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Serum osmolality and ions, and gill Na^+/K^+-ATPase of spottedtail goby *Synechogobius ommaturus* (R.) in response to acute salinity changes

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ABSTRACT

This study was carried out to determine the effects of abrupt salinity change on osmoregulatory ability of the spottedtail goby *Synechogobius ommaturus*. 720 juvenile fish (65.3 ± 11.8 g) were transferred to 200 L tanks (with 40 juveniles in each tank), in which salinities were abruptly changed from 10 to 20, 30, 40, 50 and freshwater. Survival rate, serum osmolality, electrolytes (Na^+, Cl^-, and K^+) and gill Na^+/K^+-ATPase (NKA) activity were assessed successively in 528 h. Results showed serum osmolality, ion concentrations and gill Na^+/K^+-ATPase activity increased significantly when fish were transferred to salinity 40 and 50 and all fish in these groups died by the end of the experiment. Serum osmolality, Na^+, Cl^- and K^+ in fish transferred to a salinity of 20, 30 and freshwater were not affected and no mortality was detected. Compared with the control group, a significantly decrease of NKA activity happened in the freshwater group, but the activity in 20 and 30 groups was not affected significantly. The results indicated that *S. ommaturus* could adapt rapidly and maintain homeostasis in a wide range of salinities (from freshwater to salinity 30) and this species may be suitable for aquaculture in estuarine and coastal areas where rapid salinity fluctuations commonly occur.

1. Introduction

Salinity is an environmental factor that frequently affects the physiology of aquatic organisms (Urbina & Glover, 2015). When fish are challenged by salinity change, activities like drinking rate (Evans, Piermarini, & Choe, 2005) and osmoregulation (Fielder, Allan, Pepperall, & Pankhurst, 2007) change to maintain body osmolality and ionic balance. Euryhaline fish can maintain the ionic composition and osmolality of their internal fluids relatively constant when environmental salinity changes (Anni et al., 2016). The ability to osmoregulate is essential for their survival in either freshwater or seawater environments. This regulation is achieved by a number of different ionic and osmoregulatory processes. Fish have to adjust and balance the gain and loss of water and serum ion, especially Cl^-, Na^+, and K^+. The change in plasma ion levels are used to monitor the osmoregulatory ability of fish after salinity changes (Stewart et al., 2016). The main mechanism for maintaining serum ion and osmolality balance involves gill Na^+/K^+-ATPase (NKA) (Stewart et al., 2016; Zhang et al., 2017). This enzyme is located in the branchial chloride cells (Hirose, Kaneko, Naito, & Takei, 2003) and can produce a chemical gradient to eliminate excess intra and extra-cellular Na^+ and Cl^- in a hyperosmotic environment and take up Cl^- in a hypoosmotic environment (Wood, 2011). In most teleosts when salinity changes NKA activity exhibits adaptive changes. The time course of changes in gill NAK activity in response to different environment salinities tends to vary between species. Gill NKA activity in killifish *Fundulus heteroclitus heteroclitus* (L. 1766) changed 2–3 days after environment salinity increased (Mancera & McCormick, 2000), 3–7 days were needed for a change in NKA during the breeding migration of coho salmon *Oncorhynchus kisutch* (Walbaum 1792) (Wilson & Laurent, 2002) and in the marine gilthead seabream *Sparus aurata* L. 1758 (Laiz-Carrión et al., 2005a, 2005b). Osmoregulatory ability of fishes can be evaluated by measuring the NKA activity, serum osmolality and ion levels.

The spottedtail goby *Synechogobius ommaturus* (Richardson 1862), is a large and demersal fish of the Gobiidae. It is an inshore

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species of the Asian western Pacific Ocean and is widely distributed along the coast of China, Japan and Indonesia. It grows quickly, and has a nearly linear growth curve during the first six months of the first year of its life cycle and then the growth rate slows down, but the body weight and standard length keep on increasing and achieving their maximum by the next April (Wang, Ye, Liu, & Cao, 2011, 2011b). The species had become a commercial fish in China (Wang et al., 2011). Over fishing and environment pollution pose a threat to this species and has caused sustainability problems for this species. Although in the context of improving sustainability studies about the use of salt marshes (Jin et al., 2007, 2010), genetic diversity (Song, Zhang, & Gao, 2010) and stock discrimination (Wang et al., 2011a, 2011b) of *S. ommaturus* have been conducted. However, the response of this fish to changes in salinity remain limited and the effects of different salinities on NAK activity, serum osmolality and ion levels have not been performed, despite their relevance for artificial propagation and culture. The present study contributes to better protect the species and improve aquaculture by assessing the effects of acute salinity changes on osmoregulation of *S. ommaturus*. Such information is important since the habitat of *S. ommaturus* is frequently affected by uncontrolled salinity fluctuations attributed to evaporation or inundation with rain. Gill NAK activity, serum osmolality and ion levels are used as indicators of salinity tolerance.

2. Materials and methods

2.1. Animal husbandry and experimental design

Experiments were conducted with spottedtail goby at the facilities of Shanghai Fisheries Research Institute (Shanghai, China). Fish were caught from ponds and maintained in indoor concrete pools prior to experiments. During the 2 weeks of acclimation (salinity 10) and throughout the experiment, fish were fed on commercial feed to apparent satiation once a day (10:00). After acclimation to the experimental conditions, 720 fish (65.3 ± 11.8 g) were chosen and randomly allocated between 18 independent tanks (200 L), with 40 juveniles in each tank. Fish were exposed to an ambient photoperiod of 10 L: 14D.

Five salinity levels were investigated, namely freshwater, 20, 30, 40 and 50, the culture salinity 10 served as the control. Spottedtail gobies from the different experimental tanks were abruptly and isothermally transferred from the control salinity to the experimental salinities. The effect on the spottedtail gobies of each experimental salinity was determined using 40 fish in triplicate static 200 L tank containing aerated water.

2.2. Sampling

Fish were fasted for 24 h prior to sampling. Blood and gill filament samples were taken before transfer to modified salinity and this was the 0 h sample. Three fish were randomly, chosen from each replicate and anesthetized using 100 mg/L tricaine methanesulfonate. Fish were sampled at 0.5, 1, 2, 4, 8, 12, 24, 48, 96, 216, 360, and 528 h after transfer to the experimental salinity. Blood was collected from the caudal vasculature with 1 mL heparinized syringes, then stored on ice for up to 10 h. Serum was obtained by centrifugation at 3700 *g* for 20 min at 4 °C, and the serum was stored at −80 °C until analysis. The second and third gill arch was excised and stored at −80 °C for the NAK activity test. A sample of water from each replicate was taken at the same time of blood sampling to assess its osmolality and ion content (Table 1).

2.3. Administration of the experiment

Salinity, temperature and pH were measured daily using a YSI model 30 m (Yellow Springs Instruments Inc., Yellow Springs, Ohio, USA) and YSI model pH 100, respectively. The water temperature (range 11–12 °C) and pH (range 8.0–8.6) were measured daily. Dissolved oxygen was maintained at >80% oxygen saturation throughout the experiment by continuous aeration. Ammonia-nitrogen and nitrite-nitrogen were measured weekly over the duration of the experiment and had a mean value of <0.3 mg/L and <0.1 mg/L, respectively. 70% of the tank water was exchanged daily. The water salinities tested ranged from 0 to 50 with intervals of 10 and were prepared by adding freshwater or hypersaline seawater to full seawater. The salinity was measured and adjusted by a hand-held salinity meter (YSI 30-10, Yellow Spring Instruments, Yellow Springs, OH, USA).

2.4. Biochemical analysis

Serum samples were tested after thawing at 4 °C. Osmolality was measured using a cryoscopic osmometer (Gonotec, Osmomat 030, Germany) and reported as mOsm/kg. Serum potassium, sodium and chloride concentrations were tested using an EasyLyte electrolyte analyzer (Medica Corporation, Bedford, MA, USA). Gill NAK activity was determined according to (Zaugg, 1982). The NAK specific activity was expressed as μmol P_i/(mg protein)•h.

2.5. Statistical analyses

Statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). A One-Way Analysis of Variance (ANOVA) was applied to identify significant differences in serum osmolality and ion concentrations and gill NAK activity in fish challenged with different salinities. If significant differences were detected at the levels of 0.05, then Duncan's multiple tests were used to evaluate the differences among treatments. All data were subject to a test for homogeneity of variances before analysis. The data were plotted using Microsoft Excel (Microsoft, Seattle, WA, USA) and all the values are expressed as mean ± standard deviation (M ± SD).

3. Results

3.1. Serum osmolality and ions concentration

Serum osmolality and ion concentrations were not significantly different between fish prior to the start of the salinity challenge (0 h). No mortality occurred before or immediately after transfer of fish to the salinities 20, 30 or freshwater. In contrast, 48 h after transfer of fish to 50 salinity all the fish had died and in the fish transferred to 40 salinity all died 360 h later.

Half an hour later after transfer, serum osmolality showed no significant changes (Fig. 1a). As time went by serum osmolality increased significantly in fish transferred to the 50 and 40 salinity and peaked at 48 h and 96 h, respectively. Then the osmolality in 40 decreased. The serum osmolality in fish transferred to 20, 30 salinity or freshwater was relatively stable except for a slight increase in the 30 group and decrease in the freshwater group ($P>0.05$).

Serum Cl^- concentration in fish transferred to 20 and 30 salinity remained similar to those of the control fish at 10 salinity (Fig. 1b). Fish transferred to 40 and 50 salinity had a significant increase in serum Cl^- at 96 and 48 h, respectively relative to the control ($P < 0.05$). Serum Cl^- of fish transferred to freshwater decreased at 4 h, then remained significantly lower than that of control fish ($P < 0.05$).