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ORIGINAL PAPER

Salinity-dependent changes in Na^+/K^+-ATPase content of mitochondria-rich cells contribute to differences in thermal tolerance of Mozambique tilapia

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Abstract The Mozambique tilapia (*Oreochromis mossambicus*) is prone to osmoregulatory disturbances when faced with fluctuating ambient temperatures. To investigate the underlying causes of this phenomenon, freshwater (FW)- and seawater (SW)-acclimated tilapia were transferred to 15, 25, or 35°C for 2 weeks, and along with typically used indicators of osmoregulatory status [plasma osmolality and branchial and intestinal specific Na^+, K^+-ATPase (NKA) activity], we used tissue microarrays (TMA) and laser-scanning cytometry (LSC) to characterize the effects of temperature acclimation. Tissue microarrays were stained with fluorescently labeled anti-Na^+, K^+-ATPase antibodies that allowed for the quantification of NKA abundance per unit area within individual branchial mitochondria-rich cells (MRCs) as well as sections of renal tissue. Mitochondria-rich cell counts and estimates of size were carried out for each treatment by the detection of DASPMI fluorescence. The combined analyses showed that SW fish have larger but fewer MRCs that contain more NKA per unit area. After a 2-week acclimation to 15°C tilapia experienced osmotic imbalances in both FW and SW that were likely due to low NKA activity. SW-acclimated fish compensated for the low activity by increasing MRC size and subsequently the concentration of NKA within MRCs. Although there were no signs of osmotic stress in FW-acclimated tilapia at 25°C, there was an increased NKA capacity that was most likely mediated by a higher MRC count. We conclude on the basis of the different responses to temperature acclimation that salinity-induced changes in the NKA concentration of MRCs alter thermal tolerance limits of tilapia.

Keywords Mitochondrial-rich cells · Tilapia · Laser scanning cytometry · Tissue microarray · Na^+ · K^+-ATPase

Abbreviations

TMA	Tissue microarray
LSC	Laser scanning cytometry
NKA	Na^+, K^+-ATPase
MRC	Mitochondria-rich Cell
DAPSMI	Dimethylaminostyrylmethylpyridiniumiodide
$g\ l^{-1}$	Grams per liter
°C	Degrees celcius
FW	Freshwater
SW	Seawater
PI	Propidium iodide

Introduction

We used tissue microarrays (TMA) and laser-scanning cytometry (LSC) to assess the characteristics of mitochondria-rich cells (MRCs) from the Mozambique tilapia (*Oreochromis mossambicus*) acclimated to various salinities and temperatures. Using these novel techniques, we were able to investigate the effects of these stressors on

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MRCs in cell suspension as well as in situ. Originally developed for studies of tumor tissues by clinical pathologists (Bubendorf et al. 2001), TMAs have more recently been used in comparative studies with great success (Lima and Kültz 2004). This approach allowed a high throughput analysis of up to 100 tissues under similar conditions, thereby dramatically reducing sample-to-sample variation. Together, TMA and LSC analyses provide a highly sensitive, efficient method by which we were able to directly address the hypothesis that SW-acclimated fish have a higher Na^+, K^+-ATPase (NKA) concentration within MRCs. Previous studies with tilapia have concluded this on the basis of indirect evidence; van der Heijden et al. (1997) observed that although FW-acclimated tilapia had a higher MRC density, the specific activity of NKA was similar to SW-acclimated tilapia, indicating that MRCs of SW-acclimated fish have a greater concentration of NKA, and thus a greater NKA capacity. Using the TMA technique coupled with measurements of specific NKA activity, we were able to make a direct comparison of the NKA concentration of MRCs between FW and SW-acclimated animals, as well as assess the effects of exposure to a range of temperatures.

Teleosts maintain an internal osmolality that is approximately one-third that of SW, making them hyperosmotic to FW and hypoosmotic to SW. As a result, FW-acclimated fishes have a high renal excretion rate in order to offset the osmotic water gain and also must actively absorb Na^+ and Cl^- across the branchial epithelium at the level of the MRC (Bijvelds et al. 1997; Perry 1997). SW fishes, faced with osmotic water loss and diffusive ion gains, drink SW to offset the water loss and actively absorb ions across the intestinal epithelium to drive water absorption (Wilson et al. 1996, 2002; Wilson and Grosell 2003); the excess ions are then excreted by branchial MRCs as described by Marshall (2002). The Mozambique tilapia is one of the most euryhaline teleosts and can survive salinities as high as 95 to 120 g l^{-1} (Sardella et al. 2004b; Stickney 1986) but previous studies have shown that this salinity tolerance is drastically reduced when ambient temperature fluctuates in both FW (Al Amoudi et al. 1996) and SW (Sardella et al. 2004a; Sardella and Brauner 2007). Osmotic stress resulting from temperature changes has also been shown in juvenile turbot (*Scophthalamus maximus*; Imsland et al. 2003), and Atlantic salmon (*Salmo salar*; Staurnes et al. 2001). Temperature-induced disturbances to osmotic balance have been attributed to reduced enzyme kinetics, altered phospholipid states resulting in a loss of membrane integrity, (Gonzalez and Mcdonald 1994; Handeland et al. 2000; Hochachka and Somero 2002; Johnston and Cheverie 1985), and compromises between osmoregulation and respiration at the gills (Gonzalez and McDonald 1992, 1994).

The purpose of this study was to investigate the effects of temperature acclimation on the characteristics of MRCs in SW- and FW-acclimated Mozambique tilapia. TMAs were used to measure NKA concentration of branchial MRCs, as well as the concentration per unit area within kidney sections, while LSC of MRC suspensions were used to measure cell number and size. This was the first application of these techniques in order to investigate osmoregulatory responses to disturbances resulting from temperature. Along with a classical biochemical approach (specific NKA activity) and an assessment of osmoregulatory status (plasma osmolality, hematocrit), TMAs and LSC analyses provided a detailed assessment of the responses by tilapia to salinity and temperature stresses.

Materials and methods

Fish

Mozambique tilapia (*O. mossambicus*) were reared in non-chlorinated, University of California, Davis well water at the Center for Aquatic Biology and Aquaculture, at 26°C; fish used in this study were 9.80 ± 1.1 g in size. Prior to experiments, approximately 50 tilapia were seawater-acclimated (SW) in two steps; fish were transferred from 0 to 15 g l^{-1} salinity for 4 days, and then from 15 to 33 g l^{-1}, and allowed to acclimate for 14 days. Salinity was manipulated using Instant Ocean synthetic sea salt, and no mortality was observed during SW acclimation.

Sixteen fish were acclimated to each experimental temperature (15, 25, or 35°C) for 14 days in both FW and SW. Fish were fed approximately 1% body mass per day, but food was withheld 24 h prior to sampling. Eight fish from each treatment group were removed and used to make gill cell suspensions for cytometry (see below). The remaining eight fish were sampled and used for all other analyses. During acclimation to 15°C, two FW exposed fish died, resulting in $n = 6$; all other groups were $n = 8$. Fish were killed by overdose of buffered MS-222 (Sigma) and sampled.

Live cell staining

Blood was rinsed and wiped from the gill, and the complete gill epithelium from all arches was scraped into Ca^{2+}/Mg^{2+}-free phosphate-buffered saline (PBS; 146 mM NaCl, 3 mM KCl, 15 mM $NaHPO_4$, 15 mM Na_2HPO_4, 10 mM $NaHCO_3$, pH = 7.4) and dissociated as described by Kültz and Somero (1995). Cell suspensions were stained with the mitochondrial stain dimethylaminostyrylmethylpyridiniumiodide (DASPMI; 10 μM) for 30 min and washed with PBS. After resuspension in 100 μl of PBS, 80 μl was pipetted on to a microscope slide and covered with a 60 × 24 mm^2 cover slip as described by Lima and Kültz

(2004). MRC number and size were then measured using a laser scanning cytometer.

Tissue sampling

Blood was collected into microhematocrit capillary tubes and spun in a Damon IEC MB microhematocrit centrifuge at 11,000*g* for 3 min; hematocrit was then recorded and tubes were cut so the plasma could be collected. Plasma osmolality was immediately measured using a Wescor 5520 vapor-pressure osmometer (Wescor, Logan, UT, USA). Tilapia were dissected and the second and third right gill arches and the anterior intestine, with the contents removed, were frozen in liquid N_2 for use in NKA assays, while left gill arches and whole kidney were fixed in buffered 4% paraformaldehyde for use in TMAs. Unfortunately, there was an insufficient amount of renal tissue to make both TMA and NKA activity measurements; therefore, only TMA data are available for renal tissue.

Specific Na^+, K^+-ATPase activity

Branchial and intestinal tissues were homogenized in 1 ml of SEID buffer (250 mM sucrose, 10 mM EDTA·Na_2, 50 mM imidazole, pH 7.3, deoxycholic acid (0.05%). Specific NKA activity was measured as described by McCormick (1993) and expressed as μmol of ADP per hour per μg total protein. Specific NKA activity was measured at physiological temperatures ($V_{Apparent}$; 15, 25, and 35°C) as well as at 35°C (V_{Max}). Specific activity at physiological temperature represented the level of activity in vivo, while measurements at 35°C provided an estimate of the total amount of functioning NKA present in the gill (total NKA capacity), similar to the methods of van der Heijden et al. (1997).

Tissue microarray construction and immunohistochemistry

Immunohistochemical analysis using TMAs was used to measure the concentration of NKA per unit area within individual branchial MRCs and renal tissue sections. Using the method previously described by Lima and Kültz (2004), renal and branchial tissues were fixed and embedded in paraffin using a Tissue Tek vacuum infiltration processor (Sakura Finetek, Torrance, CA, USA). Paraffin blocks were constructed with a Tissue Tek tissue embedding center (Sakura Finetek, Torrance, CA, USA). Cores 1 mm in diameter were removed from an empty paraffin block using a MTA-1 tissue microarrayer (Beecher Instruments, Sun Prairie WI, USA), and filled with cores of tissue. Tissue cores from all treatment groups were placed within either one gill or one kidney block. Cores of kidney tissue essentially encompassed the entire piece of tissue, so it was not possible to distinguish which individual section was analyzed in the end. Blocks were sectioned at 4 μm thickness using a Bromma 2218 Historange microtome (LKB, Uppsala, Sweden) and sections were floated on to a poly-lysine coated glass microscope slide; slides were dried overnight at 44°C.

Once dry, slides were deparaffinized in xylene for 5 min (3×), 100% EtOH (2×), 95% EtOH (2×), and 80% EtOH (1×). Slides were then incubated in phosphate-buffered saline containing 1% bovine serum albumin (blocking solution) for 30 min, followed by a 60-min incubation in blocking solution containing α5, an anti-NKA purified mouse IgG. Primary antibody against avian Na^+, K^+-ATPase α-subunit developed by Douglas M. Fambrough was obtained from the Developmental Studies Hybridoma Bank instituted under the auspices of the National Institute for Child Health and Human Development and maintained by the University of Iowa, Department of Biological Sciences, Iowa City, IA 52242, USA. The antibody was diluted to a concentration of 6.2 μg/μl in PBS with 1% BSA, and slides were incubated for 60 min. Next the slides were incubated with a secondary goat anti-mouse antibody covalently bound to Pacific Blue (P-10993, Molecular Probes, Eugene, OR, USA) at a final concentration of 20 μg/ml. Slides were rinsed with phosphate-buffered saline and counterstained with propidium iodide.

Laser scanning cytometry

A laser scanning cytometer (Compucyte Cambridge, MA, USA) measured the number of MRCs per fish as well as the average size of MRCs on the basis of detection of DASPMI fluorescence. For live cell suspensions, we used a 20× objective (UPlanFL 20×/0.50/∞/0.17, Olympus). Contouring and event detection variables were set for a minimal area of 50 μm and maximum area of 200 μm to avoid inclusion of accessory cells, cell fragments or artifacts using WinCyte (Compucyte, Cambridge MA, USA) as described by Lima and Kültz (2004).

The laser scanning cytometer was also used to quantify NKA concentration per unit area within individual branchial MRCs and within defined areas of renal tissue on TMAs. Immunohistochemical analysis of TMAs was conducted using a 40× objective (UPlanFL 40×/0.75/∞/0.17, Olympus, Melville, NY, USA) in combination with a UV laser (400 nm). Mitochondria-rich cells were easily detected in the interlamellar regions of the gill due to high intensity of NKA/Pacific Blue fluorescence. Within the renal tubules, individual cells could not be contoured by this method; therefore, defined areas of the tissue section were randomly selected for quantification of NKA. Contouring and event detection for TMAs was optimized as described above for cell suspensions and in Lima and Kültz (2004).

Statistical analysis

We used a two-way analysis of variance (ANOVA) to analyze the effects of temperature and salinity on all variables. Significant two-way ANOVAs were followed by a post hoc Holm–Sidak comparison. All statistical analyses were carried out using Sigma Stat version 3.0, with $\alpha = 0.05$. Values are reported as mean ± SE, $n = 6$ or 8.

Results

Of the 16 fish, 2 died during acclimation to FW at 15°C. Both FW and SW fish exposed to 15°C exhibited little spontaneous activity and FW fish rarely consumed the food that was presented to them within 24 h; however, no overt signs of stress such as discoloration of fins was observed. There was a small but significant difference in hematocrit with salinity (FW = 31.91 ± 0.94%, SW = 35.25 ± 1.22%); however, there were no differences between temperature groups. Plasma osmolality was significantly increased in SW tilapia at 15°C, and significantly reduced in FW tilapia at 15°C relative to values from the remaining treatment groups ($P < 0.001$; Fig. 1).

Branchial NKA activity at physiological temperature ($V_{Apparent}$) was significantly higher in SW-acclimated tilapia versus FW-acclimated tilapia, although the difference was not large ($P < 0.01$). We measured specific NKA activity at both $V_{Apparent}$ and at 35°C (V_{Max}). In FW, V_{Max} of 25°C-acclimated tilapia was significantly higher than in fish acclimated to 15 or 35°C ($P < 0.001$), although $V_{Apparent}$ was similar between 25 and 35°C treatment groups. $V_{Apparent}$ at 15°C was significantly reduced compared to the other treatment groups ($P < 0.001$; Fig. 2a). V_{Max} was significantly increased in SW-acclimated tilapia at 15°C relative to 25 and 35°C treatment groups ($P < 0.001$; Fig. 2b), and $V_{Apparent}$ at 15°C was significantly reduced compared to the other treatment groups ($P < 0.001$; Fig. 2b).

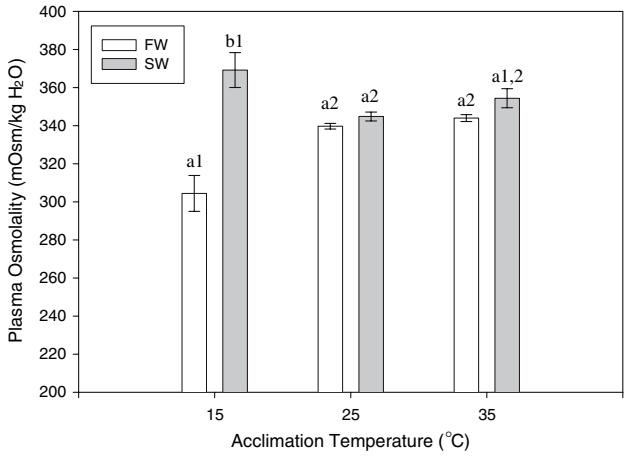

Fig. 1 Plasma osmolality in Mozambique tilapia following 2 weeks of exposure to 15, 25, or 35°C in SW (*gray bars*) and FW (*white bars*). *Letters* that differ indicate significant differences within an acclimation temperature and *numbers* that differ indicate significant differences within an acclimation salinity ($n = 8$)

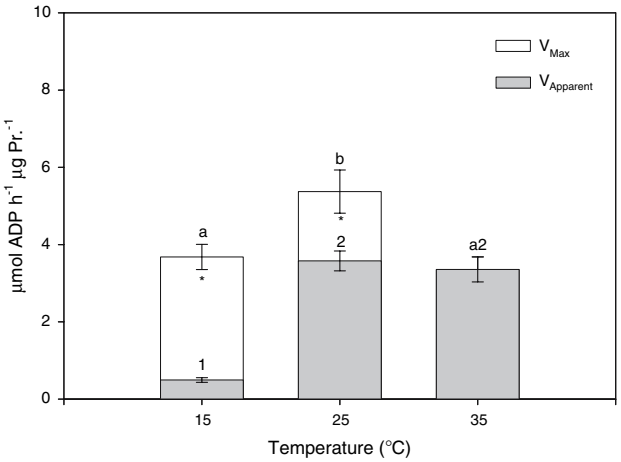

Fig. 2 Branchial NKA activity of FW-acclimated tilapia. *Gray bars* represent NKA activity measured at acclimation temperature ($V_{Apparent}$) and *white bars* represent NKA activity measured at 35°C (V_{Max}). *Numbers* that differ denote significant differences in $V_{Apparent}$ and *letters* that differ denote significant differences within V_{Max} measurements; *Asterisk* denotes differences between $V_{Apparent}$ and V_{Max} at common acclimation temperature ($n = 6$–8). At 35°C, $V_{Apparent}$ is the same as V_{Max} and only 1 bar exists

Intestinal NKA activity at physiological temperature was significantly lower in FW-acclimated tilapia compared with their SW counterparts ($P < 0.01$). In FW-acclimated tilapia at 35°C, V_{Max} was significantly greater relative to the 15 and 25°C treatment groups (Fig. 3a), while in SW-acclimated tilapia, V_{Max} was similar regardless of temperature and $V_{Apparent}$ decreased as acclimation temperature decreased ($P < 0.001$; Fig. 3b).

Mitochondrial-rich cell characteristics

Mitochondria-rich cells on the TMA were easily distinguished from surrounding cells by high intensity of NKA/Pacific Blue fluorescence in the interlamellar region (Fig. 4a). Several hundred signals were detected in each tissue section on the microarray, and average fluorescent integral and area were subsequently plotted (see Lima and Kültz (2004) for more detailed description). Signals with an area less than 50 µm^2 were discounted as they most likely represented either cell fragments or immature MRC forms (e.g., accessory cells). Signals within the limits were counted in our analysis as individual MRCs, and we averaged 111.06 ± 12.1 MRCs per fish. Salinity had a significant effect on the NKA concentration per unit area within MRCs, with SW-acclimated MRCs having more NKA per unit area (Fig. 4b; $P < 0.001$).

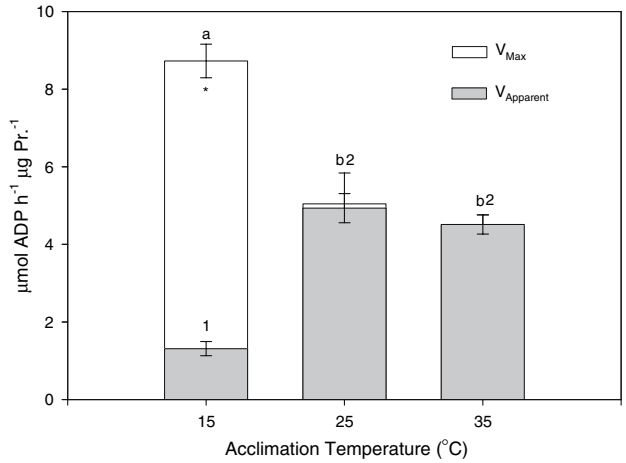

Fig. 3 Branchial NKA activity of SW-acclimated tilapia. See Fig. 2 for details

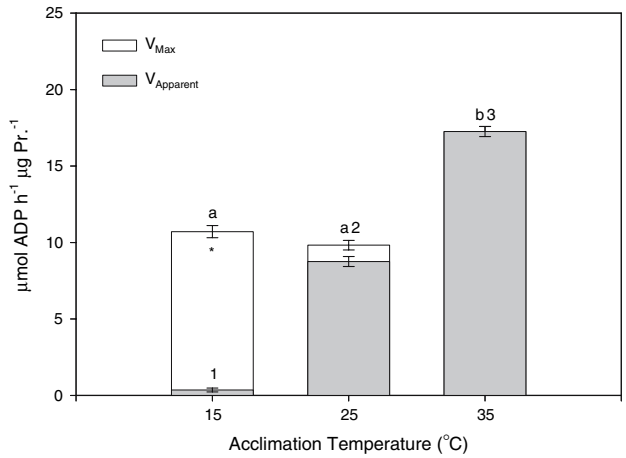

Fig. 4 Intestinal NKA activity of FW-acclimated tilapia. See Fig. 2 for details

Dissociated live MRCs detected by DASPMI fluorescence were counted as individual signals and contoured along their perimeter as described by Lima and Kültz (2004) and as described above for TMA analysis. FW-acclimated tilapia generally had a greater total number of MRCs per fish relative to SW-acclimated tilapia ($P < 0.01$), but there was no effect of temperature on cell numbers (Fig. 5). There were significant effects of salinity and temperature on MRC size ($P < 0.001$ and $P = 0.023$, respectively; Fig. 6). SW MRCs were larger than those from FW tilapia, and tilapia acclimated to 15°C in FW had larger MRCs relative to those in SW at 25 or 35°C (Fig. 6).

Changes in renal NKA

Renal NKA was identified by intense NKA/Pacific Blue fluorescence along the serosal membrane of renal tubules in section from SW-acclimated tilapia (Fig. 7a). Random

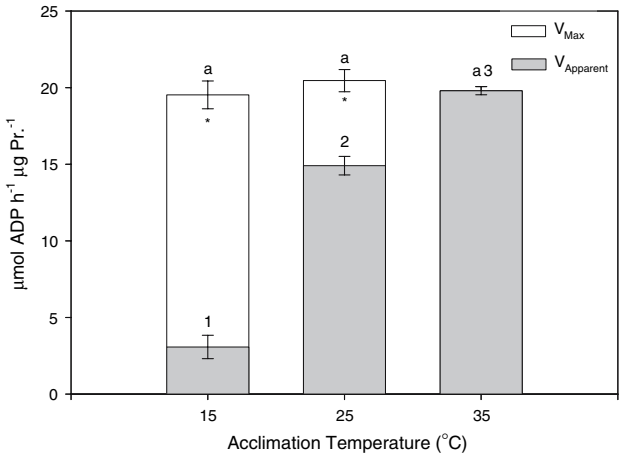

Fig. 5 Intestinal NKA activity of SW-acclimated tilapia. See Fig. 2 for details

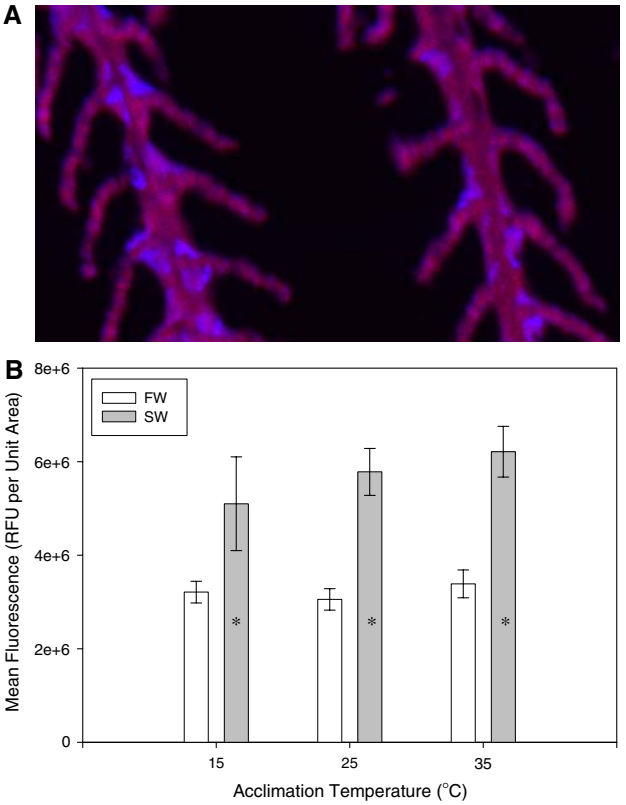

Fig. 6 **a** Mitochondria-rich cells (MRCs) labeled with α5 anti-NKA and Pacific Blue fluorescence at ×40 magnification. **b** The effect of temperature on NKA concentration (relative fluorescence) of MRCs in tilapia exposed to 15, 25, or 35°C in FW and SW, assessed with the α5 antibody using TMA. There was a significant effect of salinity (SW > FW) as denoted by *asterisk* (n = 6–8)

areas referred to as "phantoms" were assigned to scans of renal tissue, within these phantoms, the WinCyte software measured the relative fluorescence of the defined area, similar in method to the measurement within contoured MRCs in the gill scans. SW fish acclimated to 15°C had a

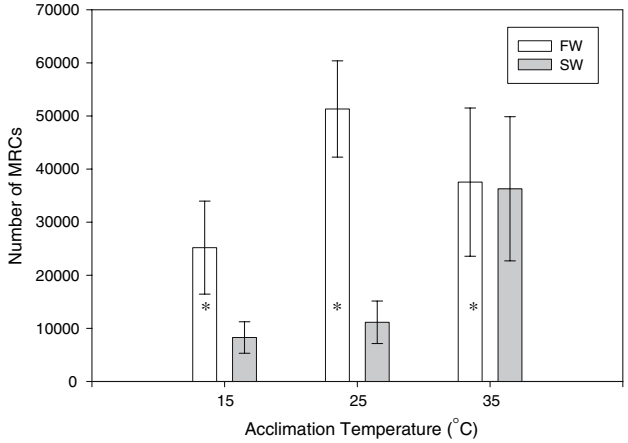

Fig. 7 The effect of temperature on the number of MRCs in tilapia exposed to 15, 25, or 35°C in FW and SW assessed by laser scanning cytometry (LSC) detection of the mitochondrial stain DASPMI. There was a significant effect of salinity (FW > SW) as denoted by *asterisk* (n = 6–8)

significantly greater concentration of NKA per unit area relative to FW fish (P = 0.02; Figs. 7b, 8, 9).

Discussion

Seawater versus freshwater

The use of tissue microarrays (TMA) allowed for a high throughput analysis of many tissues simultaneously, which minimized variation from sample to sample, and measured NKA concentration per unit area within individual MRCs directly. We have shown that the NKA concentration per unit area within mitochondria-rich cells (MRC) was significantly higher in SW-acclimated tilapia relative to FW-acclimated tilapia. To our knowledge, this is the first time that a difference in NKA concentration between SW and FW MRCs has been directly quantified, although TMAs Lima and Kültz (2004) used TMAs and observed an increase in 2.4× SW relative to FW and SW after 4 weeks in *Fundulus heteroclitus*. Previously van der Heijden et al. (1997) showed that SW-acclimated tilapia had equivalent NKA activity compared to FW-acclimated tilapia but fewer total MRCs, implying that SW MRCs had a greater overall concentration of NKA. Our specific NKA activity and cell count data agree with the findings of van der Heijden et al. (1997), but through the use of TMAs we have supported those conclusions with direct measurements.

We observed a slight but significant increase in branchial specific NKA activity between SW and FW-acclimated tilapia, similar to the observations of Dange (1985) and Kültz et al. (1992); however, other studies have shown no difference in activity between the two (van der Heijden

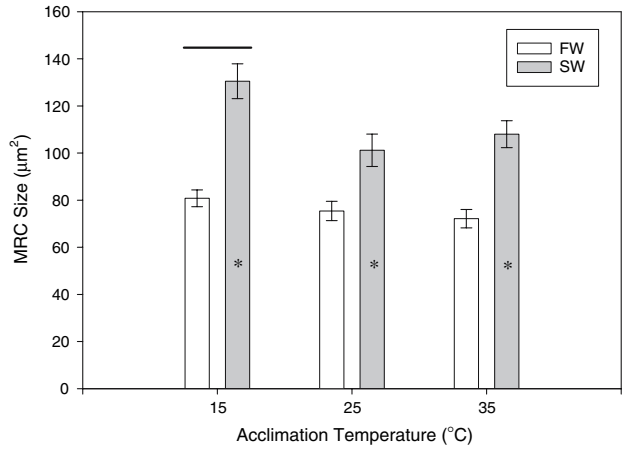

Fig. 8 The effect of temperature on the size (μm^2) of mitochondrial-rich cells in tilapia exposed to 15, 25, or 35°C in FW and SW assessed by LSC detection of the mitochondrial stain DASPMI. SW MRCs were significantly larger than FW MRCs as denoted by *asterisk*, and 15°C-acclimated tilapia had significantly larger MRCs relative to 25 and 35°C as denoted by the *bar* (n = 7)

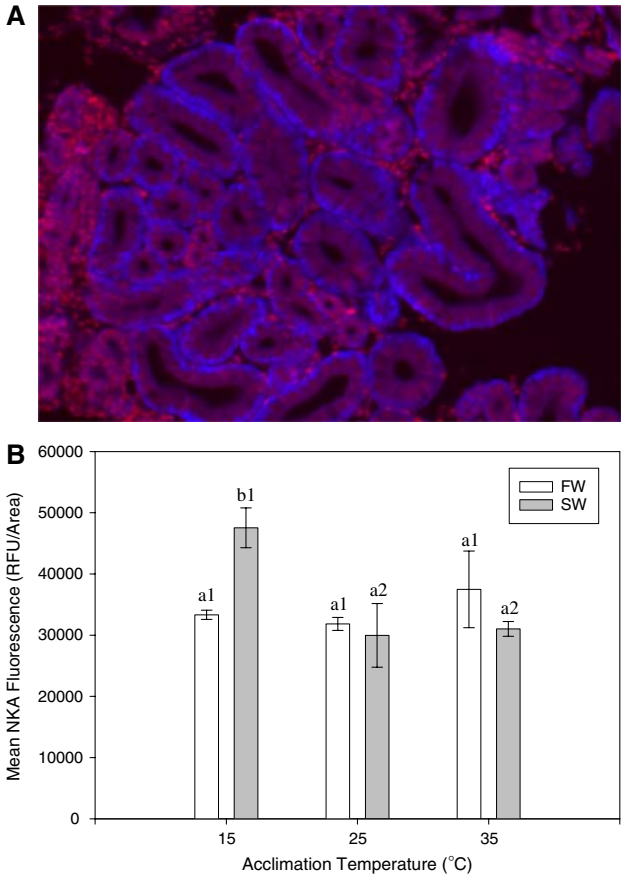

Fig. 9 **a** Renal section labeled with α5 anti-NKA and Pacific Blue fluorescence at ×40 magnification. **b** The effect of temperature on the renal NKA concentration per μm^2 in tilapia exposed to 15, 25, or 35°C in FW and SW, assessed using the α5 antibody on a TMA. Tilapia acclimated to 15°C in SW had a significantly greater NKA concentration relative to all other groups ($P < 0.001$; n = 6–8)

et al. 1997). Intestinal specific NKA activity was greater in SW relative to FW, likely due to the greater role of this enzyme in SW acclimation, where NKA provides the driving force for the absorption of salts across the intestinal wall, which draws water osmotically (Wilson et al. 1996). Furthermore, there is evidence that the osmotic gradient for water absorption from the intestine is further enhanced by the secretion of bicarbonate into the gut lumen and subsequent formation of Ca^{2+} and Mg^{2+} precipitates (Wilson and Grosell 2003; Wilson et al. 2002).

Temperature effects

In 15°C-acclimated tilapia, plasma osmolality was significantly decreased in FW and increased in SW relative to the tilapia acclimated to 25 and 35°C. This may be explained by the marked depression of branchial NKA $V_{Apparent}$ at 15°C, where it was 13.4 and 15.0% of V_{Max} in FW and SW fish, respectively. In addition to low NKA kinetics, reduced membrane fluidity has been observed in fish exposed to lower than optimal temperatures, which can result in loss of cell membrane integrity (Johnston and Cheverie 1985; Robertson and Hazel 1999) and add to osmotic disturbances. Furthermore, it should also be noted that low feeding rate in FW tilapia may contribute to low ion intake and therefore to low plasma osmolality, but this requires further investigation.

MRC size increased when SW tilapia were acclimated to15°C, but the concentration of NKA per unit area in these cells was unchanged, indicating a greater total NKA capacity relative to the other temperature groups; this was also evident by the 73 and 93% increase in V_{Max} relative to 25 and 35°C-acclimated groups, respectively. A similar relationship was observed by Dang et al. (2000) following cortisol administration, where increased MRC size correlated with increased tubular area and NKA activity. Metz et al. (2003) previously observed an increase in cell size and enhanced NKA expression as a result of temperature. Following cold water exposure, the common carp (*Cyprinus carpio*) responded in similar fashion to what was observed with tilapia in our study.

There were physiological changes in response to 15°C acclimation beyond the gill. In the intestine, NKA $V_{Apparent}$ at 15°C was only 3.3 and 15.7% of V_{Max} in FW and SW, respectively. Low NKA activity in the intestine of FW tilapia may have resulted from the low food intake by FW fish. In SW-acclimated fish, where NKA plays a much greater role with respect to osmoregulation, low activity may reduce water absorption (Wilson et al. 1996) and result in further dehydration. Lastly, there was an increase in the abundance of renal NKA per unit area in SW, but not FW-acclimated tilapia at 15°C, indicating an increased role for ion excretion at the kidney under this stressful condition.

Previously, it has been shown in FW that this species' preferred temperature range was from 30 to 35°C (Welch et al. 1989). Our results provide some physiological support for this temperature preference, as 25°C acclimation resulted in a 60% increase in V_{Max} relative to 35°C, while only achieving an identical $V_{Apparent}$. The higher total capacity is likely due to the greater total MRC count in this treatment group. This response may indicate that NKA is functioning at less-than-optimal conditions at 25°C, and that it was required for FW-acclimated tilapia to increase total NKA capacity in order to maintain the necessary activity, and thus osmotic balance, within normal limits. This response was not observed in FW fish at 15°C, where tilapia appeared to be near the point of mortality. In combination with the preference results of Welch et al. (1989), these data indicate that 25°C may be near the lower limits of prolonged tolerance of this species in fresh water, and that this species is unable to tolerate 15°C in FW for extended periods of time. This was not the case with SW-acclimated tilapia, where 25°C-acclimated fish had similar characteristics to 35°C-acclimated fish. Although it does appear from our results that 15°C is at or below the thermal limit for this species, it should be pointed out that this species inhabits both FW and hypersaline water in southern California desert areas, where over-winter water temperatures reach as low as 9°C (Sardella and Brauner 2007; Hurlbert et al. 2007).

In addition to the stressful temperatures that tilapia respond to, there was also a clear distinction between the type of response between FW- and SW-acclimated tilapia, where compensations involve increased MRC count in FW and increased MRC size in SW. These responses fit the current model for MRCs in this species where FW tilapia have a greater number of smaller cells than their SW counterparts.

In summary, we have used both novel and classical approaches to investigate the effects of salinity and temperature on the characteristics of tilapia branchial MRCs. TMA analysis continues to be a beneficial technique in that it dramatically cuts down on sample to sample variation, allows for simultaneous analysis of all treatment groups under similar conditions, and is more efficient than some other forms of analysis (e.g., Western blots). Using these techniques we have further supported previous findings that SW-acclimated Mozambique tilapia had fewer but larger MRCs relative to FW, and directly measured a greater concentration of NKA per unit area within SW- versus FW-acclimated MRCs. Furthermore, tilapia responded to 15°C acclimation in SW by an increase in total NKA capacity of the gill and kidney in order to offset a loss of catalytic efficiency, while in FW capacity was increased at 25°C. We conclude that 15°C and 25°C are at or near the lower limits for acclimation of this species in SW and FW, respectively,

and that the mechanisms of acclimation to less-than-optimal temperatures differ depending on the salinity. It involves increases in MRC size in SW and in MRC number in FW.

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