

Research Article

The non-native goby *Padogobius bonelli* in the River Tiber, Italy and its effect on the reproductive potential of the native goby, *P. nigricans*

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Abstract

The goby *Padogobius bonelli* is naturally endemic to northern Italy, but was introduced to several rivers in central Italy outside of its native range in the early 1980's. In these watercourses, the rapid expansion of *P. bonelli* coincided with the decline of the endemic (for central Italy) *P. nigricans*. The two species share the same ecological niche and in laboratory studies it was observed that *P. bonelli* successfully and consistently outcompeted *P. nigricans* for breeding sites, preventing its reproduction. However, no studies about the effects of the introduction of *P. bonelli* on the biology of *P. nigricans* have been conducted in the field. The aim of this study was to investigate some life history traits of *P. nigricans*, including size at maturity, gonado-somatic index, fecundity and egg size and compare these datasets in terms of presence and absence of *P. bonelli*. Specimens were captured in the River Aggia, a tributary of the River Tiber. The introduced goby is present only in the downstream portion of the River Aggia due to the presence of a weir; thus, below the weir *P. nigricans* and *P. bonelli* live sympatrically, while upstream of the weir only the native goby is found. A total of 780 specimens of *P. nigricans* were collected, 547 upstream and 233 downstream of the weir. Females downstream of the weir produced a significantly higher number of eggs than females upstream, in both abundance and in relation to size. Several juveniles of *P. nigricans* (0+) were recorded upstream, yet downstream no juveniles were found in the months after the breeding period, with only a few small specimens captured during the winter season. These results highlight the difficulties that *P. nigricans* encounters in reproduction, possibly as a result of the competition with *P. bonelli* which poses a serious concern for the survival of the *P. nigricans* populations.

Key words: Italian gobies, reproductive traits, non-native species, GSI, fecundity

Introduction

The Arno goby *Padogobius nigricans* (Canestrini, 1867) is a freshwater species endemic to the Tuscany-Latium district, Italy (Bianco 1987). It is limited to just six river basins and two lakes covering a range of < 2,000 km² in the Tyrrhenian catchment in central Italy. Rather common until a few years ago, the populations are now declining due to anthropogenic disturbances, such as water extraction, water temperature increase, pollution, predation and the presence of alien species (Zerunian 2004). For this reason *P. nigricans* was assessed as “vulnerable” according to the IUCN Red List of Threatened Species (Crivelli 2006) and is a named species on the ‘Red List of Italian Vertebrates’ (Rondinini et al. 2013). It is also listed in Annex II of the European Union Habitat Directive and Appendix III of the Bern Convention.

The congeneric Padanian goby *Padogobius bonelli* (Bonaparte, 1843) is counted as one of the main threats to the survival of *P. nigricans* (Zerunian 2002; Crivelli 2006). *P. bonelli* is endemic to the Padano-Venetian district, comprising southern Switzerland, southern Slovenia, the Zrmanja River in Croatia and northern Italy (Freyhof 2006). *P. bonelli* was accidentally introduced to central Italy in the early 1980's along with other fish species (Bianco and Ketmaier 2001; Zerunian 2004). In the River Tiber basin, *P. bonelli* was recorded for the first time in 1996 in the Rivers Aggia and Cerfone (Carosi et al. 1996), small tributaries of River Tiber. From there, the species has successfully colonized the upper and middle course of the River Tiber and is now spreading into adjoining tributaries (Lorenzoni et al. 2007). There is concern over the competition between *P. nigricans* and *P. bonelli* for premium reproductive territory (Gandolfi et al. 1991; Zerunian 2004; Mecatti et al. 2010).

As shown in laboratory conditions, during the reproductive period in both species, the males fight for the occupation of best breeding sites (Torricelli et al. 1988; Zerunian 1988). However, *P. bonelli* is more aggressive than *P. nigricans* (Zerunian et al. 1988; Mecatti et al. 2010). Presumably, when both species cohabit the same river, inter-specific competition for breeding sites may occur, with *P. bonelli* ultimately succeeding, thus eliminating the opportunity of *P. nigricans* to reproduce (Zerunian 2004; Mecatti et al. 2010). Furthermore, as both species are benthic and small-bodied, they share a similar trophic niche, therefore food competition can also occur where resources are limited (Pompei et al. 2014a). In some rivers of central Italy the introduction of *P. bonelli* has probably caused a decrease in *P. nigricans* populations (Zerunian and Taddei 1996; Zerunian 2004; Mecatti et al. 2010). Introductions of alien gobies throughout Europe have caused biological alterations to native communities (Copp et al. 2005). Due to higher fecundity, extended spawning periods (Grabowska et al. 2008) wide tolerance of abiotic factors and broad diets (Corkum et al. 2004; Kováč et al. 2009) alien gobies show invasive behaviour in the environment in which they are introduced. Damaging effects on native fish through both direct predation by non-indigenous gobies and competition for resources have been observed (Dubs and Corkum 1996; French and Jude 2001; Laurer et al. 2004; Karlson et al. 2007). Moreover, studies on alien gobies species indicate that nesting interference is a common aspect of behaviour (Janssen and Jude 2001; Corkum et al. 2004) that can negatively affect the recruitment of native fish species (Janssen and Jude 2001). Although reproductive interactions between the two Italian gobies have been described in laboratory observations (Zerunian et al. 1988; Mecatti et al. 2010), the possible effects of the introduction of *P. bonelli* on the reproduction of *P. nigricans* have never been studied in the natural environment. Thus, the main aims of this study were to: 1) analyse and evaluate some reproductive traits (size at maturity, reproductive period, gonado-somatic index and fecundity) of a *P. nigricans* population from the River Tiber basin and 2) compare the reproductive traits of *P. nigricans* in the presence and absence of the non-native *P. bonelli*, in order to test the hypothesis that the occurrence of the non-native species would have a negative effect on reproductive parameters of the native species.

Methods

Specimens of *P. nigricans* were collected from the River Aggia (43°24'08.57"N, 12°12'40.34"E), a tributary of the River Tiber. This sampling area supports two of the largest populations of both *P. nigricans* and *P. bonelli* (Lorenzoni et al. 2007). A weir 2.5 m high, located in the middle section of the river plays an important role in influencing the fish assemblage of the stream, since it prevents the exotic species introduced into the River Tiber from migrating upstream (Lorenzoni et al. 2007). Thus, above the weir only native species were found, which include *P. nigricans*, the Tiber barbel *Barbus tyberinus* (Bonaparte, 1839), cavedano chub *Squalius squalus* (Bonaparte, 1837), brook chub *Squalius lucumonis* (Bianco, 1983), South European roach *Rutilus rubilio* (Bonaparte, 1837) and brown trout *Salmo trutta* (Linnaeus, 1758). Below the weir, in addition to the native species already mentioned, the non-native *P. bonelli* is present with a well established, stable and abundant population (Pompei et al. 2015). A few specimens of two other non-native Cyprinidae, the South European nase *Protochondrostoma genei* (Bonaparte, 1839) and gudgeon *Gobio gobio* (Linnaeus, 1758) were occasionally found in the downstream sector.

In order to investigate the reproductive biology of *P. nigricans* with respect to absence and presence of *P. bonelli*, two sampling sites were chosen: upstream (43°24'28.28"N, 12°12'12.67"E) and downstream of the weir (43°24'15.88"N, 12°12'33.54"E). Accordingly, the two samples were: *PnU*, *P. nigricans* upstream of the weir and *PnD*, *P. nigricans* downstream of the weir.

Sampling stations were located 700 m apart with similar river characteristics; continuous dense vegetative cover on riverbanks and substrate predominantly compiled of large stones and rocks, interspersed with areas of fine sand. Both areas were characterized by very low water velocity rates, never exceeding 0.01 m³/s. Physical/chemical parameters were measured monthly in each sampling site. Water temperature, oxygen concentration, pH and conductivity were recorded directly in the field using electronic meters. Laboratory analyses involved the use of a photo-meter (photoLab S 12) to obtain concentrations of Chemical Oxygen Demand (C.O.D.), ammonium NH₄, chloride Cl, nitrate NO₃, nitrite NO₂, phosphate PO₄ and sulfate SO₄ (Table 1). For each physical/chemical parameter, the differences between the monthly values measured upstream and downstream were tested using a Mann-Whitney U test.

Table 1. Mean and standard deviation (SD) of chemical and physical parameters (monthly) in the River Aggia, upstream and downstream of the weir; results of Mann-Whitney U tests (N=observations, Z=normal probability density function; *p*=level of significance).

Parameter	N	Upstream		Downstream		Mann-Whitney U	
		Mean	SD	Mean	SD	Z	<i>p</i>
Water temperature (°C)	12	14.29	5.83	12.50	6.27	0.990	0.322
pH	12	7.46	0.66	7.33	0.69	1.126	0.260
Conductivity (µS/s)	12	547.62	89.57	557.50	90.68	0.751	0.453
Dissolved Oxygen (%)	12	80.38	22.73	78.68	24.24	0.102	0.918
Dissolved Oxygen (mg/l)	12	8.00	2.93	8.57	3.44	0.341	0.733
Ammonium (mg/l)	12	0.04	0.03	0.02	0.01	1.680	0.093
Nitrite (mg/l)	12	0.02	0.01	0.02	0.01	1.260	0.208
Nitrate (mg/l)	12	4.13	2.64	4.64	2.48	0.630	0.529
Phosphate (mg/l)	12	0.04	0.01	0.03	0.02	1.365	0.172
Sulphate (mg/l)	12	36.00	11.96	34.88	8.74	0.683	0.495
Chloride (mg/l)	12	13.38	3.93	14.63	1.30	0.473	0.637
C.O.D. (mg/l)	12	13.00	1.57	14.04	1.16	1.523	0.128

Fish samplings were conducted monthly from January 2012 to December 2012. Fishes were caught using electrofishing equipment (electro-shocker model: ELT62II-GI, direct current, 300 V, 10–100 Hz), over a standard area of 4 m wide × 60 m long, with a depth range of 0.2–0.5 m. Specimens were immediately anaesthetised with 2-phenoxyethanol and preserved in 4% formaldehyde for laboratory analysis. Total length (TL) and weight (W) were measured to the nearest 0.1 cm and 0.1 g, respectively. A sample of scales was removed from dorsolateral or ventro-lateral rows of the caudal peduncle of each individual (Miller 1975) and stored in 33% ethanol. Age was then determined from scale readings (Bagenal and Tesch 1985) using a stereo microscope; two independent age determinations were made by two different operators. When the results were different, an additional determination was made.

Fish were dissected to determine the sex by macroscopic observation of the gonads; the gonads were removed and weighed to the nearest 10⁻³ g (Wg). The reproductive state of all specimens was assessed according to the Nikolsky scale (Bagenal and Tesch 1985). Specimens with gonads at Stage I were considered juveniles; at Stage II ovaries and testes were transparent; at Stage III gonads had started to develop; at Stage IV gonads were ripe, while at Stage V, oocytes or milt could be released from genital pore with light abdominal pressure (Bagenal and Tesch 1985).

Normality of data was first assessed using one-sample Kolmogorov–Smirnov test. Comparison between the proportion of juvenile *PnU* and *PnD* was carried out using the chi-square test (χ^2). For males, females and juveniles samples, size (cms)

differences between *PnU* and *PnD* were tested using the Mann-Whitney U test. Sex ratio was investigated separately in the two samples and deviation tested from the 1:1 null hypothesis through chi-square (χ^2) analysis. Mean size at maturity was calculated for both sexes and separated for both samples (*PnU* and *PnD*) in each size class (1 cm TL intervals; Trippel and Harvey 1987) using the formula:

$$\alpha = \sum_{x=0}^w (x)[f(x) - f(x - 1)]$$

(DeMaster 1978), where α is the mean size at maturity, x is size in cm, $f(x)$ is the proportion of fish mature at size x and w is the maximum size in the sample. Gonado-somatic index (GSI) was calculated for males and females as: $GSI = 100 (Wg/W)$ (Ricker 1975). The differences between *PnU* and *PnD* in mean GSI values were calculated for both sexes on mature specimens collected during the reproductive period and were analyzed by means of the Mann-Whitney U test. Spawning period was determined by identifying monthly changes in GSI for females.

For females showing gonad Stages IV and V, eggs were counted to obtain absolute fecundity (F). Relative fecundity (RF) was expressed as the number of eggs produced (F) per unit of body weight (W): $RF = (F/W)$. The diameters of 25 randomly chosen eggs for each mature female were measured by means of the image processing program software ImageJ. Mean values (δ in mm) of the 25 diameters measured for each female were used in the subsequent analyses. Differences between *PnU* and *PnD* in F, RF and δ were tested

Table 2. Descriptive statistics of total length (TL cm) for *PnU* and *PnD* juveniles, females and males and the results of Mann-Whitney U tests (Z =normal probability density function; p = level of significance). Number of specimens (N) and percentage (%) of total samples ($PnU=547$, $PnD=233$) are indicated.

Location	Sample	N	%	TL (cm)				Mann-Whitney U	
				Mean	SD	Min	Max	Z	p
<i>PnU</i>	Juveniles	57	10.4	2.9	0.52	1.5	4.0	1.785	0.074
<i>PnD</i>	Juveniles	8	3.4	3.1	0.09	3.0	3.2		
<i>PnU</i>	Males	297	54.3	5.3	1.26	3.0	9.2	1.309	0.191
<i>PnD</i>	Males	92	39.5	5.2	1.53	3.0	9.0		
<i>PnU</i>	Females	193	35.3	5.0	0.95	2.9	7.5	2.912	0.004
<i>PnD</i>	Females	133	57.1	4.7	1.00	3.0	7.7		

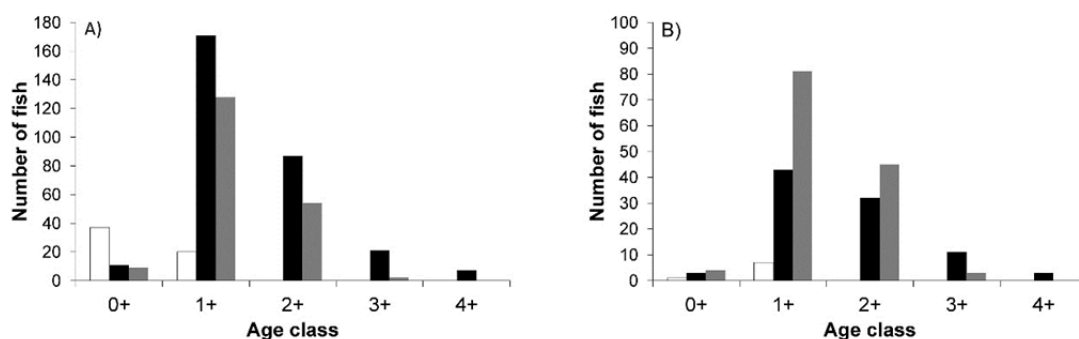


Figure 1. Age frequency distribution of specimens sampled from the Aggia River A) *PnU* and B) *PnD*; males (black), females (grey) and juveniles (white).

using ANCOVA, with TL as a covariate variable. In order to analyse whether F , RF and δ varied with the increase size (TL) of the females, the following regressions were calculated: $\log_{10}F = \alpha + \beta \log_{10}TL$, $RF = \alpha + \beta TL$ and $\delta = \alpha + \beta TL$. Equations were calculated separately for *PnU* and *PnD*.

Results

The analysis of physical/chemical parameters confirmed the homogeneity of environmental characteristics between the two stations since no significant differences were found (Table 1).

A total of 780 specimens of *P. nigricans* were collected during sampling. *PnU* was composed of 547 specimens ranging in size between 1.5 cm to 9.2 cm. The *PnD* sample ($N=233$) ranged in size from 3.0 cm to 9.0 cm. No differences in size were found when comparing males and juvenile specimens of the two samples (Table 2). *PnD* females were smaller than *PnU* females, with significant differences in mean TL (Mann-Whitney U test, Table 2).

Five age classes were found in both samples (0+ - 4+), with males having a longer lifespan

than females (Figure 1). The percentage of 0+ *PnU* individuals (10.4% of the total) was significantly higher than in *PnD* (3.4%) ($\chi^2=421.12$, $df=1$, $p<0.01$).

In *PnU* the sex ratio was heavily biased in favour of males (1.53:1; $\chi^2=22.073$, $df=1$, $p<0.01$) while in *PnD*, females were prevalent (0.69:1; $\chi^2=7.47$, $df=1$, $p<0.01$).

In the two samples most of specimens, both male and female, attained sexual maturity between 3 and 4 cm (Table 3). The mean size at maturity was 4.15 cm for males and 4.10 for females in *PnU*, and 3.83 for males and 3.81 cm for females in *PnD*. All specimens longer than 4.1 cm TL, were already mature (Table 3). However a huge difference between *PnU* and *PnD* in the proportion of mature females from the 2.1–3.0 cm size class (8.8% vs. 60%) was found (Table 3).

In *PnU* the mean GSI calculated in the breeding season ($\pm SE$) was 9.68 ± 1.05 in females and 0.92 ± 0.08 in males. In *PnD* the mean GSI value ($\pm SE$) was 11.70 ± 1.35 in females, while in males it was 1.35 ± 0.19 . The differences between *PnU* and *PnD* in mean GSI were highly significant both for males ($n_{PnU}=82$; $n_{PnD}=27$; $Z=3.66$; $p<0.01$) and females ($n_{PnU}=64$; $n_{PnD}=33$; $Z=3.06$, $p<0.01$).

Table 3. Proportion of mature males and females for each 1 cm size class in *PnU* and *PnD* samples.

Size class (cm)	<i>PnU</i>		<i>PnD</i>	
	% Mature Males	% Mature Females	% Mature Males	% Mature Females
< 2.0	all immature		no data	
2.1-3.0	3.1%	8.8%	16.7%	60.0%
3.1-4.0	76.5%	66.0%	100.0%	85.3%
> 4.1	all mature		all mature	

Table 4. Descriptive statistics (SE=standard error) of number of eggs (F), relative fecundity (RF n eggs/g) and diameter of eggs (δ in mm) in *PnU* and *PnD* samples. Analysis of Covariance (ANCOVA) with total length as covariate variable (N=number of specimens; *df*=degree of freedom, *F*=Fisher's F-distribution, *p*=level of significance).

	<i>PnU</i>					<i>PnD</i>					ANCOVA		
	N	Mean	SE	Min	Max	N	Mean	SE	Min	Max	<i>df</i>	<i>F</i>	<i>p</i>
F	49	128.69	8.13	41.00	257.00	28	160.96	9.53	69.00	355.00	1	18.898	<0.01
RF	49	56.28	2.78	25.56	105.83	28	96.41	8.23	27.78	218.00	1	18.640	<0.01
δ	49	1.23	0.06	0.45	2.07	28	1.05	0.07	0.32	1.87	1	0.256	0.27

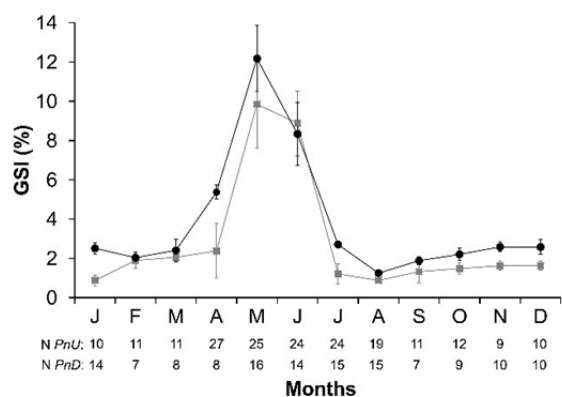


Figure 2. Monthly variations in mean gonado-somatic index (GSI) values of *PnU* (grey) and *PnD* (black) females of *P. nigricans* from the River Aggia. Sample size (N) for each month are provided. Vertical bars indicate SE.

In both *PnU* and *PnD*, from April onward GSI increased, reaching its highest mean values (\pm SE) in May (*PnU*: 9.847 ± 1.678 ; *PnD*: 12.175 ± 2.238) and in June (*PnU*: 8.880 ± 1.592 ; *PnD*: 8.335 ± 1.653). Then GSI decreased until it reached the minimum mean values for both *PnU* and *PnD* in August (*PnU*: 0.877 ± 0.053 ; *PnD*: 1.250 ± 0.148) (Figure 2).

PnD females produced more eggs, both overall ($F=160.96 \pm 9.53$) and in respect to body weight ($RF=96.41 \pm 8.23$), than *PnU* females ($F=128.69 \pm 8.13$; $RF=56.28 \pm 2.78$) (Table 4). Differences between *PnU* and *PnD* were highly significant for both F

and RF in the ANCOVA test (Covariate TL=5.36) (Table 4). *PnD* laid generally smaller eggs than *PnU*, but no significant differences were observed in the mean diameter of eggs between the *PnU* and *PnD* (Table 4).

In *PnU*, the number of eggs was positively correlated with length of females ($r^2=0.520$, $r=0.721$, $p<0.01$), meaning that fecundity increased with size, while in *PnD*, no correlation between fecundity and length of females was found ($r^2=0.003$; $r=0.002$; $p>0.05$) (Figure 3A). RF decreased as a function of size in both samples (*PnU*: $r^2=0.081$; $r=-0.285$; $p<0.05$; *PnD*: $r^2=0.625$; $r=0.791$; $p<0.01$) (Figure 3B). Regressions indicated a positive relationship between diameter of mature eggs and size of females in both samples (*PnU*: $r^2=0.071$; $r=0.298$; $p<0.05$; *PnD*: $r^2=0.294$; $r=0.542$; $p<0.01$) (Figure 3C).

Discussion

Considering the two separate populations of *P. nigricans* from the River Aggia, one upstream and the other downstream of the weir, some life-history traits were found to be similar. In both samples sexual maturity is attained at 3–4 cm corresponding to one year of age. Even the reproductive period is coincident in both populations occurring at the end of spring (May and June). Furthermore these features seem to be typical of the species, since they are consistent with data reported in literature for *P. nigricans* from different environments (Gandolfi and Tongiorgi 1974;

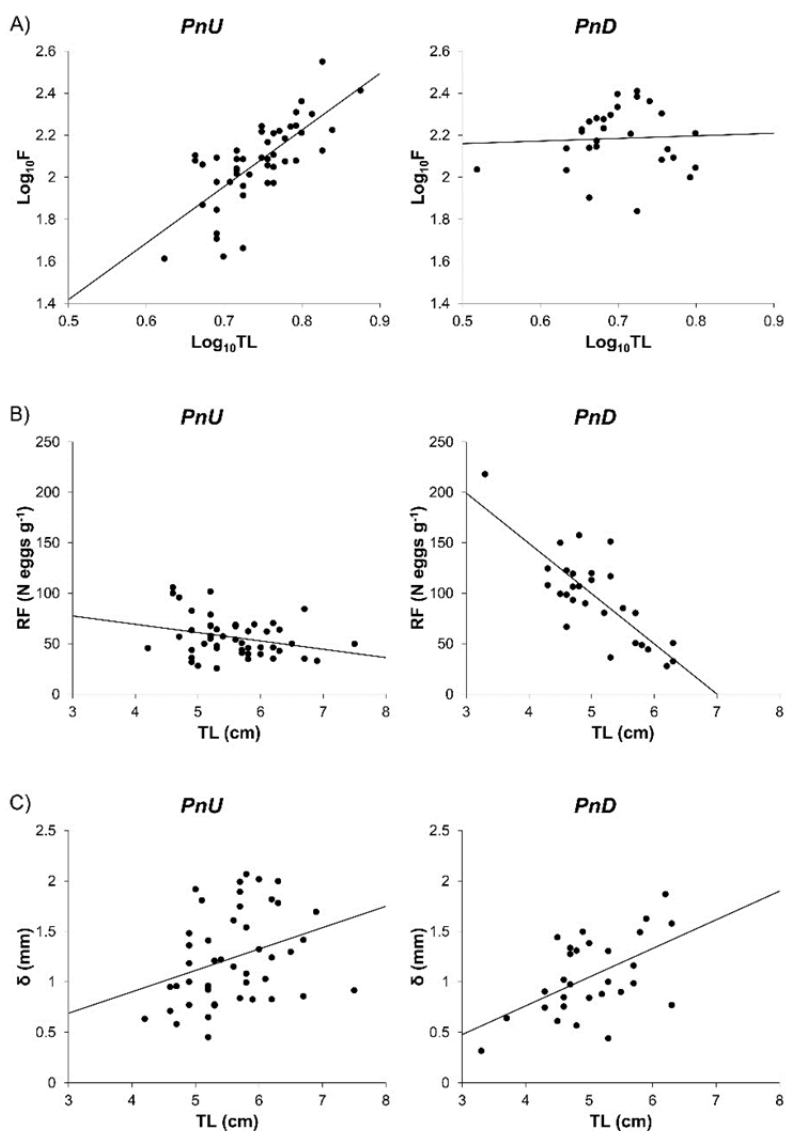


Figure 3. Correlation between total length (TL) and A) fecundity (F), B) relative fecundity (RF) and C) egg diameter (δ) in *PnU* and *PnD* females from the River Aggia.

Zerunian et al. 1988; Gandolfi et al. 1991; Scalici and Gibertini 2009). However intrapopulation variations with respect to some life history traits were found. Sex ratio was different in the two samples: females were dominant downstream, while upstream the sex-ratio clearly favoured males. The first case is the most common among gobioid fishes (Miller 1984) and several researchers who have studied the biology of gobies have noted that females outnumber males (Marconato et al. 1989; Pampoulie et al. 1999; Azevedo and Simas 2000; Malavasi et al. 2005; Gutowsky and Fox 2011; Gkenas and Leonardos 2012; Gruřa et al. 2012). The same pattern was found by Scalici

and Gibertini (2009) in a *P. nigricans* population from another river basin. This imbalance has been explained in the light of male territoriality for defending refuges: the number of males may undergo a sharp decrease since smaller males are forced to migrate, searching for alternate available territory (Scalici and Gibertini 2009). In our study, for *P. nigricans* downstream of the weir, in addition to intra-specific competition for breeding size, inter-specific competition with *P. bonelli* occurred. Thus the imbalance in favour of females may be emphasized also by the presence of the non-native goby with which a strong territorial competition for nest occupation has already been

shown (Mecatti et al. 2010). Interestingly, in the same river sector *P. bonelli* is characterized by a sex ratio shifted toward males (Pompei et al. unpublished data). Probably, density-dependent inter-specific fights among the two gobies can also result in a higher mortality of *P. nigricans* males for the bites suffered, as shown in laboratory conditions (Mecatti et al. 2010). More generally, male gobies typically display high energy investment in nest defence, which could hypothetically lead to exhaustion and death (Guo et al. 2013). Parental care is not only energetically costly but also increases the vulnerability of males to predators (Guo et al. 2013). While several explanations could support the female-shifted sex ratio downstream of the dam, it is difficult to interpret exactly the circumstances found in the river sector upstream. Usually, a sex ratio shifted toward males is observed in gobies that have invaded a new environment (Corkum et al. 2004; Tomczak and Sapota 2006; Kornis et al. 2012; Thompson and Simon 2015): a positive relationship between the percentage of survival of eggs and the preponderance of males over females has been observed (Kovtun 1980), since each male will have to guard nests with fewer eggs, resulting in a greater probability of survival of fry. Given the paucity of information on *P. nigricans*, currently, there are not enough data to affirm whether a preponderance of males is a norm for the species. Further investigations on the population dynamics of *P. nigricans* would provide valuable insight into the intrinsic characteristics of the native species, allowing clarification of the possible impact of *P. bonelli*.

Another notable difference between the two samples was observed in the proportion of mature females from the 2.1–3.0 cm size class, suggesting an earlier maturation in *PnD* compared with *PnU* females. Earlier maturation with respect to native populations has frequently been observed in freshly established invasive gobies (MacInnis and Corkum 2000; Lavrinčíková and Kováč, 2007) since it promotes population growth favouring the colonization process in a new environment (Gutowsky and Fox 2012). Moreover *P. nigricans* sampled below the dam invest more energy in reproduction than the sample upstream. Females produced a significantly higher number of eggs than females upstream, both overall and in relation to their size. Furthermore, the higher reproductive investment occurs as early as the first reproduction, since in *PnD* females fecundity is not influenced by size. The high reproductive potential doesn't compromise the quality of the eggs, since the size of the oocytes is similar into

the two samples. The greater allocation of energy in reproduction also occurs in males, as shown by the analysis of GSI. Mature males assign a higher percentage of body weight to the development of the testes where *P. bonelli* is present. Usually, individuals in a population exposed to some kind of disturbance tend to divert allocation of their resources towards reproduction to maximize the production of offspring (Fox et al. 2007). In this way the probability that the next generation will survive in the unfavorable conditions is enhanced.

A shift toward an earlier maturation and a higher reproductive investment could be a response to loosing breeding grounds caused by the non-native *P. bonelli*. Studies on variations in life-history traits are often associated with the spread of alien species, since changes in growth and reproductive properties are widely used as a predictor of potential invasiveness of a newly established species (Rosecchi et al. 2001; Vila-Gispert et al. 2005; Olden et al. 2006; Novomeská and Kováč 2009). However it seems that the population of native *P. nigricans* responded to the new stressful situation stimulated by the arrival of *P. bonelli* in the same way as invasive populations do at the beginning of invasion: earlier maturation, higher fecundity, and higher reproductive effort (Hôrková and Kováč 2015). The variations in the life-history of *P. nigricans* described in this study appear to be a demonstration of the species life-history plasticity (Záhorská et al. 2013). The ability to modify some biological traits in relation to environmental pressures could potentially enhance its chances of survival under the competition with *P. bonelli*. Nevertheless, despite the higher number of eggs, it seems that the reproduction of *P. nigricans* has been hampered overall where *P. bonelli* is present. In this regard, age frequency distribution revealed differences at the two sites, especially in the proportion of 0+ specimens. In the river sector above the weir, several 0+ fish were found (10.3%) in the months immediately following the reproductive period (August and September). In the same months, no juveniles of *P. nigricans* were found in the station below the weir and the first 0+ were caught downstream only in December, when they had already reached a length of 3.0–3.2 cm. Several biotic and abiotic factors may interfere with the reproduction of fish species (Stearns 1976; Billard et al. 1981). However in the river sector below the weir a consistent amount of 0+ *P. bonelli* specimens were captured after the reproductive period (Pompei et al. unpublished data). Therefore, since the two species are closely related and

share the same environmental tolerances, one can exclude the effect of environmental interferences in the river sector downstream on *P. nigricans* reproduction, and lean more towards the hypothesis of crucial competition with rival *P. bonelli* for spawning sites. It is therefore possible that the reproduction of *P. nigricans* was in many cases precluded, in that only a few successful specimens were able to secure a breeding territory. The paucity of juveniles may also have been compounded by the predation of *P. bonelli* on smaller specimens of *P. nigricans*, since a mutual predation between the two gobies can occur in a situation of sympatry (Pompei et al. 2014a).

In some rivers of central Italy, a progressive reduction of *P. nigricans* populations in conjunction with *P. bonelli* expansion has been noted (Zerunian and Taddei 1996; Nocita and Zerunian 2007; Mecatti et al. 2010). In the River Tiber, it has been observed that *P. nigricans* populations are shrinking and being replaced by *P. bonelli* (Pompei et al. 2014b). Furthermore the same scenario as that in River Aggia has been found in several other River Tiber tributaries, where *P. bonelli* has become more abundant with no *P. nigricans* juveniles recorded (Pompei et al. 2014b). Consequently the population of *P. nigricans* inhabiting the River Aggia below the weir, as well as other populations of River Tiber, are largely dominated by individuals that have migrated from upstream. River damming is a human activity that greatly affects freshwater environments (Baxter 1977; Dynesius and Nilsson 1994; Franchi et al. 2014). Weirs represent a barrier to the movement of fishes and penalize populations of all fish species, emphasizing the consequences of isolation and thus amplifying negative effects of all other factors of anthropogenic disturbance (Lorenzoni et al. 2006). On the other hand, small weirs could impede the spread of exotic species from the downstream reaches of rivers to upstream. In the River Tiber basin, most of the native fish species are limited to the mountain and piedmont section of the rivers (Giannetto et al. 2013), while in the downstream reaches fish communities are dominated by introduced species, which include *P. bonelli* (Lorenzoni et al. 2006). Therefore secondary small piedmont streams can play an essential role in the conservation of biodiversity, representing a reservoir of native species (Lorenzoni et al. 2006), and allowing recolonization from neighbouring sites, which prevents long-term inter-specific exclusion (Belkessam et al. 1997).

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