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Sexual and seasonal variations in osmoregulation and ionoregulation in the estuarine crab *Chasmagnathus granulatus* (Crustacea, Decapoda)

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Abstract

The estuarine crab *Chasmagnathus granulatus* (Crustacea, Decapoda, Brachyura) inhabits salt marshes along the South Atlantic coast from Rio de Janeiro (Brazil) to Patagonia (Argentina). In the present study, salinity tolerance (0–45‰; 16–1325 mOsm/kg H_2O) and hemolymph osmotic and ionic (Na^+, Cl^-, and K^+) regulation in both female and male *C. granulatus* were analyzed in summer and winter. Results showed that both female and male *C. granulatus* are euryhaline. Mortality was only observed in extremely low salinity (0‰; 16 mOsm/kg H_2O) for both sexes. For females, the LT_{50} at 0‰ salinity was similar in summer (20.1 h) and winter (17.4 h). Males were more tolerant to salinity than females in both seasons, and mortality was observed only in summer (LT_{50} = 50.9 h). Results from freshly collected crabs or long-term (16-day) osmotic and ionic regulation experiments in the laboratory showed that male *C. granulatus* is a better hyper-osmoregulator than female in summer and winter. However, a hypo-osmoregulatory ability was only observed in females experimentally subjected to salinity 40‰ (1176 ± 11 mOsm/kg H_2O) in both seasons. In both sexes, hyper-osmotic regulation was achieved by hyper-regulating hemolymph Na^+, Cl^-, and K^+ concentration. In females, hypo-osmotic regulation was achieved by hypo-regulating hemolymph Na^+ and Cl^- concentration. Long-term (16-day) osmotic and ionic regulations in different salinities were similar in males or females collected and tested in summer and winter. Despite this lack of a seasonal effect on hemolymph osmoregulatory and ionoregulatory patterns in males or females, a marked seasonal difference in the dynamics of these processes was observed for both sexes. In the first 2 days after hypo-osmotic shock (20‰→5‰; 636→185 mOsm/kg H_2O), variations in female osmolality and ion (Na^+ and Cl^-) concentration were larger and faster in winter than in summer, while in males the opposite was observed. Furthermore, a seasonal effect on the crab response to hyper-osmotic shock (20‰→40‰; 636→1176 mOsm/kg H_2O) was only

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observed in males. A new osmolality and ion (Na^+ and Cl^-) concentration steady state was faster achieved in winter than in summer. Regarding sexual differences, females showed a better capacity to hypo-regulate the hemolymph osmolality and Na^+ concentration than males, even after a sudden increase in salinity (hyper-osmotic shock) in both seasons. On the other hand, males showed a better capacity to hyper-regulate the hemolymph osmolality and Na^+ concentration than females, even after a sudden decrease in salinity (hypo-osmotic shock), especially in winter. Taken together, results reported in the present study suggest the need to consider both sex and collection season as important factors in future osmotic and ionic regulation studies in estuarine crabs.

Keywords: *Chasmagnathus granulatus*; Crab; Ionoregulation; Osmoregulation; Salinity

1. Introduction

The estuarine crab *Chasmagnathus granulatus* (Crustacea, Decapoda, Brachyura) inhabits salt marshes along the South Atlantic coast from Rio de Janeiro (Brazil) to Patagonia (Argentina) (Boschi, 1964). This species has an important ecological role in salt marshes since it is a key species in the energy transfer in this environment (D'Incao et al., 1990). Therefore, to establish populations in salt marshes and accomplish its ecological role, this crab species should present high tolerance to face the important salinity changes occurring in this environment.

The facts described above have stimulated the use of *C. granulatus* as an interesting species to be employed in ecological (Boschi, 1964; Boschi et al., 1967; D'Incao et al., 1990, 1992; Rieger and Nakagawa, 1995; Charmantier et al., 2002), toxicological (Monserrat and Bianchini, 1995, 2000, 2001; Monserrat et al., 1997, 2001; Bianchini and Castilho, 1999; Vinagre et al., 2002, 2003; Pinho et al., 2003) and physiological studies (Bromberg et al., 1995; Castilho et al., 2001; Luvizotto Santos et al., 2003). Physiological studies performed on adult male *C. granulatus* showed that this species is euryhaline and capable of hyper-osmoregulation in low salinities (freshwater and brackish water) and hypo-osmoregulation in high salinity (40‰). An important seasonal variation in the salinity tolerance and osmotic and ionic regulation of adult male *C. granulatus* was also observed. In summer, a better hyper-osmoregulatory and ionoregulatory capacity was observed.

Despite the large number of physiological studies performed in *C. granulatus*, all studies were done using adult males. So, data describing salinity tolerance, osmoregulatory pattern and ionoregulatory patterns as a function of environmental salinity, mechanisms involved in osmoregulation and ionoregulation, and seasonal variations in adult females of *C. granulatus* are not available in the literature. Furthermore, possible seasonal variations in these processes were also never considered in adult males acclimated to high salinities.

In light of the above, the objective of this study was to determine salinity tolerance and to describe the osmoregulatory and ionoregulatory patterns of adult female and male *C. granulatus* as a function of environmental salinity in different seasons (summer and winter). Results obtained from this study are quite important for a better understanding of data from ecological studies. It has been shown that *C. granulatus* follows a strategy of larval export (i.e., its larvae live under different salinity conditions from juveniles and adults). Furthermore, it has been shown that changes in the patterns in osmoregulation during larvae development match the ontogenetic changes that typically occur in the ecology of *C. granulatus* (Charmantier et al., 2002). It has also been reported that the first larval stages and juveniles of *C. granulatus* develop well in high salinities (Boschi et al., 1967; Rieger and Nakagawa, 1995), and that moderate increasing salinity and temperature correlate to the greatest abundance of both females and males (D'Incao et al., 1992).

2. Materials and methods

Adult female and male crabs in stage C or early D of the intermoult cycle (Drach and Tchernigovtzeff, 1967) were captured at the salt marshes of the Patos