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The effect of salinity on the requirement for potassium by barramundi (*Lates calcarifer*) in saline groundwater

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ABSTRACT

Significant international interest exists in utilising inland saline groundwater sources for mariculture; however potassium deficiency is a factor that may limit their use. In this study we investigated the effects of potassium supplementation between 25% and 100% of that found in equivalent salinity seawater on the growth, survival and physiological response of barramundi (*Lates calcarifer*) at hyperosmotic (45 ppt), near-isosmotic (15 ppt) and hyposmotic (5 ppt) salinities. A K-equivalence of 25% was not tested at 45 ppt because it caused mortality of barramundi in a previous study. Fish reared in 50% K-equivalence water at this salinity survived for four weeks but lost weight; whereas at 75% and 100% K-equivalences fish both survived and gained weight. Homeostasis of blood plasma potassium in these fish was maintained by buffering from skeletal muscle. That these fish exhibited muscle dehydration, increased branchial, renal and intestinal (Na^+–K^+)ATPase activity and elevated blood sodium and chloride suggests they were experiencing osmotic stress. At 15 ppt, equal rates of growth were obtained between all K-equivalence treatments. Buffering of plasma potassium by muscle also occurred at the two lowest levels of supplementation but appeared to be in a state of equilibrium. Barramundi at 5 ppt displayed equal growth among treatments. At this salinity, buffering of plasma potassium from muscle did not occur and at 25% K-equivalence blood potassium was significantly lower than at all other K-equivalence treatments but with no apparent effect on growth, survival or (Na^+–K^+)ATPase activities. These data show that proportionally more potassium is required at hyperosmotic salinities compared to iso- and hypo-osmotic salinities and also demonstrate that barramundi have a lower requirement for potassium than other species investigated for culture in inland saline groundwater.

1. Introduction

Secondary salinity affects over 380 million hectares of land in over 20 countries worldwide (Ghassemi et al., 1995; Lambers, 2003). Countries including Australia, China, India, Israel and the USA have a demonstrated interest in utilising their affected land and water resources for commercial mariculture (Ron et al., 2002; McNevin et al., 2004; Zhu et al., 2004; Barman et al., 2005; Partridge et al., 2008). In Australia, over 60% of saline groundwater sources range from 5 to 45 ppt, a range suitable for the culture of many euryhaline species (Partridge et al., 2008).

Although the ionic composition of saline groundwater generally reflects that of seawater, the exact composition varies both locally and regionally. This variability relates to the nature and timing of recharge and the nature of the weathered material between the soil surface and bedrock (George, 1990). One factor, however, that appears consistent worldwide is a deficiency of potassium, relative to equivalent salinity seawater (Fielder et al., 2001; Partridge and Furey, 2002; Saoud et al., 2003; Zhu et al., 2004; Shakeeb-Ur-Rahman et al., 2005). This deficiency is primarily caused by the fact that potassium is preferentially taken up by cation exchange sites in clay soils (Stumm and Morgan, 1996). Saline groundwater can contain as little as 5% of the potassium found in equivalent salinity seawater (i.e. K-equivalence) (Fielder et al., 2001) to as high as 75% K-equivalence (Partridge and Furey, 2002); however, in a review of saline groundwater sources, Partridge et al. (2008) reported that most of those assessed for mariculture contain approximately 20% K-equivalence.

As with all animals, potassium is the most abundant intracellular ion in fish and plays many important physiological roles including the maintenance of cellular volume and membrane potentials and the generation of nerve impulses (Epstein et al., 1980; McDonough et al., 2002). In fish, potassium plays additional critical roles in osmo- and iono-regulation and acid/base balance (Marshall and Bryson, 1998; Evans et al., 2005).

Barramundi (*Lates calcarifer*) tolerates salinities from freshwater (Rasmussen, 1991) to at least 55 ppt (Shirgur and Siddiqui, 1998) and has been identified as a suitable species for inland saline aquaculture in both Australia (Partridge et al., 2008) and India (Jain et al., 2006).

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Partridge and Creeper (2004) found that barramundi grown in a hyperosmotic (45 ppt) groundwater source with 25% K-equivalence, had elevated levels of sodium and chloride in the blood plasma and reduced potassium levels in the muscle, compared to fish grown in seawater of equivalent salinity. The buffering of blood plasma potassium with that from skeletal muscle was unsustainable, leading to death caused by severe muscle myopathy. These authors suggested that the physiological effects of potassium deficiency are dependent on salinity (whether hyperosmotic, isosmotic or hyposmotic to blood plasma) and that they would be ameliorated by potassium supplementation. In this paper, we test these predictions by measuring the survival, growth and physiological responses of barramundi at various potassium concentrations at salinities of 45, 15 and 5 ppt.

2. Methods

2.1. Experimental design

Three bioassays were conducted with a range of potassium supplementation levels outlined in Table 1.

Saline groundwater was collected from a groundwater interception scheme located on a conservation reserve 250 km east of Perth, Western Australia, and trucked to the Aquaculture Development Unit (ADU) in Fremantle. The concentrations of the eight most abundant ions found in seawater were measured in the filtered groundwater using inductively coupled plasma atomic emission spectroscopy (ICPAES). The concentration of chloride ions was determined via flow injection analysis using a method modified from APHA (2005).

The groundwater used in bioassay 1 was undiluted (45 ppt) whereas that in bioassays 2 and 3 was diluted from 45 to 15 and 5 ppt, respectively, with dechlorinated tap water. Three rates of potassium supplementation were tested in bioassay 1, ranging from 50 to 100% K-equivalence (Table 1). Unsupplemented groundwater (25% K-equivalence) was not included in this bioassay, because it caused mortality of barramundi at this salinity (45 ppt) in a previous bioassay (Partridge and Creeper, 2004). In bioassays 2 and 3, four levels of potassium supplementation, from 25 to 100%, were tested. All required potassium supplementation rates were obtained by addition of potassium chloride (Potash, technical grade 96%), with the resulting concentrations of the two elements confirmed as previously described. The additions of potassium chloride resulted in chloride concentrations increasing by no more than 1.68% compared to the unsupplemented treatments.

2.2. Measurement

Treatments in each bioassay were tested in triplicate in 180 L tanks over a period of four weeks. All tanks were held within a water bath maintained at 26 °C, and each tank operated as an independent recirculating system with water continuously airlifted through a mechanical and biological filter. Five juvenile barramundi (average weight ± SE; 41.1 ± 1.5 g, 52.6 ± 1.0 g and 38.8 ± 0.5 g, for bioassays 1, 2 and 3, respectively) were stocked into each experimental tank after a three-day acclimation period from seawater to the salinity of the water source under investigation. Fish were fed twice daily to satiety on a commercial fish diet (45% protein, 22% lipid, Skretting Australia, Rosny Park, Australia). The bottom of each tank was vacuumed three times each week and 10% of the water volume replaced. Temperature, pH, dissolved oxygen and total ammonia nitrogen were measured daily in each tank. pH was maintained above 7.5 by the addition of sodium bicarbonate as required.

Table 1
Salinity and K-equivalence treatments investigated in bioassays 1, 2 and 3

Bioassay	Salinity (ppt)	K-equivalence	Potassium (mg/L)
1	45	50%	267
		75%	401
		100%	534
2	15	25%	45
		50%	89
		75%	134
		100%	178
3	5	25%	15
		50%	30
		75%	45
		100%	59

At the end of each trial, fish were anaesthetised (40 mg/L AQUI-S) and weighed to 0.1 g. Blood was taken from the caudal vessel of a subsample of three fish in each replicate and pooled for the determination of plasma sodium, potassium and chloride using a Vetlyte ion-specific electrode analyser (IDEXX Laboratories, Maine USA). Dorsal muscle was also taken from two fish per tank and pooled. This sample was freeze-dried to determine water content and then ground. After grinding, the samples were digested with a combination of concentrated nitric acid, hydrogen peroxide and hydrochloric acid at 120°C according to McDaniel (1991). The potassium and sodium contents of the digest were determined via ICPAES and compared against reference materials of dogfish muscle (DORM2) and liver (DOLT2) (National Research Council of Canada).

Subsamples of fish from each tank were preserved in 10% formalin in seawater at the completion of each bioassay. Para-sagittal slab sections of these fish were decalcified in 10% formic acid for 6 h, vacuum embedded in paraffin, and 5 μm sections stained with haematoxylin and eosin (H&E). Sections of muscle and kidney were examined for the presence of the muscle and renal pathologies described by Partridge and Creeper (2004).

Activities of (Na^+–K^+)ATPase (NKA) in gills, kidney and intestines were determined at the end of each trial according to Zaugg (1982). Samples of tissue (50–100 mg) were thoroughly rinsed with and then frozen in 1 mL SEI buffer (0.3 M sucrose, 20 mM Na_2EDTA, 0.1 M, pH 7.1) at −80 °C. Within one week of collection, semi-purified homogenates were prepared by homogenising (Heidolph, Diax 600) thawed samples for 10 s before centrifuging at 2000 G for 7 min at 4 °C. The supernatant was discarded and the pellet resuspended in 0.5 mL of SEID (SEI with 0.1 g/L sodium deoxycholate) before homogenising again for 30 s. After a further centrifugation step of 6 min, supernatants were collected for enzyme activity and protein determination (Bradford, 1976). Enzyme activities were measured by incubating a 10 μL aliquot of semi-purified homogenate in 600 μL of either a salt solution containing potassium (KCl 50 mM, NaCl 155 mM, $MgCl_2{\cdot}6H_2O$ 23 mM, Imidazole 115 mM, pH 7.0), or the same solution without potassium and 1.67 mM of ouabain (an NKA inhibitor) for 10 min at 37 °C in the presence of 100 μL of an ATP solution (30 mM Na_2ATP, pH 7.0). After termination of the reaction by cooling, phosphate was transferred into an octanol phase, complexed with ammonium molybdate reagent and then quantified at 340 nm (Beckman Coulter DTX 880). Enzyme activities were expressed as μmol of phosphate liberated per milligram of protein per hour.

2.3. Data analysis

Growth was expressed as specific growth rate (SGR) using the following equation:

$$\mathrm{SGR}\ (\%/\mathrm{day}) = \left(\frac{\mathrm{Ln(Wf)} - \mathrm{Ln(Wi)}}{\mathrm{Time(days)}}\right) \times 100$$

Where Wf and Wi were the final and initial wet weights of the fish, averaged over all fish in a replicate. Specific growth rates, plasma electrolyte concentrations, muscle ionic and water contents, and NKA activities were compared between treatments using one-way analysis of variance (ANOVA), with a post hoc comparison of group means using Tukey's HSD test. Data were checked for heterogeneity and normality prior to analysis and transformed, if necessary. All presented