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Multi-biomarker responses to estuarine habitat contamination in three fish species: *Dicentrarchus labrax*, *Solea senegalensis* and *Pomatoschistus microps*

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ABSTRACT

Several biomarker responses were determined in three fish species, *Dicentrarchus labrax*, *Solea senegalensis* and *Pomatoschistus microps*, from two estuaries of the Portuguese coast, Ria de Aveiro and Tejo. Both estuaries have significant anthropogenic influences from multiple sources (industrial, agricultural and shipping activities), which was evident from sediment chemical characterization concerning metal (copper, zinc, nickel, lead and chromium) and polycyclic aromatic hydrocarbon (PAH) concentrations.

Spatial variability in fish responses was observed across species for most biomarkers of exposure [the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), ethoxyresorufin O-deethylase (EROD) and glutathione S-transferase (GST), and metallothionein concentrations (MT)] and effect biomarkers [lipid peroxidation (LPO), RNA to DNA ratio (R:D), protein and lipid content]. In general, the interspecific differences in biomarker responses were greater than the spatial differences, due to differences in the behavior and habitat use of the species. Nevertheless, similarities were also observed considering both chemical load and biomarker responses. In highly polluted sites fish showed in general a significant antioxidant enzyme induction, associated with decreased R:D values, while fish from the least impacted site had little enzyme induction and better condition indices (high R:D and low LPO values). EROD activity was also higher for all species in the Tejo than Ria de Aveiro estuary, despite the generally higher total PAH measured in Ria de Aveiro, most likely due to a higher proportion of 4 and 6-ring PAHs, considered more toxic than low molecular weight PAHs, in the Tejo.

In conclusion, this multi-biomarker approach considering multiple species provided improved understanding of the diverse responses and effects of exposure to contaminants and the effective risk it poses for different fish species.

1. Introduction

Coastal transition ecosystems (e.g. estuaries and coastal lagoons) sustain valuable ecologic and economic resources (Costanza et al., 1997). However, they are often vulnerable to various stressors caused by human activities (i.e. agriculture, industry, human settlements, fishing, port activities) (Kennish, 2002).

The presence of organic and inorganic contaminants in estuaries renders monitoring and risk-assessment procedures essential to ensure the preservation of their biological function (Adams, 2002) and has led to the search for improved monitoring methods that can express the biological and ecological implications of pollution beyond environmental chemical characterization (van Der Oost et al., 2003).

Biomarkers are considered useful and early measures of exposure to and/or effects of contaminants in aquatic organisms (Shugart et al., 1992; Adams, 2002; van Der Oost et al., 2003) and have been frequently used to assess habitat quality (e.g. Fernandes et al., 2002; Monteiro et al., 2007; Maria et al., 2009). Nevertheless, the presence of complex mixtures of xenobiotics in the environment and of other potentially confounding factors (i.e. life stage, abiotic natural variability) may result in further difficulties in the interpretation of biomarkers response patterns (Adams, 2002; van Der Oost et al., 2003).

A multi-biomarker approach, consisting of the combined use of different biomarkers that can both signal exposure to contaminants and quantify their effects on the health of organisms, enables a more comprehensive and integrative assessment of environmental quality (Adams, 2005; Broeg and Lehtonen, 2006; Humphrey et al., 2007). Moreover, determining responses in natural populations of different fish species encompasses diverse forms of biological integration of environmental toxicants, which can result from

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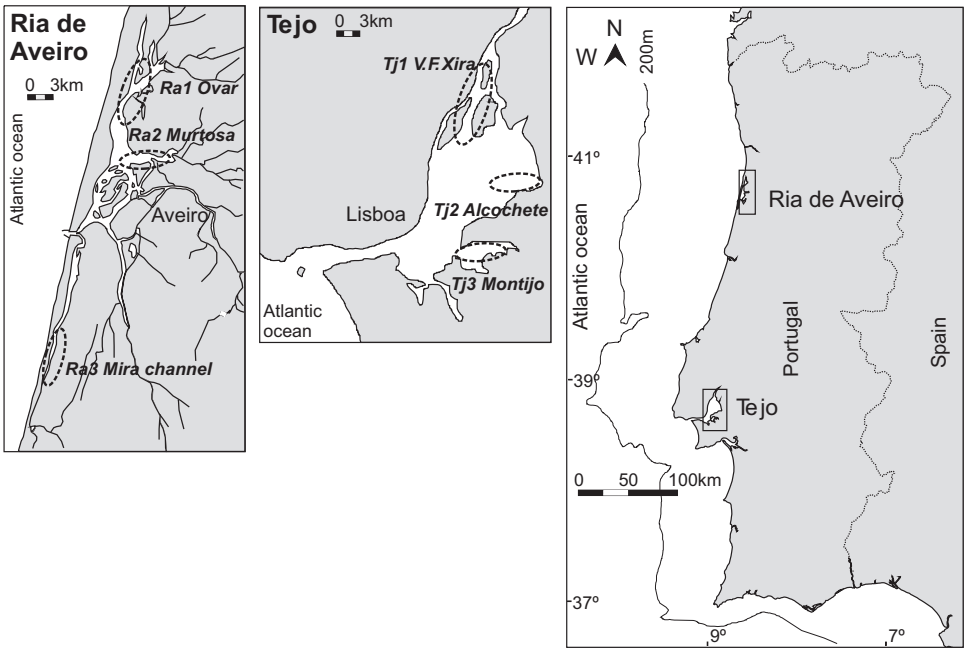

Fig. 1. Location of estuarine sites sampled in Ria de Aveiro (Ra1—Ovar; Ra2—Murtosa; Ra3—Mira channel) and Tejo (Tj1—Vila Franca de Xira; Tj2—Alcochete; Tj3—Montijo).

different physiological processes or species ecology (Solé et al., 2010).

In the present study, a multi-biomarker approach was used for three fish species with different life-history traits and strategies: common goby *Pomatoschistus microps* (Krøyer 1838), a dominant estuarine resident species with short life span (ca. 2 years) (Arruda et al., 1993; Leitão et al., 2006), and European sea bass *Dicentrarchus labrax* (Linnaeus 1758) and Senegalese sole *Solea senegalensis* Kaup, 1858, two marine species with high economic value which utilize estuaries as nursery grounds during the juvenile period (Costa and Bruxelas, 1989; Cabral and Costa, 2001; Vasconcelos et al., 2010).

The Ria de Aveiro and Tejo are estuarine systems located along the Portuguese coast, highly explored and impacted by human activities, with significant and diverse sources of aquatic contaminants due to population settlement, industrial, agricultural and shipping activities (Pacheco et al., 2005; Vasconcelos et al., 2007; Vale et al., 2008). Accordingly, several biomarkers of exposure and effect were chosen in order to account for a wide range of pollutants: (1) biotransformation enzymes [phase I ethoxyresorufin O-deethylase (EROD) and phase II glutathione S-transferase (GST) activities], that metabolize xenobiotics; (2) antioxidant enzyme activities (superoxide dismutase—SOD; catalase—CAT; and glutathione peroxidase—GPx), which reduce cellular damage (e.g. lipid peroxidation—LPO) resulting from reactive oxygen species (ROS), the levels of which in turn increase with exposure to various contaminants; (3) stress proteins, specifically metallothioneins (MT), inducible by essential and non-essential metals as well as by the presence of free oxygen radicals notably; and (4) morphological and physiological measures of the general condition of fish related to growth potential and energy reserves [condition factor K; nucleic acid ratio (R:D); protein and lipid content].

The main objective of the present work was to determine the biochemical responses of different fish species in contaminated estuarine environments, based on the spatial variability patterns (at site and estuary level) described by the set of biomarker responses for each species. The multi-biomarker approach intended to explore a wide range of fish responses to pollution in a realistic context, including biomarkers of exposure (e.g. metallothioneins; antioxidant enzyme activity) and general condition indices or biomarkers of effect (e.g. R:D ratio and lipid peroxidation, respectively) in natural populations of different fish species.

2. Material and methods

2.1. Study area and environmental chemical characterization

Two estuarine systems along the Portuguese coast were sampled in October 2008: Ria de Aveiro and Tejo. This period of the year was selected since it corresponded to the late estuarine colonization period of *D. labrax* and *S. senegalensis* juveniles, and to the occurrence period of early adults of *P. microps*. Three sites were selected per estuary based both on the presence of known anthropogenic pressures and on the composition of the fish community—i.e. in order to ensure that the three fish species were present (França et al., 2009; Vasconcelos et al., 2010; Fig. 1).

Chemical characterization of each site was based on the quantification of trace metals (cadmium, Cd; copper, Cu; zinc, Zn; nickel, Ni; lead, Pb and chromium, Cr) and polycyclic aromatic hydrocarbon (PAH) concentrations in the sediment, since sediments can serve as pollution reservoirs or sinks for particle-sorbed contaminants and as records of the anthropogenic pressures in the aquatic environment (Connor, 1984; Baumard et al., 1998). Three replicate sediment samples from each site were used for chemical characterization, and trace metals were determined in the mud fraction of the sediment (grain size <0.063 mm) to minimize the effect of different grain size composition of samples on metal distribution. Briefly, samples were sieved in 63 μm nylon sieves using Milli-Q water and the mud fraction was dried at 40 °C. Approximately 2 g of the dried and grinded sediment fraction were digested with 5 ml of 69% nitric acid (HNO_3) for 2 h at 80 °C. The digestion mixture was cooled to room temperature and 2 ml of 30% hydrogen peroxide (H_2O_2) and 3 ml of Milli-Q water were added. The mixture was heated for another hour, before the residue was allowed to cool and further diluted with Milli-Q water to a final volume of 50 ml. Samples were allowed to settle for 24 h and quantitatively transferred to 50 ml vessels for analysis.

The concentrations of Cd, Cu, Ni, Pb, Cr and Zn were determined in the supernatant using a Perkin-Elmer atomic absorption