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# Hyperosmotic shock adaptation by cortisol involves upregulation of branchial osmotic stress transcription factor 1 gene expression in Mozambique Tilapia

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ABSTRACT

The Mozambique tilapia (*Oreochromis mossambicus*) is a euryhaline species that does not survive direct seawater exposure. Cortisol is involved in re-establishing electrolyte homeostasis in seawater and is thought to play a role in allowing tilapia to cope with abrupt seawater exposure, but the mechanism(s) are far from clear. Recently, osmotic stress transcription factor1 (OSTF1) was identified as a key signaling molecule involved in hyperosmotic stress adaptation in tilapia. Consequently, we tested the hypothesis that upregulation of OSTF1 expression by cortisol is a key response for hyperosmotic stress adaptation in tilapia. Fish were exposed to different salinities over a 24 h period, while a major electrolyte disturbance and mortality was observed only with full-strength seawater exposure. Therefore, we administered cocoa butter implants of cortisol (50 mg/kg) intraperitoneally to tilapia maintained in fresh water and after three days exposed these fish to full-strength seawater. There was 50% mortality in the control fish upon seawater exposure, but this was abolished by cortisol treatment. Abrupt seawater exposure did not affect plasma cortisol levels, while, as expected, exogenous administration of this steroid elevated plasma cortisol levels both in fresh water and seawater. Cortisol treatment significantly induced OSTF1 gene expression in fresh water tilapia, and also enhanced further the seawater-induced OSTF1 mRNA abundance. Plasma osmolality decreased, while gill $Na^+/K^+$-ATPase activity was suppressed in the cortisol group in seawater compared to the sham group. This corresponded with a significant reduction in gill ionocyte size and $Na^+/K^+$-ATPase activity and protein expression after seawater exposure. Cortisol did not modify liver metabolism, but significantly suppressed gill metabolic capacity in seawater. Overall, cortisol adapts tilapia to a hyperosmotic shock associated with abrupt seawater exposure. This involves upregulation of OSTF1 gene expression and a concomitant suppression of branchial metabolism in tilapia.
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## 1. Introduction

The Mozambique tilapia (*Oreochromis mossambicus*), a euryhaline teleost, has been widely used as a model species to study ion regulation (Shepherd et al., 2006). Indeed, studies have shown extensive remodeling of gill epithelium, including changes in ionocyte (chloride cell) differentiation and upregulation of $Na^+/K^+$-ATPase activity, associated with enhanced hypoosmoregulatory capacity in seawater (Uchida et al., 2000; Evans et al., 2005). Although, Mozambique tilapia is considered a euryhaline species, it cannot survive abrupt transfer to full-strength seawater (Stickney, 1986). Consequently, most studies utilize a gradual exposure to full-strength seawater after pre-exposing fish to lower salinities (Hwang, 1987; Uchida et al., 2000; Weng et al., 2002). This pre-exposure to lower salinities is thought to provide sufficient lag time to develop ion excretion mechanisms to prevent dehydration in seawater (Hwang, 1987; Dean et al., 2003; Lee et al., 2003), thereby reducing osmotic stress. Also, it has been suggested that the elevated cortisol response seen upon exposure to lower salinities may assist with the development of these branchial ion extrusion mechanisms allowing for efficient ion regulation in full-strength seawater (Assem and Hanke, 1981; Vijayan et al., 1997; McCormick, 2001).

Indeed, cortisol is a key hormone involved in enhancing the hypoosmoregulatory capacity of fish in seawater, including modifying the number and morphology of ionocytes and elevating gill $Na^+/K^+$-ATPase activity (Dange, 1986; McCormick, 1995; Evans et al., 2005). In addition to its role in ion regulation, cortisol plays an important role in the energy substrate repartitioning that is critical for seawater adaptation. For instance, gill biogenesis and activation of branchial ion transporters, which are essential for ionregulation upon seawater exposure, are energy demanding

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processes (Mommsen, 1984). Glucose is a preferred fuel for gill metabolism (Mommsen, 1984) and, indeed this metabolite is elevated upon seawater exposure of tilapia (Vijayan et al., 1996; Fiess et al., 2007). Cortisol enhances gluconeogenesis in tilapia leading to the proposal that cortisol elevation in seawater mobilizes glucose to fuel gill metabolism that is essential for hypoosmoregulation (Vijayan et al., 1996, 1997; Mommsen et al., 1999). To this end, prior cortisol treatment of fresh water acclimated Mozambique tilapia protected the fish against mortalities associated with seawater exposure (Assem and Hanke, 1981), while the mode of action of cortisol in adapting fish to osmotic shock is far from clear.

Osmotic stress transcription factor 1 (OSTF1) is a recently discovered transcription factor that is rapidly and specifically induced in response to hyperosmotic stress in tilapia (Fiol and Kültz, 2005; Fiol et al., 2006). Although the precise role of this transcription factor in ion regulation is unknown, it is thought to be an early transducer of osmoregulatory signals (Fiol and Kültz, 2005; Fiol et al., 2006). While recent studies propose a role for cortisol in modulating branchial OSTF1 expression *in vitro* (Fiol and Kültz, 2005; Fiol et al., 2006; Choi and An, 2008; Tse et al., 2008), little is known about cortisol effect on the branchial expression of this transcription factor upon hyperosmotic shock *in vivo*. We hypothesized that cortisol protects against hyperosmotic shock-induced mortality and this involves upregulation of gill OSTF1 gene expression in the Mozambique tilapia. To test this hypothesis, we first exposed freshwater tilapia to a range of salinities, mimicking brackish water to full-strength seawater, to determine the threshold salinity concentration at which osmoregulatory dysfunction and mortality occurs in this animal. This was followed by treating fresh water tilapia with cortisol, to elevate circulating levels of this steroid, and then exposing the fish to full-strength seawater. Gill OSTF1 gene expression was used as an indicator of osmotic shock (Fiol and Kültz, 2005; Fiol et al., 2006), while plasma cortisol, glucose and ion levels as well as liver glycogen content, gill sodium pump activity and ionocyte expression and several enzymes involved in the intermediary metabolism in the gill and liver were used to determine the metabolic and ionoregulatory adjustments in response to osmotic shock in this species.

## 2. Materials and methods

### 2.1. Experimental animals

The Mozambique tilapia (193 ± 11 g body mass; 22 ± 0.6 cm length) were collected using a cast net from Tengan River in Okinawa, Japan and transported to the Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, Okinawa. Fish were maintained in fresh water (FW; 500 L capacity tank) under ambient temperature (24 °C) and natural photoperiod (14:10-h light–dark) for one week prior to transferring them to the experimental tanks. During this period, fish were fed to satiety once daily with a commercial tilapia feed (41M, Zen-no, Tokyo, Japan).

### 2.2. Experimental protocols

#### 2.2.1. Salinity exposures

Groups of six fish each were transferred to five experimental tanks (200 L) and were maintained for three weeks in FW prior to the start of the experiment. The tanks were maintained as a static system with continuous aeration and 50% water replenished every second day exactly as mentioned before (Vijayan et al., 2001). At the beginning of the experiment, half the volume of water in all tanks was drained and refilled with either FW (0 ppt) or full-strength sea water (SW; 34 ppt) in order to obtain different experimental salinities (12, 17, 24 and 34 ppt). The salinity of the water in the tanks was constantly monitored during refilling using a refractometer (Nikon, Japan) and the whole process of draining and refilling the tanks was completed within 20 min. Fish were left undisturbed in the tanks and sampled 24 h after exposing them to different salinities. Fish were not fed during the 24 h exposure period to different salinities.

#### 2.2.2. Cortisol treatment

Groups of six fish were randomly distributed into four tanks (200 L) and were acclimated 3 weeks prior to the experiment. Fish husbandry practices prior to the experiment were similar to the ones described above. Fish were lightly anesthetized with 2-phenoxyethanol (1:10,000; Kanto Pure Chemicals, Tokyo, Japan) and the fish were injected intraperitoneally with either cocoa butter alone (vehicle; control) or cocoa butter containing cortisol at 50 mg/kg body mass. The method and dosage used for cortisol treatment was exactly as described previously (Vijayan et al., 1997). Fish completely recovered from the anesthetic soon after returning to the experimental tanks. Three days after injection, water in tanks from each treatment group was replaced with either fresh water or 100% SW (34 ppt). Fish were sampled 6 h after SW exposure and this time-point was chosen because our salinity exposure study (above) showed that mortality occurred after this time period in full-strength SW. Furthermore, to assess whether cortisol had a beneficial effect on tilapia survival after abrupt exposure to seawater, we repeated the above experiment with two groups (control or cortisol treated) of 10 fish each and sampled them 24 h after SW exposure.

#### 2.2.3. Sampling

Sampling consisted of quickly netting all fish from each tank and anaesthetizing them with an overdose of 2-phenoxyethanol (1:1000). Fish length and weight were recorded and they were bled by caudal puncture into heparinized tubes and plasma collected after centrifugation (13,000g for 5 min). Plasma was stored frozen at −70 °C for later hormone and metabolite analyses, while pieces of liver and gill were dissected and quickly frozen in liquid nitrogen for mRNA and enzyme measurements. In addition, a piece of gill tissue was fixed in 4% paraformaldehyde in phosphate buffered saline (PBS, pH 7.3) overnight at 4 °C for immunohistochemistry.

#### 2.2.4. Plasma cortisol and glucose levels

Plasma cortisol concentration was measured by enzyme-linked immunosorbent assay (ELISA) following established protocols for this species (Rahman et al., 2000). Plasma glucose concentration was measured colorimetrically exactly as mentioned before (Vijayan et al., 2001).

#### 2.2.5. Plasma osmolality and ion analyses

Plasma osmolality was determined using a WESCOR vapor-pressure osmometer (VAPRO™ model 5520, Logan, Utah, USA). A polarized Zeeman atomic absorption spectrophotometer (model Z-6100, Hitachi) was used to quantify plasma ion (sodium, potassium, calcium, magnesium) concentrations, while chloride was measured using a chloride kit (Teco Diagnostics, Anaheim, CA, USA).

#### 2.2.6. Liver glycogen and liver & gill enzyme activities

Liver glycogen content was analyzed by liver glucose measurements before and after amyloglucosidase hydrolysis (Keppler and Decker, 1974). For enzyme determinations, pieces of liver and gills were homogenized (CIEDA, Japan) followed by sonication (Microson, NY) in glycerol buffer [50 mM Tris buffer (pH 7.5) and stored in a 1:10 (*w*/*v*) buffer containing 21 mmol $L^{-1}$ $Na_2HPO_4$, 5 mmol $L^{-1}$, 2-mercaptoethanol, 0.5 mmol $L^{-1}$ EDTA, 0.2% BSA, 10 μg aprotinin $mL^{-1}$, and 50% (*v*/*v*) glycerol and pH adjusted to 7.4] and stored frozen prior to enzyme measurement. The protein

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