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Evaluating the suitability of saline groundwater from Lake Toolibin, Western Australia for culturing barramundi (*Lates calcarifer*), mulloway (*Argyrosomus japonicus*) and snapper (*Pagrus auratus*)

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1. CONTENTS PAGE

2. LIST OF FIGURES

3. LIST OF TABLES

4. SUMMARY

With the view to offsetting the costs associated with their successful saline groundwater-pumping program at Lake Toolibin, the Department of Conversation and Land Management sought scientific data on the potential of this water source for commercial finfish culture. The physical and biological data required to determine commercial viability was obtained in the current project through a combination of laboratory testing and bioassays.

Laboratory testing revealed that all three groundwater sources were hypersaline (ca. 45 ppt), deficient in potassium and alkalinity and excessive in manganese, relative to 45 ppt seawater. The water also contained very high levels of ferrous iron, which oxidised on exposure to air. Filtration was required to remove this precipitate prior to conducting bioassays.

Initial bioassays revealed that snapper, mulloway and barramundi could not survive in the water sources, unless they were supplemented with potassium. The pathology of barramundi in the 'raw' (ie unsupplemented) Toolibin water revealed a condition previously only described in potassium-deficient mammals and birds.

Subsequent bioassays investigated the minimum requirements for potassium by barramundi and mulloway at various salinities, both to determine if supplementation would be cost-effective and also to provide data of relevance to other saline groundwater sources. The results of these bioassays were successful in showing that potassium requirements (expressed as K:Cl ratio) are not independent of salinity. For example, in 'full-strength' potassium-supplemented groundwater (45 ppt), the growth of barramundi increased with increasing level of K:Cl ratio. At 15 ppt, however, barramundi grew equally well across the same range of ratios. Investigations with mulloway found that at salinities between 16 and 36 ppt, this species grows equally well over the range of tested K:Cl ratios. A comparison of the response of blood electrolytes to varying levels of potassium between barramundi and mulloway suggests the possibility that the two species differ in their physiological response to low potassium concentrations.

Independent data collected over two barramundi and one mulloway bioassay suggested that another substance within the water source was having a negative impact on growth and manganese was hypothesised as the candidate. Although beyond the scope of the current project, further investigation into the effects of excessive manganese on growth are required.

The saline groundwater at Lake Toolibin would, based on the results of this project, require significant pre-treatment including degassing, filtration or flocculation of oxidised iron, buffering and potassium supplementation for it to be suitable for commercial fish culture. As the optimum salinity for many species of marine/estuarine fish is less than seawater (Partridge and Jenkins, 2002), dilution of the groundwater would also ideally occur. It is the opinion of the investigator that this level of manipulation would be cost-prohibitive for commercial culture.

Although the water from Lake Toolibin is considered unsuitable, this project has contributed greatly to the knowledge base required for the development of commercial fish culture in inland saline water. Many saline groundwater sources in the WA Wheatbelt have water quality characteristics that meet the requirements for optimal fish performance, as determined in the current project. With funding from the Avon Catchment Council, an additional bioassay was conducted during this project on a groundwater source from the Shire of Westonia. This saline groundwater source had a salinity of 16 ppt, did not require buffering or filtration and contained lower levels of manganese than that of the Toolibin sources. Although still deficient in potassium relative to seawater, the K:Cl ratio of this water was higher than that from Toolibin and equal to the minimum level found to support maximum growth of mulloway. Mulloway in the 'raw' Westonia groundwater grew at a rate equal to both the equivalent salinity control treatment and Westonia water supplemented with potassium. These results highlight the variability in quality of saline groundwater throughout Western Australia. They also demonstrate that suitable water sources, not requiring pre-treatment, are available for the culture of estuarine fish in the Wheatbelt of Western Austaralia.

5. OBJECTIVES

The primary objective of the project was to determine if the culture of marine or estuarine fish species in saline groundwater being pumped from beneath Lake Toolibin is biologically feasible. This primary objective was achieved through the following means:

1. Characterising the physical and chemical characteristics of groundwater sources from Lake Toolibin through a series of laboratory tests, designed specifically by the investigator to assess the potential of saline groundwater for fish culture.
2. Conducting bioassays in these water sources with various species of marine/estuarine fish, during which the survival, growth and other physiological parameters are compared against fish grown in seawater and seawater with an equivalent salinity to that of the groundwater.
3. Identifying any factors that may limit the potential for Lake Toolibin groundwater for commercial fish culture.
4. To increase the knowledge of inland saline water sources in the WA Wheatbelt and their potential for aquaculture.

6. QUALITY OF LAKE TOOLIBIN GROUNDWATER

6.1. Rationale

The suitability of groundwater for aquaculture is determined by its pH, ionic composition and the absence of toxic agents. Potentially toxic agents found in inland groundwater sources include certain dissolved gases, acid sulphate soils, hydrocarbons, pesticides and herbicides. As the quality of saline groundwater has been shown to vary significantly, even on small spatial scales, not all water sources will be suitable for fish culture. The author of this report has compiled a series of laboratory tests specifically for determining the suitability of saline groundwater for culturing marine/estuarine fish (Partridge and Furey, 2002). This approach has the ability to cost-effectively screen large numbers of potential water sources and can therefore save significant amounts of money conducting bioassays on those shown to be unsuitable. The aim of the current experiment was to conduct this series of laboratory tests on three saline groundwater sources from Lake Toolibin.

6.2. Methods

Water samples were collected from three saline bores from Lake Toolibin (P11, P13 and P15). Water quality analyses included measurements of salinity, pH, dissolved carbon dioxide (CO_2) and alkalinity, as well as ionic composition analyses, precipitate analyses and toxicity testing.

Levels of CO_2 and pH were measured both before and after aerating each water sample. pH was measured with a WTW pH meter and CO_2 by titration against 0.01 M NaOH to a pH end-point of 8.3 (APHA, 1995). Salinity was measured using a WTW conductivity meter. Alkalinity was measured by titrating against 0.01 M HCl to a pH endpoint of 4.8 according to Spotte (1992).

Water from the three bores was analysed for 24 ions found in seawater using inductively coupled plasma atomic emission spectroscopy (ICPAES). The concentration of chloride ions was determined via a silver-chloride titration. These parameters were compared against those present in seawater with equivalent salinity (45 ppt).

Aeration of all three saline groundwater sources resulted in the formation of an orange precipitate. The concentration of ions was therefore determined in both filtered and acid-digested samples of post-aerated water to determine the ions present in this precipitate. X-ray diffraction was employed to identify the compound/s present in this precipitate.

The use of the flocculant alum (aluminium hydroxide) was assessed as a method of removing the precipitate from solution. The effect of alum concentrations ranging from 0 to 500 ppm on the ability to flocculate geothite were assessed in 'raw' Toolibin water from bore P11 and this water buffered to an alkalinity of 100 ppm with sodium bicarbonate. The required concentrations of alum were prepared in 500 mL of each treatment water and the turbidity, pH and alkalinity of the water sources measured after 24 hours.

The toxicity of the water sources was assessed via Microtox®, a test which relies on the use of the luminescent bacteria, *Vibrio fischeri,* to measure the toxicity of water samples. These bacteria produce light as a by-product of respiration. The presence of toxins in the water inhibits the metabolism of the bacteria, which results in a decrease in the rate of luminescence. Results from this test have been found to correlate well with the survival of fish in saline groundwater (Partridge and Furey, 2002). Water samples from the three test bores were collected immediately after pumping and stored on ice until the Microtox® analysis was performed.

6.3. Results and Discussion

The pH, carbon dioxide, alkalinity and salinity of the three Toolibin water sources are shown in Table 1. Although the pre-aeration carbon dioxide concentrations in all three water sources would be lethal to fish, the process of aeration brings the concentration back into equilibrium with the surrounding atmosphere and, as such, the post-aeration levels presented in Table 1 are similar to those found in seawater. As carbon dioxide is degassed, the pH also rises to a level appropriate for fish culture. Such high carbon dioxide concentrations (and subsequent low pH) is typical of many Wheatbelt water sources, due to the interactions of the water with underground limestone. This phenomenon is not considered inhibitory to the potential of a water source to grow finfish, as the problem can be easily overcome through degassing.

	P11	P13	P15
pH^1	5.7	6.3	6.1
pH^2	7	8.2	8.2
CO_2^1 (ppm)	250	137	132
CO_2^2 (ppm)	7	7	7
Alkalinity (ppm)	50	110	130
Salinity (ppt)	45	44	47

Table 1: Water quality parameters of three saline groundwater sources from Lake Toolibin. 1 & 2 denote pre- and post-aerated values, respectively.

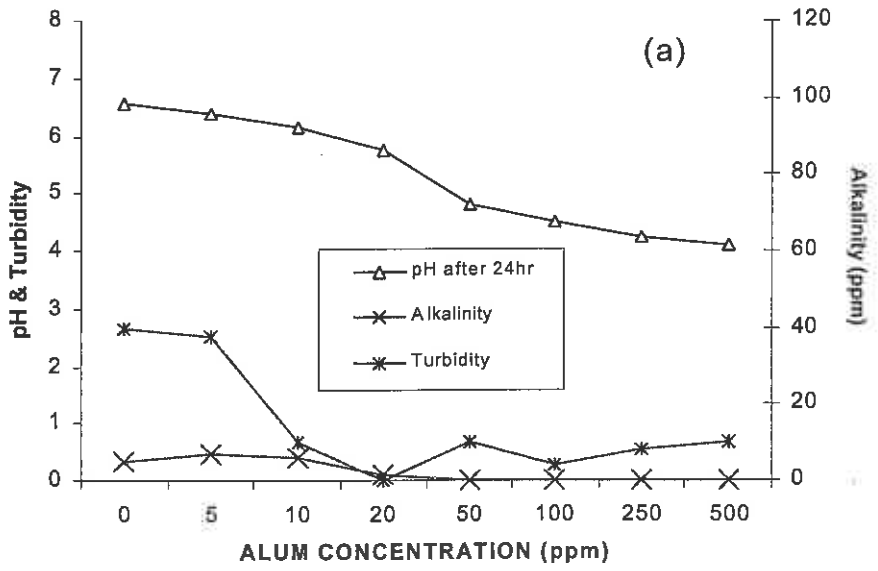
Alkalinity is defined as the capacity of a water source to resist changes in pH. Low levels of alkalinity result in large swings in pH, which can be detrimental to fish and the bacterial processes essential to maintaining water quality. Alkalinity levels greater than 100 ppm are required for maintaining stable pH (Spotte, 1992). As can be seen in Table 1, alkalinity in the Toolibin sources ranged from 50 to 130 ppm. The addition of buffering agents, such as sodium bicarbonate or lime, are effective in overcoming low alkalinity.

Aeration of all three bore water sources resulted in the formation of a thick milky-orange precipitate. A comparison of ICPAES analysis between filtered and acid-digested samples of the precipitated water sources revealed the major element in the precipitate to be iron (Table 2). Analysis of the precipitate using X-ray diffraction found the precipitate to be goethite (FeOOH). The very fine nature of the precipitate rendered settlement as an ineffective means of removal and therefore all water sources had to be filtrated via a series of 1 μm cartridge filters prior to use. The precipitate could not be left in the water column as it would likely cause irritation and/or clogging of the gills. If these water sources were to be used for commercial aquaculture, removal of this precipitate via filtration would not be cost effective and methods of facilitating settlement through the use of the flocculant, alum (aluminium hydroxide), were subsequently made.

	P11			P13			P15		
	Pre-filtered	Post-filtered	% Increase	Pre-filtered	Post-filtered	% Increase	Pre-filtered	Post-filtered	% Increase
Al	0.077	<0.003	-	<0.006	<0.003	-	0.011	<0.003	-
As	<0.02	<0.01	-	<0.02	<0.01	-	<0.02	<0.01	-
B	1.0	1.0	0	1.2	1.1	9	1.4	1.3	-
Ba	0.018	0.018	0	0.025	0.024	4	0.045	0.018	150
Ca	710	710	0	730	730	0	780	760	3
Cd	<0.002	<0.001	-	<0.002	<0.001	-	<0.002	<0.001	-
Co	0.007	0.005	40	<0.004	0.002	-	0.007	0.005	40
Cr	<0.002	<0.001	-	<0.002	<0.001	-	0.004	<0.001	-
Cu	<0.002	<0.001	-	0.011	<0.001	-	0.022	0.003	633
Fe	***15***	***0.002***	***749900***	***10***	***0.007***	***142757***	***1.5***	***0.005***	***29900***
K	98	97	1	110	110	0	120	110	9
Mg	2800	2800	0	2500	2500	0	2900	2800	4
Mn	1.4	1.4	0	1.1	1.2	-8	2.0	2.0	0
Mo	<0.01	<0.005	-	<0.01	<0.005	-	<0.01	<0.005	-
Na	13000	13000	0	12000	13000	-8	14000	14000	0
Ni	0.010	0.005	100	<0.008	<0.004	-	<0.008	<0.004	-
P	<0.04	<0.02	-	<0.04	<0.02	-	<0.04	<0.02	-
Pb	<0.02	<0.01	-	<0.02	<0.01	-	<0.02	<0.01	-
S	1500	1500	0	1300	1300	0	1600	1500	7
Se	<0.04	<0.02	-	<0.04	<0.02	-	<0.04	<0.02	-
Sn	<0.2	<0.1	-	<0.2	<0.1	-	<0.2	<0.1	-
Sr	9.7	9.8	-1	9.6	9.6	0	11	11	0
V	<0.002	<0.001	-	<0.002	<0.001	-	<0.002	<0.001	-
Zn	0.014	0.009	56	0.009	<0.001	-	0.014	0.002	600

Table 2: A comparison of ions present in acid-digested (pre-filtered) and post-filtered, samples of oxidised groundwater from Lake Toolibin.

The results of the alum trial are shown in Figure 1. In the unbuffered water (Figure 1a), pH and alkalinity decreased with increasing alum concentration. At an alum concentration of 50 ppm and greater, pH dropped below 5.0 and the subsequent increase in turbidity indicated that the precipitate was redissolving into solution. Although an alum concentration of 20 ppm in the unbuffered water resulted in clear water (turbidity = 0), the alkalinity had dropped to 1.7 ppm. At such a low alkalinity, pH could easily drop below 5.0 and the precipitate would redissolve. The use of alum in buffered groundwater gave superior results (Figure 1b). The optimum result was achieved with an alum concentration of 50 ppm. At this concentration, clear water was achieved (turbidity = 0), pH remained constant at 7.45 and alkalinity only marginally decreased to 90 ppm. The results prove the effectiveness of alum in flocculating goethite from groundwater from the P11 bore, however, as aluminium can be toxic to fish (McDonald et al., 1989), more research is required prior to it's use to determine if any residual aluminium remains in the water after treatment. In addition, due to the differences in alkalinity and iron content between the different bores (Tables 1 & 2), this trial would need to be repeated to determine the optimum dose for each water source.



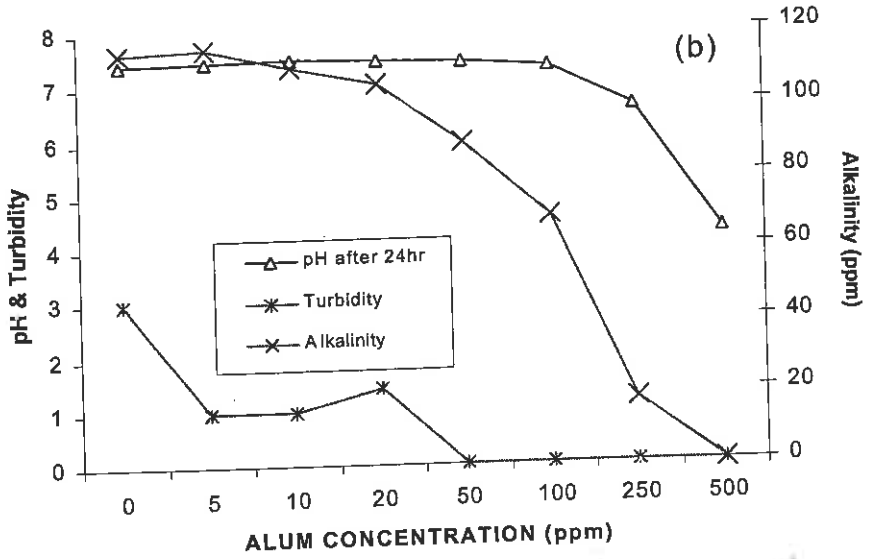


Figure 1: The effect of various concentrations of alum on the flocculation of geothite in unbuffered (a) and buffered (b) Toolibin groundwater

The ionic composition data of the filtered water sources is shown in Table 3. The concentrations of the nine most abundant ions have been expressed relative to their concentration in seawater adjusted to a similar salinity to that of the groundwater sources. The following ions were found to be deficient; potassium, boron, sodium, chloride and strontium (listed in decreasing order of deficiency). Manganese, magnesium, sulphur and calcium were present in concentrations in excess of those found in adjusted seawater.

Potassium is an essential element to fish, playing critical roles in many physiological processes, particularly in osmotic and ionic regulation (Karnkay, 1998). During an investigation into the potential of saline groundwater for fish culture, Fielder et al. (2001) found that potassium deficiency caused mortality in snapper. Although the K:Cl ratio in the Toolibin bores (0.005) is greater than that investigated by Fielder et al (0.001), it is still considerably less than seawater (0.025) and may therefore cause mortality and/or growth retardation in the species being investigated in the current project. As both mulloway and barramundi are more euryhaline than snapper, it is conceivable that they may have lower potassium requirements.

		P11	Δ	P13	Δ	P15	Δ	Seawater (36 ppt)	Seawater (45 ppt)
Major Elements	Cl	23800	***-8***	23800	***-8***	25600	***-1***	19000	25916
	Na	13000	***-13***	13000	***-13***	14000	***-7***	11000	15000
	Mg	2800	***71***	2500	***53***	2800	***71***	1200	1637
	S	1500	***29***	1300	***12***	1500	***29***	850	1159
	Ca	710	***18***	730	***22***	760	***27***	440	600
	K	97	***-85***	110	***-84***	110	***-84***	490	668
	Sr	9.8	***-2***	9.6	***-4***	11	***10***	7.3	10.0
	B	1	***-83***	1.1	***-81***	1.3	***-77***	4.2	5.7
	Mn	1.4	***>9900***	1.2	***>8471***	2	***>14186***	<0.01	<0.014
Minor Elements	Al	<0.003		<0.003		<0.003		<0.03	<0.041
	As	<0.01		<0.01		<0.01		<0.01	**<0.014**
	Ba	**0.018**		0.024		0.018		0.012	0.016
	Cd	<0.001		<0.001		<0.001		<0.001	<0.0014
	Co	0.005		0.002		0.005		<0.02	<0.027
	Cr	<0.001		<0.001		<0.001		<0.001	<0.0014
	Cu	<0.001		<0.001		0.003		<0.06	<0.082
	Fe	0.002		0.007		0.005		<0.02	<0.027
	Mo	<0.005		<0.005		<0.005		<0.05	<0.068
	Ni	0.005		<0.004		<0.004		0.040	0.055
	P	<0.02		<0.02		<0.02		<0.02	<0.027
	Pb	<0.01		<0.01		<0.01		<0.01	<0.014
	Se	<0.02		<0.02		<0.02		<0.02	<0.027
	Sn	<0.1		<0.1		<0.1		<0.02	<0.027
	V	<0.001		<0.001		<0.001		<0.002	<0.027
	Zn	0.009		<0.001		0.002		0.003	0.004

Table 3: Ionic composition of three filtered Toolibin water sources. All values are ppm. Δ represents the percentage difference in concentration between the groundwater source and seawater (45 ppt)

Boron deficiencies can effect embryonic development in fish (Eckhert and Rowe, 1999), however, there appears to be no evidence on detrimental effects to growth or survival of post-larval fish. Although the levels of sodium, chloride and strontium were also deficient, their concentrations were within 10% of seawater and are therefore considered unlikely to have any detrimental effects.

Excessive levels of manganese have been shown to disrupt sodium balance in freshwater fish. These studies have, however, focussed on acute effects (Lewis, 1978; Gonzalez et al., 1990) and extrapolation of these data to chronic effects in marine fish is not possible. It is probable, however, that manganese will also have toxic effects on marine and estuarine finfish.

Magnesium and calcium are essential elements to fish and there appears little data on any toxic effects of these ions at high concentrations, in fact both ions have been shown to be valuable in decreasing the toxicity of heavy metal ions (Miller and Landesman, 1978; Michibata et al., 1986). Forsberg et al. (1996) investigated the potential of culturing the estuarine red drum (*Sciaenops ocellatus*) in various saline groundwater sources with high sulphate concentrations and achieved good results. Other literature quoted in this report suggests that $Cl^-:SO_4^{2-}$ ratios should be at least 2.0. This ratio in seawater is 5.6 and ranges between 4.6 and 5.3 in the Toolibin water sources. Toxic effects of sulphate ions have been described in fathead minnows (*Pimephales promelas*) grown in saline water (12-13 ppt), however, these sources had a $Cl^-:SO_4^{2-}$ ratios less than 0.1 (Burnham and Peterka, 1975).

All three water sources were found to be non-toxic via Microtox®. Microtox® testing is an effective tool for determining the degree of toxicity of a water source and is particularly sensitive to pollutants such pesticides, herbicides and petrochemical pollutants (Somasundaram et al., 1990; Whale et al., 1993; Gaggi et al., 1995). Good correlation has been found between fish mortality in saline groundwater sources and a positive Microtox® result (Partridge and Furey, 2002).

7. SCREENING BIOASSAYS

7.1. Rationale

The low K:Cl ratio of all Toolibin groundwater sources suggested fish survival may be compromised. Short-term bioassays were therefore conducted on snapper, barramundi and mulloway to determine if each species would survive in these water sources, before proceeding to longer-term bioassays comparing growth rates.

7.2. Methods

During each screening bioassay, eight treatments were investigated in triplicate. These included two treatments of each of the three Toolibin water sources (6 treatments) and control treatments of 'full-strength' seawater (36 ppt) and seawater adjusted to a salinity equivalent to that of the Toolibin bores (45 ppt). This salinity was obtained by the addition of artificial seasalt (Ocean Nature®) to seawater. The two Toolibin treatments included 'raw' (unsupplemented) water and water adjusted to a K:Cl ratio equivalent to 45 ppt seawater. All Toolibin water sources were filtered and buffered prior to use. After an acclimation period to the test salinity of three days, five fish were stocked into 15 litres of each treatment water. After a period of 24 hours, survival was measured in each tank. This screening bioassay was conducted three times with snapper, barramundi and mulloway.

7.3. Results and Discussion

Complete mortality of both snapper and mulloway occurred in all of the raw Toolibin bore water sources within 24 hours. All snapper and mulloway survived in the control treatments and potassium-supplemented Toolibin bore water sources. Barramundi survived in all treatments. These data indicate that the potassium to chloride ratio in the raw groundwater sources (0.005) is insufficient for both snapper and mulloway but suggests barramundi may have a lower requirement for potassium and may therefore be suitable for culture in this water source.

8. BIOASSAYS

8.1. Rationale

Bioassays are tests in which the growth and survival of the test species in the groundwater source is statistically compared against those of fish grown in seawater (36 ppt) and seawater of equivalent salinity to that of the groundwater. Although laboratory tests are a very useful tool for narrowing down potential water sources, bioassays are always required to provide the final verification that a particular water source is suitable for the target species.

It was originally intended to conduct bioassays with snapper, barramundi and mulloway. As snapper were shown to be unsuitable for culture in the Toolibin groundwater sources (see Section 7.3) and the fact that their requirements for potassium have already been described (Fielder et al., 2001; Partridge and Furey, 2002), it was decided to focus on barramundi and mulloway in the subsequent bioassays.

8.2. General Methods

8.2.1. Water Pre-treatment

Water was pumped from saline bores at Lake Toolibin and trucked to the Aquaculture Development Unit (ADU) in Fremantle. Once at the ADU, water was transferred into a holding tank and vigorously aerated for 24 hours to precipitate geothite and to increase dissolved oxygen, degas carbon dioxide and increase pH. After aeration, geothite was removed via a series of 1 μm cartridge filters prior to use and, in those sources requiring it, the water buffered with sodium bicarbonate ($NaHCO_3$) to obtain an alkalinity of 100 ppm.

8.2.2. Bioassay System

The experimental bioassay system consisted of 18 x 180 litre tanks held within a temperature controlled water bath. The system could therefore test up to 6 treatments in triplicate. Each tank operated as an independent recirculating system with water continuously airlifted through a mechanical and biological filter.

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.

Fish were fed three times daily to satiety on a commercial fish diet (Skretting Australia, 45% protein, 22% lipid) and the amount of food consumed recorded.

In each bioassay two controls were included; 'full-strength' seawater (36 ppt) and seawater adjusted to the salinity of the groundwater source under investigation. For groundwater sources with salinity lower than seawater, the required salinity was achieved by diluting seawater with carbon-filtered scheme water. Water for control treatments with a salinity greater than seawater were prepared by the addition of artificial seasalt (Ocean Nature®) to seawater.

Prior to commencing each bioassay, experimental fish were acclimated from seawater to the salinity of the water source under investigation over a three-day period.

All bioassays were conducted over a 4-week period. At the completion of each experiment, fish were anaesthetised (40 ppm AQUI-S) and weighed to 0.1 g. Blood was taken from the caudal peduncle of a subsample of fish in each replicate and pooled for the determination of plasma sodium, potassium and chloride using a Vetlyte ion-specific electrode analyser.

8.2.3. Data Analysis

Survival, specific growth rate (SGR), food conversion ratio (FCR) and blood electrolyte concentrations were compared between treatments using one-way analysis of variance. Significant differences between treatments were identified using Tukey's HSD test. All statements of significance refer to the 0.05 level, unless otherwise stated. Specific growth is defined as follows:

$$SGR = \frac{\left(\mathrm{Ln}(W_f) - \mathrm{Ln}(W_i)\right)}{t} \times 100$$

Where W_f = Final wet weight (g)

W_i = Initial wet weight (g)

t = time (days)

8.3. Bioassay 1 – The performance of juvenile barramundi in three Toolibin water sources (45 ppt).

8.3.1. Rationale

The positive result obtained with barramundi during the screening trials warranted conducting a bioassay with this species in the three Toolibin water sources.

8.3.2. Methods

Five treatments were investigated; three 'raw' Toolibin groundwater sources (P11, P13 and P15) and two controls; seawater and seawater adjusted to the salinity of the bores (45 ppt).

Ten juvenile barramundi (8.04 ± 0.17 g) were randomly allocated to each replicate within the bioassay system (see Section 8.2.2) and the water bath set to 28°C.

Samples of fish from both the groundwater and control treatments were preserved in 10% formalin (prepared with the treatment water) for histological analysis. Para-sagittal slab sections of preserved fish were decalcified in 10% formic acid for six hours, vacuum embedded in paraffin and 5μm sections stained with haematoxylin and eosin (H&E).

8.3.3. Results and Discussion

Although barramundi survived for 24 hours during the screening bioassay, mortalities began ten days after introduction to the raw groundwater sources in this trial. At the completion of the 4-week bioassay, survival in the groundwater sources averaged 27 ± 9%. Survival in the two control treatments averaged 97 ± 3% (36 ppt) and 100 ± 0% (45 ppt). That 100% survival was obtained in the 45 ppt control, indicates that the salinity of the groundwater sources was not the cause of mortality. This is supported by Shirgur and Siddiqui (1995), who have shown that barramundi can tolerate salinities of at least 55 ppt.

The specific growth rate of surviving fish is shown in Figure 2. Those fish in the groundwater treatments lost weight over the experimental period whereas those in the control treatments grew well. Those fish held in water with salinity of 45 ppt had a significantly greater growth rate than those held in 36 ppt, giving further evidence that a salinity of 45 ppt is not detrimental to barramundi. A comparison of food intake data during the first week of the trial (when all tanks still contained equal numbers of fish) revealed that fish in the saline groundwater treatments were eating well prior to mortality commencing (Table 4). There was no significant difference in FCR between fish in the 36 and 45 ppt treatments (Table 4).

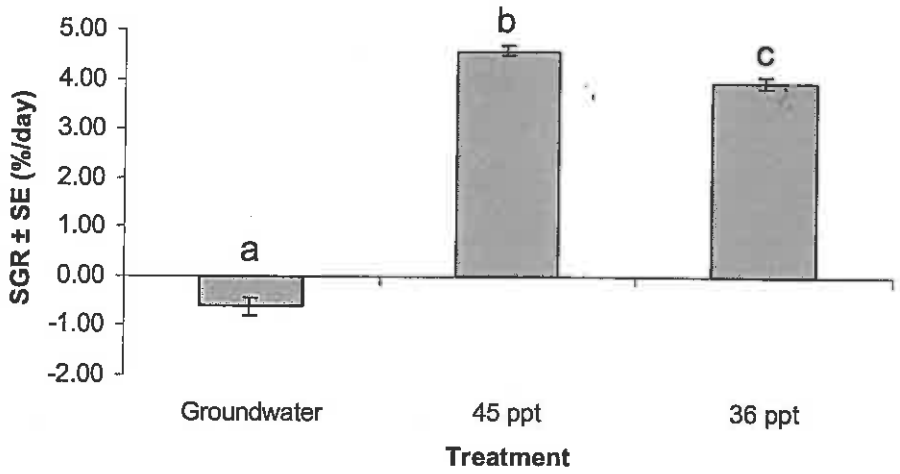


Figure 2: Specific growth rates of juvenile barramundi grown for 4 weeks in 'raw' Toolibin water sources (45 ppt) and two seawater controls. Columns sharing the same letter are not significantly different (P<0.05).

	P11 (45 ppt)	P13 (44 ppt)	P15 (47 ppt)	Seawater (45 ppt)	Seawater (36 ppt)
Food Intake (g)	11.5 ± 0.8 [b]	15.4 ± 0.8 [a,b]	14.9 ± 3.3 [a,b]	22.6 ± 0.4 [a]	14.1 ± 1.5 [b]
FCR	N/A	N/A	N/A	0.72 ± 0.01	0.68 ± 0.02

Table 4: Food intake (week 1 only) and food conversion ratios (FCR) of juvenile barramundi grown for 4 weeks in three Toolibin groundwater sources (45 ppt) and two seawater controls. Values in the same row sharing the same letter are not significantly different (P<0.05).

Histology of fish from the saline groundwater treatments revealed widespread, severe skeletal muscle degeneration and necrosis that affected both epaxial muscle groups and muscles within the pharyngeal area (Figure 3). The histological features of the muscle necrosis would approximate the duration of the lesion to be ten days, indicating that the skeletal myopathy commenced soon after entering the test water source (Jubb et al., 1993). All fish examined from the two control treatments showed no skeletal muscle degeneration.

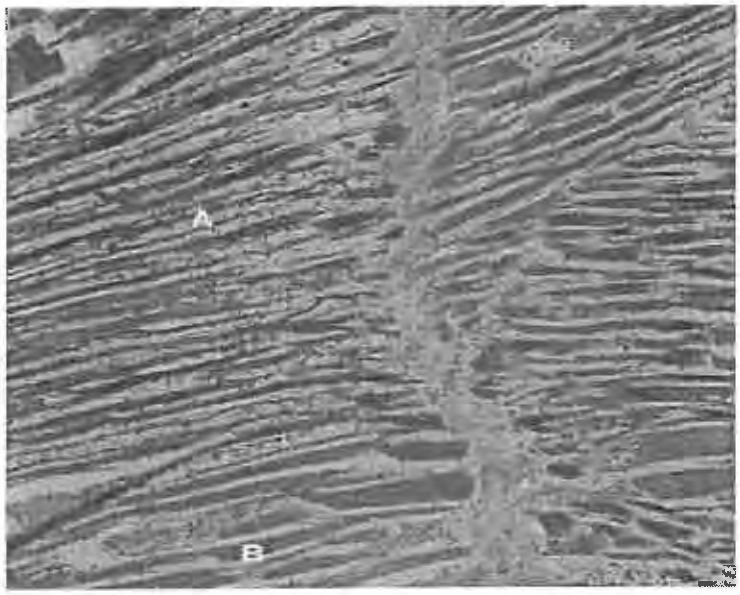


Figure 3: Muscle myopathy (H&E x40) in a longitudinal section of lateral flank muscle in barramundi cultured in Toolibin groundwater (45 ppt). There is severe, widespread dissolution of muscle fibres (B). There are few, normal fibres (A).

Skeletal myopathies caused by potassium deficiency are known as hypokalaemic muscle myopathies and have been described extensively in mammals, birds and humans (Tate et al., 1978). Rats and dogs fed potassium deficient diets, for example, suffer myopathies in skeletal muscle and also demonstrate growth retardation and death in cases where the diet is continued (De Coster, 1979; Corbett and Pollock, 1981). The myopathies seen in the barramundi have many similar characteristics to those observed in mammals, however, some differences were also noted. The lesions present in the barramundi are more diffuse and severe than those described in mammalian hypokalaemic myopathy and the fibre vacuolation that is a hallmark of mammalian lesions was not present. This appears to be the first description of hypokalaemic induced myopathies in fish and a detailed description of the pathology is therefore being prepared for the international journal, 'Journal of Fish Diseases'.

All fish examined from the saline groundwater sources showed marked chloride cell hyperplasia (Figure 4). There was patchy, mild, chloride cell hyperplasia in the 45 ppt seawater control group, whereas those in the 36 ppt seawater control treatment showed no hyperplasia.

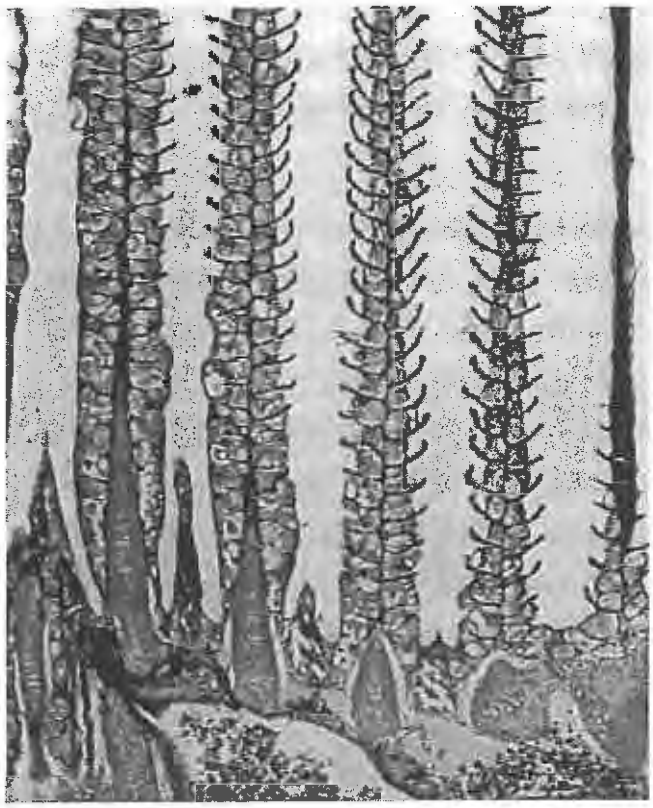


Figure 4: Gills (H&E x40) of barramundi cultured in Toolibin groundwater (45 ppt), showing severe diffuse hyperplasia and hypertrophy of chloride cells at the base of normal appearing secondary lamellae (Arrow head points to chloride cell).

The concentrations of sodium, chloride and potassium in the blood plasma of fish from the various treatments are shown in Figure 5. Due to a shortage of fish remaining in the inland saline groundwater replicates at the time of blood collection, pooling of samples was required to obtain sufficient volume for analysis. As such, the concentrations of electrolytes in fish from the groundwater treatments could not be statistically compared to those in the two control treatments. The concentration of sodium in the plasma of fish from the saline groundwater (198 mmol/l) was considerably higher than those in the 36 and 45 ppt control treatments (169 ± 3 and 169 ± 2 mmol/l, respectively). There was no significant difference in sodium plasma

content between the two controls. The plasma chloride concentration exhibited a similar pattern. There was no significant difference in plasma chloride between fish reared in 36 and 45 ppt (155 ± 3 and 153 ± 1 mmol/l, respectively), whereas the chloride plasma content of fish reared in the saline groundwater was considerably higher at 188 mmol/l. The plasma concentration of potassium in the saline groundwater treatment (14.4 mmol/l) was similar to the 36 ppt and 45 ppt control treatments (13.6 ± 0.8 and 12.3 ± 0.6 mmol/l, respectively). There was no significant difference in plasma potassium content between the two control treatments.

In teleost fish within a hyperosmotic environment, potassium plays an essential role in the branchial extrusion of both sodium and chloride (due to its role in Na^+-K^+ATPase of the sodium pump). Maetz (1969) showed that the rate of efflux of sodium across the gills is dependent on the concentration of external potassium. The transfer of flounder (*Platichthys flesus*) to seawater deficient in potassium resulted in a decrease in the rate of sodium excretion across the gills and a subsequent increase in sodium plasma concentration. That the plasma concentration of sodium was greater in the inland saline water source, compared to the two control treatments is consistent with these findings. Due to the interrelationship between the transport of sodium and chloride across the gill epithelium, increases in chloride plasma levels are also expected.

Skeletal muscle is the main store of intracellular potassium in mammals as well as fish (Jobling, 1995; McDonough et al., 2002). In mammals the ability of the skeletal muscle to rapidly buffer the potassium content of the extracellular fluid in times of potassium depletion has been demonstrated (McDonough et al., 2002). That there was little difference between the concentrations of potassium in the plasma of fish cultured in the saline groundwater and the control fish, suggests this to be the case with barramundi. With chronic potassium deficiency this buffering effect leaves the skeletal muscle deficient in potassium, which leads to vasoconstriction of the muscle. This vasoconstriction results in focal ischemia in the muscle, which subsequently leads to necrosis (Penn, 1979). Analysis of the muscle potassium content of the fish in the current trial would have been valuable to determine the relationship between the extracellular (ie. plasma) and intracellular levels (ie. muscle) of potassium.

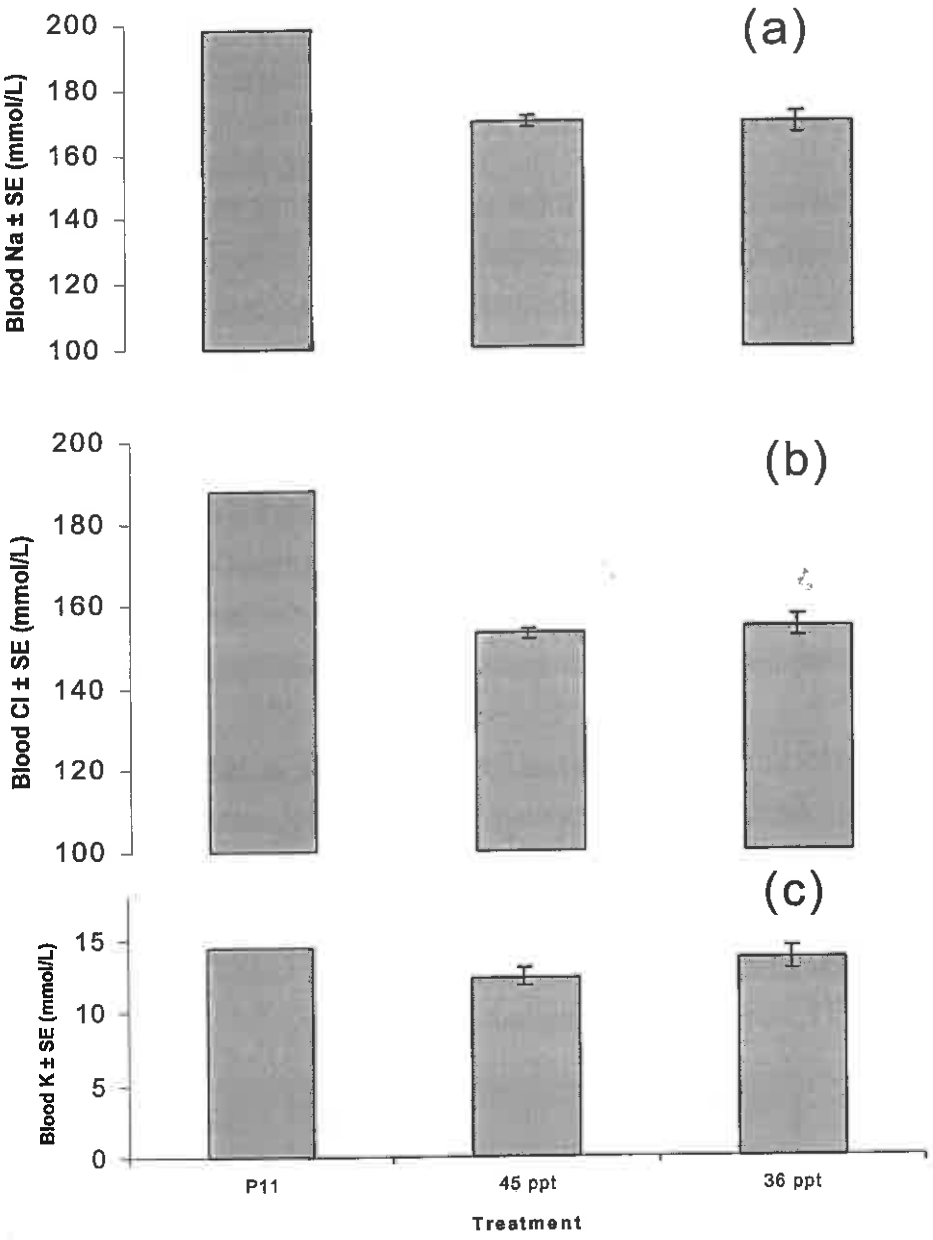


Figure 5: Concentrations of blood plasma sodium (a), chloride (b) and potassium (c) of barramundi cultured in Toolibin groundwater (45 ppt) and seawater controls. Columns within each graph sharing the same letter are not significantly different ($P<0.05$)

The extrusion of monovalent ions in marine and euryhaline teleosts occurs via chloride cells located on the gill's primary lamella. The rate of sodium and chloride secretion is directly related to the number of chloride cells and the mild chloride cell hyperplasia seen in the 45 ppt control fish is the typical physiological response to an increase in salinity (Utida and Hirano, 1973). That the chloride cell hyperplasia was much more

severe in the saline groundwater treatments is likely to be in response to the hypernatraemia and hyperchloridaemia caused by the low external potassium concentration.

The excretory portion of the kidneys in those fish cultured in the saline groundwater showed a mild nephrosis that was characterised histologically by patchy acute tubular epithelial cell necrosis and intensely eosinophilic-staining amorphous material filling tubule lumina (Figure 6). No renal lesions were identified in the two control treatment groups. Whilst renal tubular damage has been described in humans as a consequence of hypokalaemia (Emery et al., 1984), the lesion has not been described in fish. The myonecrosis seen in the affected barramundi is expected to release large amounts of myoglobin. Myoglobin is associated with nephrotoxicity in mammals (Jubb et al., 1993) and the intensely eosinophilic casts and granules present in the barramundi kidneys are histologically similar to those seen in myoglobinuric nephrosis in mammals.

Fielder et al. (2001) described mortalities in snapper cultured in potassium deficient saline groundwater. Although the pathology of these fish was not described, supplementation of this water with potassium prevented further mortality. This evidence, together with that presented from this bioassay, suggests that the concentration of potassium in the Toolibin groundwater source caused severe hypokalaemia leading to mortality in juvenile barramundi.

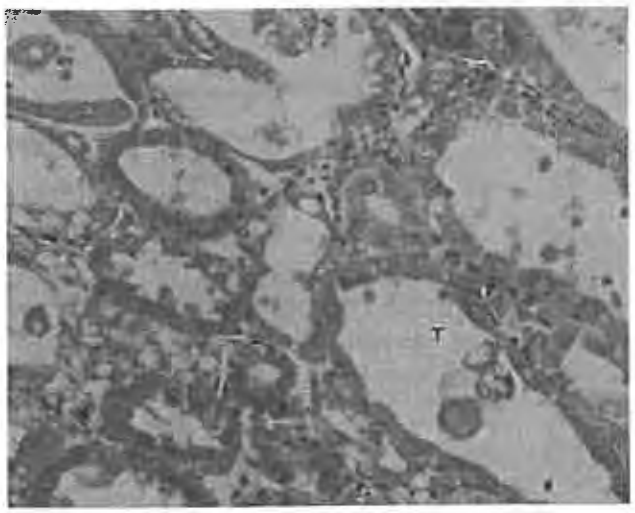


Figure 6: Kidney (H&E x200) of barramundi cultured in Toolibin groundwater (45 ppt) showing diluted renal tubules with accumulations of necrotic sloughed epithelial cells within the tubule lumen (T)

8.4. Bioassay 2 – The effect of potassium supplementation on the performance of juvenile barramundi in 'full-strength' Toolibin groundwater (45 ppt).

8.4.1. Rationale

The poor survival, growth and pathology of barramundi in the raw Toolibin sources in the previous bioassay were attributed to the low potassium concentrations of these sources. The current trial therefore aimed to determine the minimum potassium requirement of barramundi in the Toolibin water.

The design employed in the current trial allowed for only one water source to be tested and P11 was subsequently selected.

8.4.2. Methods

Saline groundwater from P11 was trucked to the ADU and aerated, filtered and buffered as described for Bioassay 1. After filtration, water was split into four separate storage tanks. To each tank was added sufficient potash (technical grade potassium chloride) to obtain K:Cl ratios of 0.010, 0.015, 0.020 and 0.025 (seawater equivalent). These levels were confirmed via ICPAES. Unsupplemented P11 water (K:Cl = 0.005) was not included as a treatment due to it causing high mortality and weight loss of barramundi in the previous trial. The same two seawater control treatments described for Bioassay 1 were also included in the current trial (36 and 45 ppt)

Five juvenile barramundi (41.1 ± 1.5 g) were stocked into each experimental tank within the bioassay system (See 8.2.2) and the exact trial protocol as described for Bioassay 1 was followed.

At the completion of the trial, a subsample of fish from each replicate were also preserved in 10% formalin (prepared with the treatment water) and analysed histologically as described for Bioassay 1.

8.4.3. *Results and Discussion*

Survival of barramundi in the current bioassay was 100% in all replicates and treatments, indicating that even the minimum level of potassium supplementation (K:Cl = 0.010) was sufficient to overcome the mortalities experienced in the previous trial. This level of supplementation was also sufficient to prevent both muscle and kidney myopathies.

There was an increase in specific growth rate with increasing level of potassium supplementation (Figure 7). Despite being sufficient to prevent myopathies and subsequent mortality, the lowest level of potassium supplementation resulted in a loss in weight of barramundi over the experimental period (SGR = -0.64 ± 0.11 %/day).

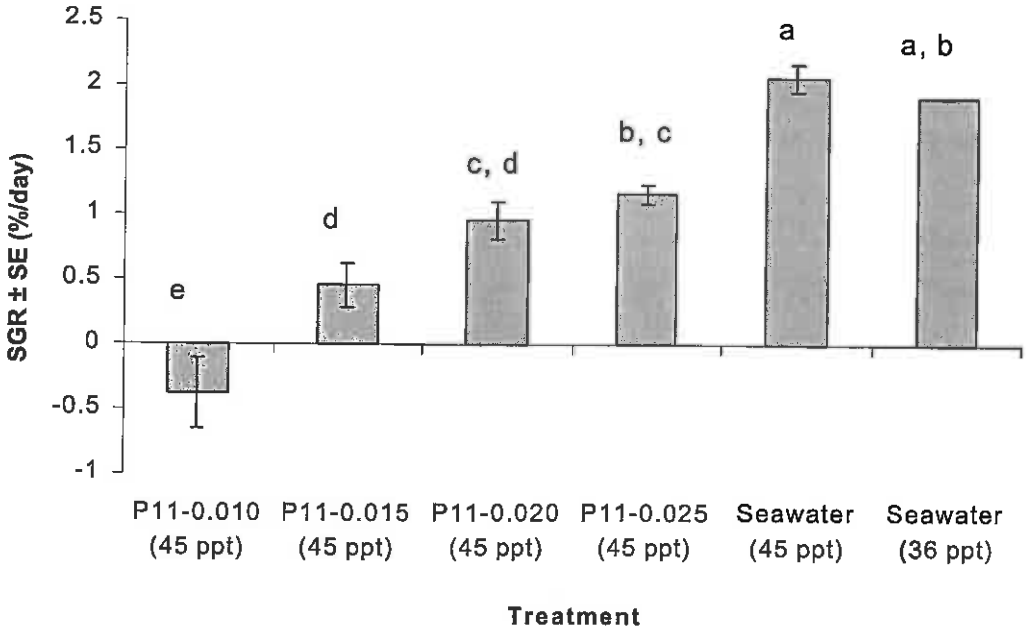


Figure 7 Specific growth rates of juvenile barramundi grown for 4 weeks in Toolibin groundwater (45 ppt) with various levels of potassium supplementation and two seawater controls. Columns sharing the same letter are not significantly different ($P<0.05$).

The SGR of barramundi grown in P11-0.025 was significantly less than those grown in the control with equivalent salinity (45 ppt). This indicates that some other factor within the Toolibin water limited growth. This could be either an excess or deficiency of another ion, or another factor not tested during the laboratory analyses. The high concentration of manganese in the Toolibin water would suggest it to be a possible candidate (Table 3). Indeed, manganese has been shown to damage gill epithelial

membranes and the gill's ability to regulate ions (McDonald et al., 1989). LC_{50} values for manganese in freshwater fish, although variable, have been reported as high as 3,000 ppm (Garg et al., 1989). The fact that high calcium concentrations have been shown to mediate the acute effects of manganese toxicity in freshwater fish (Gonzalez et al., 1990) would suggest that manganese should be less toxic in calcium-rich seawater. The chronic effects of manganese, however, cannot be extrapolated from acute data and it is possible that the levels present in the Toolibin groundwater have a long-term effect on growth. This effect could be due to the absolute concentration of manganese, or as a function of the high salinity of the water source, where damage to the gill epithelium would be more detrimental than at lower salinity. Further investigations into the chronic effects of elevated manganese concentrations and any potential salinity interaction on the growth of barramundi are clearly warranted.

The food consumption data presented in Table 5 shows that appetite was significantly less in all groundwater treatments compared to the 36 ppt seawater control. The food conversion ratio obtained in P11-0.015 was significantly higher than that obtained in P11-0.025 and both seawater controls.

	P11-0.010 (45 ppt)	P11-0.015 (45 ppt)	P11-0.020 (45 ppt)	P11-0.025 (45 ppt)	Seawater (45 ppt)	Seawater (36 ppt)
Food Intake (g)	74 ± 15^{c}	$84 \pm 3^{b,c}$	$96 \pm 9^{b,c}$	$84 \pm 6^{b,c}$	$129 \pm 16^{a,b}$	155 ± 4^{a}
FCR	N/A	4.18 ± 1.38^{a}	$1.67 \pm 0.30^{a,b}$	1.20 ± 0.09^{b}	0.83 ± 0.05^{b}	0.88 ± 0.06^{b}

Table 5 Food intake and food conversion ratios (FCR) of juvenile barramundi grown for 4 weeks in Toolibin groundwater with various levels of potassium supplementation. Values in the same row sharing the same letter are not significantly different ($P<0.05$).

On the basis of growth performance, it is difficult to make a statement on the minimum required K:Cl ratio for barramundi at the tested salinity (45 ppt), as it appears another factor, such as manganese, was effecting growth. The data presented on food utilisation, however, indicate that a K:Cl ratio of 0.015 is insufficient and a level of 0.020 may be marginal.

Results of the blood plasma analyses are shown in Figure 8. Both sodium and chloride blood plasma concentrations decreased with increasing level of potassium

supplementation. These data are consistent with those presented in Bioassay 1, where it was shown that hypernatraemia and hyperchloridaemia are due to a reduction in the excretion rates of these ions caused by the low external concentration of potassium.

There was no significant difference in blood plasma potassium concentration between any treatments. This data is consistent with that presented in the previous bioassay in which it was suggested that, like mammals, buffering of plasma potassium from the muscle occurs to maintain homeostasis of potassium concentrations in the extracellular fluid (ie plasma).

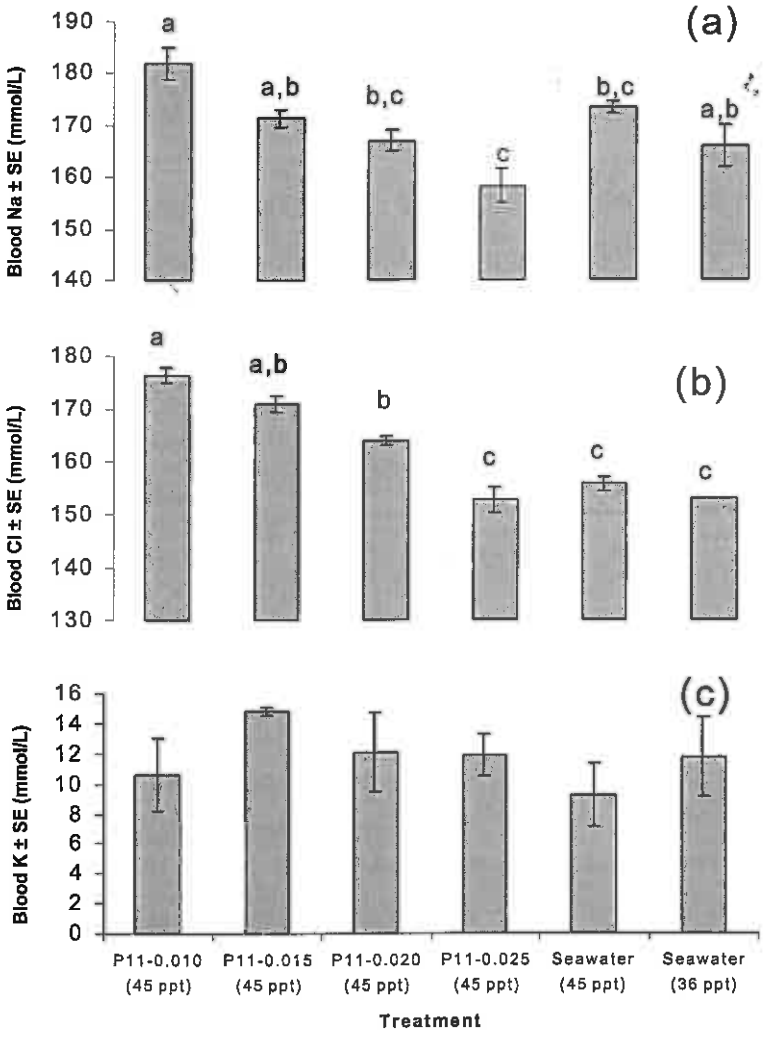


Figure 8: Concentrations of blood plasma sodium (a), chloride (b) and potassium (c) of barramundi cultured in Toolibin groundwater (45 ppt) supplemented with various levels of potassium and two seawater controls. Columns within each graph sharing the same letter are not significantly different ($P<0.05$)

8.5. Bioassay 3 – The effect of potassium supplementation on the performance of juvenile barramundi in Toolibin groundwater diluted to 16 ppt.

8.5.1. Rationale

Although the expression of potassium requirements as a ratio to chloride ions has been suggested as a means of accounting for water salinity, the physiological mechanisms regulating internal potassium in marine fish indicate that potassium requirements may decrease with decreasing salinity. That is, the requirements for potassium (expressed as K:Cl) may not be valid across all salinity ranges.

The aim of the current bioassay was to test this theory and determine the effect of total salinity on the potassium requirements of barramundi. This was achieved by repeating Bioassay 2 at the lower salinity of 15 ppt and comparing the results with those obtained at 45 ppt.

8.5.2. Methods

The methodology for this bioassay was identical to that described for Bioassay 2, with the exception that the P11 water was diluted from 45 to 15 ppt with carbon-filtered scheme water. Accordingly, the 45 ppt control treatment was also changed to one in which seawater was diluted to 15 ppt. Each treatment tank was stocked with the same size (39.3 ± 0.5 g) and number of barramundi as Bioassay 2 and the water bath maintained at the same temperature (28°C).

8.5.3. Results and Discussion

Survival in all tanks was 100%. There was no significant difference in growth of barramundi between any of the six treatments (Figure 9). This is in contrast to the result obtained in the previous trial, where the level of potassium supplementation had a significant effect on growth (see Figure 10 for a comparison of the growth data between the two trials). These data support the hypothesis that potassium requirements and salinity are not linearly correlated. Fish in a hyperosmotic

environment must constantly drink to balance the osmotic loss of water from the body (Karnaky, 1998). A consequence of drinking salt water is an uptake of ions into the body. To maintain their internal salt balance, these ingested salts (mainly sodium and chloride) must be excreted. The excretion of these ions occurs via the chloride cells located in the fishes gills; a process that requires potassium (Karnaky, 1998). As salinity declines, so too does the drinking rate and absorption of monovalent ions. It is suggested that at these lower salinities, proportionally less potassium is required to excrete sodium and chloride across the gills. ie. at lower salinities, fish have a lower K:Cl ratio requirement than at high salinities.

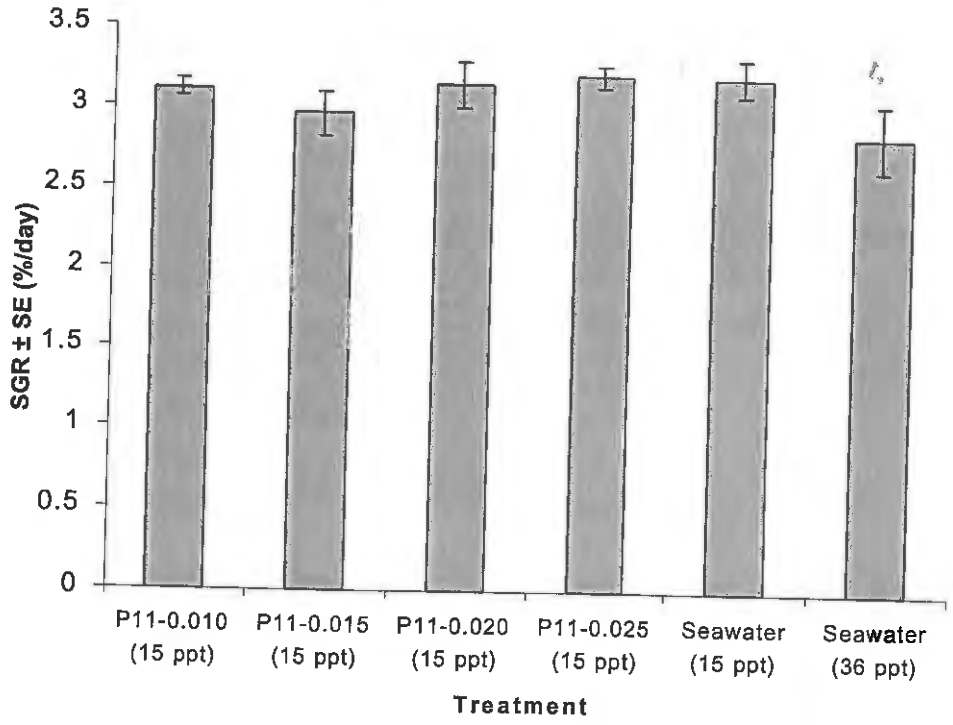


Figure 9: Specific growth rates of juvenile barramundi grown for 4 weeks in Toolibin groundwater diluted to 15 ppt, with various levels of potassium supplementation and two seawater controls. Columns sharing the same letter are not significantly different ($P<0.05$).

That there was no significant difference in growth between the 15 ppt control treatment and P11-0.025 (15 ppt) groundwater treatment supports the theories proposed in the previous bioassay that growth retardation in full potassium supplemented groundwater was the result of either the high manganese concentration, or the high Mn:Cl ratio. The data presented in this trial does not, however, provide evidence to favour either theory, as both the absolute concentration of manganese and it's ratio to salinity were reduced by diluting P11 water from 45 ppt to 15 ppt.

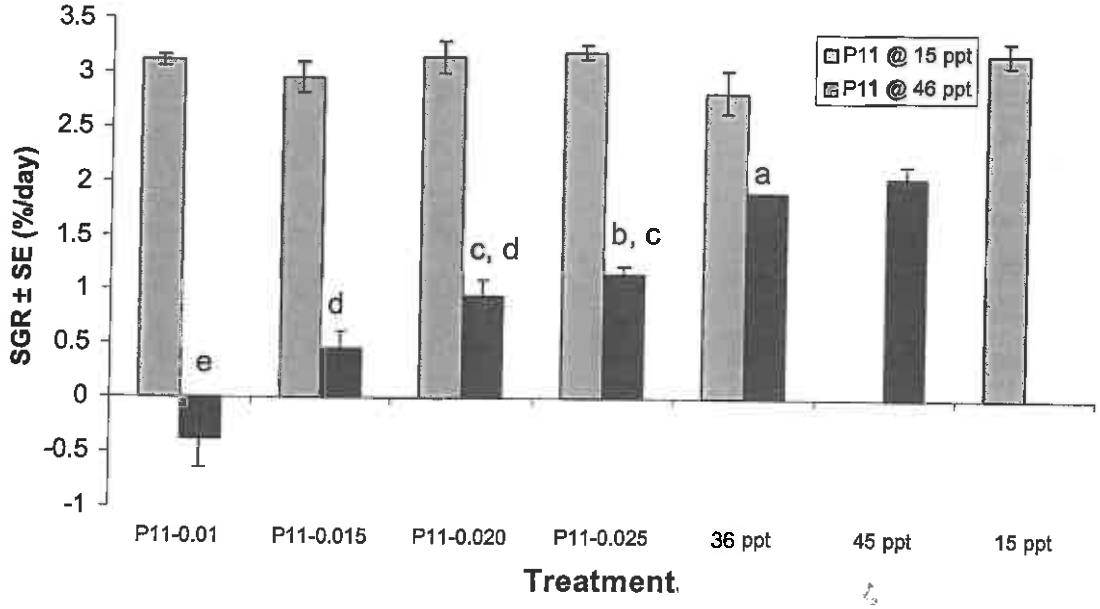


Figure 10: A comparison of specific growth of juvenile barramundi grown for 4 weeks in Toolibin groundwater with various salinities and levels of potassium supplementation. Like-coloured columns sharing the same letter are not significantly different ($P<0.05$).

Unlike Bioassay 2, there was no effect of potassium supplementation on food consumption or food conversion ratio (Table 6).

	P11-0.010 (15 ppt)	P11-0.015 (15 ppt)	P11-0.020 (15 ppt)	P11-0.025 (15 ppt)	Seawater (15 ppt)	Seawater (36 ppt)
Food Intake (g)	227 ± 11	215 ± 16	228 ± 11	235 ± 13	229 ± 7	211 ± 24
FCR	0.84 ± 0.02	0.85 ± 0.01	0.82 ± 0.02	0.81 ± 0.03	0.81 ± 0.001	0.88 ± 0.01

Table 6: Food intake and food conversion ratios (FCR) of juvenile barramundi grown for 4 weeks in Toolibin groundwater diluted to 15 ppt, with various levels of potassium supplementation and two seawater controls. Values in the same row sharing the same letter are not significantly different ($P<0.05$).

Results of the blood chemistry analyses are shown in Figure 11. There were no significant differences in blood sodium content between treatments with the three lowest levels of potassium supplementation (Figure 11a). Those fish held in P11-0.025, however, had significantly lower blood sodium content than those in P11-0.020 and both control treatments. There was no significant difference in blood chloride content between any of the six treatments. These data differ from the previous bioassays,

where a clear negative correlation between both sodium and chloride plasma concentrations and increasing level of potassium supplementation was demonstrated.

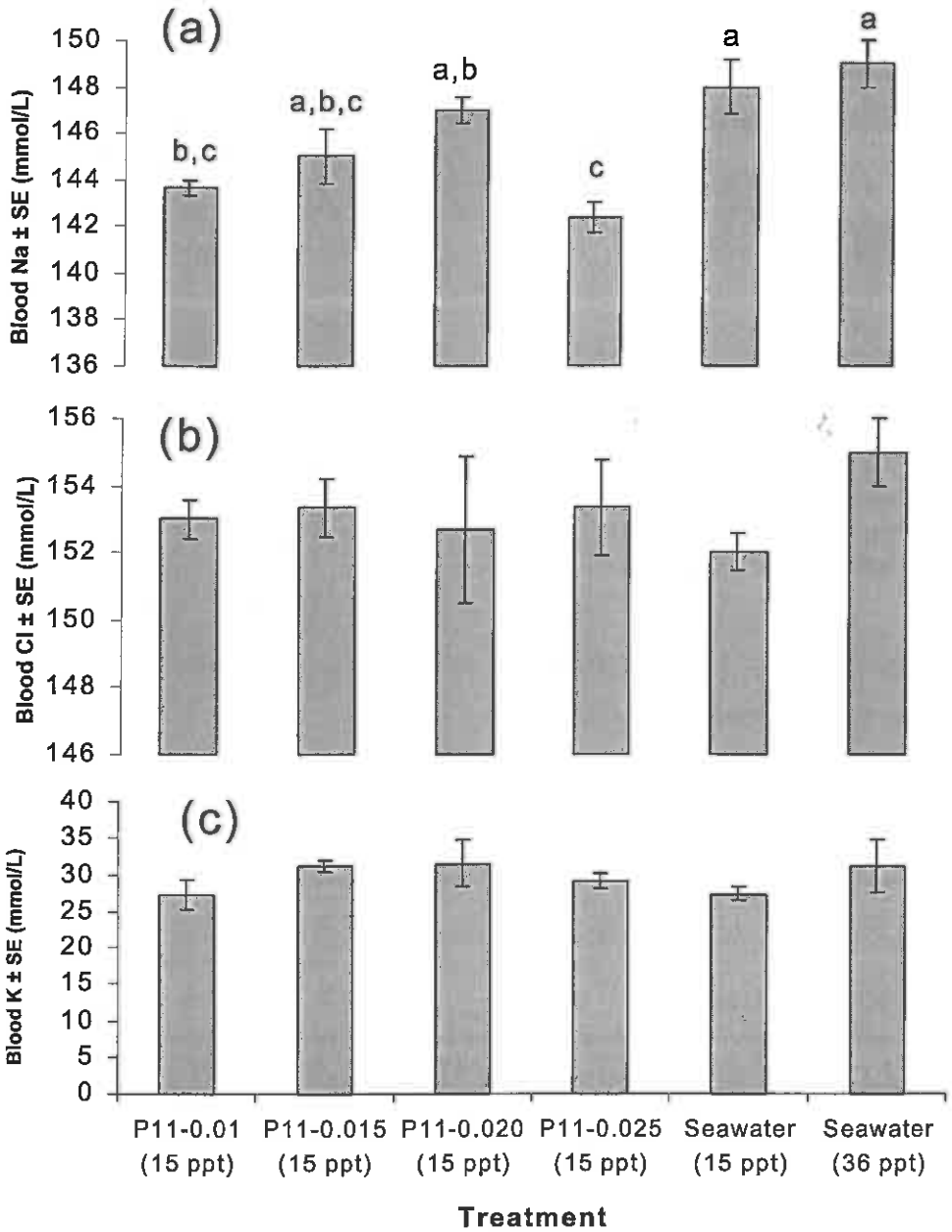


Figure 11: Concentrations of blood plasma sodium (a), chloride (b) and potassium (c) of barramundi cultured in Toolibin groundwater diluted to 15 ppt, with various levels of potassium supplementation and two seawater controls. Columns within each graph sharing the same letter are not significantly different ($P<0.05$).

Due to the close relationship between the excretion of sodium and chloride, it is expected that both should show a similar pattern with respect to external potassium

concentration (as was shown in Bioassays 1 & 2). The reason behind the significantly lower level of blood sodium in the P11-0.025 treatment and the lack of correlation between the plasma sodium and chloride is unclear.

That there were no significant differences in blood potassium concentrations between treatments is consistent with the findings from the previous barramundi bioassays.

8.6. Bioassay 4 – The effect of potassium supplementation on the performance of juvenile mulloway in 'full-strength' Toolibin groundwater (45 ppt).

8.6.1. Rationale

Results of the screening bioassay showed that mulloway do not survive in raw Toolibin water. That fish survived in potassium-supplemented water over this short-term trial suggested that mulloway culture may be possible in potassium-supplemented Toolibin water. This bioassay therefore sought to determine the potential of culturing mulloway in potassium-supplemented Toolibin water and the minimum K:Cl ratio required to maximise growth in this species at the salinity of the Toolibin groundwater.

8.6.2. Methods

The methodology for the current bioassay was identical to that described for Bioassay 2, with the exception that the controlled temperature water bath was maintained at a temperature of 23°C. Each tank was stocked with eight mulloway with an average initial weight of 40.6 ± 0.6 g.

8.6.3. Results and Discussion

All mulloway in all saline groundwater treatments died within 3 days of entering the water sources. The trial continued for two weeks to compare the performance of mulloway in the two control treatments. At the end of this period, survival in both treatments was 100% and there was no significant difference in growth between mulloway grown in 36 ppt (SGR = 1.10 ± 0.16 %/day) and those grown in the 45 ppt control (SGR = 1.20 ± 0.02 %/day). These data suggest that the mortality of mulloway was due neither to the salinity of the groundwater source, nor it's potassium content (as complete mortality also occurred in the treatment with an equivalent K:Cl ratio to seawater (P11-0.025)).

Due to the possibility that this particular batch of groundwater was contaminated during transport or pre-treatment, the trial was repeated with new water pumped from Lake Toolibin, however, the same result was obtained.

A short-term trial was undertaken in which these same water sources were diluted from 45 ppt to 36 ppt. Fish in these treatments survived and grew over the two week test period, however, no data was collected on growth rates. As with Bioassays 2 & 3, these data suggest that excessive ions (or an excessive ion:chloride ratio) in the 'full-strength' Toolibin water were diluted to a safe level on dilution of the groundwater to 36 ppt. If it is the manganese that is toxic (see Section 6.3 and 8.4.3), these data suggest that mulloway are more sensitive to high manganese concentrations than barramundi, which, unlike mulloway, survived and grew in full-strength, potassium-supplemented Toolibin groundwater.

8.7. Bioassay 5 - The effect of potassium supplementation on the performance of juvenile mulloway in Toolibin groundwater diluted to 36 ppt.

8.7.1. Rationale

The previous bioassay revealed that mulloway could not survive in 'full-strength' Toolibin water, even with potassium supplementation equivalent to seawater, however, a short-term trial indicated that dilution of the water source to 36 ppt enabled survival. This bioassay therefore sought to determine the effect of potassium supplementation on the survival and growth of mulloway at this salinity.

8.7.2. Methods

Toolibin water was diluted to a salinity equivalent to full-strength seawater (36 ppt) and the same K:Cl ratios described in Bioassay 2 were investigated (0.01, 0.015, 0.020 and 0.025). Each tank was stocked with similar sized mulloway to the previous test in which mulloway died (45.0 ± 0.6 g) and the water bath again set at 23°C.

8.7.3. Results and Discussion

Survival in this trial was 100%, providing additional circumstantial evidence that dilution from 45 to 36 ppt resulted in a toxic agent being diluted to a safe level.

The specific growth rate of these fish is shown in Figure 12. No significant differences in growth were observed between any of the six treatments. Fish in the 36 ppt control ate significantly more food than those in P11-0.020, however, there were no significant differences between treatments for FCR (Table 7).

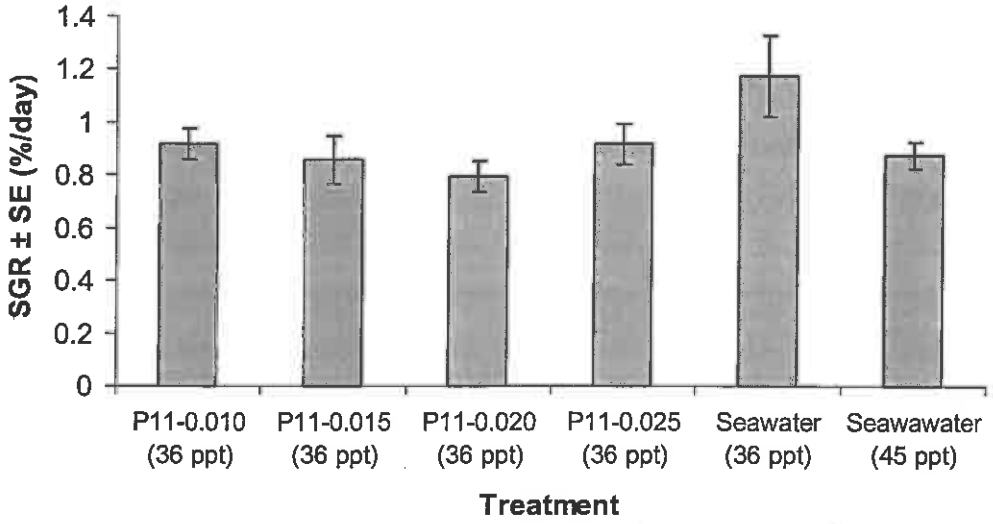


Figure 12: Specific growth rates of juvenile mulloway grown for 4 weeks in Toolibin groundwater diluted to 36 ppt, with various levels of potassium supplementation and two seawater controls. Columns sharing the same letter are not significantly different (P<0.05).

	P11-0.010 (36 ppt)	P11-0.015 (36 ppt)	P11-0.020 (36 ppt)	P11-0.025 (36 ppt)	Seawater (36 ppt)	Seawater (45 ppt)
Food Intake (g)	116 ± 4 ^{a,b}	116 ± 1 ^{a,b}	104 ± 3 ^{a}	109 ± 8 ^{a,b}	134 ± 8 ^{b}	112 ± 4 ^{a,b}
FCR	0.88 ± 0.02	0.97 ± 0.10	0.94 ± 0.08	0.82 ± 0.02	0.78 ± 0.07	0.89 ± 0.05

Table 7: Food intake and food conversion ratios (FCR) of juvenile mulloway grown for 4 weeks in Toolibin groundwater diluted to 36 ppt, with various levels of potassium supplementation and two seawater controls. Values in the same row sharing the same letter are not significantly different (P<0.05).

Analysis of blood plasma revealed patterns very different to those observed with barramundi in Bioassays 1 and 2. There were no significant differences between treatments for either blood sodium or chloride (Figure 13), whereas blood potassium increased with increasing levels of supplementation. The level of blood plasma potassium in fish from the treatment with the highest level of potassium supplementation (P11-0.025) was significantly greater than those held in water with the lowest level of supplementation (P11-0.010) (Figure 13). This is in contrast to the findings obtained with barramundi in Bioassays 1 and 2 and those of Maetz (1969). These data suggest that mulloway do not regulate their blood plasma potassium

content but do regulate both the sodium and chloride content of their plasma. Barramundi on the other do the opposite, that is regulate the potassium level of their blood over a wide range of external potassium contents, whereas their sodium and chloride content both decrease with increasing potassium supplementation. The physiological mechanism behind the approach employed by barramundi was detailed in Section 8.3.3. The mechanism employed by mulloway cannot be explained by that for barramundi and remains unclear. Noteworthy, however, is the difference in blood plasma concentrations between the two species. In all barramundi bioassays, plasma concentrations ranged from 10 to 32 mmol/L, whereas those of the mulloway have been between 3 and 5 mmol/L. Perhaps mulloway excrete sodium and chloride, without a high requirement for potassium or alternatively, they may have a mechanism that minimises osmotic water loss and they therefore drink less than barramundi. Information from gill histology and data on drinking rates of mulloway would be useful in helping elucidate the mechanism behind this approach.

Although a direct comparison between the potassium requirements of mulloway and barramundi cannot be made, due to differences in treatment salinities, the different patterns of blood plasma electrolytes in response to varying water K:Cl ratios suggest that the two species employ different approaches to dealing with potassium deficiency. The mulloway's approach can be considered as a conforming one, whilst that of barramundi is one of regulation. One approach may enable a greater tolerance to low K:Cl than the other, and a comparison between species at the same salinity and over a lower range of K:Cl is required to determine if this is the case.

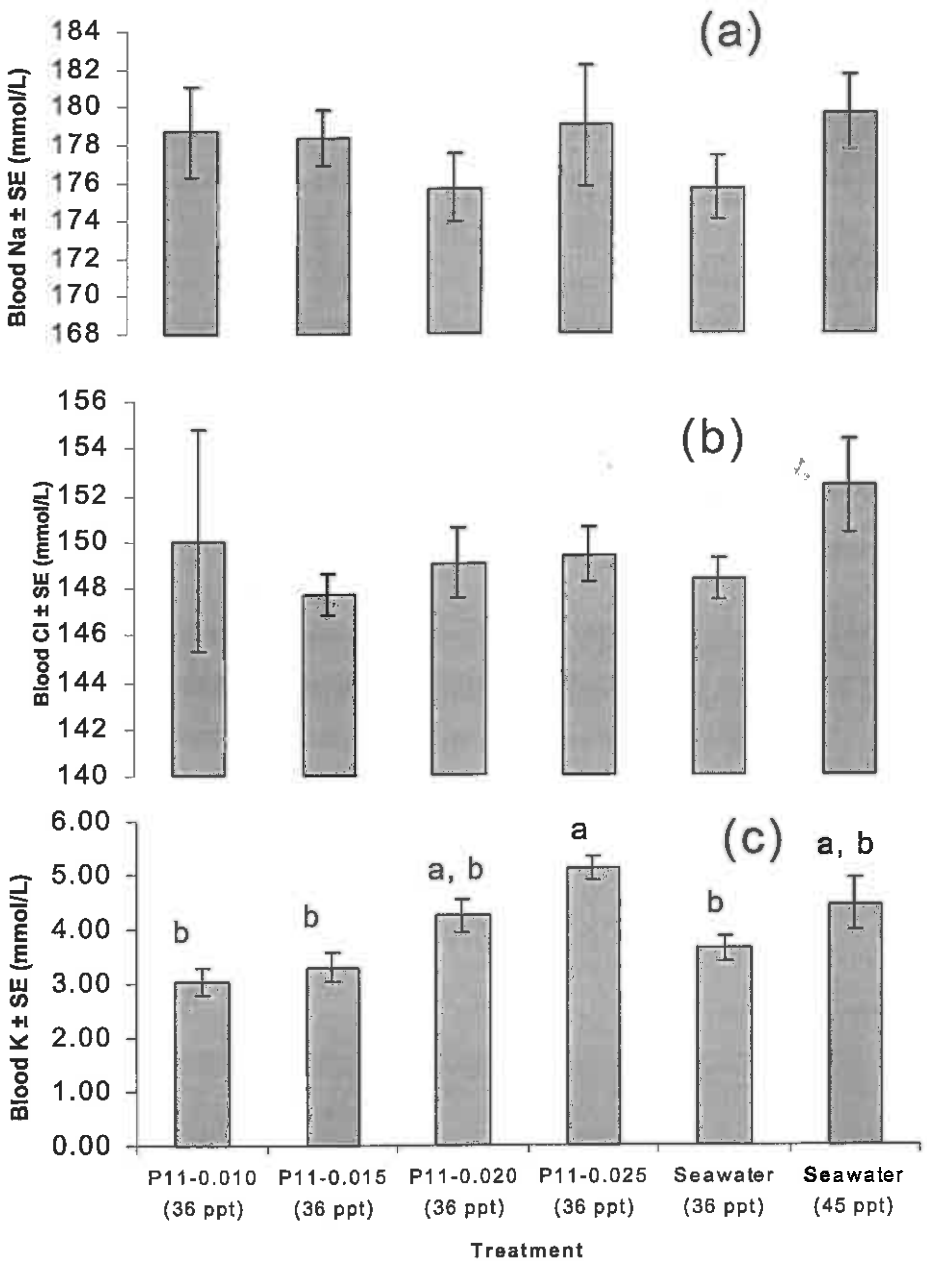


Figure 13: Concentrations of blood plasma sodium (a), chloride (b) and potassium (c) of mulloway cultured in Toolibin groundwater diluted to 36 ppt, with various levels of potassium and two seawater controls. Columns within each graph sharing the same letter are not significantly different ($P<0.05$)

8.8. Bioassay 6 - The performance of juvenile mulloway in Westonia groundwater.

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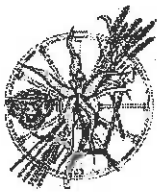
Government of Western Australia



8.8.1. Rationale.

Results of the current project have shown that groundwater from Lake Toolibin would require significant pre-treatment for fish culture to be feasible. Saline groundwater sources vary considerably in quality and, as such, sources having the water quality characteristics determined during the current project as required for optimum survival and growth are likely to exist.

During August 2003, heavy rainfall at Lake Toolibin prevented the collection of water for bioassays. The opportunity was therefore taken to assess a saline groundwater source from the Wheatbelt town of Westonia that, based on preliminary laboratory tests, showed excellent potential for fish culture. With funding from the Avon Catchment Council, detailed laboratory testing and a bioassay was therefore conducted on this water source.

8.8.2. Methods

All of the laboratory tests described in Section 6.2 were conducted on the Westonia water source (WRC002).

During the bioassay, four treatments were investigated; 'raw' WRC002 water (K:Cl = 0.01), this water supplemented with potassium to a level equivalent to 16 ppt seawater (K:Cl = 0.025) and two seawater control treatments, 'full-strength' seawater (36 ppt) and seawater diluted to the same salinity as WRC002 (ie. 16 ppt). Eight juvenile mulloway (64.7 ± 6.6 g) were randomly allocated into each of 12 tanks within the bioassay system (See section 8.2.2), which was set to a temperature 23°C.

At the completion of the trial, fish were weighed and sampled for blood electrolytes as previously described (See section 8.2.2). In addition, a sample of dorsal muscle was

taken and pooled for each replicate. These muscle samples were freeze-dried and ground, prior to digestion in concentrated nitric acid. The sodium and potassium content of the digest was determined using ICP-AES.

8.8.3. Results and Discussion

With a salinity of 16 ppt, the water from WRC002 was much less salty than the Toolibin groundwater sources (45 ppt). The water source was found to be non-toxic via Microtox®. The post-aerated CO_2, pH and alkalinity were all considered optimal for fish culture (Table 8). The K:Cl ratio (0.01) was higher than that of the Toolibin water (0.005). The manganese content of the WRC002 water (0.19 ppm) was higher than seawater but considerably lower than the Toolibin water sources (1.5 – 2.0 ppm). (Table 8). Some precipitation of iron occurred after aeration, however, was not enough to require filtration prior to use.

The specific growth rates of mulloway in the various treatments are shown in Figure 14. That there was no significant difference in growth between fish in 'WRC002 + K' and 'WRC002 raw' indicates that the level of potassium in WRC002 (K:Cl = 0.01) is sufficient for maximum growth of mulloway and supplementation is therefore not required. This data is consistent with that obtained in Bioassay 5 and shows that a K:Cl ratio of 0.010 is adequate for mulloway over the range of tested salinities (16-36 ppt).

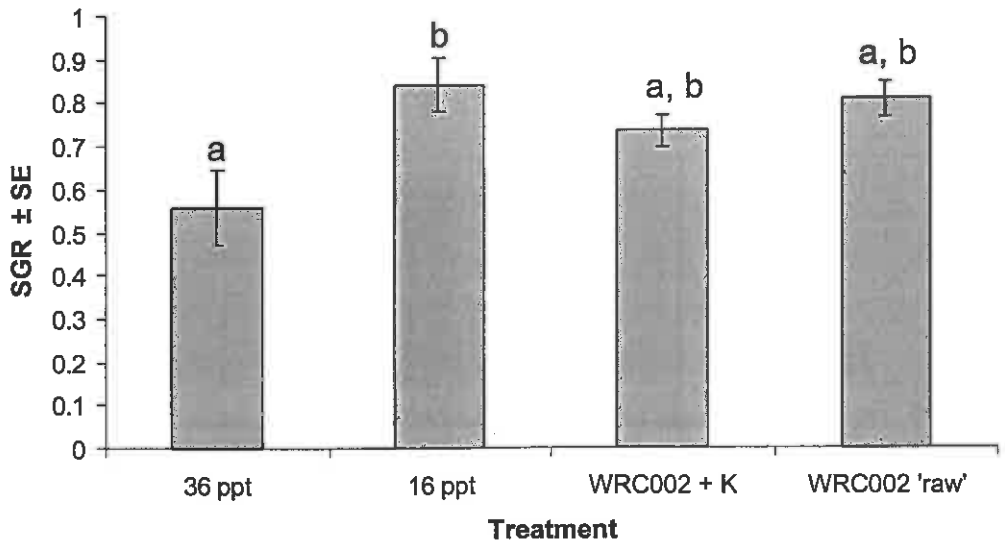


Figure 14: Specific growth rates of juvenile mulloway grown for 6 weeks in raw and potassium-supplemented Westonia groundwater and two seawater controls. Columns sharing the same letter are not significantly different ($P<0.05$)

The fact that there was no significant difference in growth between the groundwater treatments and those in the diluted seawater (Figure 14) suggests that WRC002 contains no other ions lacking or excessive to effect the growth of mulloway. Those fish grown in 16 ppt grew significantly faster than those cultured in 36 ppt, indicating that this lower salinity is preferential for maximising growth in this species. This data is supported by Fielder and Bardsley (1999) who suggested that mulloway perform best in low salinity water.

	WRC002
	Concentration
pH	7.5
CO_2	5.5
Alkalinity	225
Al	<0.006
As	<0.02
B	2.8
Ca	270
Cd	<0.001
Cl	9000
Co	0.012
Cr	0.004
Cu	<0.002
Fe	<0.002
K	96
Mg	720
Mn	0.19
Mo	<0.008
Na	5100
Ni	0.13
P	<0.04
Pb	<0.02
S	370
Se	<0.04
Sn	<0.02
Sr	3.0
V	<0.002
Zn	<0.002
K:Cl	0.011

Table 8: Water quality data from WRC002, Westonia. Values are for post-aerated water samples and are in ppm (except pH).

Results of the blood plasma analyses are shown in Figure 15. Figure 15c shows there is a significant effect of treatment on blood potassium content, but no effect on sodium

or chloride blood plasma levels (Figures 15a and 15b, respectively). Those fish cultured in 'WRC002 raw' had a significantly lower plasma potassium concentration than all other treatments and there was no significant difference in blood potassium content between the 16 ppt control treatment and the WRC002 + K.

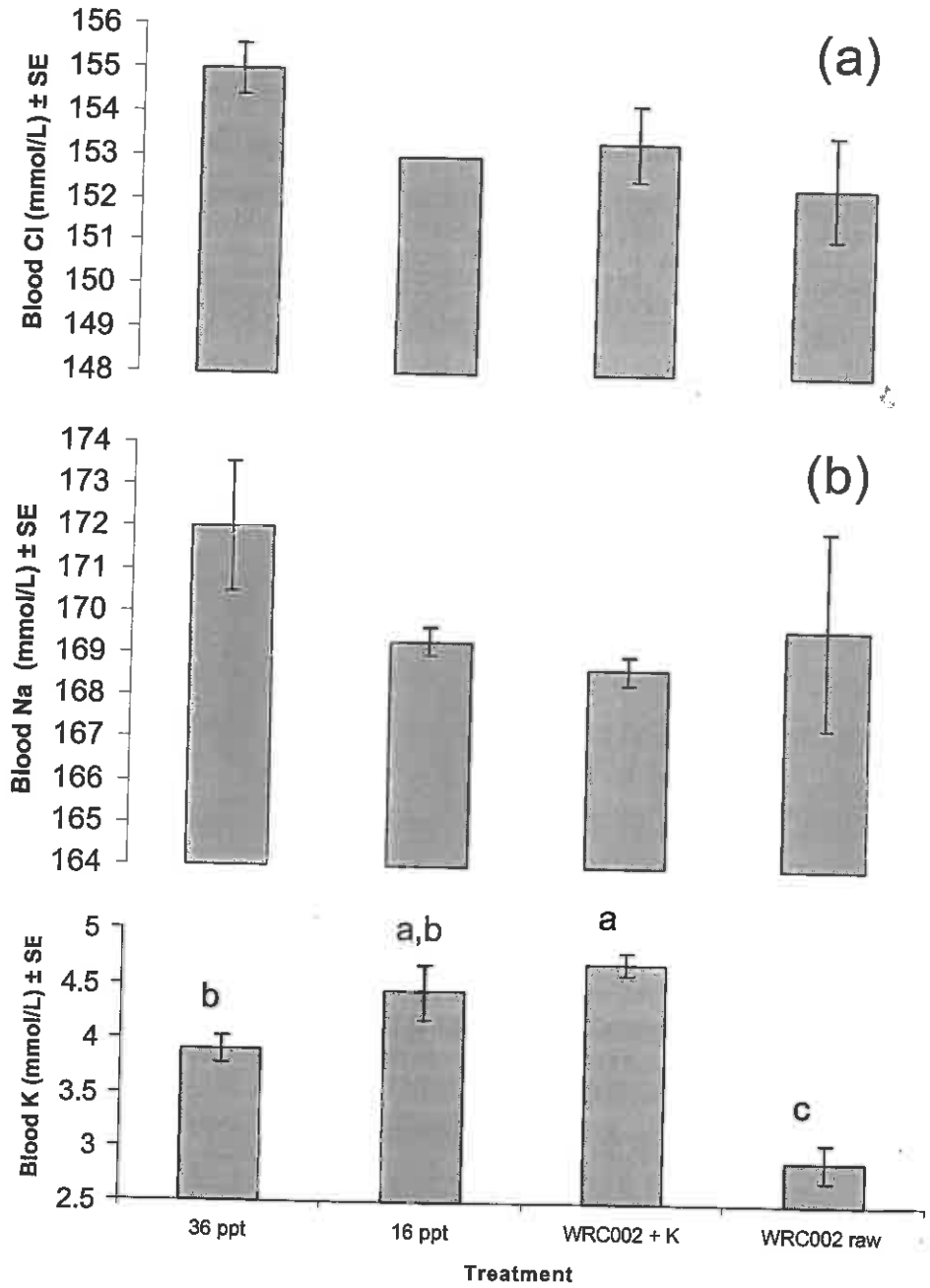


Figure 15: Concentrations of blood plasma sodium (a), chloride (b) and potassium (c) of mulloway cultured in raw and potassium supplemented Westonia groundwater (16 ppt) and two seawater controls. Columns within each graph sharing the same letter are not significantly different ($P<0.05$)

Data on muscle potassium and sodium content are shown in Figure 16. These data reveal no significant difference in muscle potassium content between treatments. The muscle sodium content was significantly higher in fish cultured in 36 ppt, however, there was no significant difference between any treatments with a salinity of 16 ppt.

The data presented on the blood and muscle potassium contents of mulloway in WRC002 are consistent with those found with mulloway in Bioassay 5. They support the theory previously presented (Section 8.7.3) that this species does not maintain homeostasis of plasma potassium through buffering from muscle, however, data from this bioassay gives no further indication on the physiological mechanism behind this quite different approach to potassium deficiency.

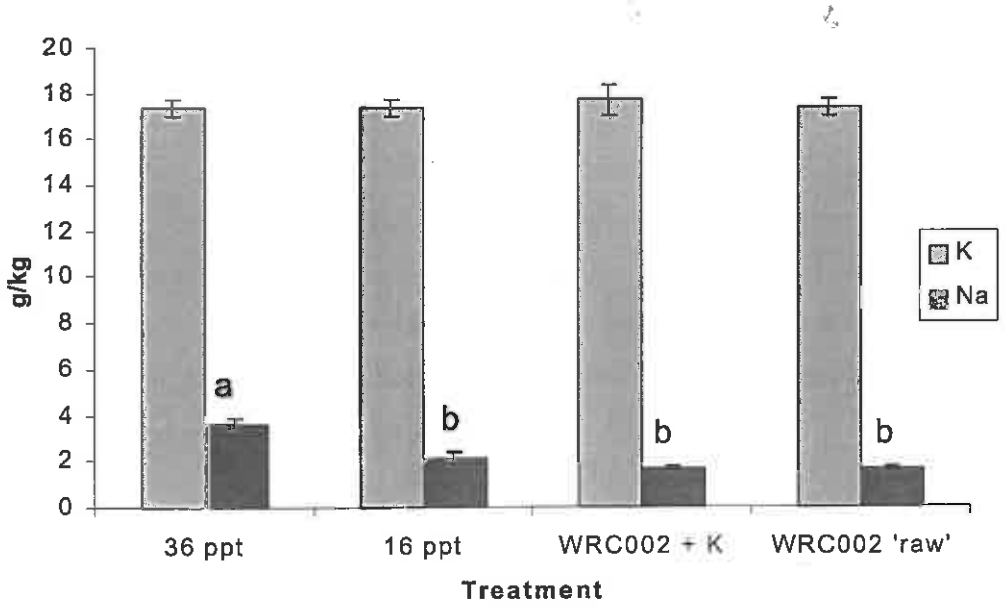


Figure 16: Concentrations of muscle potassium and sodium of mulloway cultured in raw and potassium supplemented Westonia groundwater (16 ppt) and two seawater controls. Like-coloured columns sharing the same letter are not significantly different ($P<0.05$)

9. CONCLUSIONS

Saline groundwater from Lake Toolibin is hypersaline (ca. 45 ppt), deficient in potassium and alkalinity and excessive in manganese, relative to seawater. The water also contains very high levels of ferrous iron, which oxidised on exposure to air. Filtration was required to remove this thick orange precipitate prior to conducting bioassays.

Bioassays conducted in 'raw' Toolibin groundwater resulted in mortality of snapper, barramundi and mulloway. Subsequent bioassays found that these mortalities were caused primarily by the low potassium content of the water. Circumstantial evidence also suggested that the high levels of manganese in the 'full-strength' groundwater (45 ppt) may have caused mortality in mulloway and growth retardation in barramundi.

These data show that for the Toolibin groundwater source to be suitable for commercial fish culture, substantial pre-treatment would be required including degassing, filtering/flocculation, buffering, dilution and supplementation. Due to the significant costs associated with such pre-treatment it is concluded that the groundwater from Lake Toolibin is not suitable for commercial fish culture.

Additional bioassays investigating the potassium requirements of barramundi and mulloway at various salinities were valuable in revealing the physiological mechanisms behind potassium regulation in these species and provided data valuable to assessing other potential saline groundwater sources for these species. This evidence suggests that mulloway grow equally well at K:Cl ratios from 0.010 to 0.025 at salinities between 16 and 36 ppt. Barramundi also perform equally well in this range of K:Cl ratios at low salinities, but in hypersaline water (45 ppt) require a K:Cl ratio of at least 0.020 to ensure an adequate food conversion ratio is obtained.

Although the Toolibin groundwater source was found to be unsuitable, groundwater from Westonia was found to be suitable for mulloway (and most likely barramundi) without any pretreatment. Growth of mulloway in this untreated water source was equal to that obtained in seawater adjusted to the same salinity (16 ppt).

10. BUDGET

Item	*Supplier*	*Cost*
Seasalt	Aquasonic	$ 1,122.57
Barramundi	West Beach Aquaculture	$ 315.00
Mulloway	SARDI	$ 651.20
Snapper	ADU (journal transfer)	$ 120.00
Water bath construction	ADU (journal transfer)	$ 450.00
ICPAES analyses for water and muscle samples	Marine and Freshwater Research lab	$ 2,208.30
Blood analysis	Muroch University, Clinicial Pathology	$ 1,042.00
Microtox analysis	Geotechnical Services	$ 737.00
Truck hire	Department of Agriculture, WA	$ 570.00
Miscellaneous (Couriers & Aquarium incidentals)	ADU (journal transfer)	$ 252.00
Heaters (for water bath)	ADU (journal transfer)	$ 500.00
Total		$ 7,968.07

11. DISSEMINATION

Scientific Papers

Partridge, G. J. and Creeper, J. (In prep). Skeletal myopathy in barramundi, *Lates calcarifer* (Bloch, 1790) cultured in potassium depleted saline groundwater. Journal of Fish Diseases.

Partridge, G. J. (In prep.). The effect of salinity on the potassium requirement of barramundi *Lates calcarifer* (Bloch, 1790) in saline groundwater. Aquaculture.

Popular Articles

West Australian Newspaper. 18th September 2002 "Fish in Salt Solution"

Seven Waves, September 2002. "Saline solution for fish harvest"

ProWest (Western Australia's Professional Fishing Industry Magazine). November/December 2002 "Wheatbelt barra plan wins award"

ACWA News (Aquaculture Council of Western Australia – Official Newsletter) Issue No. 41 April 2003. "Investigations into inland saline fish culture recognised by AFFA award to ADU scientist Gavin Partridge"

Austasia Aquaculture Magazine Vol 17 No. 4 "Inland Saline Research in WA"

Television/Radio

Mr Geoff Edwards from the ABC program "On-the-land" contacted the investigator with the view to doing a story on the current project. Mr Edwards has agreed to do this story in 2004, when the Science and Technology Grant awarded to the investigator and his colleague, Dr Gavin Sarre, is underway (See Section 12 – Outcomes for details on this project)

CSIRO National Science Radio "Farming the inland sea"

Workshops/Conferences

"Snapper Culture – The WA Perspective." Presentation given at the Aquafin CRC snapper workshop, 26th September, 2002, Melbourne, Victoria.

"Utilising saline groundwater for culturing marine and estuarine fish." Presentation given at the Western Inland Fisheries Co-operative's annual general meeting, March, 2003, Northam, Western Australia

"Working towards the development of a sustainable marine/estuarine finfish aquaculture industry in the WA wheatbelt. Identification of suitable species and

water sources". Presentation prepared for the State Landcare Conference, October 2003, Katanning, Western Australia.

"Inland saline aquaculture – an opportunity for farm diversification". Presentation given to the Facey Group's Women in Agriculture, Wickepin, - August 21, 2003.

The results of the project are disseminated to farmers on an on-going basis through the FarmBis sponsored course titled "Aquaculture Potential in the WA Wheatbelt", run collaboratively by CY O'Connor College of TAFE (Dr Gavin Sarre) and the Challenger TAFE (Mr Gavin Partridge).

12. OUTCOMES

At present, there is no marine fish industry in Australia utilising saline groundwater sources. The overall research goal of the investigator is to determine whether such culture is biologically and technically feasible. This objective involves research into identifying appropriate water sources, species capable of rapid growth in these sources and to investigate culture technologies appropriate to these water sources and species.

The current project was highly successful in meeting its objectives and also in contributing to the first two research objectives of the investigator outlined above. Although it was concluded that the Lake Toolibin groundwater sources are unsuitable for commercial finfish culture (See Section 4 - Summary), the results it generated are directly relevant to other salt affected areas throughout Australia. The project was successful in demonstrating that barramundi and mulloway can be grown in saline groundwater at rates equivalent to seawater, provided the water sources have the appropriate salinity, potassium concentrations and are free of toxins.

Investors are increasingly looking towards locating aquaculture enterprises in inland areas as an alternative to expensive and environmentally sensitive coastal sites. The current project, with its scientific approach to assessing potential inland saline water sources has generated significant interest in private companies, rural shires, landowners and other government agencies in the potential of saline groundwater for aquaculture. A series of other water sources around the WA Wheatbelt has subsequently been selected for similar laboratory and bioassay testing. The investigator has, and continues to, disseminate the results of this project and other bioassays to such groups to ensure they are kept up to date with current research.

As outline above, the third objective of the investigator in determining the viability of commercial marine fish farming in inland saline water is the identification of appropriate culture technology. A recent National R&D plan for inland saline aquaculture suggested that semi-intensive pond culture has the greatest prospect for commercial culture. The same report, however, states that 'no one has succeeded in growing a single crop of marine fish to marketable size in an open pond environment in Australia using inland saline water' (Allan et al., 2002). Although semi-intensive

ponds have many advantages for inland areas, a major constraint to their commercial use stems from the fact that they are prone to the boom-bust phytoplankton cycles described by Erler et al. (1999). When these blooms 'crash', oxygen is depleted and fish mortalities often occur. Such semi-intensive pond systems (which include in-pond cage culture) are therefore limited in the yields they can produce, as high stocking densities accelerate the build up of sediment and the severity of microalgal blooms. As pond sediments act as a major sink of ammonia, preventing their build-up will be an effective means of breaking this cycle and thereby allow greater densities of fish to be cultured

During the course of this project, the investigator has been working closely with industry partner, McRobert Aquaculture Systems in the design of a 'Semi-Intensive Floating Tank System' (SIFTS), which incorporates waste removal technology capable of minimising microalgal blooms. A scale-model prototype SIFTS has been constructed at Challenger TAFE's Aquaculture Development Unit and has proven the effectiveness of the waste removal system. In addition, the scale-model has suggested that increases in pond yield by a factor of at least 10 are realistically achievable in comparison to the technology currently employed.

Sufficient data and knowledge have now been collected on suitable water sources, species and appropriate culture systems. The construction of a demonstration farm in the WA Wheatbelt is the next step required to advance this research into a viable industry for rural Australia. The results of the current project have contributed to the recent obtainment of a grant from the Science and Technology Innovation Fund, which will test a commercial sized array of SIFTS. Mulloway will be used as the model species and a water body selected that has the characteristics defined in the current project to maximise growth and survival of this species. This project will also attempt to leverage commonwealth funds from the Rural Industries Research and Development Corporation (RIRDC) and the Fisheries Research and Development Corporation (FRDC) to expand this preliminary project into a larger demonstration project. In addition to move rigorous testing of the SIFTS, the proposed commonwealth project addresses the issue of sustainability and waste-water from the aquaculture facility will be used to irrigate a patented, commercially valuable halophytic plant crop (Leake et al., 2002). This research project has realistic and meaningful commercial outcomes and the potential for rapid adoption by farmers throughout salt affected Australia.

13. ACKNOWLEDGEMENTS

Special thanks to Mr Greg Jenkins, Aquaculture Manager, Challenger TAFE; Mr John Creeper, Pathologist, Department of Fisheries WA and to Mr Damon Bourket, Mr Gavin Kay and Mr Julian Seah for their technical assistance.

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