



## Modeling fish biological responses to contaminants and natural variability in estuaries



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### ABSTRACT

Understanding the factors that influence biological responses to contaminants has long been a major goal in marine environmental research. Seven estuarine sites along the Portuguese coast were sampled over a year, and different biological responses of *Pomatoschistus microps* and *Atherina presbyter* were determined: superoxide dismutase, catalase, ethoxyresorufin O-deethylase, glutathione S-transferase, metallothioneins, lipid peroxidation, RNA:DNA ratio and condition factor K. Generalized linear models (GLM) were developed for each biological variable per species in relation to sediment chemical characterization (metals and polycyclic aromatic hydrocarbons concentration) and environmental conditions (month, site, water temperature, salinity, depth and mud percentage in the sediment). GLM varied in explanatory power and in the set of predictor variables included in the models. Environmental factors were frequently selected as predictor variables. Individual metals concentration and sediment quality guidelines (integrating all metals) were the major contaminants explaining biological variability. Accordingly, models for metallothioneins and lipid peroxidation had highest explanatory power. Species-specific responses and dataset size were the basis of observed differences between GLM for the two species.

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### 1. Introduction

Estuaries are widely considered as naturally stressed ecosystems, where organisms' resilience to the inherently varying physico-chemical conditions is fundamental to their survival (McLusky and Elliott, 2007). In addition to natural variability, estuarine systems are often exposed to different types, magnitudes and mixtures of chemicals derived from human activities, making it difficult to disentangle between natural and anthropogenic-induced stress responses (Elliott and Quintino, 2007). This is particularly difficult at the ecosystem level, thus lower level changes, such as enzymes activities or specific metabolic products, have been extensively used to assess environmental quality in relation to pollutants exposure in natural populations (Stegeman et al., 1992; van der Oost et al., 2003; Walker et al., 2001).

Several abiotic and biotic factors are known to influence biomarker responses, namely water temperature, salinity, dissolved oxygen, diet, gender and reproductive stage, and can therefore confound results interpretation (Fonseca et al., 2011b;

Gourley and Kennedy, 2009; Martínez-Álvarez et al., 2005; van der Oost et al., 2003). Hence, understanding how these changes at lower levels of biological organization respond to both natural and human-induced challenges is essential to ensure their applicability and predictability towards an effective monitoring and impact assessment.

Biological inference sustaining the development of reliable and quantitative models to predict ecological outcomes, based on previously observed responses, is a promising tool widely applied to predict species distribution and community properties (e.g. França et al., 2012; Riou et al., 2001; Vasconcelos et al., 2010, 2013; Whaley et al., 2007), yet still scarcely applied to responses at lower levels of biological organization (e.g. Costa et al., 2008; Gosz et al., 2011; Napierska and Podolska, 2005).

In the present work, the activity level of several classic biomarkers of effects and exposure to contaminants, as well as condition indices, were determined in two fish species collected from five estuaries along the Portuguese coast.

Different biomarker responses were considered in accordance to major classes of contaminants [metals, polycyclic aromatic hydrocarbons (PAHs) and organometallic compounds] previously signaled as potential hazardous in many of the estuarine sites studied, namely: (1) the enzymatic antioxidant activity of

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superoxide dismutase (SOD) and catalase (CAT), that mitigate the toxicity of reactive oxygen species (ROS) originated from aerobic metabolism or from exposure to contaminants (or their metabolites) such as metals or organic pollutants (e.g. quinones, PAHs); (2) the enzymatic activity of phase I ethoxyresorufin-O-deethylase (EROD) and phase II glutathione S-transferase (GST), both mediated through the aryl hydrocarbon-receptor, in order to metabolize xenobiotics, such as PAHs, polychlorinated biphenyls (PCBs) or dioxins, and facilitate their excretion; (3) metallothioneins (MT) as indicators of metal contamination and oxidative stress; (4) and lipid peroxidation (LPO) as indicators of cellular damage (Stegeman et al., 1992; van der Oost et al., 2003; Viarengo et al., 2000; Whyte et al., 2000; Winston and Di Giulio, 1991).

Condition indices (RNA to DNA ratio – R:D, and the condition factor K) were also considered. These are not specific to environmental contamination as they have a more integrated response to the environment and provide information on individuals overall fitness (e.g. Buckley et al., 1999).

Biological responses were analyzed in two fish species that are abundant throughout the year in various estuarine systems: common goby *Pomatoschistus microps* (Krøyer, 1838), a widespread and dominant estuarine resident species (Arruda et al., 1993; Leitão et al., 2006); and sand smelt *Atherina presbyter* Cuvier, 1829, a pelagic species widespread in coastal and estuarine areas (e.g. Pombo et al., 2005). Both species have been previously tested in bioassays and contaminant monitoring studies (e.g. Fonseca et al., 2011a, 2011b; Pacheco et al., 2004; Serafim et al., 2012; Vieira et al., 2009).

The aim of the present study is to develop statistical models to describe the variation in fish biological responses to contaminants in changing environments, namely estuaries. Specifically, models will be based on measured environmental features as well as sediment chemical load, considering major classes of contaminants

concordant with fish biological responses. This approach is particularly pertinent since changes in the fish assemblage associated with environmental chemical pollution have been previously described for the sampled estuaries (Fonseca et al., in press) and changes at lower biological levels can be expected.

## 2. Material and methods

### 2.1. Study area and sampling survey

Seven estuarine sites from five estuaries along the Portuguese coast were sampled in January, April, July and October 2009: Ria de Aveiro (A, B), Tejo (C, D), Sado (E), Mira (F) and Guadiana (G) (Fig. 1). According to an anthropogenic pressure index described in Vasconcelos et al. (2007) (ranging from 0 to 1), these systems have different overall pressures, ranging from 0.14 in the Mira estuary to 0.76 in the Tejo estuary, the most pressured system. Two fish species were collected using a beam trawl – common goby *P. microps* and sand smelt *A. presbyter*. *A. presbyter* only occurred systematically in the three southern sites (E, F and G). Upon collection, fish were transported to the laboratory (following Quintaneiro et al., 2006), where they were immediately sacrificed, total length (Lt, in mm) and weight (Wt, in g) recorded. Muscle and liver tissues were dissected and stored individually in microcentrifuge tubes at  $-80^{\circ}\text{C}$ . Individuals from each species were selected aiming at ensuring a similar size range and equal sex ratio per month and site. Simultaneously with fish sampling, water temperature and salinity were measured with a multi-parameter probe (WTW). In each site three replicate sediment samples were collected with an adapted van Veen grab ( $0.05\text{ m}^2$ ) in intertidal mudflat habitat, and mean depth as well as mean mud content were also determined (percentage of dry sediment not retained in a  $0.063\text{ mm}$  calibrated sieve) (Table 1).

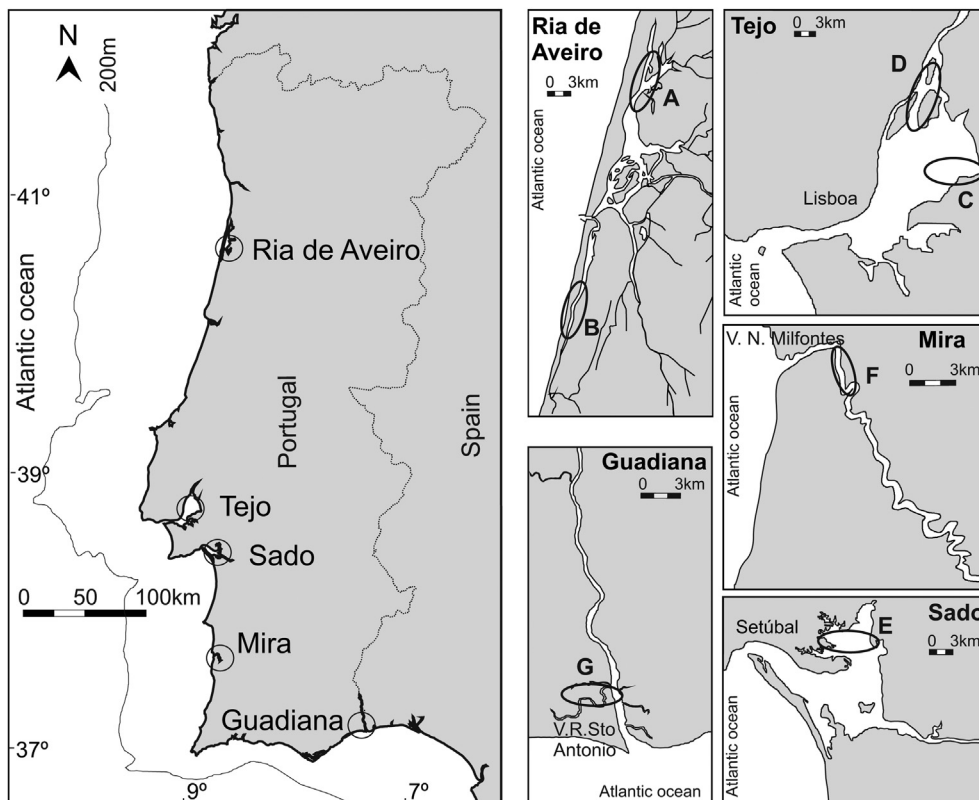


Fig. 1. Location of estuarine sites sampled (A to G) in the Ria de Aveiro, Tejo, Sado, Mira and Guadiana estuaries, along the Portuguese coast.

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