mud
	Ostracophils	O	Spawning in molluscs
	Pelagophils or live breeding	LB	Pelagic spawners or live spawners
	Polyphils	Pps	Generalist spawners
Sea spawning	Sea	Saltwater spawners	
Migration	Short	S	Within or close to the site
	Intermediate	M	Up and downstream or into flooded areas
	Long	L	Anadromous and catadromous species
Tolerance	Low oxygen tolerants	OxT	Tolerance/intolerance to low oxygen (indicatively below 3 ppm)
	Low oxygen intolerants	OxInt	
	High temperature tolerants	HTT	Tolerance/intolerance to high temperature (indicatively above 20 °C)
	High temperature intolerants	HTInt	
Habitat use	Rheophiles	Rhe	Preferring fast flowing water
	Limnophiles	Lim	Preferring slow or no current
	Eurytopics	Eur	Having no preference on current velocity
	Clear water	CW	Clear water adapted
	Turbid water	TW	Turbid water adapted
	Wide range of conditions	WR	Adapted to a wide range of water turbidity

Fish species were classified according to Kottelat and Freyhof (2007), taking into account recent taxonomic determinations and common names as listed in FishBase (Froese and Pauly 2017). Each species was categorized as native or exotic: a species was considered as native when naturally present in Italian watercourses and as exotic when human-introduced (IUCN Comitato Italiano 2019; Milardi et al. 2018a), irrespective of the time elapsed since the introduction (see Supplementary Table 1 for further details on introduction dates). Hybrid specimens or uncertain species were excluded from this study in order to avoid taxonomic asymmetries. Abundance of

each species was expressed with Moyle classes (Moyle and Nichols 1973) ranging from 1 (lower abundance, 1-2 individuals per site) to 5 (higher abundance, more than 50 individuals per site). However, numerical abundance classes tend to overestimate the ecological significance of small-bodied species and underestimate that of large-bodied ones. A weight was thus assigned to each species based on their average size published in the literature (1=small body up to ~150 g; 2=medium body ~150–400 g; 3=large body over ~400 g) and multiplied by Moyle abundance classes, in order to obtain a more ecologically-balanced representation of the

community, closer to relative biomasses (i.e. body-mass-corrected abundances, hereafter referred to simply as abundances, Milardi and Castaldelli 2018).

Water physicochemical sampling was performed with standard methods in temporal and spatial proximity with the fish sampling, by different Regional Environmental Protection Agencies (ARPAs, in Italian) for the Po, the Brenta and the rivers in the Emilia-Romagna region. The Oglio River Water Authority carried out the water sampling in the Oglio River. Eight physicochemical variables were monitored: water temperature (T ; °C), electrical conductivity (EC ; $\mu\text{S cm}^{-1}$), chemical oxygen demand (COD ; $\text{O}_2 \text{ mg l}^{-1}$), biological oxygen demand (BOD_5 ; $\text{O}_2 \text{ mg l}^{-1}$), total suspended solids (TSS ; mg l^{-1}), total phosphorus (P ; mg l^{-1}), ammonia (NH_4^+ ; mg l^{-1}) and nitrate nitrogen (NO_3^- ; mg l^{-1}). The geographical position (longitude – $Long$, latitude – Lat) and the altitude (Alt) were also considered.

Fish ecofunctional traits

In order to investigate the ecofunctional composition of fish communities, five different ecological functions were taken into account: feeding, reproduction, migration, tolerance and habitat use. These functions were included because of their ecological significance and close relationship with the environment (Noble et al., 2007; Milardi and Castaldelli, 2018). Within these ecological functions, all fish species were classified in guilds, each representing an ecofunctional trait and therefore referred as such hereafter (Table 1, see also Noble et al. (2007)).

Ecological functions, guilds and classification for most species in this study were taken from Milardi and Castaldelli (2018), where all available information was used to identify appropriate guilds for each species. Guilds were mutually exclusive (i.e. each species could be assigned to only one guild within each ecological function) and species were classified according to their prevalent adult characteristics (see also Table 1 in Milardi and Castaldelli 2018 for a more detailed explanation), thus partly discounting ontogenetic shifts (e.g. in diet or habitat) and ecological plasticity. The same methodology was applied to classify euryhaline species that were not included in the previous work (see also Supplementary Table 1).

Spatial distribution of exotic species generalist traits

We focused our analysis on generalist ecofunctional traits, with the aim to verify their spatial and community dominance within the exotic

communities, therefore selecting all areas with exotic species presence. Additionally the patterns observed for exotic communities were compared with those found in native communities over the same areas, to investigate any differences in dominance of generalist traits.

Inside each ecological functions, the ecological guilds indicative of no preference for, or no adaptation to, specific environmental conditions (i.e. generalist traits) were selected (ecological function – ecological guild): Feeding – Generalists (feeding on any source), Reproduction – Polyphils (spawning on any substrate), Tolerance – Low oxygen tolerants and High temperature tolerants (not selective for oxygen or temperature), Habitat use – Eurytopics (adapted to a wide range of current velocities) and Wide range of turbidity conditions (*idem* for turbidity).

To assess the spatial distribution of generalist ecofunctional traits, the number of sites in which generalist traits were detected was expressed as a share (percentage) of the total sites with exotic species presence (Table 2). To assess the community dominance of generalist ecofunctional traits, the share (percentage) of these traits in the communities was calculated using the abundances of all species (exotic and native) possessing each generalist trait (Table 2). Both distribution and dominance were also calculated separately for native and exotic communities, at each site (Table 2).

The community dominance of generalist traits of exotic and native species communities was also investigated in the area where exotic and native species distributions overlapped, testing the null hypothesis that exotic and native communities would not differ in generalist trait dominance. Differences in generalist trait dominance were tested using the paired t-test (paired-t, a parametric paired test) and the Wilcoxon signed-rank test (Wilcoxon, a non-parametric paired test), which investigate differences in the mean and median, respectively. Testing was performed using the PAST 3.06 software (Hammer et al. 2001).

The ArcGIS software (ESRI 2011) was used to map the spatial distribution of ecofunctional traits in exotic communities, and to investigate the spatial autocorrelation of each generalist trait, using the *Spatial Autocorrelation (Global Moran's I)* tool. The null hypothesis of the Global Moran's I analysis is that the attribute being analyzed is randomly distributed among the features in the study area. Being an inferential statistic, positive (and significant) values of

the Global Moran's I indicate spatial clustering of a generalist trait.

Table 2 – Distribution and average abundance of generalist ecofunctional traits in the area where native and exotic species distribution overlapped

Ecological Function	Trait	Exotic community		Native community	
		Distribution (% sites with trait presence)	Average dominance (% of fish community)	Distribution (% sites with trait presence)	Average dominance (% of fish community)
Feeding	Generalists	80.9	18.6	67.0	22
Reproduction	Polyphils	31.1	4.9	29.2	8.3
Tolerance	Low oxygen tolerants	98.1	95.9	84.2	53.3
	High temperature tolerants	97.6	94	84.2	49.4
Habitat use	Eurytopics	94.7	71	91.4	65.7
	Wide range of turbidity	79.4	25.3	94.3	71.9

Exotic and native species environment-ecofunctional traits relationships

Land cover data were obtained from the CORINE database (2012, <https://www.eea.europa.eu/data-and-maps/data/copernicus-land-monitoring-service-corine>). In the lowlands, where estimation of watershed areas is more difficult due to low slopes and human-regulated flow, the land cover of the whole river basin or of the administrative province was used. CORINE land cover classes were merged in five categories based on the main land use in order to better describe the study area: urban use (*Urban*), agricultural use (*Agri*), forest (*Forest*), other natural area (*OtherNat*), freshwater (*Fresh*) and brackish water (*Brack*). Land cover was expressed as the share of each of these categories in the watershed of each site.

Relationships between geographical variables, land use features, water physico-chemical variables and fish ecofunctional traits were explored using the distance-based Redundancy Analysis (db-RDA, Legendre and Anderson 1999). This analysis is normally used to analyze species-by-site data, but here it was used to analyze trait-by-site data (i.e. the total body-size-corrected abundance of all species with a certain ecological trait at each site). One of the advantages of db-RDA is that it can be used with any non-Euclidean distance measures (Legendre and Anderson 1999). Therefore, taking into account the work of de Bello et al. (2013), Gower distance measures were used because of their better suitability in detecting changes in ecofunctional diversity along

environmental gradients. Linear dependencies between variables were assessed through the Variance Inflation Factors (VIF) and variables showing high collinearity (VIF > 10) were removed from the analysis (Borcard et al. 2011). Explained variance in db-RDA was reported through adjusted r^2 values (correct constrained variance).

Matrices of traits-by-site data were Hellinger transformed (Legendre and De Cáceres 2013; Legendre and Gallagher 2001) to standardize variations among both species and community size. Environmental variables expressed as percentages were arcsine transformed, while all the other ones were log-transformed.

These analyses were performed in R software version 3.4.3 (R Core Team 2017): db-RDA analysis used the *capscale* function within the 'vegan' R package (Oksanen et al. 2017) on all species, and also separately for native and exotic species.

Ecofunctional uniqueness patterns of native and exotic species

Stream order for each site was calculated using a Digital Elevation Model (DEM) (<http://www.sinanet.isprambiente.it/it/sia-ispra/download-mais/dem20/view>), resampled into 10 m pixel size to harmonize it. Flow direction and accumulation, as well as the watershed of each sampling site, were calculated based on the DEM layer. For the entire river network generated by flow accumulation, stream order was derived with the Strahler method (Strahler 1957). This procedure was reliable for upland streams, but it was less so in the lowland, possibly due to the fact that flow direction

and magnitude in the lowlands are not always natural because of human intervention. The stream order was thus manually checked and revised when necessary in lowland rivers and streams. Rivers were grouped into four classes based on stream order: class 1 (Strahler stream order 1 and 2), class 2 (stream order 3 and 4), class 3 (stream order 5 and 6) and class 4 (stream order > 6). As drainage and irrigation canals could not be assigned into any natural class, a separate class called "Canals" was created. Canals are man-made environments, usually characterized by low habitat heterogeneity and controlled hydrology, located in the lowlands southwest of the Po River, near its delta.

The ecofunctional uniqueness of communities across sites was investigated with the Local Contribution to Beta Diversity (LCBD) and Species Contribution to Beta Diversity (SCBD) approaches, developed by Legendre and De Cáceres (2013), accounting for stream order. These analyses were originally developed to calculate the total beta diversity from the total variance of a site, partitioning the total variance into local contributions to beta diversity (i.e. LCBD), and species contributions to beta diversity (i.e. SCBD) across the area, respectively. However, for the first time, the same approach was applied here to the ecofunctional diversity of communities: high values of LCBD indicate a unique ecofunctional composition of the local community

(arising from a combination of low ecofunctional diversity, low species richness and a rare distribution in the sites) and low values of SCBD highlight those ecofunctional traits that are the most unique in the data (arising from the uniqueness of traits and their rare distribution in the dataset).

Uniqueness analyses were performed in R software version 3.4.3 (R Core Team 2017) through the 'vegan' R package (Oksanen et al. 2017) and the 'adespatial' R package (Dray et al. 2016) on all species, and also separately for native and exotic species.

RESULTS

A total of 59 fish species were detected in the study area; of these 37 were native and 22 were exotic species. Each of these species was assigned to guilds within ecological functions, as defined in Table 1 (see also Supplementary Table 1).

Spatial distribution of exotic species`generalist traits

Exotic species were rather widespread in the area (209 out of 335 sites, 62.4% of the total) and occurred mostly in the lowlands. Exotic species occasionally reached rather high abundances (abundance ≥ 40 in 12 sites, Fig. 2a, and constituting 100% of the fish community in 9 sites). Overall, generalist traits were overall widely distributed in the area occupied by

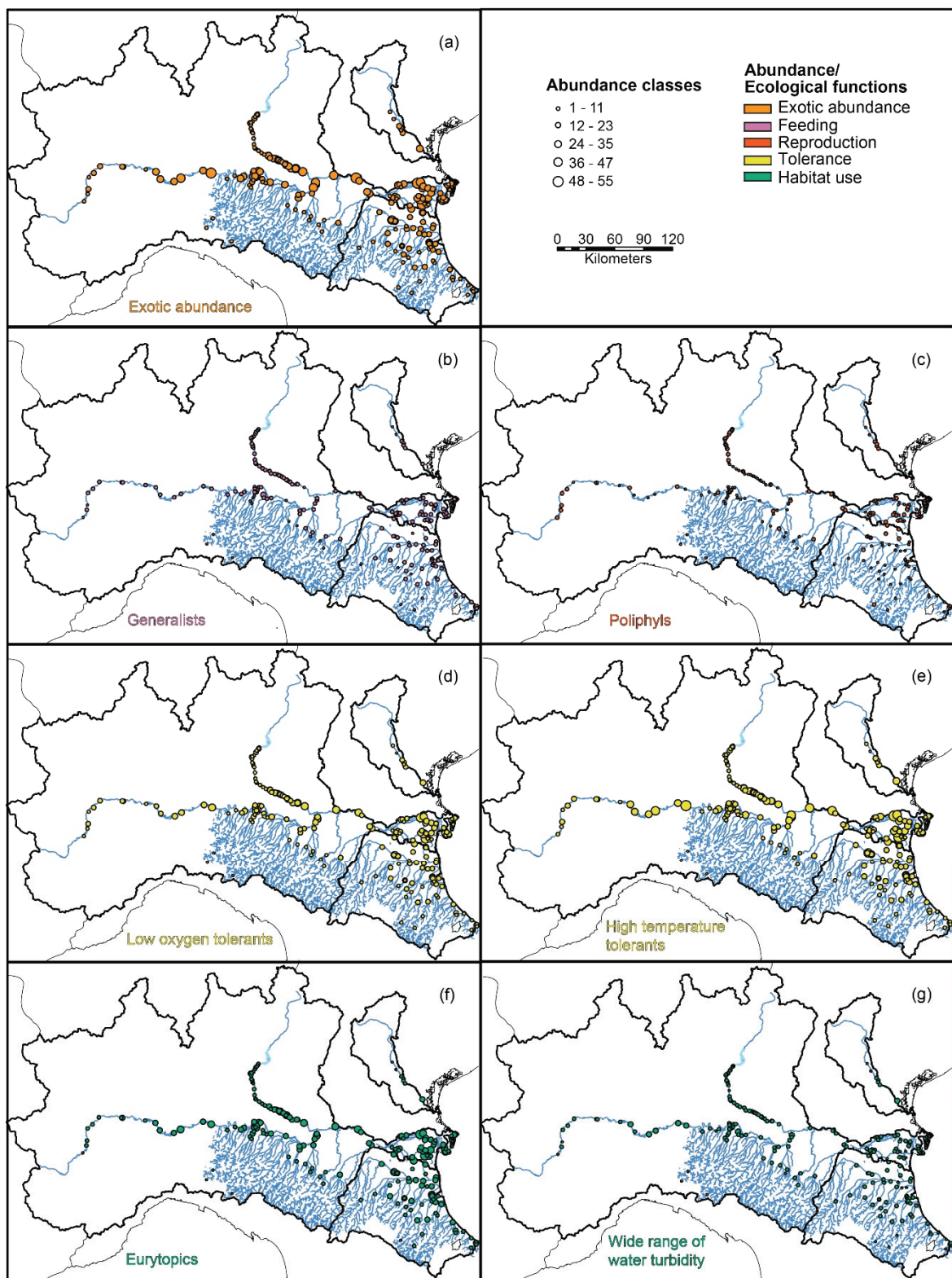


Figure 2 – Spatial distribution of exotic abundances in the study area (a) and respective abundances of generalist traits within different ecological functions. Feeding – Generalists (b), Reproduction – Polyphyls (c), Tolerance – Low oxygen tolerants (d) and High temperature tolerants (e), Habitat use – Eurytopics (f) and Wide range of turbidity conditions (g).

exotics, but not all traits were equally dominant in the exotic communities. The three most widespread generalist traits were low oxygen tolerance, high temperature tolerance and eurytopic adaptation to current velocity (98.1%, 97.6% and 94.7% of the sites, respectively, Fig. 2d, e and f, Table 2). The least

widespread traits were polyphily and adaptation to a wide range of turbidity conditions (31.1% and 79.4% of the sites, respectively, Fig. 2c and g).

Generalist feeder traits were relatively widespread (80.9% of the sites, Fig. 2b), but were a minor component of the community (18.6% of the

total abundance, Table 2), on average. Polyphil traits dominance was also very low, totaling 4.9% of the overall abundance, on average. Generalist traits were relatively slightly less widespread within the native species communities present in the same area, with the exception of adaptations to a wide range of turbidity conditions (94.3% of the sites, Table 2). However, rather surprisingly, some generalist traits were in general more dominant in native communities than exotic ones (H_1). Generalists feeders, polyphils and species adapted to a wide range of turbidity were all more dominant in native communities than exotic ones, while high temperature tolerants, low oxygen tolerants and eurytopics and were less dominant (Table 2).

Differences in dominance of generalist traits between native and exotic communities were statistically significant for adaptations to a wide range of turbidity, high temperature tolerance and low oxygen tolerance (paired-t $P < 0.01$, Wilcoxon $P < 0.01$, for all these traits), as well as for polyphily and eurytopic adaptation to current velocity (paired-t $P < 0.05$, Wilcoxon $P < 0.05$), but not for generalist feeders (paired-t $P = 0.19$, Wilcoxon $P = 0.85$). All generalist traits showed a significant spatial clustering (Global Moran's $I > 0$; P -values < 0.001 , for all traits), indicating spatial autocorrelation.

Exotic and native species environment-ecofunctional traits relationships

Environmental variables did not show collinearity ($VIF < 10$) and they were thus all included in the db-RDA analysis. The total amount of ecofunctional trait variance in the communities explained by db-RDA was 41.90% (Fig. 3a). The first axis explained 30.13% of the variance and the second axis explained 4.36% of the variance (Fig. 3a). Most ecofunctional traits were related to environmental features (e.g. rheophile (Rhe), low oxygen intolerant (OxInt) and clear water (CW) adapted species at higher elevations and vice versa), but some traits (e.g. herbivore (H) or parasite (Pa) feeding) did not clearly relate with environmental variables (Fig. 3a). Overall, the environmental factors most strongly related with ecofunctional traits were altitude, temperature and stream order class, which are all closely linked to each other and influenced both native and exotic species. The db-RDA analysis conducted exclusively on native species (50.12% of variance explained) showed a clear distribution of ecofunctional traits (Fig. 3b, the first axis explained the 29.43% of the variance and the second axis explained the 11.63% of the variance), with clear distinctions

related to environmental features, similar to those found in the general analysis. Exotic species had an overall lower linkage with environmental variables (35.46% of variance explained) (Fig. 3c, the first axis explained the 15.23% of the variance and the second axis explained the 7.77% of the variance). In general, these three analyses revealed similarities along the first ordination axis in terms of the key environmental variables (altitude, forests, temperature), whereas results differed clearly more for the second axis in terms of which environmental variables drove trait composition.

Ecofunctional uniqueness patterns of native and exotic species

According to the LCBD analysis, the degree of uniqueness of the communities' ecofunctional composition per each stream order class showed a clear decreasing pattern with increasing stream order class (Fig. 4a). Communities in lower stream orders (in the uplands) had high values of ecofunctional uniqueness, but communities in canals also showed a surprisingly high degree of uniqueness compared with other lowland sites (Fig. 4a). According to the SCBD analysis, high ecofunctional uniqueness was associated with clear water adaptation, intermediate migration and low oxygen tolerance traits (Fig. 4b). Herbivorous, planktivorous and parasitic feeding traits were associated with low uniqueness (Fig. 4b). The ecofunctional uniqueness of native communities (LCBD analysis) followed a similar pattern than that underlined in overall communities (Fig. 4c), but a comparison with exotic species (Fig. 4e) revealed that the latter contributed the least to uniqueness in higher stream orders and canals. Agreeing with our hypothesis H_3 , uniqueness patterns differed between native and exotic species.

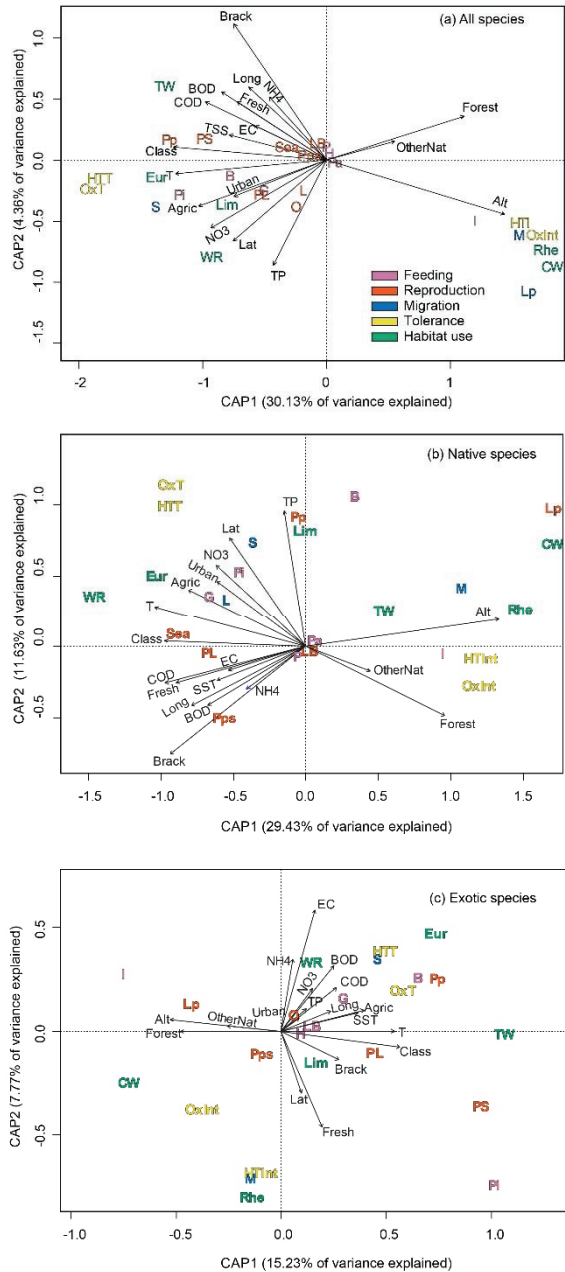


Figure 3 – Plot of distance-based RDA of all species (a), native species (b) and exotic species (c) fish ecofunctional traits composition (colored labels) using geographical variables, land cover classes, water physico-chemical variables as explanatory variables (black labels). Fish ecofunctional traits abbreviations are given in Table 1.

Among native species, lithophilic, low oxygen tolerant and clear water traits contributed the most to uniqueness according to the SCBD analysis (Fig. 4d), while planktivory, pelagic spawning and parasitism contributed the least. Among exotic species, piscivory, phytophily and benthivory were the traits that contributed the most to uniqueness according to the

SCBD analysis (Fig. 4f), while low oxygen tolerance, herbivory and pelagic spawning contributed the least.

DISCUSSION

Our results partly agreed with our initial hypothesis (H_1) that generalist traits were widespread in nearly all areas with exotic species presence, but not all generalist traits were equally abundant in the exotic communities. Moreover, only temperature tolerants, low oxygen tolerants and eurytopic traits were typically more dominant in exotic communities than native ones, suggesting that not all generalist traits are equally determinant in the invasion process and that more complex mechanisms of trait selection could be in place. Our analyses underlined strong linkages between the environment and ecofunctional traits for both native and exotic species but, as we hypothesized (H_2), environmental variables explained overall a lower proportion of exotic species traits than of native traits. This suggests that the linkage between exotic traits and environmental factors was less evident, i.e. that the environment explained less the trait composition of exotic species than that of native species, even at the late-invasion stage. This suggests that invasion dynamics could partly override habitat selectivity resulting in a partial uncoupling of environment and biotic communities, and that these effects last long. Finally, we found some differences in the ecofunctional uniqueness patterns of native and exotic species and their respective ecofunctional traits, being in line with our third hypothesis (H_3). Exotic species ecofunctional diversity hotspots were common in human-disturbed areas, suggesting that human disturbance might play a strong role in invasion patterns.

Spatial distribution of exotic species' generalist traits

It was clear from our results that exotic species presence was relatively low in upland areas, likely halted by natural gradients (e.g. temperature and habitat factors) or perhaps by physical dispersal barriers (Dynesius and Nilsson 1994; Tockner and Stanford 2002).

On the contrary, exotic species were widespread in the lowlands, therefore still exerting a significant propagule pressure in the less-invaded areas in the foothills (Lockwood et al. 2005; Simberloff 2009).

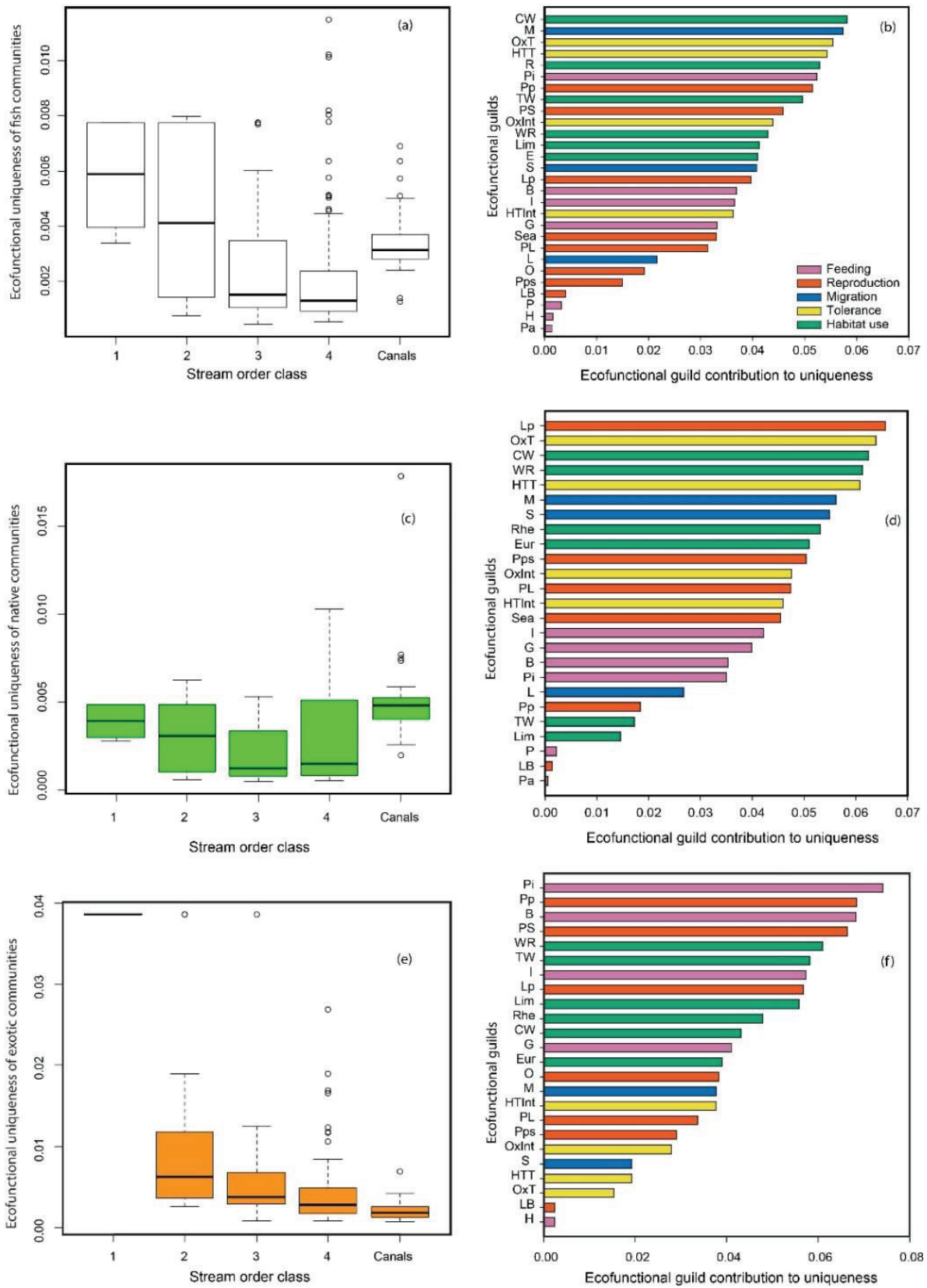


Figure 4 – Ecofunctional uniqueness of fish communities along a stream order gradient based on Local Contribution to Beta Diversity, LCBD (a) and contribution of each fish guild to uniqueness based on Species Contribution to Beta Diversity, SCBD (b). Uniqueness patterns were also analyzed separately for native (LCBD (c), SCBD (d)) and exotic (LCBD (e), SCBD (f)) species. Guild abbreviations are given in Table 1

Generalist traits were widespread in exotic communities of the lowlands, but not all traits were equally represented. Furthermore, generalist trait distribution was not the result of a random process, but different selection processes operate on different ecological functions (H_1). Some traits related to broad tolerance and flexible habitat use (temperature tolerance, low oxygen tolerance and adaptation to a wide range of water current conditions) were dominant among exotic communities (and more so than in native communities). However, reproductive polyphily and adaptation to a wide range of turbidity were more dominant in native communities over the same area, indicating that perhaps not all generalist traits are equally relevant to determine invasion success. The timeline of invasion processes could partly explain this complex result: early invasion phases could be spearheaded by true generalists, but on the long run more specialized species can also be introduced and spread in the area, overriding some of the overall invasion patterns (Nagelkerke et al. 2018). As this area is in a late invasion stage (Milardi et al. 2018a), what we observe could be the result of the complex interactions between species-specific introduction timing and mechanisms of dispersal and interaction with native species, as well as the overall outcome of longer-term environmental selection of exotic communities. These mechanisms could not be fully disentangled through our analysis, and should be further investigated in future studies, also considering that the spatial scale covered could influence the results (Taylor et al. 2019).

Exotic and native species environment-ecofunctional traits relationships

Our results confirmed that there are differences in how the environmental variables influence the distribution of traits for native and exotic species (H_2). The fact that native species ecofunctional traits are more clearly coupled with the environment confirms our initial hypothesis of a longer selection time. This tighter coupling of environmental niches was found in other taxa (Marks and Lechowicz 2005), but very few studies have dealt with this aspect in fish communities, so far (but see e.g. Buckwalter et al. 2018). Ecological theory predicts that exotic species should be equally influenced and selected by the environment, eventually, but evidently this selection could take a much longer time than that elapsed in our study area (Lambrinos 2004). The lower influence of the environment on exotic species ecofunctional traits suggests that invasion dynamics might still play a role

in the observed ecofunctional distributions (Strayer et al. 2006). Among invasion dynamics, the introduction of new species through fisheries stocking practices and active dispersal by anglers could confound spatial distribution patterns and their linkage with the environment, for both exotic and native species (Hesthagen and Sandlund 2007; Vörösmarty et al. 2010). Moreover, invasion processes are not driven by abiotic factors only, and the invasion of one species could facilitate the invasions of other species (Simberloff and Von Holle 1999), a mechanism previously suggested to be at play in this area (Lanzoni et al. 2018; Milardi et al. 2018a). Our results confirmed that exotic species are less affected by habitat filtering than native species, even at late-invasion stages.

Ecofunctional uniqueness patterns of native and exotic species

Our results (H_3) revealed a low ecofunctional trait diversity in mountain streams, which typically have lower habitat complexity and resource availability but higher habitat quality than rivers in the lowlands (Aschonitis et al. 2018; Bouska 2018). This could also be linked to a lower taxonomical diversity in these areas (Gavioli et al. 2019) as species number is known to affect uniqueness measures (Legendre 2014). Conversely, ecofunctional diversity was relatively higher in higher stream orders and in the lowlands, with the exception of canals, which had a lower ecofunctional diversity than natural rivers in the same areas. Canals should have low-heterogeneity habitats, due to their artificial nature, which can partly explain this result. However, our analysis highlighted that native species low ecofunctional diversity played a strong role in shaping this uniqueness pattern. Previous studies have underlined how freshwater fish invasions have caused a severe decrease of native taxonomical diversity at the local level, especially in canals (Castaldelli et al. 2013; Milardi et al. 2018b) and low species richness could be at the root of the low native ecofunctional diversity seen in canals. On the contrary, exotic ecofunctional diversity was highest in the lowlands and particularly in canals, which could be caused by a high number of ecofunctionally diverse exotic species. Canals in this area are indeed hotspots of exotic species diversity (Lanzoni et al. 2018) and host the highest number of exotic species in our dataset. This is somewhat counterintuitive, given the canals' simplified habitats, but could be explained by the higher degree of human disturbance. In the studied canal network, human intervention

continuously causes redistribution of fish species between different canals, thus likely increasing the spread and colonization of exotic species (Castaldelli et al. 2013). Our results suggested that exotic invasions might contribute to shape the spatial patterns of ecofunctional diversity in fish communities.

Clear water, rheophilic, intermediate migration, phytophilyc, lithophilyc and piscivorous species seem to contribute the most to the overall ecofunctional uniqueness of fish communities in the area, being typical traits of native species and most likely belonging to species with intermediate site occupancy. However, perhaps more interesting are those ecofunctional traits which are rarest in the dataset, as they highlight the presence of ecofunctionally unique and relatively rare native marine species (e.g. planktivores), temporarily entering estuarine and upstream freshwater areas. Rare ecofunctional traits also highlight the presence of hard-to-detect exotic species (e.g. herbivores, such as grass carp, *Ctenopharyngodon idella*), which were found only in canals during standard monitoring, but are also present elsewhere in the Po River basin (Milardi et al. 2017; Milardi et al. 2015). Further investigations are needed to unravel the exact contribution of species richness, ecofunctional diversity and its spatial distribution on the ecofunctional uniqueness of communities, as well as the relationship between ecofunctional uniqueness and trait diversity.

CONCLUSIONS

Our results underlined how the decoupling of the environment-ecofunctional trait relationships of exotic species is still detectable in late invasions stages, and how invasions might affect the ecofunctional diversity patterns of fish communities. However, other factors potentially affecting functional diversity (e.g. climate change and habitat loss) should also be investigated, as they might provide useful insights on the final outcome of exotic and native species interactions.

Our results also highlighted the importance of extending the analysis from taxonomical to functional diversity, when attempting to address the complexity of species interactions in invaded communities (see e.g. Colin et al. 2018; Godoy 2019; Trivellone et al. 2014). We thus advocate for a wider use of ecofunctional approaches in the future to evaluate the consequences of exotic species invasions (Griffiths

and Harris 2010; Loiola et al. 2018; Schlaepfer et al. 2011).

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4.3 How do exotic fish species affect the functional diversity?

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Exotic species invasions undermine regional functional diversity of freshwater fish

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Exotic species invasions often result in native biodiversity loss, i.e. a lower taxonomic diversity, but current knowledge on invasions effects underlined a potential increase of functional diversity. We thus explored the connections between functional diversity and exotic species invasions, while accounting for their environmental drivers, using a fine-resolution large dataset of Mediterranean stream fish communities. While functional diversity of native and exotic species responded similarly to most environmental constraints, we found significant differences in the effects of altitude and in the different ranking of constraints. These differences suggest that invasion dynamics could play a role in overriding some major environmental drivers. Our results also showed that a lower diversity of ecological traits in communities (about half of less disturbed communities) corresponded to a high invasion degree, and that the exotic component of communities had typically less diverse ecological traits than the native one, even when accounting for stream order and species richness. Overall, our results suggest that possible outcomes of severe exotic species invasions could include a reduced functional diversity of invaded communities, but analyzing data with finer ecological, temporal and spatial resolutions would be needed to pinpoint the causal relationship between invasions and functional diversity.

Biodiversity is a key element of ecosystem functioning and characterizes its resilience to different pressures¹, but suffers from a general worldwide decline². Among the causes of global biodiversity loss, exotic invasions are often placed at the top^{3,4}, but mechanisms and impact of species invasions might vary in different ecosystems, taxa and spatial scales. While a lot of attention has been devoted to the consequences of exotic invasions on species taxonomic diversity at different geographical scales⁵, hardly any regional extinctions have been recorded in aquatic taxa such as fish (with some notable exceptions, e.g.^{6,7}), but extirpation, species substitution and decrease of native biomass have all been reported as a local result of exotic invasions^{8–11}. The detection of exotic invasions' effects could thus depend on the spatial and biological detail level of the data used, so that some effects could be potentially overlooked or misinterpreted if the data is insufficiently detailed.

For example, fish introductions could enrich functional diversity at the regional scale, because, from a presence/absence perspective, they can increase regional species richness and consequently increase the number of functional traits present in that area¹². Functional diversity describes the distinctive assemblage of morphological, biochemical, physiological, structural, phenological or behavioral traits that characterizes living communities, and that is highly coupled to environmental conditions¹³. Ecofunctional diversity is a subset of functional diversity, focusing on the combination of ecological traits in communities¹⁴, but is referred to simply as functional diversity hereafter, because it is a more common term. Ecological traits in a community are usually selected by factors such as habitat diversity, geography, land use or water chemistry¹⁵, but established exotic species are often generalists¹⁶ and invasion dynamics (e.g. human-aided dispersion) could partly override habitat selectivity.

Competition with introduced exotic species could act as an additional filter for native species, sometimes stronger than environmental gradients¹⁷. Furthermore, widespread and severe biological invasions could result in the taxonomic homogenization of invaded communities^{17–19}, with few exotic species dominating heavily invaded areas, a pattern which has been detected at least in plants²⁰. Most likely, taxonomic homogenization would also affect functional diversity of invaded areas, as it leads to communities with a lower number of species and a lower variety of traits, but it remains unclear whether this is a significant element of exotic invasions.

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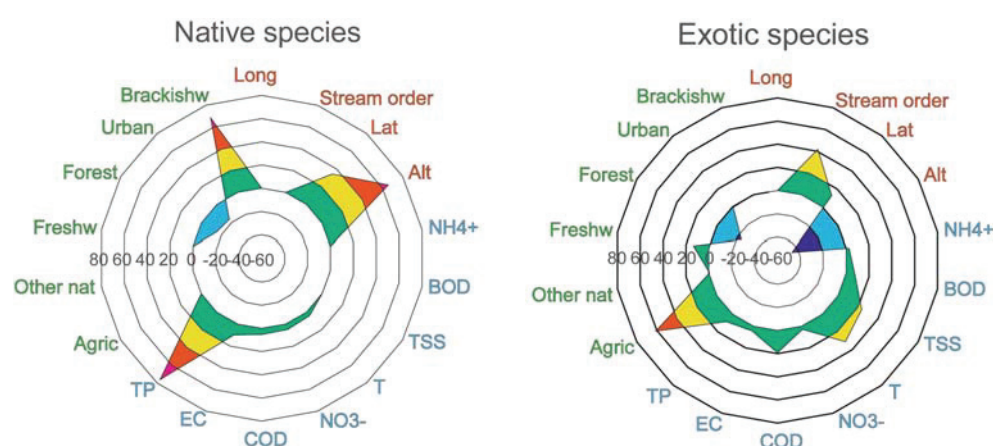


Figure 1. Boosted Regression Tree (BRT) summary showing the relative influence of geographical variables (in orange), water physico-chemical variables (in blue) and land use (in green) on freshwater fish functional diversity (calculated through the functional dispersion, FDis metric, applied to the ecological traits of species) for native (left panel) and exotic (right panel) species. The variable abbreviations stand for: *Long* – longitude, *Lat* – latitude, *Alt* – altitude, NH_4^+ – ammonia, *BOD* – biological oxygen demand, *TSS* – total suspended solids, *T* – water temperature, NO_3^- – nitrate nitrogen, *COD* – chemical oxygen demand, *EC* – electrical conductivity, *TP* – total phosphorus, *Agric* – agricultural, *Other nat* – other natural area, *Freshw* – freshwater, *Forest* – forest, *Urban* – urban and *Brackishw* – brackish water.

Exotic species are a main cause of the loss of biological diversity in the Mediterranean region^{21,22}, particularly in freshwater habitats²³. The functional structure of Mediterranean freshwater fish communities is relatively uncharted, and an ecological trait characterization of native and exotic fish species has only been recently defined for some areas^{24–26}. This finally enables further research on the linkages between biological invasions, functional diversity and the environment.

We used a spatially-broad yet very detailed dataset, comprising several river basins in northern Italy at a late invasion stage (i.e. >30 years after major invasions), as a test case to explore the outcomes of exotic species invasion in freshwater fish communities and to investigate the relationships between environmental factors, invasions and functional diversity. We used boosted regression tree (BRT) analysis to test the hypothesis (H_1) that functional diversity of exotic and native species would respond differently to environmental variables, as invasion dynamics could temporarily override habitat selectivity. We then used spatial and regression analyses to test whether (H_2) the overall functional diversity of communities would be negatively or positively affected by different degrees of exotic invasions. Our results would ultimately reveal the connections between functional diversity and exotic species invasions, on the background of habitat filtering.

Results

BRT analysis showed that environmental variables typically had different magnitude and direction of relative influence on exotic and native species functional diversity (Fig. 1). While altitude was one of the most significant variables negatively affecting native species functional diversity (and positively that of exotic ones), high temperature, low salinity or high turbidity were clearly linked to higher exotic species functional diversity (Fig. 1). Forest cover was associated to lower functional diversity of both native and exotic species, while brackish water was associated to a higher functional diversity of native species (Fig. 1).

Several sites showed minimum levels of exotic invasion (invasion degree $\leq 10\%$ for 140 sites, 41.9% of the total) and most of these sites hosted completely native communities (126 sites, 37.6% of the total). However, the majority of sites were invaded (209 sites, 62.4% of the total) and the invasion degree was relatively severe in most of them (invasion degree $\geq 50\%$ for 134 sites, 40% of the total), including some sites where the community exclusively comprised exotic species (10 sites, 3% of the total). Our spatial analysis underlined that the most severely invaded sites were mostly located on the lower stretches of most watercourses examined (Fig. 2a). Moreover, in invaded communities, there was a clear spatial overlap between the most invaded areas and the areas where functional diversity was lowest (Fig. 2b).

In invaded sites, functional diversity was also clearly negatively linked to the invasion degree (Fig. 3). At the highest invasion degrees, a decrease of nearly 50% of functional diversity, compared with less disturbed communities, could be observed.

Low-order streams (in the uplands) showed lower values of species richness and functional diversity than high-order streams (in the lowlands) (Fig. 4a,b). Species richness was low in upland streams, but these habitats showed a larger variation in functional diversity than lowland streams. Canals, in the lowlands, had lower richness and functional diversity values than natural rivers in the same area (Fig. 4a,b). The difference between native and exotic functional diversity was consistently lower than what suggested by their relative richness (Fig. 4c,d), with native species usually showing higher values of functional diversity relative to their richness. Exotic species

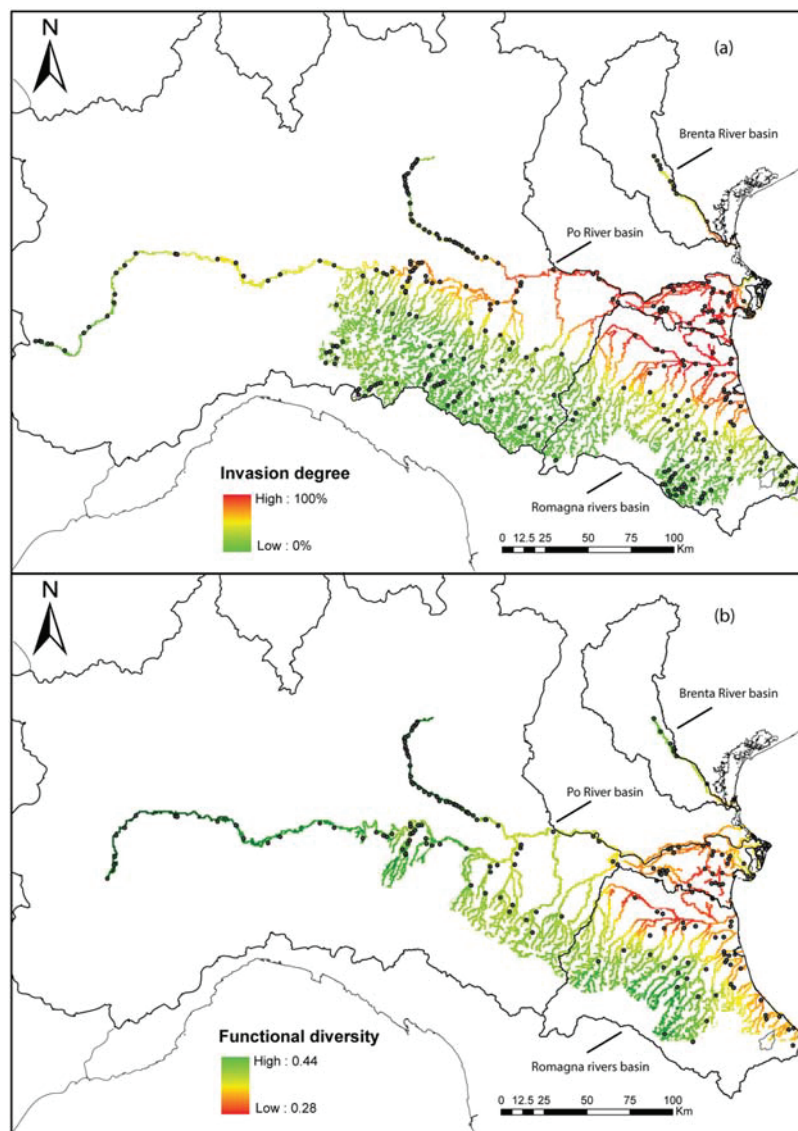


Figure 2. (a) Invasion degree in freshwater fish communities of northern Italy, and (b) spatial distribution of functional diversity (calculated through the FDis metric applied to the ecological traits of species) in the invaded sites, obtained by linear kriging. Dots represent the sampling sites used in each analysis. Colors represent the severity of invasion (% of exotic species abundance in a community) and the functional diversity of communities, respectively.

showed lower functional diversity values than native species in all natural rivers, which was most evident in higher order streams, but the pattern was reversed in canals (Fig. 4d).

Both native and exotic functional diversity in communities were linked to species richness and tended to saturate with growing richness (Fig. 5), albeit with different rates and asymptotes. However, accounting for species richness, community functional diversity for exotic species was generally lower than that of native ones (Fig. 5).

Discussion

Functional diversity of native and exotic species, as expressed by the FDis metric, responded similarly to most environmental constraints. However, confirming our first hypothesis (H_1), we found significant differences in the effects of altitude (positive effect for native and negative effect for exotic species) and in the different ranking of constraints. This suggests that invasion dynamics could play a role in overriding some major environmental drivers of functional diversity. Our results also showed that a high invasion degree corresponded to a lower functional diversity of fish communities, confirming our second hypothesis (H_2). Exotic species typically had a lower functional diversity than native species, even when accounting for stream order and species richness, which

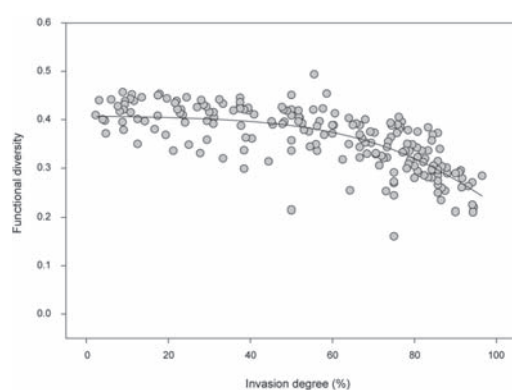


Figure 3. Italian freshwater fish communities' functional diversity (calculated through the FDis metric applied to the ecological traits of species), along the invasion gradient. Circles represent values of overall functional diversity for each invaded site, while the line represents the best-fitting non-linear regression line (see Supplementary Table 3 for best fit regression evaluation).

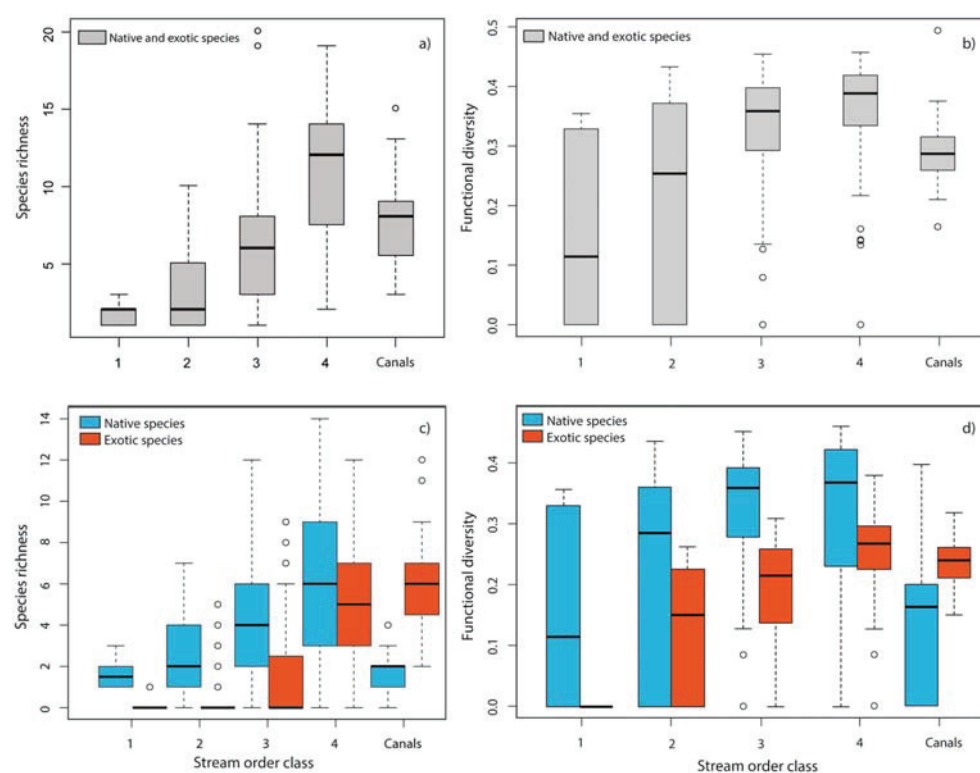


Figure 4. (a) Distribution of species richness and (b) functional diversity (calculated through the FDis metric applied to the ecological traits of species) by stream order classes, calculated on the whole fish community. The same patterns for species richness (c) and functional diversity (d) are also represented for exotic and native fish species separately. The horizontal bars in the boxes represent the median, the boxes' hinges represent the first and third quartile, and the notches represent the 95% confidence interval of the median.

could be at the root of the observed lower functional diversity of highly invaded communities. Our results thus suggest that possible outcomes of severe exotic species invasions could include a reduced functional diversity of freshwater fish communities, and that analyzing data with finer ecological and spatial resolutions could help further investigate these effects.

The different ranking of environmental drivers for exotic and native species might indicate that there are some differences in the relevance of factors shaping their respective functional diversity patterns. For example, our BRT analysis highlighted the role of native marine species entering brackish habitats in contributing to the fish

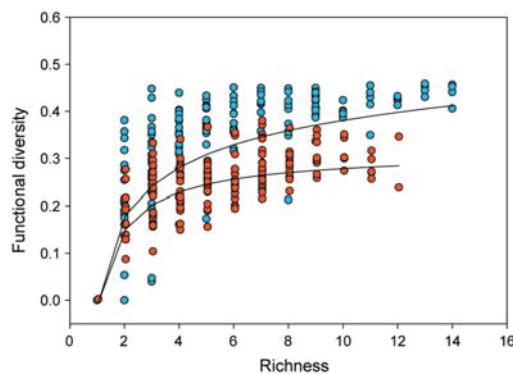


Figure 5. Native (blue circles) and exotic (red circles) functional diversity (calculated through the FDis metric applied to the ecological traits of species), in fish communities along a species richness gradient. Black lines represent best fitting non-linear regression lines for each distribution (see Supplementary Table 3 for best fit regression evaluation).

functional diversity in these areas. Our analysis results also suggested that geographical factors like altitude could play a major role to control invasion dynamics, as altitude appeared to be the main factor of divergence for native and exotic functional diversity. In addition to temperature, altitude is linked to a number of other factors, including habitat fragmentation but also habitat quality. Mountain streams typically have lower habitat complexity and resources availability but higher habitat quality than rivers in the lowlands^{27,28}. However, upland streams can be more fragmented due to the presence of dispersal barriers^{29,30}.

The high degree of invasion in the lower stretches of all basins also implies that lowland areas constitute a massive reservoir of exotic species, which could exert a significant propagule pressure towards higher altitudes^{31,32}. This pressure is held in check, at least in some cases, by impassable barriers to migration and by unfavorable ecological conditions in upstream areas³³. Habitat fragmentation and other consequences of water abstraction for human use are a worldwide issue often deeply interlinked with exotic species invasions^{34,35}, freshwater communities functional diversity³⁶, and homogenization processes³⁷. Unfortunately, habitat connectivity restoration near heavily invaded areas may not be sufficient to restore native biodiversity, as it could favor exotic invasions in upstream areas. Furthermore, some fish species can colonize upstream areas through human-mediated transport even if migration barriers are not removed^{38,39}. Our analysis did not show sharp regional invasion gradients in several non-fragmented rivers, albeit the spatial resolution of our data should have been sufficient to underline habitat fragmentation at that scale. Overall, exotic species invasion was still halted in upland areas, and we can only speculate that this could be attributed to natural gradients (e.g. temperature and habitat factors).

Perhaps the most immediate outcome of our work is a visualization of the degree of exotic freshwater fish invasion on a broad geographical scale, which highlighted the alarming decline in native species richness and abundance at most sites in the lowlands of our focus area. A high degree of invasion corresponded also to a lower functional diversity of invaded communities. Canals were among the most heavily invaded areas and a good example of this mechanism, with lower overall functional diversity than rivers in the same geographical area. This could be the result of simplified habitats, but also of the native species extinctions in this area, where only few natives remained at the time of sampling^{9–11}, but still showing higher functional diversity for the few remaining native species than for the more abundant exotic ones. Lowland rivers with high-stream order class had generally more heterogeneous habitats than canals, offering more spatial and trophic niche options to species and thus presumably allowing for a higher overall functional diversity, yet showed a similar functional diversity to that detected in canals. This pattern could potentially be caused by the presence of exotic species, which have led to local homogenizations of the fish fauna in lowland rivers and canals that are connected to them. Conversely, stream order 2 sites had a very low invasion degree (at the extreme left of Fig. 3), yet the functional diversity of these communities was not much higher than more invaded sites. This perhaps indicates that invasion in these areas is not at the initial stages, and has caused a very moderate decline (if any) of native species, thus resulting in communities where the moderate loss is compensated by a corresponding increase in functional diversity provided by exotic species. Invasion in these areas probably did not progress further because of environmental constraints (e.g. water temperature or habitat) or competition with native species¹¹.

Although there are only few documented cases of extinctions in freshwater fish^{6–8}, previous studies in this area have highlighted how freshwater fish invasions could substitute native with exotic species, resulting in a decrease of native taxonomic diversity at the local level, especially when abundances are taken into account^{9,10}. Taxonomic and functional substitutions have been previously advocated as non-detrimental or even beneficial for the environment and biodiversity^{40,41}. However, from a functional perspective, our data show that exotic species generally had a lower functional diversity than native species across similar habitats, which was evident in higher order streams and in canals. As a consequence, substitution of native species with exotic ones could decrease the overall functional diversity of the community.

It has been previously suggested that the availability of habitat and trophic niches in the environment might be the ultimate factor that defines a limit to the possible expression of functional diversity. In this framework, if

more species are added to the system functional diversity should not be positively affected, but rather show an asymptotic saturation of the available functional space⁴². While our results clearly show this asymptotic saturation, they also show that, in invaded communities, the functional diversity of exotic species was generally lower than native ones, perhaps because fewer exotic species with a limited number of traits are introduced. This could be at the root of the decrease observed in overall functional diversity of the highly invaded communities (>50% invasion degree), as the exotic component becomes predominant and drives the overall functional diversity of the community. This also suggests that a relatively smaller number of species with similar traits could be leading the invasion in the lowlands, and that high degrees of invasion might coincide with higher faunal homogenization i.e. Figure 3 of the present work,^{11,19,43}. Our view is also supported by the relatively higher functional diversity of native species in the lowlands, despite their richness not being higher than exotic ones⁴⁴. A possible explanation is the longer evolution history in the area, which allowed natives to be more functionally diverse and match the ecological niches in their native environment more closely⁴⁵, albeit no studies have dealt with this aspect in fish communities, so far. Thus, our results clearly indicate that the overall functional diversity of highly invaded communities might be diminished, resulting in an overall heavier loss of diversity and adding a new dimension to the effects of exotic invasions, which should be of great interest to conservation science and management but needs to be further investigated. Our results disagree with the outcomes of the study by¹², conducted at the macro-regional scale. This difference could stem from our different approach to the same problem: we analyzed a set of sites at a late-invasion stage rather than using a pre/post invasion approach, but also used species abundance measures and a finer spatial scale, which were advocated by¹². The divergence in our outcomes indicates that different spatial and biological resolutions, and different study setups, deeply affect the results of the analysis (see e.g.⁴⁶). It is also possible that the number and quality of functional traits investigated could further modulate the ultimate outcome of the analysis, suggesting that the role of these factors in affecting the results would need to be tested by future studies.

Given our results, we encourage further studies that deal with functional diversity and, more in general, a wider use of functional approaches before a consensus is formed on the ultimate effects of exotic invasions on diversity. Spatially- and temporally-detailed measures of species abundances would be crucial in these studies, as subtler changes in abundances could be detected before regional extinctions occur. Future studies should investigate in further detail whether a true functional loss has occurred, using a wider geographical scale and comparing areas with different invasion degrees (or theoretical reference communities). Furthermore, large-scale patterns of community diversity could be explored using measures of relative functional diversity, to express the amount of functional diversity expressed by an average species unit. We also encourage studies that explore these mechanisms in different taxa and, whenever possible, compare results across taxa. Ultimately, other aspects of functional diversity should also be investigated, as they might provide useful insights on the final outcomes of exotic and native species interactions. These are all necessary elements to fully understand the ecological consequences of functional diversity loss and its significance for ecosystem functioning at large.

Materials and Methods

Study area. Northern Italy is a dwelling area for more than 17 million humans, with consequent agricultural activities and livestock farming. This area has a Mediterranean continental climate, with an annual average precipitation of 1036 mm and a mean temperature of 12 °C. The largest river basin in Italy, the Po River basin (71,000 km²), is included in this area and we focused our investigation on the Po River itself (in all its course), the Oglio River (one of the most important hydrographic left tributaries of the Po River) and the hydrographic right tributaries in the Emilia-Romagna region. We also analyzed two additional groups of rivers outside of this basin: the Brenta River (north-east of the Po River basin) and the Romagna rivers basin (south of the Po River basin). Overall, a total of 335 sampling sites in 105 watercourses were included in this study, covering a wide range of freshwater habitats, different altitudinal zones and environmental conditions (see Supplementary Fig. 1).

Organic material originating from villages, small towns and livestock farms is the main source of river pollution for rivers in the uplands. Conversely, urbanization and intensive agriculture, causing high nutrient loads and consequent eutrophication, are the main factors affecting lowland rivers¹⁰. A network of drainage canals was established in the lowlands to support agricultural irrigation around the 19th century, with hydrological management aimed at supplying both irrigation and drainage needs^{10,47}. The study area was in a late invasion stage¹¹ at the time of sampling, since loss of native species and exotic invasion occurred already prior to 1997¹⁰, before the data analyzed here were collected.

Data collection. Fish data were collected within monitoring programs of the Emilia-Romagna region⁴⁸, the Padova Province⁴⁹, the Po River⁵⁰ and the Oglio River⁵¹ over a relatively long-term period (1999–2010). Despite this, community turnover was not a relevant factor in our study, because fish communities are typically stable over such timescales and, despite the ample interval, most of the data were collected over a limited timeframe^{44,52}. Fish sampling was performed between spring and autumn by electrofishing, combined with nets in sites of higher water depth and conductivity (e.g. lower stretches of the rivers), further details on fish sampling procedures are described in^{8,11}.

Fish species were classified according to⁵³, taking into account recent taxonomic determinations and common names as listed in FishBase⁵⁴. Each species was categorized as native or exotic: a species was considered as native when naturally present in a specific basin and as exotic when human-introduced, irrespective of the time elapsed since the introduction. Hybrid specimens or uncertain species were excluded from this study in order to avoid taxonomic asymmetries.

Abundance of each species was expressed with Moyle classes⁵⁵ ranging from 1 (lower abundance, 1–2 individuals per site) to 5 (higher abundance, more than 50 individuals per site). Unfortunately, classes of numerical abundance tend to overestimate the ecological significance of small-bodied species and underestimate that of

large-bodied ones, but direct measures of body-mass were not taken at the time of sampling. Attempting to overcome this, a weight was assigned to each species based on their average adult size in the area (1 = small body up to ~150 g; 2 = medium body ~150–400 g; 3 = large body over ~400 g, derived from FishBase and unpublished data) and multiplied by Moyle abundance classes, in order to obtain a body-mass-corrected abundance, hereafter defined simply as abundance²⁴.

Water physicochemical sampling was performed with standard methods, in temporal and spatial proximity to the fish sampling, by different Regional Environmental Protection Agencies (ARPA, in Italian) for the Po, the Brenta and the rivers in the Emilia-Romagna region. The Oglio River Water Authority carried out the water sampling in the Oglio River. The geographical position and the elevation of each site were recorded. Eight physicochemical variables were monitored: water temperature, electrical conductivity, chemical oxygen demand, biological oxygen demand, total suspended solids, total phosphorus, ammonia (NH_4^+) and nitrate nitrogen (NO_3^-).

Land cover data were obtained from the CORINE database (2012, <https://www.eea.europa.eu/data-and-maps/data/copernicus-land-monitoring-service-corine>). In the lowlands, where estimation of watershed areas is more difficult due to low slopes and human-regulated flow, the land cover of the whole river basin or of the administrative province was used. CORINE land cover classes were merged in five categories based on the main land use in order to better describe the study area: urban use, agricultural use, forest, other natural area, freshwater and brackish water. Land cover was expressed as the share of each of these categories in the watershed of each site.

Fish functional traits. In order to investigate the functional composition of fish communities, five different ecological functions were examined: feeding, reproduction, migration, tolerance and habitat use. Within these ecological functions, all fish species were classified in guilds, each representing an ecofunctional trait (Supplementary Table 1, see also Noble, *et al.*⁵⁶). Ecological functions, guilds and classification for most species in this study were taken from²⁴, where all available information was used to identify appropriate guilds for each species. The same methodology was applied to classify euryhaline species that were not included in previous work (see Supplementary Table 2). A total of 59 fish species were sampled in the study area; of these 37 were native and 22 were exotic species, relatively to the national territory.

Influence of environmental variables on functional diversity. Fish functional diversity was investigated through the functional dispersion metric FDis⁵⁷. FDis was calculated using one matrix with species abundance and another matrix of functional traits of fish communities through the *dbFD* function⁵⁸. FDis calculates the spread of traits in multidimensional space by measuring the distance of each species from a centroid weighted by the species abundance.

As a result, FDis measures the relative diversity of functional traits in a community, communities with larger FDis values are more diverse in species traits combinations, whereas low FDis values refer to communities with more traits in common. The advantages in choosing the FDis metric include that it should theoretically not be overly affected by species richness, can be computed from any distance or dissimilarity measures and take into account any number and type of traits (also qualitative traits, as in this study), is not strongly influenced by outliers and accounts for species abundances⁵⁷. FDis was calculated for both the whole fish community and for exotic and native species separately, using species abundance as weights to define the relative representation of each trait in the community.

The relative influence of geographical and land use features, as well as water physicochemical variables, on functional diversity was investigated with a machine learning method: the boosted regression trees (BRT)⁵⁹. BRT analysis is an efficient method to describe any non-linear relationships between variables (e.g. thresholds) and incorporate interactions between variables. Compared to traditional regression methods, BRT analysis combines a large number of simple tree models using the boosting technique to improve the predictive performance. BRT analysis was applied to native and exotic species separately, to investigate differences between the factors affecting the two categories. The relative influence (positive or negative) of each variable was determined by the prevalent direction of its effects on the functional dispersion of species, as no clear unimodal trends were found in our data.

Guild and species abundance matrices for each sampling site were Hellinger transformed^{60,61} to standardize variations among both species and community size, respectively. Environmental variables expressed as percentages were arcsine transformed, while others were log-transformed. All statistical analyses were performed in R software version 3.4.3⁶². FDis was calculated through the homonymous R package⁵⁸. BRT was performed with the 'gbm' R package⁶³, using standard values (Gaussian distribution, bag fraction of 0.75 and shrinkage of 0.001).

Impacts of exotic invasions on functional diversity. The degree of exotic invasion in each site was estimated through the abundance of exotic versus native species in each community (% of exotic abundance). This measure was then spatially analyzed for all watercourses within each basin in the study area, through linear kriging⁶⁴.

We also evaluated the spatial overlap and correlation between invasion degree and functional diversity (as expressed through the FDis metric calculated using ecofunctional traits⁶⁵). First, we used linear kriging to represent functional diversity, based on the whole community, for invaded sites (i.e. where both native and exotic species were present, 198 out of 335 sites, excluding an invaded site at an altitude >400 m a.s.l.). We selected these sites because we could clearly evaluate invasion effects only where both exotic and native species are present, but also, more importantly, because it allowed us to focus our analysis on a geographically uniform area (the lowlands, below 400 m a.s.l.) and thus avoid potentially-confounding geographical factors (i.e. altitude, as species-poor communities at high altitudes typically have low functional diversity, even if not invaded). This area also had a relatively homogeneous fish community at baseline conditions, so that we could consider the functional diversity of least invaded communities as a close proxy to the baseline conditions of reference communities. Following

this, we used the same sites to also explore the variations in overall functional diversity along the invasion degree gradient (between 2.3% and 96.5%).

To investigate the impact of exotic invasions on functional diversity we also examined the variations in native and exotic species richness for each stream order, alongside the respective functional diversity of each site, trying to detect patterns indicative of invasion effects. Stream order for each site was calculated using a Digital Elevation Model (DEM) (<http://www.sinanet.isprambiente.it/it/sia-ispra/download-mais/dem20/view>). Flow direction and accumulation, as well as the watershed of each sampling site, were calculated based on the DEM layer. For the entire river network generated by flow accumulation, stream order was derived with the Strahler method⁶⁶. This procedure was reliable for upland streams, but it was less so in the lowland, possibly due to the fact that flow direction and magnitude in the lowlands are not always natural because of human intervention. The stream order was thus manually checked and revised when necessary in lowland rivers and streams. Rivers were grouped into four classes based on stream order: class 1 (Strahler stream order 1 and 2), class 2 (stream order 3 and 4), class 3 (stream order 5 and 6) and class 4 (stream order > 6). As drainage and irrigation canals could not be assigned into any natural class, a separate class called “Canals” was created. Canals are man-made environments, usually characterized by low habitat heterogeneity and controlled hydrology, located in the lowlands southwest of the Po River, near its delta.

We further examined the relationship between native and exotic richness and their relative functional diversity. We used non-linear regressions, evaluated through a combination of Akaike's Information Criterion (AIC) weights⁶⁷ and R² values, to estimate best-fitting curves for functional diversity distributions versus species richness and invasion degree. Non-linear best-fitting regressions were estimated using the Curve Expert Professional 2.6 software⁶⁸ and the results of the five best regression models for each relationship were reported in Supplementary Table 3. The spatial analyses were performed with ArcGIS software⁶⁹, using its Kriging Tool and Hydrology Spatial Analyst Tool.

Data availability

Underlying data for this paper is made available also for review as Supplementary Material and will be made publicly available through OpenScienceFramework should the manuscript be accepted for publication.

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Author contributions

M.M. and A.G. conceived the idea and designed the methodology. A.G. collected and analyzed the data. M.M. led the writing of the manuscript. M.M., A.G., J.S. and G.C. contributed critically to the drafts and gave final approval for publication.

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4.3 How do exotic fish species affect the functional diversity?

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The role of species introduction in modifying the functional diversity of native communities

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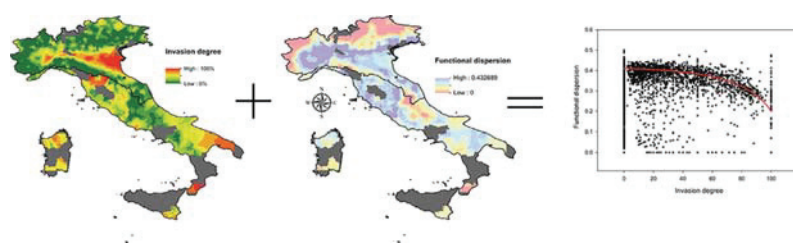
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HIGHLIGHTS

- Introduced species were widespread, and in many cases the invasion was severe, in Italy.
- Exotic and translocated fish species had different geographical distribution patterns.
- Invasion can diminish the ecofunctional diversity of host fish communities.
- Exotic species might have worse effects than translocated ones.
- Some traits were typical of invaded communities, but with regional differences.

GRAPHICAL ABSTRACT



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ABSTRACT

Although one of the most evident effects of biological invasions is the loss of native taxonomic diversity, contrasting views exist on the consequences of biological invasions on native functional diversity. We investigated this topic using Mediterranean stream, river and canal fish communities as a test case, at 3734 sites in Italy, and distinguishing between exotic and translocated species invasion in three different faunal districts. Our results clearly confirmed that introduced species were widespread and in many cases the invasion was severe (130 communities were completely composed by introduced species). Exotic and translocated fish species had substantially different geographical distribution patterns, perhaps arising from their differences in introduction timing, spread and invasion mechanisms. We also found a clear decreasing trend of functional dispersion along an invasion gradient, confirming our hypothesis that the invasion process can diminish the relative diversity of ecofunctional traits of host fish communities. Furthermore, our results suggested that exotic species might have a greater negative effect than translocated species on the relative diversity of ecofunctional traits of fish communities. This could also be linked to the fact that translocated species are more ecofunctionally similar to native ones, compared to the exotics. Our multivariate analysis of site-specific combinations of ecofunctional traits highlighted some traits characteristic of all invaded communities, while our discriminant analysis underlined how there was a substantial ecofunctional overlap between native, exotic and translocated species groups in most areas.

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1. Introduction

The ecological footprint of human presence falls heavy on worldwide ecosystems, ultimately affecting human well-being itself (Myers et al., 2013). However, despite increased knowledge on the effects of human action on worldwide ecosystems (e.g. Vörösmarty et al., 2010), the global ecosystem health is apparently still in decline (Collen et al., 2014; Sato and Lindenmayer, 2018). This trend has reflected correspondingly on biodiversity, which is the diversity of life forms that thrive in different environments. Continued efforts to reverse this decline, usually aimed to preserve and restore key species, have also failed to halt the loss of biodiversity (Butchart et al., 2010).

Direct human impacts on biodiversity can go as far as species extinctions, e.g. through excessive harvest (Anderson, 1995; Rosser and Mainka, 2002). However, also indirect impacts on the biota, e.g. habitat degradation, play a strong role in species extinctions and synergistically interact with each other (Brook et al., 2008). Indirect impacts are usually more subtle, but by no means less significant; for example, the introduction of species produces a powerful pressure on the biological component of global ecosystems (see e.g. Gallardo et al., 2016). Introduced species, i.e. species that reach a new ecosystem through human intervention (either intentionally or unintentionally), have been found to be one of the major challenges to preserve biodiversity and ecosystems functioning worldwide (Vitousek et al., 1997; Clavero and García-Berthou, 2005). Biodiversity loss is, in turn, a major driver of ecosystem change in itself as it can alter productivity and decomposition, two key processes in ecosystem functioning (Hooper et al., 2012), and can even affect human health (Díaz et al., 2006). The whole human sphere can ultimately be affected as well, through the alteration of ecosystem functioning and its ability to provide the services needed to sustain humankind (Haines-Young and Potschin, 2010; Cardinale et al., 2012). As biodiversity and ecosystem functioning are so intertwined, species loss can trigger negative feedbacks on the environment, having cascading effects on biodiversity. However, identifying and understanding the causes and mechanisms of biodiversity loss can be very challenging, as multiple pressures are at play and biodiversity is a multi-faceted concept.

Traditionally, biodiversity has been identified with species diversity, so that biodiversity loss was investigated through the analysis of species loss (Pimm and Raven, 2000; Cardinale et al., 2012). However, more recent approaches have emphasized the need to progress beyond mere taxonomical diversity, and shift more attention towards functional diversity (see e.g. Poff et al., 2006; Violle et al., 2014). The functional diversity approach privileges the ecological functioning role of species over mere species richness, and is generally considered to reflect more closely the interaction between the environment and biotic communities in space and time (see e.g. Baskin, 1994). In this perspective, changes in functional diversity can be interpreted through the analysis of the ecological-traits composition of living communities (i.e. eco-functional diversity), at different spatial scales.

Several studies established that one of the most evident effects of biological invasions is the loss of native diversity (Vitule et al., 2009; Bellard et al., 2016), which naturally leads to the inference that functional diversity could also be undermined. However, contrasting views exist on the consequences of biological invasions on functional diversity: either positive (Toussaint et al., 2018) or negative (Shuai et al., 2018) effects on native biodiversity have been found, probably as a result of different choices in functional traits, spatial scales or biological resolution considered (Jarzyna and Jetz, 2018). For example, significant differences might exist between the effects of invasions led by exotic (i.e. originally from

a distant geographic area) and translocated (i.e. originally from a nearby geographic area) species (e.g. Leprieur et al., 2008). These differences could arise from the substantially different vectors of introduction, spread and timing of exotic and translocated species, but also from the fact that translocated species are usually more ecologically similar to native species. They can thus ecofunctionally substitute native species, causing a loss of native taxonomical diversity, but not of ecofunctional diversity. However, separating these effects would require datasets where clear distinctions could be made at the geographical and taxonomical level.

To investigate the effects of species introduction on ecofunctional diversity we focused on the Mediterranean region, because previous research identified a high risk to native biodiversity, including several endemic species, from biological invasions (Médail and Quézel, 1999; Cuttelod et al., 2009; Hermoso et al., 2011). We selected fish in inland waters as model taxa, because freshwater fish diversity has been highlighted as one of the most endangered taxa in this region (Crivelli, 1995). We further selected Italy as a test area within the Mediterranean region, because it is isolated from the rest of the region by the Alps and includes several insurmountable geographical barriers defining faunal districts, thus allowing for clear distinctions of exotic and translocated species. We used the most detailed information available on community composition, to test the hypothesis that exotic and translocated species would show substantially different geographical patterns of invasion, due to their differences in introduction, spread and invasion mechanisms. We then explored the relationship between biological invasions and ecofunctional diversity, at the community level. First, we used a measure of overall ecofunctional diversity, expressed as the relative variability of traits within a community, to investigate the hypothesis that biological invasions would decrease the ecofunctional diversity of invaded communities. In particular, we hypothesized that this effect would be more severe when the invasion was led by exotic species, rather than translocated ones, because the latter would be more ecofunctionally similar to native ones. Secondly, we also analyzed ecofunctional diversity, expressed as site-specific combinations of traits, under the hypothesis that some ecofunctional traits would be common in the invaded communities of all districts.

2. Materials and methods

2.1. Data collection

We collated, for the first time, all available Italian stream fish community data from official monitoring programs run by provincial/regional administrations. Supplementary Table 1 details the sources (and the appropriate acknowledgements) of these data. We collated and often digitized data from 4126 sampling events, but we discarded all sites without fish or those sampled more than once, narrowing our dataset to 3734 sampled sites. Our dataset spanned >11 degrees of longitude (~1200 km) and 10 degrees of latitude (~1100 km), covering streams at altitudes between -4 and 2500 m above sea level.

Fish sampling was mainly performed in the warm season, following national monitoring guidelines (APAT, 2007), by direct-current electrofishing in shallow areas (e.g. upper stretches of rivers) combined with nets (a combination of trammel nets, gillnets and drop nets) in sites of higher water depth and conductivity (e.g. lower stretches of the rivers), to verify the results of electrofishing. The sampling effort was directed to ensure that all macrohabitats within each site (i.e. river stretch) were covered, and standardized based on sampled area, to allow comparisons between sites. Fish species were classified according to Kottelat and Freyhof (2007), taking into account recent taxonomic determi-

nations and common names as listed in FishBase (Froese and Pauly, 2017). A total of 99 different fish species (37 of which are exotic) were found in Italian streams. Sampling time spanned 1999–2014, but community turnover was not a relevant factor in our study, because fish communities are typically stable over such timescales and the data was collected in a restricted timeframe within each area (Korhonen et al., 2010; Gavioli et al., 2019).

2.2. Data elaboration

Species were categorized as either native or introduced according to their biogeographic origin, as established through the current scientific literature (e.g. the national IUCN red list of species (IUCN Comitato Italiano, 2019)). A species was defined as introduced when introduction was human-mediated, irrespective of the time elapsed since the introduction. Brown trout taxonomy has been under revision in this area throughout the sampling effort, and recently proposals have been put forward to split this taxon into native and exotic species (Zanetti, 2017). As surveyors could not establish the exact species at the time of sampling, we conservatively assumed that all brown trout in the dataset were native to all areas. Hybrid specimens or uncertain species were excluded from this study.

Introduced species were divided into exotic species (originally from outside of the Italian geographical barriers) and translocated species (with an origin inside of the national border, but introduced to a new area). To account for native species translocation as a form of introduction, we distinguished 3 faunal districts according to the established literature (Bianco, 1987; Bianco, 1998); these districts are separated from each other by geographical barriers (i.e. mountain chains or sea stretches) that allowed the speciation of endemic fish taxa in inland waters. With the exception of the islands, in this area translocated species are mostly endemic variants of cyprinids and gobids, which were only recently taxonomically split and are ecofunctionally very similar to other native species. Our dataset was thus composed by the Padano-Veneto district (PDV, 2418 sites), the Tosco-Laziale district (TL, 1146 sites), and the islands district (ISL, 170 sites (divided equally between the islands of Sardinia and Sicily)). The PDV district includes the largest river basin in Italy, the Po River basin (71,000 km²) within the Po River plain, limited in the north by the Alps and in the south by the Apennines. All rivers in this district flow ultimately in the Adriatic Sea. The TL district is characterized by the highest longitudinal extension; the Apennines separate the rivers that flow in the Tyrrhenian Sea, in the west Italian coast, from rivers that flow in the Adriatic Sea and Ionian Sea. The ISL district encompasses the two major Italian islands, completely isolated from the continental lands by sea stretches. Most of the rivers in the southern TL and ISL districts are not permanent, due to summer droughts.

Abundance of each species sampled during the monitoring was recorded with Moyle classes (Moyle and Nichols, 1973) ranging from 1 (lower abundance, 1–2 individuals per site) to 5 (higher abundance, >50 individuals per site). Unfortunately, direct measures of body-mass were not taken at the time of sampling and Moyle classes (as numerical abundances), tend to overestimate the ecological significance of small-bodied species and underestimate that of large-bodied ones. Attempting to overcome this, and to better describe the ecological allocation of energy in the fish community, a weight was assigned to each species (1 = small body up to ~150 g; 2 = medium body ~150–400 g; 3 = large body over ~400 g) based on the average weight of the adult stage in Italy, based on a combination of FishBase and unpublished data. This weight was multiplied by Moyle abundance classes, in order to obtain a body-mass-corrected abundance, hereafter referred to simply as abundance (Milardi and Castaldelli, 2018).

We then calculated an invasion degree, i.e. the share of introduced species in inland waters fish communities, irrespective of their origin, based on the abundance of native and introduced species. A high invasion degree equals to a high share of introduced species and a low share of native species. Similar calculations, performed using presence/absence or Moyle classes, yielded similar results in term of invasion degree (see Fig. A.1 in Appendix A). We also calculated a translocation degree, expressing the share of translocated species among the introduced species, therefore a high share of translocated species translates into a high translocation degree and equals to a low share of exotic species in the introduced community, and vice-versa.

To investigate the ecofunctional composition of fish communities, five different ecological functions were examined: feeding, reproduction, migration, tolerance and habitat use. Within these ecological functions, all fish species were classified in guilds, each representing an ecofunctional trait (Supplementary Table 2, see also (Noble et al., 2007)). Ecological functions, guilds and classification for most species in this study were taken from (Milardi and Castaldelli, 2018;), where all available information sources were used to identify appropriate guilds for each species. The same methodology was applied to classify species that were not included in previous work (see Supplementary Table 3).

Ecofunctional diversity was investigated through two different metrics based on community-specific fish ecofunctional traits: the functional dispersion metric (Laliberté and Legendre, 2010), and the functional identity metric (Mouillot et al., 2013). We selected these metrics because they could be calculated on all communities (including the species-poor ones) and because they represent two different aspects of ecofunctional diversity.

The functional dispersion metric measures the relative diversity of ecofunctional traits in a community, communities with larger functional dispersion values are more diverse in species traits combinations. This metric should not be overly affected by species richness, can be computed from any distance or dissimilarity measures and takes into account any number and type of traits (also qualitative traits, as in this study), is not strongly influenced by outliers, and accounts for species abundances (Laliberté and Legendre, 2010). We further calculated the contribution of each species biogeographic category (native, exotic and translocated species) to the overall ecofunctional diversity by fractioning the overall functional dispersion according to the abundance of each biogeographic category in the overall community.

The functional identity metric expresses the abundance-weighted average value for each trait in a community, therefore traits with higher values are more represented in the community and those with lower values are less represented. Functional identity analysis produces a complete matrix with abundance-weighted averages for each trait in every community and is thus an expression of site-specific ecofunctional diversity, providing the peculiar combinations of traits present in each community. Functional dispersion and functional identity were calculated through the *dbFD* and the *functcomp* functions (Laliberté and Legendre, 2010; Laliberté et al., 2014), respectively, using R software version 3.4.3 (R Core Team, 2017).

2.3. Data analysis

We analyzed the spatial distribution of the invasion and translocation degrees, as well as that of the ecofunctional diversity, for all the areas covered by our dataset, through linear kriging in ArcGIS software version 9 (ESRI, 2011).

We used non-linear regressions to obtain the curves best-fitting variations in ecofunctional diversity along an invasion degree gradient. The rational model regression ($y = (a + bx) / (1 + cx + dx^2)$) was ultimately selected through Akaike's Information Criterion

(AIC), and the results were graphically presented (as well as R^2 and AIC reported). Non-linear best-fitting regressions were estimated using the Curve Expert Professional 2.6 software (Hyams, 2017).

Locally-estimated scatterplot smoothing (LOESS-smoothing) was used to visualize data trends reducing noise (Jacoby, 2000). LOESS-smoothing was applied to clines of ecofunctional diversity (and its components), as well as invasion degree, to show the contribution of each component to the overall ecofunctional diversity.

We performed a linear discriminant analysis to identify differences between exotic, native and translocated species groups based on ecofunctional trait characteristics. Discriminant analysis allows to separate groups of objects based on a combination of features. The analysis derives the coefficients of a scoring function for each category from the input data, and the highest score is used to assign a case to a category (Venables and Ripley, 2013). A discriminant analysis was performed for each of the districts, using matrices of species sampled in each area, through the MASS R package. The homogeneity of within-group covariance matrices of the explanatory variables was assessed using the *betadis* function of the Vegan R package (Oksanen et al., 2017; Borcard et al., 2018).

We also carried-out a multivariate analysis, in order to investigate the relationship between ecofunctional traits and invasion/translocation degrees. A secondary aim was to reveal the relationships between invasion and translocation degrees and explore the connections between these variables and major geographical features (altitude, latitude, longitude) and to broad community diversity (species richness). Invasion and translocation degrees, geographical and community diversity variables were selected as independent variables and transformed prior to analysis: percentages were $(\arcsin X/100)^{0.5}$ transformed, while other variables were \log_{10} transformed (Legendre and Gallagher, 2001). Ecofunctional traits, expressed through the abundance-weighted averages of each trait (calculated through the functional identity metric), were selected as dependent variables. A Detrended Correspondence Analysis (DCA) was initially performed to select the most appropriate response model for gradient analysis (Lepš and Šmilauer, 2003). The dominant gradient length in DCA was always lower than 3 so the Redundancy Analysis (RDA) was finally chosen (Lepš and Šmilauer, 2003). RDA is a linear gradient analysis that

allows to quantify the variation of a multivariate data set explained by independent variables (Ter Braak and Šmilauer, 2002). The significance of independent variables was assessed with a forward selection procedure and Monte Carlo permutation tests, however all independent variables selected resulted significant. The collinearity of variables was investigated through the variance inflation factor (VIF), but no collinear variables were found ($VIF > 8$). Multivariate analyses were performed using the CANOCO 4.5 for Windows software (Lepš and Šmilauer, 2003).

3. Results

3.1. Spatial patterns of invasion

1940 sites (52% of all sites examined) showed some degree of invasion, and introduced species constituted on average 42.5% of the community in invaded sites, reaching maximums of 100% (i.e. the native community was completely lost in 130 sites, 3.5% of all sites examined). One of the invasion hotspots was located along the Po River, but other highly invaded areas existed in the central and southern part of the Italian peninsula, as well as in the islands (Fig. 1a).

Translocated species were present in 681 sites (18.2% of all sites examined), mostly located in the central and south part of the peninsula. Where present, translocated species were usually part of more invaded communities (44.2% average invasion degree in sites with translocated species presence) and constituted a large part of the introduced community (72.4% average share of the introduced community, 100% of the introduced community in 341 sites), especially in the TL district. Translocated species were a minor component of the invaded communities of the northern part of the peninsula (in the PDV district), as well as in the islands (Fig. 1b).

3.2. Relationship between biological invasion and ecofunctional diversity

Ecofunctional diversity, measured through the functional dispersion metric, generally increased from higher, species poor, alti-

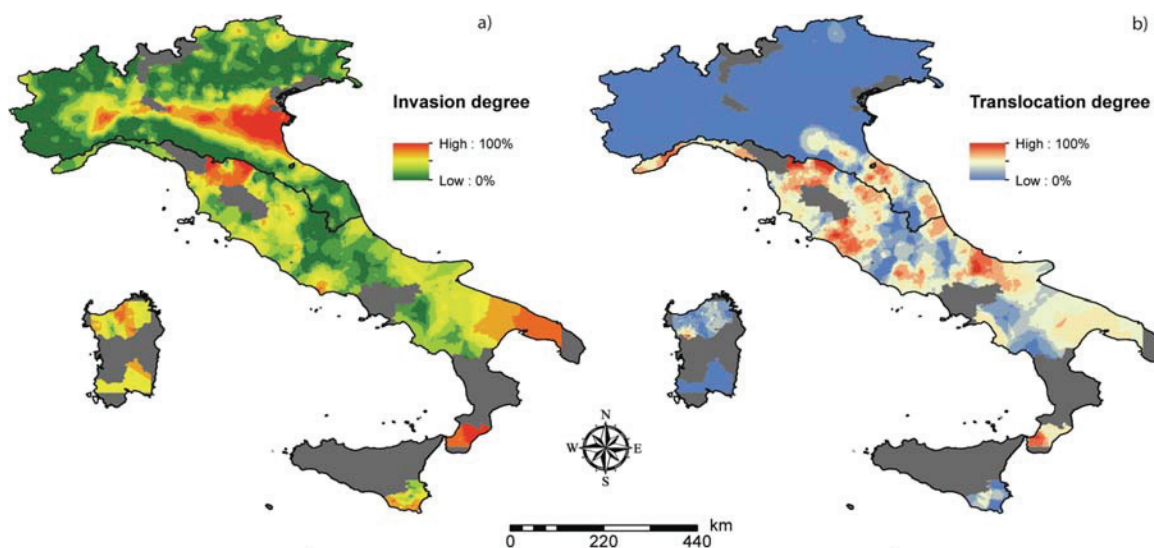


Fig. 1. Invasion (a) and translocation degree (b) in fish communities of Italian inland waters. The solid black line dividing the northern part of the peninsula represents the boundary between the Padano-Veneto district (to the north) and the Tosco-Laziale district (to the south). Grey shaded areas represent zones for which no data could be retrieved.

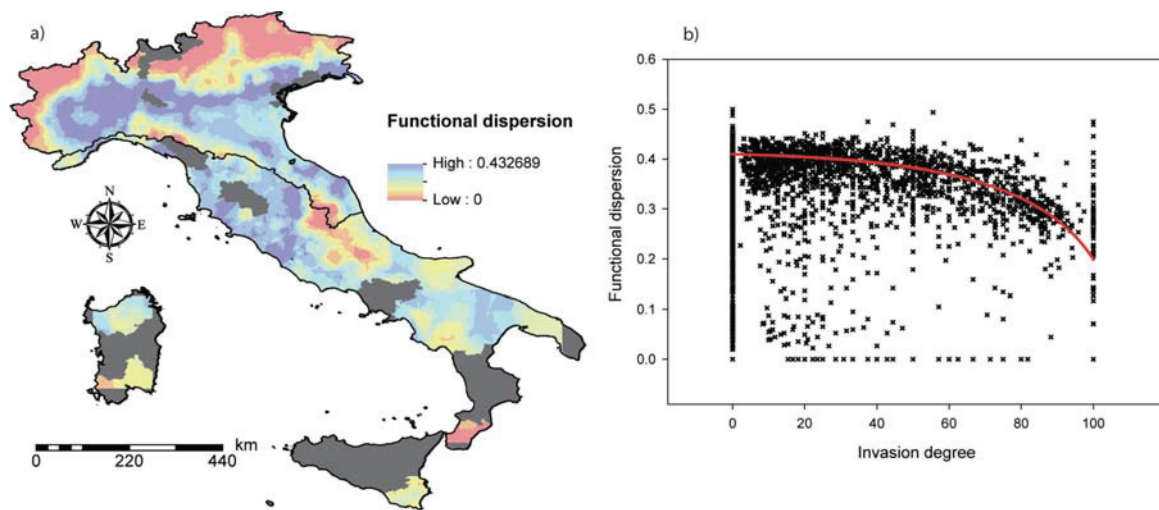


Fig. 2. (a) Spatial distribution of ecofunctional diversity, measured through the functional dispersion metric, in fish communities of Italian inland waters. The solid black line dividing the northern part of the peninsula represents the boundary between the Padano-Veneto district (north) and the Tosco-Laziale district (south). Grey shaded areas represent zones for which no data could be retrieved. (b) Relationships between ecofunctional diversity, measured through the functional dispersion metric, and the invasion degree in fish communities of Italian inland waters. The red line represents the result of the best-fitting regression analysis (rational model). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

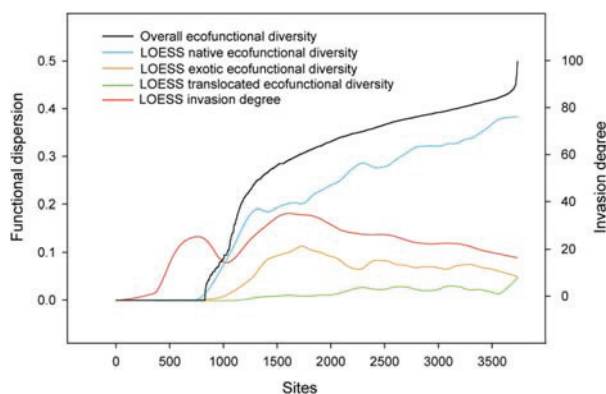


Fig. 3. Native (blue), exotic (orange) and translocated (green) species contribution to the overall ecofunctional diversity, measured through the functional dispersion metric (black line), in fish communities of Italian inland waters. Sites were sorted in ascending order of ecofunctional diversity and the distributions of native, exotic and translocated species contributions were LOESS-smoothed to reduce noise. The red line indicates the LOESS-smoothed invasion degree of each site, scaled on the right vertical axis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

tudes to lower, species rich, altitudes. However, large areas in the lowlands, corresponding to the most invaded areas (i.e. the Po River course in the northern part of the peninsula), showed decreased ecofunctional diversity compared to other, less invaded, lowland areas (e.g. in the central part of the Italian peninsula) (Fig. 2a).

That the invasion process likely undermined ecofunctional diversity was further underlined by the distribution of the functional dispersion metric along an invasion degree gradient, which showed a marked decrease in ecofunctional diversity for invasion degrees exceeding ~50% (rational model regression $R^2 = 0.23$, $AIC = -14,639.2$, Fig. 2b).

However, translocated species seemed to contribute less to the decline in ecofunctional diversity: in fact, lower values of functional dispersion were found for high invasion degrees where these were not associated to a high translocation degree (Fig. 3). Some of the highest values of community ecofunctional diversity were reached also in the presence of translocated species, but only at low invasion degrees, whereas a peak in invasion degree was found in sites completely invaded by only one species (rainbow trout), and thus with null functional diversity (Fig. 3).

Our discriminant analysis revealed that exotic, native and translocated species ecofunctional traits were highly overlapping in the PDV and TL districts (Fig. 4a and b), whereas a clearer differentiation among the three groups was present in the ISL district (Fig. 4c). The model fits/accuracies were of 80.8%, 71.2% and 87.1%, for the PDV, TL and ISL districts, respectively.

3.3. Ecofunctional traits typical of invaded communities

The RDA analysis of site-specific ecofunctional diversity, expressed through the functional identity metric, highlighted some ecofunctional traits of introduced species that were common in all districts (Fig. 5). The total variance derived from the RDA analysis, expressed as the sum of all canonical eigenvalues, was 56.7%, 31.2% and 37.7% for the PDV, TL and ISL districts, respectively. Species adapted to live in turbid waters and reproducing on sand/mud (psammophiles) were common in all invaded zones, irrespective of the district (Fig. 5). In all districts, altitude was inversely related to species richness and, to a lesser degree, to invasion degree (Fig. 5). While a clear relationship between latitude and invasion was apparent in the TL district (Fig. 5b), the PDV district showed a relationship between invasion and longitude (Fig. 5a).

4. Discussion

Our results clearly confirmed our hypothesis that exotic and translocated fish species had substantially different geographical

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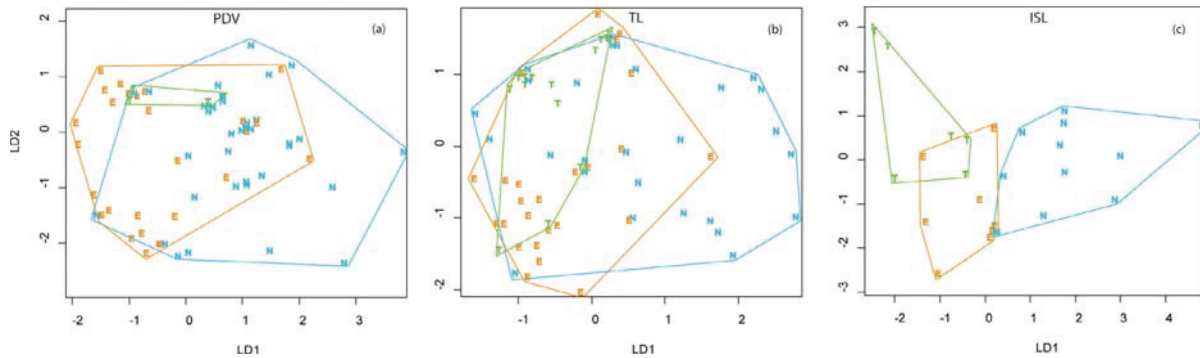


Fig. 4. Discriminant analysis of the ecofunctional overlap between native (blue), exotic (orange), and translocated (green) groups of species present in the Padano-Veneto (PDV, a), the Tosco-Laziale (TL, b) and the Islands (ISL, c) district. Solid lines represent the convex hulls around each of the groups. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

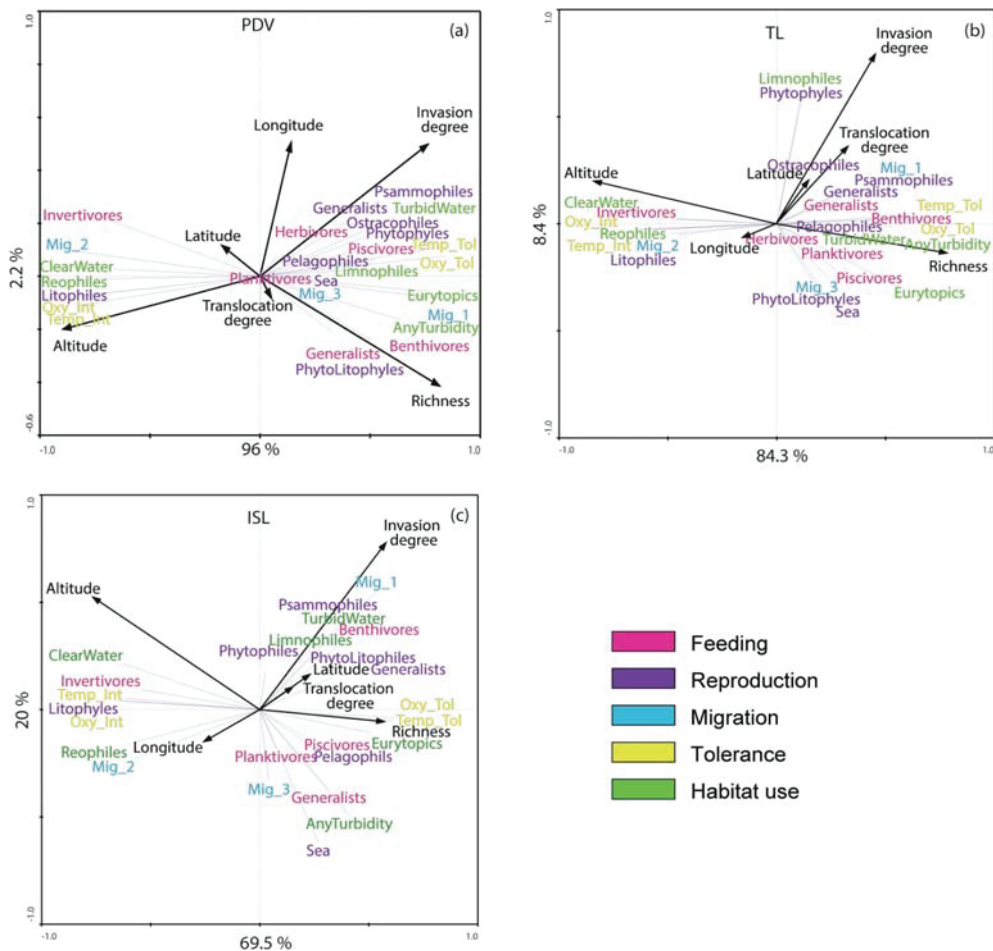


Fig. 5. Plots of RDA of site-specific ecofunctional diversity (colored labels, explored through the functional identity metric) of fish communities in Italian inland waters using geographical variables, species richness, as well as invasion and translocation degrees as explanatory variables (black arrows and labels). Each panel describes sites in a different district: the Padano-Veneto (PDV, a), the Tosco-Laziale (TL, b) and the Islands (ISL, c) district. Percentage values along each axis illustrate the proportion of variance of the traits-environment relation explained along that axis. Ecofunctional traits are color-coded to separate them in ecological functions, Supplementary Table 2 contains longer descriptions of each trait. Plots of RDA of site-specific ecofunctional diversity (colored labels, explored through the functional identity metric) of fish communities in Italian inland waters using geographical variables, species richness, as well as invasion and translocation degrees as explanatory variables (black arrows and labels). Each panel describes sites in a different district: the Padano-Veneto (PDV, a), the Tosco-Laziale (TL, b) and the Islands (ISL, c) district. Percentage values along each axis illustrate the proportion of variance of the traits-environment relation explained along that axis. Ecofunctional traits are color-coded to separate them in ecological functions, Supplementary Table 2 contains longer descriptions of each trait.

distribution patterns, perhaps arising from their differences in introduction, spread and invasion mechanisms. We also found a clear decreasing trend of functional dispersion along an invasion gradient, confirming our hypothesis that the invasion process can diminish the relative diversity of ecofunctional traits of host fish communities. Furthermore, our results suggested that exotic species might be more invasive and thus have a greater negative effect than translocated species on the relative diversity of ecofunctional traits of fish communities. This could also be linked to the fact that translocated species are more ecofunctionally similar to native ones, compared to the exotics, at least in the PDV and TL districts. Our multivariate analysis of site-specific combinations of ecofunctional traits highlighted some traits characteristic of all invaded communities, but some differences and nuances were present in different districts, perhaps due to their species assemblages or their peculiar geographical characteristics. Overall, our results depicted a dire situation for native freshwater fish communities.

4.1. Spatial patterns of invasion

Possibly, the most striking outcome of our work was the detection of the dramatic situation of native freshwater fish communities, with 130 sites where communities were solely composed by introduced species. Albeit a small percentage (3.5%) of the total sites examined, the invasion process is unlikely to be reversed in these sites, at least in the short term. Furthermore, this count is even more vivid, when considering that local extinctions in connected freshwater ecosystems have been rarely reported for fish (with few exceptions, Townsend, 1996, Kitchell et al., 1997) and that previous studies over a smaller area had underlined a critical situation for a smaller subset of sites (Lanzoni et al., 2018). However, the situation could be much worse nowadays, compared to the survey period. At this level of detail, our analysis was unable to detect smaller community variations before/after the invasion. Another possible limitation of the invasion degree calculated in the present study was that it could not discriminate between mass occurrences of a single invader or the presence of many invading species with few individuals each. Future studies that aim to disentangle the effects of species and abundances could shed further light on this crucial element of invasion dynamics. However, our results have the merit of representing a broad picture of the invasion degree gradient, using the most updated and detailed data available. The invasion was staggeringly widespread in space, spanning the entire area examined and affecting 52% of all sites. The average invasion degree in sites where introduced species were present was also considerably high (42.5%), suggesting that the invasion was not only widespread, but also very pervasive. Previous studies on brown trout genetics in the Apennines range point out that several native Mediterranean trout populations might be genetically compromised by stocking, at least in this area (i.e. most brown trout seem to be of Atlantic or Danubian origin, Caputo et al., 2004). Our conservative choice to consider all undetermined brown trout (*Salmo trutta complex*) as native, because of lack of detailed information, likely decreased the severity of our results. A similar analysis, conducted considering all reported brown trout as introduced, would have worsened our results on the invasion degree by >50%, especially at higher altitudes (Milardi, unpublished data).

Conversely, the lowlands showed the highest rates of invasion, with a multitude of introduced species present in the terminal parts of rivers, a distribution that was clearest in the Padanian plain of northern Italy. In this area, the invasion was mostly composed by exotic species, with a very low rate of translocation, if compared to the other districts. On the contrary, translocation rates were higher along the Apennines range, in the TL district, but in this area the overall invasion was relatively low. Translo-

cated species in this area were introduced from the northern part of the Italian peninsula: for example the Padanian goby (*Padogobius bonelli*), an endemic species that has been unintentionally translocated, has become invasive and detrimental to another endemic goby, the Arno goby (*Padogobius nigricans*), (see e.g. Pompei et al., 2018). The different spatial distribution of exotic and translocated species could partly be due to national mechanisms of fisheries management dating back to the early 20th century. It is around this time that some exotic species were first introduced in northern Italy and the state created aggregated farms to stock (both exotic and native) fish species of recreational value throughout the peninsula in an effort to enhance fisheries (Milardi et al., 2018a).

4.2. Relationship between biological invasion and ecofunctional diversity

From our results (e.g. Fig. 2b), it was clear that species introductions, notwithstanding their origin, undermined the ecofunctional diversity of host communities. Similar detrimental effects of exotic invasions on functional diversity were found by Shuai et al. (2018), by looking at different functional traits. Furthermore, a previous study in Italy had already underlined this mechanism, in a limited area where native and exotic species overlapped, and attributed it to the intrinsically lower ecofunctional diversity of exotic species, and partly to homogenization processes caused by the invasion process (Gavioli et al., 2019). Our present results greatly expand the basis for such conclusion and confirm such a mechanism exists over a much larger spatial scale. Local extinctions of native species, and a general decline of native abundance, have been previously linked with the interaction between native and introduced species in parts of the study area (Castaldelli et al., 2013; Lanzoni et al., 2018; Milardi et al., 2018a). Native extinctions and decline of native abundance could be partly responsible for the decline in ecofunctional diversity detected at highly invaded sites, but further work and data comparing the communities before/after invasion would be needed to confirm this. In the PDV district, the most invaded area in our dataset, richness and altitude were less inversely linked than in other districts, implying that homogenization processes underlined in a sub-area of this district (Gavioli et al., 2019) could be typical of the whole area. Biotic homogenization at the faunal district scale leads to communities dominated by few exotic species (Rahel, 2000; Leprieur et al., 2008), which are consequently less ecofunctionally diverse. Our analysis further suggested that ecofunctional diversity loss could be led by exotic, rather than translocated species (see Fig. 3). A possible explanation could be that translocated species could be ecofunctionally more similar to native species (at least in some districts, see Fig. 4a and b), because their evolutionary divergence is usually recent (i.e. the abovementioned case of endemic Italian gobies), and thus might tend to cause less ecofunctional diversity losses. Another possible explanation is that invasions led by translocated species generally reach lower levels of invasion and homogenization, thus affecting less the ecofunctional diversity of impacted communities. However, even low invasion rates of translocated species are likely to influence native taxonomic and genetic diversity, so the impact of translocation on native biodiversity should not be underestimated. Communities with diminished taxonomical or ecofunctional diversity could be less resilient to further pressures, e.g. those of climate change, if key functional groups of the communities are lost (Dunne et al., 2002). Further studies, using detailed temporal information, would be needed to assess whether biodiversity losses in the Mediterranean region have impaired the resilience of freshwater communities. Furthermore, it would be important to evaluate the respective contributions of habitat degradation and invasion processes on the functional diversity of

communities (Colin et al., 2018). Site-specific data on pristine communities would be needed to investigate the change in ecofunctional composition resulting from the invasion process (and other, multiple, pressures). Unfortunately, we could only analyze the introduction gradient over a large number of sites, which does not equal an assessment of the variation before/after the invasion in each site.

4.3. Ecofunctional traits typical of invaded communities

By analyzing this invasion gradient we were able to underline that some ecofunctional traits are dominant in highly invaded communities in all districts, for example adaptations to live in turbid waters and reproducing on sand/mud. However, our analysis could not discriminate whether some of these traits were typical of pristine communities in different invaded areas, as we lacked complete data on pristine or unimpacted communities. Not all traits highlighted in invaded communities are typical of generalist species, as suggested by previous studies (Marr et al., 2013, Gavioli et al., 2019), perhaps indicating a higher complexity of traits associated with fish invasions than previously thought. Furthermore, the differences in invasion rates among the districts, and the differences in the prevalence of some of the other traits associated with high invasion in each district (see Fig. 5), suggest that pinning down the linkages between these changes and invasion could be a rather complex task. Some of the differences that we found among districts could be attributed e.g. to the different translocation rates, or to the climatic and geographical differences between the districts. For example, the prevalence of short migration species in invaded areas of the TL and ISL districts seems to suggest that habitat degradation (e.g. habitat fragmentation by artificial barriers) could play a role in the invasion process. However, streams in these areas are generally shorter in length and naturally fragmented due to drought events in summer, so that these traits could be more associated to environmental rather than invasion factors. Furthermore, anthropogenic habitat fragmentation has been previously found to block the spread of invading species in rivers (Gavioli et al., 2018). Dissimilarities in ecological traits between native and introduced species have been previously suggested as a potential key factor of invasiveness (Divíšek et al., 2018), but our discriminant analysis underlined that, at least in the PDV and TL districts, translocated, exotic and native species ecofunctional traits had little dissimilarities. Potentially, this could be a relevant factor in the ISL district, where species groups were most ecofunctionally distinct. Despite the fact that our analysis was able to outline some of the ecofunctional traits dominant in invaded communities of different districts, the relationship between invasion success and ecofunctional traits of successful invaders would need to be further explored in dedicated studies, aiming to assess more quantitatively the differences existing among different areas. Furthermore, the validity of our conclusions should be explored in other taxa and other regions.

5. Conclusions

In summary, our results underline new aspects of a potential threat to native biodiversity, which possesses a widely-recognized conservation value (Humphries et al., 1995). However,

while biodiversity is regarded as a key characteristic of the Mediterranean region, native biodiversity has not been extensively evaluated, especially from a socio-economic perspective. Social support for native biodiversity could be too low, especially in the case of fish (see e.g. Tiberti (2018)), to develop and apply multi-species conservation actions that could be costly and unpopular. Conversely, the increasingly-popular role of several exotic and translocated species in recreational fisheries has provided a powerful stimulus in the opposite direction: the species of recreational interest are actively stocked, further spread by anglers and, in several cases, also benefit from protection measures (Milardi et al., 2018a; Milardi et al., 2018b). Perhaps as a consequence of these socio-economic pressures, national ministries of some Mediterranean countries do not strictly apply the national and international legislation aimed at limiting the presence and spread of exotic invasive species, or even allow the introduction of exotic species when they can bring economic benefits (see e.g. Anastácio et al., 2019). Despite potential economic gain, exotic species invasion can have multiple and cascading impacts on the host environment (some of which are relatively difficult to explore, see e.g. Milardi et al., 2019c), with potentially major consequences (Maceda-Veiga et al., 2017), besides negatively affecting native biodiversity. Our work could contribute to underline the critical state of native biodiversity and the relevance of the invasion process in Mediterranean inland waters, but further actions would be needed to disseminate its significance to the general population and build political support for conservation solutions.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2019.134364>.

Author contributions

M.M. conceived the study and M.M. and A.G. collected and analyzed the data. All authors critically contributed to the drafting of the manuscript.

Declaration of Competing Interest

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

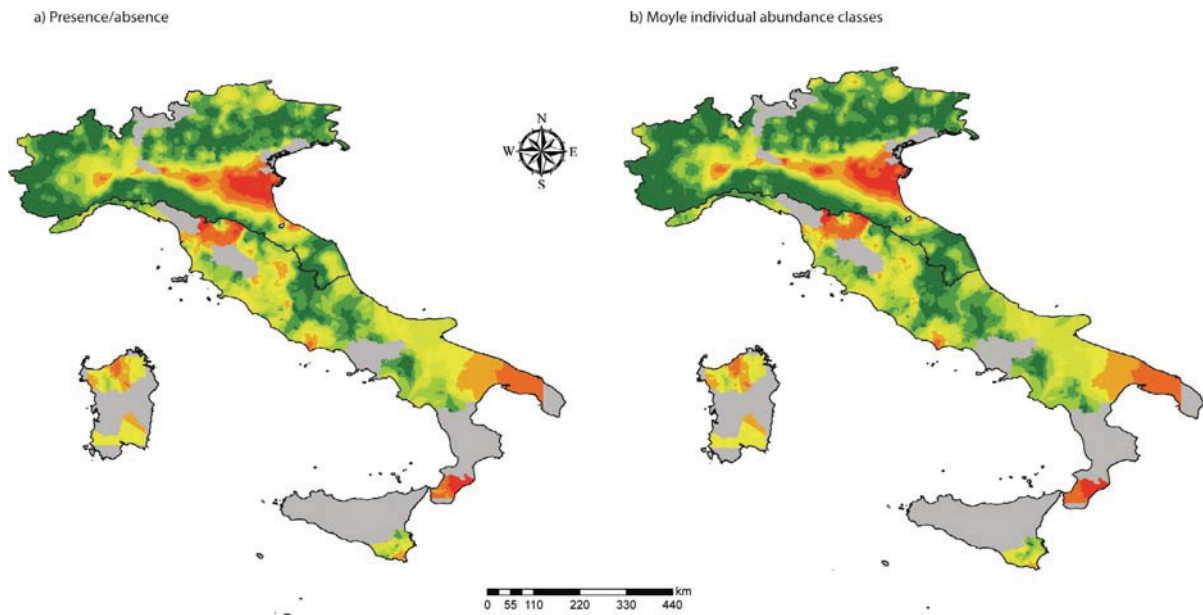
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Appendix A

Fig. A.1. The left (a) panel shows the invasion degree calculated on species presence/absence, while the right (b) panel represents the invasion degree estimated using Moyle individual abundance classes uncorrected for body size.



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