PRIMARY RESEARCH PAPER

Feeding Ecology of *Padogobius nigricans* (Canestrini, 1867) and *P. bonelli* (Bonaparte, 1846) in Aggia River (Umbria, Italy) and Their Diet Overlap

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Abstract The Arno goby *Padogobius nigricans* is an endemic species to Central Italy. The populations of this species are in sharp decline due to habitat modifications and potentially competition with Padanian goby *Padogobius bonelli*, an endemic species to Northern Italy, introduced in some watercourses of Central Italy. The purposes of this paper were to analyze dietary composition of the two species, determine the degree of overlap in their diets and investigate the feeding ecology of *P. nigricans* in terms of absence and presence of the alien species. Specimens were collected from the Aggia River, a tributary of Tiber River. Gut contents were analyzed and prey items were identified to the lowest recognizable taxa and counted. All the obtained results showed that *P. nigricans* and *P. bonelli* in the Aggia River share a similar diet. Both species fed extensively on Chironomids and no statistically significant ontogenetic changes in feeding habits were found. A high degree of diet overlap was observed. Diet of *P. nigricans* and *P. bonelli* also included fish and where the species live in syntopy, individuals belonging to *Padogobius* genus were found in stomach, suggesting that mutual predation may occur.

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Introduction

The Arno goby *Padogobius nigricans* (Canestrini, 1867) is an endemic species to Central Italy. The species is very sensitive to pollution and other anthropogenic disturbance and requires good quality waters to survive and reproduce (Gandolfi et al., 1991; Carosi et al., 1996). Its original range of distribution, restricted to the Tuscany–Latium ichthyogeographic district (Bianco, 1987), is now highly fragmented and the species is reduced to hundreds of isolated populations. The decrease of populations is mainly due to habitat modification and waters quality deterioration (Scalici & Gibertini, 2009; Mecatti et al., 2010). Thus, *P. nigricans* is listed as vulnerable both by IUCN (Crivelli, 2006) and Red List of Italian Vertebrates (Rondinini et al., 2013). A potential threat to the survival of this species is represented by the congeneric Padanian goby, *Padogobius bonelli* (Bonaparte, 1846). This species is endemic to Padano-Veneto district in Northern Italy (Bianco, 1987), but has been introduced in some watercourses of Central Italy since the early 80s as a result of restocking activities (Bianco & Ketmaier, 2001; Zerunian, 2004). *P. bonelli* has been recorded for the first time in Tiber River in 1996

(Mearelli et al., 1996). It has successfully colonized the upper and middle sectors of Tiber over the last years (Lorenzoni et al., 2006), and now it is spreading in the tributaries (Lorenzoni et al., 2010).

P. nigricans and *P. bonelli* share similar reproductive habits: both species deposit eggs on the underside of rocks that are defended by males (Gandolfi et al., 1991; Zerunian, 2004). During the reproductive period, in both species, the males fight with con-specific for the selection of the best breeding sites. However, *P. bonelli* shows a more territorial and aggressive behavior than *P. nigricans*: when the two species inhabit the same river, *P. bonelli* may outcompete *P. nigricans* for spawning sites preventing its reproduction (Zerunian, 2004). In some river basins of Central Italy (Amaseno and Mignone), *P. bonelli* has been assessed as the cause of local extinctions of *P. nigricans* (Crivelli, 2006; Nocita & Zerunian, 2007).

While mechanisms of territorial competition between the two species for the occupation of the breeding sites were deeply studied in laboratory (Zerunian, 2002; Mecatti et al., 2010), little is known about the composition of the trophic spectrum of both species (Gandolfi et al., 1991) and there is no information about the diet of *P. bonelli* outside its native range. Thus, in this study, a detailed taxonomic analysis of the diet spectrum of *P. nigricans* and *P. bonelli* populations inhabiting the Aggia River, a tributary of Tiber River, was undertaken. Specific objectives of this research were to: (1) analyze dietary composition of the two species and investigate the feeding ecology of *P. nigricans* with respect to absence and presence of the non-native species; (2) determine the niche breadth and feeding strategy of both species; (3) analyze the diet overlap where the species coexist.

Materials and methods

Study area

The Aggia River (43°24′8.57″N, 12°12′40.34″E) is a right tributary of the Tiber River, the third-longest (405 km) river in Italy and the second for watershed (17,375 km^2) (Lorenzoni et al., 2006). The Aggia river is 15.5 km long and has a low flow rate (just over 10 l/s) (Lorenzoni et al., 2010); nevertheless, the river is characterized by high quality water and it has been chosen as sampling area as it houses two of the largest

populations of both investigated species (Lorenzoni et al., 2007a). A Mediterranean climate, with maximum rainfall in autumn and minimum in summer, characterizes this biotope, so the river has a typical torrential regime. At the beginning of summer several river sectors dry up and only separate pools persist until autumn. Temperature increases, and the fishes that remain trapped in the pools are destined to die. So, to lessen the impact on fish fauna, the pools have been chosen as sampling sites.

Upstream the fish community is composed only of native species typical of the barbel zone, while downstream several exotic species introduced in Tiber River are present. Indeed a weir 2.5 m high located in the middle section of the river prevents the exotic species inhabiting the downstream sector to move upstream. Then above the weir only *P. nigricans* is present, while below the two species of goby live in syntopy. Therefore, to investigate the feeding ecology of *P. nigricans* in terms of absence and presence of the alien species, two sampling sites were chosen as representative of the river: the former located upstream (43°24′28.28″N, 12°12′12.67″E) and the latter downstream of the weir (43°24′15.88″N, 12°12′33.54″E) (Fig. 1). The sampling sites are rather close and the weir did not cause any change in environmental characteristics of the river: no differences were observed in physico-chemical, morphological, and hydrological parameters between upstream and downstream stations (Lorenzoni et al., 2007a) suggesting similar composition of benthic community and prey availability.

Collection and processing of samples

Fish of both species were collected monthly from January to December 2012 by electrofishing. The samples were immediately anesthetized with 2-phenoxyethanol and preserved in 4% formaldehyde for the laboratory analysis.

Total body length (TL) of specimens was measured to the nearest 0.1 cm with a measuring board; individual total weight (W) was recorded to the nearest 0.1 g before dissection. For age determination, a sample of scales was removed from dorsolateral or ventrolateral rows of the caudal peduncle of each individual (Miller, 1975) and stored in 33% ethanol. Fish scales were observed under a stereo microscope and the number of annuli was counted; two

Fig. 1 Map of the study area (Aggia River, Central Italy) showing the location of the sampling stations upstream (*1*) and downstream (*2*) of the weir
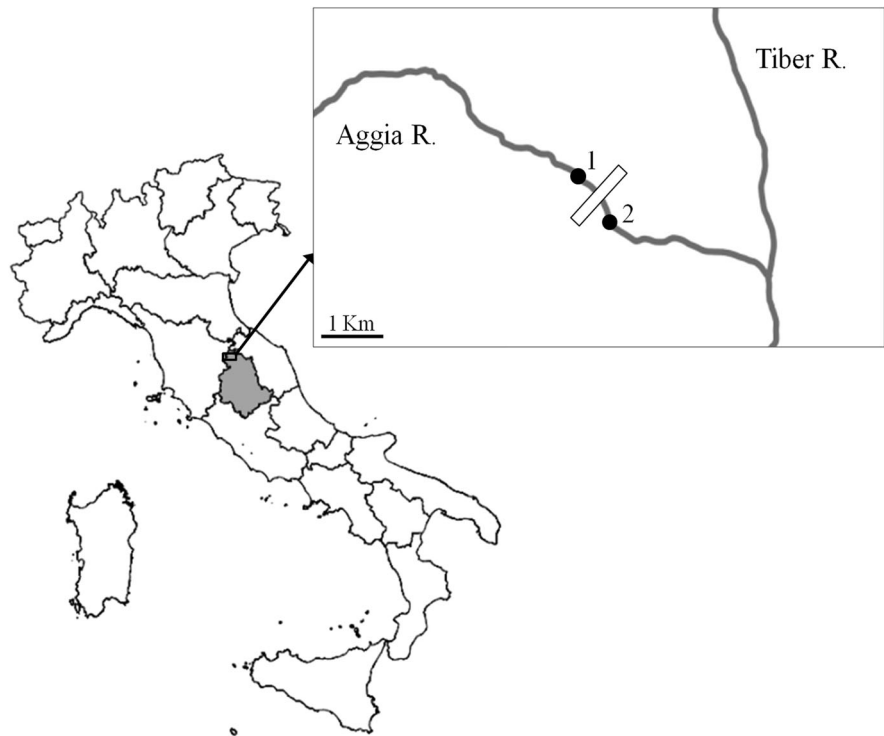
independent age determinations were made by two different operators. When the results were different, an additional determination was made. The microscopic scalimetric method was validated by means of length-frequency distribution (Bagenal, 1978). The gut contents of each specimen were removed and analyzed under a stereo microscope to identify prey categories. Prey items were identified to the lowest recognizable taxa and counted (Hyslop, 1980).

The analyses were accomplished by dividing the total sample into 3 different groups: *Padogobius nigricans* upstream of the weir (*PnU*), *Padogobius nigricans* downstream of the weir (*PnD*) and *Padogobius bonelli* (*Pb*).

Statistical analysis

The percentages of empty stomachs for the three samples were considered and comparisons between *PnU–PnD* and *PnD–Pb* were performed using χ^2 test. Season-to-season differences were evaluated by ANOVA.

Diet composition

Diet composition was expressed in terms of relative abundances (%N = the number of individuals of each food item with respect to the total number of individuals) and frequency of occurrence (%F = the number of stomachs containing each food item in relation to the total number of full stomachs) (Hyslop, 1980).

The Prominence Values (PV) of dietary component were estimated using the following formula (Hickeley et al., 1994):

$$PV = \%N \sqrt{\%F}$$

where %N = relative abundances and %F = frequency of occurrence.

Variation in prey composition was analyzed by calculating a dissimilarity index (Bray–Curtis) between all the specimens of the three groups from numerical data of individual food items. These data were used to compute a multivariate analysis of similarities (ANO-SIM) in order to test for significant differences in diet between *PnU–PnD* and *PnD–Pb*. Between-groups differences were also performed by dividing the samples in age classes and seasons. Moreover ANOSIM was carried out within each group to verify differences between age classes and seasons. ANOSIM is roughly analogous to a univariate one-way ANOVA, but it uses a non-parametric, randomization approach (Clarke & Warwick, 1994; Russo et al., 2008; Toscano et al., 2011). Where a

difference had been established by ANOSIM, this was followed by similarity percentages analyses (SIMPER) to identify which prey items could be used to discriminate between the diets of considered groups (Lek et al., 2011). The Bray–Curtis similarity measurement is implicit to SIMPER. Finally, the Bray–Curtis dissimilarities were also used in a non-metric multidimensional scaling ordination (NMDS; Schiffman et al., 1981) that provided a two dimensional map of the distances between samples in a Euclidean space based on the Bray–Curtis index (Russo et al., 2008; Toscano et al., 2011). ANOSIM, NMDS, and SIMPER were performed using the statistical software PAST (Hammer et al., 2001).

Feeding strategy and niche breadth

The Costello graphical method (1990) modified by Amundsen et al. (1996) was used to visualize the diet patterns. The diet was characterized by plotting frequencies of occurrence (%F) and relative prey-specific abundance (P_i). P_i is defined as the percentage of a prey taxon from all prey items only in those specimens in which the actual prey really occurs (Amundsen et al., 1996):

$$P_i = 100 \sum S_i \sum S_{ti}^{-1},$$

where S_i = number of stomach contents composed by prey i and S_{ti} = total stomach contents only in those individuals containing prey i. This method describes feeding strategy and niche width components of populations (Amundsen et al., 1996). The prey-specific abundance, increasing along the diagonal from the lower left to the upper right corner of the graph, provides a measure of prey importance, with dominant prey at the upper, and rare or unimportant prey at the lower end. Predators have specialized on prey items that are found in the upper part of the graph while prey items in the lower part have been ingested occasionally and indicate generalization (Amundsen et al., 1996).The Levins measure of niche breadth (B) and standardized niche breadth (BA), (Levins, 1968) were used to estimate the diet width as follows:

$$B = 1/\Sigma p^2 j$$

and

$$BA = (B - 1)/(n - 1)$$

where pj = proportion of the diet composed of prey species j; and n = the total number of prey species. The Levins measure of niche breadth (B) has a minimum of 1 when only one prey type is found in the diet and a maximum of n, where n is the total number of prey categories, each representing an equal proportion of the diet. The Levins measure of standardized niche breadth (BA) ranges from 0 (diet restricted to few prey items) to 1 (generalist diet), where low values indicate diet dominated by few prey items (Pedersen, 1999). Levins' indexes were used to calculate: (1) the average individual diet width and (2) the total diet width of the three groups (Svanback & Persson, 2004).

Diet overlap

The Diet Overlap Index (α) was evaluated using the prominence values (PV) of each food category (Lorenzoni et al., 2002) according to the following formula (Schoener, 1970):

$$\alpha = 1 - 0.5 \left(\sum_{i=1}^{n} |PV \cdot xi - PV \cdot yi| \right)$$

where n = number of food items, PV_{xi} = prominence value of food item i in species x, PV_{yi} = prominence values of food item i in species y. The index value varies from 0 (no diet overlap) to 1 (total diet overlap) and is primarily used when the prey abundance is unknown (Wallace, 1981). The degree of diet overlap between the two species was calculated for the groups *PnD* and *Pb* by dividing the samples into age-classes.

Results

Diet composition

The sample of *PnU* was composed by 170 specimens ranged in size from 2.5 to 8.9 cm TL; among all the stomachs examined, 31 were empty (18.2%). Below the weir, a total of 130 specimens of *P. nigricans* (*PnD*) were analyzed, ranged in size from 3 to 9 cm TL. The percentage of empty stomachs was 14.6%, corresponding to 19 stomachs. For *P. bonelli*, the sample ranged in total length from 2.5 to 7.7 cm. 190 stomachs were analyzed and 34 resulted empty (17.9%) (Table 1). No differences were found in the percentages of empty stomachs between *PnU* and *PnD*

Table 1 Seasonal and total percentages of empty stomachs and number of stomach examined for the three groups (*PnU* = *P. nigrigans* upstream of the weir; *PnD* = *P. nigrigans* downstream of the weir; *Pb* = *P. bonelli*)

		Winter	Spring	Summer	Autumn	Total
PnU	No. of stomachs examined	24	24	59	32	170
	% empty stomachs	42.9%	11.1%	10.6%	8.6%	18.2%
PnD	No. of stomachs examined	26	31	40	14	130
	% empty stomachs	23.5%	3.1%	9.1%	30.0%	14.6%
Pb	No. of stomachs examined	29	44	45	38	190
	% empty stomachs	23.7%	15.4%	19.6%	13.6%	17.9%

at χ^2 test ($\chi^2 = 0.69$, $P > 0.05$), neither between *PnD* and *Pb* ($\chi^2 = 0.60$; $P > 0.05$). At ANOVA, the percentages of empty stomachs among seasons were not significantly different for *P. bonelli* ($F = 1.75$, $P > 0.05$); on the contrary, for *P. nigricans* a high portion of empty stomach was found during autumn (for *PnD*) and winter (for *PnU*), and the differences between seasons were highly significant in both samples (*PnU*: $F = 22.76$, $P < 0.01$; *PnD*: $F = 10.42$; $P < 0.01$) (Table 1).

The diet composition of the two species comprised a total of 39 food items, 29 in *PnU*, 28 in *PnD*, and 30 in *Pb* (Table 2). In both species, Chironomid larvae were the most prevalent food type in terms of relative abundance, occurrence and prominence. In *P. nigricans* the second most prevalent food type was always represented by Dipteran larvae, and more specifically, belonging to the Psychodidae family ($\%N = 5.9\%$) for *PnU* and to the *Ceratopogon* genus for *PnD* ($\%N = 4.9\%$). In *P. bonelli*, instead, the second most prevalent food type was Bivalvia ($\%N = 5.1\%$) (Table 2). All the other categories contributed to the total abundance with percentages less than 4% for all the three samples. In addition to invertebrates, the diet of *P. nigricans* and *P. bonelli* also included fish: scales and remains of juveniles cyprinid, classified as "Other fish species", were found in both species (%N: *PnU* = 0.1%; *PnD* = 0.1%; *Pb* = 0.4%). Some stomachs also contained scales or entire individuals attributable to the same genus *Padogobius*. Particularly, this category was present in both species but with respect to *P. nigricans*, only in the sample downstream of the weir (*PnD*) remains of *Padogobius sp*. were found. The differences in diet were not statistically significant neither comparing *PnU* with *PnD* (ANOSIM: $r = 0.01$, $P > 0.05$) nor between *PnD* and *Pb* ($r = 0.01$, $P > 0.05$).

Analyzing the diet in the samples divided into age classes in terms of relative abundance (%N) (Fig. 2), Dipterans were an important component of diet for all age classes of both species. For *PnU* the diet of juveniles (0+) and oldest specimens (3+ to 4+) comprised only insects (Dipterans and Other Insects), while the stomach content of the intermediate age classes also included all the other categories, albeit in small quantities (Fig. 2a). *PnD* showed a more homogeneous distribution of food items between age classes: for the class 1+, though Dipterans always represented the most consumed food item (72.5%), Mollusca also reached a relevant percentage (15%). In the other age classes, after Insects (Dipterans and Other Insects), Mollusca always resulted the most preyed item, and all the other categories were found (Fig. 2b). In *Pb*, the juveniles (0+) fed only on Dipterans (80%) and Annelida (20%). In 1+ age class, Mollusca and Crustacea constitute a significant part of the diet (29.7% and 5.6%, respectively). The pattern of other age classes is rather similar to that of *PnD*, with high proportion of Insects and small quantity of all other categories (Fig. 2c). The older specimens (2+ to 4+ age classes, corresponding to 5.4–9.0 cm TL for *P. nigricans* and 2+ to 3+ corresponding to 6.0–6.7 cm TL for *P. bonelli*) preyed also on "Other fish species", while the predatory behavior toward *Padogobius* already occurred from the 1+ specimens (4.3–7.5 cm TL for *PnD* and 4.8–7.4 cm TL for *Pb*). Despite the changes observed in diet among age classes, no differences emerged between ages within the three samples (*PnU*: $r = 0.01$, $P > 0.05$; *PnD*: $r = 0.02$, $P > 0.05$; Pb: $r = 0.01$; $P > 0.05$), neither comparing *PnD* with *PnU* and *Pb* with *PnD* for each age class at ANOSIM ($P > 0.05$).

Diet composition varied considerably throughout the year and ANOSIM detected significant differences between seasons within each group (*PnU*: $r = 0.06$, $P < 0.05$; *PnD*: $r = 0.12$, $P < 0.01$; *Pb*: $r = 0.13$,

Table 2 Diet of *P. nigricans* (*PnU* and *PnD*) and *P. bonelli* (*Pb*) expressed as relative abundance (%N), frequency of occurrence (%F) and prominence value (PV) of food items (*l* larvae; *p* pupae)

Macro-categories	Food items	PnU			PnD			Pb		
		%N	%S	PV	%N	%S	PV	%N	%S	PV
Mollusca	Gasteropoda									
	Gasteropoda (ind)	0.49	3.6	<0.01	0.54	3.6	<0.01	1.57	7.83	<0.01
	Physa fontinalis (Linnaeus, 1758)				0.06	0.9	<0.01			
	Bivalvia									
	Pisidium sp.	0.07	0.72	<0.01				0.48	2.41	<0.01
	Bivalvia (ind)	0.42	3.6	<0.01	0.48	2.7	<0.01	5.13	3.01	<0.01
Annelida	Oligochaeta									
	Lumbriculus variegatus (O.F. Müller, 1774)	0.28	2.88	<0.01	0.54	6.31	<0.01	0.84	8.43	<0.01
	Hirudinea									
	Dina lineata (O.F. Müller, 1774)	0.07	0.72	<0.01	0.06	0.9	<0.01			
Crustacea	Cladocera									
	Cladocera	0.07	0.72	<0.01				0.72	0.6	<0.01
	Isopoda									
	Asellus sp.	0.28	2.88	<0.01	0.42	4.5	<0.01	0.6	6.02	<0.01
	Amphipoda									
	Echinogammarus sp.	0.14	1.44	<0.01						
Hydracarina	Hydracarina									
	Hydracarina				0.12	1.8	<0.01	0.72	4.82	<0.01
Other insects	Ephemeroptera nymphae									
	Serratella ignita (Poda, 1761)	0.35	2.88	<0.01	0.84	5.41	<0.01	0.06	0.6	<0.01
	Caenis luctuosa (Burmeister, 1839)				0.18	2.7	<0.01	0.12	1.2	<0.01
	Baetis rhodani (Pictet, 1843)	3.22	8.63	0.01	2.52	9.01	<0.01	0.84	5.42	<0.01
	Trichoptera larvae									
	Limnephilidae	0.56	5.76	<0.01	0.42	5.41	<0.01	0.06	0.6	<0.01
	Hydropsyche pellucidula (Curtis, 1834)	0.35	2.16	<0.01				0.06	0.6	<0.01
	Hydroptila martini (Marshall, 1977)	1.75	11.51	0.01	1.38	5.41	<0.01	0.78	4.22	<0.01
	Leptoceridae	0.28	2.16	<0.01	0.12	1.8	<0.01	0.78	6.02	<0.01
	Polycentropodidae	0.35	2.88	<0.01						
	Plecoptera nymphae									
	Capnia sp. Pictet, 1841	2.1	11.51	0.01	0.3	4.5	<0.01	0.66	3.61	<0.01
	Odonata larvae									
	Calopterix sp.	0.07	0.72	<0.01				0.18	0.6	<0.01
	Onychogomphus forcipatus unguiculatus (Linnaeus, 1758)				0.06	0.9	<0.01			
	Hemiptera									
	Nepa cinerea (Linnaeus, 1758)							0.12	0.6	<0.01
	Coleoptera									
	Haliplidae adult	0.07	0.72	<0.01	0.06	0.9	<0.01			

Table 2 continued

Macro-categories	Food items	PnU			PnD			Pb		
		%N	%S	PV	%N	%S	PV	%N	%S	PV
Diptera	Diptera									
	Stratiomyidae l.	0.42	3.6	<0.01				0.24	1.81	<0.01
	Tipula sp. l.	0.42	2.88	<0.01	0.24	3.6	<0.01	0.3	3.01	<0.01
	Hexatoma sp. l.	0.35	2.88	<0.01				0.36	2.41	<0.01
	Tanypodinae l.	3.01	10.79	0.01	1.02	9.91	<0.01	1.21	4.82	<0.01
	Simuliidae l.	0.28	1.44	<0.01	0.54	7.21	<0.01	1.93	9.64	<0.01
	Ceratopogon sp. l.	0.49	4.32	0	4.92	13.51	0.02	2.23	10.84	<0.01
	Psychodidae l.	5.4	16.55	0.02	3.6	14.41	0.01	3.56	13.86	0.01
	Chironomidae l.	75.4	72.66	0.64	79.9	71.17	0.67	74.59	69.88	0.62
	Athricops sp. l.				0.06	0.9	<0.01			
	Limoniidae l.	0.21	2.16	<0.01						
	Chironomidae p.	2.94	12.23	0.01	0.72	4.5	<0.01	0.91	2.41	<0.01
	Ptychopteridae p.				0.3	0.9	<0.01	0.12	1.2	<0.01
	Psychodidae p.							0.06	0.6	<0.01
Padogobius sp.	Fish									
	Padogobius sp.				0.36	5.41	<0.01	0.42	4.22	<0.01
	Padogobius bonelli				0.12	1.8	<0.01			
Other fish species	Other fish species (Cyprinidae ind)	0.14	1.44	<0.01	0.12	1.8	<0.01	0.36	3.61	<0.01

$P < 0.01$). For *PnU*, according to SIMPER analysis, differences between seasons were due to the change of proportions of three food items: Chironomid and Psychodidae larvae, that are much more abundant in spring and winter compared to autumn and summer, and *Baetis rhodani*, that is not preyed in autumn. In *PnD*, SIMPER emphasized that Chironomid larvae are abundant in all seasons except autumn and *Baetis rhodani* is preyed only in spring and summer. In *Pb*, differences were due to the proportion of Chironomid and Simuliidae larvae, more abundant in summer than in the other seasons. However, ANOSIM comparison between *PnD–PnU* and *Pb–PnD* for each season, didn't show significant differences ($P > 0.05$).

Feeding strategy and niche breadth

Visual examination of the Costello plots (Fig. 3) showed a similar distribution of prey items (pooled in the 8 categories) in the three samples suggesting a comparable feeding pattern. Dipterans proved to be the dominant category ingested in all the three groups both in terms of prey-specific abundance (%P_i) and

frequency of occurrence (%F). Most of the prey found in the stomachs showed low frequency of occurrence with low to medium levels in prey-specific abundances. However, Crustaceans for *P. nigricans* and Mollusca for *P. bonelli* were only found in a few individuals but in relative high abundances in comparison with the other categories.

Levins index (*B*) for the total samples was: 1.74 for *PnU*, 1.55 for *PnD*, and 1.78 for *Pb*. Levins' standardized index (BA) was 0.03, 0.02 and 0.03, respectively. The average individual values of *B* and BA are reported in Table 3.

Diet overlap

The value of the Schoener's index α for the two species was 0.98. A high degree of diet overlap was also observed between age classes and the values of α index were in each case higher than 0.86 (Table 4). In order to avoid that the strong dominance of Chironomids could mask the result of the overlap, the calculations were also performed excluding this food item. However, even in this

Fig. 2 Relative abundances of prey items in **a** *PnU*, **b** *PnD* and **c** *Pb* stomach samples broken down by age classes. The 39 food items were pooled in 8 categories (Table 2): *Padogobius sp.,* Other Fish Species, Hydracarinae, Crustacea, Mollusca, Annelida, Other Insects, Diptera
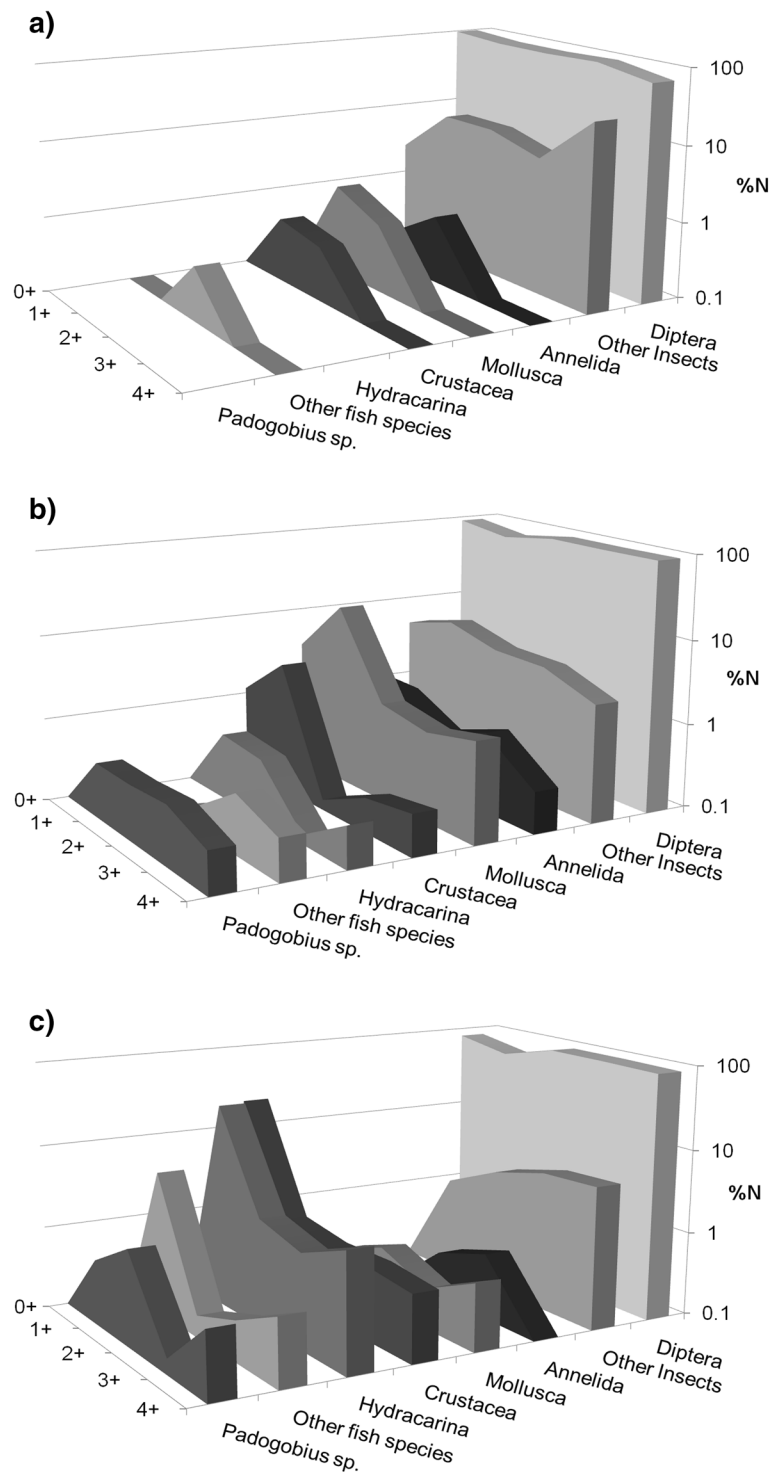
case the value of the index revealed a substantial overlap for both the total samples ($\alpha = 0.99$) and between age classes (min = 0.78–max = 1.00).

The high diet overlap between *Pb* and *PnD* is also clearly visible in the NMDS ordination plot of the Bray–Curtis distances computed on trophic data
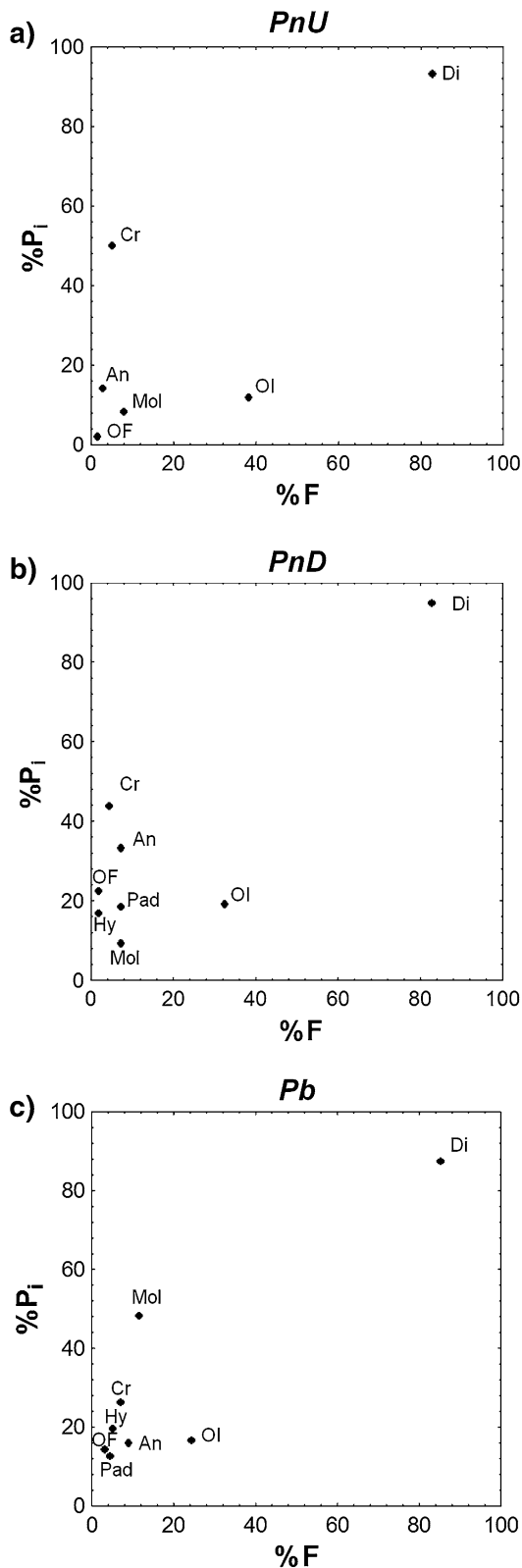
a) **PnU**

b) **PnD**

c) **Pb**

◄**Fig. 3** Modified Costello graphs (plots of prey-specific abundance %Pi and frequency of occurrence %F) showing feeding patterns of **a** *PnU*, **b** *PnD*, **c** *Pb*. (*Di* Dipterans larvae and pupae, *Cr* Crustacea, *Mol* Mollusca, *An* Annelida, *O.I.* other insects, *Hy* hydracarina, *OF* other fish species, *Pad Padogobius sp.*)

(Fig. 4a). The same analysis was conducted comparing *PnU* and *PnD* (Fig. 4b), where individuals of the two samples are rather overlapped.

Discussion

Results presented in this research indicated that *P. nigricans* and *P. bonelli* in the Aggia River share a similar diet. Both species extensively fed on Chironomid larvae and in the main, Dipterans represented the most important contribution to the total consumed food. In *P. bonelli* a significant portion of population fed on Bivalvia, while *P. nigricans* seemed to prefer other insects, especially Ephemeroptera. Slight dissimilarity in the diet of the two species occurred and dietary compositions of the two gobies in the study area didn't differ significantly neither for the total sample, nor for the samples divided into age classes. Within each species, subtle ontogenetic changes in feeding habits occurred, especially in *P. bonelli*, although no significant differences between age classes emerged. The dietary composition of both species varied through the year, mainly due to the change in proportion of Chironomids; however, no differences were highlighted in food composition between *P. bonelli* and *P. nigricans* within each season. Costello plots also suggested a comparable feeding strategy in the two species. This analysis revealed a population specialization toward a single food item (Dipterans) for both species and small proportions of other prey types were occasionally included in the diet of some individuals. A specialization of individual predators on other prey types is present: Crustaceans for *P. nigricans* and Mollusca for *P. bonelli* were found in a few individuals but in relatively high abundances. This pattern was also confirmed by the low values of niche breadth and standardized niche breadth for both species that suggested a narrow niche width, restricted to few dominant prey items.

The overall value of the diet overlap index between *P. nigricans* and *P. bonelli* inhabit the same river
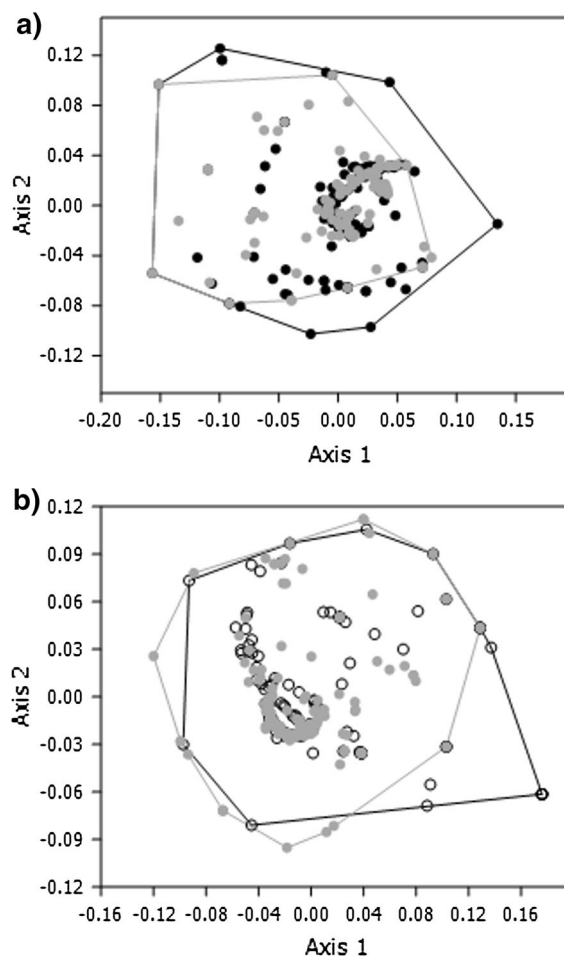
Table 3 Levins' indexes (B and BA) of *PnU*, *PnD*, and *Pb*: descriptive statistics of individual values and total diet width based on pooled data for all specimens

		N	Mean	Min	Max	SD	Pooled Levin's indexes
PnU	B	139	1.45	1.00	3.57	0.57	1.74
	BA		0.31	0.00	1.00	0.36	0.03
PnD	B	111	1.41	1.00	3.60	0.59	1.55
	BA		0.26	0.00	1.00	0.33	0.02
Pb	B	156	1.49	1.00	5.44	0.70	1.78
	BA		0.31	0.00	1.00	0.36	0.03

Table 4 Diet overlap (Schoener's index α) between the different age classes and total samples of *P. bonelli* (*Pb*) and *P. nigricans* downstream of the weir (*PnD*)

		Pb					
	α	0+	1+	2+	3+	4+	Total
PnD	0+	0.98	0.93	1.00	1.00	0.99	0.97
	1+	1.00	0.95	0.98	0.98	0.99	0.99
	2+	0.99	0.92	0.99	0.99	0.98	0.96
	3+	0.97	0.90	0.97	0.97	0.96	0.94
	4+	0.97	0.90	0.98	0.98	0.97	0.86
	Total	0.99	0.94	0.99	0.99	1.00	0.98

Fig. 4 Non-metric multidimensional scaling (NMSD) ordination plots of the Bray-Curtis distances computed on trophic data of between-groups diet variation: **a** *Pb–PnD* and **b** *PnU–PnD*. *Black marks*, *Pb*; *gray marks*, *PnD*; *white marks*, *PnU*

stretch was very high ($\alpha = 0.98$): values >0.60 should be considered biologically significant and indicative of interspecific competition if the resources are limited and values >0.80 indicate a high level of diet similarity (Zaret & Rand, 1971; Lorenzoni et al., 2007b). The value of diet overlap index found in this research was very close to one, indicating an almost total diet overlap, as also visually shown by NMDS ordination plot of the Bray–Curtis distances computed on trophic data. A significant diet overlap not necessarily entails competition if available resources are abundant (Lorenzoni et al., 2007b). But in environments with low prey densities, the chance of a strong negative interaction between the two species cannot be ruled out and it could become a considerable threat for the survival of *P. nigricans*. In general, habitat and diet are the most important niche dimensions separating coexisting fish species (Schoener, 1974); when species that have not co-evolved start to interact, the risk of competition increases, and non-native fish species may compete directly with native fauna for resources

(Moyle & Light, 1996; Karlson et al., 2007). Of the European fin fishes, Gobiidae are often associated with recent invasion (Copp et al., 2005) and many gobid species have caused biological alteration showing invasive behavior in the environment in which they were introduced, due to higher fecundity, wide tolerance of abiotic factors and broad diet (Corkum et al., 2004; Kováč et al., 2009). Dramatic ecological effects on native fish through both direct predation by non-indigenous gobies and competition for resources has been observed (Dubs & Corkum, 1996; French & Jude, 2001; Janssen & Jude, 2001; Corkum et al., 2004; Lauer et al., 2004; Karlson et al., 2007; Sokołowska & Fey, 2011). *P. bonelli* is reported as the main threat to survival of *P. nigricans* populations,

but it seems that, despite expectations, it has not disadvantaged directly the growth and the whole physiological condition of the native goby in the Aggia River (unpublished data). Further study on the composition and abundance of benthic fauna and food selectivity of the two species would help to understand the interactions between *P. nigricans* and *P. bonelli,* and knowledge on the utilization of food resources by *P. bonelli* would be also useful to predict the impact of its introduction. Currently, in the Aggia River a territorial competitor such as *P. bonelli* didn't cause a significant change in the diet of native species: despite in *PnD*, values of niche breadth and standardized niche breadth appeared lower than *PnU,* indicating a more restricted trophic spectrum, no analysis has revealed appreciable differences between the diets of *P. nigricans* with and without the co-occurring alien species. Moreover, the percentages of empty stomachs found in the different seasons proved that *P. nigricans* seems to be subjected to a feeding drop in autumn and winter. Since this behavior also occurred in the sample above the weir where *P. bonelli* is not present, is likely to be a characteristic of the native species, rather than a behavior induced by the non-native species.

An interesting result was that, for the first time, fishes are reported in the diet of the two species and remains referable to the same genus *Padogobius* were found only in individuals of the two species that inhabit the same river stretch. Because of the state of digestion, it was not always possible to identify to which species the remains belonged: this aspect will surely be deepened with further analysis. *P. bonelli* and *P. nigricans* are reported to be aggressive against each other under laboratory condition (Mecatti et al., 2010). Especially *P. bonelli* in the reproductive period can cause a high mortality in *P. nigricans* as a result of bites suffered (Mecatti et al., 2010); however, direct predation has never been reported. In the present study *Padogobius* remains are not represented by scales only, which would suggest aggressiveness toward other fish rather than predation (Stevove & Kovac, 2013), but also by almost entire individuals. In addition, aggression usually occurs against mature individuals mostly during the competition for territory and the attacks become more frequent throughout the breeding season (Mecatti et al., 2010). In this study, individuals of *Padogobius* found in the stomach were all juveniles (0+) and were preyed upon almost all year round. Moreover, further results derived from the

study on other biological characteristic of both species in the Aggia River, proved the lack of recruitment in the sample of *P. nigricans* below the weir (unpublished data). It is possible that the reproduction of *P. nigricans* has been in many cases precluded, and that only a few specimens were able to conquer breeding territory. However, this alteration in the age structure may be also worsened by predation on smaller specimens that can occur when the two species live in syntopy. In conclusion, the results of the present study suggest the existence of other possible interactions between the two species never investigated so far such as the mutual antagonism, which can result in a mutual predation.

References

Amundsen, P. A., H. M. Gabler & F. J. Staldvik, 1996. A new approach to graphical analysis of feeding strategy from stomach content data—modification of the Costello (1990) method. Journal of Fish Biology 48: 607–614.

Bagenal, T. B., 1978. Fish production in freshwater. Blackwell, London.

Bianco, P.G., 1987. L'inquadramento zoogeografico dei pesci d'acqua dolce d'Italia e problemi determinati dalle falsificazioni faunistiche. Biologia e gestione dell'ittiofauna autoctona. Atti II Convegno dell'Associazione Italiana Ittiologi delle Acque Dolci, Torino: 41–66.

Bianco, P. G. & V. Ketmaier, 2001. Anthropogenic changes in the freshwater fish fauna of Italy, with reference to the central region and *Barbus graellsii*, a newly established alien species of Iberian origin. Journal of Fish Biology 59: 190–208.

Carosi, A., M. Mearelli, G. Giovinazzo, M.L. Petesse & M. Lorenzoni, 1996. Distribuzione ed ecologia del ghiozzo di ruscello (*Padogobius nigricans* Canestrini) nella rete idrografica del bacino del F.Tevere. Atti VI° Convegno AIIAD, Varese Ligure, 6–8 giugno, 1996.

Clarke, K. & R. Warwick, 1994. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation. Natural Environment Research Council, Plymouth Marine Laboratory, Plymouth.

Corkum, L. D., M. R. Sapota & K. E. Skora, 2004. The round goby, *Neogobius melanostomus*, a fish invader on both sides of the Atlantic Ocean. Biological Invasions 6: 173–181.

Copp, G. H., P. G. Bianco, N. Bogutskaya, T. Erős, I. Falka, M. T. Ferreira, M. G. Fox, J. Freyhof, R. E. Gozlan, J. Grabowska, V. Kováč, R. Moreno-Amich, A. M. Naseka, M. Peňáz, M. Povž, M. Przybylski, M. Robillard, I. C. Russell, S. Stakėnas, S. Šumer, A. Vila-Gispert & C. Wiesner, 2005.

To be, or not to be, a non-native freshwater fish? Journal of Applied Ichthyology 21: 242–262.

Costello, M. J., 1990. Predator feeding strategy and prey importance: a new graphical analysis. Journal of Fish Biology 36: 261–263.

Crivelli, A.J., 2006. *Padogobius nigricans*. In: IUCN 2014. IUCN Red List of Threatened Species. Version 2014.1

Dubs, D. O. L. & L. D. Corkum, 1996. Behavioral interactions between round gobies (*Neogobius melanostomus*) and mottled sculpins (*Cottus bairdi*). Journal of Great Lakes Research 22: 838–844.

French, J. R. P. & D. J. Jude, 2001. Diets and diet overlap of non-indigenous gobies and small benthic native fishes co-inhabiting the St. Clair River, Michigan. Journal of Great Lakes Research 27: 300–311.

Gandolfi, G., S. Zerunian, P. Torricelli & A. Marconato, 1991. I pesci delle acque interne italiane. Istituto Poligrafico e Zecca dello Stato, Roma.

Hammer, Ø., D. A. T. Harper & P. D. Ryan, 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4, 9. Available on internet at http://folk.uio.no/ohammer/past/download.html.

Hickeley, P., R. North, S. M. Muchiri & D. M. Harper, 1994. The diet of largemouth bass, *Micropterus salmoides*, in Lake Navaisha, Kenia. Journal of Fish Biology 44: 607–619.

Hyslop, E. J., 1980. Stomach contents analysis—a review of methods and their application. Journal of Fish Biology 7: 411–429.

Janssen, J. & D. Jude, 2001. Recruitment failure of mottled sculpin *Cottus bairdi* in Calumet Harbor, southern Lake Michigan, induced by the newly introduced round goby *Neogobius melanostomus*. Journal of Great Lakes Research 27: 319–328.

Karlson, A. M. L., G. Almqvist, K. E. Skóra & M. Appelberg, 2007. Indications of competition between non-indigenous round goby and native flounder in the Baltic Sea. ICES Journal of Marine Science 64: 479–486.

Kováč, V., G. H. Copp & R. P. Sousa, 2009. Life-history traits of invasive bighead goby *Neogobius kessleri* from the middle Danube with a reflection of who may win the goby competition. Journal of Applied Ichthyology 25: 33–37.

Lauer, T. E., P. J. Allen & T. S. McComish, 2004. Changes in mottled sculpin and johnny darter trawl catches after the appearance of round gobies in the Indiana waters of Lake Michigan. Transactions of the American Fisheries Society 133: 185–189.

Lek, E., D. V. Fairclough, M. E. Platell, K. R. Clarke, J. R. Tweedley & I. C. Potter, 2011. To what extent are the dietary compositions of three abundant, co-occurring labrid species different and related to latitude, habitat, body size. Journal of Fish Biology 78(7): 1913–1943.

Levins, R., 1968. Evolution in Changing Environments: Some Theoretical Exploitations. Princeton University Press, Princeton.

Lorenzoni, M., M. Corboli, A. J. M. Dörr, G. Giovinazzo, S. Selvi & M. Mearelli, 2002. Diets of *Micropterus salmoides* Lac. and *Esox lucius* L. in Lake Trasimeno (Umbria, Italy) and their diet overlap. Bulletin Française de Pêche et de la Piscicolture 365/366: 537–547.

Lorenzoni, M., L. Ghetti & M. Mearelli, 2006. Native and exotic fish species in the Tiber River watershed (Umbria—Italy) and their relationship to the longitudinal gradient. Bulletin Francais de la Peche et de la Pisciculture 382: 19–44.

Lorenzoni, M., G. La Porta, G. Pedicillo, M.P. Spigonardi, A. Carosi, P. Vitali, G. Baldini, L. Ghetti, A. Zeetti, M. Natali, A. Biscaro Parrini, R. Dolciami, A. Mezzetti, A. Burchia, M. Di Brizio, T. Pancioni & C. Uzzoli, 2007a. Carta Ittica della Regione Umbria: Bacino del Fiume Tevere. Regione dell'Umbria, Perugia: 305

Lorenzoni, M., A. Carosi, G. Pedicillo & A. Trusso, 2007b. A comparative study on the feeding competition of the European perch *Perca fluviatilis* L. and the ruffe *Gymnocephalus cernuus* (L.) in Lake Piediluco (Umbria, Italy). Bulletin Francais de la Peche et de La Pisciculture 38: 35–57.

Lorenzoni, M., A. Carosi, L. Ghetti & R. Dolciami, 2010. La fauna ittica e i corsi d'acqua dell'Umbria. Sintesi delle carte Ittiche regionali dal 1986 al 2009. Regione Umbria, Perugia

Mearelli, M., M. Lorenzoni, A. Carosi, G. Giovinazzo & M. L. Petesse, 1996. Carta Ittica della Regione Umbria. Regione Umbria, Perugia.

Mecatti, M., M. Gualtieri & K. Gattai, 2010. Transfaunazioni invasive nel distretto ittiofaunistico tosco-laziale: prove di competizione territoriale e alimentare tra *Padogobius nigricans* e *Padogobius bonelli*. Studi Trentini di Scienze Naturali 87: 133–136.

Miller, P. J., 1975. Age-structure and life-span in the Common goby, *Pomatoschistus microps*. Journal of Zoology 177: 425–448.

Moyle, P. B. & T. Light, 1996. Biological invasions of fresh water: empirical rules and assembly theory. Biological Conservation 78: 149–161.

Nocita, A. & S. Zerunian, 2007. L'ittiofauna aliena nei fiumi e nei laghi d'Italia. Biologia Ambientale 21: 93–96.

Pedersen, J., 1999. Diet comparison between pelagic and demersal whiting in the North Sea. Journal of Fish Biology 55: 1096–1113.

Rondinini, C., A. Battistoni, V. Peronace, C. Teofili, 2013. Lista Rossa IUCN dei Vertebrati Italiani. Comitato Italiano IUCN e Ministero dell'Ambiente e della Tutela del Territorio e del Mare, Roma.

Russo, T., D. Pulcini, Á. O'Leary, S. Cataudella & S. Mariani, 2008. Relationship between body shape and trophic niche segregation in two closely related symparic fishes. Journal of Fish Biology 73: 809–828.

Scalici, M. & G. Gibertini, 2009. Freshwater goby life history in a Mediterranean stream. Hydrobiologia 628: 177–189.

Schiffman, S. S., M. L. Reynolds & F. W. Young, 1981. Introduction to Multidimensional Scaling. Academic Press, New York.

Schoener, T. W., 1970. Non-synchronous spatial overlap of lizards in patchy environments. Ecology 51: 408–418.

Schoener, T. W., 1974. Some methods for calculating competition coefficients from resource-utilization spectra. American Naturalist 108: 332–340.

Sokołowska, E. & D. P. Fey, 2011. Age and growth of the round goby *Neogobius melanostomus* in the Gulf of Gdánsk several years after invasion. Is the Baltic Sea a new Promised Land? Journal of Fish Biology 78: 1993–2009.

Stevove, B. & V. Kovac, 2013. Do invasive bighead goby *Neogobius kessleri* and round goby *N. melanostomus* (Teleostei, Gobiidae) compete for food? Knowledge and Management of Aquatic Ecosystems 401, 08.

Svanback, R. & L. Persson, 2004. Individual diet specialization, niche width and population dynamics: implications for trophic polymorphisms. Journal of Animal Ecology 73: 973–982.

Toscano, B. J., D. Pulcini, B. Hayden, T. Russo, M. Kelly-Quinn & S. Mariani, 2011. An ecomorphological framework for the coexistence of two cyprinid fish and their hybrids in a novel environment. Biological Journal of the Linnean Society 99: 768–783.

Wallace, R. K., 1981. An assessment of diet-overlap indexes. Transactions of the American Fisheries Society 110: 72–76.

Zaret, T. M. & A. S. Rand, 1971. Competition in tropical stream fishes: a support for the competitive exclusion principle. Ecology 52: 336–342.

Zerunian, S., 2002. Condannati all'estinzione? Biodiversità, biologia, minacce e strategie di conservazione dei Pesci d'acqua dolce indigeni in Italia. Edagricole, Bologna.

Zerunian, S., 2004. Pesci delle acque interne d'Italia. Ministero dell'Ambiente e Istituto Nazionale Fauna Selvatica, Quaderni Conservazione Natura no. 20.