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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

measurements are means ± standard error and statements of statistical significance refer to the 0.05 level unless otherwise stated.

3. Results

3.1. Water ionic composition

A comparison of the ionic composition of the filtered, undiluted groundwater with equivalent salinity seawater is given in Table 2. Compared to seawater, groundwater was deficient in potassium, boron and sodium, and had greater concentrations of magnesium, calcium, sulphur, chloride and strontium.

3.2. Survival and muscle and renal histology

Survival of barramundi in all treatments in all bioassays was 100%. Histological examination revealed that no fish in any of the treatments suffered from the degeneration and necrosis of skeletal muscle or the renal tubular necrosis described by Partridge and Creeper (2004).

3.3. Growth

At a salinity of 45 ppt, there was an increase in SGR of barramundi with increasing levels of potassium supplementation (Fig. 1A). At this salinity, the lowest level of potassium supplementation (50% K-equivalence) resulted in barramundi losing weight over the 4 week experimental period (SGR = −0.38 ± 0.27%/day). At a salinity of 15 ppt, the SGR of barramundi ranged from 1.86 to 2.18%/day with no significant differences among the four treatments (Fig. 1B), while at a salinity of 5 ppt, SGR ranged from 2.30 to 2.62%/day, again with no significant differences among treatments (Fig. 1C).

3.4. Blood ionic composition

At a salinity of 45 ppt, the blood plasma concentrations of sodium and chloride from fish cultured in 50% K-equivalence were both significantly greater than the corresponding plasma electrolytes from fish cultured in 100% K-equivalence, while there were no significant differences between treatments in blood plasma concentrations of potassium (Table 3). At salinities of 15 ppt and 5 ppt there were no significant differences among treatments in the blood plasma concentrations of either sodium or chloride. The blood plasma concentration of potassium also did not differ among treatments at a salinity of 15 ppt, but those fish grown in 5 ppt water with the lowest rate of potassium supplementation (25%) had a significantly lower plasma potassium concentration than those grown in both 75% and 100% K-equivalence (Table 3).

3.5. Muscle water, potassium and sodium contents

At a salinity of 45 ppt, those fish cultured at 50% K-equivalence had a significantly lower water content and muscle potassium concentration, but a significantly greater muscle sodium concentration than those cultured at 75% and 100% K-equivalence (Table 3). At a salinity of 15 ppt, there were no significant differences in muscle water content between treatments, but muscle potassium and sodium concentrations showed the same trend as at 45 ppt. The muscle potassium concentrations of fish cultured at 25% and 50% K-equivalence were significantly less than those of fish grown at 75% and 100% K-equivalence, while the muscle sodium concentrations of fish in 25% K-equivalence and 50% K-equivalence were significantly greater than those of fish grown in 100% K-equivalence (Table 3). At a salinity of 5 ppt, there were no significant differences between treatments in either muscle water content, potassium concentration or sodium concentration (Table 3).

Table 2
Concentrations of major (>1 mg/L) ions in saline groundwater, seawater of equivalent salinity to groundwater (45 ppt), and the percentage difference in ionic concentrations between these two water sources

		Goundwater	Seawater at 45 ppt	Groundwater relative to 45 ppt seawater (%)
Major elements (mg/L)	Cl	25,000	23,750	105%
	Na	12,000	13,750	87%
	Mg	2800	1500	187%
	S	1400	1063	132%
	Ca	740	550	135%
	K	140	613	23%
	Sr	9.3	9	102%
	B	1.2	5	23%

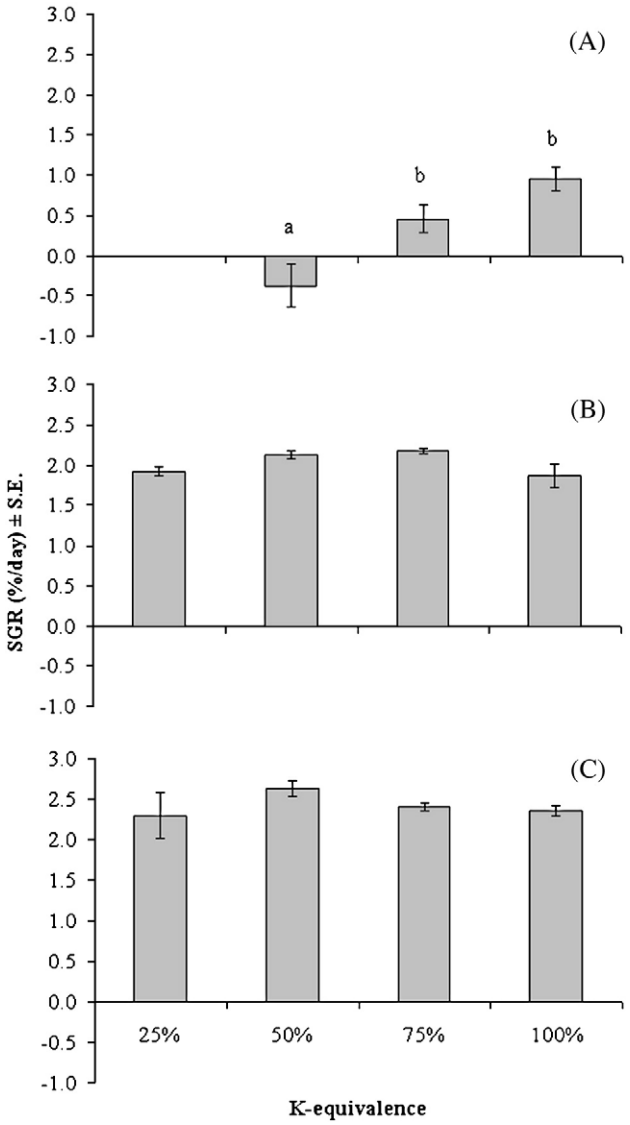

Fig. 1. Growth of barramundi cultured at various K-equivalence values in (A) 45 ppt, (B) 15 ppt and (C) 5 ppt for 4 weeks. Columns within salinity sharing the same letter are not significantly different ($P > 0.05$).

3.6. NKA activity

At a salinity of 45 ppt, those fish cultured in the lowest level of potassium supplementation (50% K-equivalence) showed a significantly higher activity of NKA in gills, kidney and intestine than those cultured in 75% or 100% K-equivalence (Table 4). At 15 ppt, there were no significant differences in the branchial or renal NKA activities

Table 3
Plasma and muscle electrolyte concentrations and muscle water contents from barramundi grown at various salinities and K-equivalence values for 4 weeks

Salinity (ppt)	K-equivalence (%)	Plasma K (mmol/L)	Plasma Na (mmol/L)	Plasma Cl (mmol/L)	Muscle water (%)	Muscle K (g/kg)	Muscle Na (g/kg)
45	50%	10.6±2.4	182±3[a]	177±2[a]	72.4±1.1[a]	11.7±0.7[a]	5.7±1.1[a]
	75%	14.8±0.2	171±2[a,b]	171±2[a,b]	77.7±0.6[b]	15.5±0.3[b]	2.2±0.2[b]
	100%	12.1±2.7	167±2[b,c]	164±1[b,c]	76.6±0.6[b]	15.8±0.2[b]	2.4±0.2[b]
15	25%	6.2±0.3	162±0	140±1	87.3±0.9	12.7±1.3[a]	5.8±0.8[a]
	50%	5.4±0.8	171±3	146±3	86.8±0.5	15.3±1.3[b]	3.8±0.3[b]
	75%	5.4±0.7	165±1	142±1	87.1±0.2	18.5±0.3[c]	2.3±0.2[b,c]
	100%	7.5±1.0	162±2	142±2	86.4±0.3	18.3±0.3[c]	2.1±0.1[c]
5	25%	3.9±0.1[a]	161±1	137±1	76.6±0.7	17.0±0.4	1.6±0.1
	50%	4.3±0.3[a,b]	161±2	135±0	77.2±0.5	17.9±0.4	1.6±0.1
	75%	5.0±0.1[b]	159±2	137±1	76.1±0.2	17.2±0.2	1.5±0.1
	100%	4.9±0.1[b]	161±1	139±1	76.1±0.1	16.8±0.1	1.5±0.0

Parameter values within each salinity sharing the same letter are not significantly different ($P>0.05$).

between treatments, but intestinal NKA activities from those fish in 25% and 75% K-equivalence were significantly greater than those at 50% and 100% K-equivalence (Table 4). At a salinity of 5 ppt, there were no significant differences in branchial, renal or intestinal NKA activities between any treatments (Table 4).

4. Discussion

In a previous bioassay, juvenile barramundi grown in hyperosmotic (45 ppt) groundwater with a K-equivalence of 25%, experienced hypernatraemia and hyperchloridaemia of blood plasma (Partridge and Creeper, 2004). Homeostasis of blood potassium was maintained via the unsustainable buffering of potassium from skeletal muscle which, within nine days, resulted in death caused by severe degeneration and necrosis of skeletal muscle (Partridge and Creeper, 2004). It was therefore hypothesised that proportionally more potassium will be required by barramundi in hyperosmotic salinities compared with iso- or hypo-osmotic salinities. The results of the current study support this hypothesis. Although the groundwater used in this study was elevated in magnesium compared with seawater (187% equivalence), we believe this was not a major contributor to the observed results, based on the positive response to potassium supplementation. In addition, red drum (*Sciaenops ocellatus*) cultured in saline water with 241% Mg-equivalence displayed no deleterious effects (Forsberg et al., 1996) and Wurts and Stickney (1989) also demonstrated that magnesium was not important for growth or survival of red drum in seawater.

4.1. Response to potassium supplementation at hyperosmotic salinity

At a salinity of 45 ppt, the lowest level of potassium supplementation (50% K-equivalence) was effective in preventing the terminal potassium depletion myopathy described by Partridge and Creeper (2004). These fish, however, lost weight over the 4-week experimental period and, compared to fish cultured at 75% and 100% K-equivalence, they had reduced muscle water and potassium content, increased muscle sodium content, elevated levels of plasma sodium and chloride (but not potassium) and increased branchial, renal and intestinal NKA activities.

It has been shown that the gills of fish in seawater are permeable to potassium (Kirschner et al., 1974) and that efflux is greater than influx (Maetz, 1969; Sanders and Kirschner, 1983). If potassium efflux accompanies the elimination of sodium through the leaky junctions between chloride and accessory cells as suggested by Kirschner et al. (1974), then it must be primarily driven by the trans-epithelium potential difference generated by the active extrusion of chloride (Evans et al., 1999), rather than an osmotic or chemical gradient for potassium. This is supported by Sanders and Kirschner (1983), who demonstrated that potassium efflux is not affected by its external concentration and by Kirschner et al.'s (1974) suggestion that potassium concentrations (in blood or seawater) are not major determinants of trans-epithelial potential difference. This would indicate that reduced uptake, rather than increased loss of potassium, is the more important factor contributing to the poor performance of fish in a 50% K-equivalence medium.

Drinking by teleosts in a hyperosmotic environment is essential to offset osmotic water loss (Marshall and Grosell, 2006). The significantly lower muscle water content of fish in the lowest level of potassium supplementation may suggest that the low potassium concentration of the imbibed seawater impedes the desalination and subsequent water uptake processes described by Ando et al. (2003) and Loretz (2001). This hypothesis is supported by Musch et al. (1982) who demonstrated that the removal of potassium from the intestinal luminal fluid of winter flounder (*Pseudopleuronectes americanus*) impeded monovalent ion uptake via the Na-K-2Cl cotransporter (NKCC). The significantly greater intestinal NKA activity at 50% K-equivalence would also be consistent with an attempt to increase potassium and water uptake by increasing the transmembrane electrochemical gradient for sodium, thereby allowing increased potassium uptake via NKCC (Loretz, 1995). The increased renal NKA activity seen in fish held at 50% K-equivalence may have also been an attempt to increase water retention via the solute-linked water transport system (Nebel et al., 2005).

The hypernatraemia and hyperchloridaemia of blood plasma in fish cultured in hyperosmotic water with 50% K-equivalence are consistent with Partridge and Creeper's (2004) findings with barramundi grown in 25% K-equivalence at the same salinity. We suggest that these increased electrolyte levels are due to elevated intestinal and renal NKA activities, which increase uptake of sodium and chloride from intestinal fluids and urine. The elevated branchial NKA activity seen in these fish is the expected response to ameliorate the elevated plasma sodium and chloride concentrations caused by the increased NKA activity in other epithelia. Under conditions of

Table 4
(Na^+–K^+)ATPase (NKA) activities (μmol/mg/h) of gills, kidney and intestines from barramundi cultured at various salinities and K-equivalence values for 4 weeks

Salinity (ppt)	K-equivalence (%)	Gills (μmol/mg/h)	Kidney (μmol/mg/h)	Intestine (μmol/mg/h)
45	50%	201.1±44.4[a]	260.9±51.8[a]	68.6±5.2[a]
	75%	88.8±4.8[b]	98.8±9.1[b]	29.6±3.4[b]
	100%	107.9±11.6[b]	66.8±9.4[b]	26.4±5.5[b]
15	25%	22.4±1.2	55.4±8.7	21.5±1.6[a]
	50%	25.7±2.3	43.7±4.1	13.0±1.7[b]
	75%	24.6±3.3	54.6±7.6	19.7±1.2[a]
	100%	17.2±2.0	48.6±10.6	13.0±1.5[b]
5	25%	29.0±3.6	33.8±8.6	12.4±1.8
	50%	24.4±2.1	5.44±6.2	16.7±2.3
	75%	20.9±1.4	48.3±5.6	13.3±1.4
	100%	22.8±1.5	57.0±0.2	15.8±1.8

Parameter values within each salinity sharing the same letter are not significantly different ($P>0.05$).

elevated electrolytes, Marshall and Grossell (2006) described reduced drinking rates, which may have either caused or further contributed to, the reduced muscle water content observed in these fish. If drinking rates were reduced, this may serve to increase monovalent ion uptake, as described in detail below. Further studies to determine if fish are capable of increasing their uptake of potassium through regulating processes such as drinking will be beneficial in furthering our understanding of the physiological mechanisms employed by fish to cope with potassium deficiency.

Under potassium deficient conditions, barramundi maintained homeostasis of potassium in the plasma. Maintaining blood potassium is essential for functions including the branchial elimination of sodium and chloride via the basolaterally located NKA and NKCC (Marshall and Bryson, 1998; Evans et al., 2005). Buffering of plasma potassium from barramundi muscle was described by Partridge and Creeper (2004) and also occurs in hypokalaemic mammals (McDonough et al., 2002). In the case of mammals, it is achieved by reducing the number of sodium pumps to reduce the active transport of potassium into the cells (McDonough et al., 2002; Clausen, 2003). The inward diffusion of sodium follows (Clausen, 1996), which may account for the significantly higher muscle sodium content of fish cultured at 50% K-equivalence. The consequence of reducing active potassium transport into cells and the subsequent increase in intracellular sodium is the disruption of the transmembrane concentration gradients for these ions. This leads to significant impairments in energy metabolism and contractile performance (Corbett and Pollock, 1981; Clausen, 1996; 2003), which have been identified as causes of the focal ischemia responsible for hypokalaemic muscle myopathy (Anderson et al., 1972; Tate et al., 1978; Corbett and Pollock, 1981; Sharief et al., 1997). Those fish reared in water with 50% K-equivalence had a muscle potassium content of 11.7 g/kg, whereas those displaying muscle myopathy in Partridge and Creeper's (2004) study had a content of 10.5 g/kg. Given the lower muscle water content and perturbations in blood chemistry and NKA activities in the various ion transport epithelia, we suggest that continued culture in this water may have further decreased muscle potassium content and led to fatal hypokalaemic myopathies over time. Longer term studies are required to confirm this hypothesis.

4.2. Response to potassium supplementation at near-isosmotic salinity

At near-isosmotic salinity (15 ppt) no hypokalaemic muscle myopathy or mortality was experienced in fish cultured in water with 25% K-equivalence for 4 weeks. This contrasts with results from Partridge and Creeper (2004) who found 100% mortality within nine days of similar sized barramundi cultured in 45 ppt water with the same potassium equivalence and therefore supports our hypothesis that proportionally more potassium is required at higher salinity. Furthermore, fish grown in 15 ppt water with 25% K-equivalence grew equally well as those cultured in 100% K-equivalence, which is in contrast to the decreased rate of growth we found for fish at 50% K-equivalence at 45 ppt. Our data are also consistent with those of Jain et al. (2006) who obtained 100% survival and equal growth of barramundi at 20% and 100% K-equivalence in saline groundwater of 15 ppt salinity.

The lower requirement for potassium at 15 ppt must be due either to a reduced loss and/or improved uptake of this ion at this salinity. Compared to hyperosmotic salinities, the loss of water from fish cultured in near-isosmotic salinities is less and these fish consequently drink less and absorb fewer monovalent ions (Sleet and Weber, 1982; Krayushkina, 1998; Wood and Laurent, 2003; Varsamos et al., 2004). The lower activity of branchial NKA obtained at 15 ppt compared with 45 ppt demonstrates the consequent reduction in ion elimination and is consistent with findings in many other species (Uchida et al., 1996; Jensen et al., 1998; Kelly et al., 1999a; Lin et al., 2004; Tipsmark et al., 2004). If potassium is lost concomitant with sodium through the leaky junctions between chloride and accessory cells as previously discussed, it follows that its loss will be significantly less at near-isosmotic salinities where the excretion of sodium is reduced. The uptake of potassium may also be more efficient at near-isosmotic salinities, as Ando et al. (2003) pointed out that lower salinities improve monovalent ion uptake across the oesophagus due to a lower drinking rate. Unlike fish cultured in hyperosmotic salinity, those in 15 ppt showed no differences in muscle water content between the various levels of potassium supplementation, demonstrating that the salt and water uptake processes were not negatively affected at the concentrations of external potassium tested.

Those fish cultured at 25% and 50% K-equivalence exhibited significantly lower potassium and higher sodium in the muscle than fish in 75% and 100% K-equivalence, demonstrating that buffering of plasma by muscle was occurring. That these fish exhibited no aberrations in muscle water content, blood chemistry or branchial or renal NKA activity, suggests that this buffering is in a state of homoeostasis. Support for this long-term equilibrium comes from our successful production of barramundi to a marketable size over a 4-month period without potassium supplementation in the same saline water source described in a previous study (i.e. 14 ppt, 42% K-equivalence; Partridge et al., 2006). Similar long-term studies growing barramundi at 25% K-equivalence are required to ensure that such buffering is sustainable at this level of supplementation.

The data presented in this study and that of Jain et al. (2006) also demonstrate that barramundi have a lower requirement for potassium than other marine/estuarine species being considered for inland saline aquaculture. Snapper (*Pagrus auratus*) experienced complete mortality within 4 days when cultured in 21 ppt groundwater with 25% K-equivalence (Fielder et al., 2001). Increasing the K-equivalence to 40% prevented mortality, but growth was still significantly lower than that obtained at 60% K-equivalence. Ingram et al. (2002) reported a survival rate of 27% with the same species in 9 ppt saline groundwater with 22% K-equivalence. Similarly, survival of mulloway (*Argyrosomus japonicus*) in 15 ppt groundwater with a potassium equivalence of 20% was significantly less than at potassium equivalents greater than 40% (Doroudi et al., 2006).

4.3. Response to potassium supplementation at hyposmotic salinity

There were no negative effects on the growth of fish at the various levels of potassium supplementation at a salinity of 5 ppt, suggesting that the lowest level of supplementation was sufficient for maintaining homeostasis.

In hyposmotic salinities, fish do not require potassium for salt excretion across the gills, but instead need to take up potassium from the water and/or diet to offset its diffusional loss to the environment (Shearer, 1988; Gardaire and Isaia, 1992; Kelly et al., 1999b). Many species of fish show increased branchial NKA activity in low salinity environments in order to absorb sufficient monovalent ions (Kelly et al., 1999a). In our study, the branchial NKA activity was equal across all potassium levels at 5 ppt, suggesting that adequate uptake of monovalent ions was occurring either from the water and/or diet without the need to increase the activity of this enzyme. Despite this, blood potassium was significantly lower at 25% K-equivalence compared with 75 and 100% K-equivalences. It appears that no attempts were made to maintain homeostasis of plasma potassium at this lowest level of equivalence through increased branchial NKA activity or buffering from skeletal muscle. These factors suggest that maintenance of plasma potassium content within a narrow range is not as crucial in hyposmotic conditions as it is for hyper- and iso-osmotic conditions. This is supported by the fact that under hyposmotic conditions, maintenance of serum potassium is not required for monovalent ion excretion via the basolaterally located NKA and NKCC. With barramundi able to thrive in freshwater (Rasmussen, 1991), they are clearly well adapted for taking up ions

in deplete media, and obtaining sufficient potassium from 5 ppt water with 25% K-equivalence appeared to create no challenges for them.

5. Conclusion

The results of this study demonstrate that barramundi require proportionally more potassium at hyperosmotic salinities than in near-isosmotic or hyposmotic salinities. This appears to be the first study to investigate the physiological mechanisms employed by teleost fish to survive in potassium deficient water sources and although the data presented are very insightful, additional studies such as drinking experiments would further advance our understanding of the exact physiological mechanisms used by fish to cope with potassium deficiency. The results of this study will aid in the selection of appropriate groundwater sources and/or supplementation regimes for the culture of this species, however, further studies to determine the lowest concentration of potassium that can be tolerated by barramundi in near-isosmotic and hyposmotic salinities are required.

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