

Selection of coastal estuarine habitats by *Atherina boyeri* larvae in laboratory experiments

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Abstract

Coastal wetlands are important nursery areas for many fish species as they provide food, refuge from predators and optimal hydrodynamic conditions. The impact of anthropogenic and environmental changes on ecological functions of these areas need to be identified as a basis for establishing adequate ecohydrological measures for the long-term sustainability of the ecosystem and its associated services. Patterns of habitat selection were examined in wild-caught larvae of *Atherina boyeri* (Risso, 1810), by means of microcosms experiments. We simulated three habitats representative of vertical marsh profile at Guadiana wetland, non-vegetated bottom (sand) and two vegetation types (*Spartina maritima* and *Sarcocornia* spp.), in experimental units and monitored fish larvae spatial distribution by video-recording. We observed a strong avoidance of both vegetated habitats suggesting that habitat choice behavior may be dependent on external stimuli such as current velocity, predator effect and food availability. Similar experiments including external factors are required to unravel the behavioral responses of *A. boyeri* to different types of habitat.

Key words: wetlands; ecosystem functions; ecosystem services; nursery habitat; behavior; fish larvae.

1. Introduction

A site is presumably selected by organisms as a nursery habitat in response to proximate factors that allow them to optimize their net energy gain, as well as avoiding predators by minimizing interac-

tions with competitors. Accordingly, areas where hydrographic conditions favour: (1) the retention of planktonic eggs and larvae; (2) protection from predators; and (3) enhanced densities of food, are usually exploited by early age fish as nursery habitats (Baltz *et al.* 1993; Azeiteiro *et al.* 2006). Those

areas often correspond to vegetated shallow waters in estuaries or lagoons, characterized by strong turbidity and elevated concentrations of organic matter. Larvae fish benefit from shallow waters, avoiding the energetic costs of maintaining position in the water column, by swimming or other means of countering negative buoyancy. Additionally, turbidity in shallow waters may be important because it can provide protection from large piscivorous fish (Baltz *et al.* 1993) and the stable water levels can offer moderate water temperatures essential for the fish larvae growth (Winward 2000). Furthermore, because food availability is a limiting factor for fish survival during early phases (Pepin *et al.* 2007; Llopiz 2013), vegetated areas recognized as a carbon source for the production of invertebrate prey that is then used by fish (Nixon 1980) may allow developing larvae to grow rapidly into strong swimmers, thus helping them to avoid predators and resist being dispersed by currents (Faria *et al.* 2011).

Along with mangroves, coral and rocky reefs, salt marshes are recognized as important fish nursery habitats (Laffaile *et al.* 2000; Beck *et al.* 2001; Salgado *et al.* 2004; Franco *et al.* 2006; Veiga *et al.* 2006). Salt marshes occur in the upper part of the intertidal zone of estuaries, being permanently exposed to the effects of tides, and consequently, to a high variability in physical (temperature, salinity, oxygen, tidal currents and direction) and biological (predation and competition) factors. As a result, critical conditions are expected for the survival of organisms in this type of environment (Veiga *et al.* 2006). Nevertheless, partially due to their composition of grasses, herbs and low shrubs, salt marshes typically present a structural complexity (sites of refuge from predators) and a high productivity (enhanced foraging opportunities) (Beck *et al.* 2001) constituting an ecotonal zone that harbours high concentrations of fishes during their critical life stages (Laffaile *et al.* 2000; Salgado *et al.* 2004; Dolbeth *et al.* 2008; Vasconcelos *et al.* 2010). This way, enhanced growth and survival of early life history stages in nursery habitats will increase recruitment success (Baltz *et al.* 1993). Their relevance as nursery areas increases when they serve economically important fish species. It can be a direct influence, when marsh habitats provide refuge from predators to commercial juvenile fish species, or indirect, if marsh provides refuge from predators for prey of the commercial fish, increasing the production of prey species and their ultimate availability to the predators (Boesch, Turner 1984).

Loss of quality and function of salt marshes has become a serious problem. They are usually located in estuarine and coastal areas, the most populated regions all over the world (aprox. 60% of the world's population) and the cumulative impact of these people within the watershed surrounding salt marshes

can be significant (Wolanski *et al.* 2006). Impacts affecting salt marshes include all modifications in the ecosystem that alter natural regimes of water flow, sediment discharges and induce pollution. Those modifications can be caused by anthropogenic and/or natural phenomena. Anthropogenic factors include agricultural activities, ship/boat movements, sewage disposal, dredging, and changes in river discharges caused by dams while natural factors comprise alterations on coastal current dynamics affecting sediment supply and transport, changes in hydrological cycle, and climate variability (Elliot *et al.* 2007). Studying the anthropogenic pressures on estuarine fish nurseries along the Portuguese coast, Vasconcelos *et al.* (2007) found that the Guadiana estuary is mostly affected by dams, but still classified it under one of the lowest pressure score. Despite this low pressure classification, Chícharo *et al.* (2006) and Sá *et al.* (2006) pointed that a reduction in mean freshwater flow, sediments, nutrients and organic matter, as well as changes in the seasonal flow and decrease in extreme flow events due to Alqueva dam operation, may seriously contribute to the degradation of fish nursery habitats. Moreover, climate change scenarios, particularly for the Mediterranean area, predict increases in the duration and frequency of dry periods, leading to more retention of river waters by dams and consequently, posing more threats to those habitats (Morais *et al.* 2009). Ecohydrology is a management tool capable to provide low cost and sustainable solutions to human impact on the environment and thus, given the scenarios for the Guadiana salt marsh area, it might be the solution for its recovery and their associated functions (Plater, Kirby 2006).

A. boyeri is classified as an estuarine dependent species with common occurrence in the Guadiana estuarine wetlands (Veiga *et al.* 2006). Similarly to many European estuaries, the tidal creeks of salt marshes in the Guadiana are dominated by 0-group fish of this ecological category, i.e., resident species that spend their entire lives in the salt marsh habitat (Sá *et al.* 2006). These larvae are eurythermal and euryhaline which enable them to exploit the high primary and secondary productivity of wetland environments, which are also characterized by important short-term fluctuations of abiotic factors to which no fish are specifically adapted (Laffaile *et al.* 2000). Gisbert *et al.* (1996), based on the analysis of stomach contents of *A. boyeri* captured on the Canal Vell Lagoon (Ebro Delta, North-east Spain), observed that during larval stages, these fish mainly feed on small prey such as copepod nauplii, copepodites and rotifers. *A. boyeri* is commercially exploited in fisheries along the Spanish, Italian and French coasts (Andreu-Soler *et al.* 2003; Mauro *et al.* 2007) although in Portugal it is mainly exploited for recreational purposes (Veiga *et al.* 2006).

A better understanding of the nursery functions of salt marshes, in particularly for important commercial fish species and their prey is, at least, expected to promote environmental awareness on wetland conservation. Ultimately, we hope that the outcome from this and similar studies will provide valuable information for decision makers react with appropriate measures to improve conservation and management of these areas.

In this study we investigated in the laboratory experiment the attractiveness of two intertidal vegetation common species in Guadiana salt marshes for *A. boyeri* fish larvae. It was hypothesized that newly fish would select vegetated habitats. This is the first study to investigate whether *A. boyeri* larvae select from habitat types such as salt marsh (*Spartina maritima*, *Sarcocornia* spp.) and non-vegetated bottom (sand).

2. Materials and methods

2.1. Experimental organisms

Wild-caught larvae of *A. boyeri* (14.9 ± 1.7 mm, SL) used in the experiments were seined from salt marsh meadows surroundings in Ria Formosa lagoon. The larvae were acclimated to laboratory conditions for one week before trials. They were maintained in 70L fibreglass aquaria supplied with filtered seawater (38 ± 0.2 ppm salinity; $23.7 \pm 0.4^\circ$ temperature) pumped from Ria Formosa lagoon, Praia de Faro, Portugal, under a photoperiod regime of 12 h light: 12 h dark. The fish were fed daily, at *ad libitum*, with a mixture of rotifers and *Artemia* nauplii.

2.2. Experimental design

Tests were performed in microcosm units adapted from Stunz *et al.* (2001). They were constructed from 2 dm^3 glass aquaria ($21 \times 15 \times 12$ cm) filled to a depth of 10cm with filtered seawater. A 2 cm height polystyrene plate was placed on the bottom of each aquarium. Water in the microcosms was maintained at salinities of 38 ± 0.2 ppm, temperatures of $23.6 \pm 0.3^\circ$, dissolved oxygen levels of $4.4 \pm 1.1 \text{ mg dm}^{-3}$, and total ammonia nitrogen (TAN) levels of $0.14 \pm 0.08 \text{ mg dm}^{-3}$.

Three common natural habitat types and one artificial reference habitat were simulated in experimental microcosms: (Sm) *Spartina maritima*; (Ss) *Sarcocornia* spp.; (Sd) sand; and (Bc) blank control (polystyrene plate). *Spartina maritima* and *Sarcocornia* spp. were chosen as natural habitats owned to their frequent occurrence in the Guadiana salt marshes. Accordingly to Simonson (2006), that salt marsh area is dominated by colonies of the cord-grass *Spartina maritima*, frequently submersed in tidal water at the edge of the creeks, passing through

the *Sarcocornia* and *Halimnion portucaloides* in the mid-marsh, to the plants of the upper marsh such as *Arthrocnemum*, *Suaeda vera* and *Limoniastrum monopetalum*. In a total area of 4605ha, *Spartina maritima* is present in 2.7 ha and *Sarcocornia* in 559.1 ha. Although a large proportion of the marshes are affected by the presence of the highly competitive exotic grass *Spartina densiflora*, the two selected species are important native vegetation, particularly in the lower and mid marsh, easily accessible to fishes (Simonson 2006).

Microcosms were divided in half, one side was occupied by a natural habitat type and the other half by the blank control. In total four possible combinations were simulated: (1) Sm vs Bc; (2) Ss vs Bc; (3) Sd vs Bc; and (4) Bc vs Bc. Placement of the microcosms in the room and of the habitat types within each microcosm were randomized. Salt marsh habitats (Sm and Ss) were constructed by collecting *Spartina maritima* and *Sarcocornia* spp. culms from Ria Formosa lagoon. Culms were washed of sediment in the field and brought to the laboratory where plants were dipped in freshwater. Subsequently, leaves and branches were wiped to remove epiphytic growth. Plants were held in place by pressing the culms through the polystyrene plate. Densities for each species were estimated from natural vegetated areas in Ria Formosa lagoon: *Spartina maritima* (1270 stems per square meter) and *Sarcocornia* spp. (3810 stems per square meter). The sand (Sd) habitat consisted of washed commercial sand and the blank control (Bc) of a polystyrene plate.

To minimize confounding factors on habitat selection, larvae were starved and no oxygen was provided during the 6h recording sessions in the experimental microcosms. Oxygen levels never dropped below 3.2 mg dm^{-3} .

2.3. Habitat selection assays

To test the hypothesis if fish larvae select among the experimental habitats, we set up eight microcosms, two replicates for each one of the four possible combinations. We ran the trial three consecutive times (2 replicates \times 3 runs = 6 replicates). For each trial, five larvae were randomly assigned to the microcosms and monitored for any abnormal behaviour for 1 h. After 1 h acclimation period, larvae behavioral responses were monitored during 6h from 10 a.m. to 4 p.m., by video recording. Larvae in the microcosms were replaced with new individuals, never reusing the same fish larvae in more than one trial. We used four video cameras connected to a switch with each camera recording two adjacent aquaria in 30sec frames. Video images were recorded in DVD's and stored for posterior image analysis. Spatial distribution was estimated by counting the number of fish larvae on each habitat, at every 30 seconds frame. This resulted in 30 counts

per hour and a total of 180 counts per session for each aquarium. Counts were transformed in percentages of occurrence. Differences in mean percentage of occurrence were tested based on 6 replicates per habitat type using a student's paired t-test.

3. Results

Percentage of occurrence in each habitat over the 6 hours of recording time were fairly stable suggesting that larvae were completely acclimated to the test units after 1 h and that food and oxygen privation were not limiting factors for their behavioral choices during the entire recording period (Fig. 1).

T-test revealed that *A. boyeri* showed no significant selection for sand-bottom (Sd) over blank control (Fig. 2). In the reference microcosm units, where no habitat choice was available (Bc vs Bc), an homogeneous distribution within the aquarium area was observed (Fig. 2). Additionally, wild-caught *A. boyeri* larvae showed strong avoidance for structured habitat types (Fig. 2), significantly avoiding *Spartina maritima* (Sm) and *Sarcocornia* spp. (Ss) over blank control (Bc). Avoidance of Sm (99.76%) was even higher than Ss (94.74%) (Fig. 1 and 2).

4. Discussion

It is well documented in other studies that salt marshes are valuable nursery habitats providing refuge from predators, enhanced food opportunities and optimal hydrographic conditions (Baltz *et al.* 1993; Laffaile *et al.* 2000; Salgado *et al.* 2004; Azeiteiro,

et al. 2006; Dolbeth *et al.* 2008; Vasconcelos *et al.* 2010). There are several *in situ* faunal assessment studies showing that larvae and juvenile fish, including *A. boyeri*, are more frequently associated with salt marshes than with unvegetated habitats. For example, Franco *et al.* (2006) confirmed the importance of marsh sites as nursery habitats by observing higher abundances of small-sized individuals of three species, including *A. boyeri*, in the salt marsh creeks of Venice Lagoon. In the Guadiana estuary, Faria *et al.* (2006) found that *Atherina* spp. larvae were among the most abundant captured species with higher concentration in the lower estuary where salt marsh areas dominate. Also Veiga *et al.* (2006) showed that *Atherina* spp. were recorded year-round in Castro Marim salt marsh, being the second most abundant species of juvenile fish caught. Although, studies done in simulated ecosystem are recognized an useful method to evaluate the impacts of habitat structure on the early life stages of fish (Bilkovic *et al.* 2002), relatively little research on habitat choice by fish larvae has been done in laboratory microcosms. To our knowledge no such studies have been done with larvae of *A. boyeri*, although, a few experiments have been conducted on other larvae and juvenile fish species.

In opposition to our results, Stoner *et al.* (2003) observed a high positive selectivity for habitats with structure over smooth sand substratum on age-0 flatfishes (Pacific halibut and Northern rack sole) while Stunz *et al.* (2001) showed significant selection for structured habitats by wild-caught red drum juveniles. Nevertheless, in agreement with our findings, Timmons (1995) observed juvenile fish avoidance of habitats with high macrophyte density,

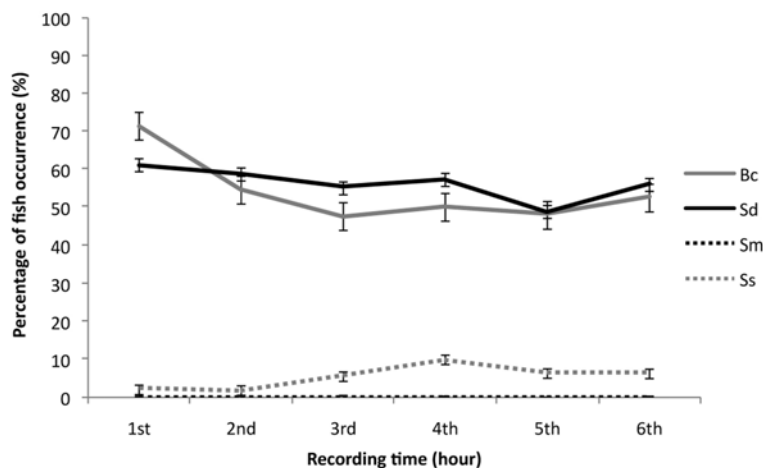


Fig. 1. Percentage of occurrence (Mean \pm SE) of wild-caught *A. boyeri* larva in each habitat type at 1 hour intervals. Black continuous line depicts percentage of larvae in the sand (Sd), grey continuous in the blank (Bc), dashed black in *Spartina maritima* (Sm), and dashed grey in *Sarcocornia* spp. (Ss) substrate. Means and SE's were calculated from 6 replicates.

in laboratory experiments. In the former case, structured habitat avoidance was justified by the anoxic events associated with high vegetation density. Nevertheless, considering that anoxic events never occurred and that there were no significant differences on oxygen levels among our microcosm units, vegetation density is unlikely to have been the cause for avoidance. Previous studies have also showed some evidence that plant stems may increase visual barriers and restrict fish movements. Manderson *et al.* (2000) and Tatrai

and Herzig (1995) observed reduced predation success by juvenile fish in structured habitats probably due to visual barriers caused by macrophytes. These observations were taken in larger fish than our larvae and, therefore, it seems unlikely that plant stems may have caused movement restrictions leading to avoidance of vegetated habitats in our study.

In other studies, no habitat selection was detected in single variable experimental unit tests. Burke (1991) only detected preference for mud substratum by southern flounder when prey was available and Stunz *et al.* (2001) showed that hatchery-reared red drum only responded to habitat selection in the presence of a predator. This suggests that habitat selection is a multifactorial problem and that “substrate type” is only part of a complex interaction among biotic and abiotic factors involved in habitat selection.

Isolated effect of environmental factors on fish behavior, in practice, can only be studied in laboratory small-scale experiments. However, as most of these factors co-vary, any behavioral response expected in natural environment will be a response to changes in the suite of factors and not just a single one of them. Therefore, extrapolating fish behavior from laboratory to nature may lead to erroneous predictions regarding fish behavior in natural settings, and thus, interpretation of results must be cautious (Baktoft 2012). In this study, a single factor behavior experiment was conducted and results contradicted our initial hypothesis. Fish larvae strongly avoided both flooded spartina (Sm) and marsh-edge sarcocornia (Ss) habitats simulated in microcosm units. Our findings suggest that *A. boyeri* larvae probably need external stimuli to make use of vegetated habitats. Similar experiments including other factors, such as: predation, food availability and flow speed are required to unravel the behavioral responses of this species to different types of habitat.

This type of investigations, enhancing the understanding of the nursery function of wetlands, are of utmost importance to provide valuable information to environmental managers and decision makers in order to adopt ecohydrological solutions for the recovery of endangered wetland areas.

Acknowledgements

This work was funded by the Ph.D. scholarship granted to Renata Gonçalves by Fundação para a Ciência e Tecnologia (SFRH/BD/47985/2008). The authors would like to acknowledge

Prof. Doctor Eduardo Barata for kindly providing all the video recording equipment. We also thank to Tiago Rocha for help with laboratory work. We are also grateful to the anonymous reviewers who greatly improved earlier drafts of this manuscript.

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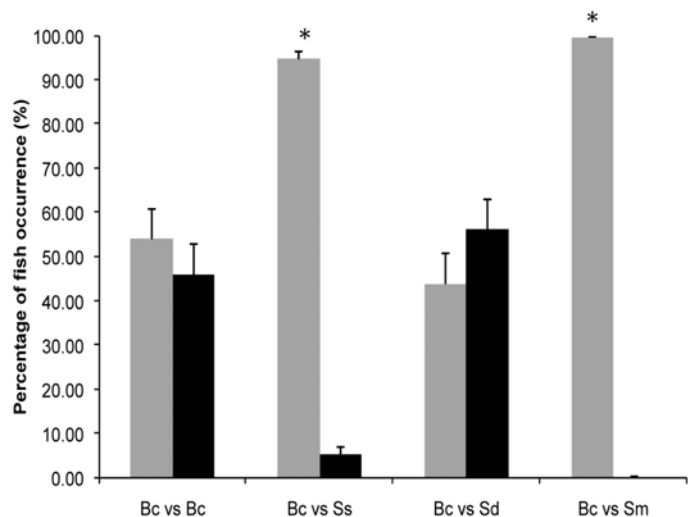


Fig. 2. Percentage of occurrence (Mean \pm SE) of wild-caught *A. boyeri* larvae in each habitat type for all possible habitat comparisons, grey bars refer to blank control and black to substrate side of microcosm units (Blank control, Bc; *Sarcocornia* spp., Ss; Sand, Sd; *Spartina maritima*, Sm). Each comparison represents six replicates. Significant results from paired Student's t-tests are indicated by * = $p < 0.001$.

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