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Maintaining osmotic balance with an aglomerular kidney

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Abstract

The gulf toadfish, *Opsanus beta*, is a marine teleost fish with an aglomerular kidney that is highly specialized to conserve water. Despite this adaptation, toadfish have the ability to survive when in dilute hypoosmotic seawater environments. The objectives of this study were to determine the joint role of the kidney and intestine in maintaining osmotic and ionic balance and to investigate whether toadfish take advantage of their urea production ability and use urea as an osmolyte. Toadfish were gradually acclimated to different salinities (0.5, 2.5, 5, 10, 15, 22, 33, 50 and 70 ppt (1.5%, 7.5%, 15%, 30%, 45%, 67%, 100%, 151% and 212% seawater)) and muscle tissue, urine, blood and intestinal fluids were analyzed for ion and in some cases urea concentration. The renal and intestinal ionoregulatory processes of toadfish responded to changes in salinity and when gradually acclimated, toadfish maintain a relatively constant plasma osmolality at environmental salinities of 5 to 50 ppt. However, at salinities lower (2.5 ppt) or higher (70 ppt) than this range, a significant deviation from resting plasma and urine osmolality as well as changes in muscle water content was measured, suggesting osmoregulatory difficulties at these salinities. The renal system compensates for dilute seawater by reducing Na^+ reabsorption by the bladder, which allowed excess water to be excreted. In the case of hypersalinity, Na^+ reabsorption was increased, which resulted in a conservation of water and the concentration of Mg^{2+}, Cl^-, SO_4^{2-} and urea. A similar pattern was observed within the gastrointestinal system. Notably, Mg^{2+}, HCO_3^- and SO_4^{2-} were the dominant ions in the intestinal fluid under control and hypersaline conditions due to the absorption of Na^+, Cl^- and water. When exposed to dilute seawater conditions, the absorption of Na^+ was greatly reduced which likely increased water elimination. As a result of decreased environmental levels and a reduction in drinking rate, Mg^{2+} and SO_4^{2-} in intestinal fluids under hypoosmotic conditions were greatly reduced. While urea did play a minor role in renal osmoregulation, toadfish appear to preferentially regulate Na^+ and to some extend Cl^- in urine and intestinal fluids.

Keywords: Toadfish; *Opsanus beta*; Intestine; Osmoregulation; Ionoregulation; Salinity; Hypersalinity; Hyposaline; Hyperosmotic; Hypoosmotic

1. Introduction

Freshwater teleost fish are hyperosmotic to their environment and as a consequence must deal with a constant osmotic influx of water and depletion of salts. To counteract these diffusive gains and losses, NaCl is replaced by active uptake across the gill (reviewed by Evans et al., 2005) and the surplus water is excreted by the kidney (Nishimura and Imai, 1982). To maintain water and salt balance, most freshwater fish have a typical vertebrate kidney nephron that includes a glomerulus, proximal tubule, distal tubule and collecting duct; the glomeruli and distal tubule being largely responsible for the excretion of water (reviewed by Beyenbach, 2004). In contrast to living in freshwater, teleost fish in a marine environment are faced with the opposite dilemma, a diffusive loss of water and a net gain of salts. In this environment, a glomeruli and distal nephron, which function to facilitate the renal excretion of water loads in the freshwater environment, become liabilities in the seawater environment where water is at a premium. Thus, to survive in seawater, glomerular shutdown to some extent becomes necessary for most teleost fish, an adaptation that reaches its pinnacle in approximately 30 fish species that are aglomerular (Lahlou et al., 1969; Beyenbach, 1986; Baustian et al., 1997). Aglomerular fish lack not only the glomeruli but also the distal tubule, dramatically reducing the urinary excretion of water (Hickman and Trump, 1969; Beyenbach, 2004). Urine formation by aglomerular kidneys occurs primarily from tubular secretion of solutes and wastes. Yet despite this rather extreme adaptation, some marine aglomerular fish are able to survive in

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dilute environments (Smith, 1953; reviewed by Beyenbach, 2004) and in doing so, must maintain osmotic balance in freshwater without the water bailing elements of a glomerular kidney.

The gulf toadfish, *Opsanus beta*, is an aglomerular teleost fish found in a predominantly marine environment. Yet, there is some speculation that the gulf toadfish, in addition to its close relative the oyster toadfish, *Opsanus tau*, is euryhaline despite its lack of glomeruli (Lahlou et al., 1969; Wood et al., 2004). In the laboratory, the aglomerular *O. tau* can survive only a few weeks in freshwater but for many months in 10% seawater and does so by increasing the renal excretion of Na^+, Cl^- and SO_4^{2-}, resulting in an elevation in urine flow rate and a reduction of plasma osmolality (Lahlou et al., 1969; Baustian et al., 1997). However, the renal excretion of these solutes contributes to the loss of valuable Na^+ and Cl^- to an already ion-depleting environment. To recover these solutes, NaCl uptake mechanisms are believed to be present in the gill and dietary salt uptake must occur across the intestine (Lahlou et al., 1969; reviewed by Beyenbach, 2004).

However, there may be factors that to date have either been underestimated or overlooked that could potentially allow some aglomerular toadfish to survive in a dilute environment better than others. One factor is the intestine, which not only plays a role in dietary salt and nutrient uptake but is directly involved in osmoregulation of fish in saline and hypersaline environments (Grosell et al., 2001; Grosell and Wilson, 2002; Wilson et al., 2002; Wilson and Grosell, 2003; Wood et al., 2004). However, the intestine may also play an important role in the osmoregulation of an aglomerular teleost in a dilute environment. Recent evidence has shown that the teleost intestine is capable of fluid secretion (Marshall et al., 2002), which may aid water balance in aglomerular fish in low salinity environments.

Another factor is urea, a nitrogenous waste produced and excreted mostly by land dwelling animals and elasmobranchs. Similar to these organisms, toadfish are one of a small number of teleost fish that have the ability to produce urea via a fully functional ornithine urea cycle (for others refer to Saha and Ratha, 1987; Randall et al., 1989; Wood et al., 1989, 1994). Almost all other aquatic organisms do not produce urea and instead excrete their waste nitrogen as ammonia, despite its high toxicity, because its high solubility allows it to be easily excreted at low concentrations into the surrounding aqueous environment (reviewed by Wood, 1993). While urea is most commonly considered the less toxic nitrogenous waste, it has many other functions (Withers, 1998). In addition to roles in buoyancy (Withers et al., 1994) and acid–base balance (Wood et al., 1995b; Wood, 2002), urea acts as a major osmolyte, contributing to the osmoconformity documented in marine elasmobranchs (Smith, 1936; Goldstein and Forster, 1971) as well as the renal medullary osmotic gradient in the mammalian kidney. In the context of the aglomerular gulf toadfish in the wake of lowered environmental salinity, could urea act as an osmolyte to facilitate the removal of excess water, minimizing the loss of valuable salts? Furthermore, when in high environmental salinties, could urea retention help alleviate water loss?

To answer these questions, the concentration of urea in the plasma and bladder urine as well as ionic composition of these two fluids were measured in toadfish acclimated to hyperosmotic and hypoosmotic environments. To evaluate whether the intestine of the aglomerular toadfish plays a role in osmoregulation in a hypoosmotic environment and to confirm the intestinal role in osmoregulation in a hyperosmotic environment, intestinal fluids were sampled from fish acclimated to a range of salinities and ionic composition was measured. In addition, the composition of muscle tissue as a representative of intracellular compartments was measured to determine the potentially increased osmoregulatory role that this tissue may have in the capability of an aglomerular fish to adapt to extreme environments.

2. Materials and methods

2.1. Experimental animals

Gulf toadfish (*O. beta*; 50 ± 3 (70) g ranging from 15 to 123 g) were captured with a roller trawl by commercial shrimpers in Biscayne Bay, Florida in the winter of 2002–2003. The toadfish were held in an outdoor tank at the shrimpers' holding facility with running sea water (ambient seasonal conditions) for no longer than 24 h following capture. Fish were treated with a waterborne dose of malachite green (final concentration $0.05\ mg \cdot L^{-1}$) in formalin ($15\ mg \cdot L^{-1}$) (AquaVet, Hayward, CA, USA) on the day of transfer to the laboratory to prevent infection by the cilate, *Cryptocaryon irritans* (Stoskopf, 1993). Before the experiment, fish were held in 50 L glass aquaria with flowing, aerated seawater and water temperature was 24–26 °C. Fish were fed weekly with squid.

2.2. Experimental protocol

2.2.1. Acclimation to hyposalinity

Gulf toadfish ($N = 56$) were separated into two 80 L tanks filled with 33 ppt (100%) seawater and held at static conditions. Debris was siphoned from the tanks and a 75% water change was performed daily. After one week (Day 7) of acclimation at 33 ppt, equal numbers of toadfish were removed from each tank and tissues and fluids were collected as described below. Immediately after sampling, the salinity of the water was reduced by dilution with reverse osmosis water and the remaining toadfish ($N = 50$) were acclimated to 22 ppt (67% SW) for one week. Sampling and salinity reduction continued on a weekly basis over the course of six weeks. Thus, toadfish ($N = 6$) were sampled on Day 14 after acclimation to 22 ppt for one week, on Day 21 acclimated to 15 ppt (45% SW; $N = 6$), on Day 28 acclimated to 10 ppt (30% SW; $N = 8$), on Day 35 acclimated to 5 ppt (15% SW; $N = 8$), on Day 42 acclimated to 2.5 ppt (7.5% SW; $N = 8$) and on Day 49 acclimated to 0.5 ppt (1.5% SW; no fish survived one week at this salinity). Consequently, the fish acclimated to the lowest salinity (0.5 ppt) had been experiencing gradual salinity reduction for six weeks. Fish were fed squid weekly to satiation on Day 3, 10, 17, 24, 31, 38 and 45.