Article

Is *Fundulus heteroclitus* Intrusion Depleting Winter Marsh Pools of Native Fish Species?

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Abstract: This work updates the characterization of winter fish communities in salt marsh areas of Guadiana estuary (SE-Portugal) and discusses the potential risks of habitat dominance by a non-indigenous species (NIS). To this effect, six field campaigns were carried out during winter season targeting the collection of fish species. Individuals from seven different families (marine and estuarine) were collected although the community was dominated by two estuarine species – the native *Pomatoschistus sp*. (goby) and the NIS *Fundulus heteroclitus* (mummichog). Goby controlled the majority of salt marsh habitats, except one specific, marsh pool, where extreme environmental conditions were registered, namely high temperature and salinity. Such conditions may have boosted the intrusion of mummichog in this area. This species is well adapted to a wide range of abiotic factors enabling them to colonize habitats where no predators inhabit. Impacts of mummichog intrusion in the Guadiana salt marsh area are still unpredictable since this is the first recorded in such high density. Nevertheless, in scenarios of increased anthropogenic pressure and, consequently, habitat degradation, there is a potential risk of mummichog spread to other habitats and therefore compete for space and food resources with native species.

Keywords: Guadiana estuary; salt marsh; non-indigenous species (NIS); anthropogenic pressures; habitat degradation.

1. Introduction

Worldwide, estuarine areas and their associated salt marsh habitats are described as highly productive and valuable aquatic ecosystems [1]. Due to high levels of primary production, large reserves of organic matter and habitat diversity, these areas are considered biochemical hotspots that offer optimal conditions for numerous birds and aquatic species [2]. These systems provide potential advantages for the growth and survival of young fish, namely high prey availability and refuge from predators [1] and, consequently, support the offshore stocks of economically valuable species [3,4]. The Guadiana estuary is no exception to this general observation since it provides an exceptionally suitable environment for fish spawning, breeding, feeding, and growth not only for estuarine but also economically important marine species, such as sardine *Sardina pilchardus*, seabream *Diplodus* sp., and sole *Solea* spp. [5,6].

Over the years, estuarine and coastal areas became increasingly affected by anthropogenic activities such as urbanization, industrialization, and tourism [7]. Also, these ecosystems have experienced degradation caused by climatic changes, namely high temperatures and low precipitation [8]. The completion of the Alqueva dam located 150 km from the Guadiana River

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mouth promoted a reduction in freshwater inflow leading to the degradation of salt marsh vegetation in the lower Guadiana estuary [9]. Moreover, climate change scenarios, predict for this region an increase in temperature, length, and frequency of dry periods [8]. Predicted lower precipitation will potentiate retention of river waters and sediments by upstream dams and consequently the degradation of water quality and lack of sediment for plant accretion on salt marsh areas of the lower Guadiana estuary.

The resulting degradation observed in salt marsh habitats *per se* are a threat to associated fish species, but also increase the risk of invasion by exotic opportunistic species [10]. In the Guadiana estuary, a community shift has been already documented, including plankton and fish [9,11,12]. In addition, first occurrences of several marine invasive species have also been recorded, with potentially detrimental effects on native biota [13]. In 2008, the invasive species *Blackfordia virginica* (cnidarian) and *Palaemon macrodactylus* (caridean shrimp) were first observed within the Guadiana estuary [13]. For both species, there is the potential competition for space and resources with native species such as *Sardina pilchardus*, *Engraulis encrasicolus*, *Pomatoschistus* sp., *Solea* sp., *Diplodus* sp., *Syngnathus* sp.. Although the sources of introduction for *B. virginica* and *P. macrodactylus* to the Guadiana Estuary remain unknown, previous research has shown that reduction of variability of river flow in estuaries has facilitated the establishment of NIS species [14].

Occurrences of Fundulus heteroclitus (mummichog) are well documented in South of Spain but remain poorly documented in the Guadiana [5,6]. Mummichog is described as an opportunistic species due to its high tolerance to extreme and highly variable environmental conditions, namely high salinity and a wide range of temperature [15] which makes mummichog a potential candidate to invasive species.

The objectives of this work are: (a) to characterize winter fish communities in salt marsh areas of Guadiana estuary and their occurrence variability according to tide regime; (b) investigate the effect of environmental factors on fish species distribution; and (c) discuss the potential risks of habitat dominance by mummichog, an NIS species.

2. Results

2.1. Composition of fish communities

A total of 2550 individuals from seven different families were collected from Guadiana estuary salt marshes. Among these, four were estuarine residents (goby, *Pomatochistus* sp.; mummichog, *Fundulus heteroclitus*; sand smelt, *Atherina* sp., and pipefish, *Syngnathus* sp.). One species was marine seasonal (sardine, *Sardina pilchardus*), the other two were marine juveniles (seabream, *Diplodus* sp. and sole, *Solea* spp.). Catches were primarily dominated by the estuarine resident goby and secondarily by mummichog, which collectively made up more than 99% of the total catch.

Marine species were caught at larval stages, except the sole that was also caught in the juvenile stage. The estuarine resident pipefish was caught at the juvenile stage, goby at both larvae and juvenile and mummichog at all stages (from egg to adult). The majority of the captured gobies were juveniles (87%) and only 13% were larvae. Mummichog catches were dominated by larvae (91%) with only 9% of juveniles (Table 1, Figure 1).

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Table 1. List of captured fish species during the study period in the lower Guadiana estuary salt marsh areas. N: total number; F (%): frequency; % of larvae; % of juveniles; Mean size (mm); Size range (mm); Spatial occupancy: MC "main channel", PIC "principal intertidal creek", SIC "secondary intertidal creek" and MP "marsh pool".

Species	N	F (%)	Larvae (%)	Juveniles (%)	Mean size (mm)	Size range (mm)	Spatial Occupancy
Atherina sp.	1	0.04	-	100.0	36.0	-	MC
Diplodus sp.	1	0.04	100.0	-	16.0	-	PIC
Fundulus heteroclitus	479	18.93	90.6	9.4	12.1	1.6 - 52.0	MP
Pomatoschistus sp.	2043	80.75	13.0	87.0	27.1	7.0 - 47.0	MC,PIC, SIC,MP
Sardina pilchardus	2	0.08	100.0	-	27.0	26.0 - 28.0	MC, SIC
Solea spp.	3	0.12	66.7	33.3	13.2	6.5 - 26.0	MC
Syngnathus sp.	1	0.04	-	100.0	92.0	-	MP

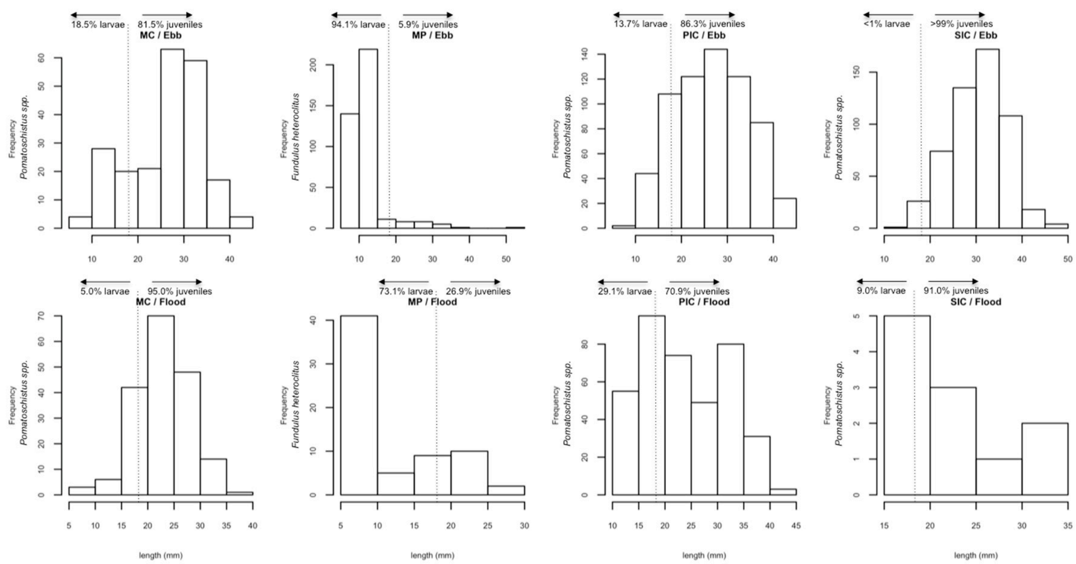

Figure 1. Size and frequency distributions of the two most abundant species per site and tide. Dotted vertical line indicates length at metamorphosis of each species (*Pomatoschistus* sp.: 17 mm; *Fundulus heteroclitus*: 18 mm).

2.2. Fish distribution among habitats

Overall, fish density was always higher during ebb tide except for MP habitat. PIC and SIC were the habitats with higher densities during the ebb tide. However, fish density in SIC abruptly dropped during the flood (Figure 2) and this habitat presented the lowest percentage of fish larvae (Figure 1). The analysis of variance performed with density data indicated that goby distribution varied significantly between habitats nested with the tide (two-way ANOVA: habitat x tide $p<0.001$, $df=3$, Table 2 and 3, Figure 2). The largest differences were found between MP and the remaining habitats. MC, PIC, and SIC were heavily used by fish during ebb tide. MC and PIC were the most and SIC the least used habitats during the flood. Gobies were present in all habitats while mummichog was exclusively observed in MP (Figure 2) justifying the extremely low habitat overlap between the two

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species in both tide stages (Opf = 0.0036 in the ebb tide and Opf = 0.0107 in the flood). Moreover, marine species were never observed in MP habitat (Table 1).

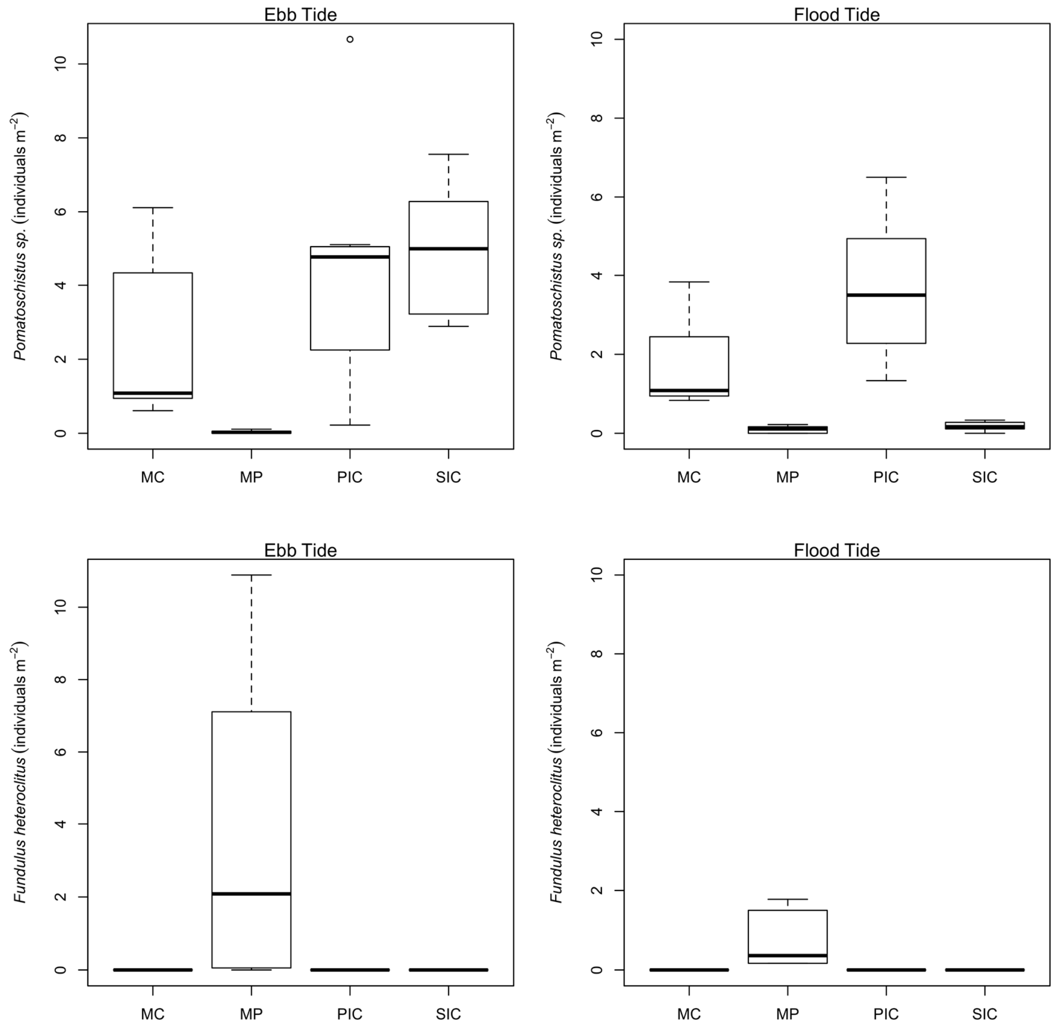

Figure 2. Box plots of fish density (no. individuals m^{-2}) of *Pomatoschistus* sp. and *Fundulus heteroclitus* per site and tide captured in the lower Guadiana estuary salt marsh area. The box includes observations from 25th to the 75th percentile, the horizontal line within the box represents the median value. Lines outside the box represent the 10th and 90th percentiles.

Table 2. Results of two-way factorial ANOVA performed on the fish density (no. individuals m^{-2}, squared root transformed) of the most abundant species (*Pomatoschistus* sp.) using habitat and tide as fixed factors. Level of significance: *p<0.001.

	df	Mean square	F ratio
Habitat	3	7.333	40.59 *
Tide	1	4.098	22.68 *
Habitat x Tide	3	2.243	12.42 *
Error	40	0.181	

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Table 3. Results of Tukey (HSD) test for habitat comparisons in each tide state. Levels of significance: ns, not significant; ***p<0.001; **p<0.01; *p<0.05.

Tide	Habitat	P value
Ebb	MC vs PIC	0.009 **
	MP vs PIC	0.000 ***
	SIC vs PIC	0.999 ns
	MP vs MC	0.000 ***
	SIC vs MC	0.041 *
	SIC vs MP	0.000 ***
Flood	MC vs PIC	0.236 ns
	MP vs PIC	0.000 ***
	SIC vs PIC	0.000 ***
	MP vs MC	0.005 **
	SIC vs MC	0.020 *
	SIC vs MP	0.999 ns

2.3. Environmental parameters

Maximum water temperature was recorded in the MP habitat during the ebb (17.1ºC) and minimum in the PIC during the flood (13.3ºC). The temperature ranges within MC, PIC, and SIC was very similar. The patterns of temperature variation among sites were analogous in both tidal stages with a slight decrease in temperature observed during the flood (Figure 3). There was a negative correlation between temperature and goby larvae density, and a positive correlation between temperature and mummichog (larvae and juveniles) density (Table 4).

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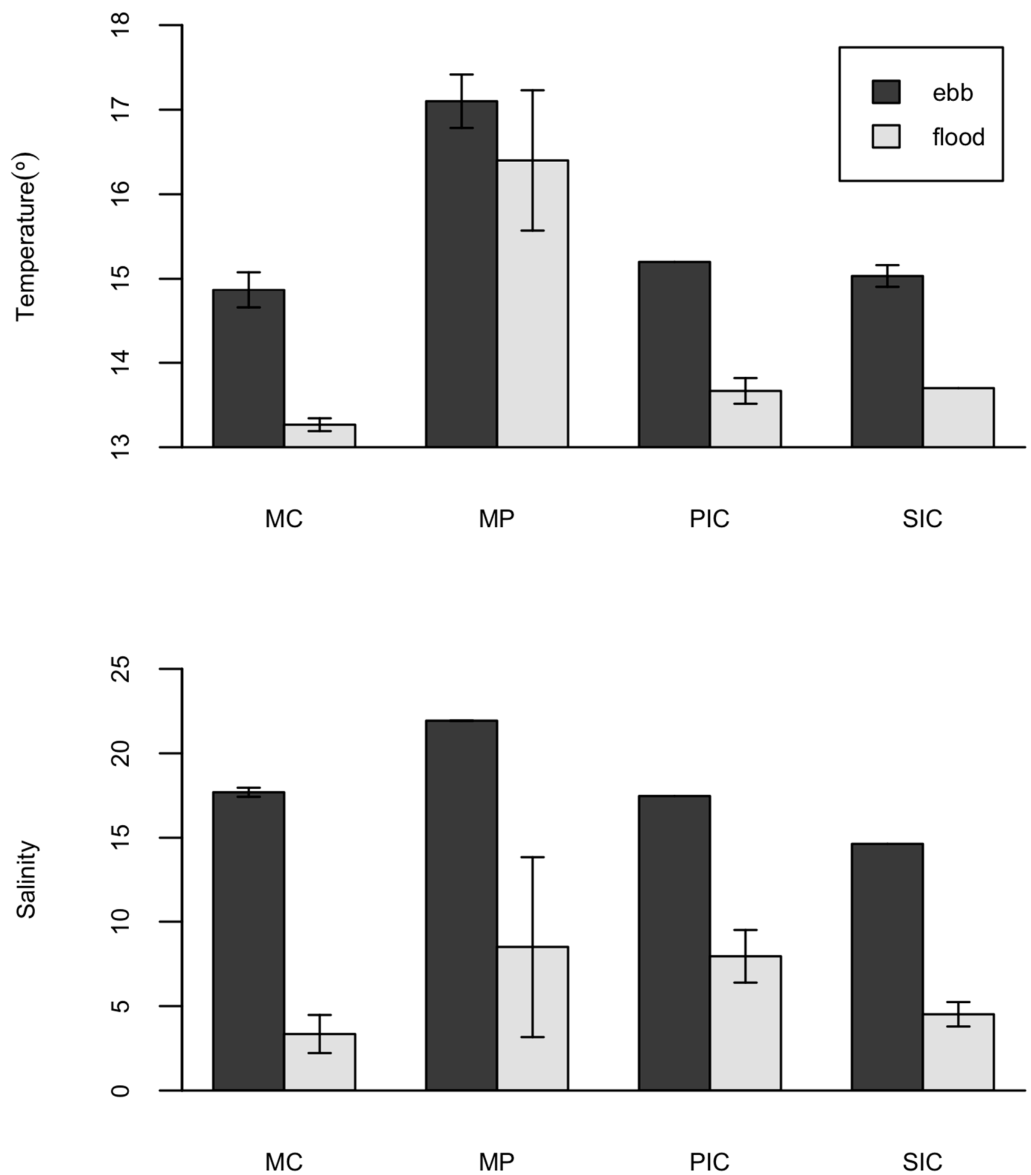

Figure 3. Spatial and tidal variation of temperature and salinity measured at each sampling site during the study period in the lower Guadiana estuary salt marsh areas.

Table 4. Results of Spearman correlation indices (rho) between larvae and juvenile densities of the two most abundant species and the environmental variables. Level of significance *p<0.05.

	Density of *Pomatoschistus* sp.		**Density of *Fundulus heteroclitus***	
	Juveniles	**Larvae**	**Juveniles**	**Larvae**
Temperature (º)	-0.244	*-0.332**	*0.564**	*0.517**
Salinity	-0.105	-0.143	0.060	0.211
Dissolved Oxygen (mgL-1)	-0.208	-0.133	-0.084	-0.137
Cha (mgL-1)	0.109	0.214	0.160	0.031
Ammonium (mgL-1)	0.017	-0.103	0.048	-0.131
Nitrate (mgL-1)	*0.467**	*0.645**	*-0.508**	*-0.703**
Nitrite (mgL-1)	0.190	*0.493**	-0.185	-0.213
Phosphate (mgL-1)	*0.489**	*0.361***	*-0.310**	-0.250

Salinity ranged between 3 and 22 with significantly higher values during ebb. A peak of salinity, as for temperature, was also registered in the MP habitat in both tides (Figure 3). There was no correlation between salinity and fish density (Table 4). Dissolved oxygen (DO) concentration ranged between 5.6 and 9.3 mgL^{-1} with higher values during the flood. The highest values (2.53 mgL^{-1}) of chlorophyll *a* (Ch*a*) were registered in the MC habitat during ebb tide and the lowest values (1.10 mgL^{-1}) in SIC also during ebb tide. There was no correlation between fish density and both DO or Ch*a*. Maximum of all nutrients concentration occurred in the PIC and minimum in the MP habitat except for nitrite (Figure 4). Nitrate concentration was extremely low in the MP during ebb and flood (0.065±0.025 and 0.050±0.022 mgL^{-1}, respectively). Goby juvenile's density was positively correlated with nitrate and phosphate and goby larvae with nitrite, nitrate, and phosphate. There was a negative correlation between mummichog (larvae and juvenile) density and nitrate. Mummichog juveniles were negatively correlated also with phosphate (Table 4).

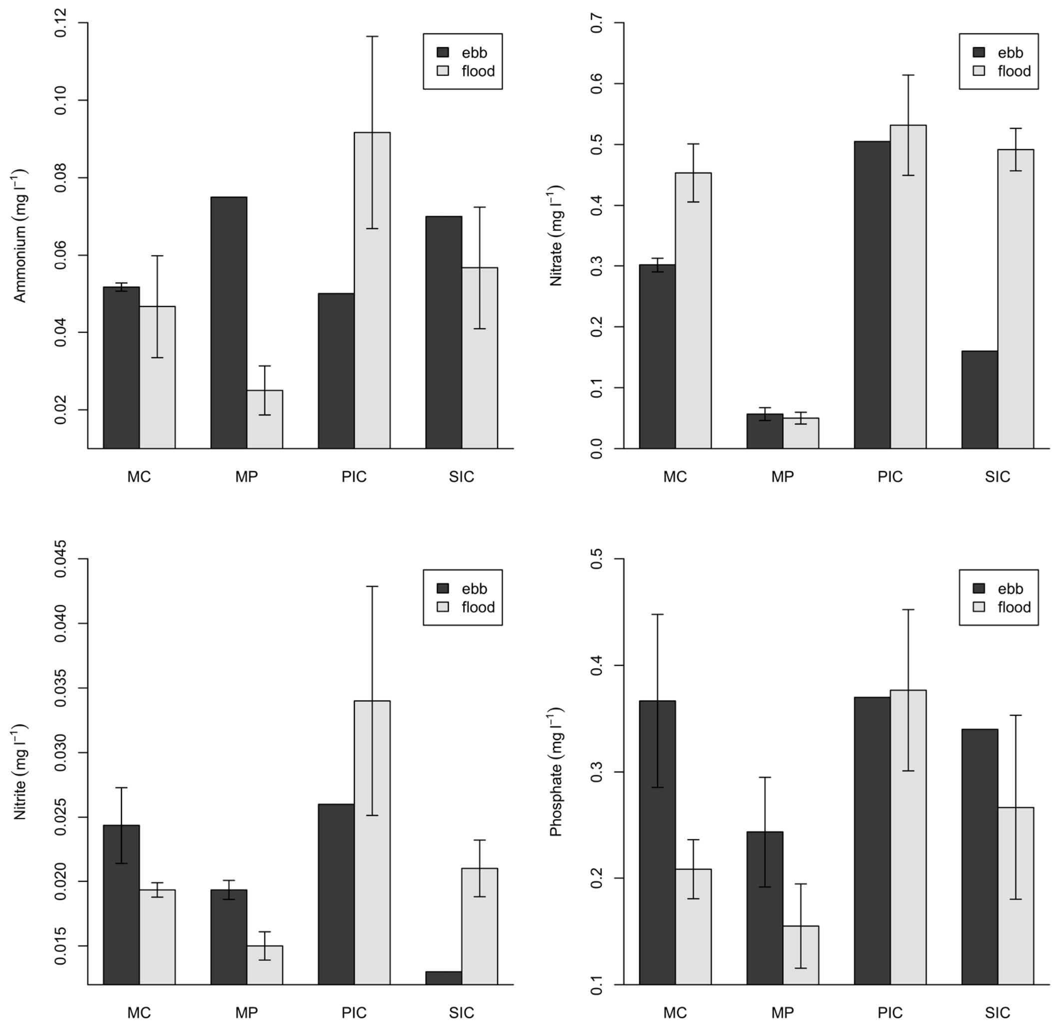

Figure 4. Spatial and tidal variation of macronutrients concentration (ammonium, nitrite, nitrate and phosphate) measured at each sampling site during the study period in the lower Guadiana estuary salt marsh areas.

3. Discussion

For the first time, the occurrence of mummichog in such high densities is reported in South Portugal and their potential risk for native species discussed. The present study was performed during winter months as it is guaranteed there is no overestimation of fish habitat colonization. Thus, fish density and diversity registered herein were relatively low, as expected for this season and region [5,6] and in other salt marsh areas of Portugal [16], North Europe [17,18] and United States [19,20]. All the seven fish genera captured had been previously observed in the study area [5,6] although mummichog in very low densities [5]. Goby and mummichog, two estuarine residents, the first being native and the later NIS, were the two dominant species. Overall, the degree of habitat overlap between this two species was very low in both tidal stages, as shown by the low values of Pianka's index. This indicates that the two species occupy different habitats with the NIS mummichog being exclusively present in an isolated habitat (MP). Gobies are among the most abundant genera all over European salt marshes [6,18,21], and are particularly successful in temperate estuarine environments [22]. The mummichog was introduced in southwestern Iberian Peninsula in 1970's apparently due to cross-contamination of ballast water of ships coming from the USA [23]. There are records of mummichog in southwest Spain [15], however, occurrences of mummichog in Portugal are poorly documented. [5] recorded their presence between 2001 and 2002 in Guadiana salt marsh subtidal creeks but as low as one individual in the total catch. In the salt marshes, marine species were mainly caught at larval stages, while estuarine residents occurred at both larval and juvenile stages. These results indicate that marine species use salt marshes mainly as a nursery area while estuarine residents depend on salt marshes during their entire life cycle [25].

Tides affected goby density in MC, PIC and SIC habitats in a similar way, with higher densities registered during ebb. [26] developed a model simulating current velocities in the lower Guadiana estuary for both tidal stages. Results indicate velocities of 5.3 cms-1 (SIC) and 12.9 cms-1 (MC) during the ebb and 8.8 cms-1 (SIC) and 15.8 cms-1 (MC) during the flood. Lower velocities in the ebb may facilitate fish spread throughout sampled salt marsh habitats especially for the younger of the year. In fact, changes in fish density according to the tide stages occur due to the movement of fish from permanently inundated areas towards the inundated intertidal areas with the flood tide [16]. It should be mentioned that even The presence of fish larvae in all sampled habitats, not only estuarine but also marine species, indicate an active habitat selection capacity since an early life stage. Previous studies performed in some of the fish genera caught during this investigation report larvae swimming speeds (Table 6) in the range of simulated current velocities by [26] supporting our hypothesis. It should be noted that although the model developed by [26] was calibrated with data recorded after the construction of the Alqueva dam, it is not guaranteed that similar velocities occurred during samplings. Results show that despite inter- and subtidal salt marsh creeks are not continuously available habitats, they play a major role not only for estuarine dependent but also marine species, and for both larvae and juvenile fish.

Table 5. Critical swimming speeds (Ucrit) of four marine fish larvae species.

Species	Ucrit range (cms^{-1})	Size range (mm)	Reference
Sparus aurata	3.0 – 19.3	6.2 – 14.1	[27]
Atherina presbyter	3.6 – 18.7	6.6 – 21.0	[28]
Solea senegalensis	0.0 – 5.0	3.5 – 7.5	[29]
Sardina pilchardus	1.6 – 9.5	7.9 – 23.4	[30]

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MP was the most dissimilar habitat, both in terms of fish composition and environmental parameters, presenting the highest temperatures and salinities and lowest macronutrients concentration, namely nitrates and phosphates. Extreme abiotic conditions registered were caused by the lack of water renewal since the MP is only partially flushed during high tides. Mummichog inhabits a wide range of salinities but prefers the most saline sites, usually above 25 [15,24,31]. They present a great euryhaline range, covering 0 to 128, [32]. Altogether with their wide thermic acclimation range, this species is able to colonize new habitats with great success [31]. MP was also the habitat with the lowest concentration of macronutrients, in particular phosphates and nitrates. According to [33], phosphate and nitrate concentrations registered at MP indicate a low impacted area, in opposition to the other three habitats (SIC, PIC, MC) that presented typical values of moderate to high eutrophic sites. Nutrient enrichment is known to stimulate primary production causing a bottom-up enrichment of the food web, fostering increased fish biomass and body size [33]. Particularly, nitrogen and phosphorus enrichment stimulates benthic algae [34], which in turn stimulates infaunal and epibenthic invertebrates [35]. Benthic algae, infaunal and epibenthic invertebrates all serve as food resources for most estuarine dependent fish species [33]. However, a nutrient over-enrichment can have deleterious consequences, namely a decrease in dissolved oxygen leading to a reduction in fish growth rates [33]. Fish such as gobies, that make use of eutrophic environments, are not likely to stay long enough to experience the negative effect of hypoxia on their growth. As observed by [36], they invade the salt marshes through tidal creeks, forage there for up to a few hours and swim back at the ebb. Such habitats are available for a limited time dependent on tides. Instead, for the short periods, they colonize salt marsh creeks and main channel edge and benefit from the high availability of food, probably influenced by the high concentration of macronutrients [35]. Accounting on the lower concentration of macronutrients observed in MP, also a lower stimulation of primary production and consequently less food available may be expected for this habitat which might explain the lower occurrence of gobies.

Gobies are described as opportunistic carnivores feeding on prey according to its availability. Most important prey items in their diet are polychaetes, mysids, isopods and decapods [22]. Mummichog is also an opportunistic species but omnivore. Their diet is mainly based on amphipods, isopods, and snails [33]. There is some overlap on feeding preferences of both species but mummichog is highly flexible easily adapting to a more herbivory diet (plant tissues) in case of animal prey reduction [33].

Mummichog growth is quite fast, being able to reproduce within the first year of life. Their eggs are also quite big and the post-hatched larvae start with great advantages due to their size [24]. Such characteristics provide this fish species an opportunistic life-history strategy effectively adapting to habitats with extreme environmental conditions as observed in MP. Although goby is described as a widespread species, relatively tolerant to fluctuations in environmental conditions [37], the establishment of mummichog in MP suggests that this species is more competitive in this type of habitat [15]. In fact, the majority of the studies observed that mummichog is occupying extreme habitats (empty niches) not previously used by native fish species [24,31,38] as it seems to be the current scenario in Guadiana salt marshes accounting on the low degree of habitat overlap (Pianka's index) between gobies and mummichog. However, a species with such an expansion capacity, along with its productivity, must have a great influence on the local fish populations. [31] reported that the expansion of mummichog in South-western Spain has already negatively affected some native endemic species like the endangered *Lebias iberia*.

At least 35 NIS fish species have been introduced into the Iberian Peninsula in the last century and, although not all of them prospered, most are now widespread in this area especially linked to degraded environments [39]. Extensive urban development has occurred in the Guadiana River basin over the past century: the consequent reduction in river flow contributed to decreases in water quality [40]. The presence of mummichog does not necessarily imply that a successful invasion has occurred. We did, however, find specimens over a wide range of sizes (1.6 – 52.0 mm) and development stages that imply local reproduction. As so, mummichog must be classified as an NIS species in Guadiana salt marsh area, i.e. a species introduced outside its natural distribution that might survive and

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subsequently reproduce. Not all NIS species turn into invasive defined as species with the potential to cause native species extinction, modify ecosystem processes and act as disease vectors [41]. However, some species out of their natural habitats lose their natural predators or control agents. As a result, they are able to increase to levels which are potentially detrimental to the native environment [41].

Mummichog establishment was recorded in an isolated and low attractive area for native species due to extreme environmental conditions registered. However, similar to what is happening in areas relatively close in South Spain, Guadiana salt marsh habitats are facing the threat of an expansion of this NIS species to close areas of great value for native species. Various measures are being taken to improve management of water bodies in the Guadiana river basin under the Water Framework Directive in conjunction with the new European Marine Strategy Framework Directive. The new strategic plan which came into force in 2016 and will be in action until 2021 provides for several measures that will potentially mitigate the risk of mummichog expansion, among them: (a) reduce or eliminate discharges of pollutants; (b) define and implement ecological flows; and (c) increase monitoring and supervising plans [42]. The authors recommend that future monitoring studies should be carried out in the study area to evaluate the effectiveness of the new measures implemented in control not only of this but other NIS species.

4. Materials and Methods

4.1. Sampling and field methodology

Larval and juvenile fishes were collected between January and February of 2013 at four sites in the Natural Reserve of Castro Marim and Vila Real de Santo António (South of Portugal) salt marsh area. The first sampling site, Main Channel (MC), was set on the edge of the main course of the Guadiana estuary; the second, Primary Intertidal Creek (PIC), a small creek directly connected to the main channel; the third, Secondary Intertidal Creek (SIC), also a small creek with secondary connection to the main channel; and the fourth, Marsh Pool (MP), an area permanently inundated due to the partially obstruction of water flow by a small dyke (Figure 5). A preliminary survey was performed in order to evaluate the adequacy of sampling points and methodologies to the characteristics of the habitats. Accessibility and representativeness were the main factors considered for the selection of sampling sites.

Animal handling was performed following the European Directive 2010/63/EU of European Parliament and of the Council of European Union on the protection of animals used for scientific purposes.

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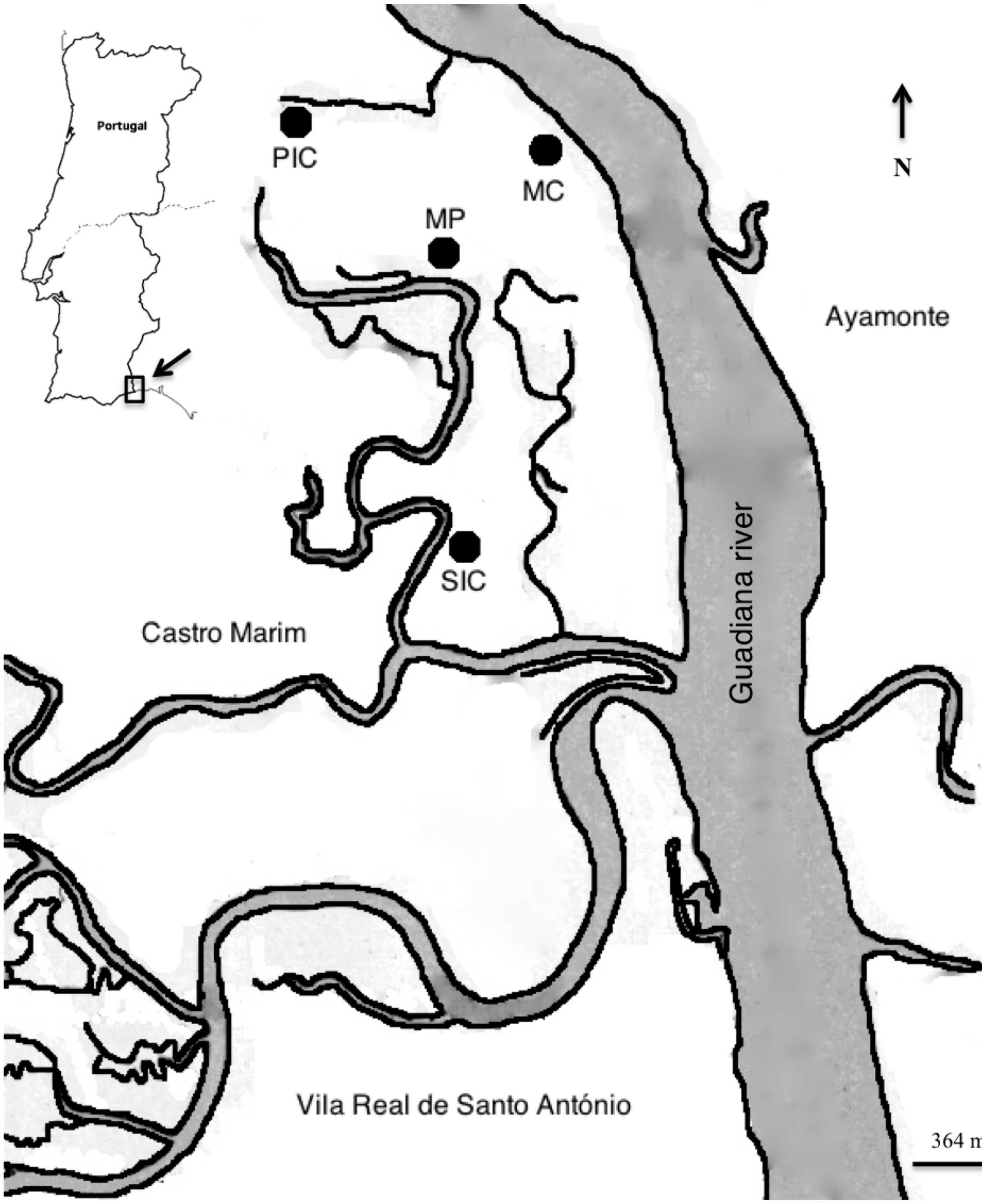

Figure 5. Geographical context of the Natural Reserve of Castro Marim and Vila Real de Santo António in Portugal and sampling sites localization in the Natural Reserve salt marsh area.

A total of six diurnal field campaigns were carried out weekly – three at the end of ebb and the other three at the end of flood tides. In each campaign and in each sampling site, larval and juvenile fish specimens were sampled for three consecutive times. Fishes were captured with a seine net (1 mm net size, 5 m length and 0.5 m height). Capture procedure consisted in encircling an area of approximately 18 m^2 near the edge of the main channel, intertidal creeks and marsh pool. One of the operators endured on a fixed point holding one of the extremities of the net, while the second one dragged the net manually in a circle, meeting the first one at the fixed point. When the two operators meet each other, the net was pulled close to the shore. The bottom of the net was equipped with weights in order to guarantee that remained in permanent and direct contact with the bottom. Each

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seine took approximately 10 minutes and was performed for three times at each sampling point. Collected samples were immediately preserved in buffered formaldehyde solution (4% final concentration) for further analysis. Additionally, physical-chemical parameters (temperature, salinity, dissolved oxygen, and pH) were recorded at each station with a Yellow Springs Instruments (YSI Model 85) probe immersed approximately 10-20 cm from the water surface. Finally, and also for each point, water samples were collected from the surface for the analysis of dissolved inorganic macronutrients (ammonium, NH_4^+; nitrate, NO_3^-; nitrite, NO_2^-; and phosphate, PO_4^{3-}) and chlorophyll *a*. Samples were immediately stored in 330 mL containers in the dark and at low temperature, until further processing.

4.2. Laboratory analysis

In the laboratory, preserved marine juvenile fishes were measured for total length (precision ± 1.0 mm) and identified according to [43] and [44]. Fish larvae were measured for total length (precision ± 0.1 mm) and identified according to [45] and [46] under a stereomicroscope (Leica S8APO, Germany). Identified fish species were classified into ecological guilds, according to their biology and behavior reported in the literature (Table 6).

Table 6. Ecological guild definition of captured fish species during the study period in the lower Guadiana estuary salt marsh areas

Ecological guild	Definition	Reference
Estuarine resident	Spend their entire lives in the estuary	[47]
Marine seasonal	Have regular seasonal visits to the estuary, mainly as adults	[47]
Marine juvenile	Use the estuary as nursery ground, usually spawning and spending much of their adult life at sea with seasonal visits to the estuary	[47]

Chlorophyll *a* concentration was determined by filtering water samples through 0.7 µm pore filters (Whatman GF/F, UK) without exceeding vacuum pressures of 100 mmHg. The filters were kept frozen until fluorimetric analysis using the Fluorimeter 10 AU Turner Designs (CA, USA). Finally, dissolved inorganic macronutrients concentrations were determined according to a spectrophotometric method using cell test photometric kits (Merck Millipore, NJ, USA) and the photometer (Spectroquant Nova 60, NJ, USA).

4.3. Data analysis

Two-way factorial analysis of variance (ANOVA) was used to test for differences in fish densities of the most abundant species (*Pomatoschistus* sp.) among sampling sites and tide stages. Both independent variables were considered as fixed factors. Fish density data was transformed to square root in order to reduce the heteroscedasticity of the data. Tukey's HSD test was used for post hoc comparisons when significant differences were detected ($p<0.01$). The Spearman correlation coefficient was used to explore patterns of association among the environmental variables and the density of larvae and juveniles of the two most abundant species (*Pomatoschistus* sp. and *Fundulus heteroclitus*). The habitat niche overlap between *Pomatoschistus* sp. and *Fundulus heteroclitus* was evaluated through Pianka's index [48], applied to the density of each species in each habitat sampled. Pianka's formula is $O_{pf} = \sum P_{ip}\, P_{if} \div \sqrt{\sum P_{ip}^2 \sum P_{if}^2}$, where Opf is Pianka's measure of niche overlap between *Pomatoschistus* sp. and *Fundulus heteroclitus*; P_{ip} is the proportion of the resource i out of the

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total resources used by *Pomatoschistus* sp.; P_{if} is the proportion of the resource i out of the total resources used by *Fundulus heteroclitus*; i could range from 1 to n, where n is the total number of habitats considered (in our case n = 4, the number of sampled habitats). The value of index O could range from 0 (no overlap) to 1 (full overlap). All statistics were applied using the open source software R version 2.15.1 [49].

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