

Fish community of the river Tiber basin (Umbria-Italy): temporal changes and possible threats to native biodiversity

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ABSTRACT

Key-words:
*introduction,
exotic species,
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longitudinal
gradient*

The introduction of exotic fish species in the river Tiber basin has probably caused a serious alteration of original faunal composition. The purpose of this research was to assess the changes occurred over time in the state of the fish communities with particular reference to the reduction of local communities of endemic species. The study area comprised 68 water-courses of the Umbrian portion of the River Tiber basin; the analyses were carried out using the data of the Regional Fish Map of 1st and 2nd level and the 1st update, respectively collected during the periods between the 1990–1996, 2000–2006 and 2007–2014, in 125 sampling stations. The results show a progressive alteration of the fish communities' structure, as confirmed by the appearance in recent times of new alien species. A total of 40 species was found, only 14 native. The qualitative change of the fish communities appear to be closely related to the longitudinal gradient of the river. The results shows that particularly in the downstream reaches, the combined action of pollution and introduction of exotic species resulted in a gradual decrease in the indigenous component of fish communities. The information collected are the indispensable premise for taking the necessary strategies for conservation of endangered species.

RÉSUMÉ

La communauté piscicole du bassin du Tibre (Ombrie-Italie) : changements temporels et menaces possibles à la biodiversité originale

Mots-clés :
*introduction,
espèces
exotiques,
communautés
de poissons,
rivière Tibre,*

L'introduction d'espèces exotiques de poissons dans le bassin du fleuve Tibre a probablement provoqué une grave altération de la composition de la faune originale. Le but de cette recherche était d'évaluer les changements survenus au fil du temps dans l'état des communautés de poissons avec une référence particulière à la réduction des communautés locales d'espèces endémiques. La zone d'étude comprend 68 cours d'eau de la partie de l'Ombrie du bassin du fleuve Tibre; les analyses ont été effectuées en utilisant les données de la carte piscicole régionale de 1^{er} et 2^e niveau et de la 1^{re} mise à jour, respectivement recueillies pendant les périodes entre le 1990–1996, 2000–2006 et 2007–2014, dans 125 stations

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*penne
longitudinale*

d'échantillonnage. Les résultats montrent une altération progressive de la structure des communautés de poissons, confirmée par l'apparition ces derniers temps de nouvelles espèces exotiques. Un total de 40 espèces a été trouvé, seulement 14 natives. Le changement qualitatif des communautés de poissons semble être étroitement lié à la pente longitudinale de la rivière. Les résultats montrent que, particulièrement dans les tronçons situés en aval, l'action combinée de la pollution et de l'introduction d'espèces exotiques a entraîné une diminution progressive de la composante autochtone des communautés de poissons. Les informations recueillies sont la prémisses indispensable pour adopter les stratégies nécessaires à la conservation des espèces menacées.

INTRODUCTION

Italy is one of the most important European areas in regard to fish biodiversity due to the presence of a high number of endemic species (Smith and Darwall, 2006). Endemic species are particularly important for the conservation of biodiversity, as being distributed on a territory generally very limited, they are potentially exposed to greater risks of extinction. These risks are particularly high for the Umbria region because the number of endemic species with particularly restricted range is higher than elsewhere (Kottelat and Freyhof, 2007). In Italy there are two ichthyogeographic districts that can be identified on the basis of the dispersion processes that have characterized the fish fauna in the course of past geological eras (Bianco, 1993), mainly in the last glaciation. The Italic-peninsular district in particular is characterized by the presence of a set of fish species with distribution range more or less wide, but that all overlap in Umbria having the river Tiber basin as its center of connection. The most representative species of this area are: *Barbus tyberinus* (Bonaparte, 1839), *Padogobius nigricans* (Canestrini, 1867), *Rutilus rubilio* (Bonaparte, 1837) and *Squalius lucumonis* (Bianco, 1982), an endemic species restricted to the Tuscany Latium district (Giannetto et al., 2013). Some of them are of particular conservational interest: *Squalius lucumonis* is listed as "species in critical risk of extinction" in red lists of the IUCN (Rondinini et al., 2013), while *Padogobius nigricans* and *Barbus tyberinus* are classified as "vulnerable species". One of the main conservation threats is represented by the introduction of exotic species that can cause extinction of endemic species and lead to a reduction of native biodiversity (Simberloff, 2010; Mejía-Mojica et al., 2015; Smith et al., 2015). Recent studies on aquatic ecosystems of the Mediterranean regions have demonstrated that the introduction of exotic species influence the functional composition of the fish communities (Marr et al., 2013). It is important in this context to investigate the mechanisms that determine the impact of exotic species on indigenous ones.

The main objectives of the present paper were to assess the presence and distribution of fish species in the river Tiber basin and to analyze the influence of alien species on the status of indigenous species. The same analysis were carried out in the past (Lorenzoni et al., 2006; Giannetto et al., 2012) and one of the purposes of this research was to evaluate the changes occurred over time.

The structure of the fish communities can be affected, as well as biotic factors such as the introduction of exotic species, also by abiotic factors such as temperature, depth, current rate (Smith et al., 2015). So another aim of the present study was to assess the environmental characterization and the role of abiotic factors in the distribution of fish species.

MATERIALS AND METHODS

The study area comprises the Umbrian portion of the river Tiber basin (8412 km²) (Figure 1); it includes many tributaries, the most important being the Nestore river (watershed = 1033 km²),

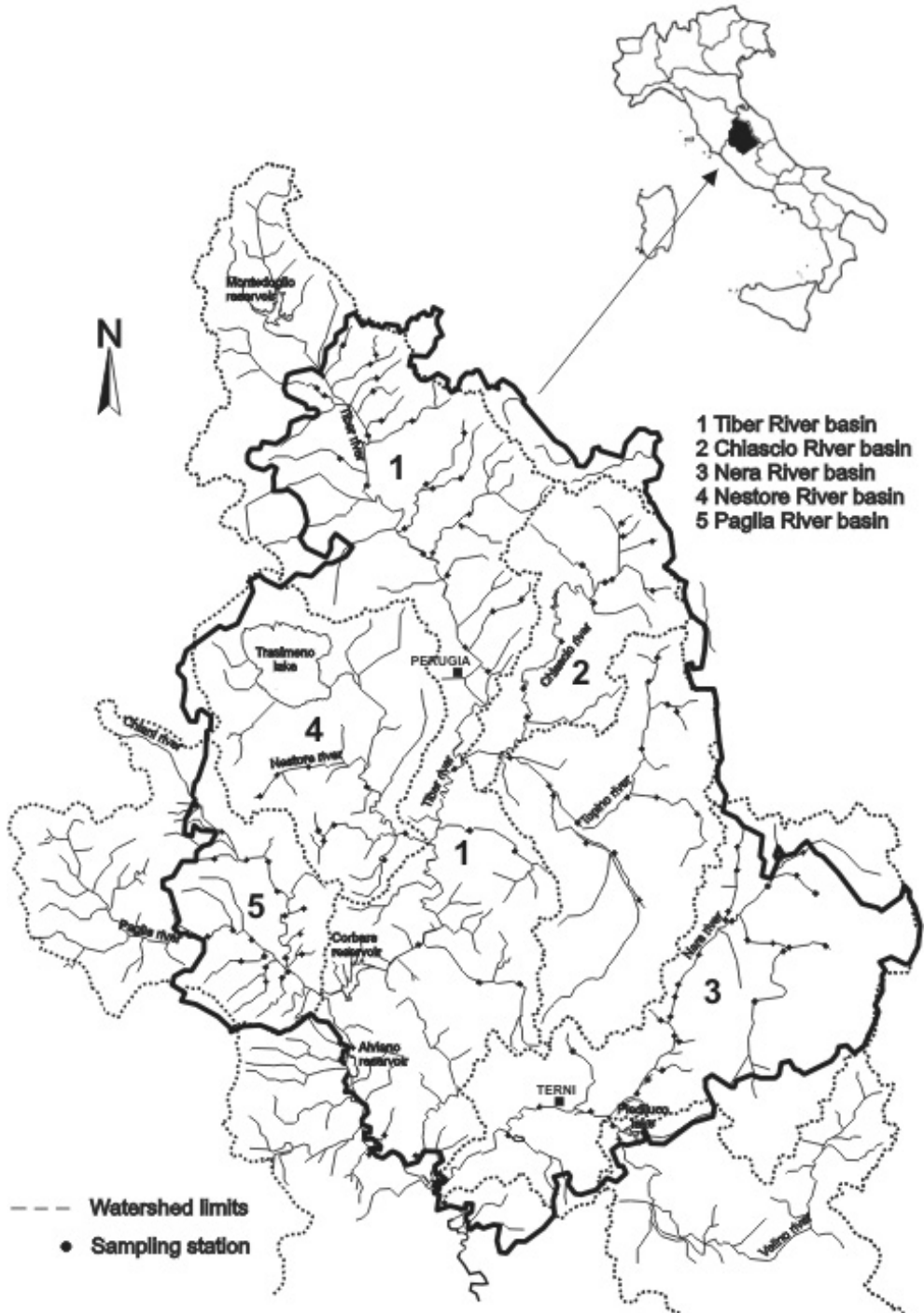


Figure 1
Study area and location of the sampling sites.

the Paglia river (1338 km²), the Chiascio river (5963 km²) and the Nera river (4280 km²). The study area was divided into five hydrographic units made up of the main sub-watersheds: the Chiascio, the Nera, the Nestore, the Paglia and the Tiber river's remainder which included the main channel and other minor tributaries. A total of 68 streams and rivers were included in the study area with 125 sampling stations. The analyses were carried out using the data of the Regional Fish Map of 1st and 2nd level and the 1st update, respectively collected during the periods between the 1990–1996, 2000–2006 and 2007–2014. All the 125 sites were

Table 1

Environmental and biological parameters.

Parameters	
Altitude (m a.s.l.)	Water temperature (°C)
Distance from the source (km)	pH
Watershed area (km ²)	Conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$ at 25 °C)
Average slope (%)	Dissolved oxygen (O ₂ mg·L ⁻¹)
Flow rate (m ³ ·s ⁻¹)	N-NH ₃ (mg·L ⁻¹)
Wetted river section (m ²)	SO ₄ (mg·L ⁻¹)
Average current speed (m·s ⁻¹)	Cl (mg·L ⁻¹)
E.B.I.	

sampled one time for each of the three census periods. The Regional Fish Map is a research promoted by the Umbria Region that relates to the census of fish species living in the catchment areas that have been investigated, the status of fish populations of greater interest and the environmental characterization of the analyzed rivers.

A census of the fish fauna was carried out at each sampling station. The fish were captured at low flow period using a continued or pulsed current electric shocker with power varying between 1500 and 4000 W. All captured fishes were identified and counted. For all specimens, the total length (TL) was measured to the nearest 0.1 cm, and weight was measured to the nearest 0.1 g (Anderson and Neumann, 1996) and a sample of scales was collected from each specimen for age determination. For all reaches, tract length was defined as 10 times the wetted channel, with a minimum and maximum length of 50 to 400 m. Some environmental and biological parameters were used to characterize the river sectors and these are reported in Table 1. The Extended Biotic Index (EBI) (Ghetti, 1986) is a biotic index used to evaluate the overall water quality based on the composition of the macrobenthic fauna. This index is based on the sensitivity of key groups to pollution and on the number of component groups in a sample: clean streams are given an index of 15 and this value decreases as pollution increases. Hydraulic variables were measured at transects within each sampling reach; depth was measured at three or more points within each transect and velocity in three positions along the vertical of the same point of depth measurement. Watershed area, distance from the source, average slope and altitude were determined from IGM topographic maps. Field measurements of specific conductivity, pH, water temperature and dissolved oxygen were made with electronic meters. The other chemical parameters of the water were determined according to APHA, AWWA and WPCF (1989) and APAT CNR IRSA (2003) specifications. The environmental variables were measured at the same time as fish sampling.

Some of the terms and definitions used are: introduced or exotic: any species voluntarily or accidentally released into an environment outside of its original range (Holcik, 1991; Cowx, 1997); introduced species are transplanted when they come from another country. Introduced species are translocated when they come from other Italian watersheds (Delmastro, 1986). An introduced species is acclimatized when it breeds and naturally maintains itself without any human interference. The level of degradation in the fish fauna was evaluated using the Zoogeographic Integrity Coefficient (ZIC) (Bianco, 1990; Elvira, 1995) expressed as the ratio between the number of native fish species and the total number of species found. This index varies from one (no exotic present) to zero (maximum level of alteration).

As in 1996 the distinction between *Barbus tiberynus* and *Barbus plebejus* Bonaparte, 1839 had not been accepted by all authors (Zerunian, 2002), in the analyses all the Po barbels caught during the Regional Fish Map were considered as Tiber barbel, also in view of the fact that the presence of this species is limited to very few sampling stations located along the main channel of the river Tiber (Lorenzoni et al., 2010).

An analysis to verify the relationships among the environmental conditions of the investigated area was conducted using principal component analysis (PCA): the matrix used 15 variables (altitude, distance from the source, watershed area, average slope, flow rate, wetted river section, average speed of current, water temperature, pH, specific conductivity, dissolved oxygen, ammonium, sulphates, chlorides and EBI) (Table 1) and 375 observations. This analysis

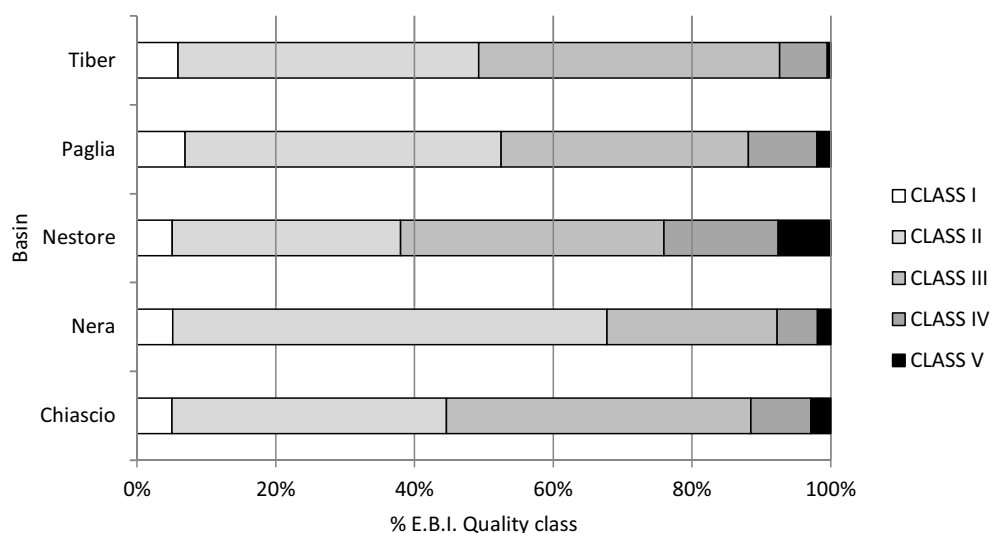


Figure 2
EBI Quality class: distribution of percentages among basins.

was performed with the STATISTICA 7 programs. All the variables (N) were transformed ($\log_{10}(N + 1)$) to normalize the distribution (Brown and Austen, 1996) and standardized to a mean of 0 and standard deviation of 1. The existing relationship between the first axis of PCA with ZIC was subsequently investigated by regression analysis. The ZIC values were converted using the arcsine transformation.

To analyze the relationships among environmental and biological data matrices was used the Canonical Correspondence Analysis (CCA) (ter Braak, 1986), a multivariate direct gradient analysis method. The CCA analysis was processed with the CANOCO statistical package for Windows 4.5. The results of CCA generate a diagram that displays approximate values of the weighted averages fish assemblage parameters (points) with respect to the supplied environmental variables. In the diagram, the environmental variables are represented by arrows that roughly point in the direction of maximum factor variation (ter Braak, 1986). The length of the arrow is proportional to the rate of change; therefore, a long arrow indicates a large change and that change is strongly correlated with the ordination axes. The position of the points in relationship to the arrows indicates the relationship between each point and the variable represented by the arrow. Those that are farthest along towards the head of the arrow are those with the largest values for that variable. To assess the statistical significance of the ordination axis we run Monte Carlo tests for 1000 permutations. An axis was considered statistically significant if the eigenvalue from the randomly permuted set exceeded the original in 50 or fewer cases $\alpha = 0.05$. The environmental matrix used included 15 variables and 375 observations (sampling stations). The fish assemblage matrix used included 39 variables (fish species) and 375 observations (sampling stations). The abundance of a fish species was coded using a scale that varies from 0 to 3 based on the population's density (0 ind/m² = absent; <0.05 ind·m⁻² = rare; from 0.05 to 0.1 ind·m⁻² = sub-dominant; >0.1 ind·m⁻² = dominant).

RESULTS

> ENVIRONMENTAL AND BIOLOGICAL PARAMETERS

The EBI values show that the water quality is very high, with the presence of many areas assigned to the first and second quality class, concerning areas with low human population, such as the Nera river basin; the worst situation refers to the areas of high incidence of human activities, such as the Nestore river basin (Figure 2). The distribution of EBI classes varied among the five basins (χ^2 Pearson = 43.325; $p = 0.001$). Compared to 1990–1996,

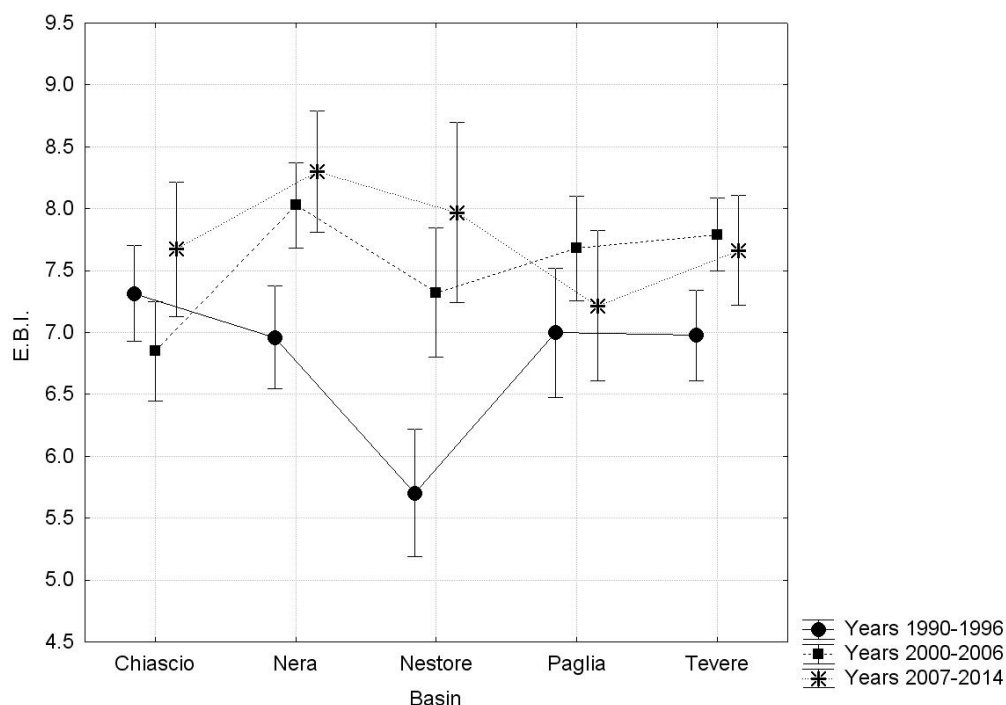


Figure 3

Distribution of average EBI values among basins in different periods. Vertical bars representing the 95% confident level.

Table II

Principal component analysis: summary statistics. In bold values for $p < 0.05$.

	Factor 1	Factor 2
Distance from the source (km)	-0.788997	-0.343403
Watershed area (km²)	-0.773475	-0.433708
Altitude (m a.s.l.)	0.643620	-0.285560
Average slope (%)	0.495417	0.158143
pH	0.179130	-0.020271
Conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$ at 25 °C)	-0.519333	0.705725
NNH₃ (mg·L⁻¹)	-0.365013	0.354319
SO₄ (mg·L⁻¹)	-0.593694	0.495176
Cl (mg·L⁻¹)	-0.450674	0.719955
Water temperature (°C)	-0.178423	-0.000444
O₂ (mg·L⁻¹)	0.247196	-0.229108
E.B.I.	0.486878	-0.330798
Average current speed (m·s⁻¹)	-0.167118	-0.617775
Flow rate (m³·s⁻¹)	-0.663165	-0.581919
Wetted river section (m²)	-0.749459	-0.460893

the environmental situation seems to have improved, especially in the case of the Chiascio, the Nera and the Nestore basins, while the situation is worsened over time in the Tiber river basin and in the Paglia river basin. The differences between the mean values among basins ($F = 3.87$; $p = 0.004$), among periods ($F = 22.16$; $p = 0.001$) and the crossing effect between the two categorical variables ($F = 4.43$; $p = 0.001$) are statistically significant to the two-way factorial analysis of variance (Figure 3).

The results of the principal components analysis on environmental parameters and EBI are reported in Tables II and III. The first factor, that explains 28.20% of the overall variability, shows the changes that occur along the upstream-downstream gradient of a water course: to the decrease of altitude and the increase of the distance of the source, there is an increase

Table III

Eigenvalues for factors 1–2 expressed as % of total variance.

	Eigenvalue	% of total variance	Cum. eigenvalue	Cum. %
Factor 1	4.230	28.203	4.230	28.203
Factor 2	2.895	19.298	7.125	47.501

of the watershed area and of the wetted river section. In the Tiber river basin this gradient is associated with a progressive decline in water quality, clearly shown by the decrease of EBI and increased conductivity and concentration of dissolved salts, such as the sulphates. The downstream reaches, where the impact of human activities is high, show a more serious pollution. The second factor, that explains 19.30% of the overall variability, indirectly shows the link between two hydraulic variables, such as current speed and flow rate, and the conductivity and the chlorides: the dilution of pollutants increases along the longitudinal gradient of a river.

> FISH FAUNA

A list of the species found is reported along with information about their origin (Table IV). *Barbus plebejus* was considered indigenous because not all authors agree to consider it as an exotic species (Bianco, 1995; Zerunian, 2002). The brown trout was considered native or transplanted because some studies have shown the presence of many indigenous populations in the study area but also the presence of populations with a degree of introgressive hybridization more or less high. The same considerations can be applied for the pike. 40 species of fish were found. Only 14 are native. Of the remaining 26 alien species, 9 come from other Italian basins, while 17 were transplanted from foreign basins. With respect to the results obtained in the Fish Map of 1st level, after a decade we can observe the emergence of 6 new alien species, of which 4 belong to the Cyprinidae family: *Barbus barbuis* (Linnaeus, 1758), *Gobio gobio* (Linnaeus, 1758), *Rhodeus sericeus* (Pallas, 1776), *Rutilus rutilus* (Linnaeus, 1758); 1 to the Salmonidae family: *Thymallus thymallus* (Linnaeus, 1758); 1 to the siluridi family: *Silurus glanis* Linnaeus 1758. In the course of the 1st update 4 more exotic species were found: *Blicca bjoerkna* (Linnaeus, 1758), *Ctenopharyngodon idellus* (Valenciennes, 1844), *Luciobarbus graellsii* (Steindachner, 1866) (Buonerba et al., 2013), *Salvelinus fontinalis* (Mitchill, 1814). The percentages of higher occurrence relate to native species: *Rutilus rubilio* (59.18%), *Barbus tyberinus* (58.19%), *Squalius squalus* (Bonaparte, 1837) (58.62%), *Telestes muticellus* (Bonaparte, 1837) (46.89%), *Salmo trutta complex* Linnaeus, 1758 (47.88%), *Padogobius nigricans* (39.12%) and *Squalius lucumonis* (35.31%). Among the exotic species higher frequency values relate to *Alburnus arborella* (Bonaparte, 1841) (30.65%), *Carassius auratus* (Linnaeus, 1758) (21.75%), *Protochondrostoma genei* (Bonaparte, 1839) (25.71%), and *Cyprinus carpio* (Linnaeus, 1758) (19.77%).

There is a progressive increase over time of the number of exotic species for sampling station (Figure 4). Comparing the Fishing Maps of 1st and 2nd level an increase in the number of native species is observed, while it can be observed an opposite pattern comparing the 2nd level with the 1st update (Figure 5). The differences between the three periods were highly statistically significant at Fisher's LSD Post Hoc test for the number of exotic species ($p = 0.001$). The differences between the years 1990–1996 and 2000–2006 of number of native species were not statistically significant at Fisher's LSD Post Hoc test ($p = 0.096$), while the differences between the years 2000–2006 and 2007–2014 were highly statistically significant at Fisher's LSD Post Hoc test ($p = 0.002$).

The relationship between the first PCA axis and the ZIC is described by a first order equation for each of the three different periods considered: $y = 1.220 + 0.123x$ ($r^2 = 0.39$, $p = 0.001$) (1990–1996); $y = 1.171 + 0.144x$ ($r^2 = 0.32$, $p = 0.000$) (2000–2006); $y = 1.169 + 0.116x$ ($r^2 = 0.24$, $p = 0.001$) (2007–2014). In all cases there is a gradual decrease in ZIC proceeding from upstream to downstream. To eliminate the effect of the longitudinal gradient the comparison of the ZIC values among the three periods was performed by ANCOVA; this analysis

Table IV
List of fish species and their origin.

Family	Species	Common name	Origin
Anguillidae Cyprinidae	<i>Anguilla anguilla</i> (Linnaeus, 1758)	Eel	Native
	<i>Alburnus arborella</i> (Bonaparte, 1841)	Bleak	Translocated
Centrarchidae	<i>Barbus barbus</i> (Linnaeus, 1758)	Danube barbel	Translocated
	<i>Barbus plebejus</i> Bonaparte, 1839	Po barbel	Native
	<i>Barbus tyberinus</i> Bonaparte, 1839	Tiber barbel	Native
	<i>Blicca bjoerkna</i> (Linnaeus, 1758)	White bream	Translocated
	<i>Carassius auratus</i> (Linnaeus, 1758)	Goldfish	Translocated
	<i>Protochondrostoma genei</i> (Bonaparte, 1839)	Italian nase	Translocated
	<i>Chondrostoma soetta</i> Bonaparte, 1840	Italian snoop	Translocated
	<i>Cyprinus carpio</i> Linnaeus, 1758	Carp	Translocated
	<i>Ctenopharyngodon idellus</i> (Valenciennes, 1844)	Grass carp	Translocated
	<i>Gobio gobio</i> (Linnaeus, 1758)	Gudgeon	Translocated
	<i>Luciobarbus graellsii</i> (Steindachner, 1866)	Ebro barbel	Translocated
	<i>Pseudorasbora parva</i> (Schlegel, 1842)	Top mouth gudgeon	Translocated
	<i>Rhodeus sericeus</i> (Pallas, 1776)	Bitterling	Translocated
	<i>Rutilus erythrophthalmus</i> Zerinian, 1982	North italian roach	Translocated
	<i>Rutilus rubilio</i> (Bonaparte, 1837)	Italian roach	Native
	<i>Rutilus rutilus</i> (Linnaeus, 1758)	Roach	Translocated
	<i>Scardinius erythrophthalmus</i> (Linnaeus, 1758)	Rudd	Native
	<i>Squalius lucumonis</i> (Bianco, 1982)	Etruscan chub	Native
	<i>Squalius squalus</i> (Bonaparte, 1837)	Chub	Native
	<i>Telestes muticellus</i> (Bonaparte, 1837)	Italian riffle dace	Native
<i>Tinca tinca</i> (Linnaeus, 1758)	Tench	Native	
<i>Lepomis gibbosus</i> (Linnaeus, 1758)	Pumpkinseed	Translocated	
<i>Micropterus salmoides</i> Lacépède, 1802	Largemouth bass	Translocated	
<i>Cobitis bilineata</i> Canestrini, 1865	Spined loach	Translocated	
<i>Cottus gobio</i> Linnaeus, 1758	Bullhead	Native	
<i>Esox lucius</i> Linnaeus, 1758	Pike	Native/Translocated	
<i>Gasterosteus aculeatus</i> Linnaeus, 1758	Three-spined stickleback	Native	
<i>Padogobius bonelli</i> (Bonaparte, 1846)	Po goby	Translocated	
<i>Padogobius nigricans</i> (Canestrini, 1867)	Arno goby	Native	
<i>Ameiurus melas</i> (Rafinesque, 1820)	Black bullhead	Translocated	
<i>Perca fluviatilis</i> Linnaeus, 1758	Perc	Translocated	
<i>Stizostedion lucioperca</i> (Linnaeus, 1758)	Pikeperch	Translocated	
<i>Gambusia holbrooki</i> Girard, 1859	Eastern mosquitofish	Translocated	
<i>Onchorhynchus mykiss</i> (Walbaum, 1792)	Rainbow trout	Translocated	
<i>Salmo trutta</i> (complex) Linnaeus, 1758	Brown trout	Translocated	
<i>Salvelinus fontinalis</i> (Mitchill, 1814)	Brook trout	Native/Translocated	
<i>Thymallus thymallus</i> (Linnaeus, 1758)	Grayling	Translocated	
<i>Silurus glanis</i> Linnaeus, 1758	European catfish	Translocated	
Ictaluridae			
Percidae			
Poeciliidae			
Salmonidae			
Siluridae			

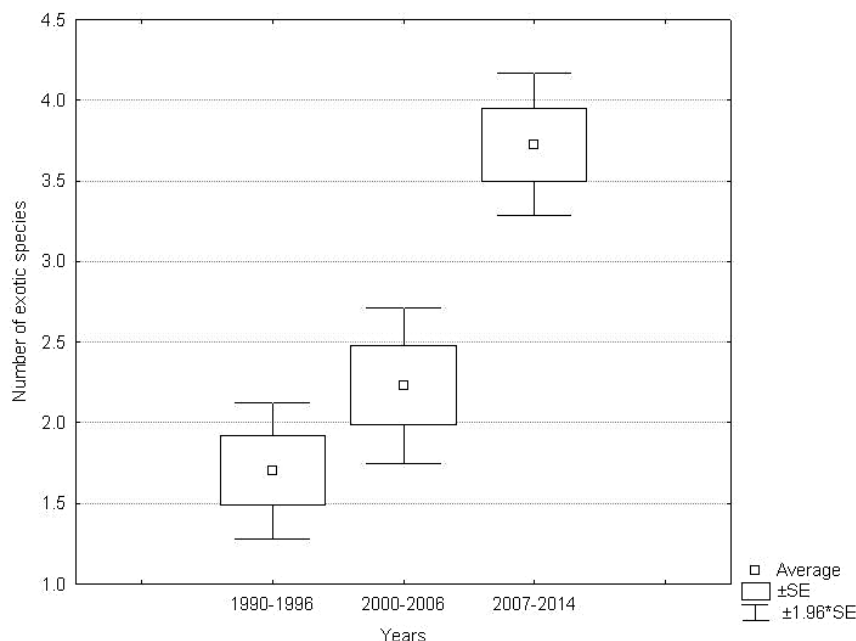


Figure 4
Trends over time of the number of exotic species.

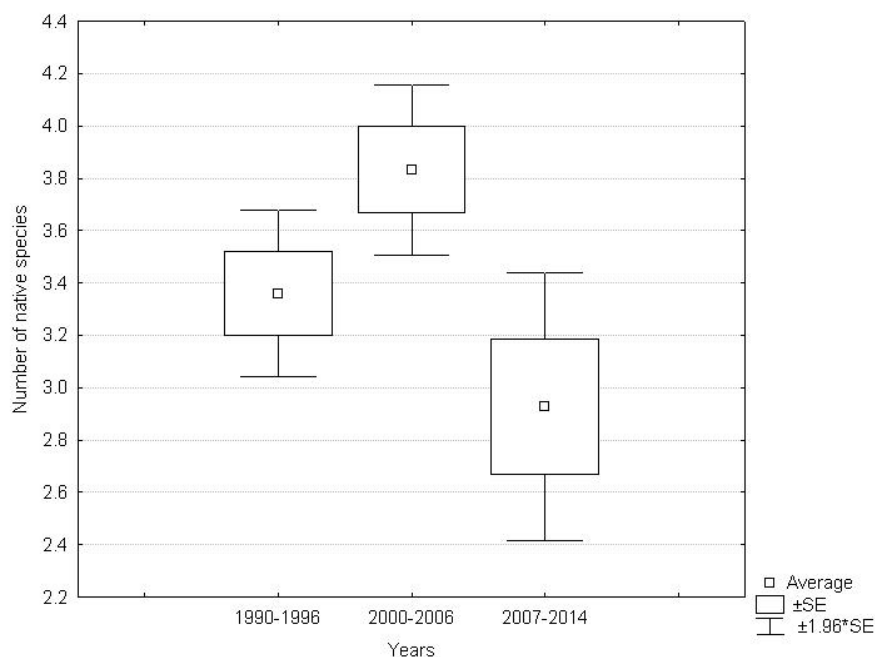


Figure 5
Trends over time of the number of native species.

was carried out using the first PCA axis as covariate, constant over time and equal to 0, and using the periods as categorical variable. Figure 6 shows that there is a progressive decrease of the ZIC over time ($F = 2.37$; $p = 0.032$), much more marked over the first two periods. Upper stream reaches have fish communities composed of indigenous species. Stream reaches that have community with a low degree of integrity, are located on the lower part of the river and show a link between water quality and status of the communities. The ZIC is higher in the mountain reaches, characteristic of small and medium sized water courses, with fish community composed of species of the trout zone and of the rheophilic cyprinidae zone.

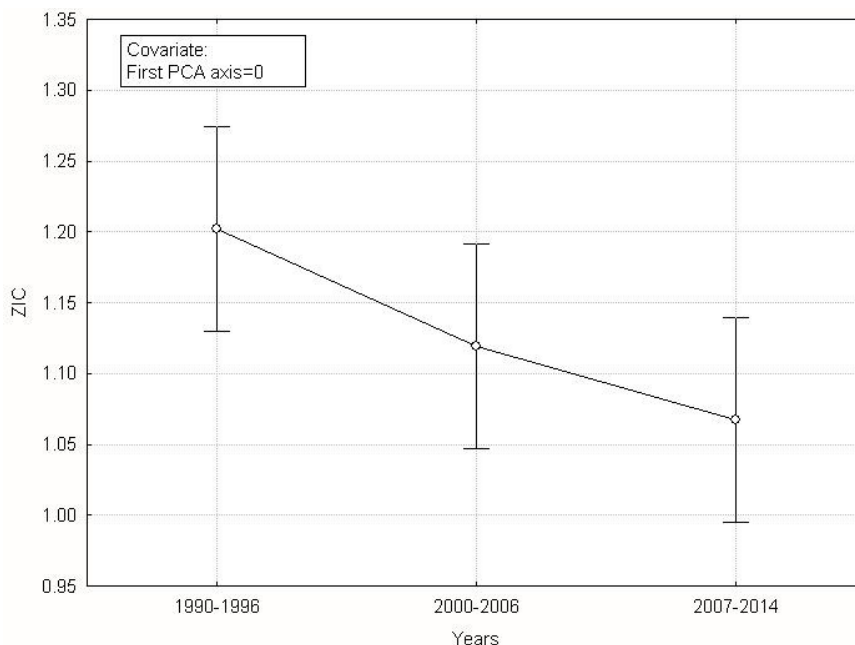


Figure 6

Trends over time of the ZIC values. Vertical bars representing the 95% confident level.

Tables V and VI shows the results of the CCA analysis: the first 2 axis explain 72.10% of the total variability (significance: $p = 0.001$); this result shows the close association between environmental variables and composition of the fish community. In Figures 7–8 are separately reported the plots of environmental variables and fish species. The first axis explains 55.10% of the overall variability (total inertia = 3.06). According with the results of the PCA analysis, the CCA analysis describes the changes of the environmental characteristics that occurs along the upstream-downstream gradient. To the decrease of altitude and average slope there is an increase in the distance from the source, the watershed area, wetted river section and flow rate. The second axis is less informative (22.10% of the overall variability) and reflects the progressive decline in the water quality along the upstream-downstream gradient: an inverse correlation between flow rate, watershed area and EBI, dissolved oxygen and chlorides can be observed. Also the position of fish species in the plot is strongly influenced by longitudinal gradient. The more distinctly rheofilic species, *i.e.* *Salmo trutta* and *Cottus gobio* Linnaeus, 1758 are positioned in the mountain portion of the investigated area; these are replaced, proceeding down the river, by an association of *Onchorhynchus mykiss* (Walbaum, 1792), *Padogobius nigricans*, *Padogobius bonelli* (Bonaparte, 1846), *Squalius lucumonis* and *Rutilus rubilio*, that characterizes the intermediate course of the analyzed rivers. In the central portion of the distribution prevail the rheofilic cyprinids and other fish species that characterize the barbel zone. At the left end of the distribution are positioned the most limnophilous fish species, typical of the downstream reaches (carp zone): *Rutilus rubilio*, *Carassius auratus*, *Scardinius erythrophthalmus* (Linnaeus, 1758), *Micropterus salmoides* Lacépède, 1802; many exotic species of recent introduction, such as *Pseudorasbora parva* (Schlegel, 1842), *Silurus glanis* and *Rutilus rutilus* also belong to this group.

DISCUSSION

In the study area the water quality is very high, with the presence of many areas assigned to the first and second EBI quality class, concerning areas with low human population, such as the Nera River basin. Compared to the past, the environmental situation seems to have improved, especially in the case of the Chiascio, the Nera and the Nestore basins. The situation

Table V

Canonical and correlation coefficients of environmental variables with axis.

Environmental and biological parameters	Canonical coefficients		Correlations with axis	
	AX1	AX2	AX1	AX2
Distance from the source (km)	-0.176	0.022	-0.451	0.306
Watershed area (km ²)	-0.156	0.534	-0.395	0.360
Altitude (m a.s.l.)	0.312	-0.201	0.508	-0.111
Average slope (%)	-0.009	-0.108	0.181	-0.106
pH	0.272	0.338	-0.006	0.047
Conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$ at 25 °C)	-0.100	0.007	-0.301	-0.039
NNH ₃ (mg·L ⁻¹)	-0.049	0.187	-0.205	0.084
SO ₄ (mg·L ⁻¹)	-0.225	0.001	-0.445	-0.036
Cl (mg·L ⁻¹)	-0.333	-0.594	-0.492	-0.219
Water temperature (°C)	-0.023	0.046	-0.050	0.029
Dissolved oxygen (mg·L ⁻¹)	0.197	-0.070	0.156	-0.048
E.B.I.	0.004	-0.221	0.205	-0.120
Average current speed (m·s ⁻¹)	0.183	0.038	0.232	0.226
Flow rate (m ³ ·s ⁻¹)	-0.008	0.107	-0.090	0.316
Wetted river section (m ²)	-0.174	-0.062	-0.180	0.245

Table VI

Eigenvalues of axis 1 and 2 expressed as % of total variance.

	Axis 1	Axis 2
Eigenvalues	0.342	0.137
Species-environment correlations	0.731	0.563
Cumulative percentage variance	55.100	77.200

is worsened over time in the areas of high incidence of human activities, such as the case of the river Tiber and of the river Paglia basins. In that areas there is the problem of water pollution from domestic and industrial point sources and from agricultural activity; also the presence of numerous water catchment for irrigation purposes aggravated the environmental situation.

The fish fauna of river Tiber basin has changed in the last 25 years with the appearance of 10 new exotic species. With the exception of *Salvelinus fontinalis*, *Thymallus thymallus*, *Ctenopharyngodon idellus* and partially *Onchorhynchus mykiss*, the other new exotic species are established with self-sustaining populations. In most cases the introduction of exotic species was unintentional and it was probably consequent to fish restocking programs: often in the past were carried out stocking programs with juvenile cyprinid fishes among which there were exotic species difficult to distinguish from native ones.

At present the 65% of the species that have been counted in the investigated area are exotic. Six native rheophilic species were detected with a frequency greater than 30% (*Squalius squalus*, *Squalius lucumonis*, *Barbus tyberinus*, *Rutilus rubilio*, *Padogobius nigricans*, *Telestes muticellus* and *Salmo trutta*). Among the exotic species higher frequency values relate to those of oldest introduction: *Alburnus arborella* (30.65%), *Carassius auratus* (21.75%), *Protochondrostoma genei* (25.71%), and *Cyprinus carpio* (19.77%).

The introduction of invasive alien species on ecosystems can determine the reduction of the natural area of distribution of native species and the extinction of native species or decline in their populations (Simberloff, 2010; Mejía-Mojica et al., 2015). In the river Tiber basin *Barbus barbus* (Linnaeus, 1758) is a threat to *Barbus tyberinus* because of competition and hybridization phenomena between the two species (Carosi et al., 2006). Also the introduction and the rapid expansion of *Padogobius bonelli* in the upper part of river Tiber basin is having a strong impact on *Padogobius nigricans* (Pompei et al., 2014). Among the most recently introduced species, *Luciobarbus graellsii* is the most concerning, because its presence

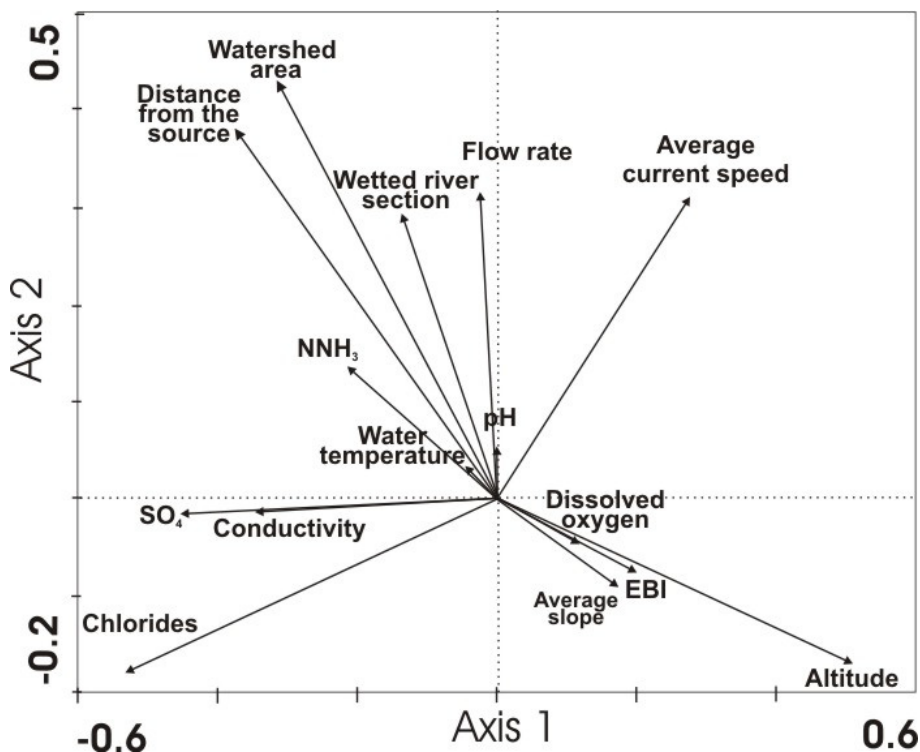


Figure 7
 Canonical correspondence analysis (CCA): plot of environmental variables.

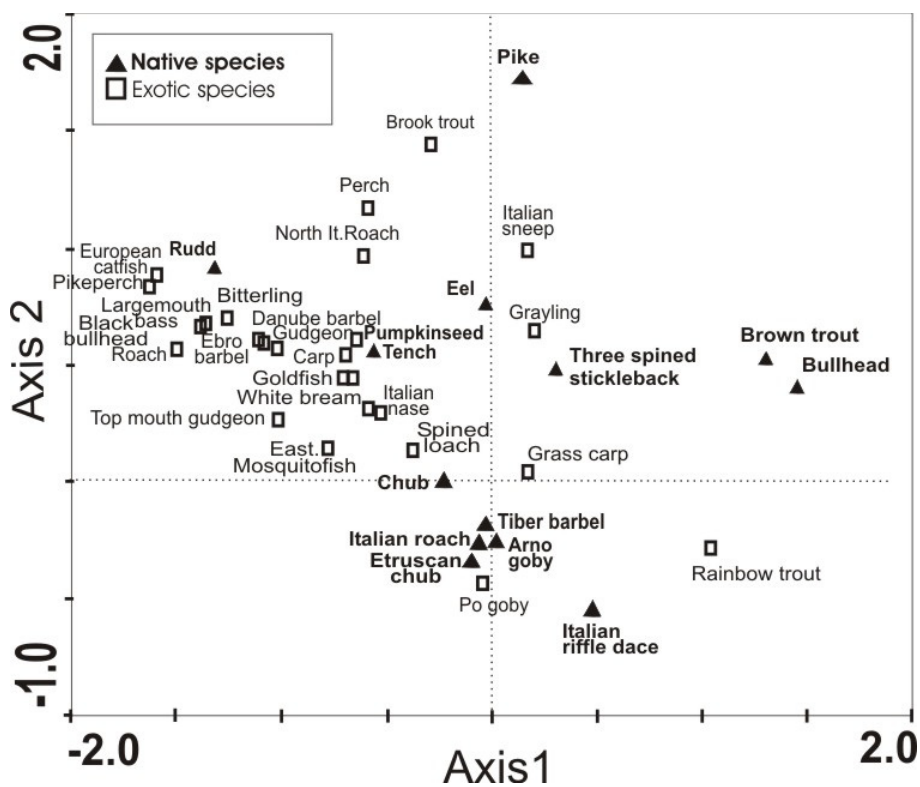


Figure 8
 Canonical correspondence analysis (CCA): plot of fish species.

has been detected in 2007 but it is already present in the river Tiber with an abundant and well-structured population (Buonerba *et al.*, 2013). Recent studies based on the use of relative weight to test the impact of the presence of exotic species on the condition of endemic native species in the Tiber river basin (Giannetto *et al.*, 2012), showed that *Barbus tyberinus* and *Telestes muticellus* represented the most sensitive species to the presence of non native species. Our results confirms the concern and show that there is a progressive increase over time of the medium number of exotic species for sampling station, while there is an opposite pattern for the ZIC index and for the number of native species, just for the last period.

Both PCA and CCA analyzes results show the changes of the environmental characteristics and the progressive decline in water quality that we can observe in the rivers of the study area along the upstream-downstream gradient. Fish assemblage composition varied along the rivers according to environmental variables (Godinho *et al.*, 2000). Numerous studies about river fish communities showed that species richness and the importance of limnophilic species increase with river size, in according to the modifications of stream habitats along the longitudinal gradient (Belliard *et al.*, 1997). In the river Tiber basin the assemblage of different fish species, proceeding from upstream to downstream, follows the zonation scheme adopted for the Umbria region (Mearelli *et al.*, 1995). The brown trout is the only species present in many mountain rivers, while the rheophilic cyprinids community is composed of an association of *Barbus tyberinus*, *Telestes muticellus*, *Squalius squalus*, *Squalius lucumonis*, *Rutilus rubilio* and *Padogobius nigricans*. The situations of best integrity were found in mountain rivers of all the basins and in the Nera river basin upstream of Terni city (Lorenzoni *et al.*, 2010). Very altered fish communities were found along the Tiber river (in the southern part of the basin) and in the upper and middle stream reaches of the rivers Nestore and Paglia. Upper stream reaches have species adapted to the cold waters and the fish community are dominated by salmonidae, intermediate reaches are characterized by the presence of native cyprinids, including many endemics, and downstream reaches are distinguished by the presence of many alien fishes adapted to warmer waters with low current rate (Marchetti and Moyle, 2001; Smith *et al.*, 2015). For the river Tiber basin the CCA results confirm that the alteration degree of the fish communities is closely linked to the longitudinal gradient and this trend, already highlighted by previous research (Lorenzoni *et al.*, 2006), it is confirmed in time. The ZIC is higher in the intermediate and high parts of rivers. Compared to the Fishing Map of 1st level we have observed a decrease in total integrity situations. Alien species are more invasive in freshwaters already altered by human activities (Baltz *et al.*, 1993; Moyle and Light, 1996; Dudgeon *et al.*, 2006). Exotic fish are dominant in conditions of low environmental quality, while native species are less tolerant to the alteration of the aquatic ecosystems (Darwall, 2009; Mejía-Mojica, 2015). Many authors reported that native fishes are associated with high water quality, flowing water and structurally complex habitats (Marchetti and Moyle, 2001). As has been reported for some Mediterranean basins (Özdemir *et al.*, 2015) also in the lower stream reaches of the investigated area the combined action of pollution and introduction of non-native species has resulted in a gradual decrease in the autochthonous component in fish communities. Increased occurrence of exotic species in lower stream reaches has also been reported in some rivers of Western North America (Smith *et al.*, 2015). In the present study, in relation to situations in which there was an improvement in water quality, the reduction of the number of native species recorded in the last decade still seems to be more related to the impact of exotic species rather than pollution.

CONCLUSIONS

The introduction of exotic species represents a serious threat to fish biodiversity, because a few species due to their large ecological plasticity, become increasingly widely distributed, while the number of indigenous endemic species reduces. The information collected in the present study are the indispensable premise for taking the necessary strategies for conservation of endangered species.

In the investigated area remain a substantial number of rivers that have not been irreversibly altered from their original state by human activities. In particular the small rivers of the Tiber river basin represent a refuge area for the indigenous fish community, which in many cases are still unchanged, and may play a key role in the maintenance of native biodiversity. In these cases, it is particularly important to establish large protected areas and to prevent the introduction of exotic species.

In some cases the presence of insurmountable natural and human made barriers preventing colonization of exotic species in the upper stream reaches and it is determinant in the conservation of native fishes; in these cases the application of the EC Water Framework Directive (2000/60) to the European water bodies, with regard to the ecological continuity, should be graduated according to the preservation of endemic freshwater ichthyofauna.

Given the torrential regime of the analyzed rivers, the maintaining of Minimum Vital Flows (MVF), plays a decisive role (Bicchi *et al.*, 2006), even considering that climate models predict future accentuations of drought phenomena as a result of global warming. Natural variability in flows and water levels is essential to underpin conservation strategies for freshwater biodiversity and their habitats (Dudgeon *et al.*, 2006). The loss of flow that occurs in drought periods can determine isolation of fishes with different thermal tolerance (Mejía-Mojica *et al.*, 2015).

The water pollution and the alteration of habitats have a decisive effect on the disappearance of the less tolerant species (Mejía-Mojica *et al.*, 2015). Especially in the lower stream reaches, the recovery of the integrity quality of the fish communities is subordinate to the recovery of water quality and the containment of exotic species. The conservation of native fish biodiversity should be evaluated in concert with fishery management (Pascual *et al.*, 2002). The angling web forums have proven particularly useful in the early detection of the introductions of exotic species, such as the case of *Perca fluviatilis* in continental Portugal (Banha *et al.*, 2015).

A decisive role in the prevention of the introduction of exotic species is played by environmental education. It is important to spread information on the impact of the exotic species and information on the possible management tools (Genovesi, 2007).

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