Ecological Indicators 76 (2017) 59–66

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Original Articles

The biochemical response of two commercial bivalve species to exposure to strong salinity changes illustrated by selected biomarkers

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ARTICLE INFO

Article history:
Received 27 September 2016
Received in revised form 17 January 2017
Accepted 19 January 2017

Keywords:
Marine bivalves
Fatty acids
Oxidative stress
Salinity
Mondego estuary

ABSTRACT

Salinity is a major controlling factor in estuarine systems whose fast change, namely during the occurrence of extreme climatic events, causes drastic alterations on aquatic communities by promoting a physiologically stressful environment. The response of fatty acid (FA) and antioxidant enzymes' activity (Glutathione S-transferase (GST) and Superoxide dismutase (SOD)) of *Cerastoderma edule* and *Scrobicularia plana* were investigated under a wide range of salinity. Species were sampled in Mondego estuary (Portugal). A set of organisms (namely "field") were stored for biochemical analysis, whereas the remaining organisms collected in the field (namely "lab") were exposed to a range of salinity concentrations. Organisms were fed daily. In general, results revealed a decrease on enzymatic activity along a set of salinity concentrations with an exception to the GST activity of *C. edule* where a trend of increase at the activity was observed at almost all treatments. *S. plana* presented a very low or null activity to both enzymes. Differences in the FA profiles of both groups were also observed, with "lab" organisms not presenting saturated FA of short chain. The diversity on FA and the quantity in unsaturated FA under different salinity concentrations presented the highest values at the extreme salinity treatments. *C. edule* directly stored from the field presented the highest diversity and quantity in polyunsaturated fatty acids (95.77%) whereas organisms of *S. plana* from the field showed the highest percentage of highly unsaturated fatty acids (20.93%). Results suggest that, under salinity stress, the consumption of food decreases and the physiological pathways are reduced. Still species can store FA recognized as of high physiological importance to animals, by reducing their activity and energy consumption. Therefore, under an extreme climatic event (e.g. drought or flood) these species may present a higher content of essential FA and, thus, a higher food quality, reducing, in general, the activity of the enzymes SOD and GST.

1. Introduction

Estuaries are adjacent coastal areas highly productive and are among the most important ecologic and socio-economic environments providing natural valuable resources to human beings, mainly to local populations. These systems are transitional areas characterised by extreme daily variations of a set of environmental parameters such as temperature, salinity and oxygen, exposing the organisms to a wide physiological stress. It is well known that salinity is one of the main controlling factors to organisms' growth causing great constrains on species productivity and diversity (Kirrolia et al., 2011; Regalla et al., 2007; Verdelhos et al., 2015).

Fatty acids are important molecules playing physiological important roles such as at the structure and functional regulation of cell membrane and on neural levels, being also important at the prevention of some diseases (Arts et al., 2009). These molecules are used as energy in all metabolic pathways of all trophic levels, being the main components of lipids. In some animals the lack of some essential fatty acids (EFA) may cause several disorders linked to mortality, low productivity and reproductive success being these

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http://dx.doi.org/10.1016/j.ecolind.2017.01.020
1470-160X/

EFA mainly incorporated through organism's diet as most animals are not able to synthesize them, or at enough amounts, to gather their physiological requirements (Arts et al., 2009; Filimonova et al., 2016). Proteins are involved in complex pathways and metabolic processes, playing an important role to repair and protect organisms from environmental induce damage. Indeed, patterns of differential protein production and expression reflect physiological responses to changing or stressful environmental conditions, including climate changes (Gotelli et al., 2012). Enzymes are the molecular biomarkers that take part in the defense of cells against oxidative stress (Almeida et al., 2010; Antunes et al., 2013). Many studies have demonstrated a close link between the activity of enzymes critical to energy metabolism, metabolic rate, and food availability (Dahlhoff, 2004). Superoxide dismutase (SOD) and glutathione S-transferase (GST) are two principal primary enzymes in antioxidant systems acting from directly scavenging free radicals, being the health status, environmental stress and dietary nutrients the main regulators of their activities (Kurutas, 2016; Li et al., 2008). These biomarkers (fatty acids and enzymes) are argued to be good bio-indicators of stress due to their high sensitiveness (Filimonova et al., 2016; Gonçalves et al., 2016). Physiological responses of aquatic organisms to salinity, mainly in lipids and fatty acids with a decrease in enzymatic activities, has also been reported in some studies (Carregosa et al., 2014; Fujii et al., 2001; Guerzoni et al., 2001; Li et al., 2008; Romano et al., 2014), still information regarding the bivalves species *Scrobicularia plana* and *Cerastoderma edule* remains unknown. *S. plana* and *C. edule* are estuarine bivalve species that present a wide geographical distribution. The former one is typically found from Norway to the Mediterranean and West Africa, whereas *C. edule* occurs from North Africa to Northern Norway and Murmansk in the Arctic, being present also on the east coast of the Atlantic (Freitas et al., 2014; Verdelhos et al., 2015). The bivalve species are able to tolerate large salinity changes related to short-term (tidal) and long-term (rainy periods) episodes, even though they do not show osmo-regulatory ability (Evans, 2009). At high (e.g. during drought episodes) or low (e.g. during flood events) salinity concentrations the stress of the organism increases and mortality rates are detected. Extreme weather events are often at the past decades worldwide, with Portugal not being an exception. At the Mondego estuary flood and drought events have already been reported with ecological impacts to aquatic communities (Cardoso et al., 2008; Gonçalves et al., 2012; Grilo et al., 2011; Verdelhos et al., 2014). Therefore, it is crucial to determine and assess lethal effects and physiological responses of aquatic organisms under the influence of salinity stress in order to predict the impacts on communities and thus at aquatic ecosystems.

The main objectives of this work are to: 1) determine and compare the sensitivity of *C. edule* and *S. plana*; 2) determine and assess biochemical response, namely fatty acids and enzymatic activity of GST and SOD, and 3) compare the nutritive value of both commercial bivalve species when exposed to a range of salinity concentrations in order to detect possible changes and predict the impacts on biochemical pathways and species traits of severe weather events (drought or flood episodes), that are foresee to be more frequent at a warmer world. Furthermore, this information will be also an available contribution to aquaculture sector on the selection of the most adequate and suitable conditions to bivalves' production.

2. Materials and methods

2.1. Study site and sampling procedures

Mondego Estuary is a small mesotidal system of 8.6 km^2, located on the western Atlantic coast of Portugal (40°08′N, 8°50′W) (Fig. 1). It comprises 2 channels, north and south, separated by the island of Morraceira which is about 7 km from the shore, and the channels join again near the mouth. Sampling of *C. edule* and *S. plana* was conducted in the north and south arms, respectively (Fig. 1). The organisms were collected using a dredge and then were put immediately in cold boxes with water from the estuary to be transported to the laboratory where the bivalves were divided by species in aquaria.

2.2. Laboratory and bioassays procedures

In the laboratory the muscle (foot) of ten individuals of each bivalve species was removed and stored at −80 ◦C until biochemical analysis, to further determination of fatty acid contents and enzymatic activity of the organisms from the field. The muscle is normally the tissue used to fatty acid analysis due to the high content stored in these molecules. Thus, and to compare the biochemical results, the muscle was also used to determine enzymatic activity. The remaining organisms were maintained in filtered sea water under laboratory conditions (T = 20 ± 2 °C; Salinity = 20; Photoperiod = 12 h^L:12 h^D, continuous aeration) and without food for depuration (Gonçalves et al., 2016; Underwood et al., 2004). The experimental saline concentrations were obtained by successive dilutions of marine filtered seawater (35 psu) in distilled water, with salinity concentrations ranging from 0 to 35 psu in order to fulfill a wide range of salinity concentrations. The optimal of salinity to these species is 20 psu, with this concentration being also referred as the control treatment at the manuscript. Each treatment was composed by ten replica with a final test volume of 1000 ml at each vial as previously described by Gonçalves et al. (2016), and guarantying a good and robust statistical design to toxic and biochemical analyses. Experimental organisms were fed daily with a frozen mixture of rotifers and microalgae previously diluted in freshly prepared salinity medium. Bivalve species were transferred to freshly prepared test solution every other day. Every day, at the same approximate hour, the species were checked for mortality and behavioral conditions (evaluation the reactions of the organism when is being fed, the activity of the valve condition and siphon). Tests were conducted under the same laboratory conditions as referred above, during 120 h. After the exposure period, all survival organisms passed by a set of phases (dissection, measurement of the weight and the body length and evaluation of their condition indices).

2.3. Biochemical analysis

Biochemical analyses were individually performed in each of the two organisms per replicate. The here-assessed parameters were the quantification of enzymatic activity (GST, SOD), the quantification of total soluble protein content and the determination and quantification of fatty acid content. Muscle tissue was stored at −80 °C until further analyses.

2.3.1. Protein quantification and enzymatic activity determination

2.3.1.1. Protein.

Protein concentration was determined according to the spectrophotometric (wavelength 595 nm) method of Bradford (1976) adapted to microplate, using Bovine Serum albumin (BSA) as a standard.

2.3.1.2. Glutathione S-transferase (GST).

The Glutathione S-transferase (GST) activity measurement is a colometric assay as described in Habig et al. (1974). The protocol was adapted to use a Multilabel Microplate Reader (Victor X3, Perkin Elmer, Villepinte, France) to detect 1-chloro-2,4-dinitrobenzene