Inland Saline Aquaculture: Overcoming Biological and Technical Constraints Towards the Development of an Industry.

By

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BSc (Hons)

A Thesis presented for the Award of Doctor of Philosophy to the School of Veterinary and Biomedical Sciences at Murdoch University, Western Australia
Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

Signed……………………………..
Acknowledgements

When I started on this journey I wasn’t even married. I now have a wife and two kids; who said it wouldn’t take long! Firstly to all of my family, Joanne, Will and Caitlin, Mum and Dad, Mark, Karen and Sarah a sincere thanks for all of your support and encouragement over the last six years and beyond.

Another big thanks to my supervisor Alan Lymbery and to all of my work colleagues, particularly to Greg Jenkins for his support, willingness to allow me to study part-time and encouragement to get it finished. Gavin Sarre for all of his assistance in the field and everyone else who provided technical assistance along the way including Gavin Kay, Damon Bourke and Rob Michael. Another big thanks to Ian McRobert and Bruce Ginbey, co-inventors of the Semi Intensive Floating Tank System (SIFTS) technology.

I would also like to thank Challenger TAFE for providing me with financial support and the opportunity to combine full-time work and part-time study. The project was also supported by Murdoch University, the Australian Department of Fisheries, Forestry and Agriculture, the Department of Conservation and Land Management, the Western Australian Department of Education and Training’s Science and Innovation Strategy, the Australian Government’s National Aquaculture Council and the Fisheries Research and Development Corporation.
Abstract

Secondary salinisation has rendered over 100 million hectares of land throughout the world, and over 5 million hectares in Australia, unsuitable for conventional agriculture. The utilization of salinised land and its associated water resources for mariculture is an adaptive approach to this environmental problem with many potential economic, social and environmental benefits. Despite this, inland mariculture is yet to develop into an industrial-scale, rural enterprise. The main aim of this study was therefore to identify and address some of the technical and biological limitations to the development of an inland finfish mariculture industry.

Three technical aspects essential to the development of an Australian inland mariculture industry were reviewed: potential sources of water, the species suitable for culture in these water sources and the production systems available to produce them. Based on factors such as their quantity, quality and proximity to infrastructure, the most appropriate water sources were deemed to be groundwater obtained from interception schemes and waters from operational or disused mines. In terms of species, mulloway (Argyrosomus japonicus) were identified as having many positive attributes for inland mariculture, including being temperate and therefore having the ability to be cultured year-round in the regions where the majority of secondary salinity occurs. Seasonal production of barramundi (Lates calcarifer) in ponds in the temperate climatic zones has potential, but may be more appropriate for those salinised water sources located in the warmer parts of the country. Rainbow trout (Oncorhynchus mykiss) were also identified as having excellent potential provided water temperature can be maintained below the upper lethal limit and also have potential for seasonal production, perhaps in
rotation with barramundi. In terms of production systems, pond-based culture methods were found to have many advantages specific to inland mariculture. Static ponds enable culture in areas with low groundwater yield and more cost-effective potassium supplementation compared with flow through ponds. Static ponds also largely overcome the issues associated with the disposal of salt-laden and eutrophied waste water; however yields from static ponds are typically low and limited by the nutrient input into the pond.

In response to the yield constraints of static pond culture, a new culture technology known as the Semi-Intensive Floating Tank System (SIFTS) was designed, patented and constructed in collaboration with the aquaculture industry and tested in a static inland saline pond in the wheatbelt of Western Australia. This technology was designed to reduce nutrient input into ponds by the collection of settleable wastes and to provide large volumes of well-oxygenated water to the target species, to ameliorate the loss of fish from low dissolved oxygen during strong microalgal blooms. The three species identified above has having excellent potential for inland mariculture (mulloway, rainbow trout, and barramundi) were grown in SIFTS held within a 0.13 ha static, inland saline water body (salinity 14 ppt) over a period of 292 days, yielding the equivalent of 26 tonnes/ha/year (total for all three species). Rainbow trout were grown with an FCR of 0.97 from 83 to 697 grams over 111 days (SGR, 1.91%/day) between June and September, when average daily water temperatures ranged from 12.3°C to 18.2°C. Over the same time period, mulloway grew only from 100 to 116 grams, however, once temperatures increased to approximately 21°C in October, feed intake increased and mulloway grew to an average size of 384 grams over 174 days with an SGR and FCR of 0.68%/day and 1.39, respectively. Barramundi stocked in November
with an average weight of 40 grams increased to 435 grams in 138 days (SGR 1.73%/day) with an FCR of 0.90. The SIFTS significantly reduced nutrient input into the pond by removing settleable wastes as a thick sludge with a dry matter content of 5 to 10%. The total quantity of dry waste removed over the 292 day culture period was 527 kg (5 tonnes/ha/yr), which was calculated to contain 15 kg of nitrogen (144 kg/ha/yr) and 16 kg of phosphorus (153 kg/ha/yr). The release of soluble nutrients into the pond resulted in blooms of macro- and micro- algae which caused large and potentially lethal diurnal fluctuations in dissolved oxygen within the pond, however, comparatively stable levels of dissolved oxygen were maintained within each SIFT through the use of air lift pumps.

It is well documented that saline groundwater is deficient in potassium which, depending on the extent of the deficiency, can negatively impact on the performance of marine species, including fish. The physiological effects of this deficiency on fish, however, have not been previously described. As such, I conducted a bioassay investigating the physiological effects of a hypersaline (45 ppt) groundwater source containing 25% of the potassium found in equivalent salinity seawater (i.e. 25% K-equivalence) on juvenile barramundi. Histopathological examination of moribund fish revealed severe degeneration and necrosis of skeletal muscles, marked hyperplasia of branchial chloride cells and renal tubular necrosis. Clinical chemistry findings included hypernatraemia and hyperchloridaemia of the blood plasma and lowered muscle potassium levels. It was concluded from this study that the principal cause of death of these barramundi was skeletal myopathy induced by unsustainable buffering of blood plasma potassium levels from the muscle. Although such hypokalaemic muscle
myopathies have been previously described in mammals and birds, this was the first description of such myopathies in fish.

It was hypothesized from the results described above that the physiological effects of potassium deficiency are dependent on salinity and that they would be ameliorated by potassium supplementation. These predictions were tested in a subsequent study which measured the effects of potassium supplementation between 25% and 100% K-equivalence on the growth, survival and physiological response of juvenile barramundi at hyperosmotic (45 ppt), near-isosmotic (15 ppt) and hyposmotic (5 ppt) salinities.

Unlike those juvenile barramundi reared at 45 ppt and 25% K-equivalence in the previous study, those reared in 50% K-equivalence water at 45 ppt in this study 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 was maintained by buffering from skeletal muscle. Fish reared in 50% K-equivalence at this salinity exhibited muscle dehydration, increased branchial, renal and intestinal (Na\(^+\)-K\(^+\))ATPase activity and elevated blood sodium and chloride, suggesting 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 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 confirmed the hypothesis that proportionally more potassium is required at hyperosmotic salinities compared to iso- and hypo-osmotic salinities and also demonstrated that barramundi have a lower requirement for
potassium than other marine and estuarine species being investigated for culture in inland saline groundwater.

In addition to ongrowing fish, saline groundwater has potential for hatchery production. Specific advantages include the vertical integration of inland saline farms and the production of disease-free certified stock through isolation from the pathogens and parasites found naturally in coastal water. To determine the potential of utilizing inland saline groundwater for hatchery production, barramundi larvae were reared from 2 to 25 days post hatch in 14 ppt saline groundwater with either no potassium supplementation (38% K-equivalence) or full potassium supplementation (100% K-equivalence). Growth, survival and swimbladder inflation of these larvae were compared against those grown in control treatments of seawater (32 ppt) and seawater diluted to 14 ppt. Those reared in saline groundwater with 38% K-equivalence exhibited complete mortality within 2 days, whilst those held in groundwater with full supplementation survived at a rate equal to both control treatments (pooled average 51.1 ± 0.5%). At 25 days post hatch, there was no significant difference in larval length or dry weight between those grown in the 14 ppt control treatment and those in the saline groundwater with full potassium supplementation. There were no significant differences in swim bladder inflation between any of the surviving treatments (average 93.3 ± 2.5%). This is the first description of rearing barramundi larvae both in low salinity seawater and in saline groundwater, and demonstrates that the requirement for potassium by larval barramundi is higher than for juveniles of the same species.

In addition to a deficiency in potassium, saline groundwater in Western Australia often contains an elevated concentration of manganese relative to seawater as a result of
anaerobic reduction of manganese oxides or the pedogenic weathering of manganese-bearing rock. The effects of elevated manganese on marine or estuarine fish have not been described and a study was therefore conducted to determine if manganese, at a concentration typical of that found in saline groundwater, has any impact on fish. The effects of 5 mg/L of dissolved manganese on juvenile mulloway at salinities of 5, 15 and 45 ppt were determined by comparing the survival, growth and blood and organ chemistry with those grown at the same salinities without manganese addition. Survival of mulloway at 45 ppt in the presence of 5 mg/L of manganese (73 ± 13%) was significantly lower than all other treatments, which achieved 100% survival. Those fish grown in seawater without manganese exhibited rapid growth, which was not affected by salinity (SGR = 4.05 ± 0.29%/day). Those fish grown at 5 ppt and 45 ppt in the presence of manganese lost weight over the two week trial (SGR -0.17 ± 0.42 and -0.44 ± 0.83%/day, respectively), whilst those at 15 ppt gained only a small amount of weight (SGR 1.70 ± 0.20%/day). Growth was therefore affected by manganese and by the interaction of manganese and salinity, but not salinity alone. Manganese was found to accumulate in the gills, liver and muscle of the fish. No gill epithelial damage or other significant histological findings were found, however, significant differences in blood chemistry were observed. Blood sodium and chloride of manganese exposed fish were significantly elevated in hyperosmotic salinity (45 ppt) and depressed at hyposmotic salinity (5 ppt) compared with unexposed fish; consistent with manganese causing apoptosis or necrosis to chloride cells. Blood potassium was significantly elevated and liver potassium significantly reduced at all salinities in the presence of manganese. These findings are consistent with manganese interfering with carbohydrate metabolism. There were no differences in blood sodium, chloride or potassium across
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Chapter 1

Finfish mariculture in inland Australia: a review of potential water sources, species and production systems

Citation:


1.1. Introduction

Secondary salinization is a major environmental problem in many arid and semi-arid areas of the world, adversely affecting up to 380 million hectares of land, including 100 million hectares of arable land (Ghassemi et al., 1995; Lambers, 2003). In the most recent assessment on the extent of salinity regions in Australia it was reported that approximately 5.7 million hectares of agricultural land and pastoral zones were under high risk of salinization through shallow water tables, unless influenced by changes to land-use and climate (NLWRA, 2001). The areas affected are primarily located in the country’s temperate zones (Figure 1-1).
Using saline groundwater for aquaculture production is a potential adaptive use of this otherwise degraded resource (Barson and Barrett-Lennard, 1995; Bell et al., 2001). Over half of the world’s aquaculture production currently occurs in marine or brackish coastal waters. These two sectors, collectively known as mariculture, are the fastest growing of the world’s aquaculture industry, which, in turn, is the fastest growing of all animal food-producing industries (FAO, 2003). Along the Australian coast, however, expansion of mariculture is limited by factors including a shortage of suitable sites and strict environmental regulations (Ogburn, 1996; Gooley et al., 2000; Kolkovski et al., 2000). These limitations, together with an abundance of salt-affected land and water resources, have led to the logical progression of investigating the suitability of these resources for mariculture. Such investigations have been underway for approximately

**Figure 1-1:** Areas affected by or at risk of secondary salinity in 2000. Modified from NLWRA (2001).
10 years and have received in-principle and monetary support from all levels of government (Smith and Barlow, 1999; Gooley, 2000; Allan et al., 2001a,b; NAC, 2004; FRDC, 2005). Despite this support and a research investment of approximately $AUD10 million, inland mariculture is yet to develop into an industrial-scale, rural enterprise. This chapter reviews the opportunities and constraints for commercial, inland mariculture in Australia in terms of the types of water resources available (including the quantity and quality of water), the species that may be grown and the culture methods that could be used to produce them. Issues of market potential, financial feasibility or industry development are not directly addressed here; these issues have been reviewed for the Australian aquaculture industry in general by Treadwell et al. (1991). Although primarily focused on Australia, the issues raised in this review are also relevant to other countries in which saline water resources have potential for finfish mariculture, including India, China, Israel and the United States of America.

### 1.2. Potential Benefits of Inland Mariculture

Inland mariculture has potential for positive economic, social and environmental outcomes. The issues are closely interlinked. From an economic perspective, the two major costs of typical pump-ashore mariculture operations are coastal land and pumping water. Salt-affected land is significantly cheaper than coastal land and of added benefit is the fact that it is typically freehold. Where inland mariculture can be successfully integrated with salinity interception schemes, then the costs of pumping water can be shared, resulting in significant savings for both enterprises (Doupé et al., 2003a). Unlike coastal mariculture developments, those located inland are not subject to tides and storms, which may produce a significant economic burden (Doupé et al., 1999). Affordable coastal sites, particularly in Western Australia, are often isolated and hence
lacking in basic infrastructure, including roads, power and population. Many salt-affected areas, on the other hand, are close to regional centres possessing these critical elements. The isolation of inland farms from pathogens and parasites found in the ocean may provide additional economic benefits through reduction or elimination of costly disease outbreaks and the production of certified disease-free seedstock.

From a social perspective, declines in crop production on salt-affected farms reduce income and decrease capital value, with the result that many farmers are opting to leave the land, thereby compromising the structure and capability of rural communities. Beresford et al. (2001) described increased incidence of stress and depression in rural communities as a consequence of the economic impacts of salinization. New, economically viable industries such as mariculture could capitalise upon an established workforce experienced in animal husbandry and agribusiness, thus helping to maintain the fabric of rural communities.

From an environmental perspective, inland mariculture production is typically an adaptive approach to salinity and will therefore not directly remediate this environmental problem. Due to the infancy of the industry, however, the opportunity exists to ensure it is managed in such a way that it does not contribute further to land and water degradation. The major potential environmental impacts from inland mariculture relate to the disposal of nutrient-enriched, saline effluent onto the land, where it may percolate back into the groundwater, or into waterways, where it may have detrimental effects on river health (Braaten and Flaherty, 2002; Starcevich et al., 2003). The value of constructed wetlands in filtering nutrients and salt from inland mariculture effluent has been addressed by Lymbery et al. (2006). Because the revenues created
from inland mariculture have the ability to offset the costs of salinity remediation measures, this may provide the impetus to implement these measures in areas in which they would not otherwise be economically viable.

1.3. Water Sources for Inland Mariculture

Water for inland mariculture may be sourced from natural (primary) salt lakes, or by utilising saline groundwater from surface and subsurface drainage systems and aquifers. Although there are many primary salinized lakes in Australia, most of these are ephemeral in nature and subsequently highly variable in salinity and volume (Gooley et al., 1997). Only two permanent, natural salt lake systems were identified during a resource inventory of potential water resources for aquaculture by Allan et al. (2001b). Utilising saline groundwater therefore offers the greatest potential for inland mariculture.

1.3.1. Groundwater Origin

The majority of groundwater interception for salinity management in Australia occurs within the irrigated horticultural areas of the Murray Darling Basin (Figure 1-1). Over 60% of the country’s irrigated agriculture, worth approximately $AUD4 billion annually, occurs in this basin (NLWRA, 2001). Because this agricultural region is spatially concentrated and highly valuable, high-cost engineering solutions to salinity management are often economically viable. The Murray-Darling Basin has the largest network of saltwater interception schemes in the world, discharging large volumes of saline water into approximately 190 evaporation basins (Simmons et al., 2000). With the majority of groundwater sources within the basin associated with either sedimentary
or superficial aquifers, individual bore yields are typically high (AWRC, 1987). The scale of saline groundwater interception in the Murray-Darling Basin clearly represents a significant opportunity for mariculture production. During an inventory of water resources with potential for inland saline mariculture, Allan et al. (2001b) identified 11 large evaporation basins within the Murray-Darling Basin as having potential for commercial mariculture, based on the quality and quantity of water, proximity of the basins to infrastructure and resources including power, freshwater, transport and population, their environmental sensitivity and opportunities for cost sharing. Despite only a small percentage of the total number of basins being identified as suitable, these cover more than 6300 ha and receive (on average) over 50 GL of saline water per year. In addition to these large scale salt interception schemes, many more private and public interception bores also exist in the Murray Darling Basin, many of which are also likely to have characteristics suitable for commercial mariculture. It is interesting to note that due to their effectiveness, pumping rates from many of these schemes have recently been decreased and the quantity of water being delivered to evaporation basins has decreased. This highlights that long-term water availability may become a significant constraint to the development or the long-term sustainability of mariculture operations relying on these water sources.

The wheatbelt region in Western Australia (Figure 1-1) accounts for over 70% of the country’s salinized land (Doupé et al., 2003a). With no major river systems or high-intensity irrigated agriculture in this region, interception schemes and managed evaporation basins do not form a large part of Western Australia’s approach to dealing with salinity. Secondary salinity is typically managed on-farm, rather than via co-ordinated regional approaches. Earthworks, including levees, banks and open drains are
therefore more common than groundwater pumping for the diversion of saline groundwater, because of their lower construction and operating costs (Trewin, 2002). The volume and quality of saline water generated by such earthworks is highly variable and unlikely to represent a significant resource for inland mariculture.

With rising salinity threatening rural infrastructure in approximately 38 rural towns in Western Australia (George et al., 2005), shallow groundwater pumped from beneath these towns may become a future source of water for inland mariculture in this state. With the majority of the wheatbelt overlying fractured rock or granite aquifers, bore yields are, however, typically only low to moderate (George, 1990). For example, the largest such scheme located in Merredin currently pumps 600 kL/day (220 ML/yr) from six bores. Dames and Moore (2001) suggested that the costs associated with groundwater pumping and disposal would exceed the financial benefits for most rural towns. This did not, however, assume any secondary use of the pumped groundwater. The shires of several rural towns are presently assessing a range of options, including mariculture, as a means to facilitate long-term groundwater pumping (Pluske, in press).

Open-cut mines for the extraction of minerals are common throughout Australia and many such mines intercept groundwater aquifers. In Western Australia alone, over 150 open-cut mines are currently operating below the water table (Johnson and Wright, 2003). Constant pumping is required from such mines to enable access to the pit-void. The intercepted groundwater ranges from fresh to hypersaline and is usually directed to purpose-built evaporation ponds. As such, these systems represent a similar opportunity for fish culture as those interception schemes pumping saline waters from beneath agricultural lands and rural towns.
The extraction of methane from coal-seams for energy production is a relatively new mining industry which also produces large volumes of water from deep aquifers (Faiz et al., 2007). Salinity of this water is typically low, with 65% of 393 currently registered bores in Queensland having a salinity between 1 and 3 ppt, and less than 1% having a salinity in excess of 18 ppt (Parsons Brinckerhoff, 2004). Pumping yields vary from 16 to 78 kL/bore/day and although the industry is still in its infancy, total water yields from Queensland alone are already in excess of 4,000 ML/year and predicted to increase to 42,000 ML/year by 2010 (Parsons Brinckerhoff, 2004).

The obvious limitation to sourcing water from operating open cut and coal seam mines is that the mariculture venture could only access this water source whilst the mine operates, unless it was willing to pay for water delivery once mining operations cease. An alternative opportunity for mariculture in association with open-cut mines emerges once the mines have ceased operation. Once dewatering stops, the voids fill with groundwater and surface run-off (Doupé and Lymbery, 2005). Water could be used within the mine lake thus formed or in purpose-built tanks or ponds adjacent to the void using water pumped from it. With over 1,800 disused voids in Western Australia alone (Doupé and Lymbery, 2005), this approach may provide a far greater opportunity than utilizing pumped groundwater from operational mines.

### 1.3.2. Groundwater Quality

The suitability of inland saline groundwater for aquaculture is dictated by its salinity, ionic composition, pH, temperature and the level of contaminants and toxins (Jenkins, 1997). Those water sources most likely to vary in terms of salinity and ionic
composition and contamination are those associated with shallow aquifers, open and
tiled drains and seepage schemes (Gooley et al., 1997; Allan et al., 2001a).

1.3.2.1. Salinity

The salinity of inland groundwater is dictated primarily by the rate of water removal by
evapotranspiration prior to recharge (Mazor and George, 1992) and has been
demonstrated to vary considerably both by depth within an aquifer and between bores,
even over short distances. George (1992), for example, measured salinities from 3.1 to
13.1 ppt from 10 bores spread over an area of approximately 50 hectares in the Western
Australian wheatbelt.

Although groundwater salinity varies from fresh through to hypersaline, the majority of
water sources are within the acceptable range for the culture of euryhaline species
suitable for farming. For example, of 88 bores tested in the wheatbelt of Western
Australia, salinity averaged 21 ppt (range 0.28 - 320 ppt), with 66% having a salinity
considered acceptable for culturing euryhaline fish (5 - 45 ppt) (Mazor and George,

In semi-arid areas where evaporation exceeds rainfall and lateral movement of
groundwater is low, evapo-concentration from water bodies can be significant. An
example of this phenomenon is the Keringal mine lake in Western Australia, where
salinity has increased from 15 to 79 ppt in three years (Johnson and Wright, 2003).
Many mine lakes are also prone to stratification of salinity (and temperature) due to
influx of fresh surface run-off, lack of wind-driven mixing, and their depth (Johnson
and Wright, 2003). Evapo-concentration is of course also a feature of the evaporation
basins associated with saltwater interception schemes and the subsequent fluctuations in salinity and temperature within these shallow, high surface-area water bodies are likely to constrain their direct use as fish production ponds.

1.3.2.2. Ionic composition

Of greater importance than salinity per se is the ionic composition of the water source. Despite the fact that the main source of salt in the Australian landscape is oceanic (Mazor and George, 1992; Zalizniak et al., 2006), the ionic composition of saline groundwater can vary considerably from seawater. Nine elements (excluding hydrogen, oxygen and nitrogen) make up 99.9% by mass of the solutes in seawater (Spotte, 1992). These elements and the major ionic forms in which they exist in seawater are shown in Table 1-1. This table also presents examples of deviations in the concentration of these ions (relative to seawater of equal salinity) in several saline groundwater sources from around Australia and the world. These data show that both deficiencies and excesses occur for all major ions except potassium; the universal deficiency of which is primarily caused by its preferential uptake over sodium by clay soils (Stumm and Morgan, 1996). Coal-seam water, in contrast to other sources of saline groundwater, is typically characterised by a lack of sulphate, calcium and magnesium ions and high concentrations of bicarbonate (Table 1-1) (Van Voast, 2003).
Table 1-1: Percentage concentrations (relative to those found in equivalent salinity seawater) of the nine most abundant elements found in seawater (excluding hydrogen, oxygen, carbon and nitrogen) from saline groundwater sources in a) India b) Australia c) United States of America. n.r. Not reported. * Coal seam water.

<table>
<thead>
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<th>Study</th>
<th>Salinity (ppt)</th>
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<th>Br</th>
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<tr>
<td>Flowers and Hutchinson (2004a)</td>
<td>18</td>
<td>89%</td>
<td>101%</td>
<td>62%</td>
<td>55%</td>
<td>158%</td>
<td>40%</td>
<td>n.r</td>
<td>n.r</td>
<td>n.r</td>
</tr>
<tr>
<td>Parsons Brinckerhoff (2004)</td>
<td>4.5</td>
<td>70%</td>
<td>114%</td>
<td>2%</td>
<td>8%</td>
<td>16%</td>
<td>n.r</td>
<td>n.r</td>
<td>n.r</td>
<td>n.r</td>
</tr>
<tr>
<td>Saoud et al. (2003)</td>
<td>16</td>
<td>101%</td>
<td>107%</td>
<td>11%</td>
<td>n.r.</td>
<td>51%</td>
<td>18%</td>
<td>n.r</td>
<td>n.r</td>
<td>312%</td>
</tr>
<tr>
<td>Ingram et al. (2002)</td>
<td>9</td>
<td>75%</td>
<td>88%</td>
<td>127%</td>
<td>165%</td>
<td>297%</td>
<td>22%</td>
<td>2%</td>
<td>260%</td>
<td>100%</td>
</tr>
<tr>
<td>Partridge and Furey (2002)</td>
<td>15</td>
<td>96%</td>
<td>111%</td>
<td>76%</td>
<td>153%</td>
<td>34%</td>
<td>68%</td>
<td>n.r</td>
<td>35%</td>
<td>112%</td>
</tr>
<tr>
<td>Fielder et al. (2001)</td>
<td>19</td>
<td>99%</td>
<td>80%</td>
<td>148%</td>
<td>79%</td>
<td>250%</td>
<td>5%</td>
<td>n.r</td>
<td>217%</td>
<td>16%</td>
</tr>
</tbody>
</table>
Potassium plays a critical role in many physiological processes and because all saline groundwater sources throughout the world are deficient in this ion, the effects of such deficiency on inland mariculture have received more attention than the effects of other major ions. Potassium deficiency has caused mortality in a number of species of cultured finfish. Fielder et al. (2001) linked mortality of snapper (*Pagrus auratus*) cultured in 19 ppt groundwater to the low concentration of potassium (5% K-equivalence). This mortality was remedied by increasing the potassium content to 40% K-equivalence; however at this concentration, growth was significantly lower than obtained at 60% K-equivalence. Doroudi et al. (2006) reported a similar response of mulloway (*Argyrosomus japonicus*) to potassium deficiency and supplementation in this same water source. The requirement for potassium may decrease with salinity, given that Ingram et al. (2002) obtained 27% survival with snapper grown in 10 ppt groundwater with 22% K-equivalence over 3 weeks, whilst those grown by Fielder et al. (2001) at a higher salinity (21 ppt) but similar potassium concentration (25% K-equivalence) died within 4 days. That Ingram et al. (2002) achieved 97% survival of silver perch (*Bidyanus bidyanus*) in the aforementioned water source also highlights that tolerance to potassium deficiency is freshwater or marine specific.

The effects of deficiencies and excesses of other ions on fish survival and growth are more equivocal. Forsberg and Neil (1997) found no deleterious effects of elevated sulphate (up to 720% SO$_4$-equivalence) or magnesium (up to 241% Mg-equivalence) on survival or growth of juvenile red drum (*Sciaenops ocellatus*) at a salinity of 3 ppt. Wurts and Stickney (1989) also found no effects of deficient magnesium on red drum at 35 ppt, but concentrations of calcium less than 44% Ca-equivalence at this salinity resulted in 100% mortality within 96 hours. By contrast, Partridge and Furey (2002)
grew snapper in 16 ppt groundwater containing only 34% Ca-equivalence without mortality for 6 weeks, suggesting either species-specific or salinity-dependant responses to deficient calcium. With 64% of 88 saline groundwater bores in Western Australia analysed by Mazor and George (1992) containing deficient calcium, further investigation is needed into the effects of such deficiency on the performance of species with potential for mariculture in Australia.

Of the minor constituents of seawater, several are essential trace elements, which are significant co-factors in enzymatic processes. Excessive concentrations of these and other elements in solution can, however, be toxic. Minor elements which are commonly found in higher concentrations in saline groundwater compared with seawater include iron, manganese and aluminium. Although there are a great deal of data on the toxicity of trace elements to freshwater fish, similar toxicity data for marine species are lacking. It is likely, however, that the toxicity of such elements is lower for marine fish because salt water is typically higher in pH, which both decreases the solubility of many toxic metals and maintains them as more hydrolysed, and therefore less toxic, forms (McDonald et al., 1989). Additionally, the high concentrations of calcium and magnesium in seawater are believed to compete with toxic metals for ligand sites on the gill surface and reduce the permeability of the paracellular pathways to toxic metals (McDonald et al., 1989).

Most studies investigating the effects of deviations in ionic composition have examined only one or two major ions at a particular salinity. Such studies may be of limited use in predicting the suitability of a particular inland water source for fish culture, particularly those that contain multiple deviations in both major and minor ions. Comparing the
growth and survival of the species targeted for culture in the proposed water source against those obtained in equivalent salinity seawater is therefore a crucial step in determining suitability, as such bioassays account for the interactions of all water quality parameters.

1.3.2.3. \textit{pH}

The optimum pH for fish production is generally considered to be in the range of 6 to 9. Many groundwater sources have pH values less than 6 for reasons including ferrolysis (Van Ranst and DeConinck, 2002; Beecher and Lake, 2004), the presence of acid-sulphate soils (Gooley and Gavine, 2003), the use of acid-forming fertilisers on agricultural lands and the presence of dissolved carbon dioxide. Not only are low levels of pH toxic to fish, but so too are the associated high concentrations of metals caused by acidic leaching from the soil matrix (Lee, 2001; Gooley and Gavine, 2003). Although acidic groundwater can be treated using a variety of chemicals including limestone, hydrated lime, sodium hydroxide and sodium carbonate (Hunt and Patterson, 2004), the cost of such treatment is likely to be prohibitive in the large volumes of water required for mariculture production.

Saline groundwater in association with carbonate-based soils may have pH as low as 4.5, due to the presence of dissolved carbon dioxide (carbonic acid). Unlike the acidification caused by mineral acids described above, low pH caused by carbonic acid is easily remedied by degassing to restore the equilibrium between atmospheric and aqueous carbon dioxide. Acidification of water also occurs in certain disused mine voids, particularly those in sulphide-rich rocks such as those associated with coal mines.
The open-cut mining of most metals, however, occurs in oxidised rock, which results in pit water of relatively neutral pH (Johnson and Wright, 2003).

Maintaining the stability of pH is also of key importance in mariculture and is achieved by maintaining a sufficient alkalinity (Spotte, 1992). In the 88 saline groundwater bores analysed by Mazor and George (1992), alkalinity ranged from 1 to 1140 mg/L (seawater = 125 mg/L). Inadequate alkalinity can result in rapid and deleterious changes in pH, whereas excessive levels of alkalinity can contribute to calcium carbonate precipitation (Spotte, 1992).

1.3.2.4. Contaminants

Both chemical (including nutrients, heavy metals, herbicides, insecticides and organic pollutants) and biological contaminants (such as diseases/pathogens and other biota) are potentially detrimental to inland mariculture. Those water sources most susceptible to contamination are surface waters, open drains and shallow aquifers (Fitzpatrick et al., 2005). The risk of contamination of surface waters and open drains is directly proportional to the size of the catchment and the land-use practices within it. Doupé et al. (2003a) pointed out that surface-fed stock-watering dams are susceptible to contamination, and Sarre et al. (1999) described fish kills associated with specific contaminants in saline water bodies. Surface waters are also highly susceptible to pesticide contamination via agricultural run-off or spray-drift, with the toxicity of the pesticide dependant on factors including its solubility, hydrophobicity, residence time and mode of action (Schulz, 2004; Scott and Sloman, 2004; Bermudez-Saldana et al., 2005). Shallow groundwater sources are also prone to contamination. Waters under urban towns, for example, are often high in nitrates and sulphates associated with
leached fertilisers, septic systems and run-off from bitumen roads (Nott et al., 2004). Contamination of groundwater with pesticides has also been described (Frank et al., 1987).

1.4. **Culture Methods for Inland Mariculture**

Culture systems for commercial inland finfish production may be divided into pond or tank based systems. Which of these culture systems is most appropriate for inland mariculture will be a function of the quantity and physicochemical parameters of the available water source, the corresponding requirements of the species to be cultured and economic considerations, including the required rate of return on investment and the subsequent level of production intensity. A summary of the advantages and disadvantages of the various culture methods discussed below is given in Table 1-2.
Table 1-2: Advantages and disadvantages of the various culture methods suitable for fish production in inland saline water.

<table>
<thead>
<tr>
<th>Culture Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pond-based</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Static         | • Water disposal minimal  
                 • Cost effective ionic supplementation | • Yields limited to approximately 6 tonnes/ha/yr  
               • Water temperatures dictated by local climate |
| Flow-through   | • Increased yields compared with static ponds  
                 • Disposal simple if adjacent to evaporation basins | • Water disposal a cause of environmental concern if not adjacent to evaporation basins  
               • Flow rates unlikely sufficient to overcome local climate effects on water temperature  
               • Ionic supplementation may not be cost effective  
               • Long term water availability may be a constraint |
| Floating cages | • Improved stock management compared with free-range fish  
                 • Lower capital cost than other in-pond technologies | • Fast water current required to provide oxygen and flush wastes  
               • Feed sinking or drifting through cages is wasted  
               • Harassment of caged fish from birds, rats and nuisance fish  
               • No ability for waste capture |
| Raceways       | • Improved stock management compared with free-range & caged fish  
                 • High fish densities within raceways. | • Plug flow results in decreasing water quality along length of raceway  
               • Fish must constantly swim at high velocity  
               • Limited ability to remove solid wastes |
| PAS            | • Improved stock management compared with free-range & caged fish  
                 • Enables high yields from static ponds  
                 • Cost effective ionic supplementation  
                 • Potential to produce valuable secondary crops | • Must be located in regions with high light intensity  
               • Suitable, local species with an ability to crop microalgae must be identified  
               • Complex management |
| **Tank Based** |            |               |
| Recirculating  | • Ability to culture fish outside their climatic range  
                 • Suitable for areas with low yielding water sources  
                 • Cost effective ionic supplementation | • High capital and operating costs  
               • Complex management |
| Flow-through   | • Lower capital and operating costs than recirculating systems  
                 • Potential to maintain the temperature of the source water | • Ionic supplementation not cost effective  
               • Water disposal a cause of environmental concern if not adjacent to evaporation basins  
               • Long term water availability may be a constraint |
1.4.1. **Pond-based Systems**

With an abundance of relatively cheap land in salt-affected areas, pond culture would be well suited to inland mariculture in Australia (Doupé et al., 2003b). Indeed, Allan et al. (2001a) suggested semi-intensive ponds to be the most prospective commercial production systems for inland mariculture in Australia. The major disadvantage of such culture lies in the fact that the majority of salt affected land and water resources are located in temperate regions, where water temperatures in open ponds vary widely between seasons. In certain cases it has also been demonstrated that water temperatures in ponds can exhibit significant diurnal variations in temperature.

Although it is possible to construct ponds by excavating directly into the water table, the water level within such ponds is dictated by the height of the water table; if the water table decreases significantly then the water level may become too low for fish culture. In addition, if the rate of lateral groundwater flow through such ponds is less than evaporation or the substrates have a low permeability, then salt will accumulate over time (Nulsen, 1997). Ponds for commercial inland mariculture should therefore be purpose built, with the capacity to completely drain. This not only facilitates harvesting but also the oxidation of organic matter via ‘sun-baking’, which is an important element in successful pond management (Boyd et al., 2002).

1.4.1.1. **Static pond culture**

Yields from static ponds are limited by the capacity of the pond to assimilate or process fish wastes via natural, in-pond processes. For example, with current management practices, the static ponds used in the US channel catfish (*Ictalurus punctatus*) industry
are operating at their maximum sustainable level of production of up to 6.7 tonnes/ha/yr (Hargreaves and Tucker, 2003). Static pond culture may be an appropriate culture technology in areas where the disposal of eutrophic, saline water is problematic (e.g. those areas without environmentally sound evaporation basins) or if ionic supplementation of the water source is uneconomical to achieve in flow-through systems. It should be noted, however, that in the case of potassium supplementation in earthen ponds, additional potassium must be added to compensate for the high affinity for this ion by clay soils in the pond substrate. The exact quantity required will be a function of the cation exchange capacity and the state of potassium saturation of the soils from which the ponds are constructed (Boyd et al., 2007a; Boyd et al., 2007b).

Despite no requirement for flow-through water in static ponds, the high net evaporation typical of the arid and semi-arid regions of salt-affected Australia indicates that relatively high volumes of water will still be required to compensate for this loss. As an example, 5 hectares of static fish ponds in the wheatbelt of Western Australia would require over 350 kL/day for 5 months of the year to compensate for evaporation (Luke et al., 1987).

Increasing production yields from static ponds can be achieved by exporting nutrients through the encouragement of denitrification (Burford and Longmore, 2001), the immobilisation of toxic nitrogen species by heterotrophically generated bioflocs (Avnimelech, 1999), the removal of bottom sludge (Hopkins et al., 1994; Burford et al., 2003), via poly-culture with filter feeders, detritivores or grazers (Lin and Yi, 2003; Yi et al., 2003; Erler et al., 2004) or by flushing (batch flow-through) (Colt, 1991).
1.4.1.2. **Flow-through pond culture**

In areas with environmentally acceptable evaporation basins, and for high yielding water sources not requiring ionic supplementation, flow-through pond culture is advantageous, as the flushing of nutrients from the pond facilitates increased production. Gooley et al. (2000), for example, reported barramundi (*Lates calcarifer*) production of 30-35 tonnes/ha/yr in flow-through ponds in North Queensland. Although the discharge of nutrients and pathogens from conventional flow-through farms into receiving waterways is a cause of environmental concern (Gooley, 1998; Burford and Williams, 2001), mariculture ventures which discharge into evaporation basins are likely to be relatively environmentally benign. It should be noted that the typical rate of water turnover in flow-through ponds is insufficient to counter climatic effects and seasonal temperature fluctuations will therefore still occur. Given that some of the large scale interception schemes in the Murray Darling Basin have recently experienced significant decreases in water yield, it is clearly of vital importance that the long-term availability of water can be secured.

1.4.1.3. **Hybrid pond culture**

Floating cages (Gooley and Gavine, 2003), floating raceways (Masser and Lazur, 1997) and Partitioned Aquaculture Systems (PAS) (Brune et al., 2003) are examples of pond-based technologies which have potential application for inland mariculture. These technologies offer varying degrees of improved stock management, yield and environmental benefits. The advantages and disadvantages of these various technologies are detailed in Table 1-2.
1.4.2. **Tank-based Systems**

1.4.2.1. **Recirculating tank culture**

Recirculating aquaculture systems are typically highly intensive and utilize a combination of mechanical, biological and chemical filtration to allow a high percentage of water reuse (Losordo et al., 1998; Piedrahita, 2003). With low water use, such systems would prove beneficial for low yielding inland saline water sources and those that require significant pre-conditioning or adjustment of their ionic composition. A major advantage of such systems is their ability to control water temperature, thereby overcoming the seasonal water temperature variations in temperate ponds.

The disadvantages of recirculating aquaculture systems are their high capital, operating and subsequent production costs. These costs are often defrayed by taking advantage of the systems’ small footprint and locating them close to niche markets where their products can command a higher price. With most inland saline water sources located in regional areas (and hence away from most niche markets), there are fewer opportunities to offset higher production costs.

1.4.2.2. **Flow-through tank culture**

The cost of production of fish in flow-through tanks is likely to be less than recirculating aquaculture systems because much of the associated capital infrastructure and subsequent operating costs are ameliorated. As for flow-through pond culture, commercial scale, flow-through tank culture is typically constrained by factors including the availability of sufficient quantities of suitable quality water, the high costs
associated with delivering this water (Pulido-Calvo et al., 2006) and environmental impacts of the effluent (MacMillan et al., 2003; Viadero et al., 2005). The latter two constraints are likely to be overcome when using cost-shared saline groundwater resources which discharge into environmentally sound evaporation basins. For flow-through culture, species need to be selected that have an appropriate thermal tolerance to the water being delivered and the ionic composition requirements of the species matched to that of the water source.

### 1.5. Species for Inland Mariculture

Although examples of inland prawn culture in saline water are well documented in Australia and internationally (Boyd and Thunjai, 2003; Saoud et al., 2003; McNevin et al., 2004; Shakeeb-Ur-Rahman et al., 2005; Boyd et al., 2007a), the majority of inland saline water reserves in Australia are located in temperate regions. Tropical prawn culture is therefore inappropriate and interest has subsequently focused on temperate finfish species.

The major criteria in selecting fish species suitable for commercial-scale, inland mariculture are essentially the same for any aquaculture industry. The selected species must be robust, have a rapid rate of growth, well established hatchery techniques and good market acceptance. Perhaps the only criterion specific to fish production in inland saline waters is that the water quality requirements for optimum performance of a species must be matched to the characteristics of an available water source (Doupé et al., 2003a). A number of fish species, including marine, estuarine, diadromous and euryhaline freshwater species, have been trialled for their suitability in inland saline waters in Australia (Table 1-3). Of the twelve species listed in Table 1-3, only
barramundi, trout and mulloway have been or are currently being produced commercially in inland saline water.

1.5.1. Barramundi

Barramundi are an ideal aquaculture candidate, meeting all of the selection criteria outlined above. In the latest Australian aquaculture production statistics, O'Sullivan et al. (2007) reported that barramundi production (using conventional water sources) in 2004/05 was 2783 tonnes. With optimal growth occurring between temperatures of 27°C and 33°C (Katersky and Carter, 2005), the majority of barramundi production occurs in the northern states in floating cages held within flow-through ponds. Approximately 25% of production occurs in the cooler southern states in recirculating aquaculture systems (O'Sullivan et al., 2007).

Based on published growth rates for barramundi (Glencross, 2007), it is conceivable this species could be grown to a marketable size over the summer months in southern Australia in open, inland saline ponds. However, with the current market preference for barramundi shifting from plate-sized fish (400-500 g) to banquet-sized (600-800 g) and fillet-sized fish (>1.5 kg) (O'Sullivan et al., 2006), seasonal production of this species to such larger sizes in open ponds may be limited.
<table>
<thead>
<tr>
<th>Common Name</th>
<th>Scientific Name</th>
<th>Habitat</th>
<th>Optimum temperature</th>
<th>Salinity tolerance</th>
<th>References related in inland saline water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australian bass</td>
<td><em>Macquaria novemaculeata</em></td>
<td>Catadromous</td>
<td>nd</td>
<td>0-35 ppt</td>
<td>Ingram et al. (2002)</td>
</tr>
<tr>
<td>Barramundi</td>
<td><em>Lates calcarifer</em></td>
<td>Catadromous</td>
<td>27-33°C</td>
<td>0 - 55 ppt</td>
<td>Jain et al. (2006)</td>
</tr>
<tr>
<td>Black bream</td>
<td><em>Acanthopagrus butcheri</em></td>
<td>Estuarine</td>
<td>22-26°C</td>
<td>0 - 60 ppt</td>
<td>Sarre et al. (1999)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sarre et al. (2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Doupé et al. (2005)</td>
</tr>
<tr>
<td>King George whiting</td>
<td><em>Sillaginoides punctada</em></td>
<td>Marine</td>
<td>nd</td>
<td>&gt;15 ppt</td>
<td>Ham et al. (1997)</td>
</tr>
<tr>
<td>Mulloway</td>
<td><em>Argyrosomus japonicus</em></td>
<td>Estuarine</td>
<td>&gt;18°C</td>
<td>&gt;5 ppt</td>
<td>Doroudi et al. (2006)</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td><em>Oncorhynchus mykiss</em></td>
<td>Anadromous</td>
<td>15-18°C</td>
<td>0-35 ppt</td>
<td>Ingram et al. (2002)</td>
</tr>
<tr>
<td>Silver perch</td>
<td><em>Bidyanus bidyanus</em></td>
<td>Freshwater</td>
<td>23-28°C</td>
<td>&lt;15 ppt</td>
<td>Ingram et al. (2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Doroudi et al. (2007)</td>
</tr>
<tr>
<td>Snapper</td>
<td><em>Pagrus auratus</em></td>
<td>Marine</td>
<td>20-28°C</td>
<td>&gt;8 ppt</td>
<td>Fielder et al. (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Partridge and Furey (2002)</td>
</tr>
</tbody>
</table>
Alternative options for barramundi production using inland saline water include the exploitation of warmer, deep aquifer water and/or the use of recirculating aquaculture systems. The extraction of warm aquifer water will only be advantageous if the temperature is adequate for barramundi growth and if heat is not rapidly lost from the culture system. Opportunities may exist to culture barramundi using warm saline water sourced from either operational mines or abandoned mine pit-voids in the warmer north west of Western Australia, particularly in the Pilbara and Murchison-Northern Goldfields regions, where the abundant metalliferous mine pit-voids typically contain brackish water of neutral pH (Johnson and Wright, 2003).

1.5.2. *Rainbow Trout*

Rainbow trout (*Oncorhynchus mykiss*) have been cultured in Australia for over 100 years (Molony, 2001). Production in 2004/05 was 2290 tonnes, of which 500 tonnes was grown in the ocean within seacages and the majority of the remainder in flow-through raceways in freshwater (O’Sullivan et al., 2007). A small quantity of rainbow trout (less than 10 tonnes per year) is produced in inland saline groundwater in the south-west of Western Australia (Starcevich et al., 2003; Lever et al., 2004). In contrast to barramundi, rainbow trout is a cold-temperate species with optimum growth occurring at 15°C to 18°C (Sumpter, 1992; Sedgwick, 1995). The upper critical thermal maximum temperature for rainbow trout has been reported from 21°C (Ojolick et al., 1995) to as high as 29°C (Currie et al., 1998), with the most common consensus being in the range of 24°C to 26°C (Molony, 2001).

Certain regions, such as the south-west of Western Australia, have suitable ambient air temperatures to facilitate the year-round production of rainbow trout in open pond
systems. Most regions with significant saline groundwater reserves, however, have ambient air temperatures which exceed 40°C (Allan et al., 2001b) and water temperature in these regions could, therefore, rise above the critical limit for trout, particularly if this water is used in open pond culture. Although seasonal production of rainbow trout in open ponds may be feasible, the lack of continuity of supply will create challenges in marketing fresh product, particularly if a number of producers are competing for limited markets at the same time. An additional disadvantage of seasonal production is that the risk of fish mortality is compounded towards the end of the growing season, when biomass (and hence oxygen demand) is greatest and increasing water temperatures approach the upper lethal limit. Potential solutions to this problem are to focus rainbow trout production in the cooler regions where year-round production can occur, or to genetically select trout with a higher temperature tolerance (Molony et al., 2004).

A major advantage of rainbow trout is that they appear to have a lower requirement for potassium in saline groundwater compared to other candidate species, perhaps due to the fact that salmonid gills are considerably less permeable to cation loss than other marine fishes (Sanders and Kirschner, 1983). Ingram et al. (2002) for example, achieved 99% survival and good growth of rainbow trout in the same evaporation basin (average salinity 16.8 ppt, K-equivalence 22%) which caused mortality of snapper. This feature makes flow-through production of rainbow trout worthy of consideration; particularly if water flow can be provided at a rate sufficient to maintain an adequately low temperature year-round. In traditional single-pass, flow-through systems, trout production ranges from 0.8 to 1.4 kg per year for each litre of water delivered per minute (Sedgwick, 1995; Summerfelt et al., 2004). Based on these figures, a farm producing 100 tonnes of rainbow trout per annum would require a water flow rate of
between 38 and 66 GL/yr; a volume similar to that delivered to all eleven evaporation basins identified by Allan et al. (2001b) as having excellent mariculture potential. To increase production per unit volume of water, many flow-through trout farms utilise multiple-pass systems whereby water flows from one raceway to another (MacMillan et al., 2003). Using this approach, production can be increased to between 5 and 18 kg/(l.min) (Summerfelt et al., 2004; Viadero et al., 2005). Based on these figures, a farm would require 3 – 11 GL/yr to produce 100 tonnes of fish; thereby potentially enabling rainbow trout production to occur in many more inland saline water sources other than just those associated with the largest interception schemes. Clearly, however, the more the water is re-used, the greater the potential for it to be influenced by external temperatures. Production of rainbow trout can be intensified to as high as 160 kg/(l.min) by the use of recirculating aquaculture systems (Summerfelt et al., 2004). The increased cost of production associated with these more intensive systems, however, would have to be carefully considered against the decreased water usage and ability for temperature control.

1.5.3. Mulloway

Production of mulloway in seacages in Australia has increased markedly over recent years, from 0.5 tonnes in 2001/02 (O'Sullivan and Savage, 2004) to 558 tonnes in 2004/2005 (O'Sullivan et al., 2007). Mulloway are well suited to culture in inland saline water. They are euryhaline, tolerating salinities down to 5 ppt (Fielder and Bardsley, 1999) and their hatchery production is well understood (Fielder et al., 1999). They have been successfully cultured on an experimental basis in various inland saline water sources around Australia (Partridge, 2003a; Flowers and Hutchinson, 2004a; Doroudi et al., 2006) and one small commercial farm is successfully growing mulloway in open
saline ponds in NSW. Unlike barramundi and rainbow trout, mulloway tolerate a wide range of temperatures, however, published data on the time taken to reach a marketable size are limited. With an initial weight of 30 grams, I have grown mulloway to 810 grams in 12 months in flowing seawater ranging in temperature from 18ºC to 23ºC (unpublished data). Mperdempes and Hecht (2002) obtained similar results growing the same species in seacages in South Africa. Flowers and Hutchinson (2004b), however, experienced negligible growth of mulloway in inland saline water at temperatures below 18ºC. In order to determine if and where year-round production of mulloway will be most viable in inland saline groundwater, further research is required to determine growth rate to market size across the range of temperatures experienced in these regions.

With mulloway performing well under a range of culture methods, including seacages, recirculating systems and open ponds (Hutchinson, 2003), the most appropriate culture methods for inland mariculture will therefore be dictated by the physicochemical characteristics of the water source and economic factors including the cost of production. With mulloway requiring a potassium concentration in saline groundwater of at least 40% of that found in equivalent salinity seawater (Doroudi et al., 2006), either a water source containing this concentration must be selected, or a production system chosen in which potassium supplementation will be cost effective.

1.5.4. Other Species

Snapper have been investigated for inland mariculture and, although technically feasible (Jenkins, 1997; Fielder et al., 2001), this species is generally overlooked in favour of faster growing, more euryhaline (and therefore more robust) species such as mulloway
and barramundi (Hutchinson, 2003). Black bream (Acanthopagrus butcheri) have been stocked into many inland water sources for recreational fishing purposes (Sarre et al., 1999; Sarre et al., 2002) and are well suited to this environment, being euryhaline (Partridge and Jenkins, 2002), robust (Doupé et al., 2005) and having well documented hatchery production techniques (Partridge et al., 2003). The major impediment to their commercial viability is a slow growth rate, but other attributes including low fillet yield and precocious maturation also contribute (Doupé et al., 2005). Australian bass (Macquaria novemaculeata) and King George whiting (Sillaginodes punctata) are similar to black bream in that they have many positive attributes for inland mariculture, yet have insufficient rates of growth to be considered for commercial culture (Ham and Hutchinson, 1997; Wilde and Sawynok, 2005). Gooley and Gavine (2003) reported the salinity tolerance of a range of Australian native freshwater fish. Because two of these species, Murray cod (Maccullochella peeli peeli), (salinity <8 ppt) and silver perch (salinity <15 ppt), are already cultured commercially in freshwater they clearly have the necessary attributes for viable culture and are therefore likely to be suitable candidates for low salinity inland waters. Indeed the potential for growing silver perch in saline groundwater has been demonstrated by Ingram et al. (2002) and Doroudi et al. (2007). In addition to those species outlined in Table 1-3, it is conceivable that more stenohaline marine species could be cultured in those inland saline water sources where salinity approximates that of seawater, provided the ionic composition is suitable for such species.

1.6. Thesis Objectives and Structure

The first objective of the current study was to review key technical aspects for the development of a commercial scale, inland mariculture industry. This review has
effectively highlighted a number of limitations to the growth of a commercially viable inland mariculture industry and the main body of this thesis addresses some of these key constraints in five research chapters. Chapter 2 describes and tests a new culture technology which I designed in collaboration with the aquaculture industry for use in static inland saline water bodies to overcome many of the limitations associated with conventional static pond culture. This technology was tested by growing three of the most promising candidate species identified in this chapter (trout, barramundi and mulloway) to a marketable size over a period of 10 months. Chapter 3 describes the physiological effects of potassium deficiency on barramundi in hyperosmotic water and puts forward the hypothesis that potassium requirements will be a function of salinity. Chapter 4 tests this hypothesis by quantifying the concentration of potassium required to culture juvenile barramundi at hyper-, near iso- and hypo-osmotic salinities. In Chapter 5, I determine the potential for using inland saline groundwater for hatchery production of barramundi to enable vertical integration of the inland mariculture industry and to provide disease free stock to coastal growout farms. Chapter 6 investigates the effects of an elevated concentration of manganese, typical of that found in inland saline groundwater, on the survival, growth and physiology of juvenile mulloway.
Chapter 2

Finfish production in a static, inland saline water body using a semi-intensive floating tank system (SIFTS)

Citation:


2.1. Introduction

A recent research and development plan for inland saline aquaculture in Australia suggested semi-intensive open ponds to be the best option for commercial fish production in such areas (Allan et al., 2001a). These systems make excellent use of the land resources in salt affected areas, however, nutrient input from fish waste, uneaten feed and microbial remineralisation of nutrients from pond sediment results in microalgal blooms (Hargreaves, 1998; Erler et al., 1999). Such blooms cause diurnal fluctuations in the concentration of dissolved oxygen and as the blooms strengthen, these fluctuations can range from supersaturated to lethally low levels. Pond systems are therefore often limited in yield by the nutrient input into the pond and the effects the subsequent microalgal blooms have on water quality (Drapcho and Brune, 2000; Avnimelech and Ritvo, 2003; Tucker and Hargreaves, 2003). Options for managing such blooms include utilising flow-through or batch water exchanges (Wang, 1990; Burford and Longmore, 2001), the cropping of microalgae within these ponds with planktivores (Shpigel et al., 1997; Drapcho and Brune, 2000; Turker et al., 2003a) and reducing the input of nutrients into the pond (Hopkins et al., 1994; Yoo et al., 1995; Burford and Longmore, 2001).
The low potassium concentration of many saline groundwater sources in Australia necessitates supplementation of this ion for many of the fish species under investigation (Fielder et al., 2001; Doroudi et al., 2003; Partridge and Creeper, 2004 (Chapter 3)). As such, the practice of using water exchange to manage algal blooms in such situations may not be cost effective. In addition, flow-through ponds have the potential to impact on receiving water bodies through the addition of nutrients and salt (MacMillan et al., 2003; Read and Fernandes, 2003; Starcevich et al., 2003).

Tilapia (Oreochromis spp.) are the most common species of planktivorous fish used for cropping microalgae in aquaculture ponds (Yi and Kwei Lin, 2001; Turker et al., 2003a; Turker et al., 2003b), however tilapia are classified as a noxious species by regulatory agencies in Australia and therefore cannot be cultured. Other fish species such as mullet have been suggested as alternatives (Erler et al., 1999), but have yet to be proven suitable. The use of other organisms including bivalves (Shpigel and Neori, 1996; Lefebvre et al., 2004; Mueller et al., 2004) and Artemia (Wang, 2003) can also effectively crop microalgae but have yet to be investigated for static inland saline aquaculture ponds.

The major source of nutrient input into aquaculture ponds is fish excretion. Up to 15% of fed nitrogen is deposited directly on the pond bottom as faeces where it undergoes microbial mineralisation to ammonia that diffuses back into the water column (Brune et al., 2003). Hopkins et al. (1994) proved that removing such sludge improves water quality and production, but Brune et al. (2003) pointed out that effective sludge removal
has not been transferred to large scale systems and as such, is not widely practised in the aquaculture industry.

The Semi-Intensive Floating Tank System (SIFTS) has been designed to reduce nutrient input into ponds by the collection of settleable wastes. In addition, the SIFTS provides large volumes of well-oxygenated water to the target species, thereby ameliorating fish loss due to low dissolved oxygen concentrations. SIFTS also increases the management capability of the cultured fish compared with those free-ranged in open ponds. This chapter describes the production of three carnivorous (i.e. non-planktivorous) fish species identified in Chapter 1 as having excellent potential for inland saline aquaculture in Australia (mulloway (*Argyrosomus japonicus*), barramundi (*Lates calcarifer*) and rainbow trout (*Oncorhynchus mykiss*)), in a prototype SIFTS held within a static, inland saline pond over a period of 10 months.

**2.2. Materials and Methods**

**2.2.1. Culture Pond**

The trial was conducted on an inland saline aquaculture farm located at Northam in Western Australia (31°39'S, 116°40'E), approximately 100 km from the coast. Saline water (14 ppt) on the farm is sourced from a bore (i.e well) at 35 m depth. Prior to use, the water was held in a 0.15 ha pond to precipitate iron.

An existing 0.13 ha earthen pond with an average depth of two metres was used for the fish production trials. The pond was not drained prior to use and had a moderate covering (ca. 60% of the pond bottom area) of the benthic macrophyte *Chara* spp. The only water added to the culture pond was to compensate for evaporation and seepage. In
the absence (at the time) of accurate data on the requirements for potassium by barramundi and mulloway, pond water was supplemented with agricultural grade muriate of potash (99% KCl) at the rate of 3 tonnes/ha to increase the potassium equivalence rate (i.e. the % of potassium found in equivalent strength seawater) from 46% to 83%.

2.2.2. Semi-Intensive Floating Tank System (SIFTS)

The experimental unit comprised four, cylindrical, 10 m$^3$ (3.1 m Ø x 1.4 m) fibreglass SIFTs (McRobert Aquaculture Systems, Welshpool, Australia) attached in a square formation to a floating platform (Figure 2-1). The top of each SIFT floated 100 mm above the pond water level. Water was pumped through each SIFT using two opposing air lifts similar to those described by Masser and Lazur (1997). Each air lift pumped 330 litres of water per minute into a vertical inlet manifold. Opposing manifolds in each SIFT created a tangential flow. Approximately 90% of the water flow exited the tank through a channel opening at the surface, with the remainder flowing via gravity from the bottom of the tank through a centrally located, overhead waste pipe. Aeration at the base of this pipe provided a secondary flow of water towards the centre of the tank, accelerating the movement of solid wastes towards the pipe as described by Timmons et al. (2002). Settleable solid wastes, including faeces and uneaten feed, flowed through this waste pipe and into the solid waste collection system, comprising a modified swirl separator similar to that described by Timmons et al. (2002).
Figure 2-1: Schematic layout of the 4 x 10 m³ SIFTs held within a 0.13 ha inland saline aquaculture pond.

Each SIFT contained an invertible PVC liner (McRobert Aquaculture Systems, Welshpool, Australia). Air may be injected behind this liner, causing it to invert and the water within the SIFT to be displaced into the pond via the channel. This consequently allowed the fish to be harvested through the channel opening during the inversion process. A variable speed, single-phase and loop-charged injected spa blower (240 V, 3.5 A) (Air Supply International, Brisbane, Australia) was used to operate all four SIFTs including all air lifts, central aeration, solid waste collection system and liner inversion.
2.2.3. *Fish*

Three species of fish were cultured in the SIFTS over the 292 day experimental period. On June 10th 2004 (Day 1), 746 rainbow trout (average weight 83 grams) were counted into each of two SIFTs and six days later 1,200 mulloway (average weight 100 grams) were counted into each of the remaining two SIFTs. Rainbow trout were harvested on September 29th (Day 112). On November 1st (Day 145) (when pond water temperature had increased to 22°C), 1,265 and 1,624 barramundi (average weight 50 and 30 grams respectively) were counted into these two SIFTs. Barramundi were harvested on March 22nd (Day 286 i.e. after 141 days of culture) and mulloway on March 29th, 2005 (Day 293).

All fish were fed daily to satiety on a sinking commercial diet (Nova ME, 45% protein, 20% lipid, Skretting, Rosny Park, Australia) and the amount of feed offered was recorded. Rainbow trout and barramundi were hand-fed twice daily at 0800 and 1700 hours. Due to their slower feeding behaviour, mulloway were fed via an automatic trickle feeder (EWOS No 130, Sandblom and Stohne, Sweden), which delivered their daily ration at 9 – 12 minute intervals throughout the day. Satiety was deemed to be reached once pellets began appearing in the solid waste collector.

At two-weekly intervals, the liner in each SIFT was partially inverted, crowding the fish into a small area that enabled representative subsamples to be taken. Twenty fish from each SIFT were anaesthetised (AQUI-S, 20 mg/L) and weighed to the nearest gram (Ohaus 4100) before being returned to the SIFT. At the completion of each crop, all fish
were harvested, counted and individually weighed to the nearest gram. Specific growth rates (SGR) were calculated as:

$$SGR \ (\%/day) = \left( \frac{\ln(W_f) - \ln(W_i)}{\text{Time (days)}} \right) \times 100$$

Where $W_f$ and $W_i$ were the final and initial wet weights of the fish, respectively.

Feed conversion ratio was calculated as the increase in biomass divided by the total amount of feed fed and did not account for fish mortality.

### 2.3. Pond Water Chemistry, Productivity and Waste Generation

Dissolved oxygen concentrations were measured constantly in the outflow (i.e. channel opening) of each SIFT using a galvanic cell probe (AquaTraul™, Dryden Aqua, Scotland UK) and were logged every 5 minutes (LookOut™, National Instruments, London, UK). Dissolved oxygen and temperature were similarly measured and logged within the pond. Salinity and turbidity (Secchi depth visibility) were measured daily.

Microalgal cell density was counted periodically throughout the trial under 40 x magnification using an Improved Neubauer haemocytometer. Samples of pond water were taken weekly for analysis of total ammonia nitrogen (TAN) according to Dal Pont et al. (1974). Alkalinity was measured routinely in filtered samples according to Spotte (1992). pH was measured and logged every 5 minutes (Yellow Springs Instruments 556 MPS) in the main pond over several days when microalgal density was high ($1.1 \times 10^6$ cells/mL; Days 191 to 194).
Solid waste collectors were emptied twice daily. For each fish species, collected waste was analysed for total volume, dry matter, total nitrogen and total phosphorus. On each sampling occasion, solid waste collectors were completely emptied prior to the evening feed and were emptied the following morning prior to the next feeding. The total volume of sludge from each collector was measured and then thoroughly mixed prior to taking four replicate 50 mL subsamples. These samples and control samples of pond water were frozen, freeze-dried and the dry weight of waste collected was calculated by subtracting the dry control weight from the dry sample weight (to correct for the total dissolved solids content of the pond water). The total dry weight of waste collected was then calculated and expressed as a percentage of the weight of feed fed. Total nitrogen and phosphorus contents of the solid waste were determined by flow injection analysis after digestion with sulphuric acid, and a catalyst of copper sulphate on a block digester in the presence of sodium sulphate at 365°C (APHA, 1998).

2.4. Results

2.4.1. Growth, Survival and Feed Conversion Ratio

During the 111-day rainbow trout culture period, average daily water temperature ranged from 12.3°C to 18.2°C and averaged 14.9°C (Figure 2-2). Over this period, rainbow trout grew from 83 to 697 ± 5 grams (Figure 2-3) (SGR = 1.91 ± 0.01%/day), with an FCR of 0.97 ± 0.03 (Table 2-1). Survival of rainbow trout was 90.4 ± 0.8% and at harvest, stocking density within the duplicate SIFTs averaged 43.1 ± 1.2 kg/m³ (Table 2-1).
Mulloway growth between June (Day 1) and September (Day 112), when water temperatures remained below ca. 19°C (Figure 2-2), was slow (100 to 116 ± 4 grams) (Figure 2-3). Growth increased from October (Day 119) until harvest in late March (Day 293), when average daily water temperatures ranged from 18.4°C to 28.4°C and averaged 24.8°C (Figure 2-2). Overall survival was 87.7 ± 0.4% and, at harvest, the average weight and stocking density of mulloway was 384 ± 3 grams and 39.9 ± 0.1 kg/m³, respectively (Table 2-1). Due to the difference in performance of mulloway over the winter and summer, FCR and SGR have been calculated for both the full 292 day culture period and the 174 day ‘summer’ period from October to March (Table 2-1).
Figure 2-2: Mean daily water temperatures recorded in the 0.13 ha trial pond.

Figure 2-3: Growth of rainbow trout, mulloway and barramundi in SIFTs held within the 0.13 ha trial pond.
Table 2-1: Growth, survival, FCR and final stocking density of rainbow trout, mulloway and barramundi grown in SIFTS within the 0.13 ha trial pond.

<table>
<thead>
<tr>
<th></th>
<th>Trout</th>
<th>Mulloway</th>
<th>Barramundi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Full</td>
<td>Summer</td>
</tr>
<tr>
<td>Days of culture</td>
<td>111</td>
<td>292</td>
<td>174</td>
</tr>
<tr>
<td>Initial weight (g)</td>
<td>83</td>
<td>100</td>
<td>116</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>697 ± 5</td>
<td>384 ± 3</td>
<td>435 ± 29</td>
</tr>
<tr>
<td>SGR (%/day)</td>
<td>1.91 ± 0.01</td>
<td>0.461 ± 0.003</td>
<td>0.649 ± 0.010</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>90.4 ± 0.8</td>
<td>87.7 ± 0.4</td>
<td>88.3 ± 2.2</td>
</tr>
<tr>
<td>FCR</td>
<td>0.97 ± 0.03</td>
<td>1.45 ± 0.02</td>
<td>1.39 ± 0.005</td>
</tr>
<tr>
<td>Final stocking density (kg/m^3)</td>
<td>43.1 ± 1.2</td>
<td>39.9 ± 0.1</td>
<td>53.6 ± 2.8</td>
</tr>
</tbody>
</table>
Barramundi grew from 40 to 435 ± 28 grams over 138 days (Figure 2-3) (SGR = 1.73 ± 0.14 %/day), with an FCR of 0.90 ± 0.05 (Table 2-1). Over this time, temperature ranged from 20.8°C to 28.4°C and averaged 25.5°C (Figure 2-2). Survival of barramundi was 88.3 ± 2.2% and the average stocking density at harvest was 53.6 ± 2.8 kg/m³ (Table 2-1).

Total fish production over the 292-day period from the 0.13 hectare pond was 2.72 tonnes (0.86 tonnes of rainbow trout, 0.79 tonnes of mulloway and 1.07 tonnes of barramundi), giving an equivalent annual pond yield of 26 tonnes/ha/yr

2.4.2. Water Quality and Sludge Removal

Feed input into the pond increased from an initial value of 44 kg/ha/day to 93 kg/ha/day by Day 103 (Figure 2-4). When rainbow trout were harvested, feed input subsequently decreased to 7 kg/ha/day and increased again when barramundi were introduced (Day 145). The maximum feed input to the pond of 133 kg/ha/day was achieved by Day 258 and remained at this level for 28 days until barramundi were harvested on Day 286.

TAN was first detected on Day 273, when daily feed input to the pond was 133 kg/ha/day (957 mg N/m²/day). On this day TAN was measured at 1.2 mg/L, and ranged between this value and 1.9 mg/L for the remainder of the trial. Alkalinity of the pond was stable at 250 mg/L and diurnal fluctuations in pH measured during the dense microalgal bloom (Day 191 to 194) were low (maximum range 8.26 to 8.79). Assuming a similar range of pH between Days 273 and 293 when TAN was measured, and a maximum pond temperature during this time of 27°C, then the concentration of unionised ammonia nitrogen (UIA-N) would have ranged from 0.13 to 0.55 mg/L.
Figure 2-4: Feed input (Nova ME, 45% protein, 20% lipid, Skretting, Rosny Park, Australia) into a 0.13 ha aquaculture pond containing a SIFTS used for culturing rainbow trout, mulloway and barramundi.

The daily turbidity (Secchi depth) of the pond throughout the year is shown in Figure 2-5. During the 111-day rainbow trout culture period, the pond remained clear (2 m Secchi depth) for the first 84 days except after two periods of heavy rainfall (Days 15 and 47) when fine clay particles entered the pond with surface run-off. During these 84 days, the cover of the macrophyte *Chara* spp. increased from the initial estimate of 60% to 90% by July and 100% by late August. In addition, other macrophytes, including *Enteromorpha* spp. and *Ruppia* spp. also colonised the pond. By Day 90, a bloom of microalgae began and gradually increased in density (Figure 2-5). The only microalgal species present during this time was an unidentified flagellated chlorophyte. After the rainbow trout were harvested the microalgal bloom subsequently decreased but restarted shortly afterwards (Day 135).
The daily profile of dissolved oxygen concentration in both the pond and the outflow of each SIFT at the height of this chlorophyte bloom (Day 107, cell density $1.1 \times 10^6$ cells/mL) is shown in Figure 2-6a. The diurnal fluctuation in oxygen concentration within the pond is typical of one in which there is a dense bloom of microalgae. During the day dissolved oxygen was supersaturated and reached a maximum level of 15.2 mg/L. At night, dissolved oxygen decreased; reaching a minimum of 1.4 mg/L and remaining below 2 mg/L for approximately 3 hours. Despite these wide diurnal fluctuations in dissolved oxygen within the pond, dissolved oxygen in the outflow of each SIFT was maintained between 4.9 and 7.0 mg/L.

From Day 193, during the barramundi/mulloway crop, a bloom of a dinoflagellate (*Heterocapsa* sp.) began. An example of the diurnal fluctuations in dissolved oxygen in the SIFTs’ outflow and pond during the height of this dinoflagellate bloom are shown in
Figure 2-6b (Day 238). On this day, dissolved oxygen in the pond reached a maximum of 32 mg/L at 1600 hours and a minimum of 0.13 mg/L at 0630; remaining below 1 mg/L for approximately 4.5 hours. Outflow oxygen concentration on this day was similar in all SIFTs, ranging from a minimum of 2.7 to a maximum of 11.0 mg/L. On seven occasions between Days 235 and 286, however, dissolved oxygen concentration in the outflow dropped below 2 mg/L for up to 3.5 hours.
**Figure 2-6:** Diurnal fluctuations in dissolved oxygen in the 0.13 ha pond and the outflow of SIFTs held within this pond on (a) Day 107 during a chlorophyte bloom and (b) Day 238 during a dinoflagellate (*Heterocapsa* sp.) bloom.
The waste collected from the solid waste collection system was a thick sludge, ranging in dry matter content from 5 to 10%. The recovery of dry solid waste as a percentage of feed fed varied from $14.4 \pm 2.7\%$ for barramundi to $26.1 \pm 5.6\%$ for mulloway (Table 2-2). Over the 292 day culture period approximately 527 kg of dry solid waste was collected (5 tonnes/ha/yr) from the 2593 kg of feed fed (i.e. 20.4\%)(Table 2-2).

The nitrogen and phosphorus contents of the dry sludge collected from each species are shown in Table 2-2. Over the 292-day trial period, a total of 15 kg of nitrogen (144 kg/ha/yr) and 16 kg of phosphorus (153 kg/ha/yr) were collected in the solid waste collectors, and subsequently prevented from entering the pond.
Table 2-2: Feed inputs to and waste collected from a 0.13 ha static, inland saline aquaculture pond stocked with rainbow trout, mulloway and barramundi within a SIFTS.

<table>
<thead>
<tr>
<th></th>
<th>Trout</th>
<th>Mulloway</th>
<th>Barramundi</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total feed fed (kg)</td>
<td>833</td>
<td>899</td>
<td>861</td>
<td>2593</td>
</tr>
<tr>
<td>Dry waste recovered in collector (a % fed)</td>
<td>20.2 ± 2.5</td>
<td>26.1 ± 5.6</td>
<td>14.4 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Total dry waste recovered (kg)</td>
<td>168</td>
<td>235</td>
<td>124</td>
<td>527</td>
</tr>
<tr>
<td>N content of dry waste (%)</td>
<td>2.97 ± 0.09</td>
<td>3.14 ± 0.29</td>
<td>2.23 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>Total N recovered (kg)</td>
<td>4.99</td>
<td>7.38</td>
<td>2.76</td>
<td>15</td>
</tr>
<tr>
<td>P content of dry waste (%)</td>
<td>3.37 ± 0.07</td>
<td>3.50 ± 0.26</td>
<td>1.95 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>Total P recovered (kg)</td>
<td>5.66</td>
<td>8.22</td>
<td>2.42</td>
<td>16</td>
</tr>
</tbody>
</table>
2.5. Discussion

2.5.1. Water Temperature, Growth and Feed Conversion Ratio

The specific growth rate of rainbow trout in the current study (1.91 ± 0.01%/day) is better than any reported under commercial conditions and equally as good or greater than experimental scale studies. Lever et al. (2004), for example, reported specific growth rates of 1.09 and 1.21%/day from intensive and semi-intensive rainbow trout farms respectively, both using saline groundwater with similar temperature and salinity regimes to the current study. With fish of similar initial size to those used in this study, Teskeredžić et al. (1989) grew rainbow trout in sea cages with SGRs ranging from 1.01 to 1.18%/day and likewise, Sahin et al. (1999) reported an SGR of 1.67%/day for similar sized fish grown in sea cages. Ingram and Pettit (1996) stated that typical growth rates under commercial conditions for 100 gram rainbow trout grown over 10 weeks were 7 – 10%/week (i.e. 1.0 – 1.4 %/day). Under small-scale experimental conditions, Okumus and Mazlum (2002) achieved an SGR of 2.0 and an FCR of 1.0 in rainbow trout grown for 22 weeks in water of 7 – 10 ppt, and the authors noted that these rates of growth were significantly greater than those achieved on commercial farms. The authors attributed the poorer performance on commercial farms to water quality, husbandry and feeding practices.

The FCR obtained by rainbow trout in the current trial (0.97 ± 0.03) is at the lower (better) end of the optimum range reported for this species (1.0 – 1.5) by Okumus and Mazlum (2002). I suggest that the ability to minimise feed wastage by using the solid waste collectors to gauge when satiety is reached would have contributed to the low FCR achieved in this trial.
Water temperature is likely to have been a contributing factor to the excellent rate of growth obtained with rainbow trout in the current trial, as the average temperature (14.9°C) equalled that identified by Sumpter (1992) as the optimum for growth of this species. That the commercial rainbow trout farms described by Lever et al. (2004) also experienced very similar temperatures but obtained much slower growth suggests that additional factors contributed to the improved growth in the current trial (see below).

Published data on mulloway growth rate are limited. Flowers and Hutchinson (2004b) reported a very low growth rate of juvenile mulloway in inland saline water when temperatures were below 18°C, supporting the data obtained in the current trial that growth is poor at such temperatures. At a constant temperature of 23°C, Doroudi et al. (2006) grew mulloway in inland saline groundwater from 11 to 270 grams over eight months (i.e. SGR of 1.3%/day). Despite an increase in growth rate in the current study between October and March when temperature averaged 24.8°C, the SGR over this period (0.69 ± 0.01%/day) was still considerably less than that obtained in the aforementioned study. That the mulloway’s FCR over the summer period was similar to that obtained over the whole year, suggests that growth was limited only by feed intake and not by any difference in conversion efficiency.

In a 56 day feeding experiment with 230 gram barramundi at 28°C, Williams et al. (2003b) obtained an SGR of 1.16%/day on a diet with a similar formulation to that used in the current study. Over this same weight and time range, but a lower average daily temperature of 25.8°C, I obtained a mean SGR of 1.10%/day for barramundi. Williams et al. (2003a) also grew 80 gram barramundi at 28°C over a 42 day period and obtained an SGR of 1.86%/day. When growth data from the current study was analysed over the
same weight and time range, barramundi grew at 1.98%/day at the lower temperature of 25.9°C. The excellent FCR (0.90 ± 0.05) that I achieved with barramundi is again likely contributed to by the minimisation of feed wastage through the use of the solid waste collectors, but also by the fact that barramundi were observed feeding on naturally occurring mosquito fish (*Gambusia holbrooki*) within the SIFTS, which were pumped from the pond through the air lift pumps. Unpublished data by the authors has shown that mosquito fish are an effective supplementary feed for barramundi, whereas mulloway have not been observed consuming mosquito fish and mosquito fish were not present in the pond during the winter months when trout were cultured.

### 2.5.2. Dissolved Oxygen, Water Quality and Sludge Removal

That the concentration of dissolved oxygen in the SIFTs’ outflow was maintained between 4.9 and 7.0 mg/L at the height of a chlorophyte bloom that caused diurnal fluctuations within the pond of 1.4 to 15.2 mg/L, highlights that the air lifts are very effective at both oxygenating the water during hypoxic conditions and degassing supersaturated levels of oxygen from solution during times of hyperoxia; a feature also noted by Yoo et al. (1995). Soderberg et al. (1983) suggested that maintaining dissolved oxygen concentrations above 5 mg/L is beneficial for rainbow trout production, and since the SIFTS maintained these levels during the rainbow trout crop, it seems likely to have contributed to their excellent growth. Several studies (Edsall and Smith, 1990; Foss et al., 2002; Person-Le Ruyet et al., 2002; Foss et al., 2003; Dabrowski et al., 2004) have investigated the effects of hyperoxia on fish growth and although none found any negative effects of hyperoxia on either growth or survival, Ritola et al. (2002) suggested that short-term fluctuations between hyperoxia and normoxia (as occurs on a
diurnal basis in pond culture) can cause stress to rainbow trout. The degassing effect caused by the air lifts may therefore be beneficial for this species.

Despite a significant difference in fish stocking density between the rainbow trout and mulloway SIFTs at the height of the chlorophyte bloom (ca. 43 and 14 kg/m$^3$, respectively), outflow oxygen concentrations between all SIFTs were similar (Figure 2-6a), suggesting that the higher density of rainbow trout was not limiting oxygen concentration in the SIFTs’ outflow. As Ojolick et al. (1995) described high mortality in rainbow trout reared at the ‘chronic high temperature’ of 21 ± 1°C, fish in the current trial were harvested when maximum daily water temperature exceeded 20°C. The data on outflow oxygen concentration presented above, however, suggests that, had temperatures remained appropriate for this species, higher stocking densities may have been achievable. Okumus and Mazlum (2002) cultured rainbow trout at densities up to 90 kg/m$^3$ with a flow rate of 3 exchanges/hour whilst achieving excellent growth and feed conversion ratios, suggesting that similar results could be achieved in the SIFTS which employ a 30% greater flow rate.

As a result of the higher water temperature and higher density of microalgae, minimum concentrations of dissolved oxygen in the pond were lower during the barramundi/mulloway crop than the trout/mulloway crop. Hargreaves and Tucker (2003) suggest that the minimum level of dissolved oxygen for good growth of channel catfish (*Ictalurus punctatus*) is 2 – 3 mg/L and this minimum level was maintained in the SIFTs’ outflows for the majority of the barramundi/mulloway crop. The occasional drop below 2 mg/L did not appear to affect the growth of barramundi, however the negligible growth of mulloway over the last 35 days of the trial suggests that these
short-term events may have affected growth in this species. Alternatively, this reduction in growth may have been the result of the high feed input into the pond over the last 28 days of the trial (133 kg/ha/day). Hargreaves and Tucker (2003), for example, suggest that at feed inputs greater than 100 to 125 kg/ha/day, the waste assimilation capacity of the pond is exceeded and water quality deterioration suppresses growth. These authors interestingly point out, however, that the actual water quality parameters responsible for decreased growth at these high rates of feed application have not been defined. That TAN was first observed in the pond during this time of high feed input suggests that either TAN or UIA-N may have been a contributing factor to the poor performance of mulloway in the final weeks. Although the concentrations of TAN and UIA-N are lower than those found to prevent growth in other species (e.g. Person-Le Ruyet et al. 1997), the reduced growth of mulloway may have been the result of the interactive effect of low dissolved oxygen and high ammonia, as described by Wajsbrot et al. (1991).

Glencross et al. (2003) determined that the dry matter digestibility of the commercial diet used in the current trial was 81.6% when fed to barramundi, indicating that the amount of faeces produced from this diet should amount to 18.4% of the diet fed. That the amount of dry waste collected from the SIFTs containing barramundi in the current trial averaged 14.4% of feed fed, suggests that the majority of the faeces produced were being collected in the solid waste collection system. The average amount of waste collected as feed fed across the three species in the current trial was 20.6% and is significantly higher than the 0.01% removed by Yoo et al. (1995) using a series of solid waste collectors attached to floating raceways. Although Masser and Lazur (1997) identified alternative waste removal technologies for floating tanks, including tube
settlers and sand and synthetic mesh filters, they did not quantify their effectiveness and also conceded that solid wastes are very difficult to remove from raceways.

The efficient removal of solid waste from the SIFTS (equivalent to approximately 5 tonnes/ha/yr) is highly likely to reduce sludge build-up on the pond bottom. This removal is beneficial because sediment is an important source of ammonia, through the mineralisation of organic matter and the diffusion from reduced sediment (Hargreaves, 1998; Burford and Longmore, 2001; Hargreaves and Tucker, 2003). Hopkins et al. (1994) proved that removing sludge from shrimp ponds significantly reduced TAN and orthophosphate concentrations, and increased the concentration of dissolved oxygen. Cole and Boyd (1986) reported a maximum TAN concentration of 4.2 mg/L in static channel catfish ponds fed 84 kg/ha/day with a 32% protein diet (430 mg N/m²/day). The maximum TAN concentration in the current study, on the other hand, was only 1.88 mg/L when fed a 45% protein feed at the rate of 133 kg/ha/day (958 mg N/m²/day). These data support the findings of Hopkins et al. (1994) that sludge removal significantly reduces TAN within the pond.

In a review of channel catfish farming in the USA, Boyd et al. (2000) stated that yields from static growout ponds averaged 6.3 tonnes/ha/yr, and Hargreaves and Tucker (2003) contended that the limits of catfish production in static ponds as they are currently configured and managed, have been reached at 5.6 to 6.7 tonnes/ha/yr. That a total fish production of 26 tonnes/ha/yr was achieved in a static water body during this study suggests that the SIFTSs’ ability to remove significant quantities of nutrient and provide high volumes of aerated water to the culture species can significantly increase fish yields from static aquaculture ponds. Continuing research is concentrating on using
phytoplankton-cropping species (e.g. *Artemia*) within SIFTS to minimise the effects of thick microalgal blooms and create secondary crops of economic value.
Chapter 3

Skeletal myopathy in juvenile barramundi cultured in potassium deficient groundwater.

Citation:


2.6. Introduction

Significant spatial variation in the ionic composition of inland saline water sources has been identified (George, 1990, Chapter 1). As such, many water sources are unlikely to be suitable for culturing fish. As indicated in Chapter 1, a crucial first step in testing the suitability of water sources for fish culture is to undertake bioassays. Bioassays are conducted to compare the growth and survival of the species under investigation against those obtained in seawater, and in seawater adjusted to the salinity of the water being tested. I have undertaken a number of such bioassays with different water sources and fish species (Partridge and Furey, 2002; Partridge, 2003b).

This chapter presents the results of an investigation into mortalities of juvenile barramundi (Lates calcarifer) obtained during two bioassays in which the suitability of a saline groundwater source was being tested.
2.7. Materials and Methods

The first bioassay was undertaken in triplicate, 180 L, independent, recirculating tanks over a period of 28 days. All tanks were held within a water bath maintained at 26°C. The test treatment was 45 ppt groundwater, sourced from an interception scheme on a conservation reserve near Narrogin, Western Australia (32°55’S, 117°36’E). Two control treatments were included; seawater (36 ppt) and seawater adjusted to 45 ppt by the addition of artificial seasalt (Ocean Nature®). Ten juvenile barramundi (8.0 ± 0.2 g) were stocked into each tank following a 3-day acclimatisation period to the salinity of the test water. Fish were fed to satiety three times each day on a commercial barramundi diet (Skretting Australia). The bottom of each tank was vacuumed three times each week and 10% of the water volume replaced.

Moribund and recently dead fish (n=6) from the saline groundwater replicates were collected on the twelfth day of the bioassay and preserved in 10% formalin (prepared with the treatment water). Unaffected fish from the two control treatments (n = 20) were also killed and preserved for comparison. Para-sagittal slab sections of preserved fish were decalcified in 10% formic acid for six hours, vacuum embedded in paraffin and 5 µm sections stained with haematoxylin and eosin (H&E).

Samples of the feed used in the trial were tested for levels of vitamin E and selenium to determine if these were the cause of the observed myopathies. Feed was saponified by the method of Bieri et al. (1961) before extraction with hexane. Vitamin E ( alphatocopherol) was measured in the extract using high performance liquid chromatography (HPLC) with fluorescence detection (McMurray and Blanchflower, 1979). The
selenium content of the feed was measured following acid digestion using hydride generation on an atomic absorption spectrophotometer (Norheim, 1986).

Analysis of the concentration of 24 ions within the groundwater source was performed by inductively coupled plasma atomic emission spectroscopy (Vista AX CCD Simultaneous ICP-AES). The concentration of chloride ions was determined via a silver-chloride titration. At the completion of the trial, blood was taken from the caudal peduncle of surviving fish and stored in heparinized (lithium) tubes on ice. The concentrations of potassium, chloride and sodium in the blood plasma (n = 22) were analysed within 2 hours of blood collection using a Vetlyte ion specific electrode analyzer.

A second, short-term bioassay was undertaken with the same treatments to provide more rigorous data on blood plasma electrolytes and also to investigate the relationship between intra- and extra-cellular potassium. Five replicates of each treatment were tested. Each replicate consisted of a single barramundi (88.1 ± 2.7 g) held in 20 L of the treatment water at 26°C for a period of 7 days. Each day, 10% of the water in each tank was replaced. At the completion of the bioassay, barramundi were killed with an overdose of anaesthetic (AQUI-S). Blood was sampled for electrolytes and fish preserved for histological analysis as described for the first bioassay. Prior to preservation, a sample of the dorsal muscle was also taken from each fish, frozen, freeze-dried and then ground. After grinding, the samples were digested with a combination of concentrated nitric acid, hydrogen peroxide and hydrochloric acid at 120°C. The potassium content of the digest was determined via ICP-AES and compared against reference materials of dogfish muscle (DORM2) and liver (DOLT2) (National
Research Council of Canada). Differences in plasma and muscle electrolyte levels among treatments were tested by one-way analysis of variance, with a post hoc comparison of group means using Tukey’s HSD test. Statements of statistical significance refer to the 0.05 level.

2.8. Results

The ionic composition of the groundwater and seawater is shown in Table 0-1. To allow a comparison of the relative ionic composition between the groundwater and seawater, each element has also been expressed relative to groundwater diluted to the salinity of seawater (36 ppt). This comparison shows that the saline groundwater is deficient in potassium and boron and has high concentrations of magnesium, calcium and sulphur, relative to seawater.

In the first bioassay, fish deaths commenced ten days after introduction to the groundwater source. Just prior to death, fish became non-responsive, dark in colour and congregated near the water surface. At the completion of the trial, survival in the groundwater averaged 27 ± 9%, whereas those in the two control treatments averaged 97 ± 3% (36 ppt) and 100% (45 ppt). Survival in the second, shorter bioassay was 100% in all treatments.
Table 0-1: 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. *Data modified from Spotte (1992).

<table>
<thead>
<tr>
<th>Major elements (mg/L)</th>
<th>Groundwater at 45 ppt*</th>
<th>Seawater at 45 ppt*</th>
<th>Groundwater relative to 45 ppt seawater (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl</td>
<td>25000</td>
<td>23750</td>
<td>105%</td>
</tr>
<tr>
<td>Na</td>
<td>12000</td>
<td>13750</td>
<td>87%</td>
</tr>
<tr>
<td>Mg</td>
<td>2800</td>
<td>1500</td>
<td>187%</td>
</tr>
<tr>
<td>S</td>
<td>1400</td>
<td>1063</td>
<td>132%</td>
</tr>
<tr>
<td>Ca</td>
<td>740</td>
<td>550</td>
<td>135%</td>
</tr>
<tr>
<td>K</td>
<td>140</td>
<td>613</td>
<td>23%</td>
</tr>
<tr>
<td>Sr</td>
<td>9.3</td>
<td>9</td>
<td>102%</td>
</tr>
<tr>
<td>B</td>
<td>1.2</td>
<td>5</td>
<td>23%</td>
</tr>
</tbody>
</table>

At the completion of the first trial, those fish surviving in the groundwater treatment weighed 15% less than their initial weight (final weight, 6.9 ± 0.5 g), whereas those in the two control treatments both increased in weight by over 200% (final weights 24.1 ± 0.6 g and 28.2 ± 1.0 g for fish in 36 and 45 ppt, respectively). No growth data were collected from the second bioassay.
2.8.1. **Histopathology**

All fish examined from both bioassays that were held in the saline groundwater exhibited widespread, severe skeletal muscle degeneration and necrosis that affected both epaxial muscle groups and muscles within the pharyngeal area (Figure 0-1). No myocardial nor pansteatitis lesions were observed. Histological examination revealed a segmental and multiphasic reaction that involved the majority of fibres in the section. Acute lesions were characterised by hypercontracted fibres, and swollen, intensely eosinophilic fibres characteristic of hyaline degeneration. There was widespread mineralisation of acutely degenerating and necrotic sarcomeres. Interspersed between degenerate and necrotic fibres were myotubes containing phagocytes and dividing subendoendomysial satellite cells. Basophilic staining fibres with centrally located myoblasts, either in clusters or short chains, characterised the regenerative response. More advanced regenerative stages were not present. These features suggest the lesions commenced soon after entering the test water source (Hulland, 1993). All fish examined from the control treatments of both bioassays showed no skeletal muscle degeneration.
**Figure 0-1:** Longitudinal section of skeletal muscle showing acute swelling of myotubes and fragmentation of myofibres (A), with oedema and mineralization of degenerate fibres (B). (H&E, × 330).

All fish examined from the saline groundwater sources from both bioassays showed marked chloride cell hyperplasia (Figure 0-2). There was patchy, mild, chloride cell hyperplasia in the saline adjusted (45 ppt) seawater controls, whereas those in the seawater control treatments showed no hyperplasia.
Figure 0-2: Gill filaments showing marked hypertrophy and hyperplasia of branchial chloride cells. (H&E, × 165).

The excretory portion of the kidneys in those fish cultured in the saline groundwater treatments in both bioassays showed a mild nephrosis that was characterised by patchy acute tubular epithelial cell necrosis with granular casts and intensely eosinophilic-staining amorphous material filling tubule lumina. Eosinophilic droplets were also present within the cytoplasm of tubular epithelial cells, particularly those tubules surrounding glomeruli. Tubular regeneration was present adjacent to necrotic tubules (Figure 0-3). No renal lesions were identified in fish from the two control treatments.
2.8.2. Feed Vitamin E and Selenium

Feed alpha-tocopherol levels were considered adequate at 1.5 mg/kg dry weight and feed selenium levels of 5 mg/kg were within normal ranges for fish feed (NRC, 1993), suggesting that low levels of these factors did not cause the observed myopathies.

2.8.3. Clinical Chemistry Results

The concentrations of sodium, chloride and potassium in the blood plasma of fish from the various treatments from the first bioassay are shown in Figure 0-4a. 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
treatment 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, however, 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), and there was no significant difference between the two control treatments.

The concentrations of blood electrolytes of barramundi from the second bioassay are presented in Figure 0-4b. There were significant differences in blood plasma sodium and blood plasma chloride concentrations among all treatments. Concentrations were greatest in the groundwater treatment (202.4 ± 0.3 mmol/L sodium; 188.2 ± 3.2 mmol/L chloride) and smallest in the 36ppt seawater treatment (173.2 ± 1.6 mmol/L sodium; 167.3 ± 0.6 mmol/L chloride). There were no significant differences in blood plasma potassium concentration between any of the three treatments.
Figure 0-4: Blood plasma concentrations (mmol/L ± SE) of sodium, chloride and potassium from barramundi cultured in saline groundwater and two seawater controls during bioassay 1 (a) and bioassay 2 (b). Columns within the same electrolyte group sharing the same letter are not significantly different (P>0.05).
The concentration of potassium in the muscle of fish held in the saline groundwater treatments in the second bioassay (10.5 ± 0.2 g/kg) was significantly less than those held in both the 45 ppt and 36 ppt controls (16.0 ± 0.3 g/kg and 15.7 ± 0.2 g/kg, respectively) (Figure 0-5).

![Figure 0-5: Muscle potassium concentration (g/kg ± SE) from barramundi cultured in saline groundwater and two seawater controls during bioassay 2. Columns sharing the same letter are not significantly different (P>0.05).](image)

2.9. Discussion

Juvenile barramundi held in saline groundwater exhibited high mortality, loss of weight and pathology of the muscle and kidneys. They also had increased concentrations of sodium and chloride in the plasma and reduced concentrations of potassium in the muscle. Based on the following evidence, I hypothesise that these factors were the result of potassium deficiency in this water source.
Although there appears to be no previous evidence of hypokalaemic muscle myopathy in fish, myopathies associated with hypokalaemia have been described in mammals, birds and humans (Tate et al., 1978; De Coster, 1979). Rats and dogs fed potassium deficient diets, for example, suffer myopathies in skeletal muscle and also demonstrate reduced growth and death if the diet is continued (Tomé, 1982). These myopathies are characterised by vacuolation of muscle fibres, necrotic and regenerating fibres and infiltration with mononuclear cells around degenerating fibres. Similar changes are also described in human hypokalaemic myopathy (Kakulas and Adams, 1985). The lesions present in the barramundi in the current trial are atypical of mammalian hypokalaemic myopathy. Lesions in the barramundi muscle 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.

Skeletal muscle is the main store of intracellular potassium in fish as well as mammals (Jobling, 1995; McDonough et al., 2002). In mammals, the ability of the skeletal muscle to rapidly buffer the potassium content of the extracellular fluid (ie. plasma) in times of potassium depletion has been demonstrated (McDonough et al., 2002). Under chronic potassium deficiency this buffering effect leaves the skeletal muscle deficient in potassium, which leads to vasoconstriction in the muscle. This vasoconstriction results in focal ischemia, which subsequently leads to necrosis (Anderson et al., 1972; Penn, 1979; Sharief et al., 1997). Barramundi cultured in potassium-depleted saline groundwater in the current trials exhibited normal levels of blood plasma potassium and reduced muscle potassium, suggesting that barramundi have the same physiological response to hypokalaemic conditions as mammals.
In fish within a hyperosmotic environment, potassium is also essential to the branchial extrusion of sodium and chloride (due to its role in Na⁺-K⁺-ATPase of the sodium pump) and it has been shown by Maetz (1969) that the rate of efflux of sodium across the gills is dependant 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 the sodium plasma concentration. That the plasma sodium concentration of barramundi held in the inland saline water source was significantly greater than those in 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, and were observed in barramundi held in saline groundwater.

The extrusion of monovalent ions in marine and euryhaline teleosts occurs via chloride cells located on the gills’ 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 fish from the 45 ppt seawater control treatment is the typical physiological response to an increase in salinity (Utida and Hirano, 1973). That the chloride cell hyperplasia was more severe in the saline groundwater treatments than in the control with the same salinity is likely to be in response to the hypernatraemia and hyperchloridaemia caused by the low external potassium concentration.

Whilst renal tubular damage has been described in humans as a consequence of hypokalaemia (Emery et al., 1984), the lesion has not previously been recognised in fish. I hypothesise that myoglobin may have been a contributing factor to these lesions.
The massive myonecrosis found in affected fish is expected to release large amounts of myoglobin. Myoglobin is associated with nephrotoxicity in mammals (Hulland, 1993) and the intensely eosinophilic casts and granules present in the barramundi kidneys are histologically similar to those seen in myoglobinuric nephrosis in mammals.

The evidence presented from these bioassays suggests that the low concentration of potassium in the groundwater source caused severe hypokalaemia in muscle cells, leading to mortality in juvenile barramundi. The data also suggest that these symptoms will be ameliorated by potassium supplementation and the amount of supplementation required will be dependant on salinity. I test these hypotheses in the following Chapter.
Chapter 4

The effect of salinity on the requirement for potassium by barramundi in saline groundwater.

Citation:


3.1. Introduction

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 (see Chapter 1, 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. (In Press) (Chapter 1) 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 (Sharigur and Siddiqui, 1998) and has been identified as a suitable species for inland saline aquaculture in both Australia (Chapter 1, Partridge et al., In Press) and India (Jain et al., 2006). In Chapter 3 (Partridge and Creeper, 2004) I 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. I 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 chapter, I 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.

### 3.2. Methods

#### 3.2.1. Experimental Design

Three bioassays were conducted with a range of potassium supplementation levels outlined in Table 3-1.
Table 3-1: Salinity and K-equivalence treatments investigated in bioassays 1, 2 and 3.

<table>
<thead>
<tr>
<th>Bioassay</th>
<th>Salinity (ppt)</th>
<th>K-equivalence</th>
<th>Potassium (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45</td>
<td>50%</td>
<td>267</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75%</td>
<td>401</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100%</td>
<td>534</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>25%</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50%</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75%</td>
<td>134</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100%</td>
<td>178</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>25%</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50%</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75%</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100%</td>
<td>59</td>
</tr>
</tbody>
</table>

The same saline groundwater used in Chapter 3 was collected and trucked to the Aquaculture Development Unit (ADU) in Fremantle.

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 3-1). Unsupplemented groundwater (25% K-equivalence) was not included in this bioassay, because it caused mortality of barramundi at this salinity (45 ppt) in the previous bioassay (Partridge and Creeper, 2004) (Chapter 3). 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 via inductively coupled plasma atomic emission spectroscopy (ICP-AES) and flow injection analysis (APHA, 2005), respectively. The
additions of potassium chloride resulted in chloride concentrations increasing by no more than 1.68% compared to the unsupplemented treatments.

3.2.2. 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.

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 six hours, 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 in Chapter 3 (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\(_2\)EDTA, 0.1 M, pH 7.1) at -80°C. Semi-purified homogenates were prepared by homogenising (Heidolph, Diax 600) thawed samples for 10 seconds before centrifuging at 2000 G for 7 minutes 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 seconds. After a further centrifugation step of 6 minutes, 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\(_2\).6H\(_2\)O 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 minutes at 37°C in the presence of 100 µL of an ATP solution (30 mM Na\(_2\)ATP, 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 μmoles of phosphate liberated per milligram of protein per hour.

3.2.3. Data Analysis

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

\[
SGR \ (\%/day) = \left( \frac{\ln (W_f) - \ln (W_i)}{\text{Time (days)}} \right) \times 100
\]

Where \( W_f \) and \( W_i \) were the final and initial wet weights of the fish, respectively. SGR, 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.

3.3. Results

3.3.1. Water Ionic Composition

The ionic composition of this water source is given in Chapter 3, Table 0-1. Compared to seawater, groundwater was deficient in potassium, boron and sodium, and had greater concentrations of magnesium, calcium, sulphur, chloride and strontium.
3.3.2. **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 in Chapter 3 and Partridge and Creeper (2004).

3.3.3. **Growth**

At a salinity of 45 ppt, there was an increase in SGR of barramundi with increasing levels of potassium supplementation (Figure 3-1a). 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 (Figure 3-1b), while at a salinity of 5 ppt, SGR ranged from 2.30 to 2.62%/day, again with no significant differences among treatments (Figure 3-1c).

3.3.4. **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 3-2). 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 3-2).
Figure 3-1: 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).
Table 3-2: Plasma and muscle electrolyte concentrations and muscle water contents from barramundi grown at various salinities and K-equivalence values for 4 weeks. Parameter values within each salinity sharing the same letter are not significantly different (P>0.05).

<table>
<thead>
<tr>
<th>Salinity (ppt)</th>
<th>K-Equivalence (%)</th>
<th>Plasma K (mmol/L)</th>
<th>Plasma Na (mmol/L)</th>
<th>Plasma Cl (mmol/L)</th>
<th>Muscle Water (%)</th>
<th>Muscle K (g/kg)</th>
<th>Muscle Na (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>50%</td>
<td>10.6 ± 2.4</td>
<td>182 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>177 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.4 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.7 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.7 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>75%</td>
<td>14.8 ± 0.2</td>
<td>171 ± 2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>171 ± 2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>77.7 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.5 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.2 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>12.1 ± 2.7</td>
<td>167 ± 2&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>164 ± 1&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>76.6 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.8 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.4 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
<td>25%</td>
<td>6.2 ± 0.3</td>
<td>162 ± 0</td>
<td>140 ± 1</td>
<td>87.3 ± 0.9</td>
<td>12.7 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.8 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>5.4 ± 0.8</td>
<td>171 ± 3</td>
<td>146 ± 3</td>
<td>86.8 ± 0.5</td>
<td>15.3 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.8 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>75%</td>
<td>5.4 ± 0.7</td>
<td>165 ± 1</td>
<td>142 ± 1</td>
<td>87.1 ± 0.2</td>
<td>18.5 ± 0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.3 ± 0.2&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>7.5 ± 1.0</td>
<td>162 ± 2</td>
<td>142 ± 2</td>
<td>86.4 ± 0.3</td>
<td>18.3 ± 0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.1 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>25%</td>
<td>3.9 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>161 ± 1</td>
<td>137 ± 1</td>
<td>76.6 ± 0.7</td>
<td>17.0 ± 0.4</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>4.3 ± 0.3&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>161 ± 2</td>
<td>135 ± 0</td>
<td>77.2 ± 0.5</td>
<td>17.9 ± 0.4</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>75%</td>
<td>5.0 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>159 ± 2</td>
<td>137 ± 1</td>
<td>76.1 ± 0.2</td>
<td>17.2 ± 0.2</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>4.9 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>161 ± 1</td>
<td>139 ± 1</td>
<td>76.1 ± 0.1</td>
<td>16.8 ± 0.1</td>
<td>1.5 ± 0.0</td>
</tr>
</tbody>
</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 muscle water content and muscle potassium concentration, but a significantly greater muscle sodium concentration than those cultured at 75% and 100% K-equivalence (Table 3-2). 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 3-2). At a salinity of 5 ppt, there were no significant differences between treatments in either muscle water content, potassium concentration or sodium concentration (Table 3-2).

3.3.6.  *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 3-3). At 15 ppt, there were no significant differences in the branchial or renal NKA activities 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 3-3). At a salinity of 5 ppt, there were no significant differences in branchial, renal or intestinal NKA activities between any treatments (Table 3-3).
Table 3-3: (Na⁺-K⁺)ATPase activities (µmoles/mg/hr) of gills, kidney and intestines from barramundi cultured at various salinities and K-equivalence values for 4 weeks. Parameter values within each salinity sharing the same letter are not significantly different (P>0.05).

<table>
<thead>
<tr>
<th>Salinity (ppt)</th>
<th>K-Equivalence (%)</th>
<th>Gills (µmoles/mg/hr)</th>
<th>Kidney (µmoles/mg/hr)</th>
<th>Intestine (µmoles/mg/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>50%</td>
<td>201.1 ± 44.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>260.9 ± 51.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.6 ± 5.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>75%</td>
<td>88.8 ± 4.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>98.8 ± 9.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.6 ± 3.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>107.9 ± 11.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66.8 ± 9.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.4 ± 5.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
<td>25%</td>
<td>22.4 ± 1.2</td>
<td>55.4 ± 8.7</td>
<td>21.5 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>25.7 ± 2.3</td>
<td>43.7 ± 4.1</td>
<td>13.0 ± 1.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>75%</td>
<td>24.6 ± 3.3</td>
<td>54.6 ± 7.6</td>
<td>19.7 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>17.2 ± 2.0</td>
<td>48.6 ± 10.6</td>
<td>13.0 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>25%</td>
<td>29.0 ± 3.6</td>
<td>33.8 ± 8.6</td>
<td>12.4 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>24.4 ± 2.1</td>
<td>5.44 ± 6.2</td>
<td>16.7 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>75%</td>
<td>20.9 ± 1.4</td>
<td>48.3 ± 5.6</td>
<td>13.3 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>22.8 ± 1.5</td>
<td>57.0 ± 2.0</td>
<td>15.8 ± 1.8</td>
</tr>
</tbody>
</table>
3.4. Discussion

In the previous chapter, I found that juvenile barramundi grown in hyperosmotic (45 ppt) groundwater with a K-equivalence of 25%, experienced hypernatraemia and hyperchloridaemia of blood plasma. 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 (Chapter 3, Partridge and Creeper, 2004). It was therefore hypothesised that proportionally more potassium would 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), I 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.

3.4.1. 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 in Chapter 3. 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 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 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 findings with barramundi grown in 25% K-equivalence at the same salinity (Chapter 3, Partridge and Creeper, 2004). I suggest 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 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 in Chapter 3 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 Chapter 3 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, I 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.

3.4.2. Response to Potassium Supplementation at 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 Chapter 3, where I found 100% mortality within nine days of
similar sized barramundi cultured in 45 ppt water with the same potassium equivalence, and therefore supports the 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 I found for fish at 50% K-equivalence at 45 ppt. My 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 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. These fish exhibited no aberrations in muscle water content, blood chemistry or branchial or renal NKA activity, suggesting that this buffering is in a state of homoeostasis. Support for this long-term equilibrium comes from the successful production of barramundi to a marketable size over a 4-month period without potassium supplementation in the same saline water source described in Chapter 2 (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).
3.4.3. 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 this 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.
Chapter 5

Larval rearing of barramundi in saline groundwater

Citation:


4.1. Introduction

In addition to ongrowing, inland saline groundwater has potential for hatchery production of barramundi and other candidate species such as those described Chapter 1. Barramundi are currently produced exclusively in coastal hatcheries using seawater; however, production in saline groundwater has several potential advantages. Local hatchery production would reduce transport costs, and/or allow vertical integration of inland saline farms. Inland saline areas have an abundance of cheaper, less environmentally sensitive land with fewer competing interests than coastal land (Doupé et al., 1999) and isolation of inland hatcheries from pathogens and parasites found naturally in coastal water could facilitate the production of disease-free certified stock (Allan et al., 2001a; Partridge et al., In Press).

The deficiency of potassium in inland saline groundwater restricts its use for mariculture (Forsberg et al., 1996; Fielder et al., 2001; Shakeeb-Ur-Rahman et al., 2005). The effects of this deficiency on juvenile barramundi have been described (Chapters 3 and 4, Partridge and Creeper, 2004; Jain et al., 2006), however, the effect on larvae is unknown. This paper describes the production of barramundi larvae in
potassium-deficient groundwater with a salinity of 14 ppt. Larval survival, growth and swimbladder inflation were measured in this groundwater source with and without potassium supplementation and compared against control treatments of seawater (32 ppt) and seawater diluted to 14 ppt.

4.2. Materials and Methods

Saline groundwater was obtained from a bore in Northam, Western Australia (31°39’S, 116°40’E), approximately 100 km from the coast, and trucked to the Aquaculture Development Unit’s (ADU) marine hatchery facility in Fremantle. The concentrations of the major ions found in seawater were measured in the groundwater using inductively coupled plasma atomic emission spectroscopy (Varian Vista AX CCD Simultaneous ICP-AES). The concentration of chloride ions was determined via flow injection analysis using a method modified from APHA (2005). The potassium concentration of the water source was 38% of that found in seawater of equivalent salinity (i.e 38% K-equivalence) (Table 4-1). Of the other major ions, strontium, boron and sulphur were also deficient (Table 4-1) and sodium, magnesium and calcium were within 20% of equivalent salinity seawater.
Table 4-1: Ionic composition (mg/L) of saline groundwater (14 ppt), seawater diluted to 14 ppt and the percentage difference in composition between the two.

<table>
<thead>
<tr>
<th>Major Ions (mg/L)</th>
<th>Saline Groundwater 14‰</th>
<th>Seawater 14‰</th>
<th>Groundwater as % of Seawater</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl</td>
<td>7800</td>
<td>7862</td>
<td>99%</td>
</tr>
<tr>
<td>Na</td>
<td>3600</td>
<td>4504</td>
<td>80%</td>
</tr>
<tr>
<td>Mg</td>
<td>630</td>
<td>539</td>
<td>117%</td>
</tr>
<tr>
<td>S</td>
<td>230</td>
<td>378</td>
<td>61%</td>
</tr>
<tr>
<td>Ca</td>
<td>190</td>
<td>172</td>
<td>110%</td>
</tr>
<tr>
<td>K</td>
<td>61</td>
<td>159</td>
<td>38%</td>
</tr>
<tr>
<td>Sr</td>
<td>2.8</td>
<td>33.5</td>
<td>8%</td>
</tr>
<tr>
<td>B</td>
<td>0.98</td>
<td>1.86</td>
<td>53%</td>
</tr>
</tbody>
</table>

Barramundi larvae were grown in four different water treatments: unsupplemented, 14 ppt saline groundwater; saline groundwater with full potassium supplementation (100% K-equivalence of 14 ppt seawater, 160 mg/L); undiluted seawater (32 ppt); and seawater diluted to 14 ppt. Potassium supplemented groundwater was prepared by the addition of potassium chloride. The ADU hatchery’s seawater bore (32 ppt) was used for the two seawater control treatments, with that at 14 ppt obtained by dilution with dechlorinated tap water. Each of the four treatments were tested with five replicates. All 180 L replicate tanks were held within a water bath maintained at 28°C. Photoperiod was maintained at 12L:12D with a surface light intensity of 170 lux.

Barramundi larvae were stocked at mouth opening, i.e. at two days post hatch (2 DPH; 2.43 ± 0.03 mm total length (TL)) at 20 larvae/L after a four hour acclimation period from seawater. The flow-through larviculture tanks were supplied with treatment water.
stored in header tanks at the rate of 150 mL/minute. *Chlorella* (Super-fresh V12, Pacific Trading Co, Japan) was maintained at 500,000 cells/mL in each tank for the first 18 days. Rotifers enriched with Rotiselco–ALG (INVE, Belgium) were fed twice daily (0830 and 1500) at 10 rotifers/mL between 2 and 5 DPH, 20/mL between 6 and 13 DPH and 10/mL between 14 and 18 DPH. *Artemia* metanauplii enriched with DHA SuperSelco (INVE, Belgium) were fed to larvae four times daily (0830, 1100, 1300 and 1530) at 10/mL/day between 13 and 19 DPH, decreasing by 1/mL/day thereafter. Weaning (Gemma Micro 300, Skretting, Australia) commenced on 16 DPH at the feeding rate of 1 gram/1000 larvae/day, increasing daily by 0.5 grams/1000 larvae.

Subsamples of five larvae per tank were taken every two to three days. Total length (TL) was measured to 0.1 mm under a stereo microscope using a calibrated eye piece graticule and the presence or absence of an inflated swimbladder was recorded. At the completion of the trial on 25 DPH, all fish were counted, their TL measured, then dried for 24 hours at 75°C to determine their dry weight. Treatment means were compared with one way ANOVA and differences detected using Tukey’s HSD test. Statements of significance refer to the 0.05 level.
4.3. **Results and Discussion**

Barramundi larvae died within 2 days in unsupplemented saline groundwater. At the end of the trial, survival rates in the remaining three treatments were 46.2 ± 7.2% (14 ppt seawater), 52.0 ± 1.6% (supplemented groundwater) and 55.0 ± 3.7% (32 ppt seawater), with no significant differences between these treatments. This rate of survival is similar to that obtained in commercial barramundi hatcheries (Bosmans et al., 2004).

The tolerance of marine fish larvae to salinities less than seawater is species specific. Fielder et al. (2005), for example, achieved 7% survival of snapper (*Pagrus auratus*) larvae reared in 15 ppt seawater, compared to approximately 25% for those reared within the optimum salinity range of 20 to 35 ppt. Australian bass (*Macquaria novemaculeata*), on the other hand, exhibits no significant difference in survival when reared at 10 or 30 ppt (Battaglene and Talbot, 1990).

The growth of larvae in the three surviving treatments is shown in Figure 1-1. At every sampling point from 4 DPH, the total length of larvae in the seawater control (32 ppt) was less than those grown in the other two treatments. At the end of the trial, the length of those larvae grown in full-strength seawater (14.3 ± 0.5 mm) was significantly less (P = 0.042) than those grown in 14 ppt seawater (15.7 ± 0.3 mm) (Figure 4-2). A similar pattern of larval dry weight was seen between treatments at 25 DPH, with those grown at 32 ppt (6.4 ± 0.5 mg) being smaller than those cultured in both seawater at 14 ppt (8.9 ± 1.1 mg) and potassium supplemented groundwater at 14 ppt (8.2 ± 0.6 mg), however these differences were not significant (P = 0.098).
Similarly to survival, the growth response of marine fish larvae to salinities below seawater varies between species. Many, including gilthead seabream (*Sparus aurata*) (Tandler et al., 1995), European seabass (*Dicentrarchus labrax*) (Johnson and Katavic, 1986), and spotted halibut (*Verasper variegatus*) (Wada et al., 2004) grow faster in such salinities, whereas others including snapper (Fielder et al., 2005), southern flounder, (*Paralichthys lethostigma*) (Moustakas et al., 2004) and black sea bass (*Centropristis striata*) (Berlinsky et al., 2004) grow either at the same rate or more slowly. Although barramundi spawn in seawater (Moore, 1982), the growth and survival results presented here demonstrate that full-strength seawater is not essential for their culture.
Figure 4-1: Growth of larval barramundi in a potassium-supplemented, saline groundwater source (14 ppt), seawater (32 ppt) and seawater diluted to 14 ppt.

Figure 4-2: Final total length (mm ± SE) of barramundi larvae at 25 DPH. Those columns sharing the same letter are not significantly different (P>0.05). GW = groundwater. SW = seawater.
The finding that no larvae survived in the unsupplemented saline groundwater (38% K-equivalence) highlights that potassium requirements for larval barramundi differ from juveniles. As described in Chapter 4, juvenile barramundi (40 grams) survived in 15 ppt saline groundwater with 25% K-equivalence and achieved growth equal to those in 100% K-equivalence. Similarly, in 15 ppt saline groundwater, Jain et al. (2006) obtained the same growth and survival of juvenile barramundi in both 20% and 100% K-equivalence waters. That barramundi larvae in the current study could not tolerate low potassium, even at near-isosomotic salinity where the requirement is relatively low (Chapter 4), effectively highlights their sensitivity to this deficiency.

This difference between larval and juvenile fish may indicate a decreased osmoregulatory capacity and/or a decreased buffering capacity in larvae. With osmoregulatory capacity in marine fish larvae generally increasing during ontogeny, as the organs responsible for maintaining hydromineral balance develop, it is likely that the low tolerance of early larval barramundi to potassium deficiency is linked to their reduced osmoregulatory capacity (Varsamos et al., 2005). In Chapter 4, I suggested that juvenile barramundi may regulate their drinking rate in potassium deficient water in an effort to increase uptake. Although it has been shown in a number of species that pre-feeding marine fish larvae actively drink seawater (Mangor-Jensen and Adoff, 1987; Tytler and Blaxter, 1988; Varsamos et al., 2004), it is generally accepted that they do not have the same ability as juveniles or adults to regulate their drinking rate in response to changing salinity (Varsamos et al., 2005). With weight-normalised drinking rates typically increasing during development (Mangor-Jensen and Adoff, 1987; Miyazaki et
al., 1998; Reitan et al., 1998), this may represent a mechanism for increased uptake of potassium as larvae develop.

The problem of a reduced osmoregulatory capacity in larval barramundi may be compounded by a reduced capacity to buffer changes in plasma potassium. In Chapter 3 (Partridge and Creeper, 2004) I showed that mortality of juvenile barramundi in potassium-deficient, hyperosmotic groundwater was the result of unsustainable buffering of blood plasma with potassium from skeletal muscle, the body’s major store of intracellular potassium. Moreover, in Chapter 4 I demonstrated that such buffering also occurs in fish at near-isosmotic salinity.

Although there appear to be no published data on the effects of deficient sulphur, strontium or boron to marine finfish, the fact there was no significant difference in the growth of barramundi larvae between the potassium-supplemented groundwater and 14 ppt seawater suggests that these ionic deficiencies have no negative effect on growth of barramundi larvae.

By 25 DPH, swimbladder inflation of barramundi averaged 93.3 ± 2.5%, with no significant differences between treatments (P = 0.139). Like growth and survival, the effect of salinity on swimbladder inflation is species-specific, with some species, including gilthead seabream and European seabass, experiencing improved swimbladder inflation at salinities less than seawater (Tandler et al., 1995). Barramundi, on the other hand, appear similar to species such as Australian bass (Battaglene and Talbot, 1993) and snapper (Fielder et al., 2005) whose rate of swimbladder inflation is as high at 15 ppt as it is in full-strength seawater.
This appears to be the first description of rearing barramundi larvae both in low salinity seawater and in saline groundwater. Although hatchery production of barramundi typically takes place in coastal hatcheries with full-strength seawater, my data suggest that rearing barramundi in diluted coastal seawater or in low salinity groundwater with a sufficient concentration of potassium should result in improved growth with no negative impacts on survival or swimbladder inflation. Although such water appears suitable for larval rearing, Moore (1982) suggested that high salinity may be required for fertilization of barramundi eggs. If this hypothesis is correct, fully integrated inland hatcheries (i.e. those holding their own broodstock) would require a groundwater source with salinity approximating seawater.
Chapter 6

Effects of dissolved manganese on juvenile mulloway cultured in water with varying salinity – implications for inland mariculture.

Citation:

Partridge, G.J., Lymbery, A.J. Effects of dissolved manganese on juvenile mulloway cultured in water with varying salinity – implications for inland mariculture. Aquaculture. ACCEPTED.

5.1. Introduction

Despite its marine origin, important deviations in ionic composition occur in saline groundwater compared to seawater. These deviations depend primarily on the nature of the groundwater systems and the regolith with which they are associated (Mazor and George, 1992). In Chapters 3, 4 and 5, I investigated the effect of reduced potassium on the growth, health and physiology of barramundi juveniles and larvae. Another common deviation in Australian saline groundwater is an elevated concentration of manganese relative to seawater. A recent survey of 315 saline water sources in the wheatbelt of Western Australia, for example, found dissolved manganese concentrations as high as 83 mg/L, with an average value of 3.1 mg/L (George, unpublished data). Sources of dissolved manganese include anaerobic reduction of manganese oxides or the pedogenic weathering of manganese-bearing rock (Howe et al., 2004; Hardie et al., 2007).

Manganese is an essential nutrient for all organisms, including fish (Underwood, 1977; Knox et al., 1981; Gatlin and Wilson, 1984; Maage et al., 2000) and plays critical physiological roles as a constituent of several metaloenzymes and as a coactivator of many other enzymes (Underwood, 1977; Cossarini-Dunier et al., 1988). Excess
concentrations of manganese, however, can have negative effects including the disruption of sodium balance (Gonzalez et al., 1990), the impairment of calcium uptake and mineralisation (Reader et al., 1988), impacts on carbohydrate metabolism (Nath and Kumar, 1987; Barnhoorn et al., 1999), effects on immune response (Cossarini-Dunier et al., 1988; Hernroth et al., 2004) and neurotoxicity (Newland, 1999; Gunter et al., 2006). Of those studies on fish, only freshwater fish have been investigated, presumably due to the susceptibility of freshwater sources to anthropogenic sources of manganese pollution. Seawater contains a very low concentration of manganese (0.002 mg/L) (Spotte, 1992) and there appears to be no documented studies on the effect of elevated manganese on marine or estuarine fish. Unpublished data I have collected from bioassays investigating the aquaculture potential of saline groundwater sources has suggested that an elevated concentration of manganese may have a chronic effect on the growth of mulloway (*Argyrosomus japonicus*).

The aim of this study was therefore to investigate the effects of an elevated dissolved manganese (II) concentration (5 mg/L) on the survival, growth, pathology, and blood and organ chemistry of mulloway. Mulloway is a temperate species considered a suitable candidate for inland saline aquaculture based on its euryhalinity, well-documented hatchery production techniques, reasonably rapid growth rates and a temperate habitat compatible with the climatic zones where most secondary salinity in Australia occurs (Chapter 1, Partridge et al., In Press). It is a member of the Sciaenid family, of which another member, red drum (*Sciaenops ocellatus*) is also being investigated for inland mariculture in the USA (Wurts and Stickney, 1989; Forsberg et al., 1996; Forsberg and Neill, 1997). Because of the wide range of groundwater salinities found throughout Australia, the effect of elevated manganese in this trial was
investigated across the range of salinities that mulloway are known to tolerate, namely 45, 15 and 5 ppt.

5.2. Materials and Methods

At each of the three salinities investigated (45, 15 and 5 ppt), the effects of an elevated manganese concentration (5 mg/L) were compared against a control (0 mg/L) in triplicate, flow-through 180 L tanks at 23°C over a period of two weeks. Prior to commencement of the trial, five juvenile mulloway (9.4 ± 0.2 g) were stocked into each tank with flowing seawater (32 ppt) then acclimatised to their respective salinity over a period of 3 days. After a further 5 days acclimation to these salinities, fish were anaesthetised and reweighed. Prior to transferring fish back to their respective tank, those tanks randomly assigned to a treatment of 5 mg.Mn/L were supplemented with laboratory grade MnCl$_2$.6H$_2$O to achieve this concentration. Water flowed through each tank at 8 L/hour from header tanks containing the treatment water. Water of 45 ppt was prepared by the addition of artificial seasalt (Ocean Nature®; Aquasonic Pty Ltd, Wauchope, Australia) to seawater, whilst 5 and 15 ppt water were prepared by diluting seawater with dechlorinated tap water. Fish were fed daily to satiety and the amount of feed consumed was recorded. Water quality parameters including temperature, dissolved oxygen and pH were measured daily. Dissolved manganese was measured four times throughout the trial in all tanks using inductively coupled plasma atomic emission spectroscopy (Vista AX CCD Simultaneous ICP-AES; Varian, Mulgrave, Australia).

At the completion of the two week trial, all fish were anaesthetised and weighed. Blood was taken from the caudal vessel of three fish per replicate and pooled for the
determination of plasma sodium, chloride and potassium using an ion-specific electrode analyser (Vetlyte, IDEXX, USA). Samples of dorsal muscle, liver and gills were taken for analysis of their ionic composition. These samples were freeze-dried, weighed to determine water content, then ground and digested in a combination of hydrogen peroxide, concentrated nitric acid and hydrochloric acid at 120°C. Digests were subsequently analysed for manganese, sodium, potassium, calcium, magnesium and iron via ICP-AES.

Samples of gill and liver were preserved in 10% formalin prepared in seawater, before embedding in paraffin then cutting 5 µm sections. Gill and liver sections were stained with haematoxylin and eosin (H&E) and liver sections with the periodic acid-Schiff (PAS) method for glycogen.

5.2.1. Statistical Analysis

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

$$\text{SGR} = \left( \frac{\ln(Wf) - \ln(Wi)}{\text{Time (days)}} \right) \times 100$$

Where $Wf$ and $Wi$ were the final and initial wet weights of the fish, averaged over all fish in a replicate. Feed intake was calculated as a percentage of the fish’s initial body weight.

The effects of dissolved manganese concentration and salinity on survival, specific growth rate, feed intake, and blood and organ chemistry were compared using two way
ANOVA followed by a post hoc comparison of group LSD means using Tukey’s HSD test. Statements of statistical significance refer to the 0.05 level. Arcsine transformations were performed where necessary to normalise data prior to ANOVA.

5.3. Results

5.3.1. Water Quality

Actual dissolved manganese concentrations were slightly less than the target value of 5 mg/L (Table 5-1). Despite concentrations between treatments differing by no more than 0.15 mg/L, the differences were significant (P < 0.01), with the concentration at 15 ppt (4.13 ± 0.01 mg/L) being significantly lower than at 5 ppt (4.28 ± 0.01 mg/L) and 45 ppt (4.24 ± 0.01 mg/L). Manganese concentrations in the control treatments were below the detectable limit of 0.01 mg/L.

Water pH (Table 5-1) was significantly affected by salinity (P < 0.001), but not manganese concentration (P = 0.24), or the interaction of manganese and salinity (P = 0.23). Water pH was significantly higher at 45 ppt (8.13 ± 0.01) than at 15 ppt (8.01 ± 0.01) and 5 ppt (8.03 ± 0.01).

5.3.2. Survival, Growth, Feed Intake and Conversion Efficiency

Survival of mulloway over the two week experimental period was 100% in all treatments except 45ppt-5mg.Mn/L, with a survival of 73 ± 13%; significantly lower than all other treatments (Table 5-1).
At all salinities, those fish exposed to manganese grew at a significantly slower rate than those without manganese (Table 5-1). Two way analysis of variance revealed that salinity did not affect SGR (P = 0.09) but both manganese concentration (P<0.0001) and the interaction of manganese concentration and salinity did (P = 0.03). Those mulloway grown in the presence of manganese at salinities of 5 and 45 ppt lost weight over the 2 week period (SGR -0.17 ± 0.42 and -0.44 ± 0.83%/day, respectively), whereas those at 15 ppt gained some weight (1.70 ± 0.20%/day). Those mulloway grown in the control treatments grew rapidly with an average SGR of 4.05 ± 0.29%/day (pooled across three salinities).

Feed intake was affected by both salinity (P = 0.02) and manganese concentration (P < 0.0001), but not their interaction (P = 0.63) (Table 5-1). The post hoc comparison of group LSD means showed that those fish at 45 ppt ate significantly less than those at 15 ppt and those fish exposed to 5 mg/L of manganese at all salinities ate significantly less feed than those in the control treatments.
Table 5-1: Water quality and performance parameters for juvenile mulloway grown in the presence or absence of manganese at various salinities for two weeks.

<table>
<thead>
<tr>
<th>Salinity (ppt)</th>
<th>Target Mn (mg/L)</th>
<th>Actual Mn (mg/L)</th>
<th>pH</th>
<th>Survival (%)</th>
<th>SGR (%/day)</th>
<th>Food intake (%BW/day)</th>
<th>FCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0</td>
<td>&lt;0.01</td>
<td>8.01 ± 0.04</td>
<td>100 ± 0</td>
<td>3.42 ± 0.1</td>
<td>4.07 ± 0.34</td>
<td>0.92 ± 0.04</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>4.28 ± 0.01</td>
<td>8.04 ± 0.01</td>
<td>100 ± 0</td>
<td>-0.17 ± 0.42</td>
<td>2.22 ± 0.28</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>&lt;0.01</td>
<td>8.00 ± 0.01</td>
<td>100 ± 0</td>
<td>3.93 ± 0.34</td>
<td>4.22 ± 0.18</td>
<td>0.81 ± 0.06</td>
</tr>
<tr>
<td>15</td>
<td>5</td>
<td>4.13 ± 0.01</td>
<td>8.03 ± 0.02</td>
<td>100 ± 0</td>
<td>1.70 ± 0.20</td>
<td>2.91 ± 0.32</td>
<td>1.55 ± 0.22</td>
</tr>
<tr>
<td>45</td>
<td>0</td>
<td>&lt;0.01</td>
<td>8.13 ± 0.01</td>
<td>100 ± 0</td>
<td>4.79 ± 0.66</td>
<td>3.40 ± 0.34</td>
<td>0.79 ± 0.07</td>
</tr>
<tr>
<td>45</td>
<td>5</td>
<td>4.24 ± 0.01</td>
<td>8.12 ± 0.01</td>
<td>73 ± 13</td>
<td>-0.44 ± 0.83</td>
<td>1.67 ± 0.24</td>
<td>-</td>
</tr>
</tbody>
</table>
Due to mulloway’s loss of weight at 5 and 45 ppt in the presence of manganese, I was unable to calculate feed conversion ratios for these fish. Fish grown in the presence of manganese at 15 ppt had a significantly higher (poorer) FCR (1.55 ± 0.22) than those cultured at the same salinity without manganese addition (0.81 ± 0.06) (Table 5-1).

5.3.3. **Histology**

No epithelial damage to the gills was observed in histological sections. PAS-positive cells in the liver were clearly more abundant in manganese exposed fish at all salinities (Figure 5-1), however, quantification could not be conducted due to their lack of uniform distribution within sections.
Figure 5-1: Liver sections stained with PAS (× 400). Arrow heads indicate typical PAS-positive cells containing glycogen.
5.3.4. Blood Ionic Composition

Concentrations of the electrolytes in the blood of mulloway are shown in Figure 6-1.
Blood potassium and chloride were significantly affected by manganese concentration (P<0.0001 and 0.0073 for K and Cl, respectively), salinity (P = 0.04 and <0.0001, respectively) and the interaction of these terms (P = 0.006 and <0.0001, respectively).
Blood sodium concentration was not affected by manganese concentration (P = 0.27) but was affected by salinity (P = 0.0005) and the interaction of manganese concentration and salinity (P = 0.002).

Because of the significant interaction terms, I conducted one-way analyses of variance, comparing blood ionic concentrations between fish exposed and not exposed to manganese at each salinity. Blood sodium was significantly greater in fish exposed to manganese at 45 ppt (P = 0.002) but significantly lower in those exposed at 5 ppt (P = 0.04). There was no difference in blood sodium concentrations between exposed and unexposed fish at 15 ppt (P = 0.11). The same pattern can be seen with blood chloride at the various salinities and manganese concentrations, i.e. a significantly greater chloride concentration in manganese exposed fish at 45 ppt compared with unexposed fish (P = 0.006), with the opposite effect at 5 ppt (P = 0.016) and no significant difference at 15 ppt (P = 0.51). Blood potassium was significantly higher in unexposed fish compared with exposed fish at all salinities (P<0.02).

In order to test the hypothesis that mulloway were osmoregulating well in the absence of manganese across the range of salinities tested, one-way analyses of variance was also conducted on the electrolyte data from unexposed fish. These ANOVAs revealed
that salinity had no significant effect on blood sodium (P = 0.97), chloride (P = 0.13) or potassium (P = 0.69).

5.3.5. **Organ Chemistry**

Exposure to manganese resulted in significantly higher concentrations of this metal in the gills, muscle and liver of the fish (Table 5-2). The manganese content of the gills and muscle were both affected by water manganese concentration (P<0.0001 for both organs), salinity (P = 0.001 and 0.01, respectively) and their interaction (P = 0.0008 and 0.015, respectively). Despite fish exposed to manganese at 45 ppt showing the poorest performance in terms of survival and growth, gill and muscle manganese concentrations at this salinity were lower than at the other salinities; significantly more so than at 5 ppt. Liver manganese concentration was not significantly affected by salinity (P = 0.65) or the interaction of salinity and manganese concentration (P = 0.70), but was significantly affected by water manganese concentration (P < 0.0001).

There were no clear trends in other organ chemistry parameters with salinity or manganese concentration, with the exception of the potassium content of liver, which was significantly lower in those fish reared in the presence of manganese (P < 0.0001) (Table 5-2). Salinity (P = 0.30) or the interaction of manganese concentration and salinity (P = 0.41) did not effect liver potassium.
Figure 5-2: Concentrations of (a) sodium, (b) chloride and (c) potassium in the blood of juvenile mulloway grown in the presence or absence of manganese at various salinities for two weeks. Columns within salinity sharing the same letter are not significantly different (P>0.05).
Table 5-2: Organ chemistry data for juvenile mulloway grown in the presence or absence of manganese at various salinities for two weeks

<table>
<thead>
<tr>
<th>Salinity (ppt)</th>
<th>Mn (mg/L)</th>
<th>Gill (mg/kg)</th>
<th>Muscle (mg/kg)</th>
<th>Liver (mg/kg)</th>
<th>Potassium (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>0</td>
<td>28 ± 4</td>
<td>2.8 ± 0.1</td>
<td>4.4 ± 0.1</td>
<td>5.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>255 ± 25</td>
<td>9.8 ± 1.2</td>
<td>19.5 ± 1.5</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>24 ± 3</td>
<td>3.3 ± 1.3</td>
<td>4.8 ± 0.4</td>
<td>5.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>315 ± 5</td>
<td>10.8 ± 2.2</td>
<td>17.5 ± 0.5</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>24 ± 3</td>
<td>3.4 ± 0.2</td>
<td>5.0 ± 2.2</td>
<td>5.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>415 ± 5</td>
<td>20.5 ± 1.5</td>
<td>21.0 ± 3.0</td>
<td>3.4 ± 0.4</td>
</tr>
</tbody>
</table>

5.4. Discussion

Dissolved manganese had a significant, detrimental effect on the growth of juvenile mulloway at all salinities tested, with this effect being greater at salinities of 5 and 45 ppt. Dissolved manganese only affected survival at the highest salinity, demonstrating that growth is the more sensitive indicator of toxicity; a finding consistent with Stubblefield et al.’s (1997) study on the effect of manganese on brown trout (*Salmo trutta*).

That I obtained equal survival, growth and blood electrolyte concentrations across the control salinity treatments demonstrates that mulloway are capable of efficient osmoregulation across this salinity range. Mulloway’s ability to tolerate 5 ppt has been described previously (Fielder and Bardsley, 1999), however this appears to be the first published data on their performance at a salinity greater than seawater.
The poor growth performance of fish exposed to manganese was concomitant with a significantly lower feed intake. Intake in the control treatments were similar to that recommended for mulloway of similar size by Collett et al. (in press). Feed intake of fish in manganese exposed treatments was approximately half this rate. Similar inhibitory effects of excess manganese on appetite have been described for pigs, cows and sheep (Cunningham et al., 1966; Underwood, 1977). The significantly higher (poorer) FCR in fish exposed to manganese at 15 ppt, compared with unexposed fish at this same salinity indicates that the reduced growth of manganese exposed fish was contributed to by poor feed utilisation efficiency as well as reduced feed intake.

The mode of action of manganese toxicity to fish has not been well defined. Nyberg et al. (1995) suggested that its toxic action is probably similar to other metals whereby it is deposited onto the gills, causing physiological disruption to the iono- and or osmo-regulatory functions of the gill. Manganese has been shown to deposit on the gills of lobsters (Baden et al., 1990), however, there appear to be no studies describing manganese precipitation onto the gills of fish and I found no evidence of damage to the gill epithelium. Although chemical analysis of gills in the current study revealed a high concentration of manganese, I was unable to determine whether this was precipitated on the gill surface, within the gills filaments or associated with the cartilaginous gill arches, which have been proposed as a long term fixation site for manganese (Adam et al., 1997).

Rouleau et al. (1995) suggested that uptake of manganese probably occurs mainly via the gills and is then transported by the blood to various organs and tissues, where it accumulates most in those tissues rich in mitochondria such as liver, pancreas and
kidney. In their study, Rouleau et al. (1995) found that manganese accumulation in brown trout was greatest in the liver, then gills, then muscle. In the current study, manganese concentration was greatest in the gills, followed by the liver then muscle. Capelli et al. (1987) also obtained higher concentrations of (naturally occurring) manganese in the gills of Atlantic bonito (*Sarda sarda*) compared with muscle and liver.

It is well documented that manganese uptake and toxicity in freshwater fish increases with decreasing pH (Bendell-Young and Harvey, 1986; Rouleau et al., 1996) and with decreasing freshwater hardness (Reader et al., 1988; Gonzalez et al., 1990; Adam et al., 1997; Stubblefield et al., 1997; Reimer, 1999). At low pH, particularly at pH values below 7, a greater proportion of manganese exists as aqueous, toxic Mn(II), whereas high pH values favour the oxidation of insoluble and inert colloidal manganese oxides (Florence et al., 1992; Rouleau et al., 1996; Zaw and Chiswell, 1999). The pH of 45 ppt water in the current study was significantly higher than at 15 and 5 ppt, and the uptake of manganese at this salinity was significantly lower than at the lower salinities, which is consistent with the above model. Although the difference in pH was significant, it amounted to less than 0.12 pH units and the pH at all salinities was well above the ‘critical’ range of 6 - 7 described by Rouleau et al. (1996). It seems unlikely, therefore, that differences in pH among salinity treatments had a major effect on manganese uptake.

The decrease in metal toxicity with increasing hardness has been attributed to the ability of calcium (and to a lesser extent magnesium) to compete with toxic metals for ligand sites on the gill surface which reduces the permeability of the paracellular pathways to
these metals (McDonald et al., 1989). This suggests that the higher calcium and magnesium concentrations in seawater should provide even greater protection than hard freshwater and that this protection should also increase with increasing salinity. Although supporting data are lacking for manganese, the uptake by fish of certain other metals, including copper and lead, is inversely proportional to salinity (Somero et al., 1977; Jezierska and Witeska, 2006) and the uptake of manganese by mussels (Mytilus edulis) has been shown to follow this trend (Struck et al., 1997). My data showing decreasing manganese content of gills and muscle with increasing salinity are consistent with this hypothesis. My data also suggest, however, that despite reduced uptake at 45 ppt, toxicity at this salinity was greater compared with lower salinities. I suggest that the greater toxicity of manganese at higher salinity, despite its reduced uptake, is due to the effects of manganese on osmoregulation.

Gonzalez et al. (1990) found that freshwater brook char (Salvelinus fontinalis) exposed to 600 mg/L of dissolved manganese experienced a 40% reduction in blood sodium concentration. Studies have also shown that copper exposure induces hyponatremia in freshwater fish caused by apoptosis and necrosis of chloride cells (Dang et al., 2000). Given that mulloway in hypo-osmotic salinities face the same osmotic challenges as freshwater fish, my findings of hyponatremia in mulloway at 5 ppt are consistent with these studies and suggest that manganese may also induce apoptosis and necrosis of chloride cells. Mulloway held at hyperosmotic salinity (45 ppt) face the opposite osmotic challenges to those in hyposmotic salinity and their chloride cells are responsible for the elimination (rather than uptake) of monovalent ions (Evans et al., 2005). My data showing elevated blood sodium and chloride in manganese-exposed fish
at 45 ppt is therefore consistent with manganese causing apoptosis and necrosis of chloride cells, as the loss of such cells would result in reduced elimination of these ions.

Given that a salinity of 15 ppt is close to iso-osmotic, uptake and elimination of monovalent ions will be minimal at this salinity and the effects of chloride cell apoptosis not as detrimental as at the more extreme salinities tested. Indeed, the differences in blood sodium and chloride concentrations between exposed and unexposed fish were smaller and statistically non-significant at this salinity, compared with 5 and 45 ppt. I therefore suggest that the reduced osmotic demands at 15 ppt are the likely reason these fish tolerated manganese exposure better than at 5 and 45 ppt. I cannot rule out the possibility that the greater tolerance for manganese exposure at a salinity of 15 ppt was due to the small, but significantly lower manganese concentration at this experimental treatment, compared to the 5 ppt and 45 ppt treatments. There are, however, two reasons why I believe that this lower manganese concentration in the 15 ppt treatment was not biologically significant. First, there was a reduction of only 0.15 mg/L at this treatment, 3% of the target value of 5 mg/L. Second, there was no relationship between manganese concentration and fish survival and growth rate in the three replicates within treatments.

Even though the fish exposed to manganese at 15 ppt performed better than those at 5 and 45 ppt, they still exhibited significantly slower growth compared with control fish at the same salinity. Given blood potassium was significantly elevated in these fish suggests there was some disruption to osmoregulation in this treatment. The reduced feed conversion efficiency seen in these fish, compared with unexposed fish at the same salinity, may therefore be the result of the repartitioning of a greater proportion of
dietary energy into osmoregulation or, alternatively, the result of impaired carbohydrate metabolism described below.

I found significant reduction in liver potassium and reduced liver glycogen in fish exposed to manganese. Manganese has been implicated in impaired carbohydrate metabolism in both fish (Nath and Kumar, 1987, 1988; Barnhoorn et al., 1999) and mammals (Underwood, 1977) and observations of decreased liver glycogen and hyperglycemia in manganese exposed fish have led to the hypothesis that manganese exposure may inhibit insulin release (Nath and Kumar, 1987) in a similar fashion to that seen in cadmium exposed rats (Ghafghazi and Mennear, 1973). Although the reduced liver glycogen that I observed in manganese exposed fish is consistent with these studies, it would also be the expected response of fish that are losing weight or growing at a sub-optimum rate. The significant reduction in liver potassium of manganese exposed fish, however, is consistent with a lack of insulin, as such a deficiency inhibits potassium absorption by liver cells in rats (Fehlmann and Freychet, 1981).

Cardeilhac et al. (1979) and Cardeilhac and Hall (1977) attributed the death of the marine fishes sheepshead (Archosargus probatocephalus) and pinfish (Lagodon rhomboides) exposed to excess copper to osmoregulatory failure and specifically an elevated concentration of blood potassium. I found significantly elevated blood potassium in fish exposed to manganese at all salinities. The blood potassium of manganese exposed mulloway at 45 ppt was within the toxic range reported for these two species and is therefore a possible cause of death of mulloway in this treatment.
Chapter 7

Conclusions

A great deal of interest has been demonstrated over the last 10 years in proving the concept of utilising Australia’s abundant saline water resources for commercial mariculture. Developing such industries could provide many economic, social and environmental benefits for those areas marginalised by salinity, but this goal is yet to be realised. The greatest technical constraints identified in this thesis are the temperate location of the majority of water sources and issues relating to water quality, including ionic composition and consistency of yield.

The wheatbelt of Western Australia has no large scale groundwater interception schemes and associated evaporation basins and water yields from its typical granite rock aquifers are only low to moderate. As such, production systems utilising low water volumes are required in this region. The Semi-Intensive Floating Tank System (SIFTS) was therefore designed to improve yields of fish from static ponds and it was demonstrated to be an effective technology for this purpose. In the production of 26 tonnes of fish (/ha/year), SIFTS effectively removed 5 tonnes of solid waste (/ha/year) via its patented waste extraction system. This solid waste contained 144 kg of nitrogen (/ha/yr) and 153 kg of phosphorus (/ha/yr). SIFTS also maintained adequate levels of dissolved oxygen to the fish, largely independent of the oxygen concentration in the pond, and enabled improvements in feed conversion efficiency and fish husbandry. Growth of barramundi and trout were as good as or better than other commercial fish production systems and yields greater than conventional static ponds.
The results of this research with SIFTS also demonstrated the ability to alternate summer and winter crops of barramundi and trout, respectively, in this region. Both species were cultured to a marketable size within the seasonal time constraints, however, issues relating to continuity of supply to market, optimum market size (particularly for barramundi) and risk of product loss at the end of the growing season (particularly for trout) were identified as constraints to this style of farming. Although mulloway demonstrated an ability to withstand winter temperatures in the wheatbelt, they exhibited very limited growth when temperatures were below approximately 18°C. Even during the warmer months, the growth rate and feed conversion efficiency of this species were considerably lower than those obtained with trout and barramundi. Given that other authors have obtained significantly better growth of mulloway in inland saline waters (Doroudi et al., 2006) than reported in Chapter 3, further investigations into this species for inland mariculture are therefore warranted.

Further research is currently being undertaken with SIFTS to maximise sustainable yields from static inland saline ponds using novel bioremediation techniques including heterotrophic pond management, the grazing of microalgal blooms with profitable invertebrate species and the irrigation of valuable halophytic crops. SIFTS are also being investigated for fish production in freshwater irrigation lakes and protected marine environments, where their ability to capture solid wastes makes them an environmentally superior alternative to conventional seacages.

Bioassays investigating the ability of barramundi to be cultured in hypersaline, potassium deficient groundwater in Chapter 3 resulted in death caused by severe hypokalaemic muscle myopathies attributable to unsustainable buffering of blood
plasma from muscle. The results presented in Chapter 4 supported the hypothesis generated in Chapter 3 that barramundi require proportionally more potassium at hyperosmotic salinities than in isosmotic or hyposmotic salinities. Increasing potassium equivalence from 25% to 50% in hyperosmotic water prevented the terminal hypokalaemic myopathies, but fish lost weight and showed evidence of osmotic stress compared with those grown in waters with either 75 or 100% potassium equivalence. Barramundi grown in near-isosmotic or hyposmotic salinity grew equally as well at 25% potassium equivalence as at 100% potassium equivalence, with no evidence of osmotic stress. This study demonstrated that barramundi have a lower requirement for potassium than other marine and estuarine species being investigated for inland mariculture and appears to be the first study to investigate the physiological consequences of culturing teleost fish in potassium deficient waters. The findings of Chapter 4 will aid in the selection of appropriate groundwater sources and/or supplementation regimes for the culture of this species. Further studies to determine the lowest concentration of potassium that can be tolerated by barramundi in near-isosmotic and hyposmotic salinities are required.

The data presented in Chapter 5 demonstrate that low salinity, inland saline groundwater can be used for successful hatchery production of barramundi, provided water contains sufficient potassium. Such culture should improve the growth rate of larval barramundi and may provide competitive advantages over coastal hatcheries, such as isolation from naturally occurring pathogens and parasites. It was also demonstrated in this chapter that larval barramundi have a greater requirement for potassium than juveniles, due to their reduced osomoregulatory capacity and/or reduced ability to buffer blood plasma from muscle. Prior to the establishment of inland
barramundi hatcheries using low salinity water, studies will be required to determine if egg fertilisation can occur at such salinities. Studies on the ontogeny of tolerance to low potassium would also be useful in determining the youngest age at which potassium supplementation could be reduced or eliminated.

The results presented in Chapter 6 demonstrate that 5 mg/L of dissolved manganese, a concentration typical of many saline groundwater sources in Western Australia, has a significant, detrimental effect on the growth, health and survival of mulloway. Selecting a water source with salinity close to iso-osmotic will minimise the effects of dissolved manganese, however, further studies are required to determine the minimum concentrations at which growth is not affected. Given that this study was conducted at relatively high pH values, the negative impacts of a similar dissolved manganese concentration can be expected to be far greater in aquaculture systems or for groundwater sources which have lower values of pH.

Given that both salinity and the pH values typical of groundwater result in very slow oxidation rates of manganese, its removal from saline groundwater is more complicated than iron; another metal often found in high concentrations in saline groundwater. Significant chemical pre-treatment with strong oxidising agents, or the temporary elevation of pH to above nine, would be required to oxidise manganese prior to its physical removal; a process that will add significant cost and complexity to the delivery of water, particularly for those production systems using large volumes. Although oxidation is slow, once it does occur, precipitation of manganese oxides onto tanks, pipes and pumps is likely to be problematic and impose a significant management issue if dissolved manganese is not oxidised and removed prior to use.
The constraints identified in this thesis will limit where viable inland mariculture enterprises can exist and will also dictate the species and culture methods appropriate for these areas. The challenge in developing successful enterprises lies in identifying complementary combinations of water source, species and production systems. That is, species and production systems must be appropriately matched to the physical and chemical characteristics of the selected water source or, conversely, an appropriate water source and production platform selected for the target species. Various technically viable permutations of these three variables will exist. For example, this study has demonstrated that rotating warm and cool water species in a static pond in the temperate regions can be used to overcome the issue of large seasonal temperature variations. Alternatively, rainbow trout could be cultured in flow-through ponds or tanks in areas of adequately cool climate using high-yielding groundwater sources, or barramundi could be produced in controlled recirculating aquaculture systems using low-yielding groundwater or in water from disused mine voids in the North West of Australia. Economic and market factors will be important in determining which of the various permutations are most viable. For example, without the capability for continuous production, detailed market analyses would be required to determine if the ‘batch culture’ strategy of seasonal production would be viable or to determine whether fish production in inland recirculating systems would be competitive with similar systems located close to the niche city markets. In addition to economic considerations, viability may also be measured by social and environmental outcomes, such as the creation of employment for the maintenance of rural communities or through enabling groundwater pumping to save rural infrastructure via resource cost-sharing.
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