



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, *Pomatoschistus 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.

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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; Delpéch 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 (Delpéch 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, 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. Blañar 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

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