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Brain as a target organ of climate events: Environmental induced biochemical changes in three marine fish species

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ABSTRACT

The present study aims to examine physiological and biochemical response of three commercial fish species (*Dicentrarchus labrax*; *Platichthys flesus* and *Solea solea*), over contrasting environmental dynamics: an extremely dry (2012) and flood (2014) years, in a shallow temperate southern European estuary, the Mondego Estuary (Portugal). Physiological and biochemical biomarkers were evaluated by a principal component analysis (PCA), which allowed to conclude that severe climatic events affected the set of fish species analyzed, revealing two distinct annual brain antioxidant responses. The drought episode affected the physiological state of the organisms, as well as increased brain antioxidant potential, strongly associated with fluctuations in environmental drivers (salinity and dissolved oxygen), however, ROS have not been effectively neutralized by antioxidant defence system causing lipid peroxidation. During flood episode was stated a general depletion of the antioxidant potential in the analyzed fish species, affected by interactions with chemical compounds, increased by a combination of high precipitation and associated runoff, probably, increasing nutrient and contaminant load at the Mondego estuary. Nevertheless, lipid peroxidation remained low, related to the action of non-enzymatic antioxidants, since that the studied fish species had optimal physiological status and high nutritive reserves. According to the present work we consider that brain enzymatic depletion may be organ-specific, looking to the greater vulnerability of brain's proteins to degradation compared to lipids. The role of enzymatic mobilization on fish brains is not extensively yet known, but our results suggest that brain seems to be metabolic sensitive to salinity and dissolved oxygen fluctuations. This is the first approach made to evaluate the physiological and biochemical responses of the brain of aquatic organisms' to extreme climatic events and to establish reference values to determine the effects of extreme climate events to aquatic species.

1. Introduction

In a world experiencing global climate change, the frequency of extreme weather events, such as droughts and periods of flood, appears to be increasing, clearly contributing to the decline in the quality of aquatic systems (IPCC, 2007).

Abiotic (e.g. temperature, salinity, dissolved oxygen, runoff, level of organic matter, etc.) and biotic factors (feeding, reproduction, age, parasitism, etc.) fluctuate throughout the year influenced by extreme climatic events (Hooper et al., 2012). Environmental factors modulate the biology of aquatic organisms, changing the bioavailability of contaminants and the chemical interactivity of them that may increase their toxicity when compared to the action of each compound alone in the aquatic system, especially in estuarine ecosystems with high retention time (Sheehan and Power, 1999).

Reactive oxygen species (ROS) are a non-viable part of aerobic life, resulting in oxidative stress if ROS generation prevails over antioxidant capacity (Lushchak, 2011). ROS cause damage to cellular proteins, lipids and nucleic acids, often leading to injury of organs (Lushchak, 2011). In order to protect against oxidative stress, organisms develop their antioxidant defence system consisting of low molecular weight metabolites (tocopherol, ascorbic acid, glutathione) and higher molecular weight proteins including antioxidant enzymes such as glutathione peroxidase (GPx), glutathione S-transferase (GST) and

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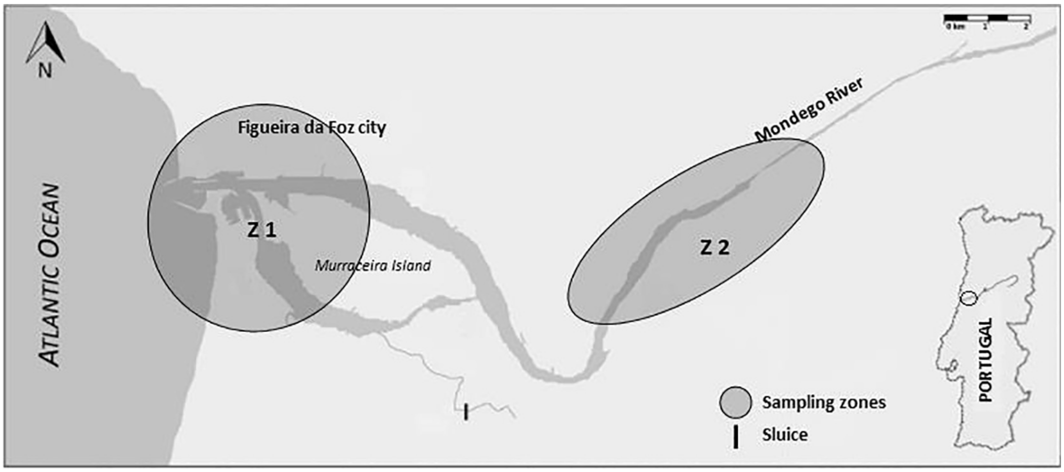

Fig. 1. Location of the sampling zones in the Mondego estuary: Zone 1 (Z1 – include mouth of the estuary and downstream areas of the north and south arms); Zone 2 (Z2 – include medium and upstream areas of the north arm).

glutathione reductase (GR), which are used globally as early warning signals of environmental stress conditions (Hellou et al., 2012). Lipid peroxidation (LPO) has been described as the major contributor to the loss of cellular function under oxidative stress, and has been usually indicated by Thiobarbituric acid reactive substances (TBARS) measurements in fish (Van der Oost et al., 2003). Temporal variations of antioxidant defences may affect the protection level of organisms and make it difficult to distinguish between the effects of pollutants and the effects of other environmental factors (Pain et al., 2007). Consequently, it is greatly important to determine the normal range of biomarkers variability in order to propose useful environmental diagnostic tools (Lushchak, 2011).

Recently, some studies have shown that the brain is a highly sensitive organ to determine oxidative stress in both fish and humans (e.g. Huang et al., 2008; Ferraro et al., 2009), associated with the decline of its richness in PUFA-n3 fatty acid (Dringen, 2000). Brain is one of the most active and sensitive metabolic organs to be altered by a wide variety of factors (Soengas and Aldegunde, 2002). Therefore, more research is necessary to verify induced oxidative damages, especially in neural tissues due to the importance of their crucial functions for the organisms' survival (Birnie-Gauvin et al., 2017). The limited number of published studies are the main reason to the scarce knowledge about the threat of oxidative stress in the fish brain, with most of them being laboratory experiments, involving the injection of toxic substances (Hellou et al., 2012). In this context, more information about the antioxidant system in the fish brain is needed in order to evaluate the susceptibility to suffer neurotoxicity, considering the crucial role of environmental factors (Pain et al., 2007). In addition to the accumulation of toxic substances, it is essential to include the temporal factor as an influence on the variation of responses to oxidative stress (Hooper et al., 2012). Measurements of biomarkers responsiveness in fish have a good potential to integrate environmental monitoring programs (Van der Oost et al., 2003). The complexity of these variables added to extreme weather events should be taken into account when interpreting the results on the field and in laboratory experiments (Hellou et al., 2012; Hooper et al., 2012).

Dicentrarchus labrax and two flatfish species, *Platichthys flesus* and *Solea solea,* were chosen for this study, because of their high economic and commercial interest, being the most abundant and representative species of the Atlantic and Mediterranean coasts, occupying a high trophic position and occurring in the most European estuaries, including the Mondego estuary. These species have been commonly used in many field and laboratory studies, proving to be viable and sensitive to environmental anomalies (Kopecka & Pempkowiak, 2008; Kleinkauf et al., 2004; Oliva et al., 2012). Extreme climatic events have been verified in the last decades worldwide, Portugal not being an exception (Gonçalves et al., 2017), with ecological impacts to aquatic communities (e.g. Gonçalves et al., 2012; Grilo et al., 2011).

Since it is crucial to determine and evaluate the physiological and biochemical responses of organisms under the influence of extreme climatic events, the main objectives of this work are to: 1) determine the physiological and biochemical responses in brain samples from the three fish species collected at the Mondego estuary; 2) extract the causal influences in variability on selected biochemical and physiological markers; 3) compare the responsiveness of the three commercial fish species and infer about the potential use of the brain and the selected battery of biomarkers to assess the specific response to climate events, contributing to the elimination of gaps in biomonitoring studies in estuarine and coastal systems.

2. Materials and methods

2.1. Study site

The Mondego estuary, situated in a warm temperate region on the Atlantic coast of Portugal (40°08′N, 8°50′W), is a small estuary of 8.6 km^2, comprising two arms, north and south, separated by an island, and at from 7 km from shore that join again near the mouth (Fig. 1). The north arm is deeper (5–10 m during high tide, tidal range 2–3 m), highly hydrodynamic, is a main navigation channel and hosts the Figueira da Foz harbour. The south arm is shallower (2–4 m during high tide, tidal range 1–3 m) and is characterized by large areas of exposed intertidal flats during low tide, with higher residence times and is almost silted over in the upstream areas. The mouth of the estuary depth ranges from 8 to 13 m, and this area is influenced by both river flow and neritic waters. Most of the river freshwater flow enter via the north arm and a small freshwater input is carried via the southern arm through the Pranto River, a small tributary system regulated by a sluice according to water needs in the surrounding rice fields. Previous studies demonstrated that singular environmental factors provide a large variety of aquatic habitats for species, mainly due to salinity gradient (Gonçalves et al., 2012).

The main environmental pressures in the Mondego estuary are: dredging and shipping in the north arm, raw sewage disposal and high nutrient input from agricultural and fish farms in upstream areas of the estuary. Combined with a high water residence time, this has led to an eutrophication process over the past two decades (Dolbeth et al., 2013). The system has been recovering from this particular eutrophication scenario since early 1990’s, showing nowadays, a significant improvement in water quality and own faunal composition (e.g. Dolbeth et al.,