

Article

The Role of Climate Changes in the Spread of Freshwater Fishes: Implications for Alien Cool and Warm-Water Species in a Mediterranean Basin

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Abstract: In running waters, under climate change conditions, the combined effect of water warming and decreasing flow rates may encourage colonisation by invasive cool and warm-water fish species. The aim of the study was to analyze the potential climate change effects on the spread of four invasive alien fishes in the Tiber River basin, taking into account the effects of river fragmentation. Fish and environmental data collected in 91 sites over the years 1998–2018, were used to analyze temporal changes in their habitat requirements. A multivariate analysis was conducted, and the hypothesis of a range expansion towards the upstream reaches has been tested. For *Barbus barbus*, *Gobio gobio*, *Padogobius bonelli* and *Pseudorasbora parva* population abundances and body condition were analyzed. Detectability, occupancy, local extinction and colonization probabilities were estimated. We showed that *B. barbus* and *P. bonelli* have significantly extended their range toward upstream. *P. parva* did not move toward higher altitudes significantly, suggesting that, at this stage, the species has probably reached an equilibrium. River fragmentation, elevation, water temperature and average current speed seem to be major determinants in colonization processes, affecting the dispersal ability of the species. Not surprisingly for species introduced in relatively recent times, the colonization probabilities were much higher than extinction probabilities. Our results provided evidence for some synergistic effects between climate changes and alien fish species invasions, in terms of species range shifts mediated by rising water temperatures, although they should be interpreted cautiously, taking into account that these species most likely were not yet stabilized.

Keywords: non-native fish species; global warming; fish range shifts; dispersal ability; river connectivity; biodiversity conservation



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1. Introduction

The negative impacts of alien fish invasions and climate changes on freshwater ecosystems have been the subject of several investigations [1–3], although often these two aspects are treated separately, overlooking their interactions [4–6]. Relatively few studies have been attempted on the role of climate factors in the spread of non-native freshwater fishes [7]. The progressive rise in water temperature as a result of global warming may encourage colonization by invasive alien species whose expansion would otherwise be limited by low temperatures [4,7,8].

The Mediterranean area, due to the particular climatic and geographic conditions, the richness of endemism, the high number of introduced alien species, in addition to other anthropogenic pressures, is particularly vulnerable to climate changes and to the loss of biodiversity [9–12].

Mediterranean rivers, in particular, as a result of both the effects of climate changes and alien species invasions, are strongly affected by the decline in native fish biodiversity [10,13].

Being ectothermic organisms, fish are particularly sensitive to changes in temperature resulting from global warming [14]. In running waters, the combined effect of increasing water temperature and decreasing flow rates may result in changes in the fish distribution along the longitudinal gradient [15–17]. Especially in the summer season, cool- and warm-water fish species may be induced to move upstream, while the cold-water species, already located near the springs, can become isolated [6,16,18,19]. Moreover, in Mediterranean watercourses numerous cool- and warm-water species are of alien origin, and can be advantaged by rising temperatures [6,11]. Many of them are pollution tolerant and may be more resilient to the indirect effects of climate change (i.e., increase of the pollutants concentration and reduced dissolved oxygen as a consequence to the decreasing flow rates and rising temperatures) than native species.

Recent studies conducted on fish communities inhabiting Italian basins provided evidence for their gradual qualitative degradation over time due to the progressive expansion of alien species [20–22]. At present, 60% of the fish species occurring in Italian freshwaters are of exotic origin [23]. Previous research carried out on fish communities inhabiting the Tiber river basin (central Italy) have shown that most of alien freshwater fish have been introduced over the last 30 years through restocking activities in favor of recreational fishery [20]; they have been initially released in multiple sites, and subsequently expanded their range using the connections of the hydrographic network [24]. Some of these species, as *Barbus barbus* (Linnaeus, 1758), *Gobio gobio* (Linnaeus, 1758), *Padogobius bonelli* (Bonaparte, 1846) and *Pseudorasbora parva* (Temminck & Schlegel, 1846), immediately after their introduction occurred in the period between 1993 and 1999, quickly acclimatized and are currently present with many self-sustaining populations [25,26].

Worryingly, the spread of these invasive species has contributed to the local extinction or range shift of some endemic ones. In particular, it has been shown that *B. barbus* represents a serious threat to the native Italian congeneric species, mainly due to introgressive hybridization phenomena leading to the loss of genetic diversity [21,27–31]. In the Tiber river basin, *B. barbus* has determined the local extinction of numerous populations of the endemic *Barbus tyberinus* Bonaparte, 1839 [26]. Additionally, *P. bonelli* exerted heavy negative impacts on a congeneric species endemic to central Italy, *Padogobius nigriscans* (Canestrini, 1867) [32]; in this case, the main mechanisms underlying the local extinction phenomena or population declines include the greater competitiveness of the alien species for environmental resources (i.e., food and habitat) compared to the native one. As regards *P. parva*, the species is listed among the worst alien species for Europe, according to its negative ecological effects exerted on the colonized environments [33,34]. Even in the Tiber basin, previous studies based on the analysis of relative weight [25,35] suggested that the presence of *P. parva* could have exerted a negative impact on the body condition of many native species, as *Sarmarutilus rubilio* (Bonaparte, 1837), *B. tyberinus*, *Squalius squalus* (Bonaparte, 1837) and *Telestes muticellus* (Bonaparte, 1837). For *G. gobio*, given the relatively recent introduction of the species, which took place in 1999, no ecological effects on the endemic species with which it was found in co-occurrence have yet been tested [24]. In accordance with the literature for temperate fish [36,37], on the basis of their thermal preferences, *B. barbus*, *G. gobio* and *P. bonelli* could be classified as cool-water species (optimum temperature $\approx 17^\circ\text{C}$), while *P. parva* could be considered as warm-water adapted species (optimum temperature $\approx 22^\circ\text{C}$).

As reported in the literature for other areas, the detrimental impacts exerted by these four alien species could be amplified by the synergistic effect with additional anthropogenic stressors, including global warming [2,38,39]. Generally, the ability to persist of introduced species is influenced by their dispersal capacity, environmental tolerance, and food and habitat availability [19]. In recent times, there have also been highlighted some effects of global climate change, in terms of rising water temperatures, which seem to encourage their further expansion and therefore their invasiveness [40]. Warmer waters would provide new suitable habitats in which warm-water adapted species could expand their range [4]. Even in central-Italian watercourses, the progressive increase in water temperature, extended

drought periods, and altered stream flow pattern seem to penalize the endemic species forcing them to go upstream [41], while at the same time it is reasonable to hypothesize the range shift of cool and warm-water alien fishes toward headwaters. In the present paper we don't analyze the climate change effects in terms of rising temperatures and decreasing precipitations because this aspect has already been described in Carosi et al. [41].

Another aspect to be taken into account in relation to the spread of alien fish is the high river fragmentation affecting the Tiber river basin. Under global warming conditions, it is important to analyze the role of connectivity in the freshwater fish range shifts put in place in order to face rising water temperatures. In fact, interruptions in river continuity may on one hand restrict the expansion of alien species [20], and on the other may preclude native species from accessing more suitable habitats, often located in the upper stream reaches [42].

The main aim of the present study was to analyze the potential climate change effects on the spread of four invasive alien fishes in the Tiber River basin using a spatio-temporal approach and taking into account the effects of river fragmentation. On the basis of long-term fish and environmental data series, temporal changes in their habitat use were analyzed, and the hypothesis of a range expansion towards upstream reaches has been tested.

2. Materials and Methods

2.1. Study Area

The study area includes the northern portion of the Tiber river basin, for a total extension of 5133 km², equal to 30% of the total catchment area (Figure 1). It is located within the Mediterranean region, where the climate is characterized by dry periods in summer and wet periods in winter [43]. These meteorological features make aquatic environments particularly vulnerable to potential climatic changes effects, in terms of rising water temperatures and decreasing precipitations [11,44]. In fact, the geological characteristics of the upper Tiber river basin, which is predominantly impermeable, make the flow rates of the watercourses closely linked to rainfalls [45]. The high-water demand for irrigation purposes contributes to aggravate the water shortage, particularly during drought periods. A recent study provided evidence for some effects of climate changes within the Tiber river basin, in terms of decreasing flow rates over the last 100 years, and increasing water temperatures over the last 15 years, with a magnitude of 0.4 °C [41]. As mentioned above, the whole hydrographic network is highly fragmented due to the presence of many weirs and dams [41].

2.2. Fish and Environmental Data Collection

Fish and environmental data were collected during three census periods, 1998–2004, 2005–2011 and 2012–2018, in 91 sampling sites spread over a total of 50 watercourses. Each sampling site was sampled once for each census period, in the autumn season. The fish fauna was sampled by two-pass electrofishing applying the removal method [46,47] (for more details, see [25]). All captured fishes were identified and counted in order to estimate the density (individual m⁻²) for each population. For all specimens, total length (TL) and weight (W) were recorded. To characterize river stretches, 21 environmental parameters that play a crucial role in the distribution and life-history strategies of freshwater fish species were used. Conductivity (µS cm⁻¹), pH (units), water temperature (°C) and dissolved oxygen (mg L⁻¹) were measured during the field activities simultaneously with fish samplings, using electronic meters manufactured by YSI Incorporated (model 55/12, Yellow Springs, OH, USA), Hanna Instruments (HI9033, Woonsocket, RI, USA) and WTW GmbH (pH 320, Weilheim, Germany). Chlorides, sulphates, phosphates, total phosphorous, COD, NNO₂, NNO₃, ammonia were subsequently measured in the laboratory following the environmental protection agencies methods [48–50]. Hydraulic variables were determined at cross sections within each sampling stretch, using an OTT MF-pro electromagnetic current meter (OTT Hydromet, Kempton, Germany). Morphological parameters, as watershed area (km²), distance from the water source (km), and elevation (m a.s.l.) were

calculated from digital maps (geographic information system (GIS)). The location of dams and weirs within the Tiber river basin was provided by the Forest, Economics and Mountain Territory Service of the Umbria Region, Perugia, Italy. The river fragmentation degree has been codified as the number of weirs with height > 80 cm occurring downstream of each sampling station, up to the main channel. To evaluate the water quality on the basis of the macrobenthic fauna composition, the Extended Biotic Index (EBI) and the IBE Quality class were used [51] (for more details, see [25]).

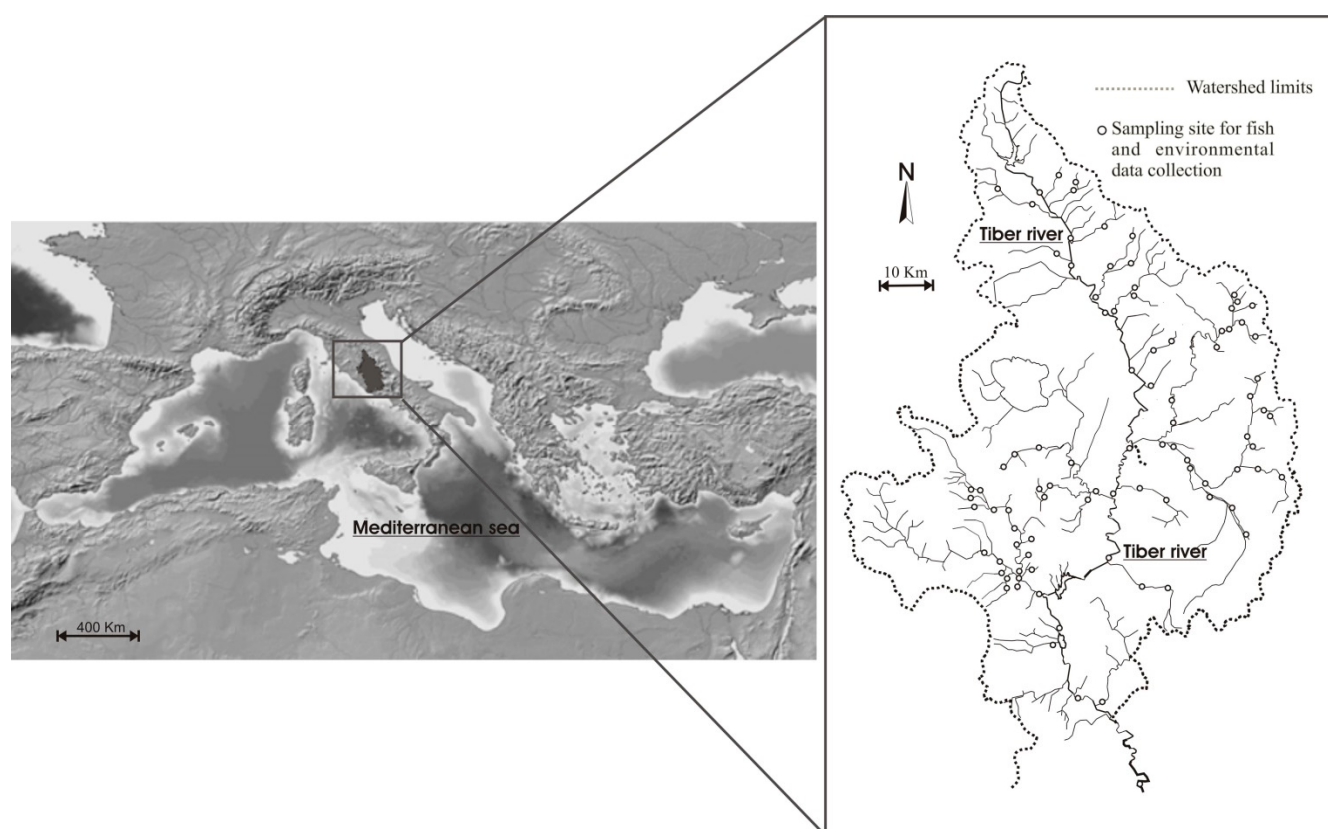


Figure 1. Study area and location of the sampling sites. Mediterranean map adapted from Isac-Cnr 2005.

2.3. Body Condition

For *B. barbus*, *G. gobio*, *P. bonelli* and *P. parva* the relative condition factor (Kn) was calculated for all individual following [52]. Primarily, the length–weight relationship equation was estimated for the total sample, applying the least-squares method [53], using the logarithmic equation:

$$\log_{10}W(g) = a + b \log_{10}TL \text{ (cm)} \quad (1)$$

The values of the regression coefficients *a* and *b* were used to estimate theoretically expected values of body weight using the equation:

$$W = a \times TL^b \quad (2)$$

Then, the relative condition factor (Kn) was calculated as follows:

$$Kn = 100W / (a \times TL^b) \quad (3)$$

This approach is based on the assumption that high Kn values characterize fish in good condition and good health, while low Kn values are typical of fish in poor body condition [54].

2.4. Statistical Analysis

To analyze the relationship between the time of introduction and spatial distribution of *B. barbus*, *G. gobio*, *P. bonelli* and *P. parva*, the Pearson correlation between the number of invaded sites and the species residence time (i.e., number of years since their first detection in the study area) was calculated. To test the ecological preferences of *B. barbus*, *G. gobio*, *P. bonelli* and *P. parva* a one-way ANOVA was performed to compare average values of environmental variables among the three sampling periods, considering only the sites in which the species were detected. Data used for the Pearson correlation and one-way ANOVA were tested for normality using the Shapiro-Wilk test.

To assess the changes over time of population densities and body condition for *B. barbus*, *G. gobio*, *P. bonelli* and *P. parva*, a one-way repeated-measures ANOVA was carried out. Data were tested for sphericity using the Mauchly test, and the Greenhouse-Geisser and Huynh-Feldt adjustments were used. ANOVA was processed with Dell STATISTICA 13 software (Dell Inc., Aliso Viejo, CA, USA) for Windows.

In order to extract the environmental variables that drive the fish species densities over the three census periods (1998–2004, 2005–2011 and 2012–2018), Canonical Correspondence Analysis (CCA) [55] was performed. The software CANOCO statistical package 4.5 for Windows (Microcomputer Power, Ithaca, NY, USA), was used to carry out the multivariate analysis. CCA was conducted using environmental variables previously selected by a Principal Component Analysis. The environmental variables more strongly associated with each of the first six components have been selected, and the final environmental matrix included 13 environmental parameters and 91 observations (sampling sites). The fish matrix included 4 variables (fish species) and 91 observations (sampling sites). Given that environmental and fish data were collected as repeated surveys of the same sites, according to Lepš and Šmilauer [56] the analysis was performed using time (i.e., census period) as a covariate. Since the CCA is a unimodal gradient analysis method, to avoid biases the sampling sites have been chosen to be distant more than one kilometer from dams or weirs. All variables (N) used in the analysis were transformed $[\log_{10}(N + 1)]$ to normalize their distributions [57] and standardized to a mean of 0 and standard deviation of 1. The Monte Carlo test based on 999 permutations was used to test the significance of the analysis, and a Generalized Linear Models (GLM) was used to fit the relationship between the fish species densities and elevation.

2.5. Detection Probability, Occupancy, Colonization and Extinction Probabilities

For *B. barbus*, *G. gobio*, *P. bonelli* and *P. parva* a multi-season model was used to estimate the detection probabilities (p_i = probability that the species will be detected at site i at time t , given its presence) [58], occupancy (ψ_i = probability that the species is present at site i) [59], colonization (γ = probability that a site unoccupied at time t is occupied in $t + 1$), local extinction (ϵ = probability a site occupied in t is unoccupied in $t + 1$) [60]. The analysis was carried out using presence-absence data collected in 91 sampling sites across three census periods (t_1 = 1998–2004, t_2 = 2005–2011, t_3 = 2012–2018). Akaike's information criterion [61] was used to select the best candidate model. Following Burnham and Anderson [62], all models within 2 AIC units were considered as a set of parsimonious models. The analysis was processed using the program PRESENCE (version 2.13.6, Vermont Cooperative Fish and Wildlife Research Unit, Burlington, VT, USA) for Windows.

Five covariates that was believed to play a key role as climate drivers for the spread a distribution of the species were considered in the analysis. Since they do not vary over time, elevation and river fragmentation degree were used as site covariates. Water temperature, average current speed and flow rate, which were all point estimated, were used as sample covariates. Covariates for which 95% confidence intervals ($\alpha = 0.05$) overlapped zero and with β /SE absolute values < 1.4 were considered [63]. The analysis was performed using the PRESENCE program for Windows.

3. Results

3.1. Distribution Pattern

Following its introduction occurred in 1998, *B. barbus* has colonized the main channel of the Tiber river and its main left and right tributaries, reaching the greater population abundances especially in more recent times (Figure 2a). The distribution of *G. gobio* was restricted to the middle and upper stretches of the Tiber river, and some of its left tributaries, where the introduction of the species took place over 20 years ago; also, in this case the highest population density values could be observed in more recent times (Figure 2b). The populations of *P. bonelli* were mainly circumscribed in the upper part of the Tiber river basin, where its introduction occurred 27 years ago, even if the sporadic presence of the species has been recently detected in the mountain portion of a left tributary, the Chiascio river; also, for *P. bonelli* it was possible to notice how the most abundant populations distinguish, above all, the most recent census period (Figure 2c). As has been observed for *B. barbus*, also *P. parva*, following its introduction occurred in 1994 in the downstream reaches of the Tiber river, has rapidly colonized the whole hydrographic network, with abundant populations that already be found starting from the years 2005–2011 (Figure 2d).

For all the four species, the number of invaded sites was closely related to residence time ($r = 0.79$; $p = 0.001$). For *B. barbus* the number of colonized sites over time was stable in the last two census period and stood at a total of 20 sites (Figure 3a). The invaded sites progressively increased for *G. gobio*, until reaching a total of 17 sites in the most recent years (Figure 3b). For *P. bonelli* the number of occurring sites remained quite stable and equal to 12 and 13 in the first two census periods, respectively, while an increase of 5 sites was observed in more recent years (Figure 3c). *P. parva* was characterized by the greater number of colonized sites compared to other species, with 31 sites invaded in the period 2005–2011; a slight decrease in the last census period was observed, when the number of invaded sites was 28 (Figure 3d).

For *B. barbus* the occupancy-elevation plot showed a prevalent distribution of the species in sites located at lower altitudes, between 100 and 260 m a.s.l.; a tendency of the species to colonize sites located at higher altitudes over time was also observed, with a difference between the maximum altitudes at which the species was recorded in the 1st (245 m a.s.l.) and 3rd census period (316 m a.s.l.) equal to 71 m a.s.l. (Figure 4a). *G. gobio* was distributed mainly in the sites of altitude between 150 and 250 m a.s.l., even if also in this case during the last two census periods the species colonized sites located at altitudes over the 400 m a.s.l. (Figure 4b). For *P. bonelli*, the altitude range of the colonized sites fell mainly between 210 and 320, even if in more recent years the presence of the species has been detected at higher altitudes, up to 460 m a.s.l.; in this case the difference between the maximum altitudes at which the species was recorded in the 1st and the last census period was equal to 140 m a.s.l. (Figure 4c). As for *B. barbus*, although the distribution of *P. parva* seems to involve more the downstream reaches located in the plain areas, at altitudes between 80 and 250 m a.s.l., in the last two census periods the species colonized sites located at 410 m a.s.l. (Figure 4d).

3.2. Population Status

For all species there was an increasing trend over time of the mean population density values, even if, except for *P. bonelli*, in all other cases the results were not statistically significant at the rmANOVA (Figure 5). *B. barbus* and *P. bonelli* were characterized by large population size especially in the last sampling period, when the mean abundance values exceeded 0.4 ind m^{-2} for both species (Figure 5a,c). In the case of *G. gobio* and *P. parva* the mean density values were always below 0.13 ind m^{-2} (Figure 5b,d).

As regards the body condition, a progressive decreasing trend over time of the mean Kn values was observed for *B. barbus*, even if the average values always remained almost close to unity, that could be considered the optimal value for this parameter (Figure 6a). On the contrary, an increasing trend in the last period of the condition factor was observed for *P. bonelli* (Figure 6c) and *P. parva* (Figure 6d), for which in the two last sampling periods the

values remained always above 1.0 unit. For *G. gobio* a fluctuating trend was observed, even if also in this case the Kn mean values remained close to 1.0 (Figure 6b). Except for *B. barbus*, for all the other species the results were not statistically significant at the rmANOVA.

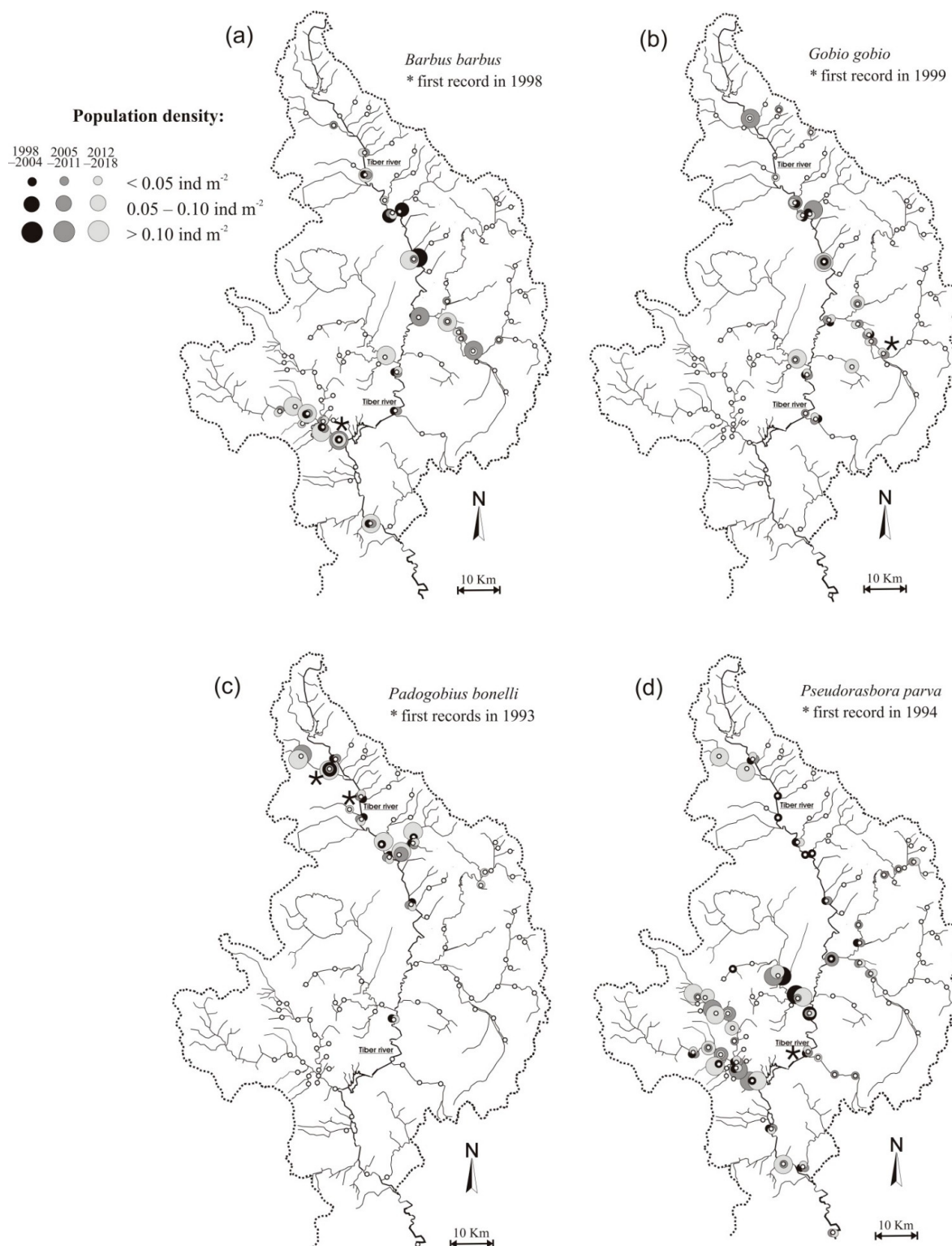


Figure 2. Comparison of the distribution and population abundances between the periods 1998–2004 (black circles), 2005–2011 (dark grey circles), and 2012–2018 (light grey circles) for: (a) *Barbus barbus*; (b) *Gobio gobio*; (c) *Padogobius bonelli*; (d) *Pseudorasbora parva*.

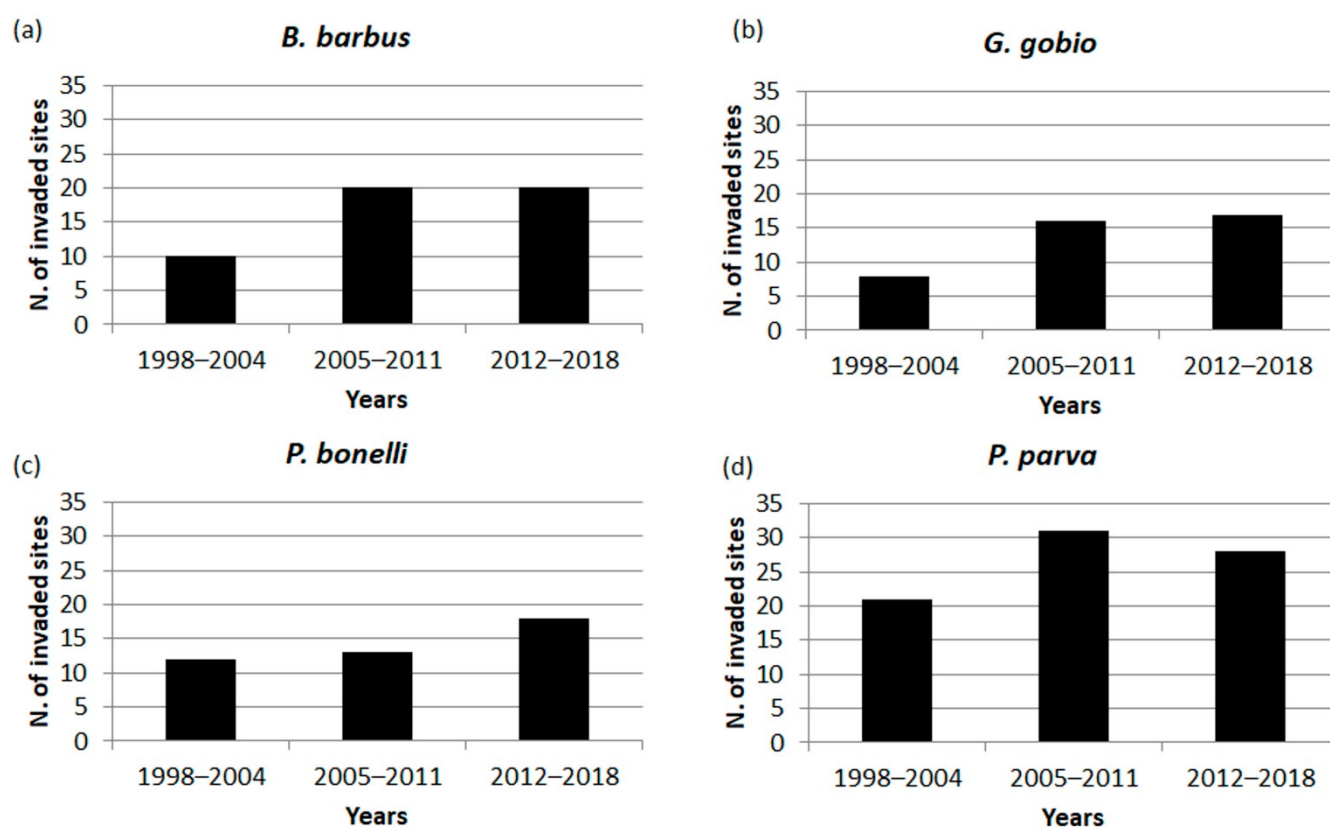


Figure 3. Number of invaded sites over the three census periods for: (a) *B. barbus*, (b) *G. gobio*, (c) *P. bonelli*, (d) *P. parva*.

3.3. Ecological Requirements

In *B. barbus* and *P. bonelli* occurring sites, a significant increase over time in the mean value of the dissolved oxygen concentration was detected (ANOVA *B. barbus*: $F = 4.11$, $p = 0.023$; *P. bonelli*: $F = 5.82$, $p = 0.006$), reaching in the last census values of around 10 mg L^{-1} for both species (Figure 7a,b). At the sites where the presence of *P. parva* had been detected, the results showed a progressive significant decrease over time in the average current speed (ANOVA: $F = 3.65$, $p = 0.033$), while especially in the last census period, a notable increase in the average values of chlorides and sulphates was observed (Figure 7c). These results, together with the significant increase in conductivity in *P. bonelli* occurring sites (Figure 6b), could reflect a worsening of water quality over time resulting from the indirect effects of reduced flow rates and current speed in the occurring sites, in terms of greater pollutants concentration (Figure 7c and Figure S1c).

The axes 1 and 2 of CCA explained 92.8% of the total variability and the analysis was highly statistically significant at the Monte Carlo test ($F = 8.50$, $p = 0.001$, total inertia = 2.028). Altitude and river fragmentation degree were significantly positively related to the first axis of CCA, which reflected the upstream-downstream gradient of the watercourses (Table 1). BOD₅, chlorides, dissolved oxygen, NNH₃, NNO₃, and watershed area showed a significant inverse relationship to Axis 2 (Table 1). The scattered distribution of points indicating *B. barbus*, *G. gobio*, *P. bonelli* and *P. parva* positions, suggested that the 4 species inhabit different river stretches with different environmental characteristics within the study area (Figure 8). For *P. bonelli* the projection point showed the close relation of the species with altitude, while *G. gobio* was placed in the river stretches located a little more downstream, where the flow rates are higher. *P. parva* and *B. barbus* preferentially colonized the downstream reaches of larger rivers, where the water quality is rather poor, and the water temperature is higher.

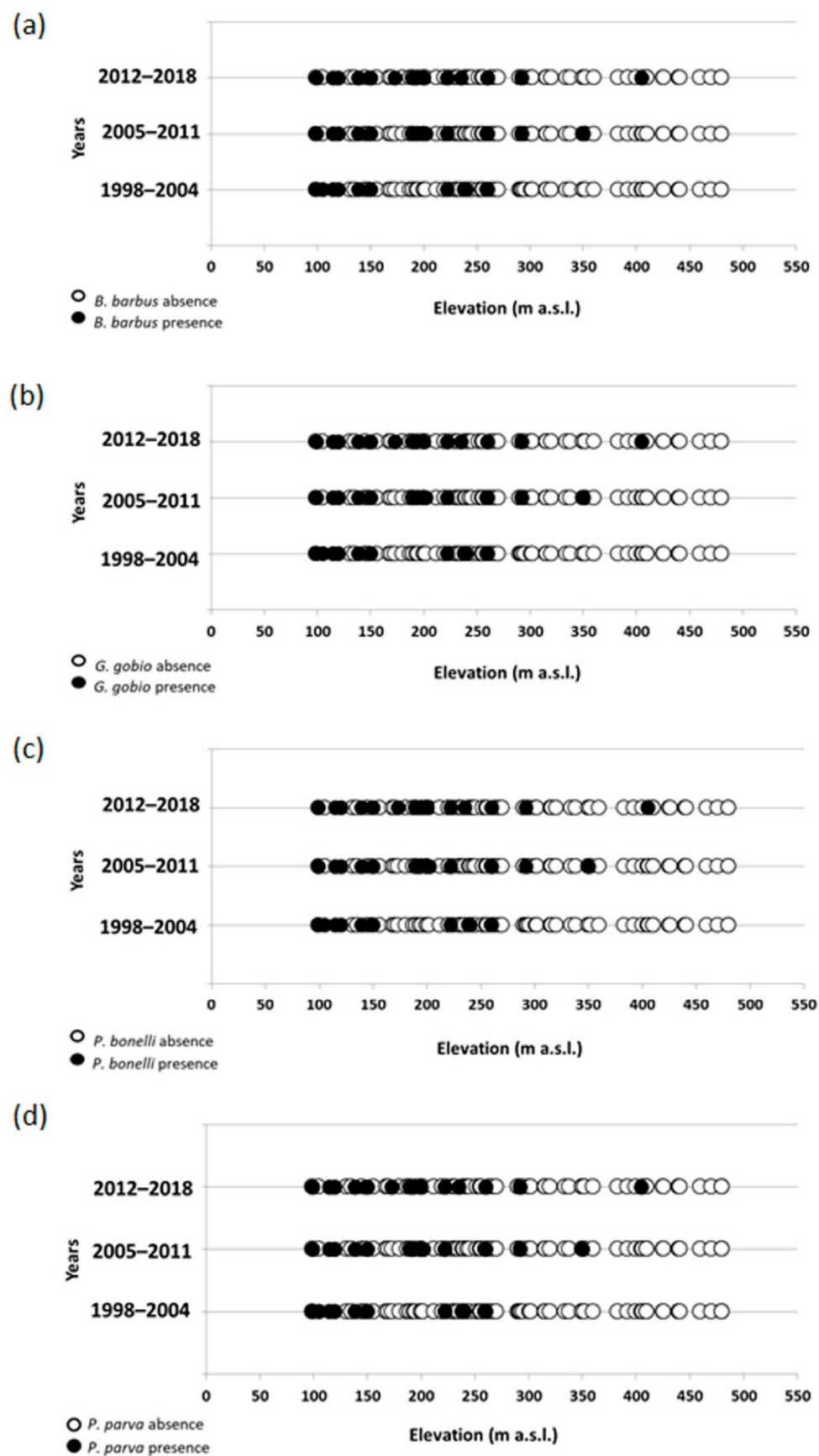


Figure 4. Occupancy elevation plots for: (a) *B. barbus*, (b) *G. gobio*, (c) *P. bonelli*, (d) *P. parva*.

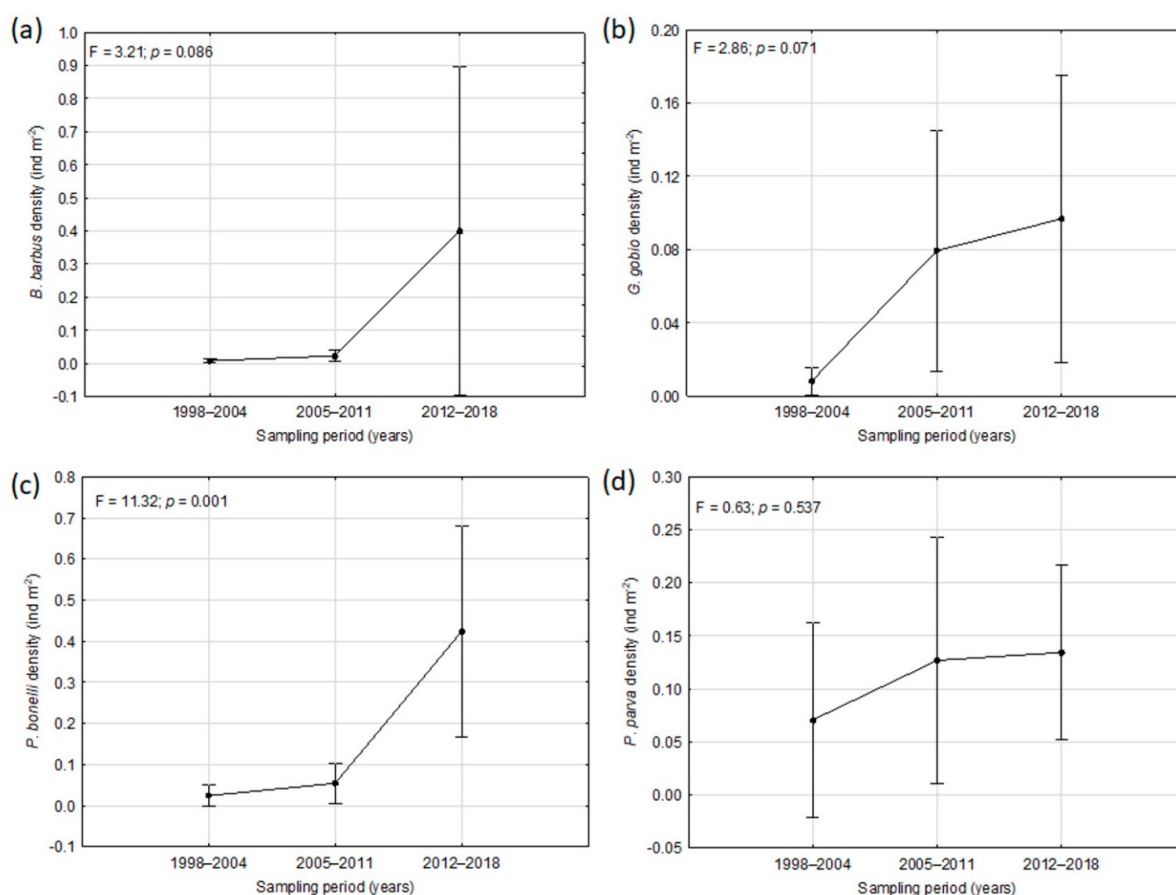


Figure 5. Trend over time of the mean population density values for: (a) *Barbus barbus*, (b) *Gobio gobio*, (c) *Padogobius bonelli*, (d) *Pseudorasbora parva*. Vertical bars denote 0.95 confidence intervals.

For all the four species the GLM results showed a progressive increase of population abundances over the three census periods (Figure 9). For *B. barbus* and *P. bonelli* a gradual shift toward higher altitudes has been also observed (Figure 9a,c). For *G. gobio* and *P. parva*, the analysis did not reveal significant changes in the elevation preferences of the species (Figure 9b,d).

Table 1. Canonical and correlation coefficients of environmental variables with axis. $p < 0.05$ is in bold.

	AX1	<i>p</i>	AX2	<i>p</i>
Altitude (m a.s.l.)	0.855	0.001	0.110	0.343
Average current speed (m s ^{−1})	−0.101	0.387	0.436	0.001
BOD ₅ (mg L ^{−1})	−0.510	0.001	0.148	0.203
Cl (mg L ^{−1})	−0.247	0.032	−0.432	0.001
Dissolved oxygen (mg L ^{−1})	−0.323	0.004	−0.798	0.001
Distance from the source (km)	−0.208	0.071	0.339	0.003
EBI Quality Class (units)	−0.037	0.748	0.207	0.073
Flow rate (m ³ s ^{−1})	−0.068	0.558	0.532	0.001
NNH ₃ (mg L ^{−1})	−0.280	0.014	0.125	0.283
NNO ₃ (mg L ^{−1})	−0.242	0.035	0.010	0.935
River fragmentation degree (units)	0.246	0.032	0.179	0.122
Water temperature (°C)	−0.004	0.976	0.092	0.430
Watershed area (km ²)	−0.316	0.005	0.438	0.001

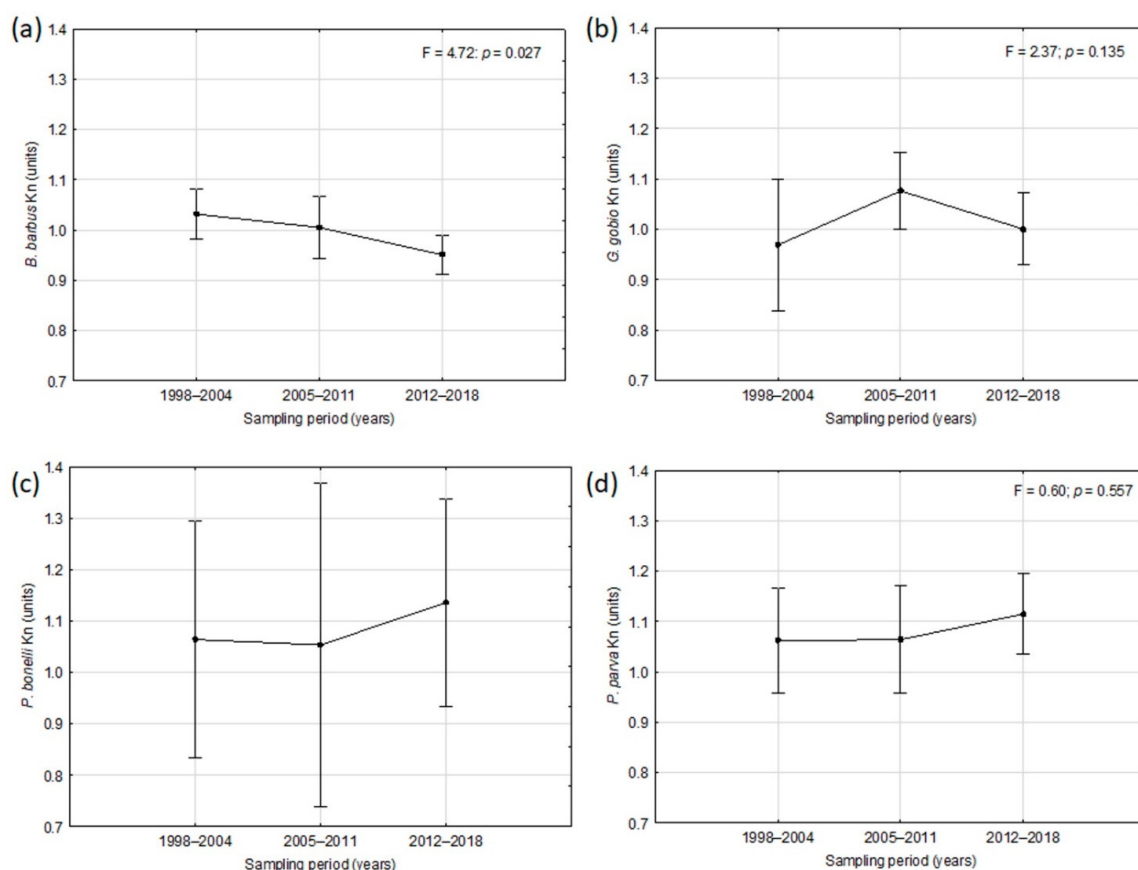


Figure 6. Trend over time of the mean condition factor (Kn) values for: (a) *Barbus barbus*, (b) *Gobio gobio*, (c) *Padogobius bonelli*, (d) *Pseudorasbora parva*. Vertical bars denote 0.95 confidence intervals.

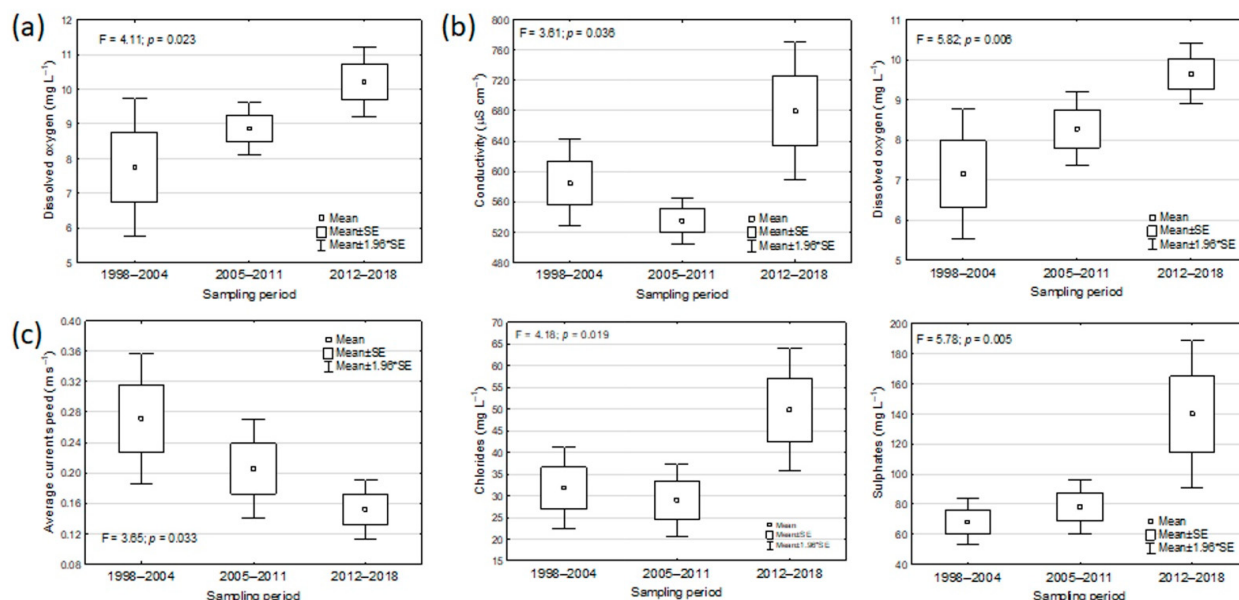


Figure 7. Ecological requirements: trend over time of the mean values of: dissolved oxygen for *Barbus barbus* (a); conductivity and dissolved oxygen for *Padogobius bonelli* (b); average current speed, chlorides, and sulphates for *Pseudorasbora parva* (c). For each species, only the occurring sites in the census periods 1998–2004, 2005–2011, 2012–2018 were considered. Only statistically significant results at ANOVA are shown.

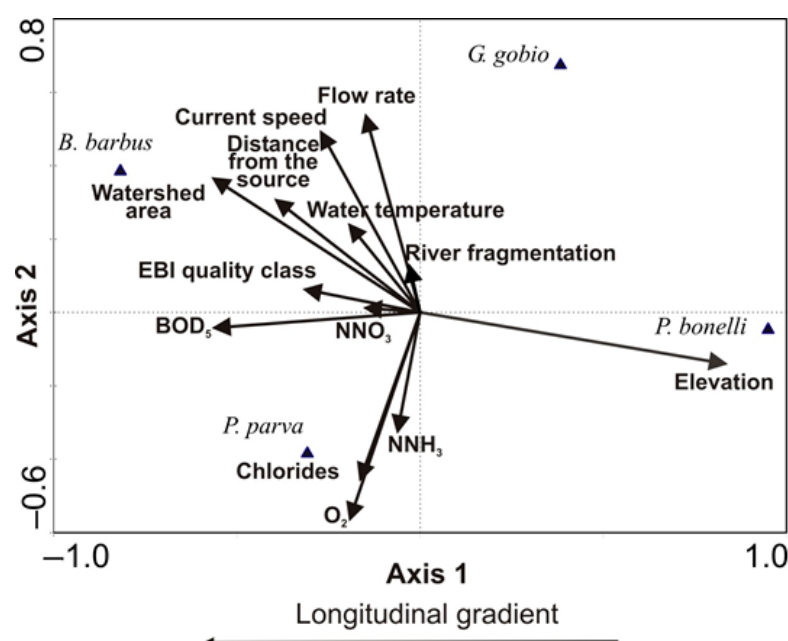


Figure 8. Canonical Correspondence Analysis results: biplot of fish and environmental variables for the census periods 1998–2004, 2005–2011, 2012–2018.

3.4. Detectability, Occupancy, Colonization, and Local Extinction Probabilities

The detection probability was quite high and never lower than 0.74, even if perfect detection (1.00) was never achieved; the highest value was observed for *G. gobio* (0.86 ± 0.06), while the lowest detection probability was observed for *P. parva* (0.74 ± 0.06). Occupancy probability values were not very high but still noteworthy, considering that they referred to alien species introduced in recent decades; the occupancy probability varied from 0.14 ± 0.04 for *B. barbus* to 0.31 ± 0.07 for *P. parva*. Colonization probabilities ranged from 0.12 ± 0.04 for *G. gobio* to 0.26 ± 0.07 for *P. parva*; these values were considerably higher than the local extinction probabilities, which for *G. gobio* and *P. bonelli* were 0.00, while the highest value referred to *P. parva* (0.18 ± 0.10) (Table 2).

Table 2. Overall occupancy (ψ), colonization (γ), extirpation (ϵ) and detection (p) probabilities ($\pm$ SE) for *Barbus barbus*, *Gobio gobio*, *Padogobius bonelli* and *Pseudorasbora parva*.

Species	Occupancy (ψ)	Colonization (γ)	Extirpation (ϵ)	Detection (p)
<i>Barbus barbus</i>	0.14 ± 0.04	0.20 ± 0.05	0.05 ± 0.01	0.79 ± 0.07
<i>Gobio gobio</i>	0.21 ± 0.04	0.12 ± 0.04	0.00 ± 0.00	0.86 ± 0.06
<i>Padogobius bonelli</i>	0.15 ± 0.04	0.10 ± 0.03	0.00 ± 0.00	0.85 ± 0.06
<i>Pseudorasbora parva</i>	0.31 ± 0.07	0.26 ± 0.07	0.18 ± 0.10	0.74 ± 0.06

The lowest values of detectability were observed during the first census period for all species (Table 3). For *B. barbus*, the detection probabilities over time varied from 0.53 ± 0.06 (years 1998–2004) to 0.80 ± 0.08 (years 2012–2018). Additionally, for *G. gobio* and *P. bonelli* an increase over time of detectability was observed, with mean values varying from 0.42 ± 0.01 and 0.67 ± 0.11 (years 1998–2004) to 0.88 ± 0.08 and 0.93 ± 0.07 (years 2012–2018), respectively. For *P. parva* the detection probabilities ranged from 0.53 ± 0.06 (years 1998–2004) to 0.71 ± 0.08 (years 2012–2018) (Table 3).

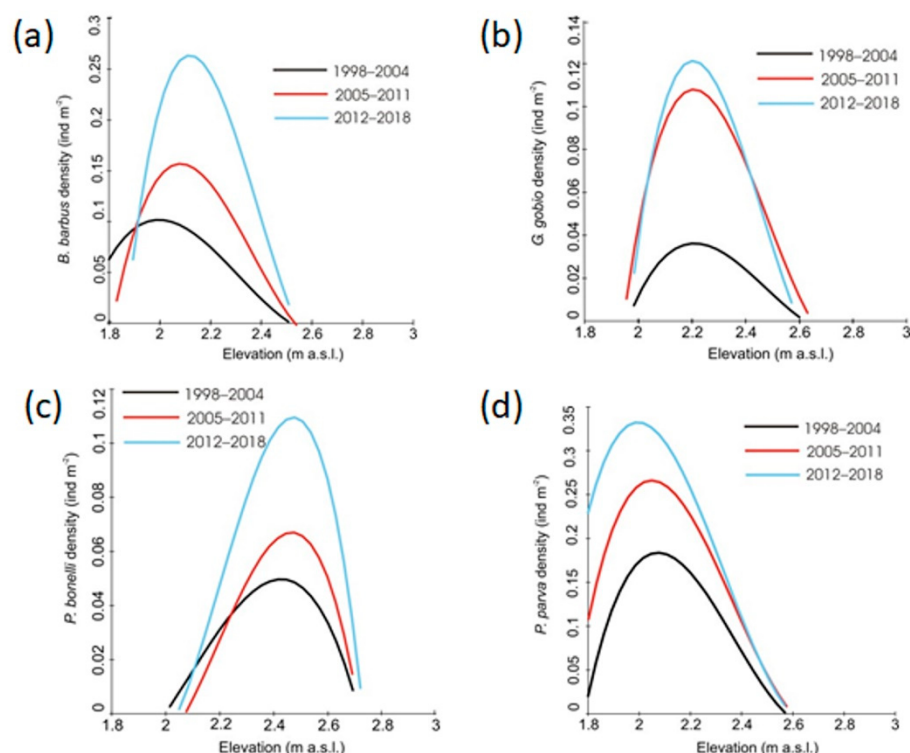


Figure 9. The effects of elevation on the density of (a) *B. barbus*, (b) *G. gobio*, (c) *P. bonelli* and (d) *P. parva* over the census periods 1998–2004, 2005–2011, 2012–2018. Solid lines indicate significant results from the general linear model (GLM).

The best multi-sampling period models, within two AIC units of the top model, for estimating occupancy, colonization, local extinction, and detection, included: elevation, fragmentation degree, water temperature and average current speed as informative variables (Table S1). For *B. barbus*, colonization probabilities progressively decreased with increasing river fragmentation degree (Figure 10a) and were highest at the warmer sites (Figure 10b). For *G. gobio* the colonization probabilities increased according to the increasing average current speed (Figure 11). As regards *P. bonelli*, colonization probability increased with elevation, while occupancy increased with river fragmentation degree (Figure 12). For *P. parva* the occupancy probability increased with elevation (Figure 13).

Table 3. Probability of detection ($\pm$ standard error (SE)) across the four time periods for *Barbus barbus*, *Gobio gobio*, *Padogobius bonelli* and *Pseudorasbora parva*.

Species	Detection Probability		
	Time Period 1 1998–2004	Time Period 2 2005–2011	Time Period 3 2012–2018
<i>Barbus barbus</i>	0.53 $\pm$ 0.06	0.79 $\pm$ 0.08	0.80 $\pm$ 0.08
<i>Gobio gobio</i>	0.42 $\pm$ 0.01	0.83 $\pm$ 0.09	0.88 $\pm$ 0.08
<i>Padogobius bonelli</i>	0.67 $\pm$ 0.11	0.72 $\pm$ 0.11	0.93 $\pm$ 0.07
<i>Pseudorasbora parva</i>	0.53 $\pm$ 0.06	0.79 $\pm$ 0.08	0.71 $\pm$ 0.08

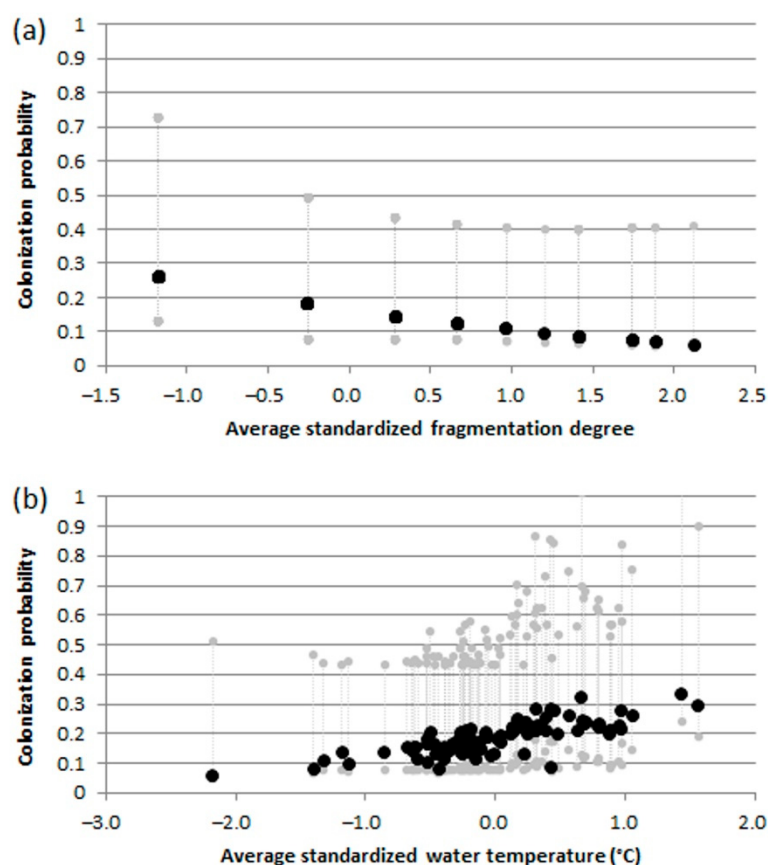


Figure 10. *Barbus barbus*: effects of fragmentation degree (a), and water temperature (b) on colonization probabilities from the best informative models. Dashed gray lines indicate upper and lower 95% confidence intervals.

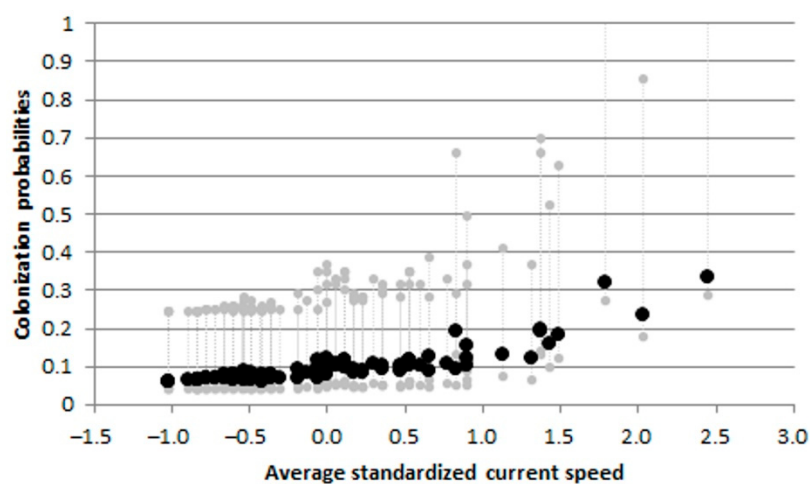


Figure 11. *Gobio gobio*: effects of average current speed on colonization probabilities from the best informative models. Dashed gray lines indicate upper and lower 95% confidence intervals.

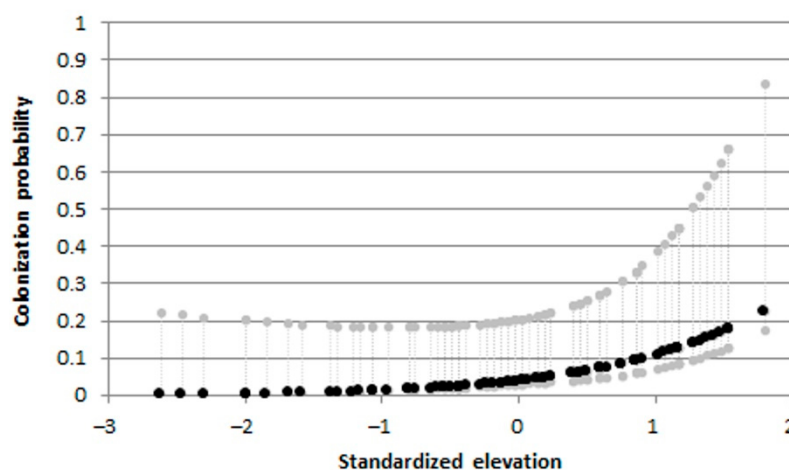


Figure 12. *Padogobius bonelli*: effects of elevation on colonization probabilities, from the best informative models. Dashed gray lines indicate upper and lower 95% confidence intervals.

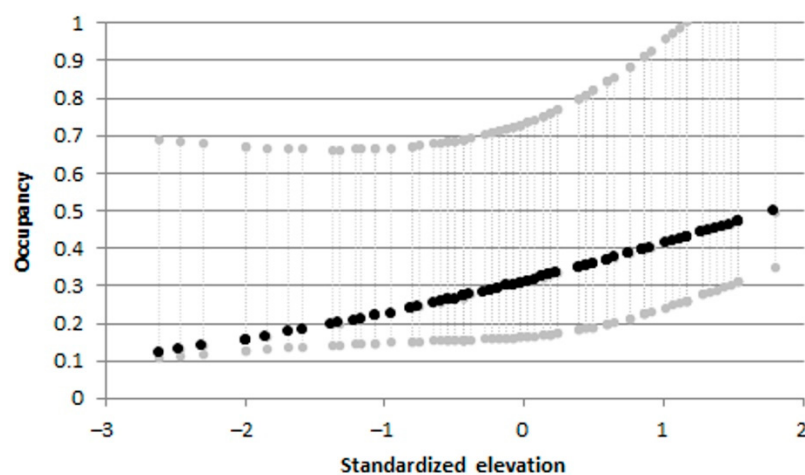


Figure 13. *Pseudorasbora parva*: effects of elevation on occupancy from the best informative models. Dashed gray lines indicate upper and lower 95% confidence intervals.

4. Discussion

As reported for other European countries [38] a close positive relationship between the time of introduction and spatial distribution of the alien species was observed in the Tiber river basin. Each species showed its own invasion pattern, in which the environmental factors that are major determinants under climate change conditions seem to be mostly represented by river fragmentation, water temperature and hydrological parameters. Most alien species are expanding their range because probably in the last twenty years they have not yet completed their dispersal process. In addition to the dispersal capability of the species, some effects of climate change, in terms of water temperature increase, seemed to favor especially cool and warm-water species, playing a key role in their expansion.

B. barbus, being among the species introduced in more recent years (1998), after having rapidly invaded numerous sites, and having formed ever more abundant populations over time [26], seemed to be still expanding its range. For the species was observed a tendency to progressively move towards the river stretches located at higher altitudes, probably favored by the water warming consequent to climate change. Moreover, the high density population values reached in the last census period may in part have contributed to the spread of the species, due to intraspecific competition phenomena [64]. Moreover, as this is a species of particular interest for sport fishing [65], secondary introductions in multiple sites through illegal stocking could have encouraged the expansion of the species [38,43]. However, a brake on the expansion of a vagile species such as *B. barbus*

can be represented by river fragmentation, as also reported in the literature for some large rivers of England and Belgium [65,66]. Thus, the presence of obstacles to migration for reproductive purposes could affect *B. barbus* response to climate change [67], especially in a dendritic hydrographic network as the Tiber river basin, where a single barrier to migration and dispersal could create an extended isolated area [68].

Also *G. gobio* resulted in rapid expansion in space and time, with a number of colonized sites progressively increasing over time, and with more abundant populations showing good body condition. The average current speed plays a key role in colonization processes, as there was a positive relationship with the colonization probabilities. Being a species introduced in the Tiber river basin in fairly recent times (first record in 1999), further research based on long-term data series are needed to highlight the ecological effects of *G. gobio* invasion, and to evaluate the resilience of the species to global warming.

For *P. bonelli* our results highlighted a resilience to climate change effects, showing a range shift towards upstream reaches, probably favored by water warming. In fact, elevation exerted a positive effect on colonization probabilities. Especially in the last census period, the number of colonized sites and the density of the populations have increased considerably, in combination with a good body condition. Contrary to *B. barbus*, *P. bonelli* is not of interest to sport fishing; however, the species may have been the subject of human-mediated involuntary translocations among different watercourses, especially during transfers of fish trapped in pools during drought periods, made by unauthorized volunteers. This would explain the appearance of the species in recent times in the Chiascio river, where *P. bonelli* could not have arrived using the natural connections of the hydrographic network. As reported for *Neogobius melanostomus* (Pallas, 1814) in the Greath Lakes, the secondary human-made traslocations within a basin could play a key role in the secondary diffusive spread of an invasive species [64]. However, as in our case these are occasional and unpredictable events, involving few individuals, it is difficult to document this kind of secondary introductions, and it is difficult to establish how much they may have influenced the expansion of the species in the Tiber river basin.

As for *B. barbus* and *P. bonelli*, also in the case of *P. parva* it is possible to hypothesize a “step and stone diffusion” dispersal pattern [25,69]; in particular, starting from the two first introduction points, the species could have colonized the hydrographic network using the natural connections and spreading both downstream and upstream, thanks to its pollution and thermal tolerance, also aided by involuntary secondary releases. Being a species of less recent introduction, *P. parva* seemed to have reached, at this stage, a temporary equilibrium in terms of distribution in the Tiber river basin. The stop in the increase in average populations density and the decrease in colonized sites in the last census period, provided some evidence that *P. parva* seems to have almost completed the dispersal process in the study area. However, being a warm-water species, this balance could be destabilized in the future due to the effects of climate change that tend to benefit species with such thermal preferences [38]. Our results confirmed that one of the major drivers for *P. parva* is represented by the average current speed, as was also demonstrated in a previous study [25]; in fact, as reported in the literature, the species inhabits lowland areas with low current speed, where it must not incur an excessive expenditure of energy for swimming, avoiding metabolic stress [70].

Investigations on the relationships between habitat and fish species are fundamental to preserve native biodiversity and to manage multi-stressed freshwater ecosystems [71]. This is the case of the Tiber river basin, where previous research has shown that changes in the composition of fish communities along the longitudinal gradient of watercourses were associated with a progressive increasing perturbation proceeding from upstream to downstream [20,72], as also highlighted by Saez-Gomez et al. [71] for other highly stressed Mediterranean basins. The 4 species considered, although belonging to the barbel zone [73], have shown different ecological requirements, with *P. bonelli* seeming to prefer environments with cleaner waters located at higher altitudes, while *P. parva* showed greater tolerance with respect to environmental degradation in terms of water pollution. *G. gobio*

was placed in river stretches showing intermediate characteristics. The increase over time in the concentration of dissolved oxygen in the sites where *B. barbus* and *P. bonelli* were present, could be linked to the range shift of the two species that are moving upstream. The progressive increase in chlorides and sulphates in *P. parva* occurring sites could reflect a worsening of water quality as an indirect effect of the progressive decrease in the average current speed.

The GLM analysis showed for *B. barbus* and *P. bonelli* range shifts towards sites located at higher altitude. These results match the hypothesis that rising water temperatures could favor the upstream colonization by cool-water adapted species, although the response curves could be influenced by the fact that the species have not yet completed the expansion process in the invaded area [43].

As might have been expected in the case of species introduced in recent decades, the colonization probabilities for the 4 species were much higher than extinction ones. The colonization processes were driven by elevation for *P. bonelli* and *P. parva*, while average current speed exerted a positive effect on colonization processes for *G. gobio*. Water temperature and river fragmentation play a key role in *B. barbus* colonization processes. In particular, for this species, colonization probabilities increased with water temperature and decreased with river fragmentation degree. This latter result confirmed that barriers (including human-made weirs and drought river stretches) could hinder the expansion of alien species [68], but at the same time should be considered that they prevent the range shift of native species to achieve thermal optimum, leading to a loss of biodiversity [43,74]. However, in the ecological evaluation of these relationships between colonization and environmental variables should be taken into account that these species were probably not yet stabilized and have not yet reached a stable equilibrium [75].

5. Conclusions

Our results provided some evidence for synergistic effects between climate change effects and alien cool-water adapted species invasions. The range shifts towards upstream reaches of *B. barbus* and *P. bonelli* allowed us to consolidate the hypothesis of a further expansion of these invasive species, favored by rising temperatures that provide new suitable habitats in which to live and reproduce. However, these results should be interpreted cautiously, taking into account that these species most likely were not yet stabilized [7,43]. The interruptions of the river connectivity can counteract the dispersal ability and resilience of invasive species by limiting the colonization probabilities. Effective conservation policy and management measures to be taken to contrast the introduction and expansion of alien fish species (i.e., early detection, eradication and removal activities, preventing secondary introductions through environmental education) [64] should be accompanied by interventions aimed at mitigating the global climate change effects on multi-stressed freshwater ecosystems (i.e., riparian restoration, maintenance of ecological flow) [43].

Supplementary Materials: The following are available online at <https://www.mdpi.com/2073-4441/13/3/347/s1>, Figure S1: Ecological requirements: trend over time of the mean values of elevation, water temperature and average current speed for: (a) *Barbus barbus*, (b) *Gobio gobio*, (c) *Padogobius bonelli*, (d) *Pseudorasbora parva*, Table S1: Models within two Akaike information criterion (AIC) units of the top model for estimating occupancy (ψ), colonization (γ), local extinction (ϵ), and detection (p) probabilities for: (a) *Barbus barbus*, (b) *Gobio gobio*, (c) *Padogobius bonelli*, (d) *Pseudorasbora parva*.

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