Comparative Biochemistry and Physiology, Part C 148 (2008) 430–436

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

The effect of elevated salinity on 'California' Mozambique tilapia (*Oreochromis mossambicus* x *O. urolepis hornorum*) metabolism ☆

Brian A. Sardella *, Colin J. Brauner

Department of Zoology, University of British Columbia, Vancouver B.C., Canada

ARTICLE INFO

Article history:
Received 27 February 2008
Received in revised form 12 May 2008
Accepted 12 May 2008
Available online 19 May 2008

Keywords:
Tilapia
Salinity tolerance
MO_2
Metabolic suppression
Euryhaline

ABSTRACT

California Mozambique tilapia (*Oreochromis mossambicus* x *O. urolepis hornorum*) are extremely saline tolerant and have been previously shown to reduce whole-animal oxygen consumption rate (MO_2) upon exposures to salinities greater than that of seawater (SW). In this study tilapia were acclimated to 15, 30, 45, 60 and 75 g/L salinity for 1, 5, 14, or 28 days. There was little change in plasma osmolality or muscle water content in salinities below 60 g/L, and branchial Na^+, K^+-ATPase (NKA) activity was low in 15 and 30 g/L relative to 60 and 75 g/L. When tilapia were exposed to 75 g/L, plasma osmolality and NKA activity were significantly increased within 5 days of exposure relative to those in 15 and 30 g/L, and remained elevated over the entire 28 days acclimation, indicating that short term salinity challenges (i.e., 5 days) are predictive of longer exposure durations in this species. MO_2 following transfer to 15 and 30 g/L was elevated, reflecting the high energy demand required for switching from a hyper- to a hypo-osmoregulatory strategy. The MO_2 of 60 g/L-exposed fish was significantly reduced at 1, 5, and 14 days, relative to 30 g/L-exposed fish; however by 28 days there were no significant differences. We investigated the potential for a metabolic basis for the salinity-induced MO_2 reduction, using forward stepwise linear regression to correlate enzyme activities of brain, liver, and kidney with MO_2. Brain NKA was correlated with MO_2 after 5 days ($p<0.01$, $r^2=0.944$) and both brain NKA and hepatic total ATPase were correlated with the reduced MO_2 at 14 days ($p=0.027$, $r^2=0.980$ and $p=0.025$, $r^2=0.780$, respectively). These results may indicate a tissue-level metabolic suppression, which has not been previously described as a response to hypersaline exposure in fishes.

1. Introduction

Teleostean fishes maintain an internal environment that is hypo-osmotic to seawater (SW), and therefore SW-acclimated fish must compensate for osmotic water loss and diffusive ion gains. These fishes drink SW and then excrete Na^+ and Cl^- at the level of the branchial mitochondrial-rich cells (MRCs) (Marshall, 2002; Marshall and Bryson, 1998). The Mozambique tilapia (*Oreochromis mossambicus*) is a euryhaline species that can acclimate to both FW and SW, and is commonly used as a model to investigate mechanisms of osmoregulation. While much is known regarding the physiological responses of this species during transfer from FW to SW (Hwang et al., 1989; Hwang, 1987), fewer studies have investigated the effects of exposure to salinities greater than SW (hypersalinity) (Kültz and Onken, 1993; Sardella et al., 2004b; Stickney, 1986). The 'California' Mozambique tilapia (*O. mossambicus* x *O. urolepis hornorum*) is found throughout southern California, inhabiting a wide range of conditions from FW irrigation canals to the hypersaline Salton Sea (approximately 44 g/L) (Costa-Pierce and Doyle, 1997). The hypersaline tolerance of this species was previously described over the salinity range from 35 to 95 g/L. Interestingly, the physiological response was biphasic depending upon the salinity (Sardella et al., 2004a). After exposure to 45 or 55 g/L for 5 days, plasma osmolality, $[Na^+]$, $[Cl^-]$ and muscle water content were unchanged relative to SW-acclimated fish, despite no increase in drinking rate, branchial Na^+, K^+-ATPase (NKA) activity, or change in MRC turnover rate. In contrast, exposure to 65, 75, 85, and 95 g/L for 5 days resulted in an elevation in NKA activity, drinking rate, and MRC turnover in proportion to salinity with an eventual elevation in plasma osmolality, $[Na^+]$ and $[Cl^-]$ at 75, 85, and 95 g/L (Sardella et al., 2004a). While the biphasic response to progressive salinity increase was evident after 5 days of exposure (the longest duration measured in that study), it remains to be investigated if length of exposure affects this pattern or the salinity at which the transition between phases is observed.

Several studies have previously documented the variable effects of ambient salinity on whole animal oxygen consumption (MO_2; for a summary see Morgan and Iwama, 1991). The majority of these studies investigated MO_2 following a FW to SW transfer, while fewer studies have investigated the effect of salinities greater than SW. Unlike the FW

☆ Contribution to the Special Issue of CBP on Chinese Comparative Biochemistry and Physiology presented at or related to the International Conference of Comparative Physiology, Biochemistry and Toxicology and the 6th Chinese Comparative Physiology Conference, October, 10-14, 2007, Zhejiang University, Hangzhou, China.

* Corresponding author. Department of Animal Science, University of California, Davis. One Shields Ave., Davis, CA 95616, USA. Tel.: +1 530 752 7322; fax: +1 530 752 0175.
E-mail address: basardella@ucdavis.edu (B.A. Sardella).

1532-0456/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2008.05.006

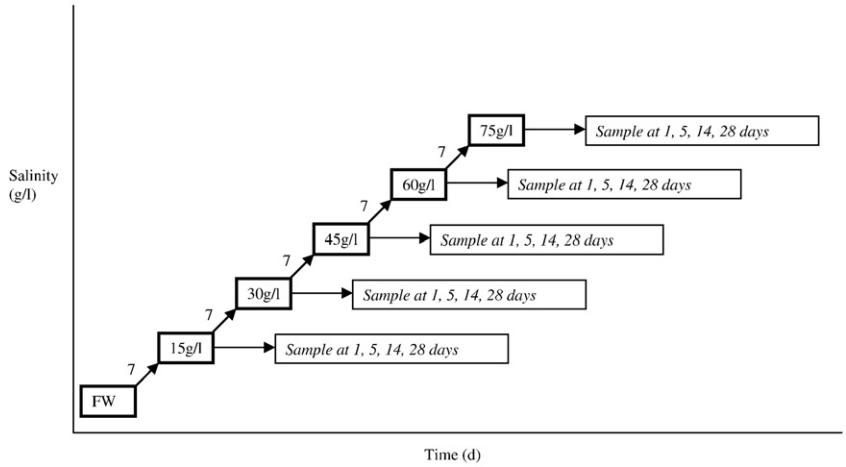

Fig. 1. Schematic representing the transfer protocol for tilapia salinity acclimation.

to SW transfer, where MO_2 is typically increased, further increases in salinity have been shown to result in an MO_2 reduction. This has been observed in 'California' Mozambique tilapia following acclimation for 14 days to a salinity range from 35 to 95 g/L (Sardella et al., 2004a), where MO_2 was reduced by 40% at 95 relative to 35 g/L. Salinity-based reductions in MO_2 have also been observed in the Dead Sea killifish (*Aphinius dispar*; Plaut (2000)), sheepshead minnow (*Cyprinidon variegatus*; Haney and Nordlie, (1997)), and milkfish (*Chanos chanos*; Swanson (1998)). It has been hypothesized that these reductions are simply due to a reduced spontaneous activity of individual fish, and furthermore are indicative of an upper limit to salinity tolerance (Plaut, 2000). Here we propose that reductions in MO_2 during exposure to hypersalinity may be biochemical in origin rather than purely behavioral, and for this species of tilapia may play a role in their impressive salinity tolerance (as high as 120 g/L; Stickney, 1986). This is the first time that changes in the activity of tissue ATPases have been correlated with metabolic rate following acclimation to salinities greater than SW.

Tilapia were acclimated to a range of salinities (15, 30, 45, 60 and 75 g/L), and the effects on osmoregulatory and metabolic parameters were measured over a 28 days acclimation period to those salinities. These experiments were conducted to address two main questions: 1) does the duration of salinity exposure affect the bi-phasic physiological response and whole animal MO_2 response that were observed previously by Sardella et al. (2004a), and 2) is there potentially a biochemical basis underlying the salinity-induced reduction in MO_2 that has been observed in this and other euryhaline species.

2. Materials and methods

2.1. Fish

Tilapia (*Oreochromis mossambicus* x *Oreochromis urolepis hornorum*, Cichlidae, Perciformes) were generously donated by Pacific Aquafarms in Niland, CA, USA. FW-reared fish were transported to the University of British Columbia, Vancouver, BC, Canada where experiments were conducted. Tilapia averaging 6.2±1.9 g were acclimated to 15, 30, 45, 60, and 75 g/L salinity (28 fish per 60 L tank). Salinity was increased every 7 days by changing 50% of the tank volume, and this was repeated every 7 days until target salinities were reached, at which time fish were maintained at that salinity and terminally sampled on days 1, 5, 14, and 28 (see Fig. 1). Previously we have showed that 5 days was sufficient for tilapia to recover from a step increase in salinity of up to 25 g/L (Sardella et al., 2004a). Tilapia used for MO_2 measurements were acclimated to the salinities using an identical protocol, but were held in separate 40 L tanks and repeated measurements of MO_2 were conducted on days 1, 5, 14 and 28 of the respective salinities. Water quality was maintained in all tanks by use of mechanical, chemical and biological filtration. During salinity acclimation and throughout the time course of exposure, temperature was held at 25 °C and fish were fed daily, however, food was withheld 48 or 24 h prior to MO_2 measurement or tissue sampling, respectively. Salinity was manipulated using Instant Ocean Synthetic Sea Salt in dechlorinated UBC tap water, and measured using a light refractometer.

2.2. Oxygen consumption measurements

Oxygen consumption rate (MO_2) was measured at 1, 5, 14, and 28 days after reaching target salinities of 15, 30, 45, 60, or 75 g/L. Tilapia were transferred to individual respirometry chambers (~360 mL) with gravity-fed water flow and allowed to acclimate for 14–16 h (overnight) before measurements of MO_2 were taken. Using killifish (*Fundulus heteroclitus*), Kidder et al. (2006) showed that exploratory movements ceased after 12 h of confinement at which time MO_2 had stabilized. To measure MO_2, water flow was stopped and the decrease in water O_2 saturation within the individual chambers was measured continuously for 25–30 min (flow was re-initiated before water O_2 level dropped below 70% saturation). The decrease in water O_2 saturation was measured using an Ocean Optics FOXY O_2 probe and Ocean Optics software (Ocean Optics Inc., Dunedin,

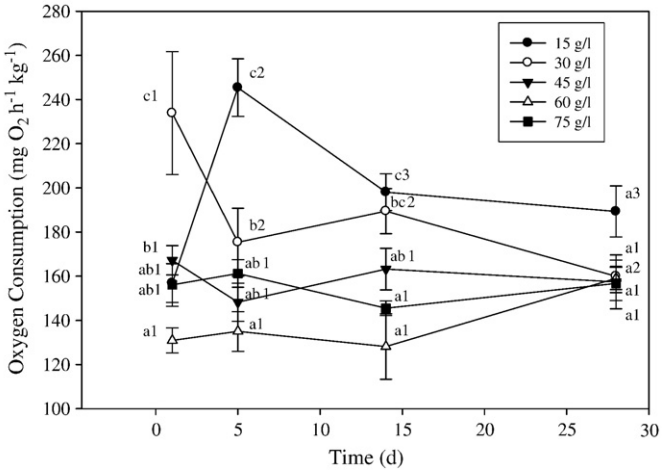

Fig. 2. A) Whole-animal oxygen consumption rate over time in California Mozambique tilapia (*Oreochromis mossambicus* x *O. urolepis hornorum*) following transfer to various salinities. Symbols indicate mean±SE. Letters that differ indicate differences between values from different salinities at a common time, while numbers that differ indicate differences with time within a given salinity ($p<0.001$; $n=7$).

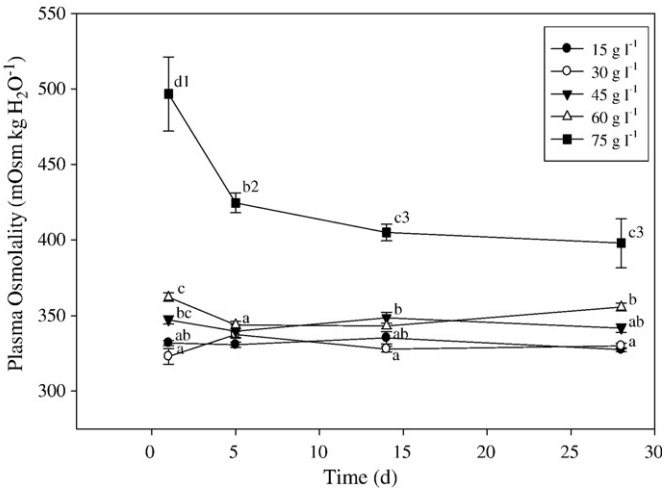

Fig. 3. Plasma osmolality over time in California Mozambique tilapia (*Oreochromis mossambicus* x *O. urolepis hornorum*) following transfer to various salinities; See Fig. 1 for details.

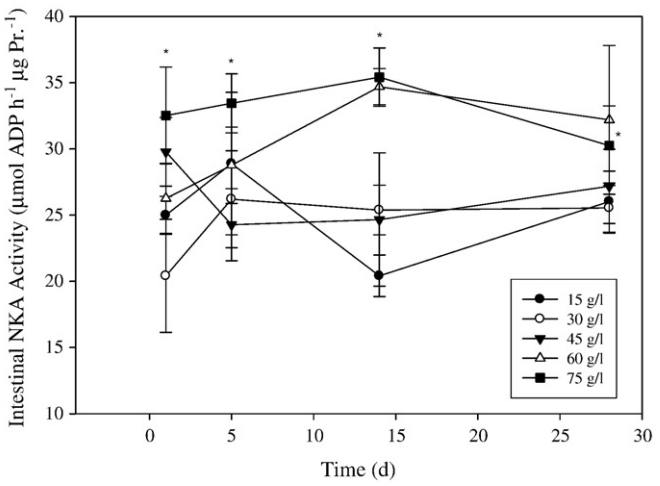

Fig. 5. Intestinal Na^+, K^+-ATPase (NKA) activity over time in California Mozambique tilapia (*Oreochromis mossambicus* x *O. urolepis hornorum*) following transfer to various salinities. There were no significant interaction effects, but there was a significant effect of salinity at 75 g/L (denoted by *$p<0.001$).

FL, USA). The oxygen electrode was calibrated to air (100%) and N_2 flow (0%) prior to, and following, each individual measurement. After measuring oxygen uptake, the weight of the fish and the volume of water in the chamber were recorded. Using the slope of the decrease in $\%O_2$ over time, the weight of the fish, the volume of the chamber, and O_2 solubility coefficients (α) adjusted for temperature and salinity, MO_2 was calculated as mg O_2/kg/h.

2.3. Fish sampling

Seven tilapia were sacrificed by a lethal dose of MS-222 at 1, 5, 14, and 28 days after reaching target salinities of 15, 30, 45, 60, and 75 g/L. The caudal peduncle was severed and blood was collected into heparinized capillary tubes, which were then centrifuged in a Damon IEC MB microhematocrit centrifuge. Hematocrit (Hct) was recorded in duplicate or triplicate, depending on available blood volume, and plasma was expelled into microcentrifuge tubes for measurement of plasma osmolality using a WESCOR 1500 vapor pressure osmometer (WESCOR Inc., Logan, UT, USA). Approximately 200 mg of left dorsal epaxial muscle was removed, separated from the skin, and weighed before and after drying at 70 °C for 96 h, to determine the % muscle water. The gills, brain, liver, intestine, kidney, and epaxial muscle from the right side of the fish were snap frozen in liquid N_2 for use in ATPase activity assays.

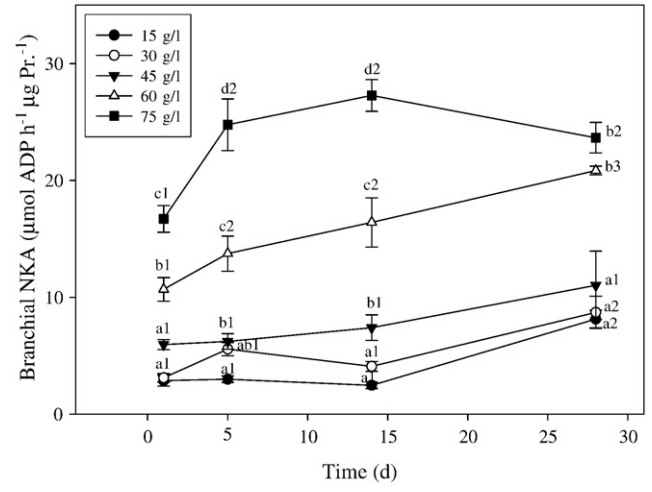

Fig. 4. Branchial Na^+, K^+-ATPase (NKA) activity over time in California Mozambique tilapia (*Oreochromis mossambicus* x *O. urolepis hornorum*) following transfer to various salinities; see Fig. 1 for details.

2.4. ATPase assays

Tissues were homogenized in approximately 1 mL of SEID buffer (250 mM sucrose, 10 mM EDTA·Na_2, 50 mM imidazole, pH 7.3, 0.05% deoxycholic acid). Na^+, K^+-ATPase (NKA) activity was determined as the ouabain-inhibited fraction of total oxidation of NADH as described by McCormick (1993) and expressed as µmol of ADP/h/µg total protein. Enzyme assays were conducted at 25 °C, and NADH oxidation was measured over a 10 min period in a 96-well microplate at an absorbance of 340 nm. When no differences between homogenates incubated with and without ouabain were observed (liver), total tissue ATPase activity was standardized to total protein content of the homogenate, which was determined using absorbance at 560 nm following 30 min of incubation with a 50:1 solution of bicinchoninic acid to $CuSO_4$. ATPase activity of muscle was also measured, but not included in the analysis because no ouabain-inhibitable fraction could be detected within the large activity. We did not standardize the total ATPase activity of muscle as we did with liver due to the inability to confirm the amount of contractile apparatus remaining in the homogenate from sample to sample.

2.5. Statistical analyses

A two-way analysis of variance (ANOVA) followed by a post-hoc Holm–Sidak multiple means comparison was used to assess the effects of time and salinity on MO_2, plasma osmolality, muscle water content, and branchial and intestinal NKA activity. In order to minimize handling stress, tilapia used in the repeated measurement of MO_2 were not marked. Because individuals could not be identified from measurement to measurement, we were unable to use repeated measures ANOVA to test for differences between groups.

Table 1
Results from the forward-stepwise linear regression test conducted for MO_2 versus brain, liver, and kidney ATPase activities at all times (*denotes inclusion into the model based on a $p<0.05$; b=brain, l=liver)

Variable	p	r^2	Standard error
Brain	0.044*	0.193	3.78
Liver	0.029*	0.368	2.62
Kidney	0.901	–	–
Model equation	MO_2=8.19(b)+6.25(l)−44.1		

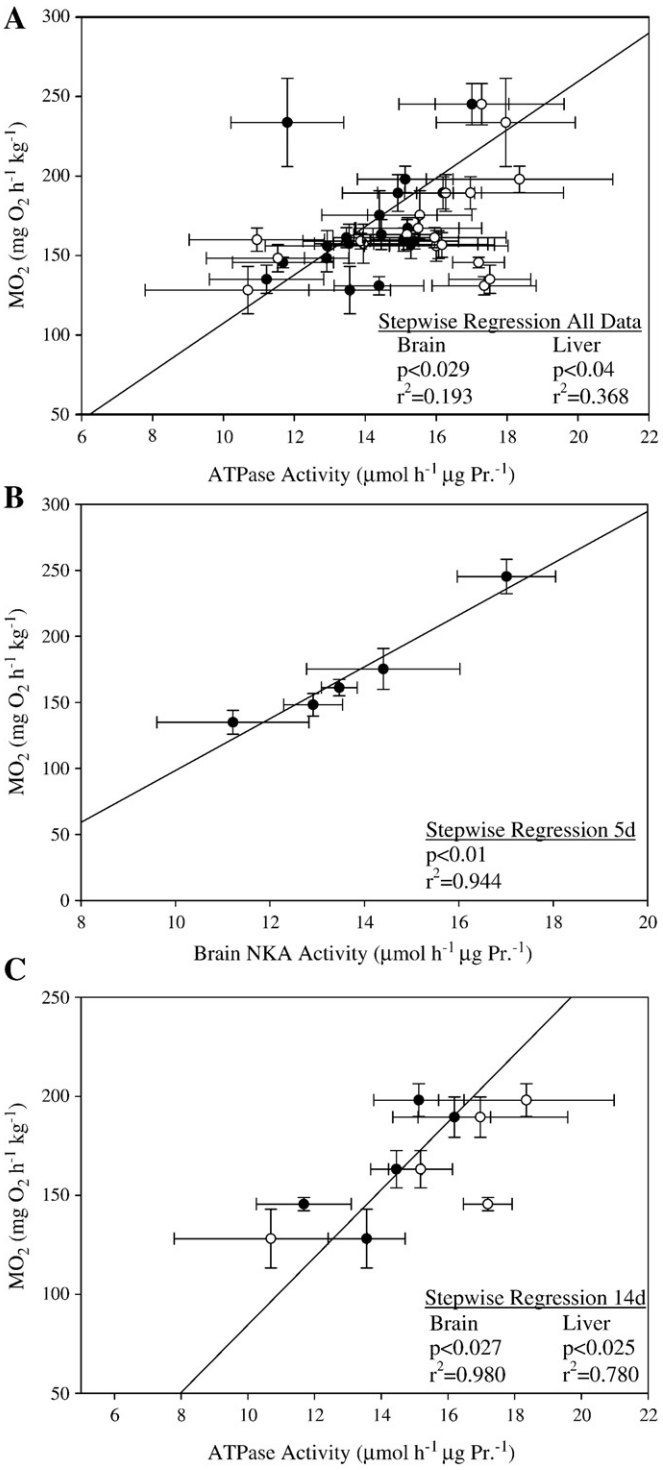

Fig. 6. Results of forward-stepwise linear regression analyses. A) Brain NKA and total hepatic ATPase with time points and salinity treatments included., B) Brain NKA at 5 days following transfer with all salinities, and C) Brain NKA and total hepatic ATPase at14 d following transfer to all salinities. Lines represent model equations; Brain (●), Liver (○).

Using forward-stepwise linear regression analysis, the *in vitro* activities of brain, kidney, and liver ATPases were plotted against whole animal MO_2 for the entire data set as well as for individual time points. For each salinity and time combination, we compared mean *in vivo* MO_2 values with the mean ATPase activity values measured *in vitro* for the respective tissues. Because MO_2 measurements and tissue activities were measured in two separate groups of tilapia, comparisons with individual fish were not possible and mean values were necessary to perform the tests. Regression provided model equations that included variables that significantly correlated with MO_2, while excluding those that did not. All statistical analyses were performed using SigmaStat version 3.0, which automatically tested data for normality and equal variance prior to running the two-way ANOVA. Plots were made using SigmaPlot version 8.0. Unless otherwise indicated, values presented are mean±SE ($n=7$; $\alpha=0.05$).

3. Results

There was no mortality during these experiments, and furthermore no observable changes in behavior were noted at the different salinities. Tilapia consistently consumed all food presented to them. Also, there was no effect of salinity or time of exposure on hematocrit (pooled value=37.1±0.5). There was a significant effect of salinity on MO_2 ($p<0.001$), as well as a significant interaction between time of exposure and salinity ($p<0.001$). The overall trend was for MO_2 to vary inversely with salinity following acclimation for 1, 5, and 14 days in salinities greater than SW, but there were no significant differences in MO_2 among salinities following 28 days of acclimation (Fig. 2). The lowest MO_2 value was measured in 60 g/L-acclimated tilapia after 14 days, where it was reduced by 35 and 32% relative to 14 days acclimation to 15 and 30 g/L-exposed fish, respectively. The highest recorded MO_2 values were in tilapia exposed to 15 g/L for 5 days and 30 g/L for 1 day.

Salinity, time of exposure, and time and salinity interaction significantly affected plasma osmolality (all p values<0.001). There were minor differences in plasma osmolarity among salinities at various times, but they were small relative to those observed at 75 g/L and well within the normal range for this species (Fig. 3). At all time points, tilapia in 75 g/L water had a significantly higher plasma osmolality relative to all other salinities. Despite the large change in plasma osmolality observed in 75 g/L-acclimated tilapia, at no time were significant differences in muscle water content observed (pooled value=79.4±0.5). Branchial NKA activity was significantly affected by salinity, time of exposure and salinity and time interaction (all p values<0.001). The greatest NKA activity levels were measured in fish exposed to 75 g/L salinity (Fig. 4), where values were significantly greater than those observed at 60 g/L at all time points except 28 days. There were other slight but significant differences as detected by two-way ANOVA, however, as with plasma osmolality, these were minor in comparison to the large increases at 60 and 75 g/L. There was a general effect of salinity on intestinal NKA activity ($p<0.001$), with values measured from 75 g/L-acclimated fish increased relative to those acclimated to other salinities, there was no effect of time or time/salinity interaction (Fig. 5).

3.1. ATPase activity and MO_2

MO_2 was reduced as salinity was increased at 1, 5, and 14 days, but not at 28 days. When all time points and salinities were included in the analysis, forward-stepwise linear regression determined that brain NKA and hepatic total ATPase activities best described whole animal MO_2, although this regression had a very high level of variation (Table 1; Fig. 6a). When the regression analyses were carried out on a by day basis, there were no significant correlations at 1 or 28 days. MO_2 was significantly correlated with brain NKA after 5 days of

Table 2
Results from the forward-stepwise linear regression test conducted for MO_2 versus brain, liver, and kidney ATPase activities at 5 days (*denotes inclusion into the model based on a $p<0.05$; b=brain, l=liver)

Variable	p	r^2	Standard error
Brain	0.006*	0.944	2.78
Liver	0.249	–	–
Kidney	0.805	–	–
Model equation	$MO_2=19.6(b)-97.81$		

Table 3
Results from the forward-stepwise linear regression test conducted for MO_2 versus brain, liver, and kidney ATPase activities at 14 days (*denotes inclusion into the model based on a $p<0.05$; b=brain, l=liver)

Variable	p	r^2	Standard error
Brain	0.027*	0.608	1.80
Liver	0.025*	0.979	1.02
Kidney	0.665	–	–
Model equation	$MO_2=10.68(b)+6.33(l)-86.07$		

exposure (Table 2; Fig. 6b), and both brain NKA and hepatic total ATPase were correlated with MO_2 at 14 days (Table 3; Fig. 6c), when reductions in MO_2 were greatest. Activity of renal NKA (pooled value=31.2±1.3 μmol h^{-1} μg $protein^{-1}$) was not significantly correlated with MO_2 at any time point and was excluded from all model equations.

4. Discussion

The 'California' Mozambique tilapia is a hybrid species that can be found throughout the waters of southern California. This species inhabits a large range of salinities, including up to 44 g/L in the Salton Sea (Costa-Pierce and Doyle, 1997). Laboratory acclimations have shown that this species, as well as the pure Mozambique tilapia, have an impressive ability to survive and acclimate to salinities much greater than that of their natural distribution (Sardella et al., 2004a; Stickney, 1986). It should be noted, however, that deviations of ± 10 °C can dramatically reduce the salinity tolerance of this species (Sardella et al., 2004b).

Although this species is one of the most tolerant to high salinity, it is not able to tolerate large magnitude increases in salinity, such as a direct transfer from FW to SW, or increases in salinity greater than 25 g/L in magnitude once acclimated to a salinity of SW or higher (Sardella et al 2004a). Consequently, fish were acclimated at intermediate salinities for 7 days in order to achieve our target salinities (see Fig. 1 for the complete schedule). One week has been shown to be sufficient for tilapia to acclimate to these salinity increases (Sardella et al., 2004a). This type of step-wise acclimation protocol added an unavoidable time effect into our experiment, as fish that were sampled in the highest salinity groups were held under experimental conditions for longer than those sampled from the lower salinity groups. In all cases, fish had been acclimated to laboratory conditions at UBC for six months, far longer than the duration of the experiment. While direct transfer to each of the salinities would be the most desirable, it was not possible, making our transfer protocol the only option to investigate the physiological responses to these salinities. Interestingly, any osmoregulatory compensation occurs within 5 days in tilapia, and extending the duration of exposure to 28 days had little additional effect. Our data therefore support the use of short-term (up to 5 days) salinity challenges for assessing salinity tolerance in tilapia, as is routinely used in salmonids to assess smoltification status (Clark and Blackburn, 1977).

MO_2 was highest at 5 days following transfer from FW to 15 g/L, and 1 d following transfer from 15 to 30 g/L; these time points represent 5 and 8 days following the first exposure to hyper-osmotic conditions (see Fig. 1.). Morgan et al. (1997) also found that MO_2 was increased in this species after 4 days of exposure to 25 g/L salinity. Foskett et al. (1981) showed that tilapia mitochondria-rich cells (MRCs) underwent hypertrophy from 4 to 7 days following SW transfer. Larger MRCs have been observed in response to SW transfer in several studies and due to increased synthesis of basolateral membrane and expression of NKA (Metz et al., 2003). In addition, other morphometric changes such as synthesis of tight junctional proteins and development of inter-cellular interdigitations also occur following a FW to SW transfer (Hwang, 1987). These biochemical and morphological changes that are associated with the development of hypo-osmoregulatory mechanisms are energy-demanding and likely played a major role in the high MO_2 values measured in tilapia exposed to 15 and 30 g/L.

4.1. The Bi-phasic response to progressive salinity increase

Although the salinity range and transfer protocol differed slightly from our previous study, current results indicated a similar bi-phasic response during longer-term salinity acclimations (28 days) and a similar transition point between phases (~60 g/L) as to what was observed following 5 days (Sardella et al., 2004a). The two phases are represented by 1) a lack of change in drinking rate, branchial NKA activity, or MRC turnover when exposed to salinities from 35–55 g/L, and 2) an increase of each during exposure to 65–95 g/L. Bi-phasic responses to hypersaline challenges have also been observed in other euryhaline species such as sheepshead minnow (*Cyprinidon variegatus*; (Nordlie, 1984) and sail fin mollies (*Poecilia latipinna*; Gonzalez et al. (2005)), although the basis for this has remained for the most part unclear.

The responses of tilapia to salinities greater than the 60 g/L transition point were more typical of an increased osmoregulatory capacity that would be expected following a transfer from FW to SW (Hwang, 1987). There was a large increase in plasma osmolality 1 day post transfer to 75 g/L, which recovered to an elevated but stable level from 5 to 28 days. The partial recovery and stabilization of plasma osmolality in 75 g/L-exposed tilapia was likely a result of the large increase in branchial NKA activity, which remained high throughout the 28 days exposure. Furthermore, intestinal NKA activity was also elevated, consistent with an increase in drinking rate following 2 weeks exposure to 75 g/L (Sardella et al., 2004a), where greater ion transport capacity for water absorption is required (Wilson et al., 1996; Wilson et al., 2002).

There were no changes in muscle water content or muscle [Na^+] regardless of salinity or plasma osmolality. In our previous study this was also the case, despite plasma osmolality values reaching as high as 450 mOsm (Sardella et al., 2004a). The maintenance of muscle water content in this species in the face of such high osmolalities was interesting. In contrast, salmonids showed a strong negative correlation between plasma ionic status and muscle water content over a much smaller range of plasma osmolalities (Brauner et al., 1994; Brauner et al., 1992). Sail fin mollies, a species with a high salinity tolerance and bi-phasic responses to salinity similar to tilapia, were unable to maintain muscle water content in salinities above 75 g/L (Gonzalez et al., 2005). More investigations regarding this maintenance of muscle water would prove interesting.

4.2. Tissue level changes associated with oxygen uptake

ATPases are a major energy sink within cells and have been shown to reduce activity under oxygen limiting conditions (Buck and Hochachka, 1993; Hochachka, 1986; Hochachka and Somero, 2002). Based on previous observations of metabolic suppression during conditions of oxygen depletion (Buck and Hochachka, 1993; Buck and Pamenter, 2006; Storey and Storey, 1990), we focused on brain, liver, and kidney ATPase activities to investigate the potential for metabolic suppression during exposure to elevated salinity. Regression analysis of all time points revealed that MO_2 was best correlated with brain NKA and total hepatic ATPase activities, although there was a high degree of variation in these correlations ($r^2=0.193$ and 0.368, respectively). The high variation was most likely a result of consistent tissue activities at 1 day and the lack of difference in MO_2 at 28 days. Variation was much lower when regressions were conducted on a by-day basis. Following 5 days of salinity acclimation, the reduction in MO_2 correlated with reduced brain NKA activity, while at 14 days it correlated with both brain NKA and total hepatic ATPase activities. The reduction of *in vitro* tissue ATPase activities are the first to support a biochemical basis underlying the reduction in whole animal MO_2 during acclimation to elevated salinity. These data suggest that ATPase

enzymes were either down-regulated or modified *in vivo*, resulting in a lower activity measurement from whole tissue homogenates. This change in biochemical status is not mutually exclusive from the previous hypotheses that MO_2 decreases due to a reduced spontaneous activity in hypersaline conditions (Haney and Nordlie, 1997; Plaut, 2000; Swanson, 1998), but it clearly shows that other mechanisms are involved.

A tissue-level suppression in ATPase activity may suggest the use of mechanisms classically described in response to low ambient oxygen (Buck and Hochachka, 1993; Hochachka, 1986) in response to increased ambient salinity. Hochachka (1986) first described the arrest of membrane ion channels and the subsequent reduction in ion pump activities as a coping mechanism for reduced ATP supply under oxygen limitation. The response of Mozambique tilapia to hypoxia has yet to be well described, but limited evidence suggests that they are relatively hypoxia tolerant (Van Ginneken et al., 1995), and members of the family Cichlidae, including the congener Nile tilapia (*O. niloticus*) have been shown to be tolerant (Chapman et al., 2000; Chapman et al., 1995). The reduction in membrane ATPase activities in response to reduced MO_2 suggests that Mozambique tilapia may be capable of reducing membrane permeability in a similar fashion to what has been described for anoxia-tolerant species such as the painted turtle, where hepatocyte NKA activity decreased by as much as 75% during exposure to anoxia (Buck and Hochachka, 1993). While the changes in tilapia *in vitro* ATPase activities and *in vivo* MO_2 are not as large as those in response to anoxia by species undergoing "facultative anaerobiosis" (Storey and Storey, 1990), it is remarkable and surprising to observe responses such as these during acclimation to hypersaline conditions in normoxic water.

We speculate that there are two possible mechanisms by which ATPase activity would correlate with reduced MO_2 during exposure to hypersalinity. The first would be that decreases in brain and liver ATPase resulted from an unknown signal (e.g., endocrine) in response to the change in ambient salinity, and the decreased ATPase activity subsequently led to a lower MO_2. This would not be in response to a change in plasma ion status however, as it was well maintained in tilapia exposed to 15–60 g/L salinity. A second hypothesis is that a reduction in gill blood and/or water flow to defend against the extreme osmotic gradient induced hypoxemia that was associated with reduced ATPase activity. That is, the response to salinity may be mediated through hypoxemia. Data at present cannot support or refute either hypothesis. In addition to salinity and hypoxia, this species is also very tolerant to aquatic ammonia (Suvajdzic et al., 2007); perhaps its tolerance to challenging environments in general is mediated through a common pathway, which is an intriguing thought.

Interestingly, the reduction in MO_2 during exposure to elevated salinity was transient, with no differences among salinity groups observed after 28 days. Kültz and Onken (1993) examined the electrophysiological effects of exposure to hypersaline conditions (up to 60 g/L) and showed that after 35 days, epithelial permeability was dramatically reduced relative to SW-acclimated fish. In their study, the number of MRCs was greatly increased in 60 g/L-exposed tilapia, but both trans-cellular and para-cellular conductances were greatly reduced. This was a radically different strategy of osmoregulation relative to what has been described for this species in SW, and it allowed for ion excretion rates to remain similar to those from SW-acclimated animals, even when exposed to twice the osmotic gradient (Kültz and Onken, 1993). While this strategy was characterized in fish acclimated for 35 days, earlier measurements were not made. We now speculate that the reduction in MO_2 up to 2 weeks following transfer to an elevated salinity is a salinity tolerance mechanism that allows time for the re-structuring of the branchial epithelium as described by Kültz and Onken (1993) to take place, and that MO_2 recovery takes place once the changes are complete. Because these changes may be metabolically expensive, whole animal metabolic rate must underestimate the degree of metabolic suppression in these animals during exposure to elevated salinity. Furthermore, if this is the case, then the changes that Kültz and Onken (1993) described must take place between 14 and 35 days following exposure; more experiments are needed to confirm these hypotheses.

5. Summary

The bi-phasic physiological response to progressive salinity increase that was previously observed by Sardella et al. (2004a) was mostly unaltered by longer term exposures (i.e. 28 days vs. 5 days), while the reduction of whole animal MO_2 was found to be transient change (up to 14 days) that was not observed by 28 days following transfer. While exposure to 15 and 30 g/L resulted in an elevation in MO_2, likely associated with the cost of the transition from hyper- to hypoosmoregulation, exposure to 60 and 75 g/L resulted in a reduction in MO_2 relative to those in 15 or 30 g/L that may be associated with acclimation to elevated salinity. Brain NKA activity at 5 days and both brain NKA and liver ATPase activities were highly correlated with the reduced metabolic rate at 14 days. These represent the first observations of a tissue-level metabolic suppression in response to salinity exposure.

Acknowledgements

We would like to thank Bill Engler and Colin Bornia of Pacific Aquafarms for their generation of tilapia for this and multiple other studies. We would also like to thank Jon Shurin of UBC for the use of his vehicle in order to deliver the fish to UBC. There are many others who contributed to this study in various ways, including Kim Suvadjzic, Milica Mandic, Jeff Richards, Patricia Schulte, and Nann Fangue, as well as two anonymous referees with valuable suggestions. Finally, this work was supported by The Josephine Berthier Graduate Fellowship, awarded to BAS, and an NSERC Discovery Grant to CJB.

References

Brauner, C.J., Shrimpton, J.M., Randall, D.J., 1992. Effect of short-duration seawater exposure on plasma ion concentrations and swimming performance of coho salmon (*Oncorhynchus kisutch*) parr. Can. J. Fish. Aquat. Sci. 49, 2399–2405.

Brauner, C.J., Iwama, G.K., Randall, D.J., 1994. The effect of short-duration seawater exposure on the swimming performance of wild and hatchery-reared juvenile Coho salmon (*Oncorhynchus kisutch*) during smoltification. Can. J. Fish. Aquat. Sci. 51, 2188–2194.

Buck, L.T., Hochachka, P.W., 1993. Anoxic suppression of Na^+, K^+-ATPase and constant membrane potential in hepatocytes: support for channel arrest. Am. J. Physiol:. Reg. Int. Comp. Physiol. 265, R1020–R1025.

Buck, L.T., Pamenter, M.E., 2006. Adaptive responses of vertebrate neurons to anoxia-matching supply to demand. Res. Physiol. Neurol. 154, 226-140.

Chapman, L.J., Kaufman, L.S., Chapman, C.A., McKenzie, F.E., 1995. Hypoxia tolerance in twelve species of east African cichlids: potential for low oxygen refugia in Lake Victoria. Cons. Biol. 9, 1274–1287.

Clark, W.C., Blackburn, J., 1977. A seawater challenge test to measure smolting of juvenile salmon. Can. Fish. Mar. Serv. Tech. Rep. 11p.

Chapman, L.J., Galis, F., Shinn, J., 2000. Phenotypic plasticity and the possible role of genetic assimilation: hypoxia-induced trade-offs in the morphological traits of an African cichlid. Eco. Let. 3, 387–393.

Costa-Pierce, B.A., Doyle, R.W., 1997. Genetic identification and status of tilapia regional strains in southern California. In: Costa-Pierce, BA, Rakocy, JE (Eds.), World Aquaculture. Worlds Aquaculture Society, Baton Rouge, LA, pp. 1–17.

Foskett, K.J., Logsdon, D., Turner, T., Meachen, T.E., Bern, H.A., 1981. Differentiation of the chloride extrusion mechanism during seawater adaptation of a teleost fish, the cichlid *Sarotherodon mossambicus*. J. Exp. Biol. 93, 209–224.

Gonzalez, R.J., Cooper, J., Head, D., 2005. Physiological responses to hyper-saline waters in sailfin mollies (*Poecilia latipinna*). Comp. Biochem. Physiol. A. 142, 397–403.

Haney, D., Nordlie, F., 1997. Influence of environmental salinity on routine metabolic rate and critical tension of *Cyprinodon variegatus*. Physiol. Zool. 70, 511–518.

Hochachka, P.W., 1986. Defense strategies against hypoxia and hypothermia. Science 231, 234–241.

Hochachka, P.W., Somero, G.N., 2002. Biochemical Adaptation: Mechanism and Process in Physiological Evolution. Oxford University Press, New York, NY.

Hwang, P.P., 1987. Tolerance and ultrastructural responses of branchial chloride cells to salinity changes in the euryhaline teleost *Oreochromis mossambicus*. Mar. Biol. 94, 643–649.

Hwang, P., Sun, C.M., Wu, S.M., 1989. Changes of plasma osmolality, chloride concentration and gill Na^+-K^+-ATPase activity in tilapia (*Oreochromis mossambicus*) during seawater acclimation. Mar. Biol. 100, 295–299.

Kidder III, G.W., Petersen, C.W., Preston, R.L., 2006. Energetics of osmoregulation II: Water flux and osmoregulatory work in the euryhaline fish, *Fundulus heteroclitus*. J. Exp. Zool. 305A.

Kültz, D., Onken, H., 1993. Long-term acclimation of the teleost *Oreochromis mossambicus* to various salinities: two different strategies in mastering hypertonic stress. Mar. Biol. 117, 527–533.

Marshall, W.S., 2002. Na^+, Cl^-, Ca^{2+}, and Zn^{2+} transport by fish gills: a retrospective review and prospective synthesis. J. Exp. Zool. 293, 264–283.

Marshall, W.S., Bryson, S.E., 1998. Transport mechanisms of seawater teleost chloride cells: an inclusive model of a multifunctional cell. Comp. Biochem. Physiol. A 119A, 97–106.

McCormick, S.D., 1993. Methods for non-lethal gill biopsy and measurement of Na^+, K^+-ATPase activity. Can. J. Fish. Aquat. Sci. 50, 656–658.

Metz, J.R., van der Burg, E.H., Wendelaar-Bonga, S.E., Flik, G., 2003. Regulation of branchial Na^+/K^+-ATPase in common carp *Cyprinus carpio* acclimated to different temperatures. J. Exp. Biol. 206, 2273–2280.

Morgan, J.D., Iwama, G.K., 1991. Effects of salinity on growth, metabolism, and ion regulation in juvenile rainbow and steelhead trout (*Oncorhynchus mykiss*) and fall Chinook salmon (*Onchorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 48, 2083–2094.

Morgan, J.D., Sakamoto, T., Grau, E.G., Iwama, G.K., 1997. Physiological and respiratory responses of the Mozambique tilapia (*Oreochromis mossambicus*) to salinity acclimation. Comp. Biochem. Physiol. A. 117, 391–398.

Nordlie, F., 1984. Osmotic regulation in the sheepshead minnow *Cyprinodon variegatus* Lacepede. J. Fish. Biol. 26, 161–170.

Plaut, I., 2000. Resting metabolic rate, critical swimming speed, and routine activity of the euryhaline Cyprinodontid, Aphanius dispar, acclimated to a wide range of salinities. Physiol. Biochem. Zool. 73, 590–596.

Sardella, B., Matey, V., Cooper, J., Gonzalez, R.J., Brauner, C.J., 2004a. Physiological, biochemical, and morphological indicators of osmoregulatory stress in California Mozambique tilapia (*Oreochromis mossambicus* x *O. urolepis hornorum*) exposed to hypersaline water. J. Exp. Biol. 207, 1399–1413.

Sardella, B., Cooper, J., Gonzalez, R., Brauner, C.J., 2004b. The effect of temperature on juvenile Mozambique tilapia hybrids (*Oreochromis mossambicus* x *O. urolepis hornorum*) exposed to full-strength and hypersaline seawater. Comp. Biochem. Physiol. A. 137, 621–629.

Stickney, R.R., 1986. Tilapia tolerance of saline waters: a review. Prog. Fish. Cult. 48, 161–167.

Storey, K.B., Storey, J.M., 1990. Metabolic rate depression and biochemical adaptation in anaerobiosis, hibernation and estivation. Qu. Rev. Biol. 65, 145–174.

Suvajdzic, K., Sardella, B.A., Brauner, C.J., 2007. Ammonia and salinity tolerance in the California Mozambique tilapia of the Salton Sea. In: Brauner, CJ, Suvajdzic, K, Nilsson, G, Randall, D.J. (Eds.), Proceedings of the Ninth International Symposium on Fish Physiology, Toxicology and Water Quality, April 24–28, 2006, Hotel San Michele, Capri, Italy. United States Environmental Protection Agency, Environmental Research Laboratory, Athens, Georgia, USA, EPA/600/R-07/010, pp. 65–72.

Swanson, C., 1998. Interactive effects of salinity on metabolic rate, activity, growth and osmoregulation in the euryhaline milkfish (*Chanos chanos*). J. Exp. Biol. 201, 3355–3366.

Van Ginneken, V., Van Den Thillart, G., Addink, A., Erkelens, C., 1995. Fish muscle energy metabolism measured during hypoxia and recovery: and in vivo 31P-NMR study. Am. J. Physiol:. Reg. Int. Comp. Physiol. 268, R1178–R1187.

Wilson, R., Gilmour, K., Henry, R., Wood, C., 1996. Intestinal base excretion in the seawater-adapted rainbow trout: a role in acid-base balance? J. Exp. Biol. 199, 2331–2343.

Wilson, R.W., Wilson, J.M., Grosell, M., 2002. Intestinal bicarbonate secretion by marine teleost fish-why and how? Biochim. Biophys. Acta 1566, 182–193.