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Effect of Salinity on Osmoregulation and Histopathology in Gills of Tilapia (*Oreochromis niloticus*)

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Abstract: Experiments on Nile tilapia *Oreochromis niloticus* were conducted to assess serum osmolalities, ions and histopathological effects induced in gill tissues of 7 days exposure to different salinities (0, 10, 15 and 20 ppt). These tissues were investigated by light microscope. Blood serum osmolality (SO), sodium (Na^+), chloride (Cl^-) and potassium (K^+) concentrations were assessed after 7 days of exposure. Serum osmolality and ionic content of exposed fish appeared differently affected by salinity throughout 7 days compared to the controls. Osmolality and Na^+ were increased at the two tested salinities (15 and 20 ppt), Cl^- increased at the three tested salinities (10, 15 and 20 ppt) but K^+ contents remained unaffected due to salinity exposure. Therefore, tilapias exposed to high salinity present the increasing of osmotic and ionic content except for K^+ contents. Na^+/K^+-ATPase (NKA) location was in the secondary lamellae in the gill of fish at 0 ppt salinity. However, NKA location was in the primary lamellae only in the gill of fish at 10 ppt salinity. The histological structure remain unaffected due to salinity changing. The osmoregulation and NKA location in gills of our findings could be a protective response of animals to the environment changing. These data provide useful information for future reference and aquaculture practice as the information of salinity effect to osmoregulation of fish.

Key words: Tilapia, salinity, freshwater, osmolality, ions, immunohistochemistry.

Introduction

Tilapia is one of the important fish species, which can face a wide range of salinity and other environmental conditions (Handayani et al., 2017). Tilapia is easily maintained in the laboratory (Yuniari et al., 2017). The increasing market demand for tilapia and the availability of brackish water (BW) and sea water resources have led the introduction of this species in large scales to reduce time consumption in tilapia cultivation as well as to reduce the effect of heavy metal in the fish body. Many authors reported that different tilapia strains can grow well in water salinities ranging from 0 to 32 g/L (Avella et al., 1993; Suresh and Lin, 1992). Physiological assay to demonstrate the fish osmoregulation condition is needed to encourage the previous study concerning tilapia is a good candidate for brackish water aquaculture.

The mechanism of active NaCl secretion by mitochondria rich cells (MRC) in seawater consists primarily of the cooperative action of three major ion transporters: basolaterally located Na^+/K^+-ATPase (NKA), $Na^+/K^+/2Cl^-$ cotransporter (NKCC) and an apical Cl^- channel (Hiroi and McCormick, 2007).

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Therefore, it is necessary to find the NKA location in the gill filament of tilapia. This fish can be an excellent candidate for culture in these water resources. The objective of the present studies was to observe their osmolalities, ions and NKA location at different salinity levels.

Materials and Methods

Experimental Fish

Nile tilapia was originally obtained from Freshwater (FW) Aquaculture (Lugang, Taiwan). Adult male tilapias (3–4 months age; n = 48; body weight = 85.8± 15.5 g; body length = 16.7 ± 3.6 cm) were maintained in FW at the university tank (National Taiwan Ocean University) with a natural light system (water temperature ranged from 19 to 24°C). Supplemental aeration and constant mechanical were provided. The fishes were fed with commercial fish food dried pellets feed *ad libitum*.

The fishes used in the current work were randomly selected from the stock culture and maintained in 600 L aquaria with a recirculation system, until the experimental treatment. The fishes were randomly divided into four groups in two replicates, maintained in FW (0 g/L) and BW (10, 15, 20 g/L) after an initial acclimation period (14 days). Five fish per tank (40 L) were exposed for 7 days.

Experimental Design

Fish from four tanks containing FW were considered the control group (C). Five fish from control and BW-treatment were sampled at day 7 of exposure, Tilapia were anesthetized with 2-phenoxyethanol, weighed, standard length measured and blood sampled. Blood was collected by puncturing the heart of the fish using 1 mL syringes and 23 G needle. Serum was obtained by centrifugation (5 min at 10,000 rpm, 4°C) and kept at -80°C for later analysis. Gill samples were collected from FW (n = 5) and BW (n = 15), then were fixed in 4% paraformaldehyde in 1X PBS for NKA location analyses.

Serum Osmolalities and Ions Measurement

Serum samples were introduced to microtubes for the assessment of serum osmolality and serum ion. Osmolality of serum was measured using an automated freezing point depression osmometer (Fiske® 210 Micro-Sample Osmometer, USA). The osmolality of the serum sample is expressed as mOsm/kg. Serum ions Na^+, Cl^- and K^+ were measured by electrolyte analyzer (SpotChem EL SE-1520).

Immunohistochemistry Analyses

Samples were obtained at the time points indicated above were fixed in 4% paraformaldehyde at least 24 hours. The tissues were then dehydrated, embedded in paraffin, cut into 6 μm thick sections on a rotary microtome RM2135 (Leica,Wetzlar, Germany) and were mounted on gelatin-coated glass slides. After sectioning, samples in slides were dewaxed in xylene and rehydrated in an ethanol series (100% ethanol to water). The sections were stained with haematoxylin and eosin (HE) for histopathological evaluation according to standard techniques. Sections were placed onto xylene-pretreated slides for the presence of NKA antigen by IHC using the avidin–biotin complex (ABC) method. Primary antibodies consisted of rabbit polyclonal to NAKα1 (Santa Cruz biotechnology, Inc). Binding of secondary goat anti-rabbit antibodies and formation of the ABC were visualized by a chromogen reaction using 3,3-diaminobenzidine-tetrachloride. The immunostained sections were counterstained with haematoxylene. For negative controls, primary antibodies were replaced by rabbit pre-immune serum. All sections were observed using an optical microscope DFC495.

Statistical Analyses

All data were expressed as mean ± standard deviation, verified their normality and homogeneity before used for statistical analysis. All statistical analyses were performed in IBM SPSS version 21 (IBM Corp., Armonk, NY, USA). If data did not meet the assumption of normality and homogeneity of variance, data were log transformed. Statistical analysis of the data was performed using one-way ANOVA followed by a Tukey's HSD post hoc comparison test to evaluate effect of different salinities on serum osmolality and serum ions. Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

Serum Osmolality

Serum osmolalities in fish exposed to 15 and 20 g/L were significantly higher than the osmolality value of the control. Serum osmolalities in fish exposed to 10 g/L were not significantly higher than the osmolality value of the control group (Figure 1). The increase appeared constant (between 15 and 25%) when fish were exposed to the BW.

Serum Ions

Sodium content in blood serum of control fish and

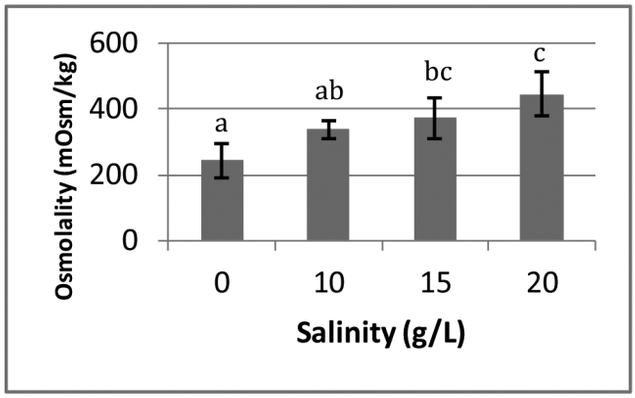

Figure 1: Blood serum osmolality concentration of *O. niloticus* exposed to different salinities. Lowercase letters indicate significant differences at different salinities. Means not sharing the same letter are significantly different (one-way ANOVA, Tukey's HSD post hoc comparison test, $p < 0.05$).

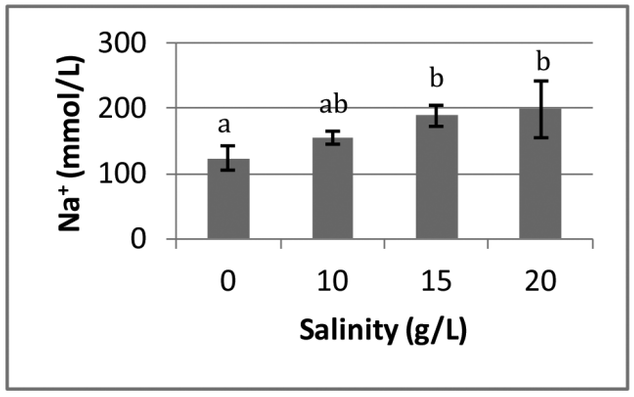

Figure 2: Blood serum sodium concentration of *O. niloticus* exposed to different salinities. Similar statistical labelling as in Figure 1.

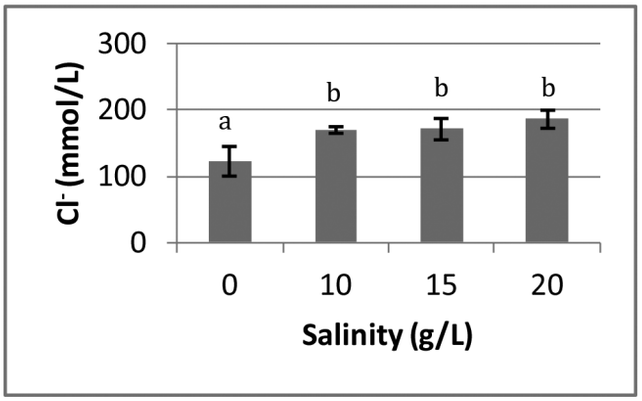

Figure 3: Blood serum chloride concentration of *O. niloticus* exposed to different salinities. Similar statistical labelling as in Figure 1.

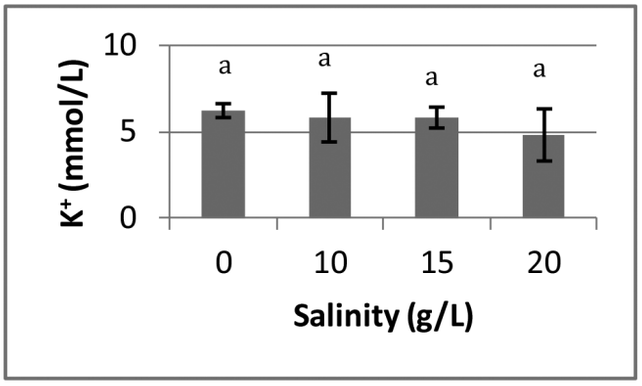

Figure 4: Blood serum potassium concentration of *O. niloticus* exposed to different salinities. Similar statistical labelling as in Figure 1.

those exposed with BW remained increase constantly (Figure 2). However, Na^+ concentration in the serum of fish exposed to 15 and 20 g/L salinity was significantly higher than the controls after 7 days exposure. Sodium concentrations at 10 g/L salinity were not significantly higher than the osmolality value of the control group.

Similarly, chloride concentration in the serum of control and exposed fish to BW remained high constantly (Figure 3), but at the 10 g/L of salinity, a significant increase in Cl^- content was observed.

However, K^+ concentrations in blood serum slightly decreased in fish exposed to BW compared to the controls. The maximum potassium content was observed after 7 days exposure in control group (Figure 4).

The concentrations of osmolality, Na^+, and Cl^- in blood serum significantly increased at highest salinity. This indicates that in the short-terms, exposure of salinity impacts the level of osmolality, Na^+ and Cl^-. Since sodium and chloride are the major ions in the body fluid, regulation of both Na^+ and Cl^- is critical for osmoregulation (Boening, 2000). If fish can adapt to the salinity exposure, their body conditions will be normal gradually as tilapia is known as euryhaline fish. It is accepted that salinity is a key factor in controlling growth in tilapia that shows better performance in brackish water (Boeuf and Payan, 2001).

Immunohistochemistry

The NKA were mainly distributed in the primary and secondary lamellae regions of the filaments. In the FW-acclimated *O. niloticus*, some NKA were also found in the basal regions of the lamellae (Figure 5). The smallest size of MRC was found in the BW group, at 20 g/L of salinity and these were significantly smaller than in FW individuals. Sodium content in blood serum of control fish and those exposed with BW remained

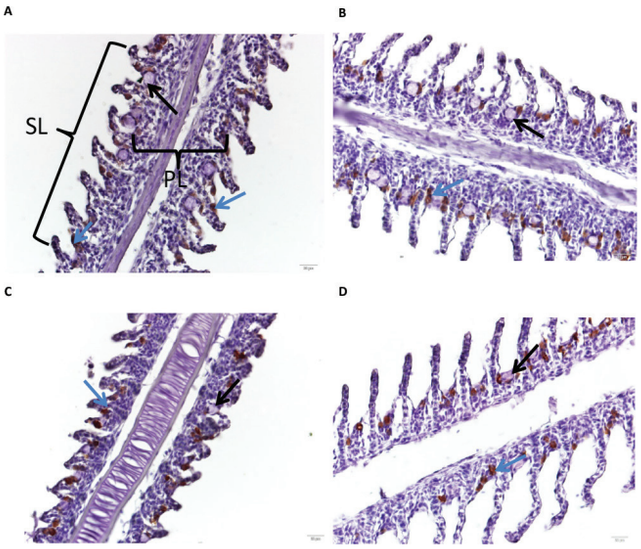

Figure 5: NKA location of *O. niloticus* exposed to different salinities (A) Control (B) 10 g/L salinity (C) 15 g/L salinity (D) 20 g/L salinity. (SL: Secondary lamellae; PL: Primary Lamellae, scale bar: 20 µm).

constantly high (Figure 2). However, Na^+ concentration in the serum of fish exposed to 15 and 20 g/L salinity was significantly higher than the controls after 7 days exposure. Sodium concentrations at 10 g/L salinity were not significantly higher than the osmolality value of the control group.

NKA location both fish in the FW and BW treatment was found in the similar location. The present study observed the MRC is much abundant in FW treatment as compared to BW treatment. Previous studies indicated that the MRC express the highest levels of NKA in teleostean gills (Hirose et al., 2003; Hwang and Lee, 2007; Karnaky et al., 1976; Lin et al., 2004; Marshall and Bryson, 1998).

Conclusion

In conclusion, tilapia can adapt the BW condition in potassium concentrations and NKA locations because both are constant. However, osmoregulation increases following salinity increasing since Na^+ and Cl^- serum concentration increase. Tilapias are capable surviving in higher salinity and maintaining the imbalance ion levels up to 7 days exposure.

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Contents