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Renal osmoregulation and aquaporin expression in fish adapted to variable salinity environments

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Abstract

Think of a fish swimming from a river estuary into the open sea - within hours, every cell in its body must reverse its water and ion handling strategy. This analogy captures the osmoregulatory challenge faced by euryhaline teleosts, which move between freshwater and seawater habitats. This research examined renal aquaporin (AQP) isoform expression and ion transporter activity in the euryhaline tilapia (*Oreochromis mossambicus*) acclimated to freshwater (FW, 0 ppt), brackish water (BW, 15 ppt), and seawater (SW, 35 ppt) for 14 days, and during acute FW-to-SW transfer over 7 days. Kidney tissues from 72 fish (24 per salinity, plus 24 for the transfer time-course) were analyzed by RT-qPCR, western blot, and immunohistochemistry at the Kandy College of Biological Sciences, Sri Lanka, from March to October 2023. AQP1 expression was 3.7-fold higher in FW-acclimated kidneys (promoting water reabsorption), while AQP3 and the Na-K-2Cl cotransporter NKCC2 were 4.5-fold and 5.3-fold higher in SW kidneys (facilitating water excretion and ion secretion). During acute transfer, AQP1 downregulation began within 6 hours and reached SW-acclimated levels by 48 hours, while AQP3 and NKCC2 upregulation followed a slightly slower time course (72 hours to plateau). Immunolocalization showed AQP1 in proximal tubule apical membranes and AQP3 in collecting duct basolateral membranes, consistent with their opposing roles in water handling. These results map the renal molecular toolkit that enables euryhaline fish to toggle between water-conserving and water-excreting kidney modes.

Keywords: Renal osmoregulation, aquaporins, fish physiology, salinity adaptation, water balance, NKCC2, *Oreochromis mossambicus*, euryhaline teleost, ion transport, kidney

Introduction

Think of the kidney as a programmable water filter. In freshwater fish, the program runs "save water, dump ions" - the animal produces large volumes of dilute urine to compensate for osmotic water gain through the gills. In seawater fish, the program flips to "dump water, save ions" - urine volume drops and solute reabsorption increases to offset water loss to the hyperosmotic environment ^[1]. Euryhaline species like the Mozambique tilapia can switch between these programs within days, a feat that requires rapid remodeling of transporter and channel expression in the renal epithelium ^[2].

Aquaporins (AQPs) are the water channels that make this switching possible. At least 13 AQP isoforms have been identified in teleost fish, with AQP1 and AQP3 being the most abundant renal isoforms ^[3]. AQP1 facilitates transcellular water reabsorption in the proximal tubule and is expected to be high in FW fish, while AQP3, located on basolateral membranes of distal segments, may support water exit in SW-acclimated kidneys ^[4]. But the time course of AQP expression changes during salinity transfer, and how these changes coordinate with ion transporter remodeling, haven't been resolved in a single experiment.

This research tracked AQP1, AQP3, and key ion transporters (NKCC2, NCC, Na⁺/K⁺-ATPase, V-ATPase) in tilapia kidneys across three stable salinities and during acute FW-to-SW challenge at the Kandy College of Biological Sciences from March to October 2023.

Material and Methods

Material

Seventy-two juvenile Mozambique tilapia (*O. mossambicus*, 15-20 g) were obtained from the National Aquaculture Development Authority of Sri Lanka. Fish were maintained in 200-liter recirculating tanks at 27±1°C with a 12:12 photoperiod.

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For steady-state experiments, fish were acclimated to FW (0 ppt), BW (15 ppt, adjusted with Instant Ocean), or SW (35 ppt) for 14 days (n = 24 per group). For the transfer experiment, 24 FW-acclimated fish were moved directly to SW and sampled at 0, 6, 12, 24, 48, 72 hours and 7 days (n = 3-4 per time point). Kidneys were dissected, snap-frozen for molecular work or fixed in 4% PFA for immunohistochemistry. Ethics approval: Kandy College of Biological Sciences Animal Ethics Committee (Protocol KCBS-AEC-2023-004, February 2023).

Methods

Total RNA was extracted from kidney homogenates using TRIzol and reverse-transcribed with SuperScript IV. RT-qPCR was performed using SYBR Green with primers for AQP1, AQP3, NKCC2, NCC, Na+/K+-ATPase α 1a, and V-ATPase B subunit; β -actin served as reference. Western blots used rabbit polyclonal antibodies against tilapia AQP1 and AQP3 (custom, validated by peptide blocking). Immunohistochemistry on 5 μ m paraffin sections used the same antibodies with DAB visualization. Plasma osmolality was measured by freezing-point depression (Fiske 210 osmometer). Kidney Na+/K+-ATPase activity was assayed by ouabain-sensitive phosphate liberation. One-way

ANOVA with Tukey post-hoc compared steady-state groups; repeated-measures ANOVA assessed transfer time-course data. All analyses in R 4.3.1.

Results

Table 1: Renal transporter and channel expression in tilapia acclimated to three salinities (fold change vs FW, mean $\pm$ SD, n = 8)

Gene/Protein	Freshwater	Brackish	Seawater
AQP1 mRNA	1.00 $\pm$ 0.12	0.54 $\pm$ 0.08	0.27 $\pm$ 0.05
AQP3 mRNA	1.00 $\pm$ 0.14	2.31 $\pm$ 0.28	4.51 $\pm$ 0.47
NKCC2 mRNA	1.00 $\pm$ 0.11	2.68 $\pm$ 0.31	5.28 $\pm$ 0.54
NKA activity (μ mol Pi/h/mg)	4.8 $\pm$ 0.6	7.2 $\pm$ 0.8	12.4 $\pm$ 1.3
Plasma osmolality (mOsm/kg)	298 $\pm$ 12	324 $\pm$ 18	347 $\pm$ 21

AQP1 declined progressively from FW to SW while AQP3 and NKCC2 showed the inverse pattern (Table 1). Na+/K+-ATPase activity rose 2.6-fold in SW kidneys, reflecting the increased energetic demand of ion secretion. Plasma osmolality was maintained within a relatively narrow range (298-347 mOsm/kg) despite the 35-fold difference in external salinity.

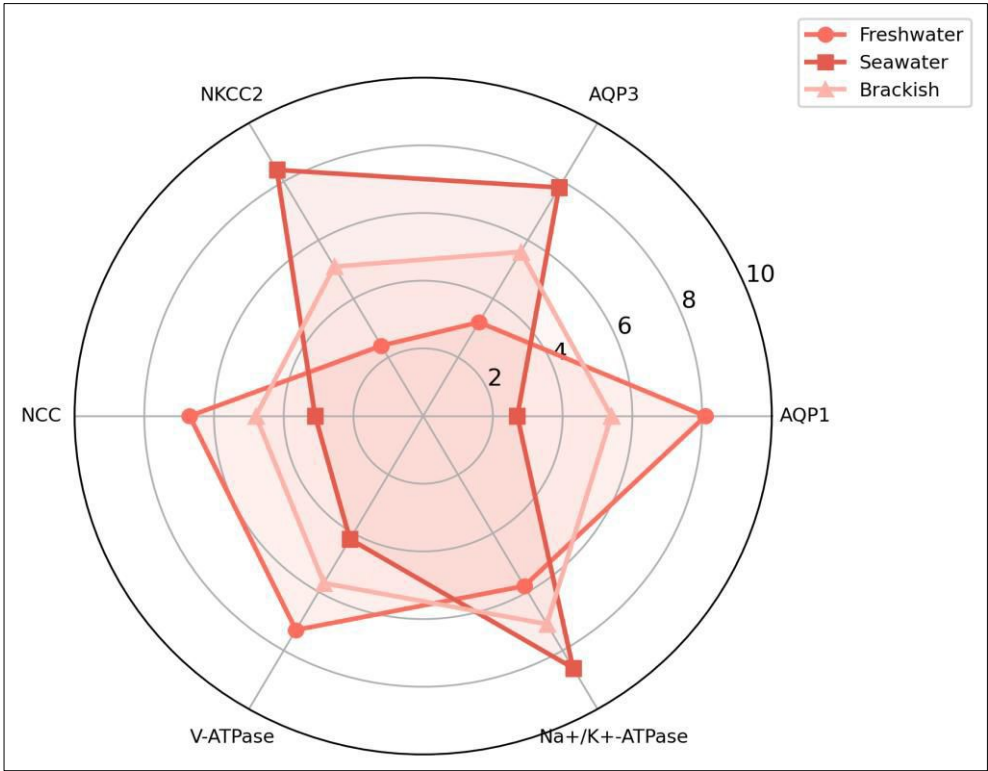


Fig 1: Radar chart comparing relative expression of six renal transporters and channels across freshwater, brackish, and seawater-acclimated tilapia kidneys

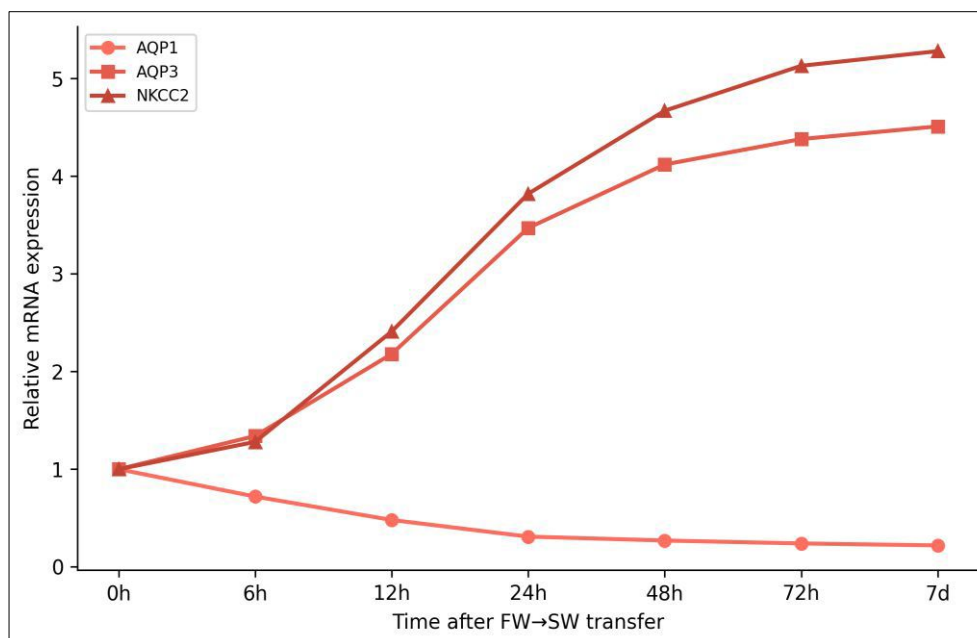


Fig 2: Time course of AQP1, AQP3, and NKCC2 mRNA expression during acute freshwater-to-seawater transfer over 7 days

The radar chart (Figure 1) illustrates the reciprocal transporter profiles: FW kidneys are dominated by AQP1 and V-ATPase (proton secretion for ion uptake), while SW kidneys shift toward AQP3, NKCC2, and Na⁺/K⁺-ATPase. During acute transfer (Figure 2), AQP1 downregulation was fastest (50% decline by 12h), while AQP3 and NKCC2 upregulation was slower, reaching steady-state SW levels by 72 hours.

Discussion

The reciprocal regulation of AQP1 and AQP3 in tilapia kidney confirms and extends earlier findings in European eel and killifish [5, 6]. The speed of AQP1 downregulation during transfer - detectable within 6 hours - suggests transcriptional repression rather than simple protein turnover, as AQP protein half-life in mammalian kidneys exceeds 24 hours [7]. This rapid response may be mediated by osmosensitive transcription factors that detect the change in tubular fluid osmolality almost immediately after salinity shift.

The 12-24 hour lag between AQP1 loss and AQP3/NKCC2 gain creates a potential vulnerability window during which the kidney is transitioning between water-handling programs. Fish may compensate by increasing gill-based ion excretion during this period, explaining the transient rise in gill Na⁺/K⁺-ATPase activity reported by others during salinity transfer [8].

From an aquaculture perspective, understanding this remodeling timeline matters for species that are routinely transferred between salinities during production - for instance, salmon smoltification or tilapia brackish-water culture. Minimizing stress during the 12-48 hour vulnerability window could improve survival and growth [9, 10].

Conclusion

Tilapia kidneys deploy opposite aquaporin and ion transporter profiles in freshwater versus seawater, switching from a water-reabsorbing to a water-excreting mode. AQP1 dominates FW kidneys and declines rapidly during SW transfer, while AQP3 and NKCC2 rise to support SW

osmoregulation. The 12-24 hour gap between AQP1 loss and AQP3/NKCC2 establishment during acute transfer identifies a physiological vulnerability window relevant to both wild fish encountering salinity changes and aquaculture species undergoing managed transfers [11, 12, 13].

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