Knowledge and Management of Aquatic Ecosystems (2016) 417, 22
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DOI: 10.1051/kmae/2016009

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RESEARCH PAPER — OPEN ACCESS

# Status of *Pseudorasbora parva* in the Tiber River Basin (Umbria, central Italy) 20 years after its introduction

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Received December 2, 2015 – Revised March 9, 2016 – Accepted March 11, 2016

**Abstract –** The aim of this research was to assess the distribution, abundance and growth of the non-native topmouth gudgeon *Pseudorasbora parva* that was recorded for the first time in the Tiber River Basin (central Italy) in 1994. The competitive interaction of *P. parva* with four native fish species was also investigated. The study area comprised 92 watercourses of the Umbrian section of the Tiber River Basin. Demographic and environmental data were collected during the period 1990−2014 in 171 sampling sites. The results of this study showed a wide distribution of *P. parva* in the study area, with records from 23.39% of all sampling sites (40 out of 171). This species inhabits the downstream reaches, where the presence of many non-native species and the poor environmental quality are associated with a decrease in native fish species. A total of 5570 specimens of *P. parva* were collected and five age classes ($0^+$ to $4^+$) were identified. The equation for the total length-weight relationship of *P. parva* was $W = 0.021TL^{2.673\pm0.015}$. For the chub *Squalius squalus*, the Tiber barbel, *Barbus tyberinus,* and the roach, *Rutilus rubilio,* the average values of the relative weight were significantly higher in the sites where *P. parva* was absent. The results of the present study suggest the need to undertake proper strategies for native biodiversity conservation.

**Key-words:** non-native species / distribution / growth / relative weight / longitudinal gradient

**Résumé – Le statut de *Pseudorasbora parva* dans le bassin du fleuve Tibre (Ombrie, Italie centrale) 20 ans après son introduction.** L'objectif de cette recherche était d'évaluer la distribution, l'abondance et la croissance de *Pseudorasbora parva* non-indigène qui a été observé pour la première fois en 1994 dans le bassin du fleuve Tibre. L'interaction compétitive de *P. parva* avec quatre espèces de poissons indigènes a également été étudiée. La zone d'étude comprend 92 cours d'eau de la région ombrienne du bassin du fleuve Tibre. Les données démographiques et environnementales ont été recueillies au cours de la période 1990−2014 dans 171 sites d'échantillonnage. Les résultats de cette étude ont montré une large distribution de *P. parva* dans la zone d'étude, avec sa présence dans 23,39% de tous les sites d'échantillonnage (40 sur 171). Cette espèce habite les tronçons aval, où la présence de nombreuses espèces non indigènes et de la faible qualité de l'environnement ont été associées à une diminution des espèces de poissons indigènes. Un total de 5570 spécimens de *P. parva* ont été recueillis et cinq classes d'âge ($0^+$ à $4^+$) ont été identifiées. L'équation de la relation longueur totale − poids était $W = 0.021TL^{2.673\pm0.015}$. Pour le chevesne *Squalius squalus*, le barbeau du Tibre *Barbus tyberinus* et le gardon *Rutilus rubilio* les valeurs moyennes du poids relatif ($Wr$) étaient significativement plus élevées dans les sites où *P. parva* était absent. Les résultats de la présente étude suggèrent la nécessité d'entreprendre des stratégies appropriées pour la conservation de la biodiversité indigène.

**Mots-clés :** espèces non indigènes / distribution / croissance / poids relatif / gradient longitudinal

## 1 Introduction

The topmouth gudgeon, *Pseudorasbora parva* (Temminck and Schlegel, 1846), is a small cyprinid species native to Southeast Asia whose expansion outside of its native range is a striking example of pan-continental invasion. In less than 50 years this species has invaded 32 countries from central Asia to North Africa (Gozlan *et al.*, 2010). *Pseudorasbora parva* was introduced in the Danube River Basin in the 60s as a result of restocking activities carried out to support grass carp populations (Benarescu, 1964; Benarescu and Nalbant, 1973). In the same period multiple introductions of the topmouth gudgeon were carried out around the Black Sea (Simon *et al.*, 2011). This species subsequently spread to many other European rivers (Lelek, 1987; Allardi and Chancerel, 1988; Bianco, 1988; Caiola and De Sostoa, 2002; Witkowski, 2009). In Italy, the first record of *P. parva* dates back to 1988, when it was reported from channels in the lower floodplains of

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Emilia-Romagna. From here, it later spread to the lower stream reaches of the Po River (Sala and Spampanato, 1990). The presence of *P. parva* in Umbria (central Italy) was reported for the first time in 1994, when it was found in one sampling site located in the middle course of the Tiber River (Lorenzoni *et al.*, 1997). Five years later the presence of *P. parva* was detected in the middle course of the Chiascio River, where it may have been introduced accidentally during fish re-stocking that were carried out to support fisheries. From these two first locations *P. parva* expanded rapidly in all directions. In 20 years this species invaded the middle and lower reaches of many watercourses of the Tiber catchment and it is still spreading throughout the Tiber River Basin (Lorenzoni *et al.*, 2006, 2010) through spontaneous successive colonizations. At the end of the 1990s, the first specimens of *P. parva* were collected in Lake Trasimeno (Ghetti *et al.*, 2007). This species has continued its expansion and currently forms an established population in this lake (Lorenzoni and Ghetti, 2012).

The rapid spreading of this species outside of its native range can be imputed to its biological and ecological characteristics: (i) omnivorous diet; (ii) short generation time; (iii) high fecundity; (iv) high tolerance to extreme environmental conditions and (v) high phenotypic plasticity (Pollux *et al.*, 2006; Kapusta *et al.*, 2008; Witkowski, 2009; Hôrková and Kováč, 2014, 2015; Masson *et al.*, 2016).

The presence of a high number of endemic species with a limited distribution (Kottelat and Freyhof, 2007) makes Umbria an important area for the conservation of biodiversity. It is known that one of the major threats for native biodiversity is the introduction of exotic species which can cause the extinction of endemic species (Simberloff, 2010; Smith *et al.*, 2015). Several studies have reported the negative impact of *P. parva* on European fish species due to predation on the eggs and larvae of native species, inter-specific competition for food, and the introduction of non-native parasites (Welcomme, 1988; Gozlan *et al.*, 2005; Pinder and Gozlan, 2005; Pollux and Korosi, 2006; Gozlan *et al.*, 2010; Spikmans *et al.*, 2013).

The objectives of this study were to analyse the distribution, abundance and growth of *P. parva* in the Tiber River Basin with the aim of assessing its competitive interaction with native fish species and to investigate the impact of an invasive non-native species on native fish fauna in the study area. The information collected in this study will form a basis on which to set up sound conservation strategies in central Italy.

## 2 Materials and methods

The study area is the Umbrian section of the Tiber River Basin (8412 km$^2$) (Figure 1) (Lorenzoni *et al.*, 2006). This area includes many tributaries of the Tiber River, the most important of which are the Nestore (watershed = 1033 km$^2$), Paglia (1338 km$^2$), Chiascio (5963 km$^2$), and Nera (4280 km$^2$) rivers. The study area was divided into five hydro-graphic areas, representing the main sub-watersheds: the Chiascio, Nera, Nestore, and Paglia rivers and a section of the Tiber River that included the main channel and other minor tributaries. A total of 92 streams and rivers and 171 sampling sites were considered in this study. The Forest Service, Economics and Mountain territory of the Umbria Region carried out research

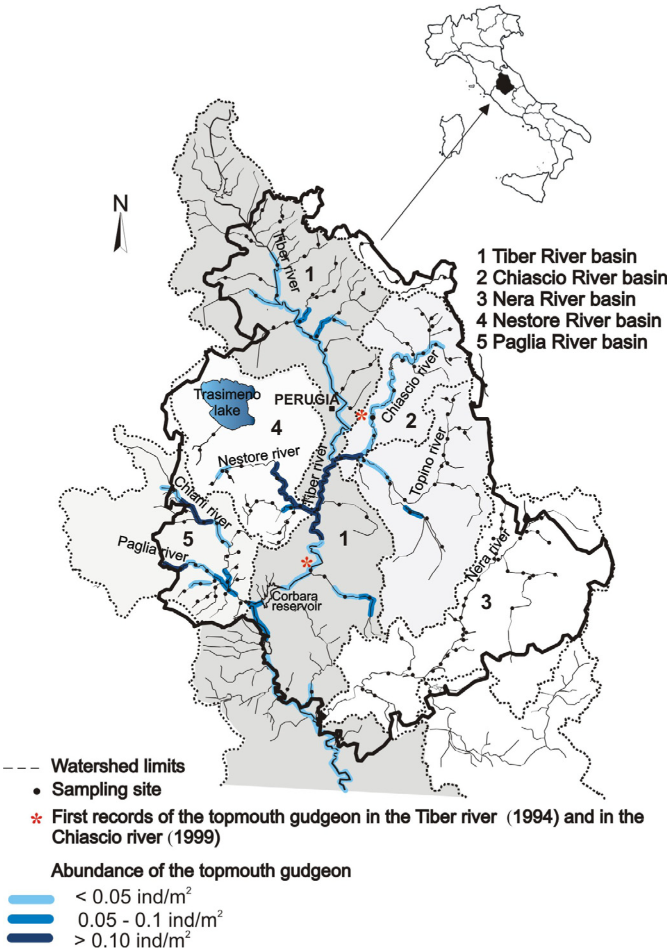


**Fig. 1.** Study area, distribution of *Pseudorasbora parva* and location of the sampling sites.

and made a Regional Fish Map that reports the census of fish species living in the catchment area, the status of fish populations of greater interest and the environmental characterization of the analysed rivers (Lorenzoni *et al.*, 2010). In the present study, data from the Regional Fish Map collected in three census periods (1990–1996, 2000–2006, 2007–2014) were used.

A census of the fish fauna was carried out at each sampling site using the removal method (Moran, 1951; Zippin, 1956). Fish were captured during low flow periods using a continued or pulsed electric current with power varying between 600 and 4500 W. All captured fish were identified and counted. For all specimens, total length (TL) and body weight were measured to the nearest 0.1 cm, and to the nearest 0.1 g (Anderson and Neumann, 1996), respectively. For all species, a sample of scales was collected from each specimen for age determination. At the end of the field activities, all the fish caught were released at the site of capture. All scales were stored in 33% ethanol and later observed with a stereo microscope using the image-analysis system IAS 2000. Age was determined using the scalimetric method (Bagenal, 1978) and further validated through the analysis of the length-frequency distribution (Britton *et al.*, 2004). For all reaches, tract length was defined as 10 times the wetted channel, with a minimum

**Table 1.** The *t*-test analysis for environmental parameters: comparison of sampling sites in which *Pseudorasbora parva* was present and those in which it was absent. $p < 0.05$ is in bold.

| Environmental parameters | *P. parva* absence | | *P. parva* presence | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *N* values | Mean (±St. Dev.) | *N* values | Mean (±St. Dev.) | Minimum | Maximum | *t* value | *p* |
| Distance from the source (km) | 382 | 20.624 ± 25.601 | 64 | 70.460 ± 61.739 | 4.000 | 216.000 | 9.643 | **0.000** |
| Watershed area ($km^2$) | 382 | 187.282 ± 432.977 | 64 | 945.810 ± 1459.871 | 15.700 | 6349.700 | 9.736 | **0.000** |
| Altitude (m a.s.l.) | 391 | 317.349 ± 143.001 | 65 | 183.803 ± 73.613 | 42.000 | 410.000 | 7.988 | **0.000** |
| Average slope (%) | 388 | 2.188 ± 1.727 | 66 | 2.05 ± 1.850 | 0.160 | 9.800 | 0.750 | 0.453 |
| pH (units) | 397 | 8.074 ± 1.110 | 68 | 8.042 ± 0.300 | 7.110 | 8.920 | 1.076 | 0.283 |
| Conductivity ($\mu s.cm^{-1}$ at 25°C) | 396 | 570.441 ± 197.189 | 68 | 777.935 ± 326.037 | 400.000 | 2220.000 | 3.840 | **0.000** |
| $BOD_5$ ($mg.L^{-1}$) | 367 | 1.749 ± 2.140 | 66 | 3.376 ± 2.817 | 0.600 | 21.000 | 6.786 | **0.000** |
| COD ($mg.L^{-1}$) | 341 | 8.501 ± 8.798 | 62 | 9.889 ± 5.556 | 0.339 | 46.000 | 2.920 | **0.004** |
| $NNO_3$ ($mg.L^{-1}$) | 389 | 1.689 ± 3.041 | 68 | 2.289 ± 2.030 | 0.057 | 10.600 | 4.114 | **0.000** |
| $NNO_2$ ($mg.L^{-1}$) | 389 | 0.033 ± 0.099 | 68 | 0.063 ± 0.112 | 0.003 | 0.760 | 2.829 | **0.005** |
| $NNH_3$ ($mg.L^{-1}$) | 390 | 0.229 ± 1.555 | 68 | 0.205 ± 0.290 | 0.006 | 12.300 | 2.952 | **0.003** |
| $SO_4$ ($mg.L^{-1}$) | 389 | 52.668 ± 59.948 | 68 | 72.217 ± 39.284 | 16.000 | 278.000 | 5.640 | **0.000** |
| $PPO_4$ ($mg.L^{-1}$) | 351 | 0.059 ± 0.098 | 59 | 0.132 ± 0.199 | 0.003 | 1.820 | 5.779 | **0.000** |
| $P_{tot}$ ($mg.L^{-1}$) | 345 | 0.073 ± 0.128 | 64 | 0.165 ± 0.224 | 0.003 | 2.200 | 5.644 | **0.000** |
| Cl ($mg.L^{-1}$) | 389 | 18.894 ± 18.929 | 68 | 31.878 ± 20.092 | 10.300 | 120.000 | 7.427 | **0.000** |
| Water temperature (°C) | 378 | 13.867 ± 4.699 | 63 | 17.380 ± 4.764 | 6.300 | 28.300 | 5.093 | **0.000** |
| Dissolved oxygen ($mg.L^{-1}$) | 383 | 9.373 ± 2.192 | 66 | 8.416 ± 2.617 | 1.970 | 17.000 | 4.196 | **0.000** |
| Extended Biotic Index (EBI) | 380 | 7.826 ± 2.018 | 65 | 6.811 ± 1.237 | 3.000 | 9.000 | 5.484 | **0.000** |
| EBI Quality Class | 388 | 2.328 ± 0.831 | 66 | 2.846 ± 0.718 | 2.000 | 5.000 | 4.258 | **0.000** |
| Width (m) | 330 | 6.531 ± 6.376 | 42 | 12.868 ± 7.359 | 0.800 | 28.500 | 6.405 | **0.000** |
| Depth (m) | 316 | 0.343 ± 0.230 | 40 | 0.493 ± 0.289 | 0.100 | 1.700 | 4.085 | **0.000** |
| Average current speed ($m.s^{-1}$) | 351 | 0.283 ± 0.272 | 45 | 0.214 ± 0.135 | 0.035 | 0.718 | 1.406 | 0.160 |
| Flow rate ($m^3.s^{-1}$) | 364 | 0.810 ± 1.533 | 47 | 1.197 ± 1.351 | 0.002 | 5.600 | 1.716 | 0.087 |
| Wetted river section ($m^2$) | 344 | 1.729 ± 2.348 | 45 | 3.904 ± 3.654 | 0.052 | 16.088 | 5.652 | **0.000** |
| Average shaded surface (units) | 394 | 2.303 ± 1.418 | 66 | 1.303 ± 1.336 | 0.000 | 4.000 | 5.656 | **0.000** |
| Cover (units) | 393 | 2.458 ± 1.150 | 66 | 2.197 ± 1.292 | 0.000 | 4.000 | 2.141 | **0.033** |
| Canopy cover (units) | 388 | 0.948 ± 1.050 | 65 | 0.923 ± 1.108 | 0.000 | 4.000 | 0.397 | 0.692 |
| Substrate size (units) | 398 | 4.848 ± 2.356 | 68 | 4.179 ± 2.480 | 1.000 | 7.000 | 2.128 | **0.034** |

and maximum length of 50 and 400 m, respectively. A total of 28 biological and environmental parameters particularly significant for the fish fauna were used to characterize the river sectors (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 macro benthic 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. Hydrologic variables were measured at transects within each sampling reach. Depth was measured at three or more points within each transect and velocity along the vertical of the same three points. Watershed area, distance from the source, average slope and altitude were determined from the topographic maps of the Istituto Geografico Militare. Field measurements of specific conductivity, pH, water temperature and dissolved oxygen were carried out with electronic metres manufactured by YSI, Hanna Instruments and WTW GmbH. Other chemical parameters of the water were determined according to APHA, AWWA and WPCF (1989) and APAT CNR IRSA (2003) specifications. Substrate size was evaluated according to the diameter of the main component present. Each size category was given an index ranging from 1 (<1 mm) to 7 (>256 mm). Cover, canopy cover and shaded areas were estimated by visual observation on a five degree scale: 0 = absent, 1 = isolated areas, 2 = frequent interruptions, 3 = few interruptions and 4 = continuous areas. All the variables 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 total length-body weight relationship ($TL$-$W$) was estimated by the least-squares method (Ricker, 1975) based on the logarithmic equation: $\log_{10} W(g) = a + b \log_{10} TL$ (cm). Theoretical growth was estimated by the von Bertalanffy growth curve model (Von Bertalanffy, 1938): $TL_t = L_\infty (1 - e - k(t - t_0))$, where $TL_t$ is the total length of the fish at time $t$, $L_\infty$ the theoretical maximum length (cm), $k$ the rate of approach to $L_\infty$, and $t_0$ the theoretical age at which $TL_t = 0$. In addition, the index of growth performance ($\Phi$) was calculated by the equation of Pauly and Munro (1984): $\Phi = \log 10k + 2 \log 10L_\infty$, where $k$ and $L_\infty$ are the growth parameters of the Von Bertalanffy model. The $TL$-$W$ relationship and theoretical growth calculations were carried out using the data recorded in September 2014 for the *P. parva* population of Lake Trasimeno.

Some of the terms and definitions used here include "non-native", meaning any species outside its original range and "introduced", meaning any non-native species deliberately or accidentally released into the wild (Gozlan *et al.*, 2010). An introduced species is "established" when it breeds and naturally maintains itself without any human interference (Gozlan *et al.*, 2010). The level of degradation in 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 ranges from one (no non-native species present) to zero (maximum level of alteration). The ZIC values were converted using the arcsine transformation.

The canonical correspondence analysis (CCA) (ter Braak, 1986) was performed to extract the environmental variables that drive the observed fish assemblages. 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 of 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 relation to the position of the arrows indicates the relationship between each point and the variable represented by the arrow. The points closest to the head of the arrow are those with the largest values for that variable. To assess the statistical significance of the ordination axis we ran 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 $\alpha = 0.05$. To minimize the potential biases affecting CCA due to differences between periods and differences in sampling sites and to make the data collected in the three census periods comparable, the fish and environmental data sets were restricted to 125 sites. Each site was sample once for each of the three census periods. The final matrix included 375 observations (sites × sampling events). The environmental matrix used included 15 variables and 375 observations (sampling sites). The fish assemblage matrix used included 12 variables (4 native and 8 non-native fish species) and 375 observations (sampling sites). Non-native fish species were selected based on the results of previous research which showed a close link between the presence of these species and the presence of *P. parva* in the study area (Carosi *et al.*, 2015). The four native species are the key species that characterize the middle and lower stream reaches of the Tiber River Basin (Lorenzoni *et al.*, 2010). 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.m$^{-2}$ = absent; <0.05 ind.m$^{-2}$ = rare; from 0.05 to 0.1 ind.m$^{-2}$ = sub-dominant; >0.1 ind.m$^{-2}$ = dominant). To fit a statistical model of change in the densities of *P. parva* and native species along axis 1, a generalized linear models (GLM) by means of normal distribution was used. The same analysis was carried out to fit the relationship between *P. parva* abundance and environmental variables.

As a contribution to the assessment of the impact of *P. parva* on native fish species, the body condition of four indigenous species was estimated, namely *Barbus tyberinus* (Bonaparte, 1839), *Squalius squalus* (Bonaparte, 1837), *Rutilus rubilio* (Bonaparte, 1837) and *Padogobius nigricans* (Canestrini, 1867). A total of 35 sampling sites were selected to estimate the body condition based on their close link with the presence of *P. parva*, as shown by the species-sampling sites plot of the CCA analysis carried out in a previous study (Carosi *et al.*, 2015). As regards *B. tyberinus*, *S. squalus* and *R. rubilio*, body condition was estimated by the relative weight $W_r$: $W_r = 100\,(W/W_s)$ where body weight = $W$ (g) and standard weight = $W_s$ (same species in good physiological condition). The relative weight ($W_r$) is a condition index based on the comparison between the real weight of an individual and the optimal weight of a specimen of the same species in good physiological condition ($W_s$). In the present study the standard weight $W_s$ was calculated separately for each species considered using the following equations calculated with the Empirical Percentile (EmP) method (Angeli *et al.*, 2010):

Tiber barbel: $\log_{10}(W_s) = -4.917 + (2.987 \log_{10} LT) - 0.003(\log_{10} LT)^2$

Chub: $\log_{10}(W_s) = -5.001 + 2.856 \log_{10} LT) + 0.067(\log_{10} LT)^2$

Italian roach: $\log_{10}(W_s) = -4.086 + 1.864 \log_{10} LT) - 0.062(\log_{10} LT)^2$

As regards the Arno goby, body condition was estimated by the relative condition factor (Le Cren, 1951): $K_n = 100W(aTL^b)^{-1}$, where $a$ and $b$ are the coefficients of the $TL$-$W$ equation estimated from the total sample.

## 3 Results

### 3.1 Overall population characteristics

A total of 5570 specimens of *P. parva* were collected, 5027 from rivers of the Tiber River Basin and 543 from Lake Trasimeno. The size of the sampled fish ranged from 2.5 to 11.2 cm (mean ± SD = 6.51 ± 1.49) and weight from 0.3 to 13.0 g (mean ± SD = 2.30 ± 1.19). Five age classes ($0^+$ to $4^+$) were identified.

### 3.2 Distribution and abundance

Fish census carried out between 1990 and 2014 showed that *P. parva* was present in 40 sampling locations (23.39% of the sampled sites) with a wide distribution in the middle and lower reaches of the Tiber river and its main tributaries, namely Chiascio, Topino, Nestore, Paglia and the Chiani rivers (Figure 1). The highest population densities were reported in the Nestore river basin (mean ± SD = 0.50 ± 0.60 ind.m$^{-2}$) and in the Paglia river basin (mean ± SD = 0.33 ± 0.61 ind.m$^{-2}$), while lower density values characterized the Tiber river basin (mean ± SD = 0.03 ± 0.03 ind.m$^{-2}$) and the Chiascio river basin (mean ± SD = 0.02 ± 0.04 ind.m$^{-2}$). In the Nestore river basin and in the Paglia river basin the density of *P. parva* clearly increased over time (Figure 2); differences between the first census period (1990−1996) and the third (2007−2014) were statistically significant ($p < 0.050$) at Fisher's LSD Post Hoc test for both basins, while differences between the first and the second period (2000−2006) were statistically significant only for the Nestore river basin. Differences between mean density

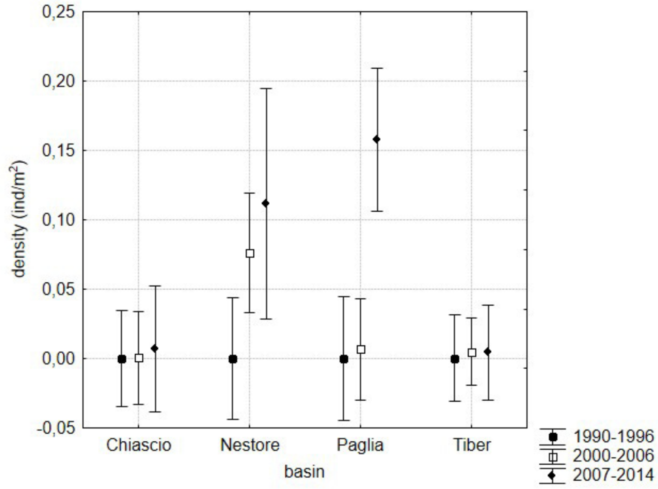


**Fig. 2.** Comparison of the mean population density values for *Pseudorasbora parva* between basins in the three census periods. Vertical bars represent 95% confidence levels.

values among river basins ($F$ = 6.31; $p$ = 0.001), among periods ($F$ = 8.07; $p$ = 0.001) and the crossing effect between the two categorical variables ($F$ = 4.14; $p$ = 0.001) were statistically significant at the two-way factorial analysis of variance.

### 3.3 Environmental parameters

The results showed that the habitat of *P. parva* was typically characterized by areas with a high concentration of dissolved salts as well as high concentrations of sulphates and chlorides (Table 1). The low class quality EBI values (mean: 2.85) showed that the presence of *P. parva* was closely linked to poor water quality and intense human activities, like in the Nestore River Basin. The watershed area was included in a wide range between 15.70 and 6349.70 km$^2$. Water temperature fluctuated from 6.30 °C to 28.30 °C, while the dissolved oxygen content ranged from 1.97 to 17.00. Water pH fluctuated from 7.11 to 8.92. The flow rate ranged from as low as 0.002 to 0.82 m$^3$.s$^{-1}$. River locations in which the topmouth gudgeon was present were also characterized by poorly shaded areas with limited canopy cover. The differences between mean values in sites where *P. parva* was present as opposed to those in which it was absent were statistically significant according to the *t*-test analysis, for all environmental parameters except average slope, pH, current speed, flow rate, cover and canopy cover (Table 1).

### 3.4 Fish community

A total of 40 species were sampled (Table 2). The relationship between fish species distribution and the environmental variables was highlighted after the reorganization of the total variability achieved through CCA analysis. The first two axes explain 77.20% of the total variability ($p$ = 0.001; total inertia = 1.12); the first axis explain 55.10% of the overall variability ($F$ = 38.20; $p$ = 0.001). The results showed that the rheophilic cyprinids and other fish species that characterize the barbel zone (the Tiber barbel, Arno goby, Etruscan chub and Italian roach) are located in the middle reaches of the rivers. *P. parva* was located in the lower stream reaches and was associated with other non-native species (*Barbus barbus, Gobio gobio, Carassius auratus, Alburnus arborella, Rhodeus sericeus, Protochondrostoma genei* and *Rutilus rutilus*) occurring in the study area. An inverse correlation between the presence of *P. parva* and EBI, altitude and dissolved oxygen was observed, while the presence of this species is directly correlated with flow rate, watershed area, distance from the source, wetted river section, chlorides, $SO_4$ and $NNH_3$ (Figure 3). All the environmental parameters except pH were significantly correlated with the first axis (Table 3). The results of the GLM analysis confirmed a linear relationship between topmouth gudgeon density and all the environmental variables. The results showed a significant negative slope between *P. parva* density and current speed ($F$ = 4.280; $p$ = 0.015), altitude ($F$ = 24.14; $p$ = 0.001), dissolved oxygen ($F$ = 6.08; $p$ = 0.014) and EBI ($F$ = 8.14; $p$ = 0.005). The abundance of *P. parva* was directly and significantly correlated with conductivity ($F$ = 7.55; $p$ = 0.006), chlorides ($F$ = 16.14; $p$ = 0.001), $NNH_3$ ($F$ = 9.06; $p$ = 0.003), sulphates ($F$ = 7.26; $p$ = 0.008) and water temperature ($F$ = 0.001; $p$ = 0.021). In the case of average slope ($F$ = 0.42; $p$ = 0.480), wetted river section ($F$ = 0.53; $p$ = 0.469) and flow rate ($F$ = 0.41; $p$ = 0.099) the relationships were not statistically significant.

The relationship between *P. parva* and native species densities and axis 1, evaluated through the GLM analysis, showed that increasing abundance of topmouth gudgeon along the longitudinal gradient corresponds to a progressive decrease in the densities of native species (Figure 4). For all the species the relationship was statistically significant.

In the lower stream reaches, the presence of many non-native species combined with poor water quality resulted in a decrease in indigenous fish communities, as demonstrated by low mean ZIC values (0.42) in sampling sites in which *P. parva* was present (Table 4). Mean differences between sites where *P. parva* was present or absent were highly significant (*t*-test analysis) for total number of fish species, number of native fish species, number of exotic fish species and ZIC.

### 3.5 Growth estimation

The $TL$-$W$ relationship for the total sample was $W = 0.021TL^{2.673\pm0.015}$ ($R^2$ = 0.86). The $b$ (slope) value of the $TL$-$W$ regressions was significantly lower than 3 ($t$ = 4.56; $p$ = 0.001), indicating negative allometric growth (Ricker, 1975). The b value calculated for the population of *P. parva* from Lake Trasimeno showed a positive allometric growth, because the b value was greater than 3. As regards the regression coefficients calculated for each sub-basin, the results showed that $b$ values were smaller than 3 for all basins (Table 5) indicating negative allometric growth. Theoretical growth parameters were calculated separately for each sub-basin and Lake Trasimeno along with $\Phi$ values. Growth of the topmouth gudgeon from the Tiber river basin can be regarded as faster and having better performance than other gudgeon populations examined.

**Table 2.** List of fish species occurring in the study area and their origin.

| Family | Species | Common name | Origin | Total number of specimens collected |
|---|---|---|---|---|
| **Anguillidae** | *Anguilla anguilla* (Linnaeus, 1758) | Eel | Native | 392 |
| **Cyprinidae** | *Alburnus arborella* (Bonaparte, 1841) | Bleak | Non-native | 10 000 |
| | *Barbus barbus* (Linnaeus, 1758) | Danube barbel | Non-native | 1001 |
| | *Barbus plebejus* (Bonaparte, 1839) | Po barbel | Native | 98 |
| | *Barbus tyberinus* (Bonaparte, 1839) | Tiber barbel | Native | 14 491 |
| | *Blicca bjoerkna* (Linnaeus, 1758) | White bream | Non-native | 1 |
| | *Carassius auratus* (Linnaeus, 1758) | Goldfish | Non-native | 2086 |
| | *Chondrostoma soetta* (Bonaparte, 1840) | Italian sneep | Non-native | 3 |
| | *Cyprinus carpio* (Linnaeus, 1758) | Carp | Non-native | 907 |
| | *Ctenopharyngodon idellus* (Valenciennes, 1844) | Grass carp | Non-native | 1 |
| | *Gobio gobio* (Linnaeus, 1758) | Gudgeon | Non-native | 1306 |
| | *Luciobarbus graellsii* (Steindachner, 1866) | Ebro barbel | Non-native | 39 |
| | *Protochondrostoma genei* (Bonaparte, 1839) | Italian nase | Non-native | 9465 |
| | *Pseudorasbora parva* (Schlegel, 1842) | Top mouth gudgeon | Non-native | 5027 |
| | *Rhodeus sericeus* (Pallas, 1776) | Bitterling | Non-native | 622 |
| | *Rutilus erythrophthalmus* Zerunian, 1982 | North Italian roach | Non-native | 171 |
| | *Rutilus rubilio* (Bonaparte, 1837) | Italian roach | Native | 36 825 |
| | *Rutilus rutilus* (Linnaeus, 1758) | Roach | Non-native | 91 |
| | *Scardinius erythrophthalmus* (Linnaeus, 1758) | Rudd | Native | 219 |
| | *Squalius lucumonis* (Bianco, 1982) | Etruscan chub | Native | 4995 |
| | *Squalius squalus* (Bonaparte, 1837) | Chub | Native | 31 286 |
| | *Telestes muticellus* (Bonaparte, 1837) | Italian riffle dace | Native | 16434 |
| | *Tinca tinca* (Linnaeus, 1758) | Tench | Native | 122 |
| **Centrarchidae** | *Lepomis gibbosus* (Linnaeus, 1758) | Pumpkinseed | Non-native | 216 |
| | *Micropterus salmoides* (Lacépède, 1802) | Largemouth bass | Non-native | 49 |
| **Cobitidae** | *Cobitis bilineata* (Canestrini, 1865) | Spined loach | Non-native | 592 |
| **Cottidae** | *Cottus gobio* (Linnaeus, 1758) | Bullhead | Native | 390 |
| **Esocidae** | *Esox lucius* (Linnaeus, 1758) | Pike | Native/Non-native | 32 |
| **Gasterosteidae** | *Gasterosteus aculeatus* (Linnaeus, 1758) | Three-spined stickleback | Native | 403 |
| **Gobiidae** | *Padogobius bonelli* (Bonaparte, 1846) | Po goby | Non-native | 1119 |
| | *Padogobius nigricans* (Canestrini, 1867) | Arno goby | Native | 6558 |
| **Ictaluridae** | *Ameiurus melas* (Rafinesque, 1820) | Black bullhead | Non-native | 160 |
| **Percidae** | *Perca fluviatilis* (Linnaeus, 1758) | Perch | Non-native | 44 |
| | *Stizostedion lucioperca* (Linnaeus, 1758) | Pikeperch | Non-native | 49 |
| **Poeciliidae** | *Gambusia holbrooki* (Girard, 1859) | Eastern mosquitofish | Non-native | 11 |
| **Salmonidae** | *Onchorhyncus mykiss* (Walbaun, 1792) | Rainbow trout | Non-native | 368 |
| | *Salmo trutta* complex (Linnaeus, 1758) | Brown trout | Native/Non-native | 18 335 |
| | *Salvelinus fontinalis* (Mitchill, 1814) | Brook trout | Non-native | 1 |
| | *Thymallus thymallus* (Linnaeus, 1758) | Grayling | Non-native | 4 |
| **Siluridae** | *Silurus glanis* (Linnaeus, 1758) | European catfish | Non-native | 15 |

### 3.6 Estimation of body condition for indigenous species

$W_r$ mean values from sites with and without *P. parva* calculated for three indigenous species (Italian roach, chub, Tiber barbel) showed that the average values of relative weight were higher in sites where *P. parva* was absent and the differences between the mean values were highly significant after the U Mann-Whitney test (Italian roach: $Z = -5.74$; $p = 0.00$; chub: $Z = -13.93$; $p = 0.00$; Tiber barbel: $Z = -6.51$; $p = 0.00$). $K_n$ mean values from sites with and without top-mouth gudgeon calculated for Arno goby showed that, in this case also, the mean value of $K_n$ was higher where *P. parva* was absent, although this difference was not statistically significant (Table 6). For all the species there was a negative correlation between *P. parva* density and the body condition, but the results were not statistically significant (Italian roach: $r = -0.20$; $p = 0.22$; chub: $r = -0.09$; $p = 0.557$; Tiber barbel: $r = -0.35$; $p = 0.09$; Arno goby: $r = 0.09$; $p = 0.70$).

## 4 Discussion

The observed distribution of *P. parva* in the investigated area suggests that this invasive species spreads following a "stepping stone and diffusion" dispersal mode (Gozlan *et al.*,

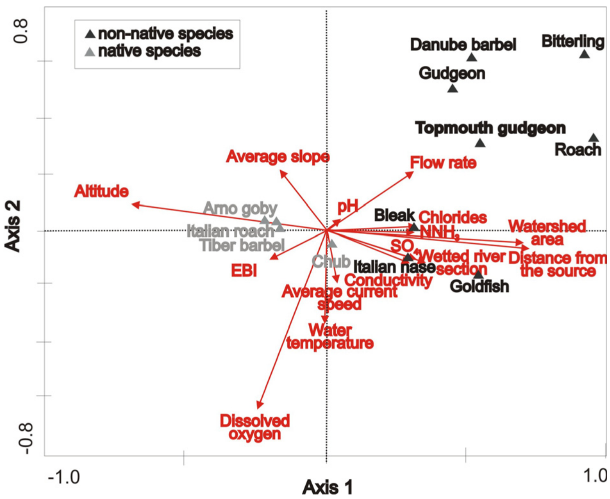


**Fig. 3.** Results of CCA analysis: biplot of the environmental variables and the densities of native and non native species. Eigenvalues of axis 1 and 2 were 0.150 and 0.027, respectively. The first two axes explained 77.20% of total variance. The length of the arrow is proportional to the rate of change; a long arrow indicates a large change and that the change is strongly correlated with the ordination axes.

**Table 3.** Canonical and correlation coefficients of environmental variables with axis. $p < 0.05$ is in bold.

| | Canonical coefficients | | Correlations with axis | | | |
|---|---|---|---|---|---|---|
| **Environmental and biological parameters** | **AX1** | **AX2** | **AX1** | ***p*** | **AX2** | ***p*** |
| Distance from the source (km) | 0.2343 | −0.3328 | 0.6723 | **0.001** | −0.2799 | **0.001** |
| Watershed area (km$^2$) | 0.3575 | 0.2081 | 0.6867 | **0.001** | −0.2684 | **0.001** |
| Altitude (m a.s.l.) | −0.4765 | 0.1254 | −0.4512 | **0.001** | 0.2826 | **0.001** |
| Average slope (%) | −0.0644 | 0.2086 | −0.1923 | **0.006** | 0.1473 | **0.035** |
| pH (units) | −0.1047 | 0.3089 | −0.0691 | 0.324 | 0.0307 | 0.661 |
| Conductivity ($\mu$s.cm$^{-1}$ at 25 °C) | 0.1562 | −0.0342 | 0.3253 | **0.001** | −0.2825 | 0.001 |
| $NNH_3$ (mg.L$^{-1}$) | 0.1372 | −0.0459 | 0.2611 | **0.001** | −0.0752 | 0.282 |
| $SO_4$ (mg.L$^{-1}$) | 0.0848 | −0.2104 | 0.3356 | **0.001** | −0.2681 | **0.001** |
| Cl (mg.L$^{-1}$) | 0.0993 | 0.1424 | 0.2815 | **0.001** | 0.1179 | 0.091 |
| Water temperature (°C) | 0.0195 | −0.071 | 0.1705 | **0.014** | −0.0128 | 0.855 |
| Dissolved oxygen (mg.L$^{-1}$) | −0.2684 | −0.5933 | −0.2341 | **0.001** | −0.1304 | 0.062 |
| E.B.I. (units) | 0.0338 | −0.1792 | −0.3151 | **0.001** | −0.2098 | **0.002** |
| Average current speed (m.s$^{-1}$) | −0.0085 | −0.6059 | 0.2055 | **0.003** | −0.0877 | 0.210 |
| Flow rate (m$^3$.s$^{-1}$) | −0.1098 | 12.152 | 0.3801 | **0.001** | −0.0029 | 0.967 |
| Wetted river section (m) | 0.1605 | −0.7876 | 0.5191 | **0.001** | −0.1506 | **0.031** |

2010). In particular, we hypothesize that this species was introduced to two different sites from which it naturally spread upstream and downstream through hydrographic connections, as reported for other European countries (Gozlan *et al.*, 2010).

Many studies have reported the ability of *P. parva* to invade polluted rivers (Pollux and Korosi, 2006; Witkowsky, 2009). In the present study the highest mean population densities of this species were recorded in the Nestore and Paglia river basins, where a progressive increase in the abundance of the species was observed over time. The hydrogeological characteristics and the high incidence of human activities adversely affect the water quality in these basins. Waterways that run through predominantly impermeable lands, such as the Nestore River, show very marked flow rate fluctuations (Lorenzoni *et al.*, 2006); in times of drought the modest flow rates produce a greater concentration of pollutants. The degradation of environmental conditions in these areas is made worse by the presence of many water catchments for irrigation purposes also aggravates in those areas (Carosi *et al.*, 2015). The Nestore River presents a broad variation in conductivity and EBI, with the latter fluctuating between III and IV water quality classifications, that indicate very high pollution levels (Lorenzoni *et al.*, 2006). Only in recent years there has been a gradual improvement of environmental quality in this area (Carosi *et al.*, 2015). The topmouth gudgeon is totally absent in the Nera River Basin (Lorenzoni *et al.*, 2010). This basin is predominantly mountainous with its rivers showing the best water quality among all the watersheds considered in the study area (Lorenzoni *et al.*, 2006). Two causes might be invoked for this absence: (i) the presence of numerous barriers in the water courses of this catchment that might impede the expansion of this species from the Tiber River; and (ii) environmental characteristics, more suited to salmonids rather than to cyprinids.

The estimated maximum age of $4^+$ is consistent with that reported for other populations of this species (Gozlan *et al.*,

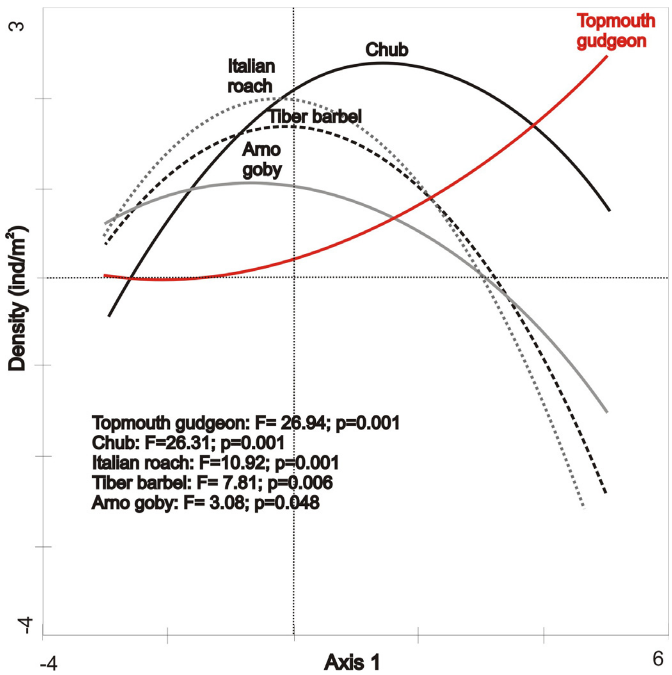


**Fig. 4.** Species response model of change of *Pseudorasbora parva* and native species densities along axis 1 evaluated through GLM analysis.

**Table 4.** The *t*-test analysis for total number of fish species, number of native and non-native fish species and Z.I.C.: comparison of sampling sites in which *Pseudorasbora parva* was present and those in which it was absent. $P < 0.05$ is in bold.

| | *P. parva* absence | | | | *P. parva* presence | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *N* values | Mean (± St. Dev.) | Minimum | Maximum | *N* values | Mean (± St. Dev.) | Minimum | Maximum | *t* value | *p* |
| Total number of fish species | 401 | 7.750 ± 3.151 | 1.000 | 16.000 | 69 | 9.652 ± 3.387 | 2.000 | 17.000 | 9.282 | **0.000** |
| Number of native fishes | 401 | 3.318 ± 2.102 | 0.000 | 9.000 | 69 | 4.116 ± 1.953 | 0.000 | 8.000 | 5.648 | **0.000** |
| Number of non-native fishes | 401 | 1.432 ± 1.962 | 0.000 | 10.000 | 69 | 5.536 ± 2.673 | 1.000 | 13.000 | 10.758 | **0.000** |
| Z.I.C. | 401 | 0.754 ± 0.311 | 0.000 | 1.000 | 69 | 0.421 ± 0.203 | 0.000 | 0.875 | 11.200 | **0.000** |

**Table 5.** Parameters of total length-weight relationship and of theoretical growth for *Pseudorasbora parva* for each sub-basin and for Lake Trasimeno.

| | TL–W relationship parameters | | | | Theoretical growth parameters | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $n$ | $b$ | $a$ | $R^2$ | $TL_t$ | $k$ | $t_0$ | $R^2$ | $\Phi$ |
| Paglia basin | 174 | 2.78 | 0.0145 | 0.90 | 15.78 | 0.192 | −1.08 | 1.00 | 1.68 |
| Chiascio basin | 3659 | 2.04 | 0.0754 | 0.99 | 17.26 | 0.161 | −0.96 | 1.00 | 1.68 |
| Tevere basin | 683 | 2.88 | 0.0110 | 0.90 | 12.52 | 0.399 | −0.57 | 1.00 | 1.80 |
| Nestore basin | 511 | 2.74 | 0.0173 | 0.85 | 16.06 | 0.192 | −0.72 | 1.00 | 1.70 |
| Trasimeno lake | 543 | 3.23 | 0.0064 | 0.90 | 15.26 | 0.191 | −1.31 | 1.00 | 1.65 |

2010; Zahorska *et al.*, 2014), although a maximum age of $5^+$ was indicated in other studies (Rosecchi *et al.*, 1993; Kapusta, 2008). The biggest specimens (11.20 cm) almost reached the maximum length reported for this species (12 cm) (Witkowsky, 2009). The $b$ (slope) value of the $TL$-$W$ regressions calculated for the total sample was lower than 3, indicating negative allometric growth, in accordance with the results reported for two populations from other Mediterranean countries (Table 7). The allometric growth is negative for all the sub-basins and positive for Lake Trasimeno, in which the individuals reach substantially higher weights at the same length. These results suggested that in Lake Trasimeno the environmental conditions (i.e. habitat conditions, food availability) may be more favourable than in the watercourses of the Tiber River Basin.

As regards the ecological preferences of *P. parva*, in the study area the presence of this species, as well as of other introduced species, is closely related to environments rich

**Table 6.** *U* Mann-Whitney test analysis: comparison of the body condition values calculated for presence and absence of *Pseudorasbora parva* for the native species. $P < 0.05$ is in bold.

| | Mean syntopy | Mean allotopy | *U* | *Z* | *p* | *N* values syntopy | *N* values allotopy | Std. Dev. syntopy | Std. Dev. allotopy |
|---|---|---|---|---|---|---|---|---|---|
| Wr Italian roach | 98.68 | 102.41 | 1450291 | −5.74 | **0.00** | 1738 | 1876 | 21.24 | 22.82 |
| Wr Chub | 94.40 | 100.20 | 1969384 | −13.93 | **0.00** | 2646 | 1958 | 16.93 | 16.02 |
| Wr Tiber barbel | 90.29 | 93.03 | 209653.5 | −6.51 | **0.00** | 902 | 581 | 13.94 | 13.91 |
| Kn Arno goby | 1.02 | 1.04 | 257754.00 | −1.03 | 0.30 | 829 | 642 | 0.24 | 0.26 |

**Table 7.** Total length-weight relationship for topmouth gudgeon populations from two other Mediterranean countries.

| Location | Country | References | Length-weight relationship |
|---|---|---|---|
| Marmara lake | West Anatolia (Turkey) | Ilhan, 2015 | $Y = 0.012x^{2.929}$ |
| River Cimiega | South eastern Poland | Rechulicz, Rechulicz | $Y = 0.032x^{2.245}$ |
| River Tiber basin | Central Italy | Present study | $Y = 0.021x^{2.673}$ |

in dissolved salts and with low dissolved oxygen concentrations. Other environmental factors that represent major drivers for the presence and the expansion of *P. parva* seem to be some hydro-morphological variables, such as current speed, watershed area and altitude.

GLM results confirm that current speed and watershed area play a key role in the presence or absence of the species in lotic ecosystems. The topmouth gudgeon seems to prefer lower stream reaches with moderate current speed and low flow rate, more suited to the feeding habits of this species. Previous studies showed that high current speeds can result in higher swimming speed and therefore a greater expenditure of energy, resulting in increased metabolic stress for the species (Sunardi *et al.*, 2007).

The absence of the topmouth gudgeon from the Nera River Basin suggests that, in some cases, the presence of barriers along the rivers prevents the colonization of the upper stream reaches by non-native species and plays an important role in the conservation of endemic species (Carosi *et al.*, 2015).

Regarding the interactions between *P. parva* and other fish species, our results confirms the association of the topmouth gudgeon with other non-native species. These non-native species seem to take advantage of their wider tolerance and preferentially colonize downstream reaches, where the water quality is poor and the environment is less suited to the most sensitive native species. Compared with the topmouth gudgeon, the four indigenous species considered in the present study mainly inhabit the middle stream reaches where the environmental quality is better than in the downstream reaches. Nevertheless in several cases native species and *P. parva* coexist. In these cases, competitive interactions are likely to occurr.

It is known from the literature that *P. parva* constitutes a threat to the native fish fauna (Pollux and Korosi, 2006; Witkowsky, 2009). However, current information on the interaction of *P. parva* with native fish species is limited. Interspecific competition for food between *P. parva* and native fish species has been observed in many European water bodies (Rosecchi *et al.*, 1993; Pollux and Korosi, 2006; Gozlan *et al.*, 2010; Didenko and Kruzhylina, 2015). The body condition estimates for the indigenous species that inhabit the middle and lower reaches of the Tiber River Basin was particularly useful in assessing the negative impact of *P. parva* on native species (Giannetto *et al.*, 2012). Infact, this parameter highlighted the presence of competitive interaction. In accordance with a previous research based on the use of relative weight (Giannetto *et al.*, 2012), our results show that native species are sensitive to the presence of non-native ones, probably because of the occurrence of high diet overlaps between the topmouth gudgeon and many other cyprinids. The average relative weight of native species decreased with the increase of the topmouth gudgeon density, even if the two parameters were not significantly correlated. Although we cannot exclude the influence of other factors, such as the presence of other exotic species, the analysis was designed to minimize this influence by: (i) selecting the sites with a strong link between *P. parva* and native species; (ii) considering only the sites with similar environmental characteristics, to eliminate the potential effect of longitudinal gradient. The results obtained with this approach, which still requires further testing and verification, suggest the possibility that the presence of *P. parva* constitutes a threat for native fishes, with serious consequences for biodiversity conservation in the study area. For example, the Italian roach is a species endemic to central Italy and is of particular conservation interest, being listed as "near threatened" in the Red list of the IUCN (Rondinini *et al.*, 2013).

In conclusion, the results of this study may represent a basis to develop strategies for biodiversity conservation and to control invasive species. Such strategies could involve: (i) the establishment of refuge areas in those watercourses where alien species are absent; (ii) the use of native bait fish coming only from the same basin where they will be used; and (iii) the prohibition of fish translocation from basins where exotic species are present. Many creeks, located mainly in the mountain areas of the basin, have been preserved as fish communities composed only of native species. These watercourses may be also very important for the maintenance of native biodiversity, thanks to the presence of barriers that prevent non-native species from moving from downstream to upstream. These refuge areas, identified on the basis of the results of the present research, should be the object of "continuous surveillance systems", as provided by the Regulation (EU) 1143/2014 on the prevention and management of the introduction and spread of invasive alien species. In case of the introduction of the topmouth gudgeon, early detection and the quick application of eradication programmes may be effective in the maintenance and recovery of native fish biodiversity and in preventing further dispersal of this species in the study area.

*Acknowledgements.* The authors are grateful to the anonymous referees for their valuable comments that considerably improved the original version of the manuscript. The authors would also like to thanks all the people who participated in the field activities to collect data for the Regional Fish Map of the Umbria Region and all the students and collaborators who joined the project. Moreover, they would like to thank Massimo Giovannotti for his valuable help with the critical revision of the text.

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**Cite this article as:** A. Carosi, L. Ghetti, M. Lorenzoni, 2016. Status of *Pseudorasbora parva* in the Tiber River Basin (Umbria, central Italy) 20 years after its introduction. *Knowl. Manag. Aquat. Ecosyst.*, 417, 22.