

Structure, growth and production of a remarkably abundant population of the common goby, *Pomatoschistus microps* (Actinopterygii: Gobiidae)

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Abstract The common goby, *Pomatoschistus microps*, is a relevant species from estuarine food webs, playing important roles as predator of polychaetes and crustaceans and as prey for larger fishes and crustaceans. The Minho estuary (NW Portugal) is a relatively well-preserved and productive system. To assess the population structure and production of *P. microps* in this estuary, monthly samples were undertaken in three different areas along an estuarine gradient in the lower estuary. The density of *P. microps* varied considerably among seasons and sampling stations, with higher densities occurring in summer and autumn. The lowest densities were found closer to the sea. In general, the density of females was higher than the density of males in all sampling stations, while juveniles were more

abundant within a salt marsh area. Compared with other European estuaries, our data showed a remarkable higher density and production values of *P. microps*. This may be related to the high freshwater input and the low salinities found in this estuary. In addition, we hypothesize that the lower density of the sympatric species *P. minutus* and the high availability of bivalve shells observed in the Minho estuary may have also contributed to the present results, once *P. minutus* and *P. microps* often display a diet overlap and the bivalve shells are crucial for the common goby reproduction.

Keywords *Corbicula fluminea* · Life cycle · Minho · *Pomatoschistus minutus* · Secondary production

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Introduction

Estuaries contain diverse aquatic habitat types, including seagrass, kelp and shellfish beds, muddy and sandy environments, among others (Nelson and Monaco 2000). These are highly biologically productive ecosystems that provide important forage, spawning, refuge, and nursery habitat for several fish species at various stages in their development (Chambers 1992; Nelson and Monaco 2000; Able 2005).

The Minho estuary (NW Iberian Peninsula) is a relatively well-preserved system where several aquatic species present higher values of density and/or secondary production compared with other systems (see Sousa et al. 2008a; Freitas et al. 2009; Dolbeth et al. 2010; Freitas 2011; Souza et al. 2013). In this estuary,

five species of Gobiidae are present, namely, the common goby, *Pomatoschistus microps*, the sand goby *P. minutus*, the painted goby, *P. pictus*, the transparent goby, *Aphia minuta* and the black goby, *Gobius niger*, with *P. microps* being largely more abundant than other Gobiidae species (Antunes pers. comm.; Antunes and Rodrigues 2004; Costa-Dias et al. 2010).

The common goby is a small-bodied demersal euryhaline species that usually occurs in high densities, from Norway to Mauritania, including the Canary Islands, western Mediterranean and Baltic Sea, spanning ca. 44° of latitudinal range (Froese and Pauly 2010). This species is of great importance in marine and estuarine ecosystems as it links low and high levels of the food-web, by acting as a mesopredator, thus connecting microbenthos to larger predators such as birds and fishes (Doornbos 1984; Moreira et al. 1992; Cabral 2000).

The ecology of *P. microps* has been the focus of several studies, which include population structure (Arruda et al. 1993; Pampoulie 2001; Leitão et al. 2006; Dolbeth et al. 2007), breeding behavior (Magnhagen 1998; Pampoulie et al. 2000; Pampoulie 2001) and diet (Mehner 1992; Leitão et al. 2006; Jackson and Rundle 2008). However, few studies have focused on *P. microps* populations from the Atlantic coast of Southern Europe (but see Arruda et al. 1993; Leitão et al. 2006; Dolbeth et al. 2007). Besides, in this area, nearly 55 % of published studies regarding the species focused on ecotoxicology (e.g., Monteiro et al. 2007; Vieira et al. 2008, 2009; Guimarães et al. 2012; Oliveira et al. 2012).

The common goby is often reported as one of the most abundant fish in estuaries (Martinho et al. 2007; Dolbeth et al. 2010), and it is frequently found in areas where the sympatric species *P. minutus* co-occurs. Both species are often found in equal or slightly different densities (e.g., Arruda et al. 1993; Leitão et al. 2006; Dolbeth et al. 2007; Martinho et al. 2007). However, in the Minho estuary, the common goby seems to be much more abundant than the sand goby (pers. obs.; Costa-Dias et al. 2010).

In this context, the present study aims at assessing the population structure, density, production and growth of *P. microps* in the Minho estuary. By focusing on the distributional patterns of *P. microps* within a system where the species seems to be especially abundant, we expect to increase the understanding of the ecology of this core species in several estuarine systems across Europe.

Materials and methods

Study area and sampling procedures

This study was conducted in the Minho estuary (NW of the Iberian Peninsula – 41°53'N 8°50'0), which ranges up to 40 km (considering the upstream limit of spring tides), covering a total area of 23 km². The Minho estuary is a very shallow system (Moreno et al. 2005), with a mean depth of 2.6 m (Freitas et al. 2009); it is characterized as a mesotidal and partially mixed system, although it tends towards a salt wedge estuary during periods of high river flow (Sousa et al. 2005).

This study was carried out during 18 months, from February/09 to July/10, with months being grouped into four seasons, namely winter (January, February and March), spring (April, May and June), summer (July, August and September) and autumn (October, November and December). Sampling was carried out in 3 nearby stations within the first 8 km of the Minho estuary, considering a gradient of distance to the river mouth (Fig. 1). The 3 stations (S1, S2 and S3) were chosen in order to assess possible dissimilarities on the population dynamics related to differences in habitat type and environmental parameters. S1 was closer to the river mouth (ca. 1.5 km), and characterized by the presence of soft bottoms, often densely covered by debris such as drifting seaweeds, dead leaves and empty mollusks' shells (Souza et al. 2011). S2 was located inside a salt marsh (ca. 3.5 km upwards from the river mouth), which is a relatively small system characterized by the presence of narrow channels, typically bordered by the seagrass, *Spartina maritima*. The channel's soft bottoms are often sparsely covered by debris and empty peppery furrow shells, *Scrobicularia plana* (Souza et al. 2011). S3 was located ca. 5 km upstream from S2, and characterized by high densities of the Asian clam, *Corbicula fluminea*, with the soft bottoms often sparsely covered by debris and submerged vegetation (Sousa et al. 2008b, c; Souza et al. 2013). In each site, with monthly periodicity, 3 replicates were collected using a 1 m beam trawl (5 mm mesh size) towed at constant speed (2 km.h⁻¹) for 2 to 3 min, during daylight at the high tide of spring tides. Previous studies in the Minho estuary indicated that the sampling area covered by a 3-min tow, at constant speed, was equivalent to 100 ±4 m² on average (Freitas et al. 2009). Density data was standardized to the same scale (ind. 100 m⁻²) prior to the

statistical analyses. Additionally, at each site, water temperature, salinity, pH and oxidation reduction potential (ORP) were measured at 20 cm above the bottom with a multiparameter probe YSI 6820 (two replicates).

The monthly river discharge data measured at Foz do Mouro hydrometric station between February/09 and July/10 was obtained from the INAG – Instituto da Água, I.P. (<http://snirh.inag.pt>).

Laboratory procedures

At each sampling date, the density of *P. microps* was determined by counting all sampled individuals. The size (total length - TL) of each fish was measured (0.01 mm precision) using a digital caliper, and subsequently assigned into one of the following seven size classes: A (< 15.00 mm), B (15.01 to 20.00 mm), C (20.01 to 25.00 mm), D (25.01 to

30 mm); E (30.01 to 35.00 mm), F (35.01 to 40.00 mm) and G (> 40.01 mm).

Fifty randomly selected individuals of each sample were observed under a magnifying glass for sex distinction based upon dimorphic features on their morphology and gonads (Whitehead et al. 1986). Individuals smaller than 26 mm of TL showed no clear morphological signs of their sex, and thus were considered sexually immature and classified as juveniles (see Bouchereau et al. 1989). Females with advanced stage of gonad development (i.e. presence of eggs) were assigned as ovigerous females. The total density of juveniles, males and females was estimated based on the percentage of contribution of each group within the fifty randomly selected fishes at each sample.

Additionally, 291 randomly selected fish from the three sampling stations were wet weighted in a precision scale to the nearest 0.001 g in order to determine the length-weight relationship. The

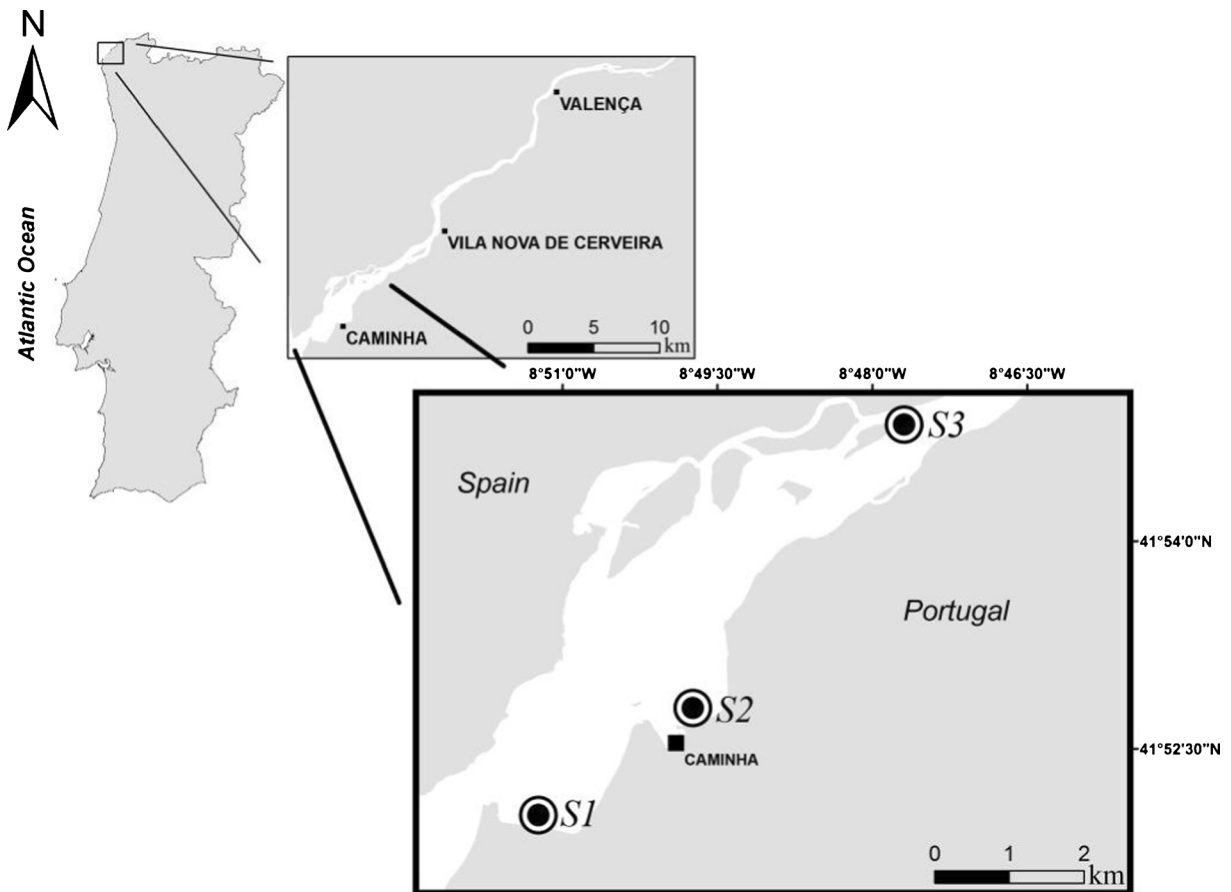


Fig. 1 Sampling stations on the lower Minho Estuary (NW Iberian Peninsula)

condition factor of *P. microps* was calculated using the allometric equation:

$$W = a.L^b \quad (1)$$

where W is the wet weight of the fish (in grams), L is the total length of the fish (in centimeters) and a and b refer to the allometric coefficients obtained through the length-weight relationship (Reiss 1989).

Data analysis

Prior to the analyses of abiotic data, all variables were normalized. Posteriorly, a resemblance matrix based on the Euclidean distance was calculated. Principal Components Analysis (PCA) was performed in order to detect habitat differences among sampling stations based on environmental data.

A two-way SIMPER routine was applied to determine the contribution of each abiotic variable for the dissimilarities among groups of stations and seasons.

Afterwards, a Permutational Multivariate Analysis of Variance (PERMANOVA) (type-III) was employed in a two-way crossed design, with station as a fixed factor (3 levels: S1, S2 and S3) and season as a fixed factor (4 levels: winter, spring, summer and autumn), in order to test the statistical significant differences among sampling stations and seasons using all abiotic variables (multivariate approach) and comparing each variable separately (univariate approach). The PERMANOVA analysis is capable of handling unbalanced statistical designs, such as in this study (due to unequal number of samples collected in each season), and calculates an identical F statistic that would be produced using traditional ANOVA and it is not affected by non-normal distribution of data (Anderson 2001).

A similar analysis procedure was adopted to investigate the common goby data. Prior to the analyses, all variables were normalized, and resemblance matrices based on Euclidean distance were calculated. The overall density, the density of each gender (juvenile, male and female), the density of ovigerous females, the density of *P. microps* size classes, the size of juveniles, males and females and the size of ovigerous females were statistically tested using a two-way PERMANOVA (type-III) in a two-way crossed design, with station as a fixed factor (3 levels: S1, S2 and S3) and season as a fixed factor (4 levels: winter, spring, summer and autumn),

using both multivariate and univariate approaches (comparing each variable separately).

In PERMANOVA, the statistical significance of variance components was tested using 9999 permutations of residuals under a reduced model. When the possible number of permutations was lower than 150, the Monte Carlo- p value was considered. Subsequently, significant terms and interactions were investigated using a posteriori pairwise comparisons to determine which pairs of stations and seasons were significantly different.

In order to assess the influence of the abiotic variables on the abundance and distribution of *P. microps*' males, females and juveniles, a distance-based linear modeling (DistLM) was performed (Anderson et al. 2008). The selection criterion adopted was the AIC (Akaike Information Criterion), and a step-wise selection procedure was used, which optimizes selection of variables explaining most variation in the biotic data. The direction and magnitude of the relationship between abiotic variables and males, females and juveniles were displayed using distance based redundancy analysis (dbRDA) biplots (Legendre and Anderson 1999).

The PCA, the SIMPER, the PERMANOVA, the DistLM and the dbRDA analyses were performed using PRIMER v 6.1.11[®] (Clarke and Gorley 2006) with PERMANOVA+1.0.1. add-on package (Anderson et al. 2008).

The population structure of *P. microps* was defined by tracking recognizable cohorts from the successive sampling dates. Spatial samples were pooled together and analyzed using the size frequency distribution of successive dates. The mixture analysis method was used to identify cohorts within size frequency distributions constructed from population samples in each month using the PAST[®] software. The mixture analysis is a maximum-likelihood method for estimating the parameters (mean, standard deviation and proportion), of univariate normal distributions, based on a pooled univariate sample. This software uses the EM algorithm described by Dempster et al. (1977) and considers a histogram of frequency as a mixture of probability density functions. The number of modes in each month was determined by visual analysis of the histogram.

Absolute growth rates (AGR, mm day⁻¹) were determined for each cohort according to:

$$AGR = \frac{L_{t2} - L_{t1}}{t2 - t1} \quad (2)$$

where L_{t2} and L_{t1} are the total length at time t_2 and t_1 respectively.

Finally, the secondary production was computed using the size-frequency method (Hynes method). This method calculates production by summing the biomass lost between size classes (Krueger and Martin 1980). In order to determine the production of *P. microps* in the lower Minho estuary, the Krueger and Martin (1980) equation was applied:

$$P = \sum_{j=1}^{a-1} 0.5 [(Y_{i,j} + Y_{i+1,j}) - (Y_{i,j+1} + Y_{i+1,j+1})] \cdot (W_j \cdot W_{j+1}) \cdot (t)^{-1} \tag{3}$$

where P is the annual production of the species, $Y_{i,j}$ is the mean density (ind.m⁻²) of the size class j in the date i , $Y_{i+1,j}$ is the mean density of the size class j in the date $i+1$, $Y_{i,j+1}$ is the mean density of the size class $j+1$ in the date i , $Y_{i+1,j+1}$ is the mean density of the size class

$j+1$ in the date $i+1$, W_j is the biomass (g.m⁻²) of the size class j , W_{j+1} is the biomass of the size class $j+1$ and t is the time between i and $i+1$. Population production estimates correspond to the sum of each size class production (P). Negative production values were not included in the overall estimates and were regarded as zero production.

Annual production was calculated by a cross-multiplication, based on the values obtained for the overall production (18 months).

Results

Abiotic data

The two-way PERMANOVA for stations and seasons of all abiotic variables was significant for both main

Table 1 Values of the five environmental variables (mean ± SE) measured in the three sampling stations throughout the seasons in the lower Minho estuary, NW Iberian Peninsula and the two-way

PERMANOVA results on the effects of sampling stations and seasons and their interaction term

	Seasons				Two-way PERMANOVA		
	Winter	Spring	Summer	Autumn	Stations	Seasons	Interaction
Depth							
S1	4.2±0.3 ^{ac(A)}	3.1±0.3 ^{b(A)}	3.7±0.2 ^{ab(A)}	4.3±0.1 ^{c(A)}	Pseudo- <i>F</i> =57.78**	Pseudo- <i>F</i> =6.87**	Pseudo- <i>F</i> =0.97 ^{ns}
S2	1.9±0.2 ^{a(B)}	1.3±0.2 ^{a(B)}	1.8±0.1 ^{a(B)}	1.8±0.1 ^{a(B)}			
S3	2.7±0.4 ^{a(B)}	1.9±0.3 ^{a(B)}	2.8±0.3 ^{a(C)}	2.1±0.1 ^{a(B)}			
ORP							
S1	180.8±37.6	156.1±28.9	144.4±36.3	169.2±27.0	Pseudo- <i>F</i> =0.09 ^{ns}	Pseudo- <i>F</i> =0.57 ^{ns}	Pseudo- <i>F</i> =0.02 ^{ns}
S2	189.1±45.6	153.6±32.0	159.5±41.5	173.4±19.0			
S3	201.2±52.2	159.9±31.5	167.2±43.7	170.6±26.7			
pH							
S1	7.8±0.2 ^a	7.8±0.3 ^a	8.5±0.3 ^a	8.0±0.2 ^a	Pseudo- <i>F</i> =0.39 ^{ns}	Pseudo- <i>F</i> =8.77**	Pseudo- <i>F</i> =0.20 ^{ns}
S2	7.8±0.2 ^a	8.0±0.2 ^{ab}	8.8±0.3 ^b	8.1±0.3 ^{ab}			
S3	7.7±0.1 ^a	8.1±0.2 ^{ab}	8.9±0.3 ^b	8.1±0.2 ^{ab}			
Salinity							
S1	25.8±6.5 ^{a(A)}	26.0±2.9 ^{a(A)}	32.0±1.0 ^{a(A)}	31.8±1.9 ^{a(A)}	Pseudo- <i>F</i> =22.83**	Pseudo- <i>F</i> =5.77*	Pseudo- <i>F</i> =0.55 ^{ns}
S2	12.8±7.8 ^{a(AB)}	15.9±6.2 ^{a(A)}	30.8±1.9 ^{a(A)}	23.0±6.9 ^{a(AB)}			
S3	0.4±0.2 ^{a(B)}	0.4±0.2 ^{a(B)}	19.1±4.2 ^{b(B)}	4.3±4.2 ^{ab(B)}			
Temperature							
S1	11.9±0.9 ^{a(A)}	14.6±0.5 ^{b(A)}	15.0±0.6 ^{b(A)}	15.8±0.4 ^{b(A)}	Pseudo- <i>F</i> =0.27 ^{ns}	Pseudo- <i>F</i> =21.63**	Pseudo- <i>F</i> =2.17 ^{ns}
S2	11.7±0.8 ^{a(A)}	16.4±0.9 ^{b(A)}	15.9±0.5 ^{b(AB)}	15.1±1.5 ^{b(A)}			
S3	9.6±0.8 ^{a(A)}	16.4±1.2 ^{b(A)}	18.3±1.2 ^{b(B)}	14.7±1.8 ^{b(A)}			

*= $p < 0.01$; **= $p < 0.001$; ^{ns} = non-significant. Different letters indicates significant differences among them. Lower case letters refers to comparisons among seasons, while upper case letters in brackets refers to comparisons among sampling stations (Two-way PERMANOVA, pairwise test: $p < 0.05$)

effects (stations: pseudo- $F=8.83$, $p<0.001$; seasons: pseudo- $F=6.81$, $p<0.001$), but was not significant for the stations \times seasons interaction (pseudo- $F=0.53$, $p=0.97$).

Depth was significantly higher in S1 during all seasons. On the other hand, pH was significantly higher during summer compared to winter in S2 and S3. Salinity was higher in S1 during all seasons; and in S3, salinity was significantly higher during summer compared with other seasons. Temperature varied seasonally, with significant lower values being recorded during winter in all sampling stations. The ORP values did not vary among sampling stations and seasons (Table 1).

The two-way SIMPER results revealed that 60 % of the dissimilarity between S1 and S2 was explained by depth and ORP values; while the dissimilarity between S1 and S3 was mostly explained by salinity and depth. On the other hand, dissimilarity between S2 and S3 was mainly related to differences in ORP and salinity. This analysis also revealed that temperature was the abiotic parameter that most varied among seasons (ESM 1).

Additionally, the PCA routine indicated that the first two axes explained 62.3 % of the variance among samples (PC1: eigenvalue=1.71, explain 34.2 % of variation; PC2: eigenvalue=1.40, explain 28.1 %) and revealed marked differences between sampling

stations, with S1 differing from the other stations, mainly due to its lower values of temperature and pH and higher values of depth and salinity (Fig. 2).

Common goby data

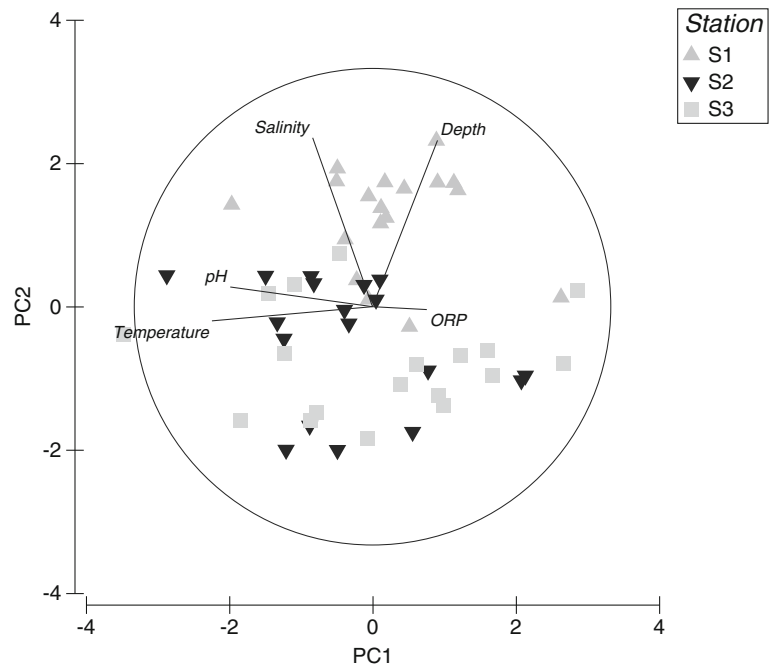
A total of 15,001 fishes were sampled (S2=59.9 %, S1=21.4 % and S3=18.7 %), with 36.6 % of these being caught during autumn, 33.0 % in winter, 18.3 %, in spring and 12.1 % in summer.

Density patterns

The mean density of *P. microps* in the Minho estuary, considering the entire sampling period and the 3 stations, was 118.3 ± 14.7 ind. 100 m^{-2} (mean \pm SE). The density of *P. microps* varied throughout the sampling period, with higher values being found during the autumn (429.0 ± 140.0 ind. $\cdot 100\text{ m}^{-2}$ in December/09) (Fig. 3a).

The density of *P. microps* varied significantly through space and time (stations: pseudo- $F=40.14$, $p<0.001$; seasons: pseudo- $F=18.89$, $p<0.001$; stations \times seasons interaction pseudo- $F=10.11$, $p<0.001$), with each sampling station presenting a different pattern. In S1, the density of *P. microps* was lower during the summer (17.8 ± 4.8 ind. $\cdot 100\text{ m}^{-2}$) compared with other seasons (pairwise test: $p<0.05$). In S2 the density of

Fig. 2 PCA showing the differences of the environmental variables among sampling stations in the lower Minho Estuary, NW Iberian Peninsula



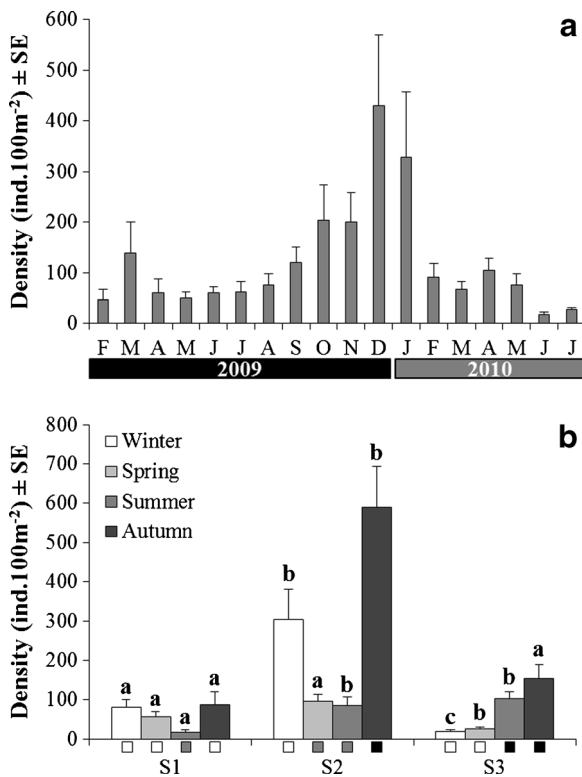


Fig. 3 **a** Mean density (\pm SE) of the common goby, *Pomatoschistus microps*, during 18-months study period in Minho estuary. **b** Comparisons on the densities (mean \pm SE) of *P. microps* between sampling stations and seasons. Different letters (a, b or c) indicates post-hoc differences among sampling stations within the same season. Post-hoc differences among seasons within each sampling station are indicated by squares of different colors (white, grey or black) (Two-way PERMANOVA pairwise tests: $p < 0.05$)

individuals was higher during the autumn (591.0 ± 102.0 ind·100 m⁻²) and winter (302.9 ± 78.3 ind·100 m⁻²) compared to spring and summer (pairwise test: $p < 0.05$); whereas in S3, the common goby was more abundant during autumn and summer (pairwise test: $p < 0.001$). Overall, the density of *P. microps* was higher in S2 during all seasons, except during summer, when S3 presented the highest density (102.0 ± 18.4 ind·100 m⁻²) (Fig. 3b).

The estimated densities of juveniles, males and females varied significantly through space and time [stations: pseudo- $F=25.08$, $p < 0.001$; seasons: pseudo- $F=11.79$, $p < 0.001$; stations \times seasons interaction (pseudo- $F=7.03$, $p < 0.001$)]. Males and females were more abundant in S2 during all seasons, except in summer, when density was higher in S3. Juveniles, on the other hand, were denser in S2 during all seasons

but spring, when the higher density was recorded in S1; with the lowest density value being recorded during summer in S1, spring in S2 and winter in S3 (Table 2).

The sampled population was dominated by females (53.0 %), with males and juveniles contributing with a smaller portion of individuals (26.3 % and 20.7 % respectively). The sex ratio (M/F) was not significantly different for stations (pseudo- $F=0.62$, $p=0.55$), but was significant for seasons (pseudo- $F=10.34$, $p < 0.001$) and for the stations \times seasons interaction (pseudo- $F=2.49$, $p < 0.05$). The highest (spring: 1.7 ± 0.4) and the lowest (autumn: 0.2 ± 0.1) values of sex ratio were recorded in S2 (ESM 2).

A total of 349 ovigerous females were caught in 18 months, with 20 individuals in autumn (5.7 %), 41 in winter (11.8 %), 190 in spring (54.4 %) and 98 in summer (28.1 %) with an average density of 2.2 ± 0.3 ind·100 m⁻² (mean \pm SE). The density of ovigerous females also varied through space and time (stations: pseudo- $F=2.14$, $p=0.12$; seasons pseudo- $F=5.91$, $p < 0.01$; stations \times seasons interaction (pseudo- $F=2.71$, $p < 0.05$), with higher densities being recorded during spring (S1 and S2) and summer (S3) (Fig. 4).

Size and recruitment patterns

The range of TL varied between 9.3 and 71.4 mm, with an average value of 30.4 ± 0.1 mm (mean \pm SE). The size of males did not vary significantly among sampling stations, but larger males were found during winter and autumn. On the other hand, the size of females varied significantly among sampling stations and seasons, with larger females being recorded in S3 during all seasons, except in autumn, when larger females were recorded in S1. Juveniles also presented significant differences among sampling stations and seasons, with smaller fish being recorded in S3 during all seasons, except in autumn, when smaller gobies were found in S1 (Table 3).

The TL of the ovigerous females varied between 26.8 and 46.9 mm, with an average value of 35.9 ± 0.2 mm. The two-way PERMANOVA indicated that size of ovigerous females varied significantly among seasons (pseudo- $F=6.72$, $p < 0.001$), but not among stations (pseudo- $F=0.11$, $p=0.73$); the stations \times seasons interaction was also non-significant (pseudo- $F=1.02$, $p=0.41$) (Table 4).

The density of the different size classes of *P. microps* varied through space and time (stations:

Table 2 Comparisons on the estimated density (mean \pm SE) of males, females and juveniles of the common goby *Pomatoschistus microps* among three sampling stations and four seasons in Minho estuary, NW Iberian Peninsula

	Seasons				Two-way PERMANOVA		
	Winter	Spring	Summer	Autumn	Stations	Seasons	Interaction
Male							
S1	20.5 \pm 4.6 ^{a(A)}	19.4 \pm 4.1 ^{a(A)}	6.0 \pm 1.5 ^{b(A)}	15.6 \pm 6.0 ^{ab(A)}	Pseudo- <i>F</i> =22.04*	Pseudo- <i>F</i> =7.13*	Pseudo- <i>F</i> =5.38*
S2	51.0 \pm 11.6 ^{a(B)}	47.4 \pm 7.9 ^{a(B)}	6.2 \pm 1.4 ^{b(A)}	61.3 \pm 10.1 ^{a(B)}			
S3	4.7 \pm 1.5 ^{a(C)}	8.3 \pm 2.5 ^{a(C)}	19.1 \pm 4.0 ^{b(B)}	32.1 \pm 7.4 ^{b(A)}			
Female							
S1	46.6 \pm 12.2 ^{a(A)}	29.9 \pm 7.9 ^{a(A)}	7.4 \pm 2.0 ^{b(A)}	57.7 \pm 25.0 ^{a(A)}	Pseudo- <i>F</i> =37.42*	Pseudo- <i>F</i> =22.18*	Pseudo- <i>F</i> =11.64*
S2	156.1 \pm 42.4 ^{a(B)}	43.3 \pm 8.9 ^{b(A)}	43.5 \pm 18.0 ^{ab(B)}	370.0 \pm 63.8 ^{c(B)}			
S3	12.1 \pm 3.0 ^{a(C)}	12.0 \pm 3.3 ^{a(B)}	59.4 \pm 13.3 ^{b(B)}	80.7 \pm 21.1 ^{b(A)}			
Juvenile							
S1	12.7 \pm 4.2 ^{ab(A)}	7.1 \pm 2.2 ^{ab(A)}	4.1 \pm 1.8 ^{a(A)}	14.1 \pm 4.3 ^{b(A)}	Pseudo- <i>F</i> =19.31*	Pseudo- <i>F</i> =8.88*	Pseudo- <i>F</i> =5.36*
S2	95.9 \pm 30.6 ^{ac(B)}	6.3 \pm 2.5 ^{b(A)}	29.9 \pm 9.1 ^{a(B)}	160.2 \pm 53.4 ^{c(B)}			
S3	1.5 \pm 0.9 ^{a(C)}	4.8 \pm 2.0 ^{a(A)}	23.6 \pm 4.8 ^{b(B)}	40.4 \pm 24.5 ^{b(A)}			

*= $p < 0.001$. Different letters indicates significant differences among them. Lower case letters refers to comparisons among seasons, while upper case letters in brackets refers to comparisons among sampling stations (Two-way PERMANOVA, pairwise test: $p < 0.05$)

pseudo- $F=17.61$, $p < 0.001$; seasons: pseudo- $F=11.47$, $p < 0.001$; stations \times seasons interaction: pseudo- $F=5.06$, $p < 0.001$). Results indicated that most of the size classes were more abundant in S2, except size class A, which was more abundant in S3. In S1 and S3, the most abundant size classes were E, D and F respectively, while classes D, E and C were the most abundant in S2 (Fig. 5).

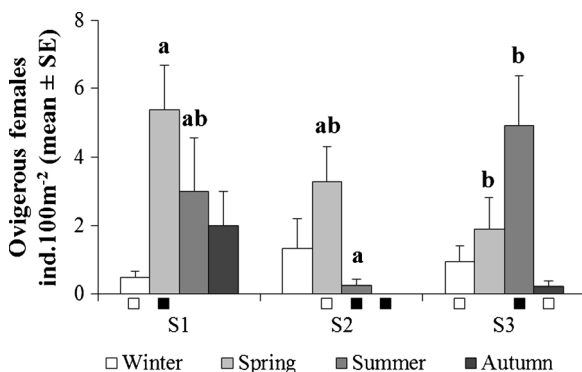


Fig. 4 Comparisons on the density (mean \pm SE) of ovigerous females of the common goby, *Pomatoschistus microps*, among three sampling stations and seasons in lower Minho estuary, NW Iberian Peninsula. Different letters (a or b) indicates post-hoc differences among sampling stations within the same season. Post-hoc differences among seasons within each sampling station are indicated by squares of different colors (white or black) (Two-way PERMANOVA pairwise tests: $p < 0.05$)

The mixture analysis indicated that the recruitment of *P. microps* occurred three times during the sampled period, twice in 2009 (May and October) and once in 2010 (June) (Fig. 6).

Fish-habitat associations

The DistLM results indicated that 3 of the 7 abiotic variables significantly influenced the abundance of *P. microps* males, females and juveniles. The river discharge, depth and distance from the river mouth were the best combination of predictor variables identified by the model and accounted for 22.6 % of the total variance (Table 5).

The dbRDA explained less than ¼ of the variance, and within this scope, the model suggest that *P. microps* males were more abundant in shallow areas closer to the river mouth during months with high river discharge. On the other hand, *P. microps* females and juveniles showed little evidence of association with the significant abiotic variables (ESM 3).

Length-weight relationship, growth and secondary production

The calculated length and wet weight relationship of the *P. microps* presented a high allometric b value ($b=3.6489$) and low allometric a value ($a=0.000001$).

Table 3 Comparisons on the total length (mean ± SE) of males and females of the common goby *Pomatoschistus microps* among sampling stations and seasons in Minho estuary, NW Iberian Peninsula

	Total length (mm)				Two-way PERMANOVA		
	Winter	Spring	Summer	Autumn	Station	Season	Interaction
Male							
S1	36.7±0.5 ^{a(A)}	34.0±0.3 ^{b(A)}	33.1±0.5 ^{b(A)}	37.3±1.0 ^{a(A)}	Pseudo- <i>F</i> =1.46 ^{ns}	Pseudo- <i>F</i> =40.51*	Pseudo- <i>F</i> =3.84*
S2	37.4±0.4 ^{a(AB)}	34.5±0.3 ^{b(A)}	34.3±0.6 ^{b(A)}	36.8±0.8 ^{a(A)}			
S3	38.6±0.50 ^{a(B)}	36.1±0.4 ^{b(B)}	31.8±0.3 ^{c(B)}	36.8±0.6 ^{b(A)}			
Female							
S1	33.1±0.3 ^{a(A)}	34.7±0.3 ^{b(A)}	32.5±0.6 ^{a(A)}	34.6±0.4 ^{b(A)}	Pseudo- <i>F</i> =86.87*	Pseudo- <i>F</i> =30.47*	Pseudo- <i>F</i> =7.06*
S2	32.4±0.2 ^{a(A)}	32.7±0.3 ^{a(B)}	30.1±0.3 ^{b(B)}	32.3±0.3 ^{a(B)}			
S3	35.5±0.4 ^{a(B)}	36.4±0.3 ^{b(C)}	33.1±0.3 ^{c(A)}	33.4±0.3 ^{c(C)}			
Juvenile							
S1	25.7±0.4 ^{a(A)}	24.6±0.6 ^{a(A)}	21.8±0.4 ^{b(A)}	22.3±0.8 ^{b(A)}	Pseudo- <i>F</i> =21.06*	Pseudo- <i>F</i> =26.87*	Pseudo- <i>F</i> =49.04*
S2	24.2±0.3 ^{a(B)}	24.5±0.6 ^{a(A)}	23.5±0.3 ^{a(B)}	23.8±0.3 ^{a(A)}			
S3	23.6±0.5 ^{a(B)}	14.8±0.4 ^{b(B)}	21.3±0.4 ^{a(A)}	26.8±0.5 ^{c(B)}			

^{ns} = non-significant; *=*p*<0.001. Different letters indicates significant differences among them. Lower case letters refers to comparisons among seasons, while upper case letters in brackets refers to comparisons among sampling stations (Two-way PERMANOVA, pairwise test: *p*<0.05)

Growth of the C2 and C3 cohorts was similar, while individuals from the C4 cohort grew faster than others cohorts (Table 6).

The estimated overall secondary production of *P. microps* was 5.38 g·WWm⁻², with an annual production of 3.83 g·WWm⁻²·year⁻¹.

Discussion

Common goby has a great capacity of adaptation, which allows it to successfully occupy habitats with different environmental characteristics (Bouchereau and Guelorget 1998). However, it seems that in the Minho estuary, the species preferentially occupied the salt marsh area (S2), since the density of *P. microps* at this station was constantly higher compared with its

density at the river mouth (S1) and the upper station (S3). In the salt marsh area, most of the fishes were small-bodied, suggesting that this area may serve as a refuge or shelter site for juveniles and subadults. Actually as reported in other studies, the high complexity and productivity of salt marshes’ biological communities might provide an ideal environment for feeding, sheltering and growing for the common goby (Koutsogiannopoulou and Wilson 2007). In contrast, at the river mouth (S1) most of the fishes were larger, with ca. 70 % of the individuals being sexually mature throughout the year. In the upper station (S3), the density of gobies varied considerably throughout seasons, with lower values being recorded during winter and spring, compared with summer and autumn. Such variations on *P. microps* density suggest that the upper station presented important seasonal oscillations in

Table 4 Comparisons on the total length (mean ± SE) of ovigerous females of the common goby, *Pomatoschistus microps*, among sampling stations and seasons in Minho estuary, NW Iberian Peninsula

Station\Season	Winter	Spring	Summer	Autumn
S1	40.6±1.5 ^{a(A)}	35.9±0.4 ^{b(AB)}	34.9±0.7 ^{b(A)}	38.5±0.9 ^{a(A)}
S2	35.7±0.9 ^{a(B)}	34.9±0.5 ^{a(A)}	32.3±1.8 ^{b(A)}	–
S3	37.9±1.2 ^{a(AB)}	37.1±0.8 ^{a(B)}	35.3±0.5 ^{b(A)}	38.3±2.8 ^{ab(B)}

Different letters indicates significant differences among them. Lower case letters refers to comparisons among seasons, while upper case letters in brackets refers to comparisons among sampling stations (Two-way PERMANOVA, pairwise test: *p*<0.05)

abiotic variables and might be located near the upstream limit of distribution of the species in winter. Our results suggest that the area occupied by the common goby in the estuary might vary throughout the year due to different degrees of saline intrusion. During summer, the saline plume often reaches upstream areas of the estuary, noticeably influencing the distribution of the species. The saline intrusion in the Minho

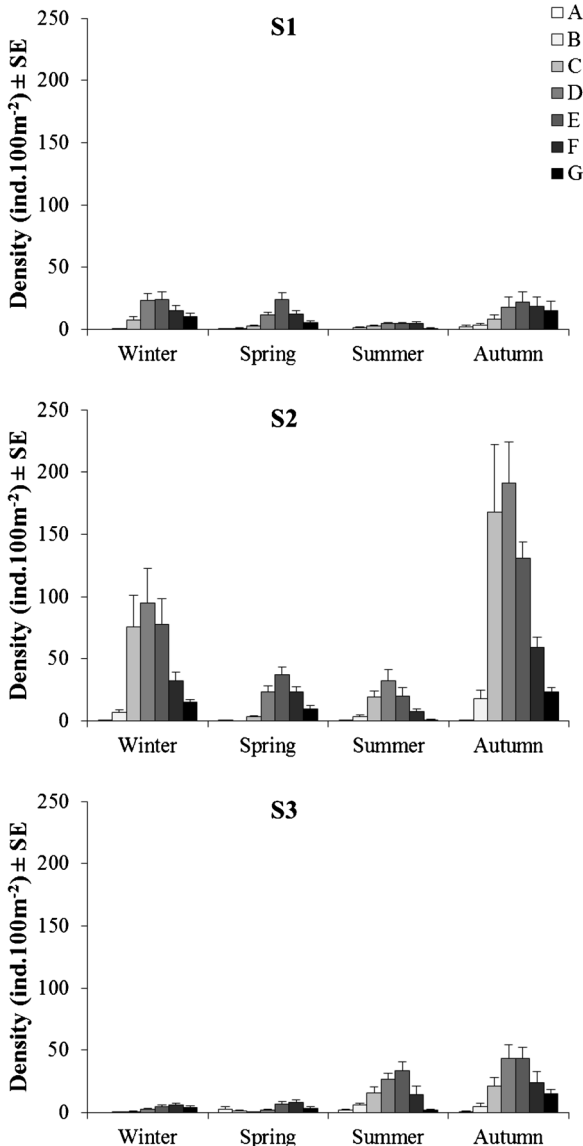


Fig. 5 Mean density (\pm SE) of the seven size classes of the common goby, *Pomatoschistus microps*, among seasons in each sampling station of the lower Minho estuary, NW Iberian Peninsula. Size classes: **a** (< 15.00 mm), **b** (15.01 to 20.00 mm), **c** (20.01 to 25.00 mm), **d** (25.01 to 30 mm), **e** (30.01 to 35.00 mm), **f** (35.01 to 40.00 mm) and **g** (> 40.01 mm)

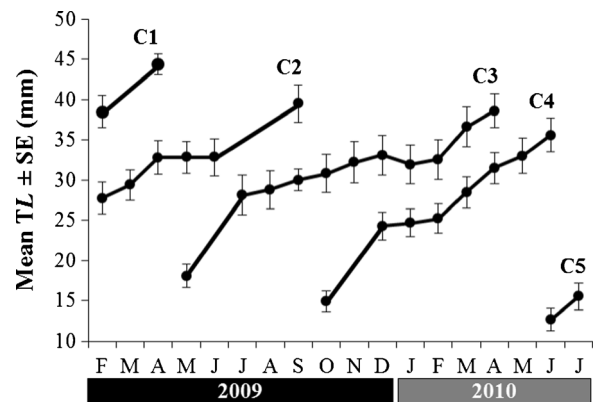


Fig. 6 Mean cohort length of the common goby, *Pomatoschistus microps*, in lower Minho estuary, with indication of the five cohorts identified by the mixture analysis (C1, C2, C3, C4 and C5)

estuary fluctuates between 10 km during winter and 20 km during summer, exceptionally ranging up to 30 km in extreme conditions (Antunes pers. comm.). In fact, during summer this species can be found in a much wider spectrum within the estuary, being recorded *ca.* 13 km upstream from the river mouth; whilst during winter, *P. microps* is usually restricted to the lowest areas of the estuary (*ca.* 9 km) (pers. obs.).

The numerical dominance of females over males is relatively common in *Pomatoschistus* spp. populations (e.g., Bouchereau et al. 1993; Fouda et al. 1993; Koutrakis and Tsikliras 2009) and may be related to different behaviors exhibited by sexes. According to Miller (1984), during the breeding season, there is a marked reduction in the proportion of males due to their nest guarding behavior. *P. microps* males guard eggs under shells or stones, consequently males are less easily caught. In fact, males were less abundant during summer at the river mouth and at the salt marsh.

Differently from Leitão et al. (2006), which noted that 0+ group associated with high temperatures, in our study, juveniles associated with colder waters, indicating that juveniles recruited in different months in Mondego and in Minho, two estuaries from the Atlantic coast of Portugal. According to the present study, recruitment occurred twice per year (in late spring/early summer and in the autumn), while in Mondego, *P. microps* recruited three times per year (winter, spring and summer) (Leitão et al. 2006). Notwithstanding, a third pattern was reported for the Minho and Mondego estuaries, with recruitment occurring once per year in Minho (in early summer), and

Table 5 DistLM model based on the abundance of *P. microps* males, females and juveniles and fitted abiotic data

Sequential tests					
Variable	AIC	SS (trace)	Pseudo- <i>F</i>	<i>p</i>	Prop. (%)
River discharge	57.32	14.04	5.04	< 0.05	8.8
Depth	54.23	13.05	5.05	< 0.05	8.2
Distance from the mouth	52.43	8.95	3.64	< 0.05	5.6

Prop. = proportion of variation explained

twice (spring and summer) or three times (winter, spring and summer) per year in Mondego (Dolbeth et al. 2010). Actually, the recruitment of fishes in the Minho estuary often takes place later and growth rates are lower compared with the Mondego estuary, and those differences probably are related to differences in temperature between estuaries (Dolbeth et al. 2010). Also, in the present study, we found different growth rates among *P. microps* cohorts, with the autumn recruits (C4) growing faster than the spring ones (C3). Different growth rates between fish cohorts in a given population are common and often related to environmental conditions, but their ecological causes and implications are still uncertain (e.g., Fuiman et al. 2005 and the references therein). However fast growing is often related to low temperatures (e.g., Dolbeth et al. 2010), which could explain the faster growth of autumn recruits compared with spring ones; since in spring water was slightly warmer than in autumn.

The observed differences in the density of ovigerous females may also indicate that breeding grounds varied seasonally within the estuary, probably due to differences in the temperature and in the availability of substrate for egg attachment, once each sampling station presented different substrate characteristics. The

Table 6 Absolute growth rates (AGR, mm.day⁻¹) for the common goby, *Pomatoschistus microps*, cohorts

Cohort	Growth±CI
C2	0.045±0.002
C3	0.046±0.001
C4	0.069±0.001

The AGR and the 95 % confidence interval (CI) were computed only when the cohort was recorded for more than 3 months

presence of bivalve shells is important for the species, since *P. microps* use them during the reproduction (Nyman 1953), and also for shelter. Thus, the high density of empty bivalve shells found in S3, can be related with the high density of ovigerous females found in this station during the summer, when most of the Asian clams *C. fluminea* die due to salinity increase (see Ilarri et al. 2011). In a small spatial scale experiment, Ilarri (2012) found that the densities of *P. microps* and *C. fluminea* were positively correlated, suggesting that the common goby prefers to inhabit areas with higher availability of bivalve shells in the Minho estuary.

P. microps is an iteroparous species (Miller 1984), being able to spawn several times along the reproductive cycle, normally spawning when water temperature is between 15 and 20 °C (Wiederholm 1987). In the Minho estuary, the water temperature in the salt marsh and in the upper station varied within the optimal levels for *P. microps* reproduction from May to November, whereas in the river mouth, the optimum range of temperature occurred for a shorter period of time (from September to December). These results suggest that the reproduction of the common goby may occur in different periods of time in each estuarine section. It is also possible that the optimal temperature range for *P. microps* reproduction varies with latitude, once this species has a great plasticity on reproductive traits (Pampoulie et al. 2000; Dolbeth et al. 2010); however, due to the lack of data, this hypothesis cannot be confirmed or contradicted, and hence further studies are necessary to investigate if the optimal values suggested by Wiederholm (1987), based on individuals from the Bothnian Sea, are valid for *P. microps* populations in southern Europe.

Differences in recruitment periods obtained in this and other studies may also be related to the duration of the breeding season. In higher latitudes, breeding season is typically shorter than in lower latitudes, but in certain cases, breeding season can last longer in lower latitudes (see Bouchereau and Guelorget 1998). This seems to be the case of the Minho estuary, where the common goby presented a longer breeding season (6 months) than in estuaries located at higher latitudes (e.g., Miller 1975; Rogers 1988). Nevertheless, breeding season in the Minho estuary was also longer than in systems located in lower latitudes (e.g., Arruda et al. 1993; Leitão et al. 2006). A longer breeding season might have an important effect on the *P. microps* population, due to the improvement of its reproductive

success (Bouchereau and Guelorget 1998), which would also help to explain the high densities recorded in the Minho estuary. In fact, the densities recorded in this study are much higher than those reported in southern (e.g., Leitão et al. 2006; Dolbeth et al. 2007; Martinho et al. 2007; Almeida et al. 2008; França et al. 2008) and in northern European estuaries (e.g., Norte-Campos and Temming 1994; Selleslagh and Amara 2008) (Table 7). Latitudinal comparisons in *P. microps* density are difficult to be made, once the sampling strategies, techniques and periodicity are often different in each study. However, considering only studies that have adopted similar sampling methods, latitudinal trends become clearer. For instance, in studies which used encircling nets or traps (throw and drop), densities appear to be higher in northern compared with southern systems. Similarly, in studies that used beam trawls, fish density seems to be higher in higher latitudes. On the other hand, the densities recorded in the Minho estuary were much higher than elsewhere, suggesting that in this system, *P. microps* might find a combination of factors which helps it to achieve higher densities than would be expected, given the latitude of this estuary.

The common goby is sympatric with the sand goby *P. minutus* in several estuaries, and presents some degree of habitat and diet overlap (Leitão et al. 2006;

Złoch and Sapota 2010). Nevertheless, the sand goby is less tolerant to salinity and temperature fluctuations than the common goby, preferring to inhabit estuarine zones with higher salinities or the sea, whereas *P. microps* is preferentially found in brackish waters (Fonds and Van Buurt 1974; Pampoulie et al. 1999). In the Minho estuary, during winter, salinity can reach extremely low values even near the river mouth. For instance, in January/10, salinity at the bottom reached 0.12 in S1. The occurrence of such low salinities within the estuary may benefit *P. microps* by potentially expanding its favorable habitat and also by reducing the detrimental effects of competition with its congener *P. minutus*, which is much less abundant in areas with low salinities in this estuary (Souza et al. unpubl. data).

Also, the sampling strategy adopted in the present study may have contributed to the observed differences between population density in Minho and other estuaries, since we performed diurnal 1-m beam trawl catches, while other studies performed night catches using 2-m trawls. Changes in trawling time can result in differences on fish density (Rotherham et al. 2008) or not (Ribeiro et al. 2006); and as far as we know, there are no published studies that compare the efficiency of 1-m and 2-m beam trawls for *P. microps*.

Table 7 Mean density of the common goby, *Pomatoschistus microps*, in different Atlantic estuaries

Location	Mean density·100 m ⁻²	Study period	Seasons	Day period	Sampling gear	Reference
Sylt-Rømø Bight, Germany	230.0–1070.0 (seagrass); 30.0–200.0 (sand)	2003	Summer	Diurnal	Portable drop trap	Polte et al. 2005
Wadden Sea, Germany	6.0	1991/92	Summer	Diurnal and nocturnal	Beam trawl	Norte-Campos and Temming 1994
Canche estuary, France	8.2	2006/07	All seasons	Diurnal	Beam trawl	Selleslagh and Amara 2008
Minho estuary, Portugal	118.3	2009/10	All seasons	Diurnal	Beam trawl	This study
Minho estuary, Portugal	60.1	2006	Summer	Diurnal	Beam trawl	Costa-Dias et al. 2010
Mondego estuary, Portugal	0.8	2003/06	All seasons	Nocturnal	Beam trawl	Martinho et al. 2007
Mondego estuary, Portugal	0.8	2003/04	All seasons	Nocturnal	Beam trawl	Dolbeth et al. 2007
Mondego estuary, Portugal	0.8	2004/05	All seasons	Nocturnal	Beam trawl	Leitão et al. 2006
	0.5	2005/06	All seasons	Nocturnal	Beam trawl	
	0.8	2003/04	All seasons	Nocturnal	Beam trawl	
Tagus estuary, Portugal	60.0 (upper intertidal); 17.1 (lower intertidal); 89.9 (subtidal)	2005	All seasons	Diurnal and nocturnal	Encircling nets	França et al. 2008
Ria Formosa, Portugal	115.0	2001/02	All seasons	–	Throw trap	Almeida et al. 2008

Other studies also reported remarkable high densities of aquatic species in the Minho estuary, for instance, the Asian clam, *Corbicula fluminea* (Souza et al. 2008a), the European flounder, *Platichthys flesus* (Cabral et al. 2007; Freitas et al. 2009; Souza et al. 2013) and the shore crab, *Carcinus maenas* (Souza et al. unpubl. data). These high densities may be linked to the particular abiotic profile of the Minho estuary, namely the high river discharge and possibly the high availability of nutrients and organic matter. These characteristics may lead to a scenario of dominance of a few species, highly tolerant to freshwater in detriment of species with higher affinity with salt water, suggesting that in stressful environments certain species can be benefited.

Secondary production can be used to infer the population fitness (Rigler and Downing 1984) and therefore, it may represent an interesting proxy with regard to the functional responses of populations to environmental stressors (Dolbeth et al. 2012). The annual production of *P. microps* in the Minho estuary was notably higher than in other systems (see Dolbeth et al. 2010 and the references therein), suggesting that this system provide advantageous environmental conditions for this particular species to thrive. Nonetheless, in a previous study, Dolbeth et al. (2010) recorded production values four times lower for *P. microps* in the same estuary, suggesting that the common goby production might have increased over the years, probably due to the fluctuations on the abiotic conditions caused by drought events that occurred in this estuary recently (see Dolbeth et al. 2010; Ilarri et al. 2011). Alternatively, differences in production values obtained for *P. microps* in the Minho estuary can be the result of different sampling strategies and production estimation method adopted in each study.

Conclusions

In summary, our results highlight that the common goby population in the Minho estuary is remarkably dense and productive. This is probably explained by a combination of factors found in the system, such as the low salinity, the prolonged breeding season of *P. microps*, the low density of the competing species *P. minutus*, and the copious availability of suitable substrate for reproduction. In conclusion, the common goby may have found in the Minho estuary an area

with a combination of factors that created optimal conditions for it to thrive.

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