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Patterns of shelter usage and social aggregation by the vocal Lusitanian toadfish

**Maria Clara Pessoa Amorim · José Miguel Simões ·
Paulo Jorge Fonseca · Vitor C. Almada**

Abstract In some marine fishes, males migrate from deeper to littoral water to breed, where they defend nests and provide parental care. In this study, we describe seasonal patterns of shelter occupation in estuarine shallow waters and assess social aggregation in the Lusitanian toadfish (Batrachoididae), a vocal species with male parental care. Occupation of intertidal shelters was restricted to the breeding season but adult fish remained in subtidal regions during the non-reproductive season and they produced sounds throughout the year. Intertidal shelters were aggregated with up to five shelters found per 2-m segment of an 80-m transect. This aggregation probably resulted from social attraction since many segments were found unoccupied. Moreover, shelters contained on average two fish (maximum of nine). Based on size, shelters with multiple fish seemed to contain females, or possibly sneakers, but also more than one type I male inside, indicating a high social tolerance for conspecifics.

Introduction

In many marine inshore fish species in which males guard the offspring, the preferred sites to establish nests often

M. C. P. Amorim (✉) · J. M. Simões · V. C. Almada
Unidade de Investigação em Eco-Etologia,
Instituto Superior de Psicologia Aplicada,
Rua Jardim do Tabaco 34, 1149-041 Lisbon, Portugal
e-mail: amorim@ispa.pt

P. J. Fonseca
Departamento de Biologia Animal e Centro de Biologia
Ambiental, Faculdade de Ciências da Universidade de Lisboa,
Bloco C2, Campo Grande, 1749-016 Lisbon, Portugal

differ from those used for feeding or shelter. In many temperate species, individuals move from overwintering deeper water to more littoral habitats where they breed (Almada et al. 1994). Even in true rocky intertidal resident species, there are often movements to places where structures favourable to serve as nest holes are available. Parental males typically stay in the nests during the guarding period whereas females visit the nest sites to spawn but return to other habitats for foraging and shelter (Almada and Faria 2004).

Marine teleosts of the family Batrachoididae include toadfishes and midshipmen and are established vertebrate models for the study of acoustic communication and audition (Bass and McKibben 2003) as well as in other areas such as ethology and endocrinology (Palazón-Fernández et al. 2001). Batrachoidids seasonally migrate from deeper coastal and estuarine waters to intertidal and subtidal zones where males dig burrows under rocks to prepare a nest for spawning (Gray and Winn 1961; Brantley and Bass 1994; Costa 2004; Cotter 2008). Ripe females are attracted to the males by the low-frequency advertisement calls (boatwhistles in toadfishes and hums in midshipmen) that result from the contraction of sound-producing muscles attached to the swimbladder (Skoglund 1961; Bass and McKibben 2003). Females are known to lay a single batch of eggs on the roof of a nest and depart soon after spawning is completed (Modesto and Canário 2003; Sisneros et al. 2009). Parental males continue calling to attract more females until the nest substrate is fully covered with multiple clutches. The nest holder fans and guards the eggs until the young reach the free-swimming stage (DeMartini 1988; Brantley and Bass 1994; dos Santos et al. 2000). This species presents an alternative mating strategy with a second male morphotype (type II males) that sneak egg fertilizations but does not defend nests or court females acoustically. Type I males are

larger in body size and invest in sonic muscles and associated neural circuitry for acoustic communication used in courtship and agonistic interactions whereas type II males are smaller and mainly invest in producing larger testes (Bass 1996; Modesto and Canário 2003).

The Lusitanian toadfish, *Halobatrachus didactylus*, is an eastern Atlantic member of the family Batrachoididae that inhabits estuaries and coastal lagoons and has its northernmost limit of distribution in the Tagus estuary, Portugal, appearing occasionally up to the Bay of Biscay (Roux 1986). Females release a low number (hundreds) of large eggs (5–8 mm diameter) (Palazón-Fernández et al. 2001; Costa 2004) in a single batch (Modesto and Canário 2003) whose high survival is assured through male parental care. The spawning season extends from March to August with a peak in May–June in the Bay of Cádiz, Southern Spain (Palazón-Fernández et al. 2001), but in Portugal it extends from May to July (Modesto and Canário 2003; Costa 2004). The main constraint for reproduction in *H. didactylus,* as in other batrachoidids, is water temperature, and breeding activity is only observed with temperatures higher than 18/19°C (Costa 2004).

Although Costa (2004) and Cotter (2008) studied temporal variations in the distribution, abundance, population structure and activity of Lusitanian toadfish populations in Mira and Tagus estuaries (western coast of Portugal) little is known on the usage of shelters in shallow water including the intertidal zone. Previous studies suggest that males form breeding aggregations in the subtidal since calls from several individuals can be detected in close proximity to a registering hydrophone placed in shallow water, resulting in a very conspicuous acoustic output (dos Santos et al. 2000; Amorim et al. 2006). The use of intertidal cavities as nests has also been documented in this species (Amorim et al. 2008). However, the dynamics of shelter occupation during and outside the breeding season, including whether parental males exclude other conspecifics from their nests, has not been clarified. In this paper, we describe seasonal patterns of shelter occupation in shallow waters of the Tagus estuary and assess the level of nest and social aggregation in the Lusitanian toadfish.

Materials and methods

Intertidal shelters

Cais do Seixalinho

Shelter occupation was monitored approximately twice a month from May 2006 to September 2007, in an intertidal area of the Tagus estuary (Portugal, Montijo, 38°42′ N; 8°58′ W). This study site is alongside a ferry boat station

(Cais do Seixalinho) and has been previously identified as a Lusitanian toadfish breeding area (Amorim et al. 2006; Amorim and Vasconcelos 2008). Next to the landing platform for the ferries there is a secondary pier where standby ferries moor, and along the river-bank there is a rock revetment on a sand/silt substrate. The rocks of the revetment and the ones at the secondary pier pillars provide nesting places for Lusitanian toadfish. In particular, the revetment is an excellent site to monitor natural occupation of intertidal shelters since they get exposed at low spring tides but never during neap tides. We monitored an 80-m-long (~1 m wide) transect, running along the shoreline and centred in the secondary pier. In low spring tides, we systematically inspected every crevice and small refuge beneath the rocks to ascertain that a similar inspection effort was kept throughout the study.

For each 2-m segment of the transect, we recorded the number of occupied shelters and the number and size of individuals in each shelter. Size was estimated from the mouth width (MW, equivalent to the head width in this species), and three categories were considered: A (MW < 100 mm), B (100 mm ≤ MW < 150 mm) and C (150 mm ≤ MW < 200 mm). Mouth width was measured with a ruler precise to the nearest mm. To establish a correspondence between MW and body length, 143 males and 39 females were obtained from local fishermen from the Tagus estuary, and MW and total length (TL) were measured to the nearest mm. Sex was determined from the internal examination of gonads. Mouth width increased with TL (Fig. 1). Class A contained both genders with males ranging in MW from 39 to 99 mm (mean = 79.1 mm) and TL from 174 to 368 mm (mean = 289 mm) and females
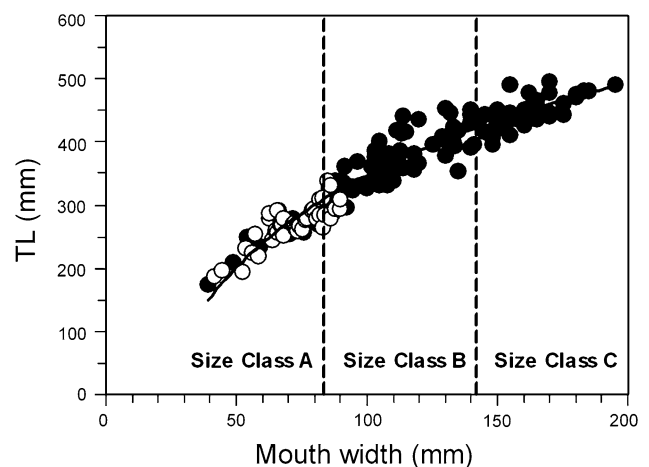
Fig. 1 Relation between total length (TL) and mouth width (MW) in male (*filled circles*) and female (*open circles*) toadfish. The three size classes considered are depicted: *A* (MW < 100 mm), *B* (100 mm ≤ MW < 150 mm) and *C* (150 mm ≤ MW < 200 mm). The best fit for a logarithmic regression is shown ($y = 210.82\mathrm{Ln}(x) - 624.43$). Sample size: A—$n_{♀} = 39$, $n_{♂} = 42$; B—$n_{♂} = 54$; C—$n_{♂} = 47$

ranging in MW from 42 to 90 mm (mean = 71.6 mm) and TL from 187 to 338 mm (mean = 269 mm). Class B and C were only represented by type I males, with B-sized animals ranging in MW from 100 to 148 mm (mean = 120.4 mm) and TL from 326 to 452 mm (mean = 387 mm) and C males ranging in MW from 150 to 195 mm (mean = 164.1 mm) and TL from 410 to 495 mm (mean = 452 mm). The fact that larger fish (B and C) were only represented by parental males (type I) is consistent with previous studies carried out with Iberian Lusitanian toadfish populations (Palazón-Fernández et al. 2001; Modesto and Canário 2003).

The distance between the two closest occupied shelters in each 2-m segment was also measured to the nearest cm to check for nest aggregation. During the transect inspection, temperature from the estuary water was also registered to the nearest 0.5°C.

Artificial shelters

Because it was difficult to check for the presence of eggs/embryos in Cais do Seixalinho's shelters due to the size of the boulders, we deployed 60 artificial concrete shelters in three rows along the shoreline in a nearby intertidal area (Military Air Force Base 6 (AFB6), also at Montijo, 38°42′ N; 8°58′ W). These shelters (internal dimensions: 50 cm long, 30 cm wide and 20 cm high) had a hemicylinder shape capped at one end, were placed ∼1.5 m apart from each other, and were also only exposed to air at low spring tides. The inspection of shelters at AFB6 was also carried at low spring tides from May to August 2006, to check for shelter occupation and the presence of offspring. We killed the occupiers of two shelters to ascertain their sex. We further dissected four fish found just outside occupied shelters. Killing was restricted to few individuals for ethical reasons. All other fish used for measurements of size were, as stated above, obtained from local fishermen.

Both study sites were subject to human intervention, and it was common to see local fishermen capturing fish from shelters so that in each sampling period new shelter occupiers were likely observed.

Subtidal area

To ascertain the presence of adult males in the subtidal zone, we additionally monitored acoustic activity from Cais do Seixalinho's secondary pier approximately twice a month during the same study period. Five-minute recordings were carried out in five different locations in the pier separated by at least 4 m. Water depth of the recording locations varied approximately between 2 and 6 m depending on tide. Sounds were registered with a High Tech 94 SSQ hydrophone (High Tech Inc.; sensitivity of −165 dB

re 1 V/μPa, frequency response within ±1 dB from 30 Hz up to 6 kHz) and an Edirol R4 digital recorder (Roland; 16 bit resolution, 44.1 kHz acquisition rate per channel). The number of sounds (boatwhistles) was counted per minute and per recording location. Sound analysis was carried out with Adobe Audition 2.0 (Adobe Systems Inc.) and Raven 1.2.1 for Windows (Cornell Laboratory of Ornithology).

Statistical analyses were performed with Statistica 8.0 for Windows (StatSoft, Inc., 1984–2008, Tulsa, USA). Non-parametric tests were used when assumptions of parametric analysis were not met.

Results

Intertidal shelters

Cais do Seixalinho

The occupation of intertidal shelters at Cais do Seixalinho coincided with the Lusitanian toadfish breeding season and was significantly higher in May–July than in the remaining months (Fig. 2a; Mann–Whitney test, $U = 7.0$, $P < 0.001$). Shelter occupation peaked in May and declined thereafter till the end of July in 2006 and the end of August in 2007, showing null values after these dates. In 2007, fish were recruited to these intertidal shelters in April with three of the 40 segments of the transect having one occupied nest in the 1st of April and 15 out of 40 segments had one to three occupied shelters in the 19th of April 2007. Shelter occupation was not related with water temperature (Spearman rank correlation, $n = 28$, $r = 0.33$, $P > 0.05$) as water temperature was relatively high all year round (Fig. 2a) and was always above the minimum required for reproduction activities in this species (Costa 2004). An average of 0.6 and 0.7 shelters was occupied per 2-m segment of the transect during both breeding seasons of 2006 and 2007, and a maximum occupation reached four and five shelters per segment, respectively, in 2006 and 2007. The coefficient of variance of shelter occupation was high (CV = SD × 100 × mean^{-1}, 155% in 2006 and 123% in 2007 breeding seasons) reflecting the fact that there were many segments with no occupation and others with several occupied shelters.
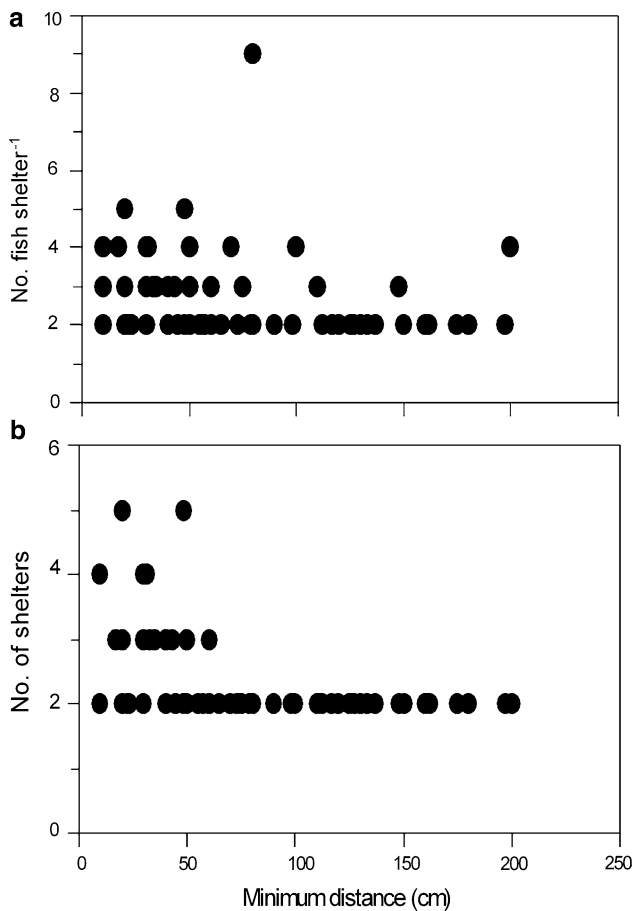
The minimum distance between used shelters was on average ($\pm$SD) 86.2 ± 53.2 cm (range 17–200 cm) in 2006 and 65.2 ± 42.1 cm (range 10–180 cm) in 2007 (Fig. 2b). This distance did not differ among sampling dates (ANOVA, $F_{9,69} = 0.61$, $P > 0.05$) indicating that it does not change with the progression of the breeding season. However, the minimum distance between shelters shortened with the increase of occupation of the 2-m segments, i.e. with the number of fish per shelter (Fig. 3a; Spearman Rank
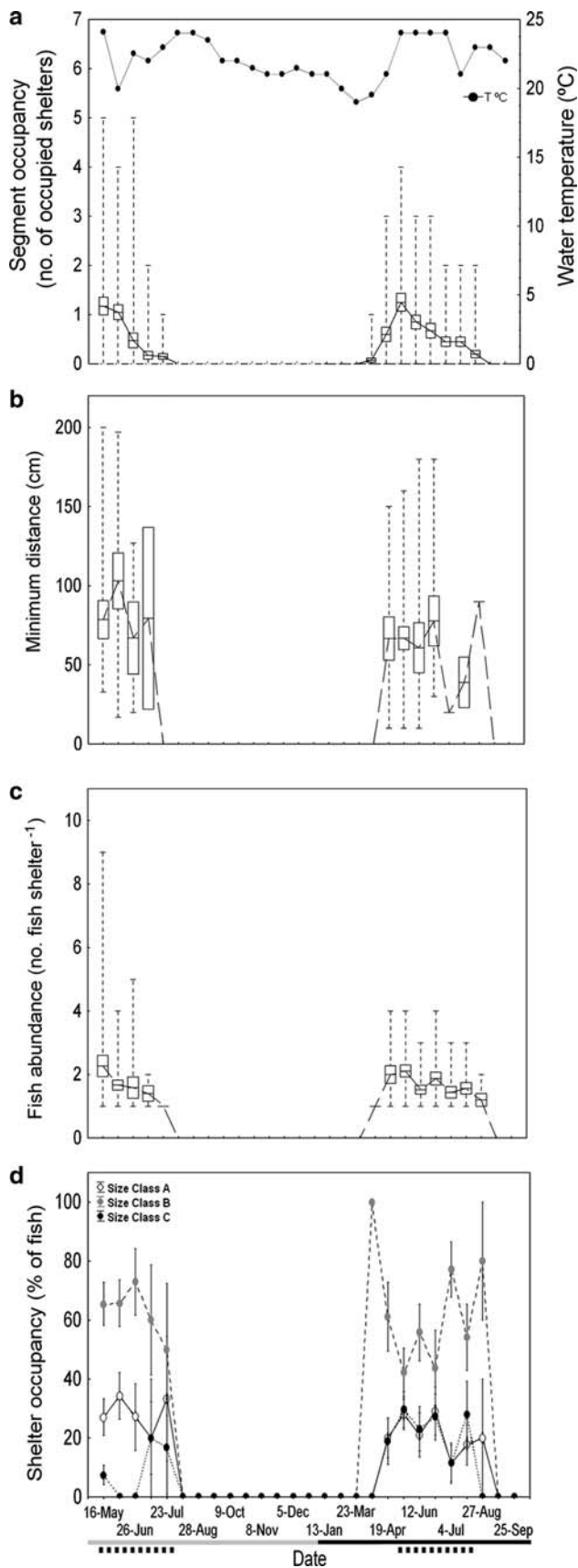
◄ **Fig. 2** Seasonal variation in the **a** number of occupied shelters in the 2-m segments of the 80-m transect (seasonal variation of water temperature is also depicted), **b** minimum distance between shelters, **c** number of fish found in shelters and, **d** percentage of different size classes (A—*open circles*, B—*filled grey circles*, C—*filled black circles*), observed at Cais do Seixalinho from May 2006 to September 2007. Mean ± SE is represented by *boxes* in **a–c** and by *circles* and *whiskers* in **d**. *Whiskers* in **a–c** are min–max values. The *grey/black bar* in the *bottom* represents the years 2006/2007, and the banded bars depict the Lusitanian toadfish's breeding season

Correlation, $n = 79$, $r = -0.27$, $P = 0.02$) and with the number of occupied shelters (Fig. 3b; $n = 79$, $r = -0.42$, $P < 0.001$). These correlations were however weak ($r < 0.5$). Figure 3b illustrates that the minimum distance between shelters was ≤ 60 cm when there were three or more used shelters in a segment but that these short distances were very common even when there were only two used shelters (observed in 44% of the cases). Taken together, these data suggest that Lusitanian toadfish tend to aggregate during shelter occupation. To further explore this hypothesis, we tested whether during the reproductive months the number of occupied shelters per 2-m segment exhibited a clumped pattern. The distribution of shelter per 2 m did not follow a Poisson distribution (chi-square test, $\chi^2 = 13.66$, $df = 2$, $P < 0.001$) suggesting that nests are not randomly distributed and adjusted well to a negative binomial (chi-square test, $\chi^2 = 5.48$, $df = 3$, $P > 0.05$) pointing to a distribution of nests in a clumped fashion. We also compared the number of occupied shelter between the 2-m segments to ascertain whether there are preferred segments or others that are avoided consistently due to habitat characteristics. Considering both years, there was no difference among segments (Kruskal–Wallis test, $n = 400$, $H = 47.25$ $P > 0.05$), indicating that any segment could be equally occupied.

The number of fish in an occupied shelter averaged 1.8 (range 1–9) in 2006 and 1.7 (range 1–4) in 2007 and did not differ among sampling dates (Fig. 2c; ANOVA, $F_{9,69} = 0.78$, $P > 0.05$). Approximately half of the occupied shelters had only one fish (52.6% in 2006 and 48.7% in 2007), one-third had 2 fish (32.9% in 2006 and 34.5% in 2007) and the remaining had three or more occupiers (14.5% in 2006 and 16.8% in 2007). Fish of the size class B were the most frequent occupiers of intertidal shelters and had a similar seasonal pattern of shelter usage as class A and C (Fig. 2d). When the number of occupiers per shelter increased all size classes augmented (Spearman rank correlation: $n = 212$, $r_A = 0.66$, $r_B = 0.32$, $r_C = 0.39$, $P < 0.001$), but mainly fish size A, as shown by its highest correlation coefficient. Figure 4 depicts histograms of the frequency of the number of fish found per shelter (Fig. 4a) as well as the percentage of fish of each size class observed per shelter,

Fig. 3 Relation between the number of fish per shelter (**a**) and the number of shelters (**b**) with the minimum distance between shelters in each 2-m segment of an 80-m transect. Data are pooled observations from repeated sampling along the same transect carried out during the periods of nest occupation

i.e. the number of fish of a given class divided by the total number of fish per shelter × 100 (Fig. 4b, d). Toadfish of size classes A and C typically shared shelters with a conspecific but also occupied shelters alone, since the histograms show a bimodal distribution of observations with peaks at 50 and 100% (Fig. 4b, d). Note that as in the majority of cases there were either one or two fish per shelter (Fig 4a), 100% usually indicates that the fish occupied the shelter alone while 50% suggests that it shared the space with another fish. Medium-sized fish (B), on the other hand, were usually the single occupiers of shelters but occasionally shared shelters with conspecifics (Fig. 4c).

Artificial shelters

Eggs and resident fish were found in the shelters of AFB6 during the months of May and June 2006 but not in July or thereafter (Fig. 5). In late June (27/6/06), clutches were mostly composed by embryos. The occupiers of two nests that contained more than one individual were killed to

ascertain their gender. In one nest, there was a type I male of 450 mm TL (MW = 165 mm) and a female of 332 mm TL (MW = 861 mm). The occupiers of the second nest were one type I male of 490 mm TL (MW = 155 mm) and two females of 264 cm TL (MW = 83 mm) and 284 mm TL (MW = 84 mm). In both cases, the parental males would be classified as C and the females as A. We further measured residents from another 14 occupied artificial shelters. These were also large fish with mean TL of 440 mm (270–490 mm). Except for one fish that had 270 mm (MW = 135 mm, i.e. size B), all others were above 400 mm in TL (MW = 150–195 mm, i.e. all size C). Eight of these shelters had eggs. Six shelters had one specimen just outside the shelter, typically against its side. From these, three were outside shelters with eggs and three outside shelters with no eggs. Fish found outside the shelters averaged 367 mm in TL (340–397 mm) and were all size class B (MW: mean = 128 mm, range 110–145 mm). Four of these fish found outside nests were killed and three were type I males and the fourth was a type II male.

Subtidal area

The rate of boatwhistle emission (number of boatwhistles per minute) showed a pronounced seasonal variation presenting significantly higher values during the breeding season than in the remaining months (Fig. 6; Mann–Whitney test, $U = 0.0$, $P < 0.001$). However, boatwhistles were surprisingly heard all year round (Fig. 6), indicating that the subtidal area was occupied throughout the year. Boatwhistle rate was positively correlated with the average number of occupied shelter per 2-m segment of the intertidal transect (Spearman rank correlation, $n = 28$, $r = 0.79$, $P < 0.001$) and the average number of occupiers per shelter ($n = 28$, $r = 0.78$, $P < 0.001$).

Discussion

Batrachoidids have traditionally been described to live in deep waters during the winter months and to migrate to shallow waters for the breeding season (Gray and Winn 1961; Brantley and Bass 1994; Costa 2004). Consistently, our study showed a clear seasonal usage of intertidal shelters by the Lusitanian toadfish that coincided with its breeding season (May–July). Shelter occupation started prior to the breeding season in April in 2007, peaked in May and declined until the end of the breeding season in both years. Previous studies based on bottom trawling conducted at various depths in the Tagus and Mira estuaries are consistent with our results and indicate that the Lusitanian toadfish seems to follow the same seasonal migration pattern as other batrachoidids (Costa 2004; Cotter 2008). These
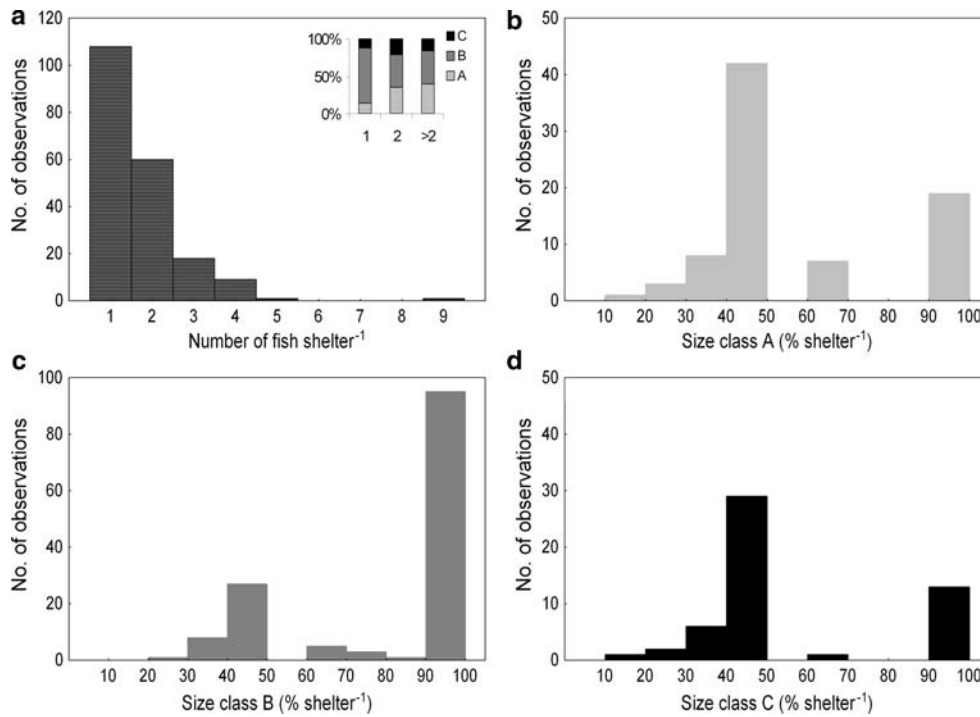
Fig. 4 **a** Frequency of observations for the number of fish found per shelter. Embedded is the proportion of each size class in shelters with one, two or more than two occupiers. Size classes were based on mouth width (MW): A (MW < 100 mm), B (100 mm ≤ MW < 150 mm) and C (150 mm ≤ MW < 200 mm). **b–d** Percentage of each size class observed per shelter, i.e. the number of fish of a given class divided by the total number of fish per shelter × 100
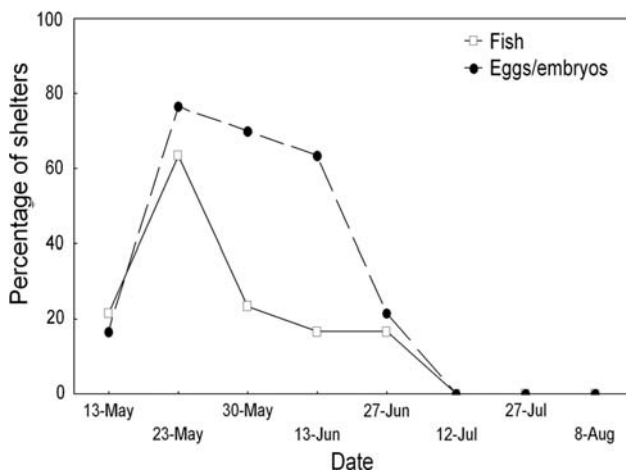
Fig. 5 Percentage of shelters with resident fish and with offspring versus time (sampling date) in 40 artificial intertidal shelters during the breeding season of 2006
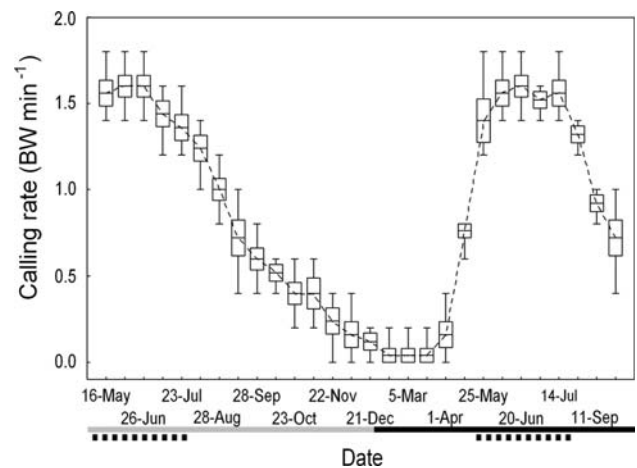
Fig. 6 Seasonal variation of boatwhistle emission rate recorded from a pier at Cais do Seixalinho from May 2006 to September 2007. *Boxes* depict mean ± SE and *whiskers* min–max values. The *grey/black bar* in the *bottom* represents the years 2006/2007, and the *banded bars* depict the Lusitanian toadfish's breeding season

results also conform to the pattern of shelter occupation in the intertidal zone in other teleosts with male parental care such as gobies or blennies of temperate coasts (e.g. Zander 1990; Almada et al. 1994). Males of these fishes migrate to the intertidal to defend nests that they use to receive spawns from multiple females and leave the area when the breeding season is over. Almada et al. (1994) suggested that in temperate areas, environmental pressures (e.g., caused by tides, temperature, food availability and other factors) permit

only seasonal utilization of nearshore, shallow water habitats. Hence, many species are found to migrate to deeper waters to search for suitable habitat and for better feeding sites. Likewise, in batrachoidids the periodic migration to shallow waters is likely explained by the availability of spawning substrate and appropriate temperature for reproductive behaviour and offspring development, and the

return to deeper waters at the end of the breeding season is driven by feeding and temperature needs (Gray and Winn 1961; Costa 2004).

The occupation of the artificial shelters placed in the second study site (AFB6) and the presence of offspring also followed a similar seasonal trend. The presence of eggs confirmed that intertidal shelters were being used as nesting places that are abandoned once reproduction is over. The decline in shelter usage and in the presence of eggs/embryos in the nests observed during the progression of the breeding season probably also reflected a decreasing number of males initiating reproduction since local fishermen continuously removed *H. didactylus* from the intertidal shelters at low spring tides at both sites. At AFB6, shelters were often found full of fresh eggs but with no occupant because males had been recently removed by recreational fishermen. Hence our data likely represents an underestimation of shelter occupation, especially at Cais do Seixalinho, where the confirmation of shelter usage could not be easily assessed by the presence of eggs.

In contrast with the intertidal zone, acoustic monitoring from the pier at Cais do Seixalinho showed that at least some adult male vocalizing Lusitanian toadfish remain in very shallow water and in close proximity to the shore throughout the year. Indeed, in shallow water sound attenuation is very high and boatwhistles would barely be recorded at distances greater than 4 m from the hydrophone (Amorim and Vasconcelos 2008; also see Fine and Lenhardt 1983). There were however similar seasonal trends both in subtidal acoustic activity and intertidal shelter usage. The increase of acoustic activity observed during the breeding season could reflect both an increase in sound production related to reproductive activities (i.e. a higher sound production rate per individual) but also a seasonal variation in fish density at the site. This last hypothesis is consistent with the registered acoustic activity being positively correlated with intertidal shelter occupation at Cais do Seixalinho suggesting a major migration to shallow waters at the start of the reproductive period. In a study with the gulf toadfish, *Opsanus beta*, Barimo et al. (2007) deployed artificial shelters in shallow waters (1–2 m of depth) and observed a similar seasonal pattern of shelter occupation with a full occupation rate (100%) in the reproductive season that markedly diminished to 20% in the non-reproductive season. The trend of shelter usage paralleled the one of offspring presence in shelters indicating that shelters were primarily being used as nests, although some animals still remained in the shelters in the non-reproductive season. Barimo et al. (2007) also found no seasonal variation for the abundance of gulf toadfish collected outside shelters, either on or buried in the sediment, consistent with our findings for the presence of Lusitanian toadfish in the subtidal zone in the non-reproductive period. Also,

Gray and Winn (1961) have observed that although most specimens of the oyster toadfish (*Opsanus tau*) move to deeper water in the winter, some fish stay in waters of less than 3 m deep during the non-reproductive season. Costa (2004) conducted a study in the Mira estuary (Portugal) based on trawl captures and found that the Lusitanian toadfish generally avoid depth of less than 2 m: juveniles prefer depth from 3 to 4 m and adults are usually found at depths higher than 5 m, with larger animals preferring deeper waters. The present study detected individuals all year round at Cais do Seixalinho from 2 to 6 m deep that were not juveniles since the emission of boatwhistles is characteristic of adult reproductive males (dos Santos et al. 2000) which in Tagus estuary corresponds to a minimum age of 6 years and body lengths larger than 35 cm TL (Pereira 2006). The presence and activity of adult males in the subtidal zone in the winter months probably reflect the fact that Montijo contains the highest densities of Lusitanian toadfish in the Tagus estuary (Cotter 2008) and the relatively high water temperatures registered in winter months of 2006/7 (Costa 2004).

Interestingly, boatwhistles are considered to be a mate attraction call, and its production is described to be restricted to the breeding season (e.g. Gray and Winn 1961; Bass 1996; Amorim et al. 2006). In the present study, boatwhistles were also heard well outside the reproductive season, suggesting they might have other social functions. Accordingly, Tavolga (1958) has also mentioned that the boatwhistle may also be produced outside the reproduction context in *O. tau*.

The Lusitanian toadfish occupied shelters as close as 10 cm apart. Although an average of less than one occupied shelter was found per 2-m segment, the variability in shelter occupation was quite high in both breeding seasons (CV = 123–155%), indicating that some segments had no occupied shelters and others had several. Also, there were no significant differences in the rate of shelter occupation among 2-m segments, i.e., there were no systematically preferred segments or others that were consistently avoided. Taken together, the results indicate that, in a given sampling date, nests were aggregated and that aggregation did not result from a lack of nesting places but probably from social attraction. Consistently, nest aggregation has been found in other batrachoidids, such as in *Porichthys notatus* (Bass 1996; Bass et al. 1999).

We have also observed tolerance for the presence of conspecifics inside the shelters. An average of ca. two fish was found per shelter, reaching a maximum of nine specimens in one shelter. The number of fish per shelter also did not differ among sampling dates, indicating that it did not decrease towards the end of the breeding season, when the quantity of breeding animals declines as revealed by the reduction in the occupied shelters (Fig. 2a). Our results also

demonstrate that an increase in the number of shelter occupiers was mainly caused by the presence of more small fish (size class A), which are likely females or sneakers, but also by and increase of size classes B and C, which are most probably parental males. In other words, shelters with several fish seemed to have often several females, or possibly sneakers, but also more than one type I male inside. This shows tolerance for females and for other males since they are not excluded from the shelters at least during low tide, and further supports the social attraction hypothesis. Tolerance of females in the nest during low tide is also common in batrachoidids especially because spawning may take up to several hours (Brantley and Bass 1994). Indeed, Barimo et al. (2004) found only parental *O. beta* males in nests (*n* = 41) but in three occasions (7%) the males were accompanied by one gravid female. Likewise, Gray and Winn (1961) sampled *O. tau* nests over two breeding seasons and found that 91% of occupied nests (*n* = 718) had a single fish that was typically a male, while the remaining nests had a pair of fish, sometimes with eggs. These two batrachoidid species seem to follow a different pattern of that found for *H. didactylus* since in these species parental males are typically alone in the nest or with one gravid female. However, in both aforementioned studies (Gray and Winn 1961; Barimo et al. 2004), small artificial structures were used as nests which might have limited the number of occupants. Tolerance for other males in the nests has been reported by DeMartini (1988), for *P. notatus*, who found a mean of 1.4 (max = 4) males in large artificial shelters and an average of ca. 1.2 (max = 3) males in natural shelters. This author also found more males per shelter when the available spawning area was larger; suggesting that when nests are large enough to accommodate clutches of more than one male, they tolerate the presence of other nest-holders, which could also be the case in *H. didactylus*. In these two batrachoidid species, a benefit from the presence of other males in the nest may possibly occur if they would attract more females or if they would cooperate in nest defence. For example, other fish species are known to tolerate satellite males when they cooperate in nest defence (e.g. Oliveira et al. 2002). In *H. didactylus* it is also feasible that other conspecifics are only tolerated at extreme low tides, when nests become exposed, and occupiers need to face extreme conditions of high water temperature and oxygen depletion. Patterns of nest aggregation and of tolerance for other conspecifics in the nest, especially males, deserve further investigation.

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