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Biochemical and physiological effects of nickel in the euryhaline crab *Neohelice granulata* (Dana, 1851) acclimated to different salinities

Michele da Silva Aires, Christianne Lorea Paganini, Adalto Bianchini*

Programa de Pós-Graduação em Ciências Fisiológicas, Instituto de Ciências Biológicas, Universidade Federal do Rio Grande – FURG, Av. Itália km 8, 96.201-900 Rio Grande, Rio Grande do Sul, Brazil

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ABSTRACT

The estuarine crab *Neohelice granulata* was maintained under control condition or exposed to sublethal concentrations of dissolved Ni (measured: 128 and 1010 μg/L) for 96 h at different salinities (2 and 30 ppt). After metal exposure, whole-body oxygen consumption was measured and tissue (hemolymph, gills, hepatopancreas and muscle) samples were collected. Control crabs acclimated to 2 ppt salinity showed lower hemolymph concentrations of Na^+ (33%), Mg^{2+} (19%) and K^+ (30%), as well as increased LPO levels in anterior gills (379%), posterior gills (457%) and hepatopancreas (35%) with respect to those acclimated to 30 ppt salinity. In crabs acclimated to 2 ppt salinity, Ni exposure increased whole-body oxygen consumption (75%), hemolymph K^+ concentration (52%), hemolymph (135%) and hepatopancreas (62%) LDH activity. Also, it reduced hemolymph Cl^- concentration (16%) and muscle LDH activity (33%). In crabs acclimated to 30 ppt salinity, Ni exposure increased LDH activity in hemolymph (195%), hepatopancreas (126%) and muscle (53%), as well as hemolymph osmolality (10%), Cl^- (26%) and Ca^{2+} (20%) concentration. It also reduced hepatopancreas lipid peroxidation (20%) and hemolymph Mg^{2+} (29%) and K^+ (31%) concentration. These findings indicate that *N. granulata* is hyper-osmoregulating in 2 ppt salinity and hypo-regulating in 30 ppt salinity, showing adjustments of hemolymph ionic composition and metabolic rates, with consequent higher oxidative damage to lipids in low salinity (2 ppt). Ni effects are associated with metabolic (aerobic and anaerobic) disturbances in crabs acclimated to 2 ppt salinity, while osmotic and ionoregulatory disturbances were more evident in crabs acclimated to 30 ppt salinity.

1. Introduction

Nickel (Ni) is an essential trace metal occurring naturally in all ecosystems around the world. Domestic sewage, industrial effluents, incineration of wastes, mining, and marine drilling activities contribute to increasing Ni concentrations in both terrestrial and aquatic systems (Bryan, 1984). In estuaries, Ni concentration generally varies between 1 and 75 μg/L (Eisler, 1998).

Like Cu and Zn, bivalent Ni is the predominant form in aquatic environments (Förstner and Wittmann, 1983). However, in contrast to these metals, Ni bioavailability, toxicity and mode of action in invertebrates is not well understood (Keithly et al., 2004; Leonard et al., 2011; Blewett et al., 2015, 2016). In vertebrates, Ni is reported as a Ca^{2+} channel blocker, inducing changes in intracellular Ca^{2+} concentration (Zamponi et al., 1996; Lee et al., 1999). Ni is also referred to as an oxidative stress inducer, causing increased cellular levels of reactive oxygen species (Bal and Kasprzak, 2002; Chen et al., 2003), with consequent damage to lipids (Chen et al., 2002) and DNA (Lynn et al., 1997). In euryhaline invertebrates, failure to develop a standard shape in larvae of the New Zealand sea urchin (*Evechinus chloroticus*) suggests skeletal impairment after acute exposure to waterborne Ni. Also, it is demonstrated that sea urchin development is highly sensitive to Ni via a mechanism that involves ionoregulatory disturbance (Blewett et al., 2016). In the shore crab *Carcinus maenas*, the simultaneous presence of three modes of sub-lethal Ni toxicity in exposed animals was recently demonstrated. Ni toxicity was shown to involve ionoregulation impairment and/or respiratory gas exchange, as well as oxidative stress (Blewett and Wood, 2015; Blewett et al., 2015). Acute respiratory effects associated with Ni exposure were also observed in the rainbow trout *Oncorhynchus mykis*. These effects were followed by increased blood lactate levels and hematocrit values (Pane et al., 2003a). In the freshwater flea *Daphnia magna*, significantly reduced oxygen consumption and impaired whole-body Mg^{2+} content was observed after acute and chronic exposure to waterborne Ni (Pane et al., 2003b).

Based on the findings described above, it is expected that Ni exposure would induce respiratory impairments, changes in energy

* Corresponding author at: Universidade Federal do Rio Grande (FURG), Instituto de Ciências Biológicas, Av. Itália km 8, Campus Carreiros, 96.203-900Rio Grande, RS, Brazil.
E-mail address: adaltobianchini@furg.br (A. Bianchini).

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metabolism, and oxidative stress followed by damage to molecules such as lipids and DNA in euryhaline invertebrates. A disturbance in the regulation of divalent cations, especially Ca^{2+}, would be also expected. However, these effects would depend on the water salinity, since it is well recognized that this water parameter largely influences metal bioavailability and the consequent bioaccumulation and toxicity of metals, including Ni, in euryhaline invertebrates (Bianchini et al., 2004; Pedroso et al., 2007; Pinho et al., 2007; Lauer and Bianchini, 2010; Pinho and Bianchini, 2010; Leonard et al., 2011; Blewett and Wood, 2015; Blewett et al., 2015). Also, it is known that water salinity changes markedly influence the biochemistry and physiology of estuarine invertebrates, including crabs (for review: Bianchini et al., 2008).

The euryhaline crab *Neohelice granulata* inhabits estuarine and coastal areas of South America, being distributed from Southeastern Brazil to Argentina. In the Patos Lagoon estuary (Rio Grande, RS, Southern Brazil), it is subjected to frequent and marked changes in water salinity. *N. granulata* shows a suite of biochemical and physiological mechanisms involved in ionic and osmotic regulation to cope with environmental changes in water salinity. In fact, this euryhaline crab can survive in a wide range of salinities (from freshwater to hypersaline water), being considered as a hyper-hypo-osmoregulator. Anterior gills are mainly involved in gas exchange while posterior gills are involved in ionic and osmotic regulation (Bianchini et al., 2008). In this context, it is important to consider that water contamination with metals can induce structural changes in anterior gills altering their ability to exchange gases, with consequent changes in the aerobic metabolism and energy production. In turn, the Na^+,K^+-ATPase activity present in posterior gills is considered the major process involved in the NaCl uptake across the gills, playing an extremely important role in both ionic and osmotic regulation. Exposure to elevated concentrations of essential metals, such as Cu, was shown to inhibit gill Na^+,K^+-ATPase activity in *N. granulata* (Bianchini et al., 2008). In general, juvenile and adult *C. granulata* are reported to be tolerant to divalent metals, such as Cu (Bianchini et al., 2003; Ferrer et al., 2006) and Zn (Bianchini and Castilho, 1999; Ferrer et al., 2006), when compared to other crustaceans from the same ecosystem (Bianchini et al., 2003; Pinho and Bianchini, 2010). Also, it is well known that its tolerance to these divalent metals is dependent on water salinity. Indeed, *N. granulata* is much more sensitive to divalent metals in dilute media (Cu: 0.1% seawater = 0.028 mg/L, Bianchini et al., 2003; Zn: 2 ppt salinity = 15.7 mg/L, Bianchini and Castilho, 1999) than in brackish water (Cu: 15 ppt salinity = 14.9 mg/L; 30 ppt salinity = 91.2 mg/L; Bianchini et al., 2003) and seawater (Cu: 35 ppt salinity = 130.1 mg/L; Zn: 35 ppt salinity = 51.0 mg/L; Ferrer et al., 2006). Unfortunately, there are no values (LC_{50}) of acute or chronic lethal toxicity available for *N. granulata* exposed to Ni in any water salinity condition.

Considering the background described above, the aim of the present study was to identify the possible mechanism(s) of Ni toxic action in euryhaline crustaceans. Therefore, the response of some biochemical and physiological parameters associated with metabolism, oxidative status, and ionic and osmotic balance were evaluated in the estuarine crab *N. granulata* after acute exposure to sub-lethal concentrations of waterborne Ni. Experiments were performed using crabs acclimated to two different salinities (2 and 30 ppt) to evaluate the influence of salinity on the biochemical and physiological parameters analyzed.

2. Material and methods

2.1. Crab collection and acclimation

Adult male crabs *N. granulata* (13.2 ± 1.41 g wet body mass) were collected in salt marshes from the Patos Lagoon estuary (Rio Grande, RS, southern Brazil), transferred to the laboratory, and acclimated for two weeks under controlled conditions (temperature: 20 °C; photoperiod 12 h light:12 h dark). Crabs were progressively acclimated to two different water salinities (2 and 30 ppt), which were prepared from natural sea water filtered (0.45-μm mesh filter; PVDF membrane, Durapore, Millipore, São Paulo, SP, Brazil) and diluted with Milli-Q water to obtain the desired salinities. Crabs were fed until apparent satiation with ground beef three times a week.

2.2. Ni exposure

All glassware employed were previously washed in 10% HNO_3 and thoroughly rinsed with distilled water before use. Exposure media were prepared using natural sea water adjusted to the desired experimental salinities (2 and 30 ppt) as described above. Nominal concentrations of Ni (0, 100 and 1000 μg Ni/L) were obtained from a stock solution prepared with $NiCl_2.6H_2O$ (Merck, Haar, Germany). Experimental media were prepared 24 h before crab introduction in the test chamber to allow Ni to completely equilibrate with salt water. Crabs (n = 5) were individually exposed to Ni in 5-L glass aquaria in the absence of food for 96 h. Every 24 h, the experimental media were completely renewed and crab survival was monitored.

After Ni exposure, whole-body oxygen consumption was measured as described below. Crabs were cryoanesthetized and hemolymph samples were collected by puncture of the hemolymph sinus at the basis of the 3rd or 4th pair of pereiopods. Crabs were then killed by removal of the exoskeleton and had their gills (anterior and posterior), muscle and hepatopancreas dissected and frozen (− 80 °C) for further analyses, as described below.

Filtered (0.45-μM mesh filter) and non-filtered samples (10 mL) from the experimental media were collected daily for measurements of dissolved and total Ni concentrations, respectively. All samples were acidified with 50 μL of 65% HNO_3 (SupraPur, Merck, USA) and desalted before Ni analysis as described by Nadella et al. (2009). Ni concentration was determined by graphite furnace atomic absorption spectrophotometry (HR-CS GF AAS, Analytic Jena, Germany) using a standard curve built with standard solutions prepared from a certified standard solution (Tritisol, Merck, USA).

2.3. Oxygen consumption

After 96 h of Ni exposure, whole-body oxygen consumption of crabs was measured using a 140-mL static respirometer mounted over a magnetic agitator and connected to an oximeter (Digimed, São Paulo, SP, Brazil). Every 5 min, dissolved O_2 content was measured. After 30 min, crabs were weighed (wet body mass) and the oxygen consumption calculated based on changes in oxygen content in the experimental medium, respirometer volume, time elapsed and crab wet body mass. Therefore, results were expressed as mg O_2/g wet body mass/h.

2.4. Hemolymph osmolality and ionic concentration

Hemolymph osmolality was measured using a vapor pressure osmometer (VAPRO 5600, Wescor, USA) and was expressed as mOsmoles/Kg H_2O. Na^+ and K^+ concentrations were measured by graphite furnace atomic absorption spectrophotometry (HR-CS GF AAS, Analytic Jena, Germany) using certified standard solutions (Tritisol, Merck, USA). Cl^-, Ca^{2+} and Mg^{2+} concentrations were measured using commercial reagent kits (Reference 49, Reference 95-2/50 and Reference 50, respectively; Labtest Diagnóstica, Lagoa Santa, MG, Brazil). Hemolymph ion concentrations were expressed in mM.

2.5. Lipid peroxidation (LPO)

Gill (anterior and posterior), muscle and hepatopancreas samples were weighed and homogenized (1:9 w/v) in a solution containing KCl (1.15%) and 35 μM butylhydroxytoluene (BHT). The supernatant obtained was collected and used for analysis. LPO measurements were performed following the procedures described by Ohkawa et al. (1979).