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Is parasitism in fish a good metric to assess ecological water quality in transitional waters? What can be learned from two estuarine resident species?

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ABSTRACT

Fish-based indices are becoming important bioassessment tools for estuaries since the European Water Framework Directive included fish as a biological quality element to be monitored for the assessment of ecological status of those systems. In particular, data on species composition and abundance of the ichthyofauna must be used to evaluate the ecological status of European estuaries, but other factors like measures of fish health are also considered important metrics. These indicators of fish health include infections by parasites. The common goby, *Pomastoschistus microps*, and the Lusitanian toadfish, *Halobatrachus didactylus*, are fairly sedentary fish, very abundant in Portuguese estuaries, and were used to investigate if parasitism in fishes in transitional waters is a good metric to reflect anthropogenic impacts in the environment. No significant relationships were observed between the parasite levels in these two species and the intensity of human pressures, either at a large or small spatial scale. Results obtained also are contrary to the generalized idea that a higher proportion of parasitized fish necessarily reflects a decrease in their condition as a result of habitat degradation. A high degree of temporal and ontogenetic variability in the parasite levels of both *P. microps* and *H. didactylus* was detected, which make it difficult to establish the scores for this hypothetical metric even if significant relationships between parasite levels and human pressures are identified in the future. Therefore, the present work does not uphold the hypothesis that parasitism in fish is a good metric to assess ecological water quality in transitional waters.

1. Introduction

In European estuaries, fish-based indices are becoming important water quality bioassessment tools since the Water Framework Directive (WFD) indicated that for those systems' evaluation of ecological status, fish are a biological element to be monitored as part of the process (e.g. Borja et al., 2004; Breine et al., 2007; Coates et al., 2007; Delpech et al., 2010). Although some of the initial indices were based on a single criterion (e.g. Cooper et al., 1994; Ramm, 1988), most recent tools are multimetric (Coates et al., 2007), deriving from Karr (1981) original concept of Biotic Integrity. They consist of a combination of several metrics, which can be defined as measurable factors that represent various aspects of biological assemblage, structure, function, or other community component (Delpech et al., 2010). Thus, multimetric indices are expected to provide information about various aspects of fish assemblages, and lead to a more holistic, integrative, and functional approach (Roset et al., 2007). According to the USEPA (2000), data on species composition and abundance of the ichthyofauna must be used to report the ecological status of estuaries, but other factors like the presence and/or abundance of tolerant and sensitive species, and indicators of fish health also can be considered important metrics to perform that task. These indicators of fish health include tissue contamination by pollutants, lesions, tumours, deformities, diseases and infections by parasites. However, very few fish-based multimetric indices for transitional waters consider individual health indicators. In fact, only the EBI (Deegan et al., 1997), developed for North-Eastern USA, and the AFI (Borja et al., 2004), developed for the Basque Country (Spain), use the percentage of diseased fish (including parasitized) as an evaluation metric. In both cases, no clear justification was furnished for the metric scores, and only its rationale was presented: a higher proportion of diseased fish reflects an increase of habitat degradation.

Recent reviews have compiled data on parasites as bioindicators of environmental impact (e.g. Blanar et al., 2009; Marcogliese, 2005; Sures, 2004; Vidal-Martínez et al., 2010). Investigations

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performed in the last two decades have demonstrated significant effects and interactions between parasite levels and the presence and concentration of various pollutants and/or environmental stressors (Vidal-Martínez et al., 2010). However, changes in parasite abundance are usually influenced by stochastic changes in a population or community (Sures, 2004). Moreover, there is conflicting evidence regarding the impact of habitat degradation on aquatic parasite abundance (Sures, 2008a). Lafferty (1997) and Poulin (1992) suggested that heavy metals and generalized disturbance negatively affect parasites, while eutrophication has positive effects. Furthermore, depending on the species, numerical or physiological responses to pollutants can be positive, negative, or neutral (Sures, 2008b). On the other hand, directly exposed (external parasites and the free-living transmission stages of internal parasites) and freshwater taxa are more susceptible to a wider range of pollutants than indirectly exposed (internal parasites) and marine taxa (Blanar et al., 2009). Therefore, the circumstances under which parasites can be used as indicators of anthropogenic impact have not yet been demonstrated conclusively.

The main goal of this work is to determine if parasitism in fish in Portuguese transitional waters is a good metric to reflect anthropogenic impacts in the environment. Specific objectives include investigating (1) if parasite levels are related to human pressures; (2) if the degree of parasitism is a good indicator of fish condition; and (3) if temporal and ontogenetic variations in parasite levels may induce important bias in any potential relationships detected.

Only macroparasite assemblages and estuarine resident fish were considered in the study herein because the time necessary for searching and identifying microparasites would be too great to include them in an effective monitoring tool, and fish that complete the entire life cycle within an estuary would better reflect its environmental conditions, especially if a fairly sedentary behaviour is displayed. According to França et al. (2009), in main Portuguese estuaries the common goby, *Pomatoschistus microps* (Krøyer, 1838), the two-banded sea bream, *Diplodus vulgaris* (E. Geoffroy Saint-Hilaire, 1817), the sand goby, *Pomatoschistus minutus* (Pallas, 1770), the Lusitanian toadfish, *Halobatrachus didactylus* (Bloch & Schneider, 1801), and the black goby, *Gobius niger* (Linnaeus, 1758), are the most abundant fish species, by order of decreasing importance. From this group only the two-banded sea bream uses estuaries as nursery grounds (Branco et al., 2008), constituting the other species the bulk of resident fish in Portuguese transitional waters (França et al., 2009). However, only for the common goby and the Lusitanian toadfish, the data available on parasitism (Alves, 2010; Costa et al., 2001; Freitas et al., 2009; Marques et al., 2005) were adequate to relate to potential anthropogenic pressures and, therefore, these were the species studied in the present work. Together, they comprise more than 40% of all fish captures when considering the most important Portuguese estuaries (França et al., 2009).

The distribution of *P. microps* ranges from the coast of Norway to the Gulf of Lion, in the Mediterranean (Bouchereau et al., 1993). It is a small benthic fish, measuring up to 64 mm on British coasts (Jones and Miller, 1966), and up to 53 mm in the Mediterranean (Bouchereau et al., 1989), and attaining a maximum age of approximately two years (e.g. Miller, 1986; Moreira et al., 1991). Although in Portuguese estuaries the species is present along the entire saline gradient, it shows higher densities in middle and upper reaches (Costa, 2004; Leitão et al., 2006). In northern European estuaries, the common goby migrates downstream during the breeding season (Miller, 1975), but in the Mediterranean it tends to be less mobile (Pampoulie et al., 2000). Some evidence seems to indicate that Portuguese populations exhibit an intermediate behaviour (Arruda et al., 1993; Caçador et al., 2012). At this latitude, reproduction occurs in the winter and early spring, with a peak of recruitment in late spring (Arruda et al., 1993; Leitão et al., 2006). The diet is mainly composed by benthic meio- and macro-fauna like foraminifera, annelids, bivalves, and small crustaceans, and also by some nekton-benthic prey like mysids (e.g. Leitão et al., 2006; Salgado et al., 2004).

H. didactylus is typical of the Eastern Atlantic subtropical realm occurring from Cabo Carvoeiro (central Portugal) to the Gulf of Guinea (Bauchot, 1987). Despite being mainly a marine littoral species, this benthic fish is secondarily adapted and limited to brackish water systems in the northern region of its distribution area due to thermal and hydrodynamic constraints on offspring development (Costa et al., 2003). Its head and mouth are very wide, and the body is robust and quite large, reaching some specimens more than 500 mm in total length and an age of twelve years, although their size and longevity are typically shortened in estuaries (Costa, 2004; Palazón-Fernández et al., 2010). In brackish water systems this species appears mainly in the middle and lower reaches (Costa and Costa, 2002). The Lusitanian toadfish was considered by Roux (1986) to exhibit sedentary behaviour, but Campos et al. (2008) and Costa (2004) found that some individuals might perform important displacements (more than 10 km). Adult specimens show an increased activity during the reproductive period, which occurs in spring and early summer (Costa and Costa, 2002; Palazón-Fernández et al., 2001), leading to a peak of recruitment in late summer and early autumn (Costa, 2004). In contrast, *H. didactylus* individuals become quite inactive during winter as a result of the decrease in water temperature (Costa et al., 2000). This species occupies a top position on the estuarine food webs, and exhibits a high degree of trophic plasticity, adapting its feeding habits to prey availability (Cárdenas, 1977; Costa et al., 2000). Crabs, fish, shrimp, molluscs and anomura are the main food of adults, where younger individuals consume mostly small benthic and nekton-benthic organisms like amphipods, isopods, and mysids (Costa et al., 2000, 2008).

2. Materials and methods

2.1. Data acquisition

Data used in the present work were collected and partially analysed in the scope of previous studies. Most of the samplings were performed in the euhaline zone although some of them were conducted in less saline areas (polihaline, mesohaline and oligohaline zones).

In the case of *P. microps*, data were obtained by Freitas et al. (2009) between May and July 2006, and by Alves (2010) between July and October 2009, on both studies at the Tejo, Sado, Mira, and Guadiana estuaries and the Ria de Aveiro coastal lagoon (Fig. 1). For the 2006 study, a single sampling representative of each system was conducted with a beam trawl, while for the 2009 study collections were performed twice (in July and October) at two different sites per system (Fig. 1), using a beam trawl or a purse seine net. Fishes captured in 2006 were frozen and kept at −20 °C until further examination, and after defrosting ectoparasites were recovered from the body surface, fins, oral cavity, and gills using a stereoscope. The digestive tract of fishes collected in 2009 was removed in the field, tagged and frozen at −20 °C for posterior parasitological examination under a stereomicroscope.

Regarding *H. didactylus*, data were obtained by Costa et al. (2001) at the Mira estuary between July 1991 and November 1996, and by Marques et al. (2005) between September and November 2000 at the Tejo, Sado, Mira, and Guadiana estuaries and the Ria Formosa coastal lagoon (Fig. 1). In the 1991–1996 study, fishing operations were performed with a beam trawl, and samples were obtained monthly until June 1992 and with irregular frequency afterwards, in four different sites (Fig. 1). Fishes were categorized according to their total length (±1 mm), and the total weight (±0.01 g) of a

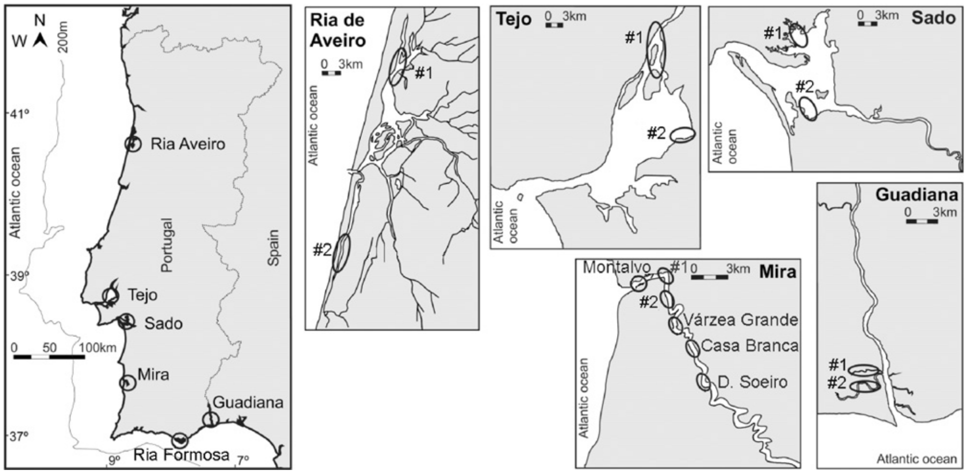

Fig. 1. Brackish water systems studied, and location of sampling sites on those estuaries where small spatial scale analyses were performed. Sites #1 and #2 were sampled by Alves (2010) for *P. microps*; and sites D. Soeiro, Casa Branca, Várzea Grande and Montalvo in the Mira estuary were sampled by Costa et al. (2001) for *H. didactylus*.

subsample of specimens was registered in order to estimate their condition. The detection of ectoparasites was carried out by visual inspection of tegument, fins, and oral cavity. In the 2000 study, a single sample representative of each system was obtained by trawl. After capture, fishes were frozen at −20 °C and later examined for parasites collection using a stereoscope. Ectoparasites were obtained by inspecting tegument, fins, oral cavity, and gills, and endoparasites by observing the liver, digestive tract, and ventral musculature.

In the four studies, all parasites were identified to the lower taxonomic level possible and counted.

2.2. Data treatment

Following Bush et al. (1997), the parasitological indices prevalence (percentage of infected fishes) and mean abundance (mean number of parasites per fish examined) were computed for each set of data as measures of the degree of parasitism.

Data from Freitas et al. (2009) and Alves (2010) were used to investigate the relationship between the parasite levels in *P. microps*, and the intensity of human pressures at a large spatial scale (different estuarine systems), in the first case considering ectoparasites, and in the second case, endoparasites. Data from Marques et al. (2005) were used with the same objective for *H. didactylus*, either for ecto- or endo-parasites. The index described by Vasconcelos et al. (2007) to assess the global intensity of human pressures, here designated as Anthropogenic Pressures Assessment Index (APAI), was used in these analyses as a proxy of human pressures in each estuary. It ranges between 0 (low pressure), and 1 (high pressure), and considers different metrics like dams, wastewater treatment, human population, industrial loads, water and sediment quality, industry, dredging, port activities, aquaculture, agriculture, bank regulation and fishing.

In order to perform a similar analysis but at a smaller spatial scale (considering in separate different sites within the estuaries), data from Alves (2010) and Costa et al. (2001) were used for the common goby and the Lusitanian toadfish, respectively. In the first case, were used as proxies of human pressures the concentration of polycyclic aromatic hydrocarbons (PAHs) and the Metal Pollution Index (MPI) (Usero et al., 1997), both obtained for the sediments of each site at the same time that fish collection. The heavy metals considered for MPI computation were Cd, Cr, Cu, Ni, Pb, and Zn. The sampling and analytic procedures employed to obtain the data on sediment contamination are described in Fonseca et al. (2011). As for relating *H. didactylus* parasitism with anthropogenic pressures within the Mira estuary, water/sediments quality and the condition of macroinvertebrates communities were used as proxies of human pressures. In both cases, the criteria defined by Chainho et al. (2008) were applied and the quality status of sites classified as high/good, moderate and poor/bad. Chemical status was based on the concentration of heavy metals in the sediment, nutrient concentrations in water (DIN), and percentage of bottom dissolved oxygen, and status of benthic communities obtained by the conjugation of Shannon-Wiener, Margalef, and AMBI indices.

Temporal variations on the parasite levels of both species were analysed considering the data gathered by Alves (2010) and Costa et al. (2001), which in the latter case allowed also investigating ontogenetic variations in parasitic relationships and relating the degree of parasitism with fish condition.

Relationships between prevalence or mean abundance of parasites and the human pressures indicators were investigated by means of Spearman correlation tests (Siegel and Castellan, 1988). Values of prevalence were compared using the RxC independence G-test with Williams' correction (Sokal and Rohlf, 1995), or the Wilcoxon signed-ranks test, while those of mean abundance were compared using the last statistical procedure or the Kruskal–Wallis test (Siegel and Castellan, 1988). Weight of specimens of *H. didactylus* with different parasite levels (not parasitized, single parasitized, and multiparasitized) were compared by means of Analysis of Covariance (ANCOVA), using total length as covariate (Sokal and Rohlf, 1995). The RxC independence G-tests were conducted using the BIOMstat software (Version 3.0), whereas the other tests were performed using the SPSS statistical package (version 16.0). The level of significance used in all analyses was 0.05.

3. Results

3.1. Parasites of P. microps and H. didactylus

Only one ectoparasite taxon, a platyhelminthe Digenea belonging to the genus *Cryptocotyle*, was found in *P. microps*, mostly on the base of the fins. Endoparasites of this species included 10 different taxa, composed of eight Digenea and two Nematoda. The Digenea fauna were represented by *Lecithochirium musculus* (Looss, 1907), *Hemiurus appendiculatus* (Rudolphi, 1802), *Zoogonoides viviparous* (Olsson, 1868), three species of the genera *Prosorhynchus*, *Acanthostomum* and *Diplostomum*, as well as one unidentified Diplostomatidae, and one undetermined taxa in the metacercariae stage. Nematodes found in the digestive tract of

Table 1
Parasitological indices for parasites present in *Pomatoschistus microps* in Portuguese estuaries (Alves, 2010; Freitas et al., 2009) and Anthropogenic Pressures Assessment Index (APAI) computed for each system (Vasconcelos et al., 2007).

Estuary	*Cryptocotyle* sp.			Endoparasites			APAI
	Prevalence (%)	Mean abundance	Nr fishes	Prevalence (%)	Mean abundance	Nr fishes	
R. Aveiro	74	12.4	31	36	2.4	230	0.42
Tejo	43	13.4	30	59	4.7	279	0.76
Sado	54	12.0	39	59	6.4	180	0.49
Mira	24	28.0	38	42	3.8	76	0.14
Guadiana	54	16.5	35	54	4.9	144	0.21

Table 2
Parasitological indices for parasites present in *Halobatrachus didactylus* in Portuguese estuaries (Marques et al., 2005) and Anthropogenic Pressures Assessment Index (APAI) computed for each system (Vasconcelos et al., 2007).

Estuary	*H. aduncum*		*P. dasyatidis*		*N. orbignyi*		Nr fishes	APAI
	Prevalence (%)	Mean abundance	Prevalence (%)	Mean abundance	Prevalence (%)	Mean abundance		
Tejo	83	3.8	0	0.0	3	2.5	65	0.76
Sado	12	1.0	3	1.0	14	1.1	57	0.49
Mira	3	1.0	0	0.0	0	0.0	37	0.14
R. Formosa	2	1.0	2	2.0	0	0.0	44	0.32
Guadiana	14	2.3	8	14.0	29	2.2	49	0.21

the common goby were *Raphidascaris acus* (Bloch, 1779), and one unidentified species of the genus *Contracaecum*.

Three species of parasites were found in *H. didactylus* in the collections performed in 2000: *Hysterothylacium aduncum* Rudolphi, 1802, *Progrillotia dasyatidis* Beveridge et al., 2004, and *Nerocila orbignyi* Guérin-Méneville, 1829. *H. aduncum* is a Nematoda Anisakidae, *P. dasyatidis* is a Cestoda Trypanorhyncha, and *N. orbignyi* is an Isopoda Cymothoidae. *H. aduncum* and *P. dasyatidis* were found in the digestive tract of fishes, while *N. orbignyi* was observed attached to their external tegument. This isopod was also the only ectoparasite found in the 1991–1996 samples of Lusitanian toadfish obtained in the Mira estuary. It was detected both on the tegument and fins of fishes.

3.2. Parasites and human pressures

At the estuary level, no significant relationships were found between the prevalence ($r_s = 0.205$; $N = 5$; n.s.) or the mean abundance ($r_s = -0.700$; $N = 5$; n.s.) of the ectoparasite *Cryptocotyle* sp. in the common goby, and the APAI values (Table 1). The same output was obtained for endoparasites, either for each taxon, subsets of taxa, or the whole parasitic community ($-0.900 < r_s < 0.900$; $N = 5$; n.s.). The results verified for *H. didactylus* were very similar to those obtained for *P. microps*, with no significant relationships observed between the degree of parasitism by *H. aduncum* (prevalence: $r_s = 0.500$; mean abundance: $r_s = 0.447$; $N = 5$; n.s.), *P. dasyatidis* (prevalence: $r_s = -0.103$; mean abundance: $r_s = -0.205$; $N = 5$; n.s.), and *N. orbignyi* (prevalence: $r_s = 0.205$; mean abundance: $r_s = 0.616$; $N = 5$; n.s.), and the APAI values (Table 2).

The results obtained at the site level were not different from those observed for the estuaries as a whole, either for the common goby or the Lusitanian toadfish. In fact, no relationships were found between the prevalence or the mean abundance of all combinations of *P. microps* endoparasites and the MPI values ($-0.391 < r_s < 0.391$; $N = 19$; n.s.), or concentration of PAHs ($-0.564 < r_s < 0.564$; $N = 10$; n.s.) in the sediments (Table 3). On the other hand, although the biological/chemical quality status of the areas studied in the Mira estuary ranged from poor/bad in the uppermost reaches to high/good near the mouth, no significant differences were observed between those sites regarding the prevalence ($G_W = 7.406$; df = 3; n.s.) and mean abundance ($H_{adj} = 0.723$; $N = 4$; n.s.) of *N. orbignyi* in *H. didactylus* populations (Table 4). Furthermore, there was a tendency for higher parasite levels being observed in the area exhibiting better chemical conditions, and for a lower degree of parasitism being noticed in the area with worst biological conditions.

3.3. Parasites and fish condition

In the Mira estuary, specimens of *H. didactylus* multiparasitized by *N. orbignyi* presented an average corrected (by ANCOVA) weight of 31.7 ± 1.9 g, while those with only one parasite or with no parasites exhibited average corrected weights of 35.3 ± 1.2 g and 35.4 ± 0.2 g, respectively. These variations were significant ($F = 4.920$; df = 2, 742; $p < 0.01$), but only the group of fishes infected

Table 3
Endoparasites parasitological indices for *Pomatoschistus microps* in different locations of Portuguese estuaries (Alves, 2010) and Metal Pollution Index (MPI) and polycyclic aromatic hydrocarbons (PAHs) concentration ($ng\,g^{-1}$ of dry weight of sediments) in each site (unpublished data).

Site	July			October			MPI		PAHs
	Prevalence (%)	Mean abundance	Nr fishes	Prevalence (%)	Mean abundance	Nr fishes	July	October	July
R. Aveiro #1	29	1.0	55	65	3.9	69	23.4	20.6	9.9
R. Aveiro #2	39	3.7	33	14	1.4	73	14.2	10.5	9.1
Tejo #1	31	0.7	61	66	2.9	67	26.2	25.3	20.4
Tejo #2	51	6.0	89	92	8.7	62	23.3	23.2	40.4
Sado #1	50	4.5	62	68	7.1	60	19.1	23.9	18.4
Sado #2	54	7.3	13	62	7.9	45	20.4	19.5	15.9
Mira #1	27	0.4	30	82	15.2	11	19.2	33.2	20.6
Mira #2	25	0.6	16	58	5.4	19	17.0	30.2	53.3
Guadiana #1	48	2.8	58	80	11.1	44	20.7	24.1	66.1
Guadiana #2	36	1.4	42	–	–	–	26.4	–	5.6

Table 4
Parasitological indices for the parasitic isopod *Nerocila orbignyi* in *Halobatrachus didactylus* in different locations of the Mira estuary (Costa et al., 2001) and chemical and benthic communities quality status for each site (Chainho et al., 2008).

Site	Prevalence (%)	Mean abundance ($\times 10^{-2}$)	Nr fishes	Chemical status	Benthic communities status
D. Soeiro	2	2.0	1388	Moderate	Poor/bad
Casa Branca	3	4.8	1957	Moderate	Moderate
Várzea Grande	2	3.1	708	Moderate	Moderate
Montalvo	3	5.1	585	High/good	Moderate

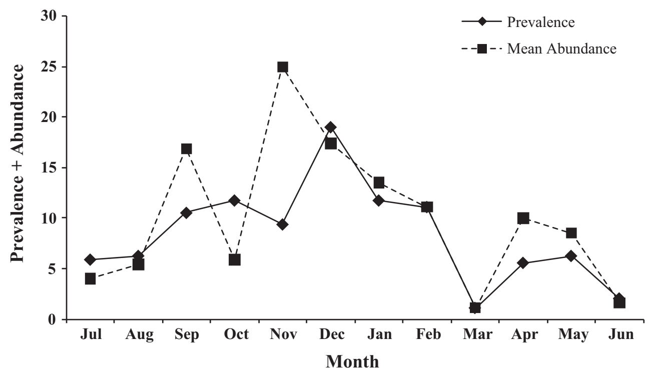

Fig. 2. Variation of prevalence (%) and mean abundance ($\times 10^{-2}$) of the parasitic isopod *Nerocila orbignyi* in the Mira estuary *Halobatrachus didactylus* population between July 1991 and June 1992 ($N = 682$; data from Costa et al., 2001).

with two or more parasites differed from the other groups in terms of biomass.

3.4. Temporal and ontogenetic variations in the parasitism

Although no significant variations ($Z = -0.771$; $N = 9$; n.s.) were observed in the contamination levels of sediments by heavy metals in Portuguese estuaries from July to October 2009, there was a significant increase in the prevalence ($Z = -2.310$; $N = 9$; $p < 0.05$) and mean abundance ($Z = -2.310$; $N = 9$; $p < 0.05$) of *P. microps* endoparasites in that period (Table 3). On the other hand, no relevant variations in human pressures seemed to occur in the Mira estuary between July 1991 and June 1992, but during that period the infection of *H. didactylus* by *N. orbignyi* was much higher (prevalence: $G_W = 11.460$; df = 11; $p < 0.01$; mean abundance: $H_{adj} = 26.694$; $N = 12$; $p < 0.05$) in winter compared to spring and early summer (Fig. 2). Moreover, highly significant differences on the prevalence ($G_W = 42.260$; df = 5; $p < 0.001$) and mean abundance ($H_{adj} = 106.571$; $N = 6$; $p < 0.001$) of this isopod parasite in the Lusitanian toadfish specimens were detected from year to year in the Mira estuary (Table 5), once again with no apparent relationship to noticeable variations on anthropogenic pressures in that system. Finally, in this water body the prevalence of *N. orbignyi* in *H. didactylus* increased significantly ($G_W = 115.920$; df = 4; $p < 0.001$) with the size of fishes (Fig. 3).

Table 5
Variations between 1991 and 2000 on the parasitological indices of the parasitic isopod *Nerocila orbignyi* in the Mira estuary *Halobatrachus didactylus* population (Costa et al., 2001; Marques et al., 2005).

Year	Prevalence (%)	Mean abundance ($\times 10^{-2}$)	Nr fishes
1991	3	4.3	951
1992	2	2.7	1464
1993	2	3.1	2543
1994	7	8.3	180
1996	23	6.1	43
2000	0	0.0	37

4. Discussion

In the present work no significant relationships were observed between the parasite levels in the two species of estuarine resident fishes studied, and the intensity of human pressures, either at a large or small spatial scale. It could be argued that the quality of the data used as proxies of anthropogenic pressures may not have always properly reflected the degree of degradation of the estuaries and sites investigated, or that in some analysis the number of study areas was reduced. In fact, at a smaller spatial scale, the accuracy of the analyses performed had been greatly improved if a wider range of proxies of human pressures were available. On the other hand, an increased number of sampling sites certainly had improved the strength of the statistical procedures employed, especially at a larger spatial scale. However, it is much relevant that in so many analyses no clear pattern or at least any significant relationship was found between the prevalence or the mean abundance of parasites in both fish species, and a wide set of human pressures. It is also remarkable that this phenomenon was observed not only for endoparasites but for ectoparasites too, which are usually considered more prone to external pressures because they do not benefit from the homeostatic and contaminant transport and detoxification mechanisms of their hosts (Blanar et al., 2009). On the other hand, although the later authors infer that in the marine environment the concentration of PAHs is the most important anthropogenic factor for parasite proliferation, the data analysed in the present work concerning *P. microps* do not corroborate such a relationship. Furthermore, it has been reported that heavy metal contamination may produce negative impacts on some groups of parasites because these elements are particularly toxic to them (Blanar et al., 2009; Lafferty, 1997; Poulin, 1992), but that feature was not confirmed in this study either, although a wide range of contamination by heavy metals was observed among the sediments of the sites analysed for the common goby. It is important to remember that *P. microps* and *H. didactylus* represent more than 40% of the captures in main Portuguese estuaries (França et al., 2009). Therefore, different results should not be expected if more species had been included in these analyses, especially since most of the estuarine fish species are not resident in those systems, and some

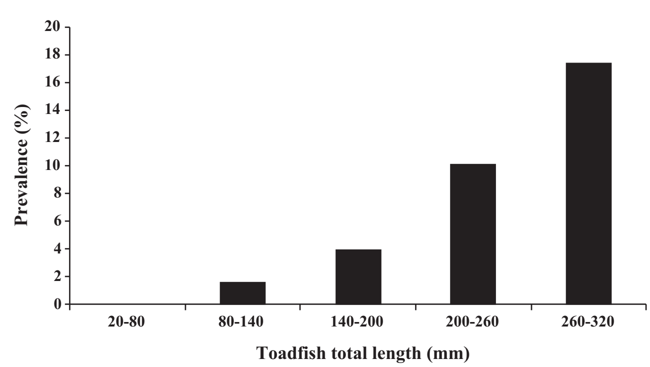

Fig. 3. Variation of prevalence of the parasitic isopod *Nerocila orbignyi* in the Mira estuary *Halobatrachus didactylus* population, according to the host size ($N = 4615$; data from Costa et al., 2001).

of them are highly mobile. This assertion is corroborated by the fact that Neto (2008) also failed to obtain any relationship between the prevalence and mean abundance of the nematode parasite *Anguillicoloides crassus* Kuwahara et al., 1974 in the European eel, *Anguilla anguilla* (Linnaeus, 1758), and the heavy metal concentration in the Tejo estuary sediments.

Nevertheless, it is important to stress that none of the estuaries studied in this work was highly eutrophized (Bettencourt et al., 2003), where eutrophication is the anthropogenic factor that most seems to favour parasite proliferation in continental waters (Blanar et al., 2009; Lafferty, 1997; Zander and Kesting, 1998). It should be noted, however, that eutrophication is not a major problem in Portuguese transitional waters (Bettencourt et al., 2003), except in the case of the Mondego estuary, particularly in the south branch (e.g. Marques et al., 1997), but even there some relatively recent management measures have permitted the system to initiate an important recovery (e.g. Ferreira et al., 2004). On the other hand, according to Blanar et al. (2009), marine parasites are normally less susceptible to anthropogenic pressures than freshwater taxa, mostly because marine environments are generally more complex than those of freshwater. This complexity derives from a number of factors, including tidal movements, pronounced spatial variation in key water chemistry variables such as salinity and temperature, and large scale currents. Furthermore, dilution effects due to the open nature of the ecosystem may reduce impacts in the marine environment. In brackish waters, parasites, and especially ectoparasites, should be even less susceptible to human pressures given their adaptation to this complex and naturally stressed environment as a result of the high degree of variability in its physical–chemical characteristics, for example, oxygen, temperature and salinity in the water column, and bed sediment dynamics (Elliott and Quintino, 2007). According to Moller (1978), there is a reduction in the parasitic fauna of estuaries due to the stenohalinity of the majority of the parasites and that of the hosts. In fact, it is expected that parasites able to survive in estuaries have the capacity to experience and acclimate to stress without relevant effects. In addition, although being residents in Portuguese estuaries, both *P. microps* and *H. didactylus* specimens seem to perform important movements inside those systems (e.g. Arruda et al., 1993; Campos et al., 2008). Therefore, the reduced eutrophication of Portuguese transitional waters, the natural characteristics of the estuarine environment, and the behaviour of the hosts probably contributed to the absence of a relationship between human pressures and parasite levels in the fish studied. The secondary adaptation of the Lusitanian toadfish to brackish water environments along the Portuguese coast (Costa et al., 2003) may have also contributed to the outcome of the results because in this species, the taxa richness of metazoan parasites is reduced in estuaries (Marques et al., 2005).

In the Mira estuary, specimens of *H. didactylus* parasitized by one individual of *N. orbignyi* showed the same condition as specimens without parasites, but multiparasitized fishes were in a worse condition. This is a clear indication that in this case susceptibility to parasitism is not a consequence of fish weakness, but instead, that the decrease in fish condition is a result of the infection by the parasite (fishes with a single parasite showed still a normal condition and only those with more parasites became weaker). So, in this parasitic relationship the infection by the parasite is not a good indicator of fish weakness, contradicting the generalized idea that a higher proportion of parasitized fish necessarily reflects a decrease in their condition as a result of habitat degradation (Deegan et al., 1997; Borja et al., 2004). As indicated by Hudson et al. (2006), a healthy ecosystem could be in fact rich in parasites, contrasting with recently disturbed or invaded systems, where parasitic diversity is often reduced.

This work also indicated a high degree of temporal and ontogenetic variability in the parasite levels of *P. microps* and *H. didactylus*. Seasonal and inter-annual variations in parasitic communities are common in brackish water environments, and typical of *P. microps* parasitic fauna (Kesting et al., 1996; Zander, 2004; Zander and Kesting, 1998). According to these authors, such variability is related to changes in environmental conditions, and also to the life cycles of hosts and parasites. Incrementing parasite levels in the common goby in Portuguese estuaries from July to October could reflect its increased sedentary behaviour after the reproduction and settlement of juveniles, which end in early and late spring, respectively (Arruda et al., 1993; Leitão et al., 2006). Seasonal variations in the infection of *H. didactylus* specimens by *N. orbignyi* in the Mira estuary appear more complex with species interdependencies. According to Bragoni et al. (1984), this parasitic isopod infects mainly grey-mullets (Mugilidae). The increase in the Lusitanian toadfish parasitism by *N. orbignyi* in winter is apparently related to the reproduction of the thin-lipped grey mullet, *Liza ramada* (Risso, 1826). This is the most abundant mullet species in the Mira estuary, and performs its catadromous migration between November and February, when *H. didactylus* is more inactive and exhibits a better condition, stimulating the change of hosts by this mobile parasite (Costa et al., 2001). According to these authors, smaller specimens of Lusitanian toadfish are less prone to infection by *N. orbignyi* because they have not attained a large enough size to support this relatively large ectoparasite. These results clearly indicate the need to consider temporal and ontogenetic variability in the parasite levels when establishing scores for this potential metric of ecological water quality evaluation.

Although parasites have an enormous potential as bioindicators of anthropogenic degradation (Vidal-Martínez et al., 2010), with the current available data no relationship could be established between the prevalence or mean abundance of fish parasites and human pressures in Portuguese estuaries. However, investigations should proceed with the objective of detecting significant relationships between the proliferation of specific parasites and human pressures; and temporal and ontogenetic variations in the degree of parasitism should be accounted for in those studies.

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