Science of the Total Environment 639 (2018) 457–464

Contents lists available at ScienceDirect

Science of the Total Environment

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

Redox and metabolic strategies developed by anterior and posterior gills of the crab *Neohelice granulata* after short periods of hypo- or hyper-osmotic stress

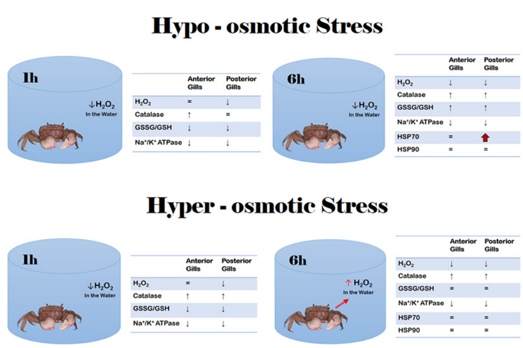
F.A. Fernandes [a,b], B.K. Dutra [b], F. Mosele [c], A.S.R. Araujo [c], G.D. Ferreira [d], A. Belló-Klein [c], L.C. Kucharski [a], A.S. Vinagre [a,*], R.S.M. Da Silva [a]

[a] *Laboratório de Metabolismo e Endocrinologia Comparada (LAMEC), Departamento de Fisiologia, Institute of Basic Health Sciences (ICBS), Federal University of Rio Grande do Sul (UFRGS), Brazil*
[b] *Laboratório de Ictiologia, Instituto Federal de Educação, Ciência e Tecnologia Farroupilha – Campus São Vicente do Sul (IFFAR), Brazil*
[c] *Laboratório de Fisiologia Cardiovascular, Departamento de Fisiologia, ICBS, UFRGS, Brazil*
[d] *Departamento de Fisiologia, Federal University of Pelotas (UFPEL), Brazil*

HIGHLIGHTS

- In crab gills rapid osmotic challenges induced redox and metabolic strategies.
- Redox and metabolic strategies differ between anterior and posterior gills.
- Osmotic stress type and time of exposure change the redox and metabolic strategies.
- Na^+/K^+ATPase activity decreased in both gill sets when facing osmotic stress.
- HSP70 expression increased after hypo-osmotic stress only in posterior gills.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 February 2018
Received in revised form 11 May 2018
Accepted 13 May 2018
Available online xxxx

Editor: Henner Hollert

Keywords:
Crustacean
Osmotic stress
Gills
Oxidative stress
Heat shock proteins
Na^+/K^+ATPase activity

ABSTRACT

The aim of this study was to identify the response pattern of redox balance, Na^+/K^+ATPase activity and HSP70 expression in the posterior and anterior gills of the crab *Neohelice granulata* submitted to hypo- or hyper-osmotic stress for 1 h and 6 h. After 1 h of either type of osmotic stress, there was an increase in catalase activity, but a decrease in GSSG/GSH ratio (oxidized to reduced glutathione ratio) and Na^+/K^+ATPase activity in both gill sets. H_2O_2 levels decreased only in the posterior gills. H_2O_2 levels and Na^+/K^+ATPase activity remained reduced after 6 h of exposure to either type of osmotic stress in both gill sets. The GSSG/GSH ratio returned to initial levels after 6 h of hyper-osmotic stress, whereas it increased 10 times in both gill sets after hypo-osmotic stress. Furthermore, HSP70 protein expression increased in posterior gills after 6 h of hypo-osmotic stress. H_2O_2 levels in tank water decreased after hypo-osmotic challenge and increased after 6 h of hyper-osmotic stress, indicating increased H_2O_2 excretion. Therefore, *N. granulata* gills have redox, metabolic and molecular strategies to deal with rapid osmotic challenges, an important environmental parameter that influences juvenile and adult crab distribution and abundance within different populations.

* Corresponding author at: Departamento de Fisiologia, Institute of Basic Health Sciences (ICBS), Federal University of Rio Grande do Sul (UFRGS), Rua Sarmento Leite no 500, CEP 90050-170 Porto Alegre, RS, Brazil.
E-mail address: anapaula.vinagre@ufrgs.br (A.S. Vinagre).

https://doi.org/10.1016/j.scitotenv.2018.05.162
0048-9697/

1. Introduction

Estuaries are open bodies of water characterized by a longitudinal salinity gradient that can run just above zero parts per thousand (ppt) near the river to 40 ppt close to the ocean (Freire et al., 2011a; Henry et al., 2012). Superimposed upon this gradient, tidal movements of sea water, seasonal changes in rainfall and intermittent storms and hurricanes can also alter water salinity on daily or seasonal patterns that can be accompanied by relevant oscillations in temperature, dissolved O_2, pH and food availability (Henry et al., 1994; Freire et al., 2008; Thibodeaux et al., 2009; Lüchmann et al., 2015). The ecological and evolutionary advantage of being able to survive in such a harsh habitat lies in the fact that estuaries are nutrient-rich environments, so that any species able to tolerate the physical challenges of the estuary can exploit a habitat that is rich in resources and low in competition (Henry et al., 2012). Estuarine resident animals evolved structural and functional adaptations in multiple physiological processes that enable their survival in this challenging environment (Thibodeaux et al., 2009; Nakano et al., 2014; Chaurasia et al., 2016). However, global warming and increasing anthropogenic pressures are affecting these natural oscillations and altering the chemical and physical characters of the estuaries, leading to higher stress levels on the resident animals (Filiciotto et al., 2014; Nakano et al., 2014; Banni et al., 2015; Lüchmann et al., 2015; Chaurasia et al., 2016).

The cellular respiratory chain, which takes places in the mitochondrion, is the most important redox reaction in which reactive oxygen species (ROS) are produced (Niforou et al., 2014). Since they are important redox messengers involved in intracellular transduction signaling, a sensible balance between oxidant and antioxidant agents is required to maintain ROS physiological levels (Niforou et al., 2014; Espinosa-Diez et al., 2015). Oxidative stress (OS) occurs when antioxidant defenses are overcome by pro-oxidant components (Freire et al., 2011b; Espinosa-Diez et al., 2015). Once oxidative stress is established, oxidative damage, such as lipid peroxidation, protein carbonyls or alterations in the glutathione (GSH):glutathione disulfide (GSSG) ratio may appear (Freire et al., 2011b; Espinosa-Diez et al., 2015). As a result, the oxidative stress response (OSR) is activated to increase the expression and activity of antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) (Dayalan Naidu et al., 2015; Espinosa-Diez et al., 2015). In estuarine environments, variations in temperature and/or salinity interact to modulate endogenous ROS production, affecting the OSR of estuarine residents (Freire et al., 2011b). In the green crab *Carcinus aestuarii*, two weeks after transference to a hypo-osmotic medium, ROS levels and CAT activity increased both in the anterior and posterior gills while SOD activity increased only in the posterior gills (Rivera-Ingraham et al., 2016). After this crab was transferred to a hyper-osmotic medium, ROS levels, SOD and CAT activities were not altered in either type of gills (Rivera-Ingraham et al., 2016). When the crabs *Callinectes danae* and *C. ornatus* were exposed to osmotic challenges, the more euryhaline species (*C. danae*) displayed higher constitutive activities of antioxidant enzymes while the less euryhaline species (*C. ornatus*) exhibited activation of these enzymes when exposed to air or hyper-salinity (Freire et al., 2011a). These results suggest that either an increase or a decrease in salinity may affect the redox balance in different ways in each crustacean species (Freire et al., 2011b).

Heat shock response is another pathway activated by different types of environmental stresses, and is characterized by increase in the expression and activity of heat shock proteins (HSPs) (Niforou et al., 2014; Cottin et al., 2015; Chaurasia et al., 2016). These proteins function as chaperones that assist in protein folding, unfolding and remodeling (Niforou et al., 2014; Cottin et al., 2015). In particular, HSP70 is described as a biomarker of the stress response in crustaceans exposed to many types of environmental stress, such as heat (Qian et al., 2012; Frenkel et al., 2008; Cascella et al., 2015; Cottin et al., 2015), immunological agents (Yan et al., 2014; Chaurasia et al., 2016), metal exposure (Qian et al., 2012; Mazzei et al., 2015), salinity alterations (Chang, 2005; Bao et al., 2014) and water shortage (Frenkel et al., 2008).

Neohelice granulata is a euryhaline and semi-terrestrial crab that inhabits the mid- and supralittoral zones of estuaries along the South American coast from the Araruama Lagoon (Brazil) to the San José Gulf (Argentina) (Spivak, 2010). The climate in the Brazilian region is classified as humid subtropical oceanic with hot summer (cfa) according to the Koeppen Climate classification (Alvares et al., 2013). Therefore, circadian variations in temperature and rainfall are common and can cause rapid salinity alterations in the water. *N. granulata* is considered a strongly hyper-osmoregulator in diluted salinities and hypo-osmoregulator in salinities more concentrated than seawater (40‰), thus being regarded as an ideal model for studying the impact of salinity alterations (Luquet et al., 2005). The anterior gills of this crab are equipped with a respiratory epithelium and do not undergo significant structural changes when faced with salinity variations in the medium (Luquet et al., 2002a, 2002b; Genovese et al., 2004; Freire et al., 2008). Posterior gills, with a thick epithelium and mitochondrial-rich chloride cells, are the primary site of ion transport in crustaceans (Luquet et al., 2002; Genovese et al., 2004; Freire et al., 2008). Depending on the salinity in the medium in which *N. granulata* lives, the NaCl transport process may be stimulated (if in a hypo-osmotic medium) or inhibited (if in an hyper-osmotic medium) (Castilho et al., 2001; Bianchini et al., 2008; Freire et al., 2008). When *N. granulata* was acclimated to low salinity for a long period, Na^+/K^+ATPase activity increased in anterior and posterior gills, while when this crab was exposed to hyper-osmotic stress for long periods, the enzyme activity decreased significantly (Castilho et al., 2001; Freire et al., 2008). Even though there are studies about the impact of long periods of osmotic stress on *N. granulata* (Bianchini et al., 2008), we still lack information about the impact caused by short-term alterations of salinity on the metabolism and redox status of this crab. Thus, in this study, we focused on identifying the metabolic and redox strategies developed by *N. granulata* anterior and posterior gills to cope with 1 h and 6 h (short periods) of a hypo- or hyper-osmotic medium.

2. Materials and methods

2.1. Animals

Young male adults *Neohelice granulata* crabs weighing 15.5 ± 1.06 g at stage C of the intermolt cycle, according to morphological criteria (Drach and Tchernigovtzeff, 1967; Barcelos et al., 2007), were collected (IBAMA license 39062-1) in the Tramandaí Lagoon (29°58′S, 50°08′W) in the state of Rio Grande do Sul, Brazil.

2.2. Experimental procedure

In the laboratory, the animals were placed in aerated aquaria at 20‰ of salinity, 25 °C of temperature and natural photoperiod. The animals were fed ad libitum with raw meat (50 g per 25 crabs) once a day in the late afternoon. After 15 days of acclimation period, the crabs of the control group remained at a salinity of 20‰ and the other crabs were transferred to aquaria containing water at a salinity of 34‰ (hyper-osmotic group) or 0‰ (hypo-osmotic group).

After the experimental period, the crabs were anesthetized by chilling on ice for 15 min to withdraw hemolymph and gill samples. Hemolymph samples were collected from the base of the chelipeds with a syringe (1 mL) and osmolarity was immediately recorded with a Wescor 5520 Vapor Osmometer (Table 1). Anterior and posterior gills were carefully removed, weighed, and rinsed in cold (4 °C) buffer (374 mM NaCl; 10 mM KCl; 8.8 mM H_3BO_3; 10 mM $MgCl_2$; 10 mM HEPES; 0.1 mM phenylmethylsulfonyl fluoride (PMSF, Sigma) and 25 mM $CaCl_2$, pH 7.8).