Comparative Biochemistry and Physiology, Part A 178 (2014) 121–131

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Effects of salinity on metabolic rate and branchial expression of genes involved in ion transport and metabolism in Mozambique tilapia (*Oreochromis mossambicus*)

Aris Zikos [a], Andre P. Seale [b], Darren T. Lerner [b,c], E. Gordon Grau [b], Keith E. Korsmeyer [a,*]

[a] Department of Natural Sciences, Hawai'i Pacific University, Kāne'ohe, HI 96744, USA
[b] Hawai'i Institute of Marine Biology, University of Hawai'i, Kāne'ohe, HI 96744, USA
[c] University of Hawai'i Sea Grant College Program, University of Hawai'i, Honolulu, HI 96822, USA

ARTICLE INFO

Article history:
Received 7 June 2014
Received in revised form 28 July 2014
Accepted 18 August 2014
Available online 1 September 2014

Keywords:
Osmoregulation
Respirometry
Osmolality
Mitochondria-rich cells
Mitochondrial biogenesis
NKCC
NCC
PGC-1α
NRF-1
COX

ABSTRACT

This study investigated the effects of two rearing salinities, and acute salinity transfer, on the energetic costs of osmoregulation and the expression of metabolic and osmoregulatory genes in the gill of Mozambique tilapia. Using automated, intermittent-flow respirometry, measured standard metabolic rates (SMRs) of tilapia reared in seawater (SW, 130 mg O_2 kg^{-1} h^{-1}) were greater than those reared in fresh water (FW, 103 mg O_2 kg^{-1} h^{-1}), when normalized to a common mass of 0.05 kg and at 25 ± 1 °C. Transfer from FW to 75% SW increased SMR within 18 h, to levels similar to SW-reared fish, while transfer from SW to FW decreased SMR to levels similar to FW-reared fish. Branchial gene expression of Na^+–K^+–$2Cl^-$ cotransporter (NKCC), an indicator of SW-type mitochondria-rich (MR) cells, was positively correlated with SMR, while Na^+–Cl^- cotransporter (NCC), an indicator of FW-type MR cells, was negatively correlated. Principal Components Analysis also revealed that branchial expression of cytochrome c oxidase subunit IV (COX-IV), glycogen phosphorylase (GP), and a putative mitochondrial biogenesis regulator in fish, peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), were correlated with a higher SMR, plasma osmolality, and environmental salinity, while expression of glycogen synthase (GS), PGC-1β, and nuclear respiratory factor 1 (NRF-1) had negative correlations. These results suggest that the energetic costs of osmoregulation are higher in SW than in FW, which may be related to the salinity-dependent differences in osmoregulatory mechanisms found in the gills of Mozambique tilapia.

1. Introduction

Osmoregulation may constitute a substantial portion of the total energy budget in fishes, with estimates varying by species, from less than 10% to greater than 30% of total metabolic cost in teleosts (Bœuf and Payan, 2001; Ern et al., 2014). In teleost fishes, mitochondria-rich (MR) cells, also termed ionocytes or chloride cells, of the gill epithelium are the main site of osmoregulatory ion exchange (Hwang and Lee, 2007; Evans, 2008), and are also likely to be a major site of energy consumption, with high mitochondrial densities and ATPase activity (Perry and Walsh, 1989; Kaneko et al., 2008; Sardella et al., 2008). In euryhaline fishes, the gill epithelium has the functional plasticity to switch between mechanisms supporting ion uptake in fresh water (FW) and ion extrusion in seawater (SW) (Tipsmark et al., 2011). Mozambique tilapia, *Oreochromis mossambicus* (Peters, 1852), for example, can osmoregulate over a wide range of salinities, from deionized water (0 psu) to three times normal seawater (~105 psu) (Stickney, 1986; Choi et al., 2010). Previous studies investigating the bioenergetics of osmoregulation in tilapia have found that routine metabolic rates (RMR) are greater in fish reared in, or acclimated to, FW (~0.1 psu) than in those reared in, or acclimated to, SW (~35 psu) (Ron et al., 1995; Iwama et al., 1997; Sparks et al., 2003). It has been suggested that this difference in energy expenditure may account for higher growth rates in SW-acclimated fish relative to those of FW-reared fish (Kuwaye et al., 1993; Sparks et al., 2003).

Different energetic responses have been found, however, when fish are acutely transferred between different salinities. Mozambique tilapia transferred from FW to 75% SW (~25 psu) show significant increases in RMR at 24 h (Kammerer et al., 2010) and after 4 days (Morgan et al., 1997). It is not clear to what extent these increases in metabolic rate are due to direct osmoregulatory costs in the different salinities, or to the added energetic demands of the physiological and biochemical changes involved in the acute phase of salinity acclimation (Kammerer et al., 2010). To our knowledge, no similar studies have examined acute transfers in the other direction, from SW to FW.

* Corresponding author at: Department of Natural Sciences, Hawai'i Pacific University, 45-045 Kamehameha Hwy, Kāne'ohe, HI 96744, USA. Tel.: +1 808 236 5862.
E-mail address: kkorsmeyer@hpu.edu (K.E. Korsmeyer).

http://dx.doi.org/10.1016/j.cbpa.2014.08.016
1095-6433/

Four different types of MR cells (I–IV) have been identified in the branchial epithelium of tilapia. They are classified based on the presence or absence of certain ion-exchange pumps in their membranes, and their relative abundance in the gill changes according to acclimation salinity (Hiroi et al., 2005; Kaneko et al., 2008; Inokuchi and Kaneko, 2012). SW-type MR cells (types III and IV) are ion-secreting and express basolateral Na^+–K^+–$2Cl^-$ cotransporter (NKCC). On the other hand, apical Na^+–Cl^- cotransporter (NCC) is expressed only in FW-type ion-absorptive MR cells (type II). Type I MR cells express neither genes, and are not salinity-specific (Kaneko et al., 2008). Therefore, high levels of branchial NCC expression are associated with acclimation to FW, while high levels of NKCC expression are an indicator of SW acclimation (Hiroi et al., 2008; Inokuchi et al., 2008; Kaneko et al., 2008).

In addition to the energetic costs of active ion exchange, acclimation to a different salinity leads to extensive remodeling of the MR cells in the branchial epithelia, which may require additional energy (Kammerer et al., 2010; Hwang et al., 2011; Inokuchi and Kaneko, 2012). Energy for MR cells partially comes from glucose supplies in adjacent glycogen-rich cells, or from stores in the liver (Chang et al., 2007; Tseng et al., 2007). Changes in cell content of glycogen are reflected by changes in gene expression of the antagonistic cytosolic enzymes, glycogen phosphorylase (GP) and glycogen synthase (GS), which regulate glycogen breakdown and synthesis, respectively (Chang et al., 2007; Tseng et al., 2007).

With changes in the energetic costs of osmoregulation, we would expect responses in the energy-producing mechanisms of MR cells, including those that require the mitochondria and enzymes involved in providing ATP. In mammals, mitochondrial biogenesis is controlled by a group of regulatory transcription factors, including nuclear respiratory factor (NRF)-1 and -2 and their coactivators, peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α and -1β (Puigserver and Spiegelman, 2003; Kelly and Scarpulla, 2004). Coactivators PGC-1α and PGC-1β form complexes with NRF-1 and NRF-2 that regulate the expression of mitochondrial transcriptional factor A (mtTFA), a nucleus-encoded gene with a key role in the replication of mtDNA (Lin et al., 2003; Puigserver and Spiegelman, 2003; Kelly and Scarpulla, 2004). In a few fish species (*Carassius auratus*, *Danio rerio*, and *Gasterosteus aculeatus*), expression of these genes has been studied in response to cold temperatures, exercise, and dietary stress (McClelland et al., 2006; LeMoine et al., 2008; O'Brien, 2010). To our knowledge, however, the effects of salinity changes on gene expression of these putative regulators of mitochondrial biogenesis in fish have not been investigated.

The purpose of this study was to make precise measurements of standard metabolic rate (SMR), to best reflect differences in osmoregulatory costs, in Mozambique tilapia reared in either FW or SW, and after acute transfer to 75% SW or FW, respectively. In addition, we examined any correlations of metabolic rate with the expression of genes involved in ion transport, cellular respiration pathways, and putative regulators of mitochondrial biogenesis, in the gills. We hypothesized that differences in SMR would be positively correlated with the branchial expression of genes involved in elevated metabolism and mitochondrial biogenesis.

2. Materials and methods

2.1. Fish rearing and experimental protocol

Adult male Mozambique tilapia (*O. mossambicus*) were obtained from populations maintained at the Hawai'i Institute of Marine Biology, University of Hawai'i (Kāne'ohe, HI, USA). One group was spawned and reared in FW (14 mOsm kg^{-1}; ~0.2 psu) and had no prior exposure to SW. The other group was spawned and reared in full-strength SW (1042 mOsm kg^{-1}; ~35 psu) and had no prior exposure to FW. The fish in both groups were maintained in outdoor tanks (700 L) with continuous flow of either FW or SW and exposed to natural photoperiod and water temperatures (24–26 °C). Fish were fed ~5% of their body mass daily with Trout Chow (Skretting, Tooele, UT, USA).

The tilapia selected for experiments were unfed during the 24 h prior to introduction to a respirometer for measurement of SMR. The total duration of each experiment was approximately 48 h. Fish were weighed and introduced into the respirometer around mid-day, and initial salinity was the same as rearing conditions. Handling was minimized to reduce additional metabolic expenditure by the fish and the respirometry chamber was covered with black plastic to reduce stress due to visual stimuli. After the first night of oxygen consumption rate measurements in the rearing salinity, some fish (Table 1) were exposed to an acute change in salinity, approximately 24 h after the beginning of each experiment. FW-reared fish were transferred to 75% SW, and not full-strength SW, due to high rates of mortality of adult tilapia observed after direct SW transfer (Morgan et al., 1997). For the transfer from FW to 75% SW (~25 psu), the ratio of FW:SW inflow was adjusted to approximately 1:3 by measuring the salinity of water samples with a handheld refractometer. Complete salinity transfer required approximately 1 h. Control groups (Table 1) were maintained in the original salinity throughout the experiments. Respirometry was then conducted over a second night (post-salinity transfer). Tissue collection occurred after the end of the respirometry measurements the following day, and approximately 24 h after the salinity transfer. All experiments were conducted in accordance with the principles and procedures approved by the Institutional Animal Care and Use Committee, University of Hawai'i.

2.2. Respirometry

Automated intermittent-flow respirometry (Steffensen, 1989) was conducted in indoor tanks (100 L) supplied with air-equilibrated water at a flow-through rate of approximately 2 L min^{-1}. Water temperature was maintained (~25 °C, Table 1) with the use of thermostated submersible heaters. The respirometer was comprised of a submerged, cylindrical, acrylic chamber (1.170 L or 1.993 L, selected according to fish size) connected to a submersible pump that recirculated the water past an optical dissolved oxygen (DO) sensor and a conductivity probe to measure salinity. Water pH was measured with a probe suspended in the water bath. All probes were calibrated prior to experiments according to the manufacturers' specifications, and connected to an Orion 5-Star Portable pH/ORP/ISE/DO/Conductivity Multimeter (Thermo Fisher Scientific Inc., Waltham, MA, USA), which automatically corrected DO content for temperature and salinity. DO, salinity, temperature and pH were continuously recorded (0.2 Hz) to a computer (LabView 2012, National Instruments Inc., Austin, TX, USA). Respirometry measurements consisted of consecutive 10-min recording cycles with alternation between an open, flushing period (4 min) and a closed, recirculating period (6 min). The decline in oxygen for each cycle was measured during the last 5 min.

Only overnight measurements (from 18:00 to 06:00 h), when the fish were least active, were used to calculate standard metabolic rates (SMRs). Post-transfer SMRs (including the control groups) were measured over the second night in the respirometer. To account for background oxygen consumption by microbial activity in the respirometer, blank runs (~1 h) were also conducted both before and after the experiments. Following each experiment, the respirometry setup was cleaned thoroughly to prevent microbial buildup.

Oxygen consumption rates ($\dot{M}O_2$) for each measurement cycle were calculated from the slope of a linear regression fit to the decline in oxygen content over time, using the formula:

$$\dot{M}O_2 = s \times V_{resp}, \quad (1)$$

where s is the slope, and V_{resp} is the volume of the respirometer system minus the volume of the fish (assuming 1 g ≈ 1 mL).