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Integrated biomarker responses of the invasive species *Corbicula fluminea* in relation to environmental abiotic conditions: A potential indicator of the likelihood of clam's summer mortality syndrome

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ABSTRACT

The aim of this study was to investigate the variation of several biomarkers in wild populations of *Corbicula fluminea* in relation to abiotic condition changes to identify environmental factors associated with increased stress in this species potentially leading to massive mortality events. The study was carried out from July to October in the freshwater tidal areas of the estuaries of Minho and Lima Rivers (NW Iberian Peninsula). Monthly, 7 biomarkers (biotransformation, energy production, anti-oxidant defenses and lipid peroxidation damages) were determined in *C. fluminea* and 17 abiotic parameters were determined in water or sediments in 4 sampling sites: M1, M2 and M3 in Minho (up = > downstream); and L in Lima estuaries. The results of biomarkers were integrated using the Integrated Biomarker Response (IBR), Index and also analysed in relation to environmental parameters by Redundancy Analysis (RDA). Overall, the findings of the present study indicate that July and August are particularly stressful months for the studied *C. fluminea* populations, especially at downstream sites; the increase of nutrients and ammonium water concentrations, water temperature and conductivity are major contributors for this increased stress; the biomarkers indicated that in July/August *C. fluminea* is exposed to oxidative stress inducers, environmental chemical contaminants biotransformed by esterases and glutathione S-transferase enzymes, and that organisms need additional energy to cope with the chemical and/or thermally-induced stress. The findings of the present study stress the importance of biomonitoring the health condition of *C. fluminea* because it may allow determining the likelihood of summer/post summer mortality syndrome in this species.

1. Introduction

The summer/post-summer mortality syndrome in bivalves is a periodical event in temperate regions that may have significant adverse ecological and economical impacts (Tomaru et al., 2001; Mouthon and Daufresne, 2006; Werner and Rothhaupt, 2008; Vohmann et al., 2009; Weitere et al., 2009; Ilarri et al., 2010). In wild ecosystems, the sudden input of high amounts of organic matter from dead bivalves and its degradation often leads to considerable changes in nutrient cycles, energy fluxes, a deep reduction of water dissolved oxygen and massive mortalities of several other species, with important changes in ecosystem functioning and potential biodiversity losses (Cooper et al., 2005; Baur and Schmidlin, 2007; Sousa et al., 2007, 2008b, 2008c). The dimension and severity of such events are especially high if the primary affected bivalve species is a keystone species of the ecosystem and/or if it has a considerable biomass (Power et al., 1996). Furthermore, in ecosystems invaded by non-native invasive species (NIS), massive mortality events may have a decisive influence on the competition between the bioinvasor and its native competitors, often acting in favour of the NIS. This is believed to occur because NIS generally recover faster from such adverse events than their native competitors (McMahon, 2002).

Several factors and conditions have been pointed as possible causes for the summer/post-summer mortality syndrome in bivalves, including extreme events such as draughts, temperature increase, decrease of oxygen concentration in the water, increase of pollution, post-spawning stress among several others (Tremblay et al., 1998; Urrutia et al., 1999; García-esquivel et al., 2001; Morgan et al., 2003; Cooper et al., 2005; Mouthon and Daufresne, 2006; Dégremont et al., 2007; Vohmann et al., 2009; Weitere et al., 2009; Cotter et al., 2010; Huvet et al., 2010; Ilarri et al., 2010). However, the phenomenon is not yet completely understood (Vohmann et al., 2009; Rosa et al., 2011) and more research is needed.

The goal of the present study was to investigate the variation of a set of biomarkers in wild populations of *Corbicula fluminea* in relation to abiotic conditions changes to identify environmental factors associated

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with increased stress in this species potentially leading to massive mortality events. This species was selected for this study mainly because it is one of the 100 worst invasive species in Europe (DAISIE, 2009); massive mortality events in their populations have been having considerable economic and ecological negative impacts (Cherry et al., 2005; Cooper et al., 2005; Sousa et al., 2008b, 2011; Ilarri et al., 2010), and these events have being pointed as decisive contributors to the decline of several native bivalve competitors.

2. Material and methods

2.1. Chemicals

All the chemicals used were of analytical grade and purchased from Sigma-Aldrich (Germany), Merck (Germany) or Bio-Rad (Germany).

2.2. Sampling estuaries, sites and C. fluminea populations

The populations of the tidal freshwater area (TFA) of two neighbour estuaries, those of Minho and Lima Rivers (NW Iberian coast), were selected for this study because summer massive mortality events have been reported for the former (Sousa et al., 2007; Ilarri et al., 2010) but not for the latter. *C. fluminea* is present in the Minho estuary at least since 1989 (Araújo et al., 1993). At present, the population is spread over all the TFA and has a very high density (more than 4000 individuals per m^2 in some sites) and biomass (more than 400 g of AFDW per m^2) (Sousa et al., 2005). In the TFA of the Minho estuary, 3 sampling sites were selected (Fig. 1) based on Sousa et al. (2008d) identification of three main areas with distinct environmental characteristics (upstream to downstream): M1 (N42°03′09.37″ W8°33′42.73″), a relatively low impacted area with low organic matter (OM) and environmental contamination, and residual tidal influence; M2 (N42°01′25.12″ W8°39′24.49″), located about 3 km (downstream) far from the mouth of the Louro River, a tributary of the Minho River that is one of the most contaminated rivers in Galicia (Filgueiras et al., 2004; Concha-Graña et al., 2006; Farkas et al., 2007; Lavilla et al., 2010), with a high content of OM; and M3 (N41°54′41.25″ W8°47′36.59″), with a greater tidal influence. *C. fluminea* was recorded for the first time in the Lima estuary in 2002 and has a relatively low density in the TFA (mean of 60 individuals per m^2 and 26 g AFDW per m^2) (Sousa et al., 2006a, 2006b). This estuary has been considered more contaminated than the Minho estuary, with impacts from a paper mill industry, urban settlement, agriculture crops, and harbour activities (INAG, 2000; Cairrão et al., 2004; Sousa et al., 2006a; Guimarães et al., 2009; Reis et al., 2009). Due to the low density of *C. fluminea* in the TFA, only one sampling site was selected (Fig. 1), hereafter indicated as L, in a restricted area about 15 km upstream from the estuary mouth (N41°42′07.03″ W8°44′37.05″).

2.3. C. fluminea sampling

C. fluminea specimens were collected monthly from July to October 2011 in the subtidal area (about 40–90 cm deep), at low tide using a hand rake with a net. Clams were transported to the laboratory within 2 h after their collection, being maintained in water from the local where they were collected in thermally isolated boxes regularly open to allow water oxygenation. These conditions were found adequate for the transport of the clams and did not influence significantly the biomarkers'determinations in preliminary studies (data not shown).

2.4. Abiotic parameter determination

Monthly, at each sampling site and simultaneously to *C. fluminea* collection, water temperature, conductivity, dissolved oxygen and pH were measured in triplicate using a multiparameter probe (WTW 340i). In addition, water samples were collected, in triplicate, and maintained in appropriate bottles on ice until arrival to the laboratory where the following parameters were determined using colorimetric methods following the Photometer Systems for Water Analysis protocols (Palintest 7000 interface photometer): hardness ($CaCO_3$), turbidity (Turb) and the concentration of ammonium (NH_4^+), nitrates (NO_3^-), nitrites (NO_2^-), phosphates (PO_4^-), iron (Fe), phenol (C_6H_5OH), and silica (SiO_2).

Sediment samples were collected in triplicate with a 100 ml syringe to a 50 ml polyethylene centrifuge tube. Thirty milliliters of acetone (90%) and 0.2 ml of magnesium carbonate (1 g 100 ml^{-1} ultra pure water) were added to each tube, and the mixture was shaken and maintained on ice in a thermally isolated box until arrival to the laboratory. Once in the laboratory, samples were kept at 4 °C for 24 h. After 24 h, samples were centrifuged (in Kubota 5400 centrifuge) for 3 min at 2330 *g*, mixed and centrifuged again for 10 min in similar conditions; 3 ml of each sample supernatant was carefully collected, put in a glass spectrophotometer cuvette and its absorbance was read in a spectrophotometer (Jenway 6405 UV/VIS) at 480, 630, 645, 647 664, 665 and 750 nm; after addition of 0.2 ml of a HCl 1 N solution directly to the cuvette, agitation and 4 min waiting, the absorbance was read again at 665 and 750 nm; the concentration of chlorophyll *a*, chlorophyll *b* and chlorophyll *c* was calculated according to Jeffrey and Humphrey (1975).

The quantification of organic matter was obtained after combusting during 24 h at 550 °C in a muffle furnace (Fisher Scientific, Isotemp Muffle Furnace). Values are expressed in percentage relatively to the weight loss on ignition of each sample analysed.

2.5. Determination of biomarkers

In the laboratory, the anterior–posterior shell length of the clams was measured with a calliper (0–150 mm). Animals (20) with a shell

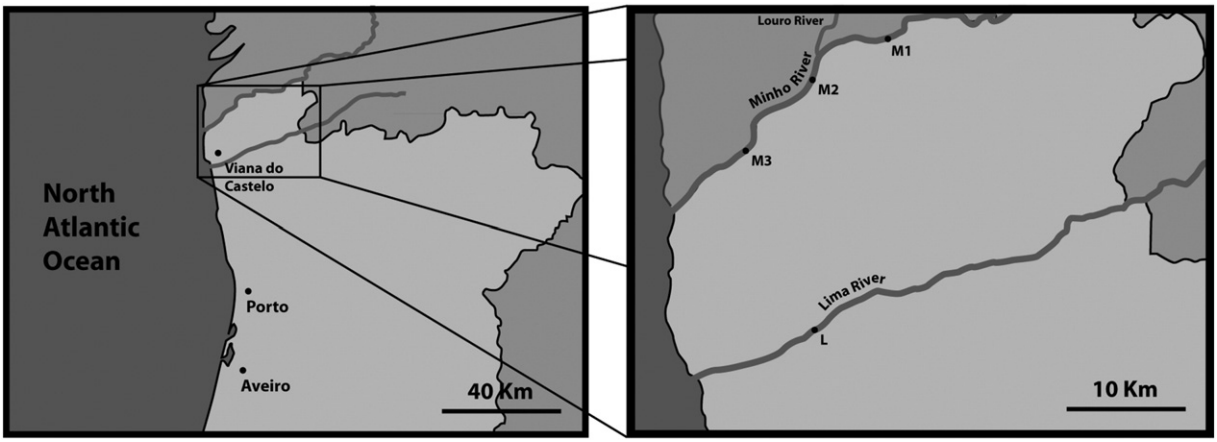

Fig. 1. Map of Minho and Lima Rivers showing the four sampling sites location.