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Sustainable development of barramundi cage aquaculture at Lake Argyle

Final FRDC Report and KSRP Report – Project 2003/026

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Fish for the future

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1.0 Overview

1.1 Non-Technical Summary

2003/026 Sustainable development of barramundi cage aquaculture at Lake Argyle

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Objectives:

1. To determine methods for improving flesh and skin quality attributes in barramundi.
2. To develop disease management plans and options for Lake Argyle.
3. To optimise feed management strategies for optimal efficiency.
4. To determine the environmental sensitivity of the Lake Argyle ecosystem to additional nutrients.
5. To develop carrying capacity assessment models of the Lake Argyle ecosystem.

- A preliminary flavour evaluation study confirmed the presence of a muddy flavour taint issue in the barramundi farmed at Lake Argyle. This was examined by studying the flavour properties of a series of samples of fish from Lake Argyle (purged and unpurged), wild (estuarine) and marine-farmed barramundi. No significant differences in flavour attributes and/or acceptability attributes were detected between the wild and farmed barramundi, provided the barramundi was either marine-farmed or purged. However, it was determined that a clear "muddy" flavour and odour could be detected in the unpurged Lake Argyle fish.
- Expansion of the flavour work led to the establishment of a trained sensory panel at the Queensland Department of Primary Industries (QDPI) Centre for Food Technology (CFT) for the assessment of flavour-taint in barramundi. From this cross-referencing to some untrained sensory work was also undertaken and showed that similar results could be achieved from such panels.
- It was demonstrated that there was a significantly greater muddy-flavour effect in large (~2000g) compared to small (~400g) barramundi.
- It was found that flavour taint was highest in the "belly-cut" of the fillet and lower in the "tail-cut" and "shoulder" of the fillet and that there is a strong correlation of flavour taint with fat levels in the various fillet cuts.
- Assessment of the influence of flavour taint in the presence or absence of the compounds geosmin (GSM) and 2-methylisoborneol (MIB) identified that at Lake Argyle, it was likely that MIB was the primary compound causing the problem. Assessment of cyanobacteria and phytoplankton species present in the water at the lake identified several known MIB producers that are likely to be the source of the problem.

- The rates of GSM and MIB uptake were examined with a significant increase in muddy-flavour was detectable by sensory evaluation after as little as 1 hour, with no significant further increase in muddy flavour noted after 6 hours.
- For fish purged in tanks with flow rates at either 8 L/min or 16 L/min, the muddy-flavour of the fish halved after 24 hours of purging and further reduced again with each successive day up to five days of purging.
- A range of methods of preparing geosmin and MIB-free water were examined. It was identified that simple aeration of the water was among the most effective methods. The use of an algaecide in conjunction with aeration accelerated to removal of MIB. The use of a flocculant also reduced the MIB concentration in the water, but this was not as effective as the other two methods.
- Designs for a commercial-scale purging unit were created based on knowledge gained from the findings in this project.
- An outbreak of the bacterium *Streptococcus iniae* occurred during the project, in January 2004. The occurrence of this outbreak was characterised by the Fish Health Laboratories using standard tests. This outbreak provided in important case study for the development of a management framework for quarantining farmed barramundi stocks.
- The potential for a vaccine to *Streptococcus iniae* was considered in a review of the disease management strategies for the barramundi cage aquaculture industry at Lake Argyle.
- It is notable that other major barramundi producers in Australia have now begun using vaccines to this bacterium, sourced from a company in Singapore.
- Growth data obtained from the industry and some laboratory sources allowed the development of a revised growth and feed utilisation model. The development of the revised growth and feed utilisation model for barramundi allowed the development of a series of revised feed tables for different diets (based on different digestible energy density) and fish sizes from 10g to 3000g over temperature ranges of 20°C to32°C.
- Assessments of some of the key assumptions of the model were also examined. These assumptions were found to be generally consistent with the model and where necessary allowed subtle adjustments to be made to improve its robustness
- Versions of this model are already being used by some industry sectors.
- Based on an agreed clause in the project relating to the scale of activity at Lake Argyle, the environmental work (objectives 4 and 5) was not initiated.

1.2 General Summary

- A preliminary sensory evaluation study was conducted to confirm the presence of a taint issue. Prior to this the issue was reliant on purchaser feedback and was not verified independently. To examine the issue of flavour taint a series of barramundi samples were collected from Lake Argyle (purged and unpurged), wild (estuarine) and marine-farmed barramundi were assessed. No significant differences in flavour attributes and/or acceptability attributes were detected between the wild and farmed barramundi, provided the barramundi was either marine-farmed or purged. However, it was determined that a clear “muddy” flavour and odour could be detected in the unpurged Lake Argyle fish, but not in any of the other samples. From this finding it was decided to establish an independent

professional sensory panel to assess a range of key factors that may influence the sensory perception of the muddy taint issue. A number of other differences in flesh colour and texture were also observed.

- The project also resulted in the establishment of a trained sensory panel at the Queensland Department of Primary Industries (QDPI) Centre for Food Technology (CFT) for the assessment of flavour-taint in barramundi. From this, cross-referencing to some untrained sensory work was also undertaken and showed that similar results could be achieved from untrained panels.
- Assessment of the effect of fish size on muddy-flavour demonstrated that there was a significantly greater muddy-flavour effect in large (~2000g) compared to small (~400g) fish. Small fish were also perceived to be sweeter and fresher in their sensory characteristics.
- A sample of unpurged Lake Argyle fish were assessed for variability in flavour taint in different sections of the fillet. It was found that flavour taint was highest in the "belly-cut" of the fillet and lower in the "tail-cut" and "shoulder" of the fillet.
- It was shown that there is a strong correlation of flavour taint with fat levels in the various fillet cuts. Highest fat levels and flavour taint were observed in the belly-cut of the fillet and lower fat and taint levels in the tail-cut of the fillet.
- Assessment of the influence of flavour taint in the presence or absence of the compounds geosmin (GSM) and 2-methylisoborneol (MIB) identified that at Lake Argyle, it was likely MIB was the primary compound causing the problem. Assessment of cyanobacteria and phytoplankton species present in the water at the lake identified several known MIB producers that are likely to be the source of the problem.
- Assessment of the sensory thresholds for GSM and MIB was constrained by vagaries in the assessment of GSM and MIB from the test water samples. A test based on the serial dilution of depurated and tainted water was undertaken, with barramundi placed within each treatment and subsequently evaluated for their sensory characteristics. A significant increase in the sensory detection of muddy flavour was observed at a level of 60% taint affected water. This translated to a water MIB concentration of between 3.5 and 5.5 ng/L. It was not feasible within the project budget constraints to develop an in-flesh chemical test for either GSM or MIB.
- The rates of GSM and MIB uptake were examined in purged fish returned to GSM and MIB affected water. A significant increase in muddy-flavour was detectable by sensory evaluation after as little as 1 hour, with no significant further increase in muddy flavour noted after 6 hours. The greatest muddy-flavour was noted 48-hours after immersion of the fish in the tainted water.
- The reverse of this effect is the rate of depuration. Fish were purged in tanks with flow rates at either 8 L/min or 16 L/min. The muddy-flavour of the fish halved after 24 hours of purging and further reduced again with each successive day up to five days of purging. No significant improvements were noted after 48 hours of purging. The flow rate of the water was found to have no effect on the rate of change in muddy-flavour of the fish.
- A wide range of phytoplankton and cyanobacteria species was identified in Lake Argyle. Total counts were dominated by cyanobacteria. Of the species identified numerous are known geosmin and 2-methylisoborneol producers and some were also known toxin producers.
- A range of methods of depuration were examined and it was identified that simple aeration

of the water was among the most effective methods. The use of an algaecide in conjunction with aeration accelerated to removal of MIB. The use of a flocculant also reduced the MIB concentration in the water, but this was not as effective as the other two methods. The aeration procedure was also useful in decreasing the level of geosmin in the water. Different Aeration methods, such as diffusers and mushroom sprayers were also observed to influence the rate at which depuration occurred. Primarily, the greater the level of aeration the faster the MIB concentration in the water reduced.

- Designs for a commercial-scale purging unit were created by a consulting engineering company, which were based on knowledge gained from the findings in this project. Design plans created are included in this report.
- An outbreak of the bacterium *Streptococcus iniae* occurred during the project, in January 2004. The occurrence of this outbreak was characterised by the Fish Health Laboratories using standard phenotypic and biochemical tests. This outbreak provided a significant case study for the development of a management framework for quarantining barramundi stocks based on the key strategies of limiting the impact of clinical disease should it occur and also limiting the exposure of farmed fish to any pathogen. A decision flow-chart was developed to allow ease of response by farm operators in the occurrence of a potential outbreak.
- The potential for a vaccine to *Streptococcus iniae* was considered in a review of the disease management strategies for the barramundi cage aquaculture industry at Lake Argyle. Although production levels at the lake are now not warranting development of a specific autogenous vaccine to the strain of *S. iniae* that caused significant losses at Lake Argyle in 2004, it is notable that other major barramundi producers in Australia have begun using autogenous vaccines to this bacterium, sourced from a company in Singapore.
- Growth data obtained from the Lake Argyle farm, some other farms and some laboratory studies allowed the development of a revised growth model. This model superseded earlier published models in that it allowed the development of a model that better reflects growth rates seen in Australian barramundi production conditions (i.e. water temperatures 24°C to 34°C) and also accommodates the use of the larger fish sizes produced in Australian barramundi farms.
- An assessment of the assumption of energy utilisation efficiency effects between fish of different sizes determined, that irrespective of transformation exponents used, the efficiency with which dietary digestible energy is used by small (15g) and large (410g) fish was marginally, but significantly different. Maximal protein deposition rates were also different, but the efficiency at which this occurred in either fish size class was not significantly different.
- The development of the revised integrated factorial growth and feed utilisation model for barramundi allowed the development of a series of revised feed tables for different diets (based on different digestible energy density) and fish sizes from 10g to 3000g over temperature ranges of 20°C to32°C. This same revision also allowed the iterative development of a series of potential diet specifications for fish over a wide size range, based on energy demand and nutrient to energy ratio requirements. These iteratively determined specifications are similar to empirical data obtained in other, independent studies with barramundi.

1.3 Introduction and Need

Barramundi aquaculture is presently one the fastest growing aquaculture sectors in Australia and specifically in Western Australia. One of the key places in Western Australia where development has occurred is at Lake Argyle, in the Kimberley region in the far-north of the state (Figure 1.1). Within the lake, several sites have been developed for barramundi cage-culture production (Figure 1.2). Maintaining environmental quality has also long been seen as an important facet in sustainable aquaculture production. Indeed environmental issues have been notable in recent developments in the Australian prawn, salmon and tuna industries. In partial recognition of this, the Australian aquaculture industry has developed a draft code-of-practice, incorporating the recognition of the industry's environmental responsibilities. To proactively address this issue for the barramundi industry in Lake Argyle, it is likely that the development of accurate carrying capacity estimates and waste discharge models will be required. The viability of such models will depend on the availability of a range of accurate data on both fish physiology and environmental variability and its relevance to the various farming systems. The Department of Fisheries, in being proactive in the development of this industry in WA, has already implemented some smaller projects examining the development of waste excretion models and lake hydrodynamics studies. In addition, a provisional discussion document for the development of a series of management "zones" in the northern end of Lake Argyle has also been prepared. Presently this document proposes that no single zone be allowed a licence for production of greater than 500 tonnes per annum in lieu of data to the contrary that supports that greater tonnages are viable. Key environmental studies are required to validate some of the underlying assumptions in this discussion document.

Principally, there are three strategies to manage environmental issues concerning aquaculture; these are through management of (1) location of the operation, (2) the volume of fish produced or feed inputs at any specific location, and (3) the type of feed used to produce the fish. While the industry at Lake Argyle has a basic growth and nutritional model for barramundi production, and has used it to assist management of feeding and production, it is apparent that its reliability is diminished at larger fish sizes and higher water temperatures. Importantly these larger sizes are the more sensitive parts of the production process from both an environmental and economic perspective. It is therefore important that some revision of this model, a version of which is also being used by barramundi producers in the Northern territory, is undertaken to ensure its relevance.

The economic viability of the industry has also been threatened as market feedback has, for some time, indicated that the barramundi farmed in Lake Argyle have a muddy or earthy taste/ odour in the flesh that distinguishes them from saltwater farmed and wild-caught barramundi. The extent of this taint/odour is reported to vary with time of year. Anecdotally, the problem is worst late in the dry season or during the wet season. A recent survey of customers indicated that this issue is the most important single factor for lower than expected sales, particularly in large fish where there is greater competition and an increasing availability of saltwater farmed fish. Taint/odour is not reported to be a significant problem in the market for plate size fish; however this may change as saltwater-farmed plate size fish become increasingly available. The fish have also been noted to be much darker in colouration of both their skin and fillet than fish that is saltwater farmed and wild-caught. This too reduces market acceptance.

Disease management has also recently become another important issue at the Lake Argyle barramundi farm, with several outbreaks of the pathogenic bacteria, *Streptoccocus iniae*. This bacterium is endemic to tropical Australia, but becomes problematic when allowed

to proliferate unmanaged. Losses due to this problem have already been substantial and management protocols are required.

Therefore a series of research needs for sustainable development of the barramundi industry at Lake Argyle have been identified. Some of these, such as the disease and fish energetics work have broader application beyond Lake Argyle, while others are more specific to needs inherent to production at Lake Argyle.

1.4 Project Strategy and Background

The environmental work planned as part of this project was seen as strategic, underpinning further development of the industry, if and when it expanded. However, because of the high-cost nature of this component of the project a decision clause was in-built to the project to justify this part of the project. At the end of year-1 of the project (June 2005) an assessment was to be made on the development status of the industry and whether such an environmental research program was warranted. Should there not be sufficient industry activity present or imminent, then the environmental components of the project would default and not be undertaken.

However, the most imminent problem to the viability of the barramundi aquaculture operation in Lake Argyle is the incidence of a "muddy taint" in the fish being produced. The fish produced are also highly pigmented and the dark colour is less well received by the market than a paler silvery coloured fish. These problems are causing significant market acceptance issues with the fish, even at discounted prices. Two terpenoid molecules, geosmin (GSM: Figure 1.3) and 2-methyl-iso-borneol (MIB: Figure 1.4), in the ambient water have been implicated in episodes of earthy/muddy flavours in freshwater fish, although farmed fish may also have off-flavours other than earthy/muddy ones resulting from chemicals other than GSM and MIB. The most likely source of the GSM and MIB are micro-organisms, particularly phytoplankton and/or cyanobacteria in the water. The presence and intensity of off-flavour/odour in fish usually varies seasonally and is associated with blooms of cyanobacteria and/or phytoplankton, usually in the warmer months. A wide range of cyanobacteria and phytoplankton species have been identified as producing geosmin and MIB. The chemical structures of geosmin and 2-methylisoborneol are presented in Figures 1.3 and 1.4 respectively.

Other freshwater fish farming industries facing this same problem have addressed the issue by using various purging regimes. Current R&D at Lake Argyle is also suggesting that purging regimes might also be appropriate for reducing the "muddy taint" problem, but commercial application of early tank trials needs to be undertaken. These purging studies have also suggested that there is potential for manipulation of skin and/or flesh colour. Practical resolution of these problems will require an improved knowledge of when the "muddy-taint" issue is at its peak and how this relates to the diversity and abundance of known geosmin and MIB producing phytoplankton. Additional tank trials to optimise purging regimes, with accessory chemical and sensory evaluation will be required. The final outcome being the development of the potential use of in-cage liners to purge fish on-site.

Management of the pathogenic bacteria, *Streptoccocus iniae* has also become another important issue at the Lake Argyle barramundi farm, with several outbreaks occurring in 2004. The losses due to this problem have already been substantial and a series of proactive management protocols are required. These management protocols will rely on identification of key constraints such as the level of transmissibility of the bacterium within Lake Argyle and the conditions under which an outbreak event occurs or its potential is exacerbated. Beyond this,

examination of the potential for a vaccine against *Streptoccocus iniae* is seen as a priority and there may be a need to consider the requirements for its subsequent development.

Basic growth and nutritional models for barramundi production exist and are being used by industry, but are largely based on the energetics and growth of small (< 200 g) fish. Because of the cost-sensitivities associated with large (3000 g) fish production, it is important that such models are also refined to accommodate the energetics and growth demands of larger (> 200 g) fish. Such models allow substantial improvements to feed management efficiency and thereby assist with the environmental and economic sustainability of the operation. Improvements to such growth models also allow an increased degree of control of stocking density management. Therefore a reassessment of the growth model and some key assumptions in the differences between large and small fish energetics is required.

1.5 Contracted Objectives

1. To determine the environmental sensitivity of the Lake Argyle ecosystem to additional nutrients [Not undertaken – Trigger clause of insufficient industry activity at Lake Argyle]
2. To develop carrying capacity assessment models of the Lake Argyle ecosystem [Not undertaken – Trigger clause of insufficient industry activity at Lake Argyle]
3. To determine methods for improving flesh and skin quality attributes in barramundi [Achieved]
4. To develop disease management plans and options for Lake Argyle [Achieved]
5. To optimise feed management strategies for optimal efficiency [Achieved]

1.6 Planned Outcomes

- *Confirmation of flavour taint problem in Lake Argyle produced barramundi*

Comparison of the sensory values of wild, salt-water farmed, Lake Argyle and purged barramundi confirmed that there was a muddy/earthy flavour taint issue in the Lake Argyle produced fish. However, it was noted that there was no appreciable difference in acceptance of salt-water farmed and purged fish and also with either of these fish against wild fish. Further assessment of the effect of two alternative purging regimes identified that it was possible to mitigate the problem. Further assessment of the influence of flavour taint in the presence or absence of geosmin and 2-methylisoborneol (MIB) identified that at Lake Argyle, it was MIB that was the probable primary compound causing the problem. Assessment of cyanobacteria and phytoplankton species present in the water at the lake identified several known MIB producers that are likely to be the source of the problem. It was determined that the problem was more profound in larger fish than smaller fish and that the belly region of the fish was more susceptible to the muddy-flavour taint problem. It was shown that there is a high degree of correlation between flesh fat content and muddy-flavour taint.

- *Reduction in flavour taint in lake Argyle produced barramundi*

A method for reduction in flavour taint was developed and key parameters (fish size, fillet section, water geosmin and/or MIB levels) determined that influenced the occurrence of muddy-flavour taint identified. After the presence of a problem was first confirmed, independently of

the market and producer by a consumer sensory panel, this was followed with the establishment of a professional sensory panel to further assess different aspects of the problem. The rate of uptake of muddy flavour taint by purged fish placed in lake water was shown to occur rapidly with no further significant increase in muddy flavour after 6 hours immersion. In contrast, the removal of the taint, by placing the muddy-tainted lake fish into water free of geosmin and MIB was shown to halve after 24 hours, then reduce to 25% of the initial taint level by 96 hours and continued to decline up to 120 hours after the fish were placed in the untainted water. However, the most appreciable effects were noted within the first 48 hours.

- *Producing water suitable for depuration at Lake Argyle*

The key issue for the viability of removing the muddy-taint from fish at Lake Argyle is the ability to access MIB-free water sources. An assessment of the levels of phytoplankton and cyanobacteria indicated that cyanobacteria dominated. Among the cyanobacteria and phytoplankton identified a wide range of known geosmin and MIB producers were identified. A range of methods of depuration was examined and it was identified that simple aeration of the water was among the most effective methods. The use of an algaecide in conjunction with aeration accelerated to removal of MIB. The use of a flocculant also reduced the MIB concentration in the water, but this was not as effective as the other two methods. The aeration procedure was also useful in decreasing the level of geosmin in the water. As an outcome from this component of the project, plans for a commercial scale purging system were designed.

- *Maintaining and quarantining barramundi stocks to prevent the spread of Streptococcus iniae.*

Strategies for management of disease outbreaks in Lake Argyle were developed based on some of the known pathologies of likely diseases to occur in barramundi cage culture. From a case study that occurred at Lake Argyle during the time of this project, *Streptococcus iniae* was identified as one the primary pathogens of concern. In 2004 a single outbreak resulted in significant mortalities to the industry at Lake Argyle (ca. 125 tonne lost). However, the occurrence of this outbreak also provided a useful disease model for development of management strategies for any future outbreaks. Key aspects of the developed management strategy included aspects of limiting the impact of clinical disease should it occur and also limiting exposure of fish to potential pathogens. To enable this to be implemented on farm a simple, decision flow-chart was developed for use by farm staff to guide them in the daily actions required to carry out disease management monitoring. Other options such as use of vaccines, immunostimulants and probiotics were also considered.

- *Modelling the growth performance and feed utilization of barramundi*

The potential of using a bio-energetic approach to managing feed use was reviewed and revised. Use of the preliminary bio-energetic model to manage feed rationing per cage, per week was undertaken by the industry partner with promising results, though given that an early version of the model was used, it identified that refinements were required, particularly at larger fish sizes and higher water temperatures (> 26°C). An experiment was undertaken within this project, which coupled with the use of further farm-based data, allowed the revision of the growth model. Specifically, the lab-based experiment was undertaken to examine the effects of fish size on a series of bio-energetic parameters. This work identified that some assumptions were not necessarily valid, but the use of some generalised parameters allowed the development of a more robust model. This included refined assessments of maintenance protein and energy demands and a better understanding of the changes in protein and energy utilisation

efficiency that occur with changes in fish size and feed (protein and energy) intake. From these refinements a series of improved feeding tables and diet specifications were derived. However, like all models, the one presented has the potential to be useful, but is still far from optimal and requires significantly more work to become a highly robust model. Therefore caution must be applied when using features of the model or applications derived from it.

- *Established expertise in barramundi R&D, environmental management and tropical based research presence.*

The entire practical component of the purging research was carried out on-site, at Lake Argyle in the Kimberley region of WA. Not only did this establish a consolidated research presence in the northern part of WA, but also the long-term co-location of a research technician with the farm management and staff had direct benefits to both industry and the research sectors. Similarly, the mid-term co-location of a project scientist at the industry site had direct benefits to both industry and the research sectors by increasing exposure of both sectors to pertinent issues, constraints and needs.

- *Extension of Results*

Direct communication with barramundi aquaculturists was undertaken regularly on-site at Lake Argyle and through the developed relationship, frequent remote (phone, fax, email) linkages were maintained. Communication of the findings, from the aspects with broader application, has been made to feed companies and the Australian Barramundi Farmers Association.

Some of this work has previously been presented at national conference venues, including Australasian Aquaculture 2004 and an ACIAR Masterclass course in Bangkok, Thailand in 2006.

A popular article, “Farming Fish on the Frontier” was prepared for incorporation in Western Fisheries Magazine. This article summarized the recent developments at Lake Argyle, publicising the research presence, the environmental management being undertaken and the expanding activities of the industry.

As a consequence of the subcontracted independent sensory tests done by the Centre for Food Technology (CFT) at the Queensland Department of Primary Industries (QDPI) these researcher also developed a near infrared spectrometry (NIRS) assessment method for determining the presence of geosmin and MIB in flesh of barramundi, and correlating that with their sensory assessment. For this work Dr Heather Smyth and her team received an award. While not specifically an objective of the project, a clear outcome of this work has been the establishment of a centre of skill in the assessment of muddy-taint issues in fish flesh in Australia.

The preparation of scientific publications from this work is presently being explored.

Figure 1.1 The Kimberley region in northern Western Australia. Circled is Lake Argyle.

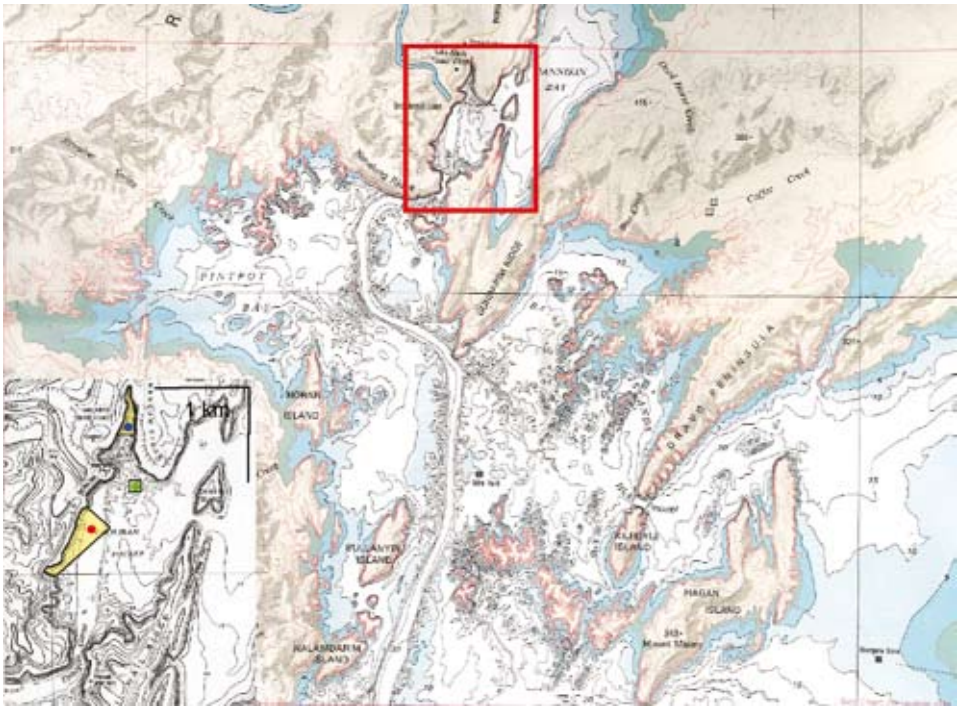

Figure 1.2 Lake Argyle, showing the northern end of the lake with an inset magnified map of Coolibah Pocket (identified within red-rectangle), where farming operations (lease sites in yellow, grow-out site marked red-dot, transfer site green-dot, nursery site blue-dot) were based and key sites identified.

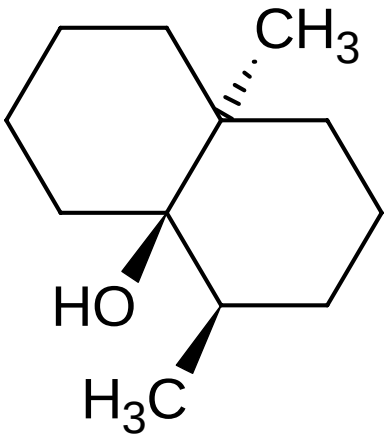

Figure 1.3 The chemical structure of geosmin (GSM).

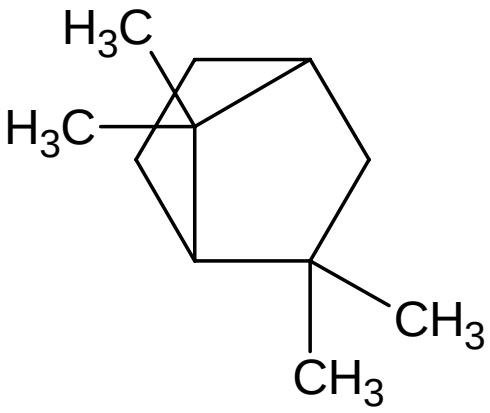

Figure 1.4 The chemical structure of 2-methylisoborneol (MIB).

2.0 Identifying flavour taint in farmed barramundi

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2.1 Introduction

With increasing production of farmed barramundi from Lake Argyle, an incidence of flavour taint has been reported from the market (Glencross, 2006). The taint is reputedly muddy and earthy in flavour, which is characteristic of the presence of the compounds geosmin (GSM) and/or 2-methyl-isoborneol (MIB) (Howgate, 2004). Problems with such flavour taint are well documented in other fish species produced from freshwater systems (Lovell, 1983; Bett, 1997; Zimba and Grimm, 2003; Grimm et al., 2004; Howgate, 2004; Robertson et al., 2005). The GSM and MIB are noted metabolites produced from algae and cyanobacteria found in freshwater systems (Brown et al., 1982; Bett, 1997; Howgate, 2004).

Problems with a similar muddy/earthy taint in freshwater farmed fish has been reported in a range of species, including: largemouth bass (Schrader et al., 2005), white sturgeon (Schrader et al., 2005), Tilapia (Yamprayoon and Noonhorm, 2000), Channel catfish (Lovell, 1983; Zimba and Grimm, 2003; Grimm et al., 2004), shrimp (Lovell and Broce, 1985) and Rainbow trout (Robertson et al., 2005; Robin et al., 2006). Sensory thresholds in water have been reported at 15 and 35 ng/L for GSM and MIB respectively (Howgate, 2004). In fish flesh the threshold appears to vary among fish species with values for GSM ranging from 250 and 10,000 ng/kg and for MIB threshold values ranging from 100 and 700 ng/kg (Yamprayoon and Noonhorm, 2000; Grim et al., 2004; Robertson et al., 2005). The lipid content of the fish is also reported to affect the uptake of GSM and MIB (Howgate, 2004). This feature may be an important aspect of the species variation in GSM and MIB taint, but could also cause variable uptake in the same species, but in fish of different sizes and in also different parts of the fish fillet.

Chemical assessment of GSM and MIB from water is relatively well established in several laboratories in Australia. However, no laboratory in Australia has established a reliable method for the assessment of GSM and MIB in fish flesh. Assessment of other studies from the literature show that chemical assessment of flesh levels of GSM and MIB is generally unreliable, with recoveries of the order of 30% to 89% being typical among the data reported (Lovell et al., 1986; Yamprayoon and Noonhorm, 2000; Robertson et al., 2005). Because of this variability in the assay from fish, and the cost of developing a chemical analysis/test for GSM and MIB, sensory assessment of samples was used as the primary means of assessment from fish samples. Where there have been comparisons of sensory and chemical analysis, they have shown sensory evaluation to be a quite robust and reliable method of assessment (Grimm et al., 2004). In some cases, the use of trained animals has also been explored for such assessment (Shelby et al., 2004; 2005).

The first part of this study was to confirm the extent of a flavour taint problem in farmed barramundi from Lake Argyle. This involved the assessment of a range of issues including:

- Comparison of barramundi from different sources to confirm the existence of a flavour taint problem
- Determination of the sensory threshold of flavour taint

- Characterisation of the variability in flavour taint within the fillet
- Characterisation of the variability due to fish size

2.2 Materials and Methods

2.2.1 Preliminary taint detection trial

Variation of taint among five different fish samples was compared in blind sensory assessment by an untrained sensory panel of 22 people (mixed sex, age range 21 to 60) at the Department of Fisheries Research Laboratories. The fish samples included farmed saltwater barramundi (sourced Catalanos Pty Ltd, Bassendean, WA), wild barramundi (sourced Sealanes Pty Ltd, Fremantle, WA), unpurged Lake Argyle barramundi and Lake Argyle fish purged using one of either two treatments. For purging, fish (~2000 g) from cages in the lake were transferred to the Lake Argyle Industries Pty Ltd (LAI) enclosed hatchery, where they were placed into 2000L fibreglass tanks, with a white interior. The two purging methods used were using bore water (< 1ng/L both MIB and geosmin) for five days (treatment 1: T1) and a second treatment of fish purged in bore water plus 20 g/L salt for three days followed by 30 g/L salt for two days (treatment: T2).

This assessment was done to confirm the presence of a taint problem. Each sample was compared and ranked (0: not present to 5: extreme) against itself and the other four samples by every taster in a pair-wise assessment. This allowed for not only an assessment of each sensory characteristic, but also a degree of direct comparison among each of the samples. All fish samples were provided as fillets and were prior frozen, before being thawed overnight at 4°C prior to preparation and cooking for sensory assessment. All fish samples, of a similar weight and thickness, were microwaved inside plastic oven bags for the same period of time. Each sample was taken from the dorsal muscle group in each case.

Each sample was provided whilst warm (~75°C) with a 3-digit blinding code to allow identification of each sample during analysis. Sensory attributes of odour (muddy, weedy, musty), flavour (sweet, sour, bitter, salty), colour (white, brown, yellow, grey), texture (oily, dry, mushy, chewy) and overall acceptance were evaluated (Figures 2.1, 2.2, 2.3, 2.4, 2.5, 2.6). A minimum of five fish from each treatment were used. Each panellist was provided with purified water and plain water crackers to cleanse their palate between samples.

2.2.2 Trained sensory assessment

For most of the sensory assessment studies, it was decided that using a professional, trained sensory panel would provide the most robust and independent data. Sensory analysis by a trained panel was undertaken, under contract, at the Centre for Food Technology (CFT, Hamilton, QLD), coordinated by Dr Heather Smyth. The panel consisted of 10 female judges, aged between 30 and 61, who were experienced with sensory descriptive analysis of foodsand beverages.

The panel were trained over four sessions, each of approximately two hours, to rate a number of defined sensory attributes. A series of 14 aroma, flavour and aftertaste descriptors were chosen. The attributes and sensory analogues that were chosen by the panel to rate the barramundi fillets are given in Table 2.1. In addition, an ‘other’ attribute for aroma, flavour and aftertaste was included for the panel to rate if they thought they could detect a property which was not covered by the chosen list of terms.

Frozen samples were thawed overnight at 2°C prior to preparation for assessment. Slices of barramundi fillet (no skin) were cut from dorsal to ventral direction across the fillet to give a ~20 g portion of fish. Samples were cut starting from the anterior end, such that any unused fillet always remained at the tail end of the fish.

In preparation for sensory assessment fish samples were weighed into foil dishes and covered with aluminium foil sheets (shiny side down) that were pre-numbered with the blinding code. The samples were prepared up to 1 hour ahead of time and kept chilled in a refrigerator at 2 - 4°C prior to cooking. Samples were cooked no more than 30 minutes prior to serving. Samples were cooked on an oven tray, in a pre-heated fan-forced oven, at 200°C for 6 minutes. After cooking, samples were transferred to a warming oven at ~75°C until served.

Only three samples were presented to each panellist at any one time so that all the samples would still be hot for sensory assessment. Samples were presented warm (~75°C) to each panellist in a randomised order. Where there was sufficient flesh from one fish to serve the whole panel, one fish (of two fillets) was treated as one individual sample.

Panellists were asked to first evaluate the aroma of the sample and then to taste the sample and assess flavour, and finally aftertaste. For each sample, panellists were asked to rate the intensity of each of the attributes listed in Table 2.1 on a scale of 0 to 100, anchored from low to high. An 'other' term was also provided for panellists to rate any aroma, flavour and aftertaste not characterised by the listed attributes. Plastic forks were used to taste the samples and a fresh fork was used for every sample tasted. Panellists were forced to wait 60 seconds between samples, and were asked to leave the booths after every set of three samples to take a 5 - 10 minute extended break. Each panellist was provided with purified water, plain water crackers and slices of granny smith apple to use for palate cleansing between samples.

2.2.3 Comparison of purged and unpurged fish by trained sensory assessment

To further confirm the taint issue and establish the trained sensory assessment panel a simple two-way comparison study was undertaken. Lake Argyle barramundi and Lake Argyle fish purged through bore water (< 1ng/L both MIB and geosmin) for 5 days were used for evaluation. Fish sampled were killed by ice immersion, filleted and frozen prior to being sent to the Centre for Food Technology for sensory assessment by a trained sensory panel (Figure 2.8).

2.2.4 Threshold trial

To determine the threshold of sensory detection of GSM and MIB taint in barramundi exposed to these compounds a study was undertaken by placing fish previously purged in bore water known to be free from GSM and MIB based on earlier studies, into water tainted with GSM and/or MIB. Ranges of dilution levels (0%, 20%, 40%, 60%, 80%, 100%) of the bore water were used, representing various dilutions between an upper and lower concentration. Water samples were taken from each dilution and sent the Australian Water Quality Centre for analysis of GSM and MIB respectively. GSM concentrations in the depurated and tainted water were 0.0 ng/L and 2.0 ng/L respectively (Table 2.2). MIB concentrations in the depurated and tainted water were 0.0 ng/L and 5.0 ng/L respectively (Table 2.2). Two fish were then sampled after a 12 h period. Fish sampled were killed by ice immersion, filleted and frozen prior to being sent to the Centre for Food Technology for sensory assessment by a trained sensory panel.

2.2.5 Differences within fillet

Variation of taint levels within the fillet was examined by comparing three sections when assessed by a trained sensory panel (Figure 2.11). Six ~2000 g fish were harvested from cages that had ambient GSM and MIB levels (GSM: 1 ng/L; MIB: 13 ng/L). Fish harvested were killed by ice immersion, filleted and frozen prior to being sent to the Centre for Food Technology for sensory assessment by a trained sensory panel. Variations in sensory scores among each fillet section are presented Figure 2.12. The three fillet sections used are depicted in Figure 2.13.

A parallel study using the fish from the same batch, but an untrained panel of six people (3 men: 3 women aged 32 – 52) was also undertaken at the Department of Fisheries Research Laboratories using the methodology reported in section 2.2.1. Flesh samples (see Figure 2.13) were also taken from six sections within the fillets of five different ~2000 g fish from the same stock. Two flesh samples were taken from each fillet section used for sensory evaluation. Each sample was frozen before being freeze-dried prior to total lipid analysis. The total dry matter of each wet sample was also determined by oven-drying at 105°C for 24 h. Total lipids were determined gravimetrically after a chloroform: methanol (2:1) extraction (Folch et al., 1957).

2.2.6 Differences between large and small fish

The differences in sensory qualities of fish of different sizes were examined to determine how influential this factor was on perception of the muddy/earthy flavour issue. Six ~ 2000 g fish and 18 x ~500 g fish were harvested from cages that had ambient GSM and MIB levels (GSM: 1 ng/L; MIB: 13 ng/L). Fish harvested were killed by ice immersion, filleted and frozen prior to being sent to the Centre for Food Technology for sensory assessment by a trained sensory panel. Sensory assessment methods used were as described in section 2.2.2. Variation in sensory scores between each fish size is presented Figure 2.12.

2.2.7 Geosmin and 2-methyl-isoborneol analysis

The method used was based on Method 6040B contained in Standard Methods for the Examination of Water and Wastewater, 19th Edition with in-house modifications. The process involves pre-concentrating a 1L sample by Closed-Loop-Stripping analysis. 2-methylisoborneol and geosmin are removed from the water by a recirculating stream of air and adsorbed onto a carbon filter from which they are then extracted using dichloromethane. The extract is then quantitatively analysed by gas chromatography/mass spectrometry using Selected Ion Monitoring.

2.2.8 Statistical analysis

A one-way analysis of variance (ANOVA), blocking for Panellist Effect, was conducted for each sensory attribute rated, to determine if there were significant differences between treatments. The software used for statistical analysis for the preliminary confirmation work at the Department of Fisheries was Statistica v6 (Statsoft®, Tulsa, OA, USA). The software used for remaining statistical analysis work at CFT was GenStat Seventh Edition, Lawes Agricultural Trust.

2.3 Results

2.3.1 Taint detection

The LAKE farmed fish had a muddy odour that was significantly more noticeable than that in all the other treatments (Figure 2.1 and 2.2). There were no other differences in the muddiness between the different treatments. The LAKE farmed fish also had a weedy odour that was significantly more noticeable than in both the WILD and SALT treatments, but not the two purging treatments (T1 and T2). The LAKE farmed fish also had a noticeable musty odour that was significantly more noticeable than in the T1 purging treatment (LAKE fish purged using treatment 1) but there were no other significant differences between the other treatments. Notably some of these senses were determined on tasting through volatilisation of compounds in the mouth. Although arguably sensed as flavours, they are technically odours not flavours, as there are only five flavour senses (sweet, sour, bitter, salty, umami) of which these odour characteristics do not conform.

There were few significant differences in true discrete flavour attributes (sweet, sour, bitter, salty) noted among the treatments (Figure 2.1 and 2.3). The only significant difference being that the T1 purged fish were sweeter than the LAKE fish.

The colour of the WILD fish were typically whiter than all the farmed fish, with each of the farmed fish also being significantly greyer than the WILD fish (Figure 2.1 and 2.4). However, one of the purging treatments (T1) also produced fish with whiter flesh than some of the other farmed fish treatments and also less grey. The WILD and T1 fish were less brown than the T2 and SALT farmed fish. No significant differences in the yellowness of any of the fish samples were noted.

The texture of the farmed fish (all treatments except WILD) were typically more oily and mushier than WILD fish (Figure 2.1 and 2.5). However WILD fish were considered much more chewy and dry than the farmed fish. The LAKE fish provided some exceptions to these observations, and were not significantly drier than the WILD fish, nor significantly mushier.

The overall perception of the different fish samples indicated that there was no preference for WILD fish over farmed fish, provided the lake-farmed fish were purged (T1 and T2) or were farmed in SALT water (Figure 2.1 and 2.6). However, unpurged LAKE grown fish were less preferable to all other options.

During the purging process a distinct difference in skin colour was noted between the purged fish and those removed directly from the cages in the lake. Those fish purged took on a much paler and silvery colour, while the cage sourced fish were much darker in skin colour (Figure 2.1 and 2.7).

2.3.2 Trained panel sensory assessment trial

An assessment of purged and lake fish by a trained sensory panel confirmed the earlier results by an untrained consumer panel (Figure 2.1 and 2.8). Significant differences in a range of sensory parameters between the purged and lake fish were observed. Most noticeable were the differences between the two treatments in muddy flavour and muddy aftertaste, which were both greater in the lake fish than the purged fish. Other key differences were the greater fresh flavour and salty sea breeze aroma in the purged fish relative to the lake fish.

2.3.3 Threshold trial

The results showed that the there was no significant threshold for geosmin as determined by the presence of muddy/earthy flavour in the fish samples. A significant effect of GSM on fishy flavour was observed, though this was highest in the starting fish, but generally showed a positive correlation with GSM concentration in the water (Figure 2.9). Stronger relationships between the sensory attributes and MIB levels were found (Figure 2.10).

A significant effect of MIB on muddy/earthy flavour was noted. Unfortunately, despite several attempts accurately diluting GSM and MIB compounds was found to be extremely difficult. The results reflect other research however, that suggest the threshold for off-flavour in water and fish is in the range of 5-7 ng/L (either GSM or MIB alone or in combination). On-site taste tests found it difficult to detect muddy/earthy flavours when lake water concentrations of MIB were 7 ng/L.

2.3.4 Differences within fillet

The muddy/earth flavour was most perceptible in the belly section, though not significantly ($P>0.05$) more so than the dorsal section, but significantly more so than the tail section of the fillet. The fresh flavour of the belly region was significantly ($P<0.01$) more so than both the dorsal and tail region of the same fillet. The “milkiness” of each section showed that the belly region was also significantly ($P<0.01$) milkier in flavour than both the dorsal and tail region of the same fillet. The belly section was also assessed as being sweeter than both the dorsal and tail section of the same fillet.

The Department of Fisheries Research Laboratories study with an untrained sensory panel of 6 people also identified that the muddy taint was significantly greater in the belly region of the fish than in the other two cuts of the fillet. Despite only 6 people being used (3 men and 3 women) it was also shown that women were more likely to detect differences between the different fillet sections.

Total lipid analysis of the different cores from the fillet (Figure 2.13 and 2.14) showed that on a wet-tissue basis (Figure 2.13) the total lipid content was highest in the belly cut, in particular the anterior core (29.6% total lipid) within the belly cut. Both the dorsal and tail fillet regions were relatively low in total lipids, particularly the tail section, which had a mean level of 1.8% total lipids. The lowest total lipid level was found in the anterior sample (1.3% total lipid) of the dorsal cut of the fillet.

2.3.5 Differences between large and small fish

The findings of the sensory comparison of large and small fish fillets showed that there were perceptible differences in flesh flavour attributes between fish from the same source, but of different sizes. The large fish had a significantly more perceptible muddy/earthy flavour ($P<0.001$) and aftertaste ($P<0.05$) than small fish, but the small fish were significantly sweeter ($P<0.05$) and had a fresher ($P<0.05$) flavour than the large fish.

2.4 Discussion

2.4.1 Taint detection trial

The initial trial was undertaken to verify the presence of a muddy-taint problem in farmed barramundi from Lake Argyle and to also demonstrate that purging of farmed Lake Argyle fish would produce flesh characteristics that are similar to saltwater farmed and wild caught barramundi. Based on the studies of other researchers, two purging regimes were tested in 2000L tanks at Lake Argyle Industries Pty Ltd hatchery at Lake Argyle (Howgate, 2004; Robertson et al., 2005). Barramundi were sourced from lake (LAKE) cages and transferred into the tanks. The first group were purged in flow through bore water (< 1ng/L both MIB and GSM) for five days (T1). A second group of fish were purged in bore water plus 20 ppt salt for three days followed by 30 ppt salt for two days (T2).

Independent taste panel tests undertaken at Department of Fisheries (WA) were unable to detect significant differences between either group of purged fish (T1 and T2), saltwater-farmed barramundi (SALT) or wild caught barramundi (WILD). However, farmed barramundi that was taken directly from lake (LAKE) cages had a muddy odour that was significantly more noticeable than that noted in the other fish. The overall ranking of the samples showed no preference for wild caught over farmed fish, provided the fish were farmed in saltwater or lake-farmed fish were purged. Other studies examining farmed barramundi have found limited effect of feed source on sensory attributes (Williams et al., 2003). No preference was attributed to the addition of salt in purging regimes for lake fish.

Purged fish also changed from the usual dark skin colour of lake (LAKE) fish to a silver colour typical of saltwater barramundi when purged in indoor tanks that had white walls (see Figure 2.7). This observation is similar to the effect observed with some other fish species when removed from ultraviolet light exposure and placed in the presence of a lighter background (Booth et al., 2004).

Further assessment of the difference in sensory attributes by a trained sensory panel confirmed the presence of flavour taint, and also identified that it could be rectified through a purging process (Figure 2.8). This was consistent with observations that had been reported through purging with other fish species (Zimba and Grimm, 2003; Grimm et al., 2004; Howgate, 2004; Robertson et al., 2005).

There have been several studies comparing the chemical and sensory analysis of flavour taint in fish and in most cases there is little advantage to be gained by using chemical analysis. The chemical analysis procedure, whilst requiring significant infrastructure investment, still has poor and variable recovery rates to be considered a reliable assay (Lovell et al., 1985; Robertson et al., 2004; 2005). Other researchers have also noted this limitation and have resorted to using sensory assessments as their key way of assessing muddy taint issues, or in some cases resorted to training dogs to detect the smell (Shelby et al., 2004; Robin et al., 2006). This supports the strategy used in the present study, where the use of both an untrained consumer and a trained professional sensory panel reached similar conclusions when given paired samples (i.e. the same samples sent to both groups).

2.4.2 Threshold trial

In studies with rainbow trout and channel catfish either MIB or GSM respectively have been found to be the primary causative agent of muddy-flavour in these fish (Lovell et al., 1986; Grimm et al., 2004; Howgate, 2004; Robertson et al. 2005). It was not known which of these two compounds was responsible in the case at Lake Argyle, or whether they both act in combination. In the present study a clear significant effect of the water MIB concentration on muddy/earthy flavour in fish was noted.

The results showed that the there was no significant threshold for GSM as determined by the presence of muddy/earthy flavour in the fish samples. A significant effect of GSM on fishy flavour was observed, though this was highest in the starting fish, but generally showed a positive correlation with GSM concentration in the water. A minor, non-significant increase in muddy-flavour was noted with increasing GSM level in the water.

In the present study no direct assessment of the concentration of either GSM or MIB in barramundi flesh was undertaken. However, assessments of GSM content within fish flesh in other studies have reported that GSM levels in channel catfish ranged from 3.7 to over 200 µg/kg of flesh and rainbow trout ranged from negligible to 7.2 µg /kg of flesh (Lovell et al., 1985; Robertson et al., 2006). A flesh sensory threshold for GSM in channel catfish of 250 - 500 ng/kg and MIB of 100 – 200 ng/kg was reported (Grimm et al., 2004). A flesh sensory threshold for GSM in rainbow trout of 900 ng/kg was reported (Robertson et al., 2006). The water concentration of GSM, shown to have caused muddy taint in earlier studies was ~25 ng/L. In the present study a water MIB threshold of 3 ng/L was suggested, based on an assessment of the calculated MIB concentration of 60% dilution treatment and a 0% and 100% level of 0 ng/L and 5 ng/L respectively. In contrast, a much higher level of 78 µg /kg in the flesh was observed with shrimp indicating that there is some species-specific variation (Lovell and Broce, 1985). This may be reflective of differences in lipid content of the flesh within each species, with the two muddy-taint compounds known to be lipophilic.

2.4.3 Differences within fillet

There are few other studies examining the variation of muddy-taint within the fillet. Indeed, this study is the only specific example that was identified. The results of the study assessing the different sections of a fillet of barramundi from unpurged lake fish showed that the muddy flavour was most perceptible in the belly section. However this effect was not significantly more so than the dorsal section of the fillet but was more so than the tail section, which had the least muddy flavour taint. However, the belly section was also perceived as being fresher, sweeter and milkier in flavour than the other fillet sections. It is suggested that this difference is generally reflective of differences in fat levels of flesh between the fillet sections. If these observations of muddy flavour taint being related to fat levels are valid, then a similar, fat level related effect should be noticeable between small and large fish. Typically larger fish have higher fat levels than smaller fish (Glencross et al., 2002). Therefore it was hypothesised that the muddy taint problem would be greater in the larger fish than the smaller fish.

In the present study, the use of both an untrained consumer and a trained professional sensory panels both provided significant outcomes and when given paired samples (i.e. the same samples sent to both groups). Both sensory panels tested for the same issues independently, and the same outcome was arrived at. This confirmed that the Lake Argyle fish do have a problem with muddy taint of their flesh, and that the problem varies with portion of the fillet assessed. Analytical assessment of key compositional differences between each fillet portion show that

the dominant difference between each fillet portion is the level of fat. It has been hypothesised that flesh lipid levels significantly influence detection of muddy-taint, with both geosmin and MIB being likely to sequester into the fat tissue within the flesh (Howgate, 2004).

2.4.4 Differences between large and small fish

Similar to the study on variation within the fillet, limited information was available on the effect of fish size on degree of muddy-taint effect. However, based on the observations of a possible relationship between lipid content and muddy-taint, it was hypothesised that larger fish should be more susceptible to muddy-taint problems. The findings of the comparison of large and small fish showed that there were perceptible differences in flesh flavour attributes between fish from the same source, but of different sizes. Importantly, the larger fish showed a significantly more perceptible muddy/earthy flavour and aftertaste than small fish, but the small fish were notably sweeter and had a fresher flavour than the large fish. This observation was consistent with the proposed hypothesis that this difference is reflective of differences in fat levels of flesh between the different fish sizes. Studies on many other fish species have also confirmed that larger fish have a higher concentration of fat in their total body mass and also their flesh (Lupatsch et al., 2001; Johnston et al., 2006).

From the observations of the differences in taint among the fillet sections and their corresponding fat content, it can be postulated that this relationship is also consistent with the differences observed in the present study between taint of small and large fish. From earlier studies, a distinct effect of fish size on total animal fat content has been observed and provides some basis for the nature of this effect (Glencross et al., 2002) (Figure 2.14). Given that the MIB and GSM molecules are lipophillic substances and therefore more likely to be associated with fattier fish portions and also larger fish. Examination of their chemical structures (Figure 1.1 and 1.2) supports this notion.

2.4.5 Determining issues with muddy-taint in barramundi

A range of aspects of the muddy-taint issue were determined from a series of studies on the barramundi from the Lake Argyle farm (LAI). Importantly these consisted of repeated, independent sensory assessments of the freshwater-farmed Lake Argyle fish, against fish known to not have had a history of the problem of muddy-taint. This included samples of saltwater-farmed fish and wild-caught barramundi from the Northern Territory. Additional treatments included some preliminary purging assessments, to have a provisional examination of the potential of this fish management technique with this species.

The comparison of wild, saltwater farmed, freshwater farmed and purged freshwater farmed barramundi is the first such assessment to be presented. The evidence produced in this study provides sound evidence that there is no consumer preference, from a sensory perspective, for wild over any form of the farmed barramundi, other than those with a muddy-taint problem. In this case, this was limited to the unpurged freshwater-farmed fish. Some discernable differences were noted though between the wild and farmed fish, with a key feature being the observed difference in the colour of the fillet, with farmed fish having a higher degree of “greyness”, presumably through some melanisation process. This effect has been noted previously (Glencross, 2006).

The threshold of detection, from a geosmin (GSM) and MIB concentration in the fish flesh was not able to be determined from this study, because no direct measurement of GSM or MIB in the fish flesh was undertaken. However, an assessment of the effect of GSM and

MIB concentrations in the ambient water on the sensory aspects of the fish was undertaken. This allows for some guide as to water GSM and MIB concentrations that are likely to be problematic from a sensory quality perspective. For MIB the deterioration in sensory value (as determined by the significant increase of muddy-taint) of the barramundi occurred at a water MIB concentration around 3 ng/L. For GSM, the effect was never really a significant one, although a numerical increase in sensory detection of muddy-flavour was detected at a water GSM concentration of around 0.4 ng/L.

The variation of sensory attributes within the fillet is something new in the context of other work that has been undertaken on the issue of muddy-taint in fish. However, consistent with other reports, the results were highly consistent with the variation in lipid content in the flesh (Howgate, 2004). This finding also explains the variation in sensory attributes observed between the different fish sizes. Again, this too is another aspect of the study that does not seem to have been repeated with any other species. However, there is a wide-ranging level of sensory thresholds reported for geosmin and MIB and mostly this seems to revolve around species differences. One of the key aspects of those species differences being the difference in fat levels within the flesh of each species.

Many of the other studies discussed the muddy-taint problem as a seasonal issue, with it predominating in the warmer months. Presumably this was due to the growth of the GSM and MIB producing cyanobacteria and algae being exacerbated during this period (Brown and Boyd, 1982). While anecdotal observations also suggest the same occurrence with barramundi production in Lake Argyle, some confirmation of the seasonal variability species and abundance of cyanobacteria and phytoplankton in the Lake Argyle system, would be of some value in determining if there are periods when there is likely to be an increase prevalence of the muddy-taint problem. While many of the GSM and MIB producing cyanobacteria and phytoplankton are well known in Australian water quality circles the possibility of lesser known GSM and MIB producers being present in the Kimberley region of Australia is a possibility. Therefore the proposed assessment of cyanobacteria and phytoplankton species diversity and abundance would be a good avenue to benchmark such an issue.

Central to determining the operational constraints with depurating barramundi of muddy-flavour taint induced by MIB accumulation will be a better understanding of the rate kinetics of MIB uptake and depuration. Once these functions are defined the timeframe over which flavour problems develop and can be resolved can be better understood. Therefore the logical next step now that the nature of the problem has been defined is to assess the time frame over which flavour-taint accumulates and dissipates and the conditions that optimise each parameter.

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2.7 Tables and Figures

Table 2.1 Sensory attributes and standard descriptors used by Centre for Food Technology in the assessment of aroma, flavour and aftertaste attributes of barramundi.

Attribute	Descriptor / Analogue
Aroma	
Milky	Similar aroma to a 20 mL solution (33%) of boiled milk served in a small glass vessel
Steamed	Similar aroma to a strip of hot, freshly steamed (with 33% milk solution) chicken breast fillet served in a small glass vessel
Salty Seabeeze	Similar aroma to a mixture of sand, shell grit and seaweed served in a small covered plastic cup
Fresh	No Standard - Defined as smell of recently cooked fresh, white-fleshed fish
Fishy	Similar to aroma of 20 mL of mackerel fillet in brine solution served in a small coverred plastic cup
Muddy/Earthy	Similar to aroma of 20 g of mud after a shower of rain served in a small coverred plastic cup
Other	As defined by individuals as case arises
Flavour	
Sweetness	No Standard - Defined as sweet flavour experienced when sample in mouth
Milky	No Standard - Defined as the flavour of warm, diluted milk experienced when sample in mouth
Fresh	No Standard - Defined as the fresh flavour of recently cooked white-fleshed fish experienced when sample in mouth
Fishy	No Standard - Defined as the fishy flavour of old white-fleshed fish experienced when sample in mouth
Muddy/Earthy	No Standard - Defined as the flavour of mud/potting mix/earth experienced when sample in mouth
Metallic	No Standard - Defined as the tingly metallic sensation/flavour that might be caused by a metal spoon experienced when sample in mouth
Other	As defined by individuals as case arises
Aftertaste	
Muddy	No Standard - Defined as the lingering muddy/potting mix flavour after the sample has left the mouth
Fishy	No Standard - Defined as the lingering flavour of old white-fleshed fish after the sample has left the mouth
Other	As defined by individuals as case arises

Table 2.2 Geosmin and 2-methyl-isoborneol concentrations of threshold treatments.

	0%	20%	40%	60%	80%	100%
Geosmin - measured (ng/L)	0.0	0.0	0.0	1.0	0.5	2.0
Geosmin - estimated (ng/L)	0.0	0.4	0.8	1.2	1.6	2.0
2-Methyl-isoborneol - measured (ng/L)	0.0	1.0	3.5	0.5	5.5	5.0
2-Methyl-isoborneol - estimated (ng/L)	0.0	1.0	2.0	3.0	4.0	5.0

Concentrations determined from duplicate water samples

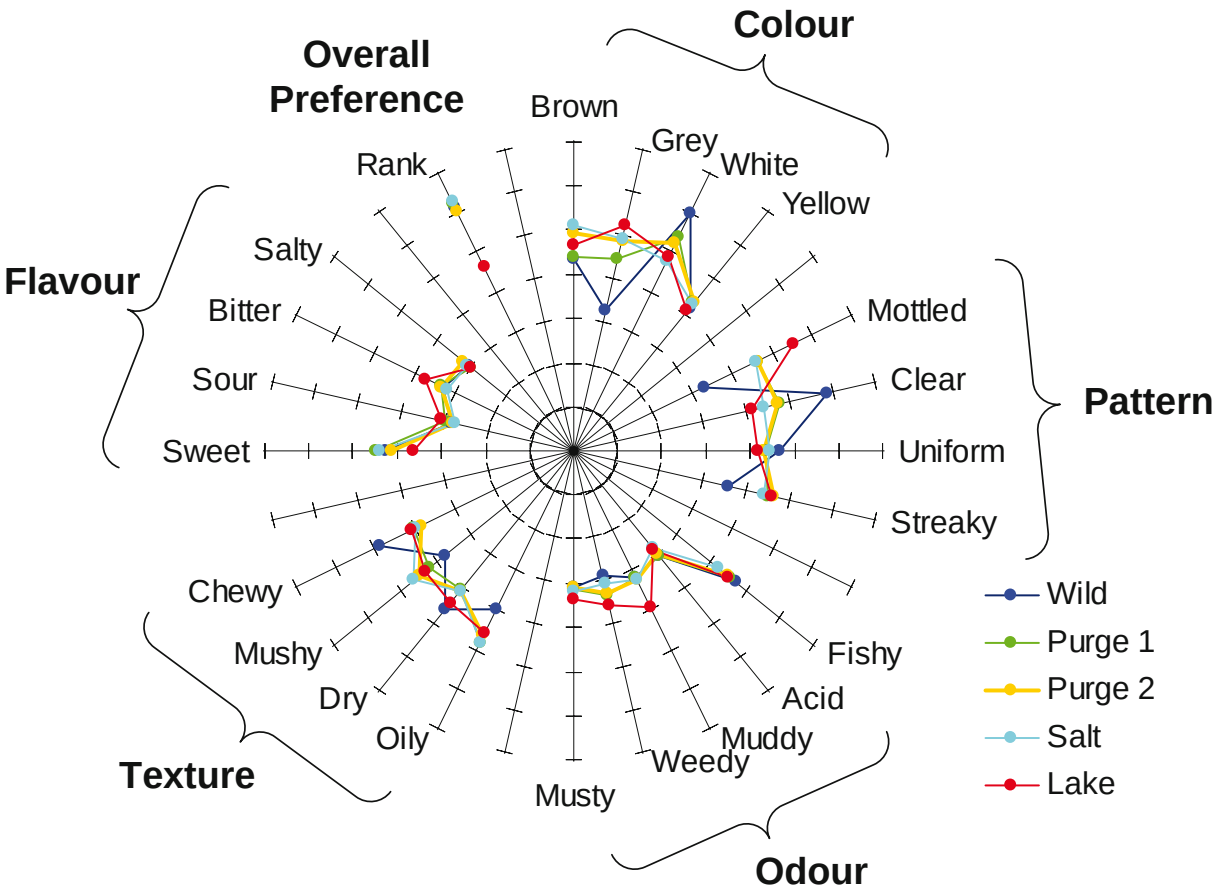

Figure 2.1 Summary of the sensory attributes (visual (pattern and colour), odour, flavour and texture) of the different fish assessed by an untrained panel. Treatments were - Wild: Wild fish caught in estuarine waters in the Northern territory, T1: purging treatment 1, T2: purging treatment 2, Salt: Saltwater farmed fish, Lake: Fish direct from commercial cages in Lake Argyle.

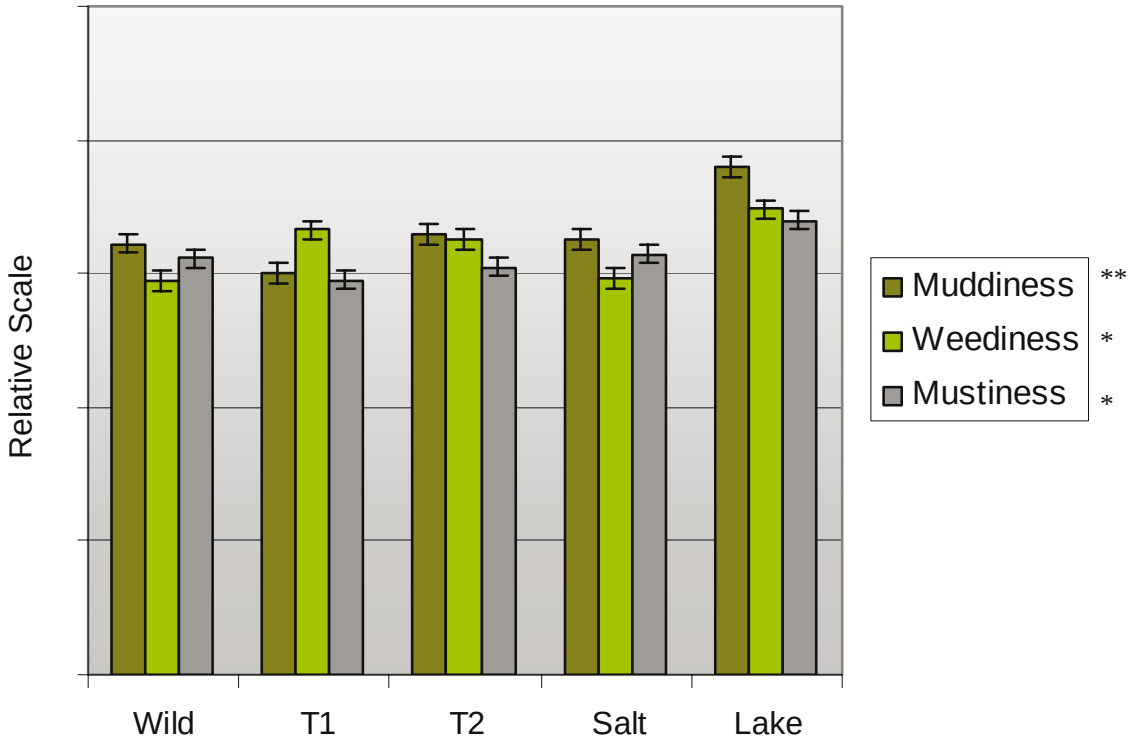

Figure 2.2 Odour of fish from the five treatments examined to confirm the presence of a muddy/ earthy flavour taint problem. Wild: Wild fish caught in estuarine waters in the Northern territory, T1: purging treatment 1, T2: purging treatment 2, Salt: Saltwater farmed fish, Lake: Fish direct from commercial cages in Lake Argyle. Significant differences (*P<0.05, **P<0.01, ***P<0.001) between treatments are indicated.

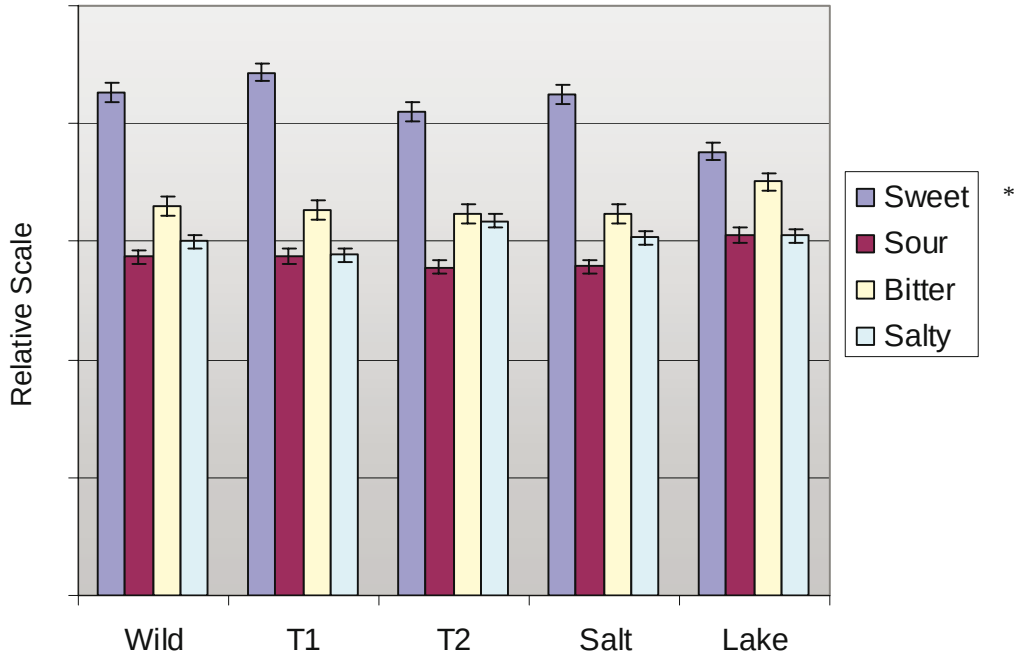

Figure 2.3 Flavour of fish from the five treatments examined to confirm the presence of a muddy/ earthy flavour taint problem. Wild: Wild fish caught in estuarine waters in the Northern territory, T1: purging treatment 1, T2: purging treatment 2, Salt: Saltwater farmed fish, Lake: Fish direct from commercial cages in Lake Argyle. Significant differences (*P<0.05, **P<0.01, ***P<0.001) between treatments are indicated.

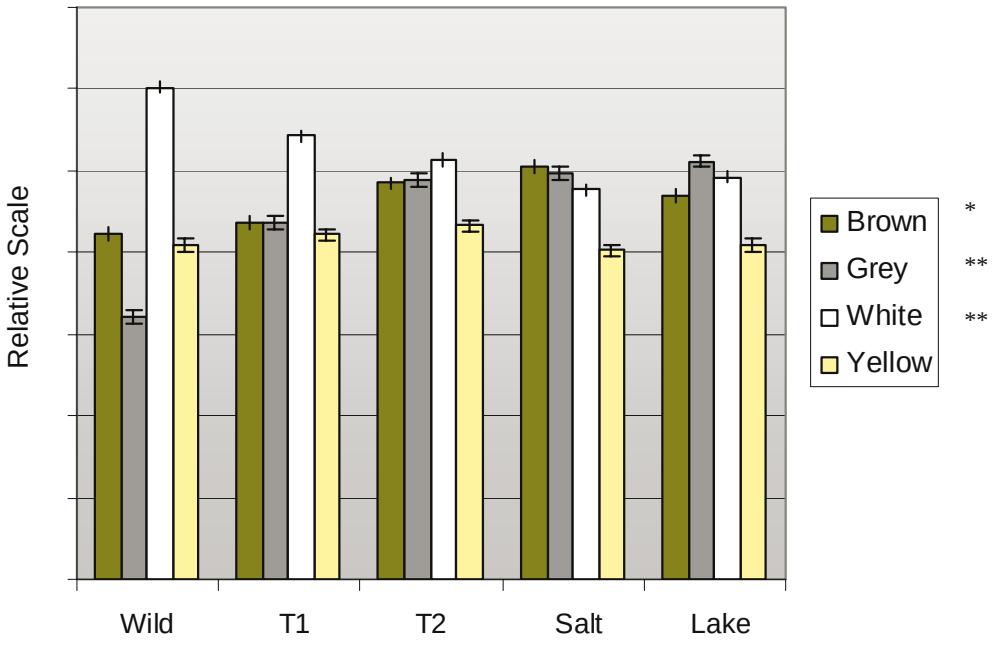

Figure 2.4 Colour of fish from the five treatments examined to confirm the presence of a muddy/ earthy flavour taint problem. Wild: Wild fish caught in estuarine waters in the Northern territory, T1: purging treatment 1, T2: purging treatment 2, Salt: Saltwater farmed fish, Lake: Fish direct from commercial cages in Lake Argyle. Significant differences (*P<0.05, **P<0.01, ***P<0.001) between treatments are indicated.

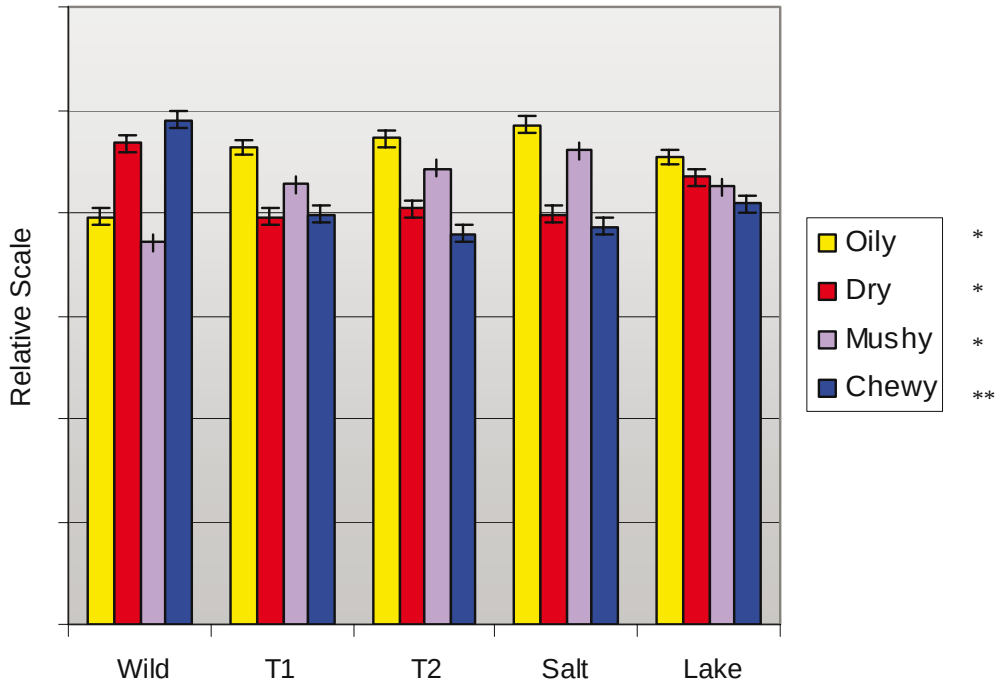

Figure 2.5 Texture of fish from the five treatments examined to confirm the presence of a muddy/ earthy flavour taint problem. Wild: Wild fish caught in estuarine waters in the Northern territory, T1: purging treatment 1, T2: purging treatment 2, Salt: Saltwater farmed fish, Lake: Fish direct from commercial cages in Lake Argyle. Significant differences (*P<0.05, **P<0.01, ***P<0.001) between treatments are indicated.

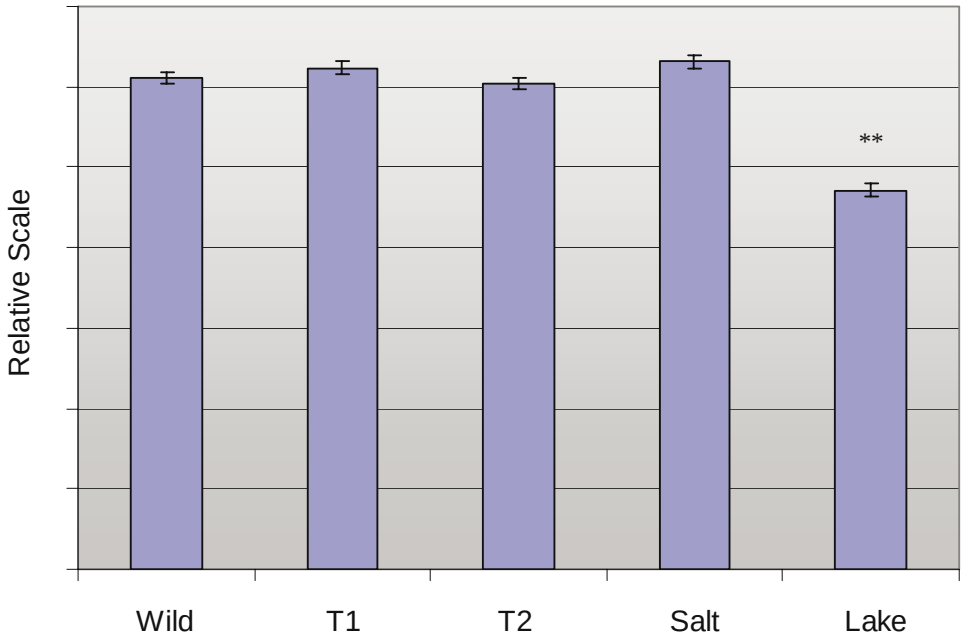

Figure 2.6 Overall ranking of fish from the five treatments examined to confirm the presence of a muddy/earthy flavour taint problem. Wild: Wild fish caught in estuarine waters in the Northern territory, T1: purging treatment 1, T2: purging treatment 2, Salt: Saltwater farmed fish, Lake: Fish direct from commercial cages in Lake Argyle. Significant differences (*P<0.05, **P<0.01, ***P<0.001) between treatments are indicated.

Figure 2.7 Comparison of skin colour of purged (top three fish) and unpurged (bottom three fish) fish directly from cages in the lake. All fish had been harvested within 6 hours and kept on ice prior to the photograph being taken.

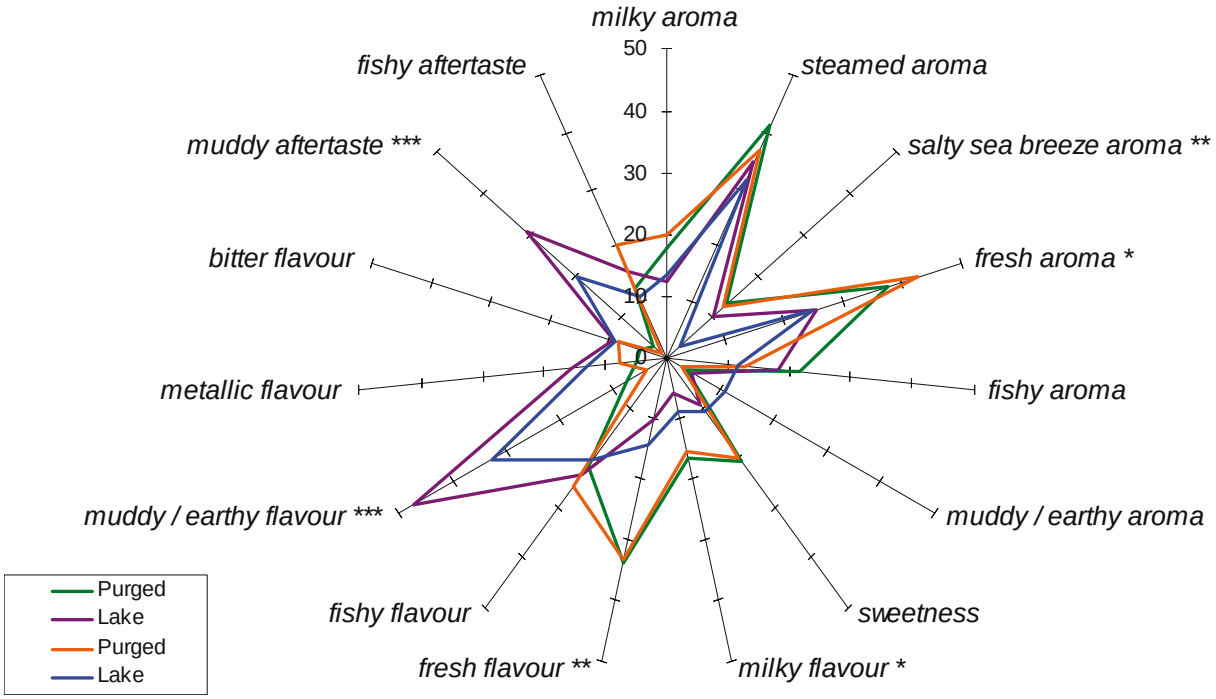

Figure 2.8 Comparison of sensory parameters of lake and purged fish undertaken by a trained sensory panel. Two fish from each treatment were used and assessed for a wide range of sensory characteristics in a pairwise comparison test. Significant differences (*P<0.05, **P<0.01, ***P<0.001) between treatments are indicated.

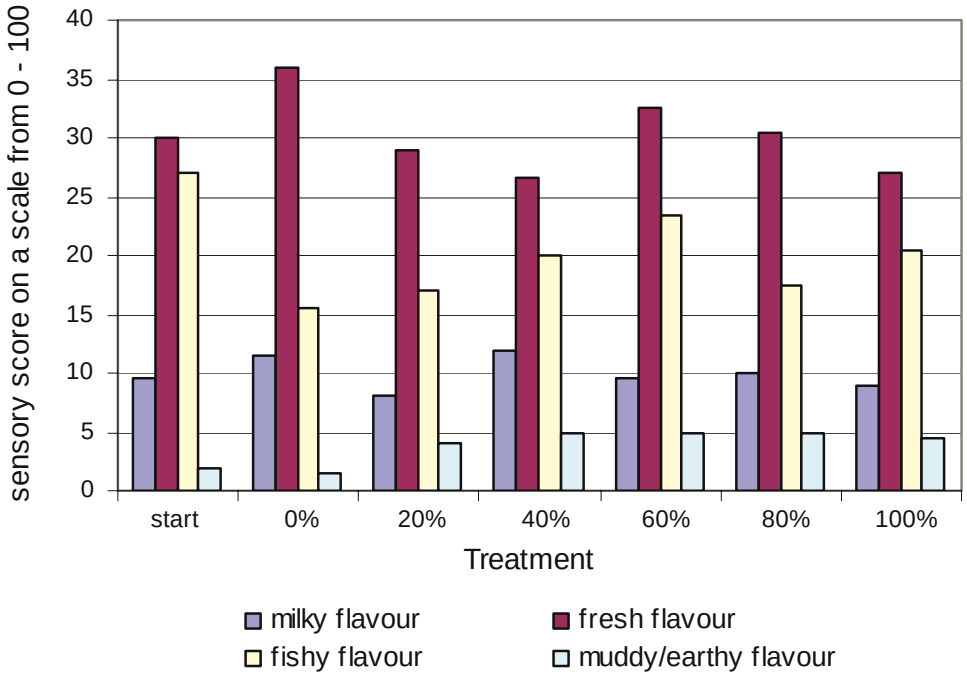

Figure 2.9 Average sensory scores by trained sensory panel for *fresh flavour and muddy / earthy flavour* for each water concentration treatment in the geosmin threshold trial. Attribute scores are calculated as the mean value for each time point (mean of 10 judges and 2 replicates).

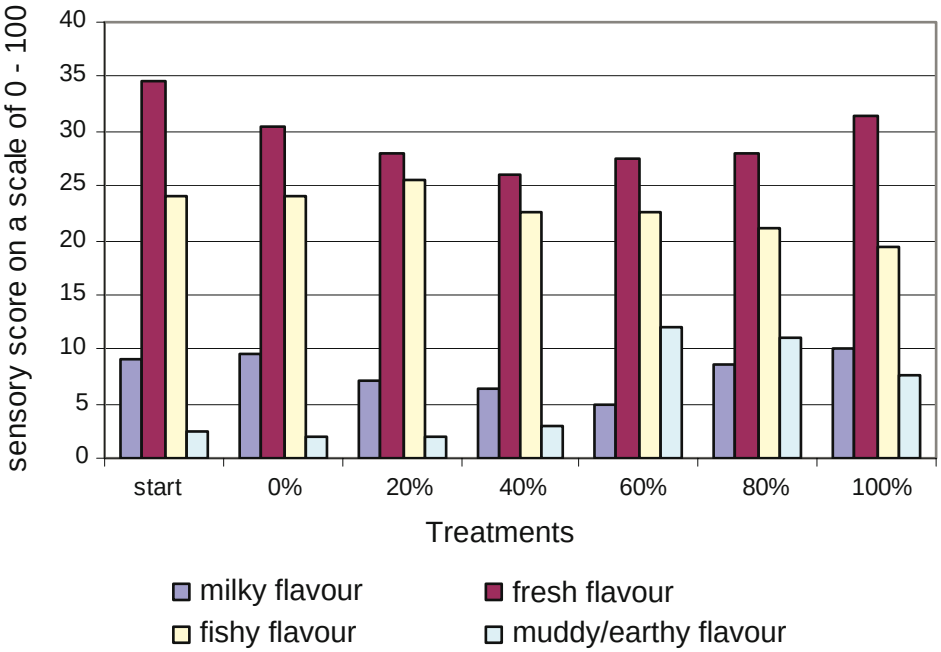

Figure 2.10 Average sensory scores by trained sensory panel for *fresh flavour* and *muddy / earthy flavour* for each water concentration treatment in the methyl-isoborneol threshold trial. Attribute scores are calculated as the mean value for each time point (mean of 10 judges and 2 replicates).

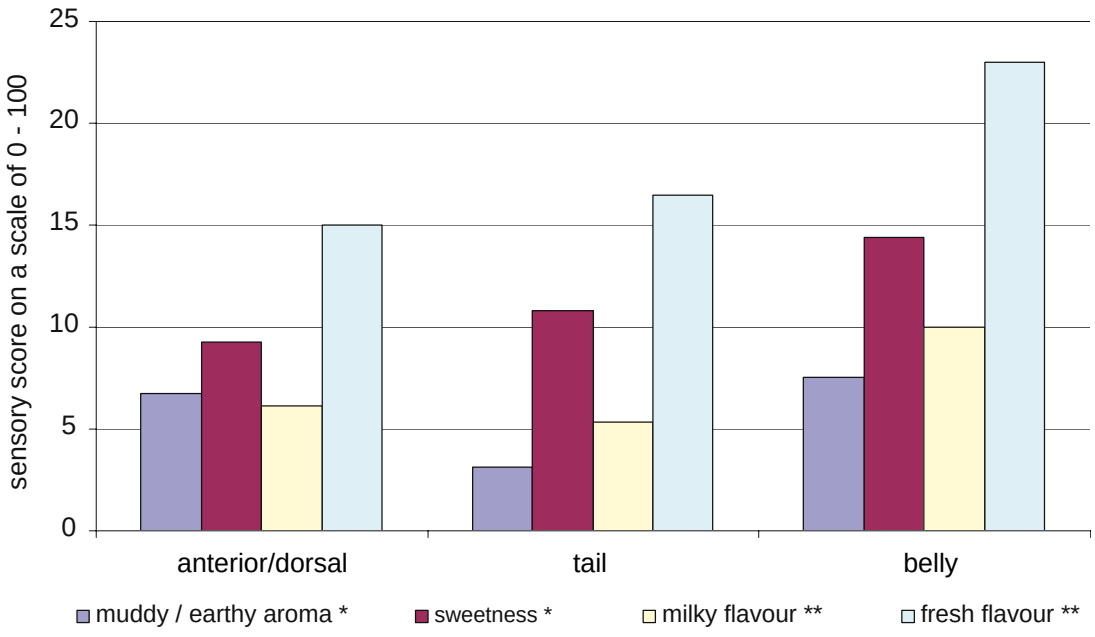

Figure 2.11 Average sensory scores by trained sensory panel for attributes where significant differences were observed between different sections of the fillet. Attribute scores are calculated as the mean value for each time point (mean of 10 judges and 5 replicates). Significant differences (*P<0.05, **P<0.01, ***P<0.001) between treatments are indicated.

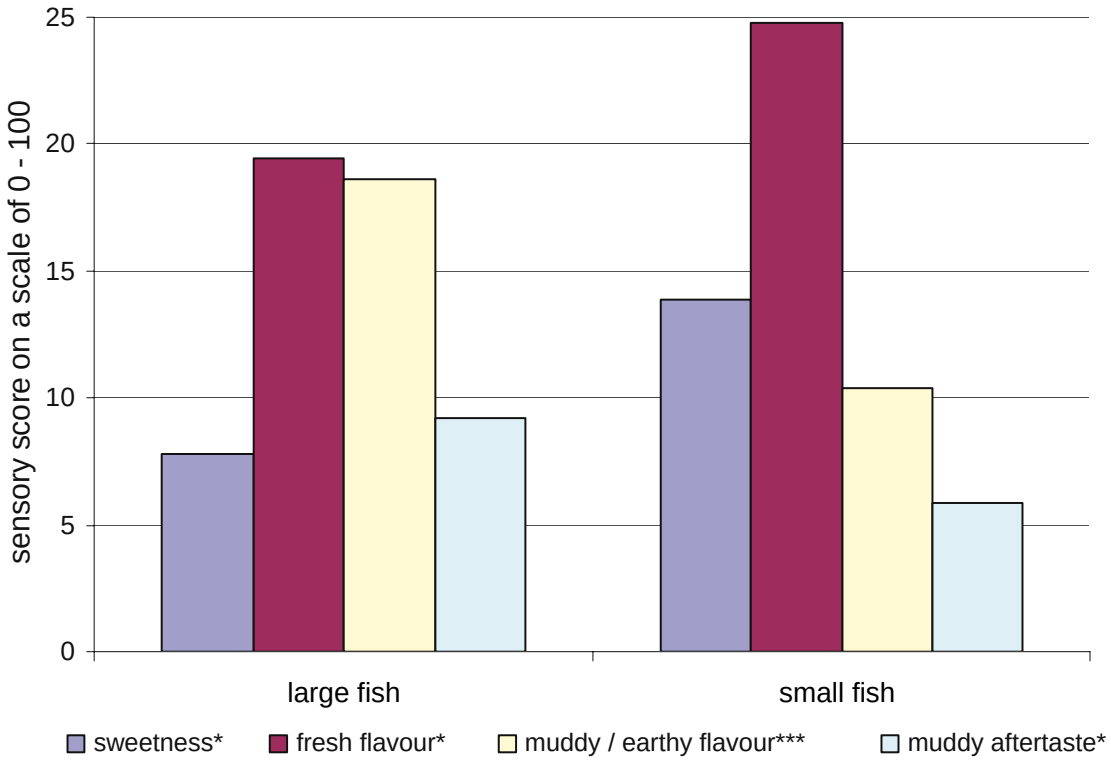

Figure 2.12 Average sensory scores by trained sensory panel for attributes where significant differences were observed between fish of different sizes. Attribute scores are calculated as the mean value for each time point (mean of 10 judges and 7 replicates). Significant differences (*P<0.05, **P<0.01, ***P<0.001) between treatments are indicated.

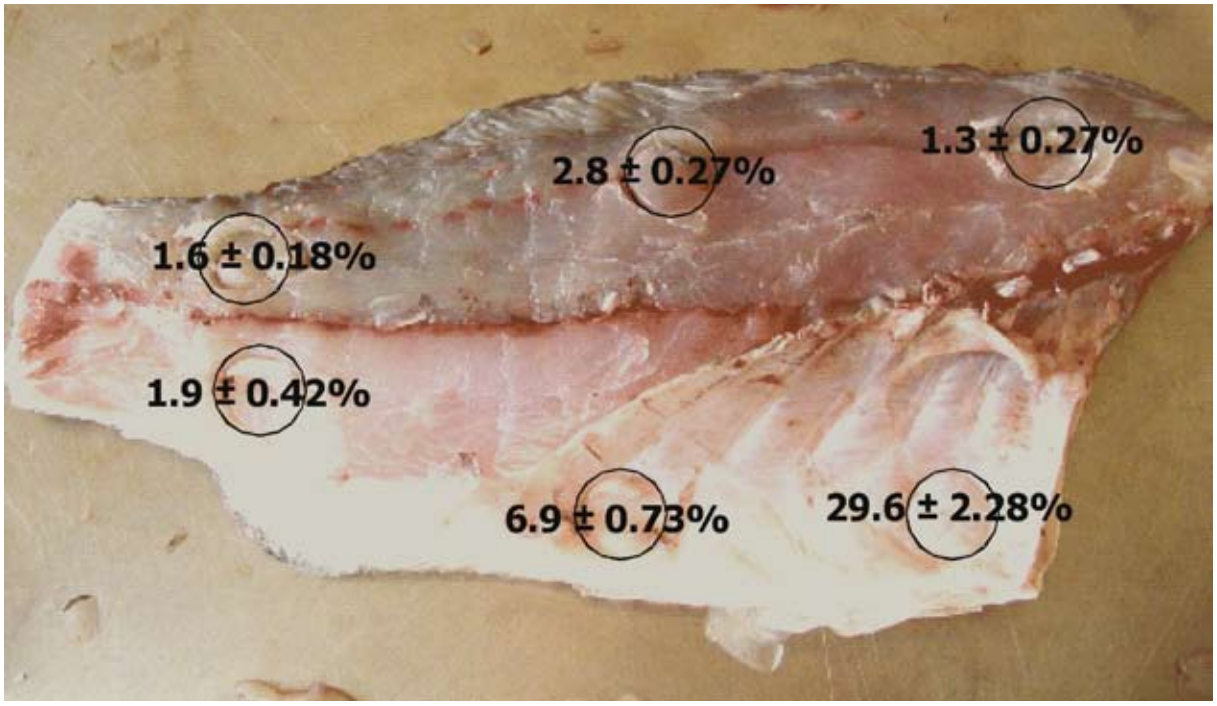

Figure 2.13 Variability in fat content (% live-weight) within the fillet of 2 kg barramundi. Shown are the fat levels (%) determined from within the sample taken from the area marked by the respective ring. Also shown are the three fillet sections used on the intra-fillet sensory evaluation work (Dorsal, Tail, Belly).

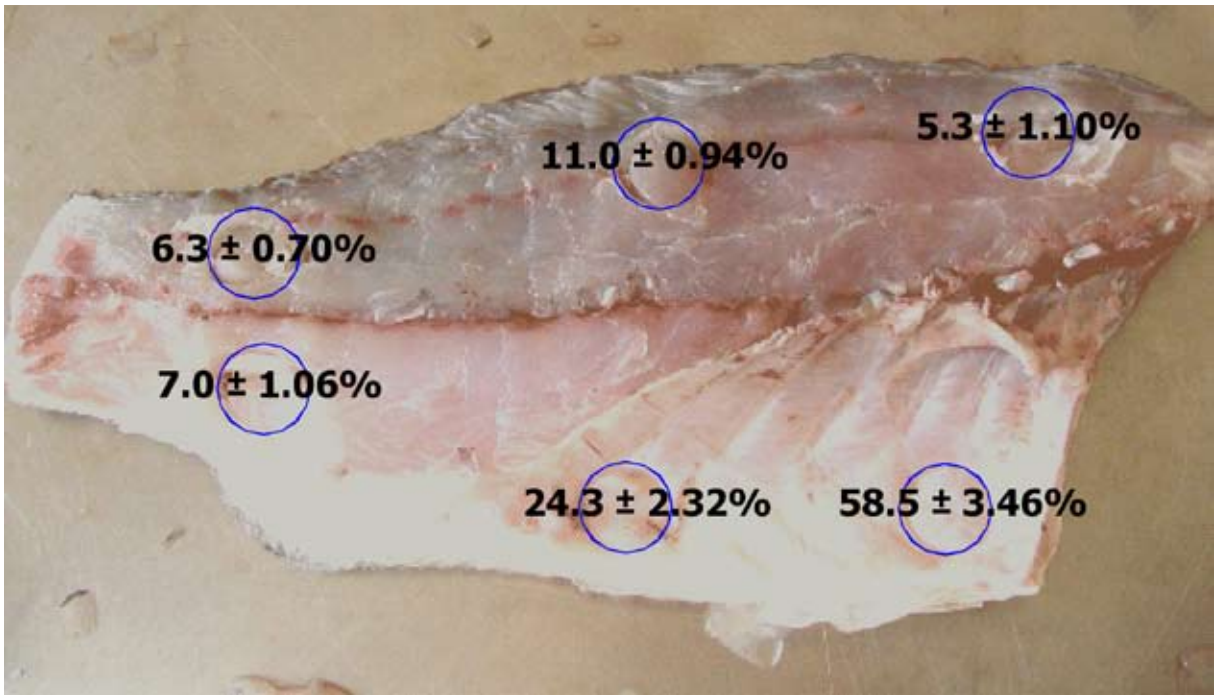

Figure 2.14 Variability in fat content (% dry-weight) within the fillet of 2 kg barramundi. Shown are the fat levels (%) determined from within the sample taken from the area marked by the respective ring.

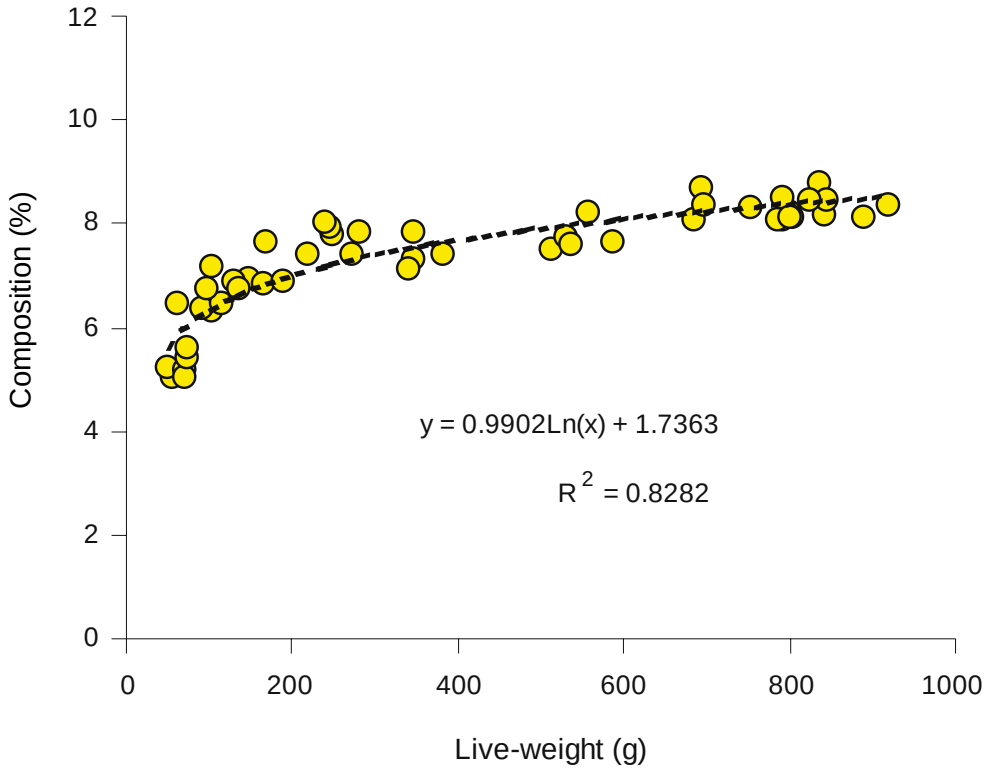

Figure 2.15 Variation in fat content of whole barramundi over the size range examined (51 g to 918 g). Reproduced from Glencross et al. (2003).

3.0 Reducing flavour taint in farmed barramundi

Steve Percival [a], Paul Drabsch [b] and Brett Glencross [c]

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3.1 Introduction

Once the flavour taint issue has been confirmed as being related to geosmin (GSM) and more predominantly to 2-methylisoborneol (MIB) levels, the issue then becomes how to reduce the levels of these compounds in the fishes flesh. In fish flesh the critical threshold appears to vary among fish species with values for GSM ranging from 250 and 10,000 ng/kg and for MIB threshold values ranging from 100 and 700 ng/kg (Yamprayoon and Noonhorm, 2000; Grim et al., 2004; Robertson et al., 2005). The lipid content of the fish has also been related to the uptake of GSM and MIB, and this is also consistent with our own findings of variability within the fillet and between fish of different sizes (Howgate, 2004).

Studies on other fish species have shown that placing the muddy flavour tainted fish in water free of GSM and MIB reduces the muddy flavour taint problem (Robertson et al., 2005). However, one of the key commercial variables of importance is the time taken to purge the fish. Studies on other fish species have shown this to take up to 16 days (Yamprayoon and Noonhorm, 2000). It is also of interest to know how quickly the problem can develop, but there is limited data on rates of uptake of either GSM or MIB in the literature (Howgate, 2004).

Reviews on the topic suggest that the kinetics of uptake and depuration are single-compartment kinetic processes and well described by exponential decay and/or rate-kinetic functions (Howgate, 2004; Robertson et al., 2005). While there are likely to be many underpinning factors that influence these processes, including concentration of MIB and GSM in the water (both in uptake and depuration), the size of the fish, the fat content of the fish and the duration of exposure (both in uptake and depuration).

To examine the issue surrounding the reduction of muddy-flavour taint in barramundi a series of issues to be resolved were identified. In essence these are primarily based on understanding the kinetics of change in muddy flavour taint in farmed barramundi.

- What is the rate at which taint is gained in taint-free fish?
- What is the rate at which taint is lost in tainted fish?
- What critical environmental conditions need to be considered for holding barramundi in confinement if they are being purged?

3.2 Materials and Methods

3.2.1 Uptake Rates

Uptake of flavour taint by barramundi exposed to geosmin and methyl-isoborneol was undertaken by placing fish previously purged for five days in an indoor facility in 2000L tanks containing bore water known to be free from geosmin and methyl-isoborneol based on earlier studies. The fish (~2000g) were then reintroduced to tanks containing fresh lake water (from

Lake Argyle) that had ambient geosmin and methyl-isoborneol levels (Geosmin: 1 ng/L; 2-methylisoborneol: 13 ng/L). Two fish were then sampled at various time points over a 72 h period. Fish sampled were killed by ice immersion, filleted and frozen prior to being sent to the Centre for Food Technology for sensory assessment by a trained sensory panel. Results are presented in Figure 3.4.

3.2.2. Purging trial

The purging of geosmin and 2-methylisoborneol taint from barramundi was undertaken by placing fish from the lake into water in an indoor facility in 2000L tanks containing bore water known to be free from geosmin and methyl-isoborneol based on earlier studies. Bore water was supplied to the tanks at either a low (8 L/min) or high (16 L/min) flow rate. Fish (~2000g) were stocked at 100 kg/m^3 in 500 L tanks. The water in the purge tanks was also passed through a separate tank at 8L/min and 16L/min respectively to re-oxygenate the water and partially dissipate any taint compounds through vigorous aeration. Two fish were then sampled daily for five day period. Fish sampled were killed by ice immersion, filleted and frozen prior to being sent to the Centre for Food Technology for sensory assessment by a trained sensory panel.

3.2.3 Water quality studies

In association with the five day purging trial, studies were also conducted on the dissolved oxygen, ammonia, and pH in the tanks holding barramundi (average weight ~2000 g) in 500L of water at five different stocking densities (44, 66, 88, 110, 132 kg/m^3; Figure 3.5). Water samples were collected every 12 h for analysis. Dissolved oxygen was measured using an Oxyguard™ oxygen probe (Oxyguard, Birkerod, Denmark). Ammonia and water pH were both measured using a colorimetric test kits (Aquasonic, Wauchope, Australia).

A second water quality trial was undertaken to examine in more detail the key water quality parameters at a greater range of lower flow rates. This study examined the dissolved oxygen and ammonia in tanks holding barramundi (average weight = 730 g) in 2000L of water at six different flow rates of 3, 5, 8, 10, 12, 15 L/min (Figure 3.6). The water temperature was maintained at 30^o C during the trial. Water quality parameters were measured once every 24 hours for seven days using the above-mentioned methods.

A third trial was also conducted on the dissolved oxygen, ammonia, CO_2 and pH in replicate tanks holding barramundi (average weight = 900 g) in 500L of water at five different stocking densities (Figures 3.7, 3.8 and 3.9). The flow rate in all tanks was the same at 15 L/min and water temperature ranged from 30-33^o C during the trial. Water quality parameters were measured once every 12 hours for four days using the above-mentioned methods. Dissolved CO_2 was measured using a colorimetric test kit (Aquasonic, Wauchope, Australia).

3.2.4 Geosmin and 2-methyl-isoborneol analysis

The method used was based on Method 6040B contained in Standard Methods for the Examination of Water and Wastewater, 19th Edition with in-house modifications. The process involves pre-concentrating a 1L sample by Closed-Loop-Stripping analysis. 2-methylisoborneol and geosmin are removed from the water by a recirculating stream of air and adsorbed onto a carbon filter from which they are then extracted using dichloromethane. The extract is then quantitatively analysed by gas chromatography/mass spectrometry using Selected Ion Monitoring.

3.2.5 Trained sensory assessment

For most of the sensory assessment studies, it was decided that using a professional, trained sensory panel would provide the most robust and independent data. Sensory analysis by a trained panel was undertaken, under contract, at the Centre for Food Technology (CFT, Hamilton, QLD), coordinated by Dr Heather Smyth. The panel consisted of 10 female judges, aged between 30 and 61, who were experienced with sensory descriptive analysis of foods and beverages.

The panel were trained over four sessions, each of approximately two hours, to rate a number of defined sensory attributes. A series of 14 aromas, flavour and aftertaste descriptors were chosen. The attributes and sensory analogues that were chosen by the panel to rate the barramundi fillets are given in Table 2.1. In addition, an 'other' attribute for aroma, flavour and aftertaste was included for the panel to rate if they thought they could detect a property which was not covered by the chosen list of terms.

Frozen samples were thawed overnight at 2°C prior to preparation for assessment. Slices of barramundi fillet (no skin) were cut from dorsal to ventral direction across the fillet to give a ~20 g portion of fish. Samples were cut starting from the anterior end, such that any unused fillet always remained at the tail end of the fish.

In preparation for sensory assessment fish samples were weighed into foil dishes and covered with aluminium foil sheets (shiny side down) that were pre-numbered with the blinding code. The samples were prepared up to 1 hour ahead of time and kept chilled in a refrigerator at 2 - 4°C prior to cooking. Samples were cooked no more than 30 minutes prior to serving. Samples were cooked on an oven tray, in a pre-heated fan-forced oven, at 200°C for 6 minutes. After cooking, samples were transferred to a warming oven at ~75°C until served.

Only three samples were presented to each panellist at any one time so that all the samples would still be hot for sensory assessment. Samples were presented warm (~75°C) to each panellist in a randomised order. Where there was sufficient flesh from one fish to serve the whole panel, one fish (of two fillets) was treated as one individual sample.

Panellists were asked to first evaluate the aroma of the sample and then to taste the sample and assess flavour, and finally aftertaste. For each sample, panellists were asked to rate the intensity of each of the attributes listed in Table 2.1 on a scale of 0 to 100, anchored from low to high. An 'other' term was also provided for panellists to rate any aroma, flavour and aftertaste not characterised by the listed attributes. Plastic forks were used to taste the samples and a fresh fork was used for every sample tasted. Panellists were forced to wait 60 seconds between samples, and were asked to leave the booths after every set of three samples to take a 5 - 10 minute extended break. Each panellist was provided with purified water, plain water crackers and slices of granny smith apple to use for palate cleansing between samples.

3.2.6 Statistical analysis

A one-way analysis of variance (ANOVA), blocking for Judge Effect, was conducted for each sensory attribute rated, to determine if there were significant differences between treatments. The software used for graphical presentation was Microsoft Excel. The software used for remaining statistical analysis work at CFT was GenStat Seventh Edition, Lawes Agricultural Trust.

3.3 Results

3.3.1 Taint Uptake

In this study fish that were previously depurated in bore-water were returned to lake water with a 2-methylisoborneol concentration of 13 ng/L and a geosmin concentration of 1 ng/L. Uptake of the geosmin and/or 2-methylisoborneol, as determined by the presence of a muddy/earthy flavour, by the barramundi occurred rapidly, with a significantly noticeable increase in muddy/earthy flavour observed within 1 h of exposure. The muddy-flavour plateaued within 3 h. The sensory detection of muddy-taint flavour peaked at 48 h after the introduction of purged fish to the lake water. After 72 h the taint had declined from that determined after 48 h.

Concomitant with the increase in the muddy-flavour was a decrease in the fresh-flavour of the fish. Changes in fresh-flavour were essentially the inverse of the muddy-flavour observations. The lowest fresh-flavour was observed at 48 h. Decrease in fresh-flavour was rapid and had plateaued within 1 to 3 h.

Both the uptake of the muddy-flavour taint and loss of fresh-flavour by the purged fish placed in the geosmin and 2-methylisoborneol tainted water, were consistent with typical first-order rate-kinetic mediated transfer of the compounds. In this report we have described this as a logarithmic function. The increase in muddy-flavour taint was described by the logarithmic function of:

$$Y_{taint\ uptake} = 2.1613*LnX + 13.857\ (R^2 = 0.9279)$$

Where Y is the muddy-flavour score and X is time. The decrease in fresh-flavour was described by the logarithmic function of:

$$Y_{freshness\ loss} = -1.2295*LnX + 24.734\ (R^2 = 0.8400)$$

Where Y is the fresh-flavour score and X is time.

3.3.2 Taint Depuration

In this study fish from the lake were placed in geosmin and 2-methylisoborneol free water and fish sampled every 24 hours to assess their level of muddy-flavour taint by sensory evaluation. Essentially, this study showed that the rate of depuration was substantially longer than that of uptake. The results show that the biggest effect of purging was on the muddy/earthy flavour of the fish and that the response was rapid, mostly occurring in the first 24 h.

Further reduction was achieved with additional purging time, with an inverse exponential/logarithmic relationship evident between time and sensory score. The decline in muddy-flavour taint was consistent with rate-turnover kinetics (and exponential function) and showed that the taint halved approximately every 36 hours. The decline in the sensory score with increasing purging time could be described by a common exponential decay function of:

$$y = 28e^{-0.3568x}$$

where y is the sensory score, x is time and the value of 28 is the initial sensory score based on untainted fish and is an average of the two flow-rates studied. There was a marginal improvement in the “fresh” flavour of the barramundi with increasing purging time, but there was little effect observed in the other key parameters over time, or with differing flow-rates.

3.3.3 Water Quality with fish Holding

Water oxygen concentrations in the first five-day purging study were initially found to be low and with time rose to around 6.0 mg/L and stabilised. No effect of flow-rate was observed. Variability in ammonia concentrations was negligible, and although initially higher at the lower flow rate, over time no difference was observed. The water pH was relatively stable for the duration of the purging period.

In the second water quality trial a more detailed examination was made of the key water quality parameters at a greater range of lower flow rates (3, 5, 8, 10, 12, 15 L/min) (Figure 3.6). Ammonia concentrations were lowest in all treatments on day one, generally highest on day two after which they declined in most treatments. There was a secondary resurgence in ammonia concentrations on day 7 (Figure 3.6). Among treatments ammonia concentrations were consistently higher in the 3 L/min flow-rate treatment, followed by the 5 L/min treatment. Ammonia concentrations were consistently lowest in the 15 L/min flow-rate treatment. Water dissolved oxygen concentrations were highest in the 15 L/min flow-rate treatment and lowest in the 8 L/min flow-rate treatment, which was generally lower than that the dissolved oxygen concentrations observed in both the 5 and 3 l/min flow-rate treatments. There was also a general decline in the dissolved oxygen concentrations from around 6.7 to 6.2 mg/L on day 1 to 6.0 to 6.2 mg/L on day 5.

The third trial, which focussed on different stocking densities (44, 66, 88, 110 and 132 kg/m^3) of 900 g fish with a comparison also made to conditions in cages in the lake (Figures 3.7, 3.8 and 3.9). For this study the same water quality parameters as the previous study were tested, but at a constant flow rate (15 L/min). Generally water ammonia concentrations were related to the stocking density, with higher stocking densities producing higher water ammonia concentrations. Water ammonia concentrations were highest in the 132 kg/m^3 treatment, peaking at around 0.9 mg/L, but fluctuated over the four-day period of the study (ranged from 0.35mg/L to 0.9 mg/L), with cyclical peaks every 36 h. A similar scenario was also observed in the 88 kg/m^3 treatment. The ammonia concentrations in the lake samples were consistently the lowest.

Dissolved oxygen (DO) concentrations varied with the different stocking densities. The DO concentrations were consistently lowest (3.5 to 5.4 mg/L) over the four-day period in the 132 kg/m^3 treatment and highest in the lake treatment (6.5 to 7.6 mg/L), followed by the 44 kg/m^3 treatment (6.0 to 6.7 mg/L). With increasing stocking density, there was generally a corresponding decline in DO concentrations. No consistent pattern in the variability in DO concentrations was noted in any of the treatments.

Dissolved carbon dioxide (CO_2) concentrations varied more than the DO concentrations with the different stocking densities. The CO_2 concentrations were consistently lowest (consistent at 0 mg/L) over the four-day period in the lake treatment, followed by the 44 kg/m^3 treatment (0.0 to 2.5 mg/L). and highest in the 132 kg/m^3 treatment (2.1 to 5.4 mg/L). With increasing stocking density, there was generally a corresponding increase in CO_2 concentrations. The CO_2 concentrations were consistently higher at the first measurement point in all of the treatments. This declined over the following 36 h period before a brief rise and fall over the following 36 h period.

3.4 Discussion

Off-flavour and muddy-taint flavours have been recognised and reported in aquaculture production systems for some time (Lovell, 1983; Bett, 1997; Howgate, 2004). However, there is surprisingly limited information describing the kinetics associated with either uptake or depuration of the flavour influencing compounds (geosmin and 2-methylisoborneol) in fish (Howgate, 2004; Robertson et al., 2005). Studies also vary as to the proposed compound causing the problem, with most studies considering both geosmin (GSM) and 2-methylisoborneol (MIB), and some only GSM (Bett, 1997; Robertson et al., 2005; 2006; Robin et a., 2006). For barramundi, there are only few studies on sensory evaluation and none specific examining the issue of muddy-taint flavours and the depuration rates appropriate for this species (Williams et al., 2003; Glencross, 2006).

3.4.1 Taint Uptake

The uptake of muddy-flavour taint by purged barramundi placed in GSM and MIB tainted water was consistent with a typical first order rate-kinetic mediated transfer of the compounds. This is consistent with most rate-transfer processes where the rate of transfer is dependent on the primary concentration of the compound being transferred. In this study we have described this as a logarithmic function. From the data in this study it was noted that the uptake of the muddy-flavour taint was rapid, with a plateau reached within three hours of the fish being placed in the tainted water. The deterioration in "fresh" flavour was essentially the inverse of that observed of the muddy-taint flavour.

The rate of uptake of muddy-taint flavour in the present study was considerably quicker than that reported by Lovell and Sackey (1973), who reported in 50 g channel catfish (*Ictulurus punctatus*) that they developed a distinct earthy-musty flavour within two days, with a peak in flavour intensity being reached after ten days. Interestingly the control treatment in this study also developed an off-flavour suggesting that either or both GSM and MIB, were present in the control water source. Based on the findings of chapter 2 it is possible that the rate of uptake is affected by the fat levels in the fish and it is likely that the 50 g fish used in the Lovell and Sackey (1973) study, had substantially lower fat levels than the barramundi (~2000 g fish) used in our study. Based on these discrepancies it would be of value to examine the differences in depuration rate between plate size (500 g) and fillet size (2000 g) fish. This may also provide some indication if there is a difference in depuration potential that may affect the economic viability of the depuration process. In a different study on rainbow trout (*Oncorhynchus mykiss*), the sensory threshold for muddy-flavour taint by GSM presence was estimated to be ~0.9 µg/kg (Robertson et al., 2005). Uptake of this muddy-taint was observed within 3 h, similar to the rates observed in the present study, with maximum uptake recorded 6 h after exposure.

3.4.2 Taint Depuration

The reciprocal of the uptake study was the rate of depuration study. In this study barramundi from the cages in Lake Argyle were placed in GSM and MIB free water and fish sampled every 24 hours for five days to assess their level of muddy-flavour taint by sensory evaluation. This study showed that the rate of depuration was substantially longer than that of uptake. The decline in muddy-flavour taint was consistent with rate-turnover kinetics (and exponential function) and showed that the taint halved approximately every 36 hours. In retrospect, the inclusion of a long-term purged fish as a control/reference in this study would have allowed

some indication of how close the fish were after a defined period of purging, to a muddy-flavour taint-free fish.

In other fish species, the depuration rate for GSM has been reported to vary between 96 to 150 h and for MIB, 150 to 500 h when channel catfish (*Ictulurus punctatus*) were placed in 2000L tanks of depurated bore water, similar to the strategy used in the present study (Dionigi et al., 2000). In a different study on rainbow trout (*Oncorhynchus mykiss*), the authors used exponential rate equations to describe the depuration process, similar to that reported in the present study (Robertson et al., 2005). These authors also examined three classes of fish that they categorised according to their taint level (mildly tainted, tainted and grossly tainted). The time taken to achieve an acceptable sensory threshold (0.9 µg/kg) varied among the different taint-levels. The time taken for the mildly tainted, tainted and grossly tainted fish was 48 h, 72 h and 120 h respectively (Robertson et al., 2005). These rates are more consistent with the results obtained in our studies than those obtained by Dionigi et al. (2000) with channel catfish. The extent/severity of the taint-level of the barramundi in the present study is not known, but it is likely that a similar scenario of depuration rates depending on flesh geosmin and 2-methylisoborneol levels will also exist within barramundi.

Differences between uptake and depuration rates are possibility indicative of a couple of scenarios. The uptake may be facilitated by an active uptake process and the depuration dependent on simple half-life turnover effects. Alternatively the lipophilic chemistry of the compounds GSM and MIB may mean that they have a greater affinity for the fish than the media (water) and therefore the uptake rate is quicker and the depuration rate slower. Unfortunately there is no evidence to support or refute either hypothesis from the present study.

3.4.3 Water quality constraints for holding barramundi while purging

Because of the necessity to hold fish in a confined water volume for the purging process, accessory studies on water quality during the purging experiments were also undertaken. Key aspects of each study were the oxygen, ammonia and pH levels associated with each purging regime studied.

In the initial five day purging study, the oxygen concentration was initially found to be low and with time rose to around 6.0 mg/L and stabilised. This effect was independent of flow-rate of the purging tank. The initial lower concentration is suspected to be attributed to higher oxygen consumption of the fish immediately after handling, as other studies have shown that at least 5 to 6 hours is required for the fish to return to a basal oxygen consumption rate (Neill and Bryan, 1991; Glencross and Felsing, 2006). Ammonia build-up in the tank was negligible, although initially higher at the lower flow rate. Over time it also showed no difference in concentrations. This is consistent with what is widely known of unfed fish, in that post-feeding ammonia excretion increases during the 6 to 12 hour period following feeding, but is otherwise maintained at a basal level (Hepher, 1988). At all points of this study, the ammonia concentrations were below those recommended as safe for fish culture (Hepher, 1988; Russo and Thurston, 1991). The water pH was relatively stable for the duration of the purging period and well within regions acceptable for fish culture (Tucker and Martin, 1991).

The second water quality trial included a more detailed the examination of the key water quality parameters at a greater range of lower flow rates (3, 5, 8, 10, 12, 15 L/min) (Figure 3.6). Ammonia concentrations were lowest in all treatments on day one, generally highest on day two after which they declined in most treatments. Given that this was in flow-through

tank systems the variability in ammonia levels is difficult to explain. However, it is suggested that the low levels on day one are indicative of water that had not been holding fish for long, while the high levels of day two indicative of ammonia excretion by the fish as they metabolise their previous meal (probably the day prior to being placed in the tanks, i.e. Day 0) (Phillips et al., 1991). The secondary resurgence in ammonia concentrations on day 7 is probably indicative of a build-up of faecal waste in the tank, given that with a flow through system the soluble ammonia should be being diluted as ammonia excretion decreases since time from last feeding increases (Figure 3.6). That the ammonia concentrations were consistently higher in the 3 L/min flow-rate treatment, followed by the 5 L/min treatment is consistent with this notion of soluble ammonia excreted by the fish being diluted by increasing flow-rates. The ammonia concentrations observed in the low flow rates on day 2 and day 7 were marginally above the recommended levels for fish culture, but still below the levels where fish kills are likely to occur (Hepher, 1988; Russo and Thurston, 1991). Generally there was only nominal variability in the dissolved oxygen concentrations. However, the oxygen concentrations were highest in the higher flow-rate treatments and lowest in the lower flow-rate treatments. The general decline in the dissolved oxygen concentrations from around 6.7 to 6.2 mg/L on day 1 to 6.0 to 6.2 mg/L on day 5 is unusual, but may be explained by a build-up of organic matter (faeces) increasing the biological oxygen demand (BOD) of the water body, thereby reducing the oxygen levels in the water (Phillips et al., 1991).

The third trial, which focussed on different stocking densities (44, 66, 88, 110 and 132 kg/m^3) of 900 g fish with a comparison also made to conditions in cages in the lake (Figures 3.7, 3.8 and 3.9). As was generally anticipated the water ammonia concentrations were related to the stocking density, with higher stocking densities producing higher water ammonia concentrations (Russo and Thurston, 1991). The variability in ammonia concentrations over time is similar to that observed in the second study, which given that the fish were unfed whilst in the purging system, is likely to be a build-up of residual excretion from prior feeding and subsequent faecal loading (Phillips et al., 1991). Dissolved oxygen (DO) concentrations varied with the different stocking densities and were consistent with the biomass demand for oxygen (Glencross and Felsing, 2006). The variability in the DO concentrations show no consistent patterns and is most likely the result of inherent variability in the analysis. The high dissolved carbon dioxide (CO_2) concentrations at the commencement of the study are indicative of a high-energy expenditure of the fish post-handling. It is well known that fish, when stressed will increase their energy utilisation and as a consequence oxygen consumption will increase and carbon dioxide excretion also increase (Schreck and Li. 1991). Consistent with the data obtained for the oxygen consumption, with increasing stocking density, there was generally a corresponding increase in CO_2 concentrations. With the exception of the highest stocking densities, the CO_2 levels were always below the recommended thresholds for fish production and were always well below levels where fish kills are known to have occurred (Schrek and Li, 1991).

3.4.4 Reducing flavour taint in barramundi - Conclusions

The optimal purging regimes required will be a balance between the volume of MIB and GSM "free" water necessary to purge fish effectively, the time period over which the fish can be kept in a confined water volume prior to harvest and the volume and flow of water necessary to hold barramundi without impacting on fish health. All these parameters are also dependent on fish size and water temperature (Glencross and Felsing, 2006). However, at Lake Argyle the key issue was the ability to obtain adequate MIB and GSM free water as this step was likely to be key economic consideration to the cost of production.

Based on the findings in this study there would be some value in examining the effect of different MIB and GSM concentrations on the uptake rate and also the effect it has on the accumulation and subsequent depuration process. It is suspected, that based on the observation of the process being a likely first-order rate kinetic process, that at higher concentrations the uptake will be quicker and therefore over the same time period accumulated at greater concentrations in the fish flesh. This is likely to increase the time taken to depurate the fish when returned to MIB and GSM free water, but remains to be validated.

One factor constraining the present project is the lack of a reliable and validated test for MIB and GSM in fish flesh. Enquiries were made to the Australian Water Quality Centre and other analytical chemistry groups throughout Australia about the possibility of undertaking analyses for MIB and GSM. However, no lab approached had a reliable, validated method. This was, primarily due to the highly volatile characteristics of the compounds, which make them difficult to accurately extract from tissue samples. While methods have been used overseas for testing MIB and GSM in fish flesh, relatively low and variable recoveries have been reported (Lovell et al., 1985; Grimm et al., 2004; Robertson et al., 2004). Several international laboratories were also approached without success. A recent response from the Chemistry Centre of WA has indicated that the test may be developed for in the order of $20,000, but with no guarantee of the likely recoveries from any assay developed. However the contracting for the development of an assay was not within the scope of the project budget provided. As a result, increased reliance was placed on use of subjective taste testing panels (Centre for Food Technology – Brisbane / Department of Fisheries - Perth).

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3.6 Tables and Figures

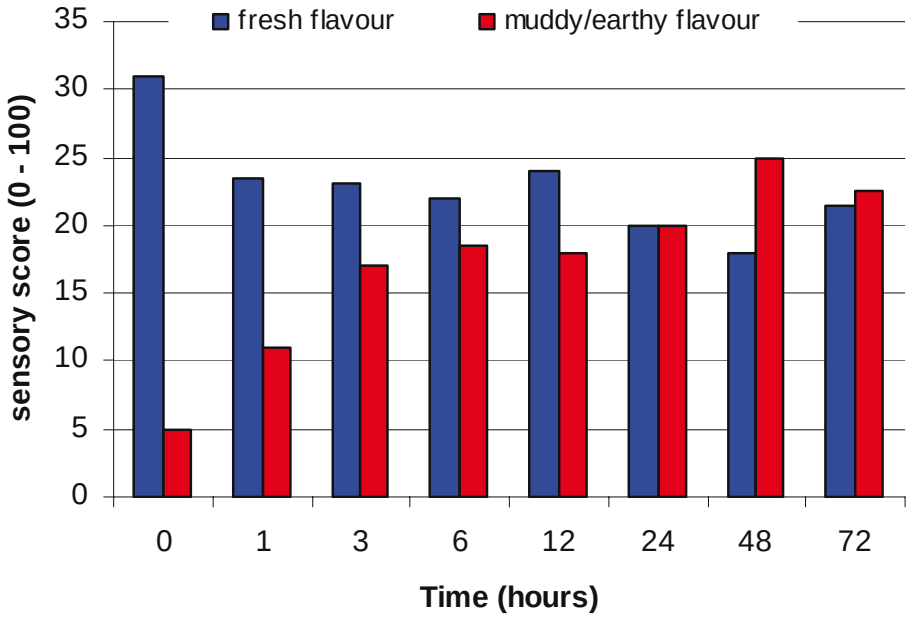

Figure 3.1 Average sensory scores for *fresh flavour* and *muddy / earthy flavour* for each time point in the uptake trial. Attribute scores are calculated as the mean value for each time point (mean of 10 judges and 2 replicates).

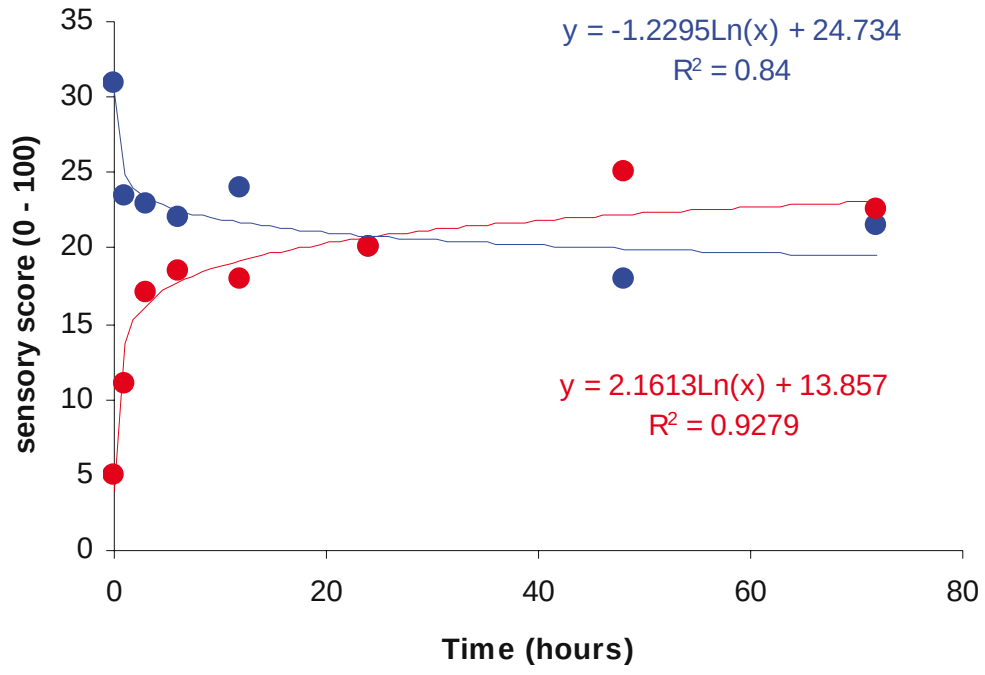

Figure 3.2 Regression relationships for *fresh flavour* and *muddy / earthy flavour* over the time period studied in the uptake trial. Attribute scores were calculated as the mean value for each time point (mean of 10 judges and 2 replicates). A maximum sensory score for muddy/earthy flavour accumulation (red) of ~23 was determined. A minimum sensory score for fresh flavour (blue) of ~18 was determined. Exposure time to reach half the final values in each case was around 1 to 2 hours. This figure is a reinterpretation of Figure 3.1.

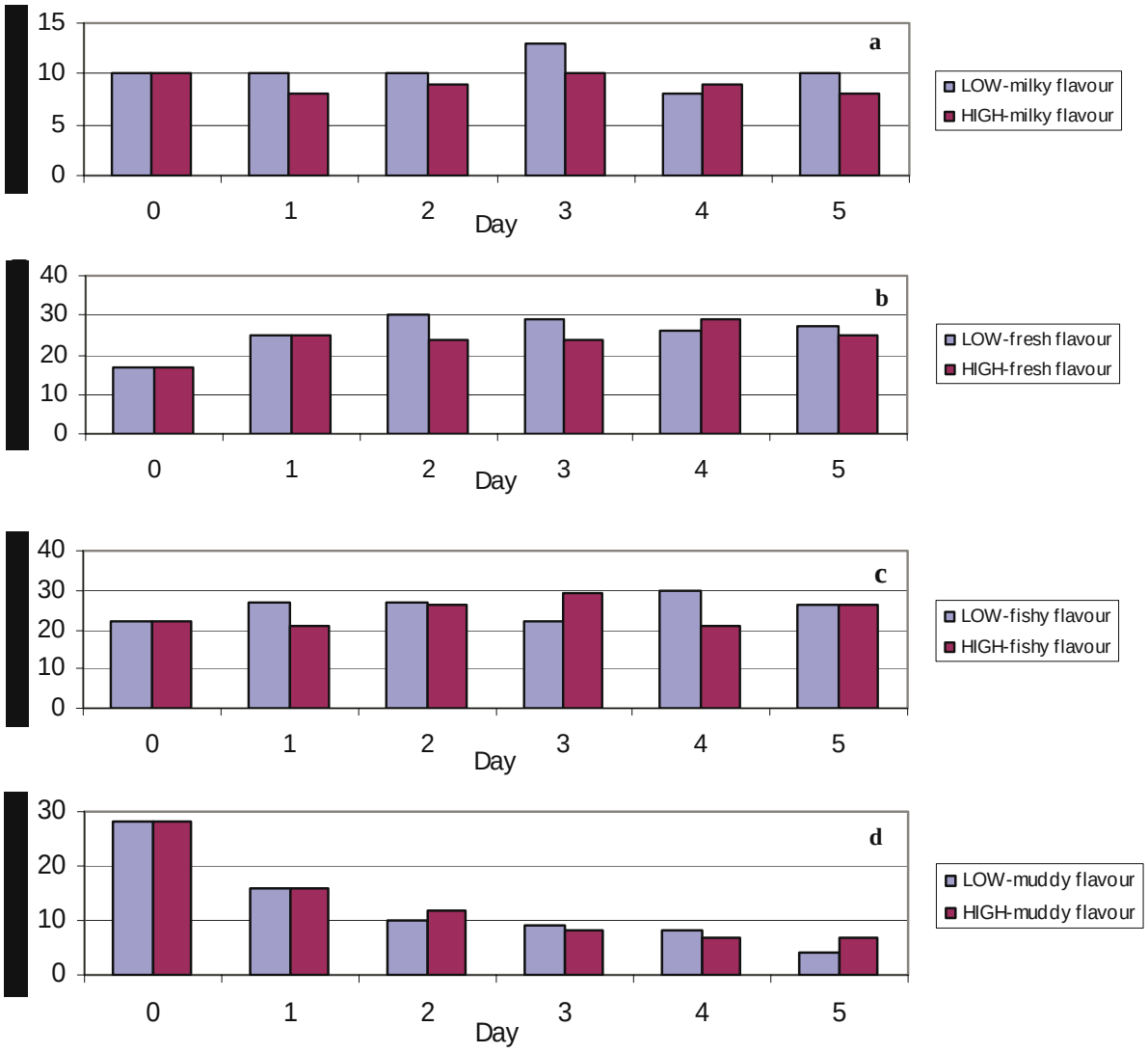

Figure 3.3a, b, c, d. Effect of purging at low (LOW: 8 L/min) or high (HIGH: 16 L/min) flow rates on milky, fresh, fish and muddy/earthy flavours in barramundi flesh as determined by a trained sensory panel.

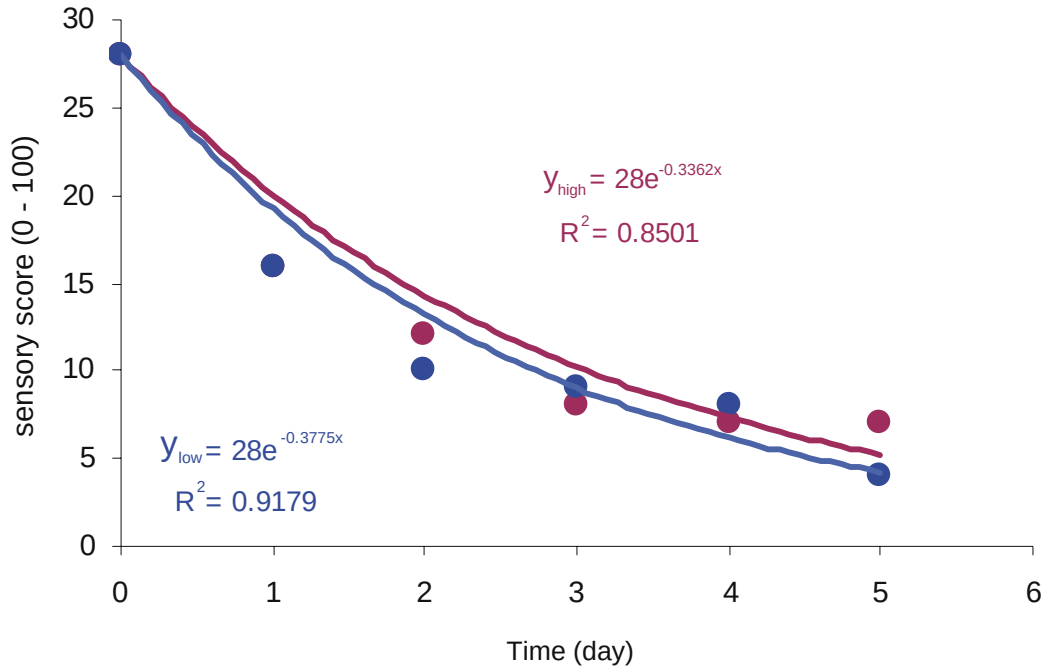

Figure 3.4 Exponential clearance functions for the decrease in muddy-flavour taint in barramundi over time at either low (8 L/min: blue) or high (16 L/min: purple) flow rates. Flow rate had no effect on the rate of clearance as evidenced by the similar exponents for both exponential equations. Half life of muddy taint clearance was just under 2 days.

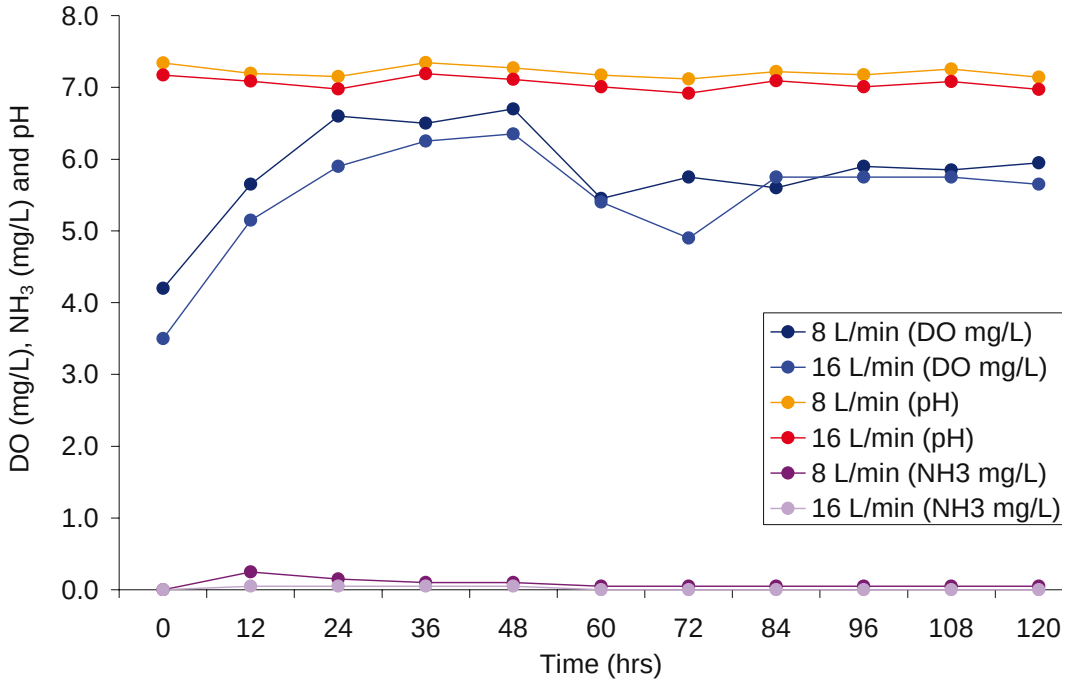

Figure 3.5 Dissolved oxygen (mg/L), total NH_3 (mg/L) and pH over the 5 days of the purging trial.

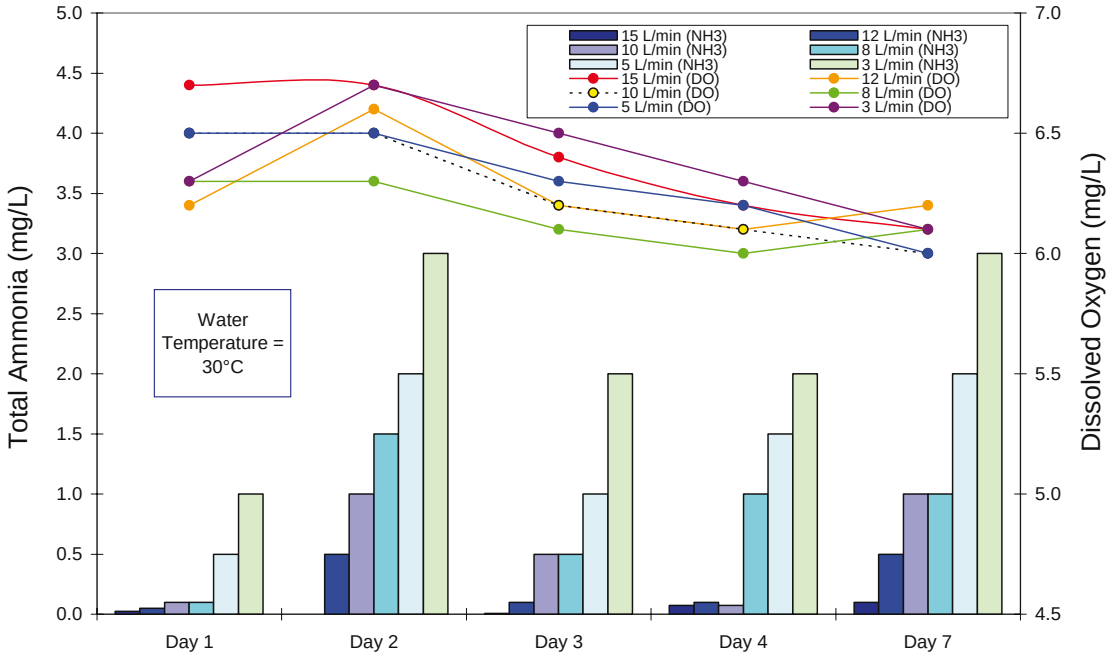

Figure 3.6 Total ammonia and DO levels with different flow rates in 2000L tanks holding barramundi (av. wt. = 730 g) at 30 kg/m^3.

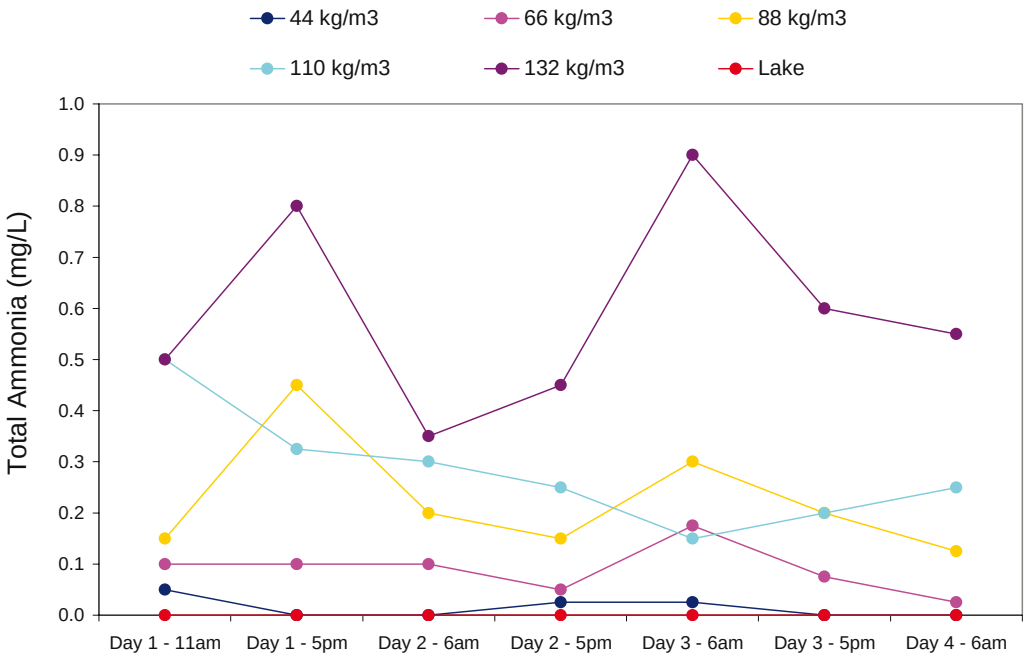

Figure 3.7 Total ammonia levels (mg/L) in tanks holding barramundi (av. wt. = 900 g) in 500L of water at five different stocking densities (Flow rate = 15 L/min).

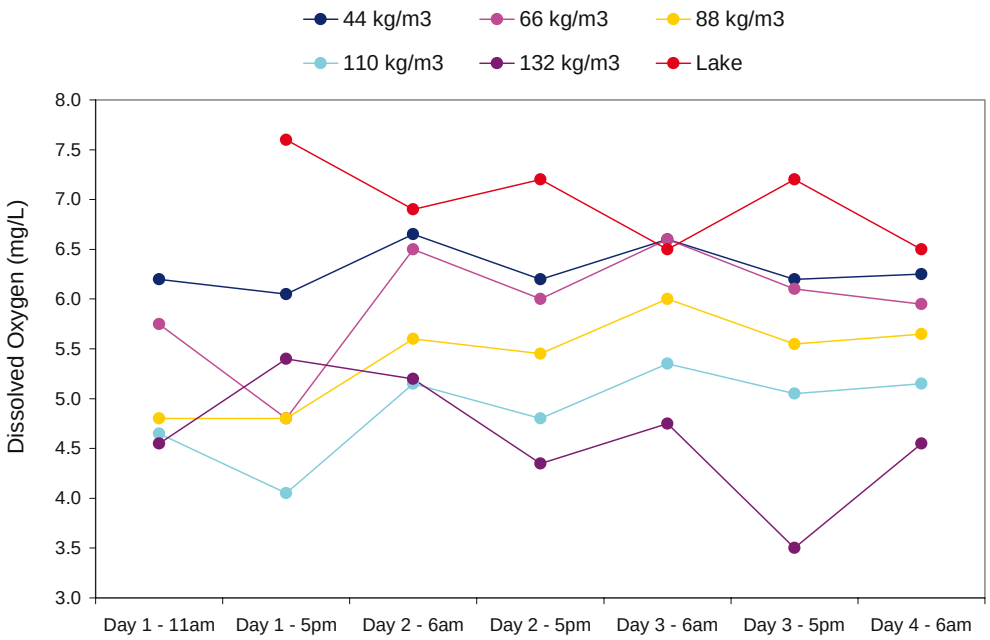

Figure 3.8 Dissolved oxygen levels (mg/L) in tanks holding barramundi (av. wt. = 900 g) in 500L of water at five different stocking densities (Flow rate = 15 L/min).

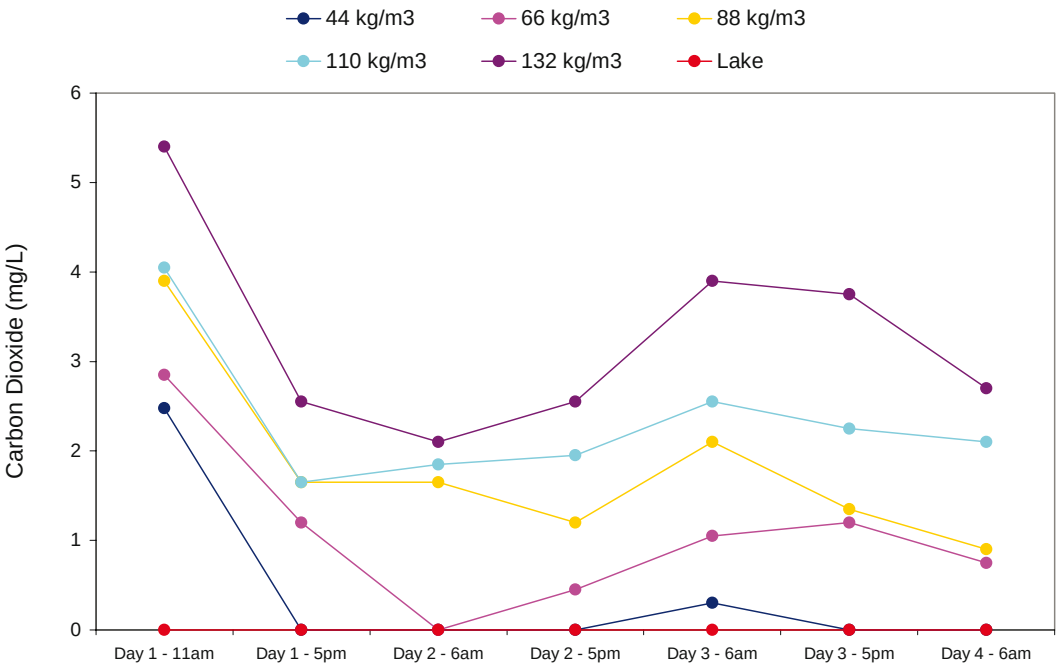

Figure 3.9 Carbon dioxide (mg/L) levels in tanks holding barramundi (av. wt. = 900 g) in 500L of water at five different stocking densities (Flow rate = 15 L/min).

4.0 Producing water suitable for depuration at Lake Argyle

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4.1 Introduction

Earlier work identified that placing barramundi with flavour taint in water free of 2-methylisoborneol and geosmin resulted in an improved sensory profile, including a reduction in muddy and earthy flavour taints. The earlier work in this report also demonstrated that this change was rapid, with uptake of taint occurring within a few hours of immersion in the geosmin / 2-methylisoborneol contaminated water. Purging of muddy/earthy taint from the fish took somewhat longer with no significant improvements with longer than 3 days purging, but significant improvements with each day up until day 3.

To achieve this purging effect, water that is free from geosmin and 2-methylisoborneol is required. At Lake Argyle several likely sources of geosmin and 2-methylisoborneol were identified and these are reported on in this chapter. Geosmin and 2-methylisoborneol are terpenoid metabolites produced by cyanobacteria and micro algae (phytoplankton) present in freshwater systems (Lovell, 1983; Armstrong et al., 1986; Bett, 1997; Robin et al., 2006). There are numerous species of cyanobacteria and micro algae that are known geosmin (GSM) and 2-methylisoborneol (MIB) producers (Izguirre et al., 1982; Dionigi and Ingram, 1994; Robin et al., 2006). The removal of cyanobacteria and microalgae were seen as obvious ways to potentially reduce the problem, consistent with what has been used in other industries (Blevins et al, 1995; Bruce et al., 2002; Howgate, 2004; Jung et al., 2004). The use of algicides and flocculants has been reported as options in controlling cyanobacteria and microalgae causing GSM and MIB problems in other situations (Dionigi et al., 1991; Dionigi, 1995; Schrader et al., 2005). Other possibilities also include the removal of GSM and MIB directly using either oxidation or filtration (Lawton et al. 2003; Elhadi et al., 2004; Howgate, 2004; Jung et al., 2004; Klausen et al., 2004; Kelly et al., 2006).

An improved understanding of the variability in environmental conditions present in the lake, principally the rainfall/inflow of water into the lake was seen as one aspect to examine and how this related to the variability in GSM and MIB in the water. The other key environmental parameter relating to the concentrations of GSM and MIB in the water is the diversity and abundance of phytoplankton and cyanobacteria throughout the lake.

A series of simple studies designed to characterise some of the key environmental parameters influencing the levels of GSM and MIB in the water were undertaken. In addition to this several potential water treatment options were examined for depurating the water from Lake Argyle for use in purging systems. Key issues examined in this chapter include:

- What species of phytoplankton and cyanobacteria are present in the Lake Argyle system?
- Can the levels of GSM and MIB in the water be reduced using chemical additives?
- Can the levels of GSM and MIB in the water be reduced using aeration?

4.2 Materials and Methods

4.2.1 Assessment of variability in geosmin and 2-methylisoborneol levels

Assessment of the variability in geosmin (GSM) and 2-methylisoborneol (MIB) levels in the Lake Argyle were undertaken by both direct assessment of water samples and also the assessment of the presence of phytoplankton and cyanobacteria known to produce these compounds.

Samples were collected at the surface, 15 metres depth and approximately one metre from the bottom at a number of locations in the northern Lake Argyle basin Water samples were collected weekly, by hand for surface samples and by Niskin-bottle for sub-surface samples, from early May 2004 to early January 2005. These water samples were prepared by chilling (4°C) and sent to Dalcon Environmental (Belmont, WA, Australia) for determination of total phytoplankton and total cyanobacteria counts. Species within the different phytoplankton classes *Dinophycae, Prasinophycae, Bacillariophycae, Chlorophycae, Chrystophycae, Cryptophycae* and *Euglenophycae* were identified. Species within the cyanobacteria counts were also identified.

Water samples were collected monthly from May 2004 to April 2005 using the method described earlier. Samples were collected at the surface, 15 metres depth and approximately one metre from the bottom at a number of locations in the northern Lake Argyle basin. These samples were prepared by chilling (4°C) and sent to the Australian Water Quality Centre (AWQC) for analysis of GSM and MIB.

GSM and MIB concentrations in each water sample provided to the AWQC were determined based on Method 6040B contained in Standard Methods for the Examination of Water and Wastewater, 19th Edition with in-house modifications. The process involves pre-concentrating a 1L sample by Closed-Loop-Stripping analysis. MIB and GSM are removed from the water by a recirculating stream of air and adsorbed onto a carbon filter from which they are then extracted using dichloromethane. The extract is then quantitatively analysed by gas chromatography/ mass spectrometry using Selected Ion Monitoring.

4.2.2 Analysis of rainfall and lake inflow data

Data on the ambient rainfall received at Argyle meteorological station (16.64 S, 128.45 E) were obtained from the Bureau of Meteorology (www.bom.gov.au). The cumulative monthly rainfall at this site was obtained as it is not only one of the closest meteorological stations to the farm site, but is also central to the main catchments of the lake and therefore provides a good indication of likely lake inflow volumes on a relative basis. These data were collated and presented against the maximum and minimum rainfall data available for each month to provide additional perspective.

4.2.3 Pilot testing with various treatments - Trial 1

Four liners of 2 m x 2 m x 2 m and two of 6 m x 5 m x 2 m dimensions were manufactured from PVC and fixed to a cage structure at Lake Argyle near the main fish production site in Coolibah Pocket. Lake water was pumped into each liner and various treatments designed to reduce the GSM and MIB levels were then applied to the liners (Table 4.3, Figure 4.5 and 4.6). Unreplicated treatments in the small liners (2m x 2m x 2m) included aeration of the water using a diffuser aeration system, the addition of a flocculant (polyaluminium chloride : PAC) at 16 mL/m3, aeration (using a diffuser) with addition of algicide (cupricide and coptrol) added at

10 mL/m^3 and water left static as a control. Algicide and flocculant concentrations were based on manufacturers recommendations. The two large liners (6m x 5m x 2m) were aerated with a diffuser or a diffuser plus a mushroom sprayer. Water samples were collected from each liner daily for five days, chilled and sent the Australian Water Quality Centre for analysis of geosmin and 2-methylisoborneol. GSM and MIB levels were analysed as detailed above in section 4.2.1.

4.2.4 Testing of aeration and algaecides on geosmin and 2-methylisoborneol levels - Trial 2

Lake water was pumped into each of two 6 m x 5 m x 2 m liners. One liner was aerated with a diffusion aeration system while in the other liner algicide was added at 10 mL/m^3 in addition to aeration (Figure 4.7). This trial tested the effectiveness of the two most promising methods from Trial 1 in reducing GSM and MIB in larger volumes of water. Water samples were collected from each liner daily for five-days, chilled and sent the Australian Water Quality Centre for analysis of GSM and MIB. GSM and MIB levels were analysed as detailed above in section 4.2.1.

4.2.5 Testing of aeration on geosmin and 2-methylisoborneol levels - Trial 3

Water was brought from Lake Argyle to the onshore hatchery facility at Lake Argyle Tourist Village. The lake water was transferred into 2000L tanks and either aerated for five days in the hatchery, kept for five days in the hatchery with no aeration, kept for five days in darkness or kept in the open (exposed to sunlight) for five days. Additional samples collected for the assessment included local bore water, local scheme supply (chlorinated) water and the initial lake water. Water samples were collected from each treatment after five-days, chilled and sent to the Australian Water Quality Centre for analysis of GSM and MIB. GSM and MIB levels were analysed as detailed above in section 4.2.1.

4.2.6 Testing of a flocculant on geosmin and 2-methylisoborneol levels - Trial 4

Lake water was pumped into each of two 6 m x 5 m x 2 m liners. To one liner polyaluminium chloride (PAC) was added at 16 mL/m^3 in addition to aeration, while to a second liner PAC was added at 80 mL/m^3 in addition to aeration (Figure 4.9). This trial tested the effectiveness of higher concentrations of PAC in reducing geosmin and 2-methylisoborneol. Water samples were collected from each liner after two and seven days, chilled and sent the Australian Water Quality Centre for analysis of GSM and MIB. GSM and MIB levels were analysed as detailed above in section 4.2.1.

4.2.7 Depuration time course of geosmin and 2-methylisoborneol - Trial 5

Using the large-scale liners an unreplicated study was undertaken to examine the time-course effect of aeration on MIB and GSM depuration. The liners were initially filled and aerated to produce MIB and GSM free water (Figure 4.10a). The depurated water was then spiked with MIB and GSM (Sigma Chemicals, Missouri, USA), and aeration applied to the water in the liners (Figure 4.10b). Water samples were collected from each liner daily for seven-days, chilled and sent the Australian Water Quality Centre for analysis of GSM and MIB. GSM and MIB levels were analysed as detailed above in section 4.2.1.

4.2.8 Development of a commercial scale purging system

Based on the outcomes of the different water treatment strategies examined in this chapter, and the purging parameters required to reduce the geosmin and 2-methylisoborneol content of the fish, a series of design criteria were given to an engineering contractor (SEMF Pty Ltd) to design a commercial scale purging system. The critical design criteria given included:

- Water source to be obtained from within the lake surrounding the production cages and depurated by aeration.
- Containment volume to hold and purge 10 tonne of fish per week (suggested volume of a 1000m^3 facility).
- Aeration of water for up to five days in duration, though three days likely to be generally sufficient.
- Containment period for up to five days in duration, though three days likely to be generally sufficient.
- That the purging system had to operate independent of mains supplied power.
- That the system had to minimise the handling and transport requirements for maintaining live fish.

The final report provided by the engineering firm is included as appendix 3.

4.3 Results

4.3.1 Variability in rainfall, phytoplankton, geosmin and 2-methylisoborneol

The rainfall that preceded the occurrence of the sensory problems encountered in Lake Argyle in late 2003, early 2004 was among the highest recorded for the region (Figure 4.1). These same high rainfall events also corresponded with the *Streptococcus iniae* outbreak that also occurred at Lake Argyle around the same period.

Over 110 species of phytoplankton, from 8 families were identified from water samples collected near the barramundi farm site. In addition, around 25 species of cyanobacteria were also reported. Of both the phytoplankton and cyanobacteria, about 48% and 40% respectively of all species identified are known GSM/MIB producers. Two of the cyanobacteria species are also known toxin producers (Table 4.1)

The total phytoplankton and cyanobacteria levels observed in samples collected at monitoring sites from May 2004 to December 2004 ranged from ~75,000 cells per mL, to less than 1000 cells/mL (Figure 4.2). For most of the year total cell counts were around the 10,000 cells/mL range. Of the total cells counted the clear majority were cyanobacteria, which was generally in excess of 70% of the total cell counts (Figure 4.2). The highest levels of phytoplankton and cyanobacteria were observed in May 2004 and the lowest in December 2004. Other peaks in total cell counts throughout the year occurred in June 2004, July 2004 and October 2004. None of these secondary peaks were of a similar scale to the single massive cell proliferation event recorded in May 2004 (Figure 4.2).

Some variability in total phytoplankton and cyanobacteria levels was observed with the depth at which the samples were collected (Figure 4.3). Total phytoplankton and cyanobacteria levels

were highest at the surface (~2000 cells/mL) and lowest (~500 cells/mL) near the bottom. Cyanobacteria dominated cell counts at all depths, but were only about 50% of the total cell counts in the 15 m and bottom samples.

GSM and MIB levels were measured in water samples collected monthly at a wide range of sites (Table 4.2), throughout the northern area of Lake Argyle from May 2004 to April 2005. Some spatial variability was observed throughout the lake, though variability was generally more pronounced as a function of sample depth than the location at which it was collected. A more intensive assessment was undertaken near the main barramundi production site (Figure 4.4). Levels of MIB were consistently higher than those of GSM throughout the survey period, ranging from 8 ng/L to 19 ng/L. Peaks in MIB greater than 12 ng/L were observed in May 2004, September 2004 and March 2005. Limited variation in GSM levels was observed throughout the study period.

4.3.2 *Chemical additives and aeration to reduce water geosmin and 2-methylisoborneol levels*

From the pilot study, the comparison of the six treatments (Table 4.3, Figures 4.5 and 4.6) showed that the use of a flocculant (Polyaluminiumchloride: PAC) and algicides (Coptrol and Cupricide) both had dramatic effects on the water MIB levels (Figure 4.6), but their effect on GSM was somewhat limited by the inherent low levels of GSM present at the time of the study (Figure 4.5). Levels of both GSM and MIB were reduced dramatically within 24 hrs, with further reductions after this initial period occurring more slowly. The addition of algicide resulted in lower levels of both GSM and MIB at all sampling points.

Aeration of the water (Figure 4.6 and 4.7) showed substantial decrease in MIB levels within one day and this was marginally improved with the use of an algicide (Figure 4.6 and 4.7). The use of a flocculant also improved MIB levels within one day, but this was not as effective as aeration alone. The control (Figure 4.6) also showed a slow decline in MIB levels over time. With the large-scale liner (5 m x 6 m x 2 m), the use of a diffuser and mushroom sprayer was more effective than a diffuser alone (Figure 4.6).

In a second study using the large-scale purging liner, the use of an algicide in conjunction with aeration compared to aeration alone, showed that there was no significant advantage obtained by the use of algicides over the reduction in MIB levels achieved through aeration. The reduction in GSM levels was greater and faster with the use of the algicide, but low levels of GSM in the water that were present at the start of the trial, close to the analytical threshold, may have clouded the assessment process (Figure 4.7).

Tank based studies using lake water brought to the indoor hatchery facility examined the effect of light (or the lack thereof) and aeration over a five-day period (Figure 4.8). A control treatment included a similar volume of water was placed outside, but with full aeration. After the five-days the control treatment had no measurable level of MIB remaining. After the same time period, the treatment kept indoors with no aeration had reduced MIB levels to about 55% of that from the initial lake water sample. By comparison, a tank kept in darkness for the five days had the same MIB levels as the treatment that was kept indoors with no aeration. A tank kept in sunlight for five days, but without aeration had slightly lower MIB levels, at about 35% that of the initial sample. For reference, the bore water obtained from the Lake Argyle Tourist Village had no measurable MIB, while the scheme/town water supply, which was also chlorinated, had MIB levels of about 5 ng/L, which was about half of that of the initial lake water samples.

Examination of the dosage effect of PAC showed that no reduction in MIB was achieved with increasing the PAC dosage from 16 mL/m^3 to 80 mL/m^3 (Figure 4.9). Over the time course of the study substantial reductions in water concentrations of MIB were observed after 24 hours and again after 168 hours for the 16 mL/m^3 dosage rate. A similar decrease in MIB was observed with the 80 mL/m^3 dosage rate after 24 hours as was observed with the 16 mL/m^3 dosage, but after 168 hours the reduction in MIB was greater with the 16 mL/m^3 dosage rate. Changes in GSM were difficult to assess objectively due to the low levels in the initial water sample.

Using the large-scale liners, a study was initiated to examine the time-course effect of aeration on MIB and GSM depuration in this large-scale system (Figure 4.10a). The liners were initially filled and aerated to produce MIB and GSM free water. The depurated water was then spiked with MIB and GSM and water samples collected every 24 hours for eight days. Initial measured spike levels of MIB and GSM were 90 and 50 ng/L respectively. An exponential decrease in both compounds was seen over the ensuing seven days. The exponential loss in GSM occurred at a faster rate than that of the MIB (Figure 4.10b).

4.4 Discussion

One of the key issues surrounding the development of commercial scale purging system is the ability to obtain adequate volumes of depurated water at a cost-effective rate. While in earlier experiments bore-water and town-scheme-treated water were both used, it was realised that for a commercial-scale purging system to be cost-effective, then some treatment of the lake water was probably the best option. This of course being dependent on the nature of the water treatment required.

4.4.1 Sources and variability of Geosmin and 2-Methylisoborneol in Lake Argyle

Over 110 species of phytoplankton, from 8 families were identified from water samples collected near the barramundi farm site. An additional 25 species of cyanobacteria were also identified. Almost half of the observed phytoplankton species identified are known GSM and/or MIB producers (Harris and Baxter, 1996). About 40% of the cyanobacteria species identified are known GSM and/or MIB producers. This suggests that the presence of these compounds in the Lake Argyle system is widely endemic and that broad scale water treatment is probably unviable. In addition two of the cyanobacteria species are known toxin producers.

The rainfall preceding the occurrence of the sensory problems encountered at Lake Argyle in late 2003, early 2004 were among the highest recorded for the region (Figure 4.1). This high rainfall event preceded a clear incidence of phytoplankton and cyanobacteria blooms that occurred a few months later (Figure 4.2). These blooms also corresponded with elevated levels of MIB, but no changes in the levels of GSM (Figure 4.4). This high MIB level in the water was also consistent with a period where fish harvest also had a distinct muddy-flavour and some negative market feedback was received by the company. The relationship to the rainfall event is based on one of an exacerbation of conditions predisposing the lake to algal blooms (Harris and Baxter, 1996). It is hypothesised that the high rainfall and subsequent inflow events dramatically increase the nutrient flux into the Lake Argyle system and as a consequence the blooms resulted. In light of this, future farm operations should be mindful of the potential impacts of heavy rainfall events and the consequences they may bring. In this case, there appeared to be a delay in bloom and muddy-taint by a period of two to three months. This delay is suggestive of the time course taken for inflow from some distance

upstream in the catchment before it reaches the embayment within Lake Argyle in which barramundi farming is based.

The monthly collection of water samples throughout the northern region of Lake Argyle showed that there was some spatial variability throughout the lake, though variability was generally more pronounced as a function of sample depth than location at which it was collected (Table 4.2). The more intensive assessment undertaken near the main barramundi production site showed that concentrations of MIB were consistently higher than those of GSM throughout the survey period, ranging from 8 ng/L to 19 ng/L (Figure 4.4). Peaks in MIB greater than 12 ng/L were observed in May 2004, September 2004 and March 2005. Limited variation in GSM levels was observed throughout the study period. These observations are consistent with data from earlier studies in this report that indicate that MIB is more influential in causing the muddy-flavour problem with barramundi in Lake Argyle.

4.4.2 Using chemical additives to reduce Geosmin and 2-methylisoborneol

Algicides have been reported to reduce the productivity of certain geosmin and 2-methylisoborneol producing phytoplankton in other studies (Dionigi, 1995; Schrader et al., 1998; Schrader et al. 2004; Schrader et al., 2005). Some of these compounds are reputed to have selective toxicity to cyanobacteria based on the use of copper sulphate compounds (Schrader et al., 1998; Schrader et al., 2005).

Floating liners were also used to compare the effectiveness of aeration, flocculants and algaecides (Cupricide and Coptrol – both registered for use in potable water sources) in reducing 2-methylisoborneol/geosmin levels in larger volumes of lake water. However, despite PAC, Cupricide and Coptrol being registered for use in potable water sources, methods that effectively reduce 2-methylisoborneol and geosmin levels without the use of chemicals will be preferable for both product assurance and economic reasons.

The studies in this chapter also investigated the effectiveness of a flocculating agent to reduce the level of MIB and GSM in lake water. A flocculant works by causing the aggregation of certain particles within a solution, facilitated by an aqueous cation solution (e.g. magnesium, aluminium, iron or calcium) to enable their removal by either settlement or faster filtration. In this study a flocculant was used to attempt the removal of the phytoplankton and cyanobacteria that produce the MIB and GSM in the lake water. Polyaluminium chloride (Al_2O_3) (PAC) is one such chemical and it was used as it is registered for use in potable water sources where it causes aggregation of the phytoplankton to allow for its easier removal. An initial study examined the comparative impact of PAC over a five-day period against other treatment options (Figure 4.5 and 4.6). In this study the PAC was added at 16 mL/m^3. At this level of PAC addition to the water the least impact on MIB was observed of all the proactive treatments, with only the water left static not reducing its MIB levels faster (Figure 4.6). A second study tested the effectiveness of a higher concentration of PAC to reduce the levels of MIB and GSM in lake water samples (Figure 4.9). Following the addition of the PAC, the water was aerated for 2 hours to promote maximum contact between the flocculant and suspended particles. The results showed that addition of PAC at 80 mL/m^3 did not provide any advantage over addition of PAC at 16 mL/m^3. As with other studies in this report, GSM levels were generally too low to provide an objective assessment of the effects of the treatments. From these studies it can be concluded that there is little point in the use of PAC to reduce the levels of MIB in the lake water for depuration.

The pilot comparison of six treatments (Table 4.3, Figures 4.5 and 4.6) showed that the use of aeration was the most effective strategy in depurating lake water, but that the use of algaecides (Coptrol and Cupricide) did provide some further reduction in the levels of MIB. The algaecides both had significant effects on water MIB levels (Figure 4.6), but their effect on geosmin was somewhat limited by the inherent low levels of GSM present at the time of the study (Figure 4.5). Levels of MIB were reduced most substantially within the first 24 hrs, with further reductions occurring more slowly. The addition of algaecide resulted in lower levels of both compounds at all sampling points, but the benefits of using it in addition to aeration require careful consideration. This was further verified in a second study using the large-scale purging liner, where the use of an algaecide in conjunction with aeration, compared to aeration alone showed that there was no advantage obtained in the reduction of MIB levels (Figure 4.7).

4.4.3 *Using aeration to reduce Geosmin and 2-methylisoborneol*

The results from the studies examining the use of flocculants and algicides showed that while they did in some cases assist the process of reducing water MIB concentrations, that the same effect could generally be achieved through aeration of the water alone. Therefore, based on the findings of the present study, it is suggested that using persistent aeration of the water for 24 hours or longer is the preferred option to depurate the water for use in purging of barramundi in Lake Argyle.

The initial tank based studies using lake water brought to the indoor hatchery facility showed that the presence or absence of light had little impact on the MIB levels in the lake water. Only aeration over the five-day period had any appreciable effect on the MIB levels, with it being undetectable in the water after five days of aeration (Figure 4.8). The bore water obtained from the Lake Argyle Tourist Village had no measurable MIB, while interestingly the scheme/town water supply, which was chlorinated, had MIB levels of about 5 ng/L, which was about 50% of that of the initial lake water samples. The water for the town water supply is obtained directly from the lake prior to chlorination. That measurable levels of MIB were present in the town water supply shows that simple filtration and chlorination methods used in water treatment do not necessarily remove MIB from the water.

The aeration studies also showed that use of both a diffusion and mushroom sprayer systems resulted in a faster decrease in MIB than use of just a diffusion system alone (Figure 4.6). However it remains to be examined if this effect was actually an effect of the mushroom sprayer per se or just a higher level of aeration of the water allowing a greater rate of depuration.

The time-course effect of aeration on MIB and GSM depuration in the large-scale liner systems showed an interesting difference between the depuration rates of the two compounds (Figure 4.10a). There was an exponential decrease in both compounds over the ensuing monitoring period, with the half-life of GSM being substantially shorter than that of the MIB, with the former having a half-life of around 24 hours and the later closer to 36 hours. By 96 hours both compounds were below 10 ng/L and by 144 hours essentially non-existent. However, it should be noted that the spiked levels of both compounds added to the water at 0 hours was considerably higher than any of the levels measured in the survey component of this work.

4.4.4 Designing a commercial-scale purging system

The system designed by the consulting engineers (SEMP Pty Ltd) had further specific operating procedures detailed. In the proposed operating procedure the fish are to be purged for two days in a two-stage process. It was suggested that the quality of water required for the first purge didn't need to be as good as that required in the second purge. Allowing for a 5-hour turnover, a total of three 1,000,000 L stores of depurated water will be required for each of the two-day purging processes. The actual purging processes are to be conducted within three smaller 100,000 L submersible liners. Based on a one-week production operation, the proposed procedure is:

a. Day 1, 8am – Commence filling three 1 ML storages.

b. Day 2, 8am – Continue filling. Commence aeration of all three water storages.

c. Day 3, 8am – Dose water storages with an algaecide if required. Continue aeration.

d. Day 4, 8am – Continue aeration.

e. Day 5, 8am – Continue aeration. Prepare purge liners 1, 2 and 3 and begin in-liner aeration. Transfer graded fish (for example plate-size, banquet and fillet) into each of three purge liners.

f. Day 6, 8am – Commence purging fish within the purge liners.

g. Day 7, 8am – Continue purging fish in purge liners with water form storage 2 and 3, commence refill of storage 1 and start water depuration (aeration) again.

h. Day 8, 8am – Harvest fish from the purge liners, refill water storage 2 and 3 and commence aeration (repeat cycle using shortest aerated storage first for purging the purge liner. This means that the last storage used will have received maximum aeration.

As can be seen from the suggested operating procedure a seven-day cycle can be implemented to enable a weekly rotating harvest system to be used. A range of system options were proposed in the engineering report (see appendix 3) that considered the more detailed design, system operations and constraints and provisional costings for system construction. A design layout of the water depuration system is also included (Figure 4.11).

Such a depuration system is consistent with the operations used by the channel catfish industry in the USA (Lovell, 1983; Lovell et al., 1985; Zimba and Grimm, 2003). However, despite the implementation of a purging/depuration system for fish quality management it is still important to consider the use of routine sensory evaluation of fish before and after purging (Howgate, 2004; Robertson et al., 2005; 2006). With barramundi, a prudent strategy would be to:

- Have an ongoing assessment system for MIB and GSM in the lake water.
- To harvest larger barramundi prior to purging and undertake taste-testing of its belly-region meat for muddy-taint. This may provide some indication of the severity of the problem at a given point in time.
- Irrespective of sensory outcome, purging should be considered mandatory to a\ensure quality control.
- Following purging, again harvest a larger barramundi and undertake taste-testing of its belly-region meat for muddy-taint. If any incidence of muddy taint is detected then further purging is required.

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4.6 Tables and Figures

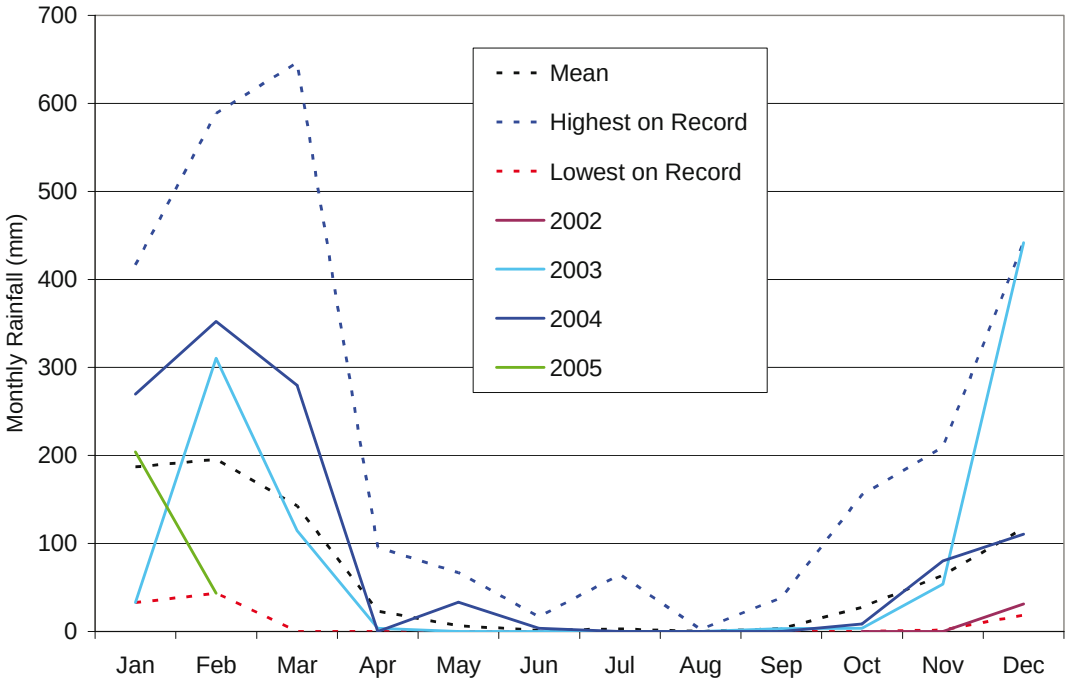

Figure 4.1 Variation in rainfall at Argyle meteorological station.

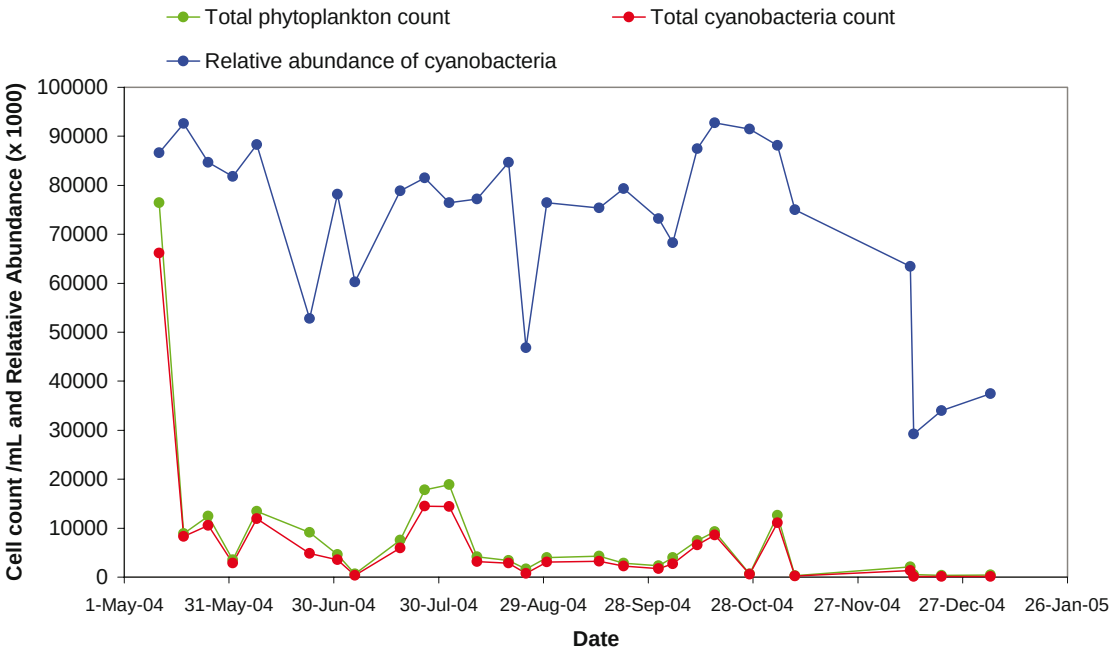

Figure 4.2 Variability in total phytoplankton and cyanobacteria counts from May 2004 to January 2005.

Table 4.1 Classes and species of phytoplankton and cyanobacteria found at Lake Argyle. Indicated in yellow are known 2-methylisoborneol and/or geosmin producers and in orange are known toxin producers.

Bacillariophyceae	Chlorophyceae	Chlorophyceae cont…	Chrysophyceae	Cryptophyceae
Achnanthes brevipes	*Ankistrodesmus spiralis*	*Pediastrum simplex*	*Chrysophyte 039*	*Chroomonas sp. 001*
Achnanthidium minutissma	*Botryococcus sp.*	*Pediastrum spp.*	*Mallomonas sp. 002*	*Cryptomonas sp.*
Amphora sp.	*Chlamydomonas sp. 001*	*Pediastrum sp. 006*	*Mallomonas sp. 003*	*Cryptomonas sp. 001*
Amphora sp. 007	*Chlamydomonas sp. 002*	*Pediastrum sp. 011*		*Cryptomonas sp. 004*
Amphora ovalis	*Chodatella sp. 001*	*Pediastrum sp. 012*		*Cryptomonas sp. 009*
Aulacosira granulata	*Cladophora sp.*	*Pediastrum sp. 013*		*Cryptomonas sp. 016*
Aulacosira sp.	*Closterium sp. 005*	*Pediastrum sp. 014*		
Aulacosira sp. 002	*Coelastrum sp. 001*	*Scenedesmus sp. 001*		
Aulacosira sp. 004	*Coelastrum sp. 005*	*Scenedesmus sp. 002*		
Cocscinodiscus sp.	*Coelastrum sp. 006*	*Sphaerocystis sp. 001*		
Cyclotella sp. 003	*Cosmarium sp. 001*	*Sphaerocystis sp. 002*		
Cyclotella spp.	*Cosmarium sp. 010*	*Staurastrum spp*		
Cylindrotheca closterium	*Cosmarium sp. 017*	*Staurastrum sp. 001*		
Cymbella spp.	*Cosmarium sp. 019*	*Staurastrum sp. 002*		
Diatom 003	*Cosmarium sp. 020*	*Staurastrum sp. 005*		
Diatom 022	*Cosmarium sp. 024*	*Staurastrum sp. 009*		
Gomphonema sp.	*Dictyosphaerium sp. 002*	*Staurastrum sp. 013*		
Mastogloia ellliptica	*Dictyosphaerium sp. 003*	*Staurastrum sp. 015*		
Navicula spp.	*Dictyosphaerium sp. 004*	*Staurastrum sp. 017*		
Nitzschia spp.	*Dispora sp. 001*	*Staurastrum sp. 021*		
Nitzschia sp. 020	*Elakatothrix sp. 001*	*Staurastrum sp. 022*		
Synedra crystallina	*Elakatothrix sp. 002*	*Staurastrum sp. 024*		
Synedra sp.	*Hyalotheca sp. 001*	*Staurastrum sp. 029*		
Synedra sp. 003	*Kirchneriella sp. 002*	*Staurastrum sp. 043*		
Urosolenia sp. 002	*Micractinium sp. 004*	*Staurastrum sp. 045*		
Urosolenia sp. 004	*Nephrocytium sp. 001*	*Tetraedriella sp. 001*		
Urosolenia sp. 005	*Oocystis sp. 002*	*Tetraedron minimum*		
	Oocystis sp. 005	*Tetraedron sp. 011*		
	Oocystis sp. 006	*Tetrallantos sp. 001*		
	Oocystis sp. 008			
	Oocystis sp. 009			

Dinophyceae	Prasinophyceae	Euglenphyceae	Cyanobacteria	Unidentified
Dinoflagellate 004	*Prasinophyte 002*	*Euglena sp. 025*	*Anabaena sp. 001*	*Unknown 060*
Dinoflagellate 005	*Prasinophyte 006*	*Euglena sp. 019*	*Anabaena bergii var. limnetica*	
Dinoflagellate 009		*Euglena sp. 027*	*Aphanocapsa sp. 001*	
Dinoflagellate 014		*Trachelomonas sp. 006*	*Aphanocapsa sp. 002*	
Glenodinium sp. 002			*Aphanothece sp. 002*	
Glenodinium sp. 003			*Chroococcus sp. 001*	
Glenodinium sp. 004			*Chroococcus sp. 007*	
Peridinium sp. 001			*Cyanodictyon planktonicum*	
Peridinium sp. 002			*Cylindrospermopsis raciborskii*	
Peridinium sp. 005			*Dactylococcopsis sp. 002*	
Peridinium sp. 006			*Lyngbya sp. 001*	
Peridinium sp. 007			*Merismopedia sp. 001*	
			Merismopedia sp. 003	
			Merismopedia sp. 006	
			Microcystis aeruginosa	
			Microcystis wesenbergii	
			Oscillatoria sp.	
			Planktolyngbya contorta	
			Planktolyngbya subtilis	
			Phormidium sp.	
			Pseudanabaena sp.	
			Pseadanabaena sp. 001	
			Pseudanabaena sp. 002	
			Synechocystis sp. 001	
			Synechocystis sp. 002	

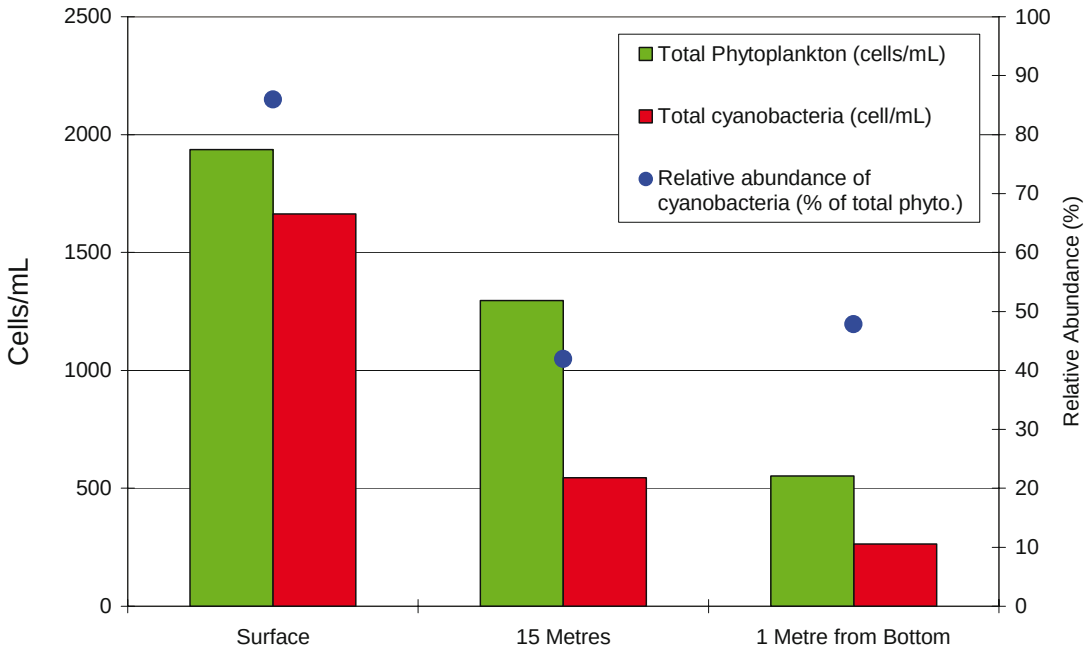

Figure 4.3 Variability in total phytoplankton and cyanobacteria counts at various depths in Lake Argyle.

Table 4.2 Variability in geosmin and 2-methylisoborneol levels throughout the northern part of Lake Argyle.

Sample Site	17th May 04		17th Oct 04		13th Jan 05		31st Jan 05		9th Feb 05		11th Mar 05		8th Apr 05	
	MIB	Geosmin	MIB	Geosmin	MIB	Geosmin	MIB	Geosmin	MIB	Geosmin	MIB	Geosmin	MIB	Geosmin
Central Coolibah Pocket[a] (surface)	-	-	8	1	8	1	7	1	7	1	11	1	14	1
Central Coolibah Pocket (15 M)	-	-	9	1	-	-	-	-	-	-	-	-	-	-
Central Coolibah Pocket (1 M from bottom)	-	-	<1	<1	4	1	6	1	2	1	5	2	2	< 1
Central Coolibah Pocket (disturbed sediment)	-	-	<1	<1	-	-	4	1	-	-	-	-	-	-
Fish cages site[b] (surface)	-	-	-	-	7	2	7	1	10	1	11	3	-	-
Fish cages site (1 M from bottom)	-	-	-	-	14	<1	10	<1	12	1	14	<1	-	-
Fish cages site (disturbed sediment)	-	-	-	-	-	-	8	1	-	-	-	-	-	-
Lake Argyle Spillway[c] (surface)	19	3	5	3	-	-	-	-	-	-	-	-	-	-
Ord River[d] (surface)	-	-	3	1	-	-	-	-	-	-	-	-	-	-
Harvest site[e] (surface)	-	-	-	-	-	-	-	-	-	-	11	2	13	3
Harvest site (insde cage)	-	-	-	-	-	-	-	-	-	-	-	-	14	8
Main Lake[f] (surface)	-	-	-	-	-	-	-	-	-	-	-	-	14	< 1
Main Lake (1 M from bottom)	-	-	-	-	-	-	-	-	-	-	-	-	3	< 1
Pelicans Bay[g] (surface)	-	-	-	-	-	-	-	-	-	-	-	-	13	< 1
Pelicans Bay (1 M from bottom)	-	-	-	-	-	-	-	-	-	-	-	-	2	< 1
Main Lake Channel[h] (surface)	-	-	-	-	-	-	-	-	-	-	-	-	13	< 1
Main Lake Channel (1 M from bottom)	-	-	-	-	-	-	-	-	-	-	-	-	2	< 1

a Routine site remote from fish cages (approx. 300 M)
b Western Coolibah Pocket
c Overflow point for Lake Argyle (approx. 6 km from fish cages)
d Ord River immediately below the dam wall
e Site in Bamboo Cove (northern Coolibah Pocket) with a few stocked cages for harvest
f Middle of Lake Argyle far removed from fish cages, but out of main channel
g Eastern end of Lake Argyle far removed from fish cages, and out of main channel
h Middle of Lake Argyle far removed from fish cages, directly in main channel

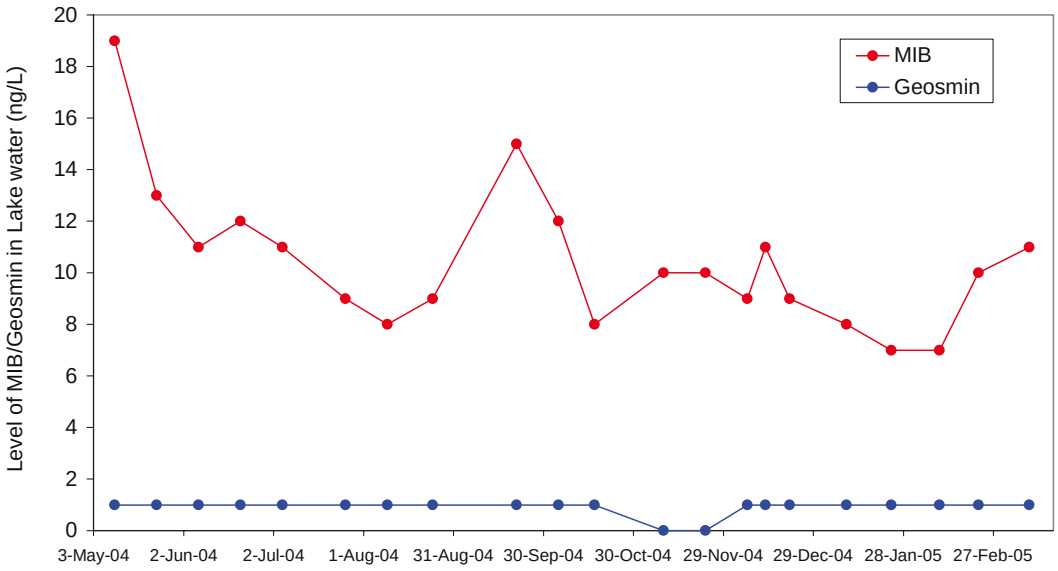

Figure 4.4 Variability in geosmin and 2-methylisoborneol (MIB) levels from May 2004 to March 2005.

Table 4.3 Water treatment descriptions for pilot testing – Trial 1.

Treatments
Liner 1 - Aeration (diffuser) only in 2m x 2m liner
Liner 2 - Flocculant in 2m x 2m liner
Liner 3 - Aeration (diffuser) + algicide in 2m x 2m liner
Liner 4 - static (no treatment) in 2m x 2m liner
Liner 5 - Aeration (diffuser) only in 6m x 5m liner
Liner 6 - Aeration (diffuser and mushroom sprayer) only in 6m x 5m liner

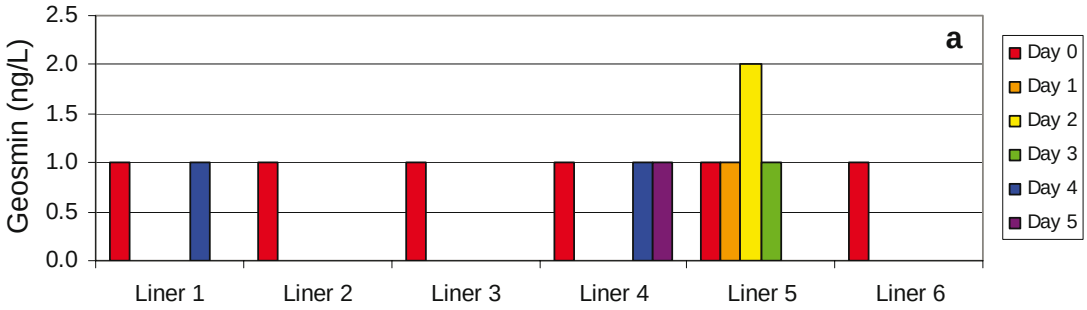

Figure 4.5 Concentrations of geosmin over a 5-day period in each of the treatments.

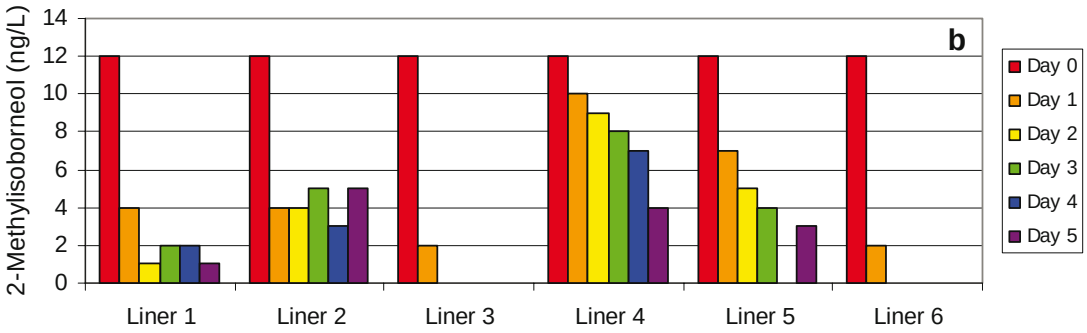

Figure 4.6 Concentrations 2-methylisoborneol over a 5-day period in each of the treatments.

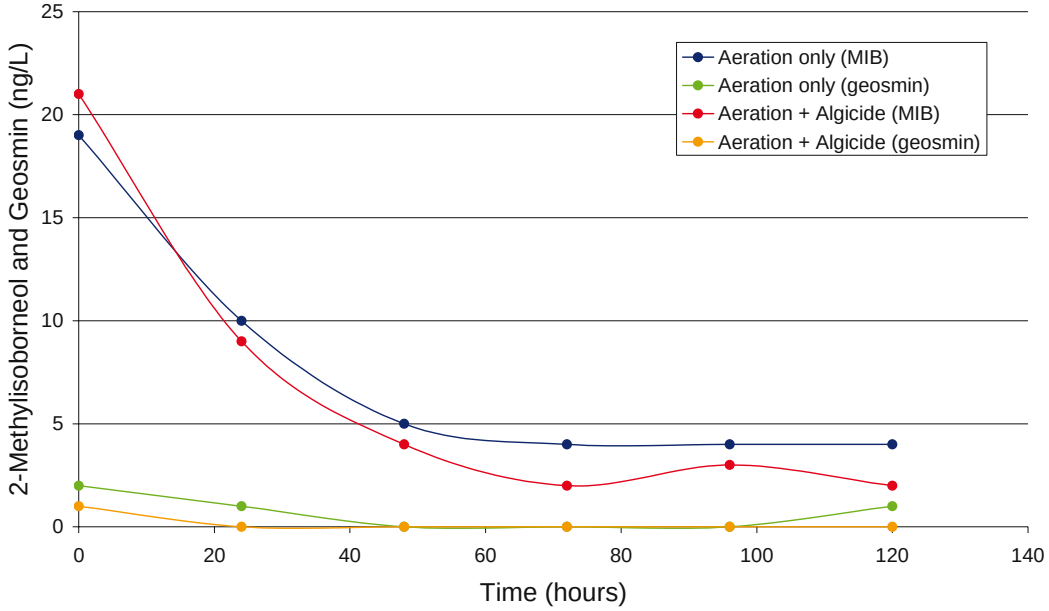

Figure 4.7 Level of 2-methylisoborneol and geosmin in 6m x 5m x 2m liners over time with aeration only and aeration + algicide.

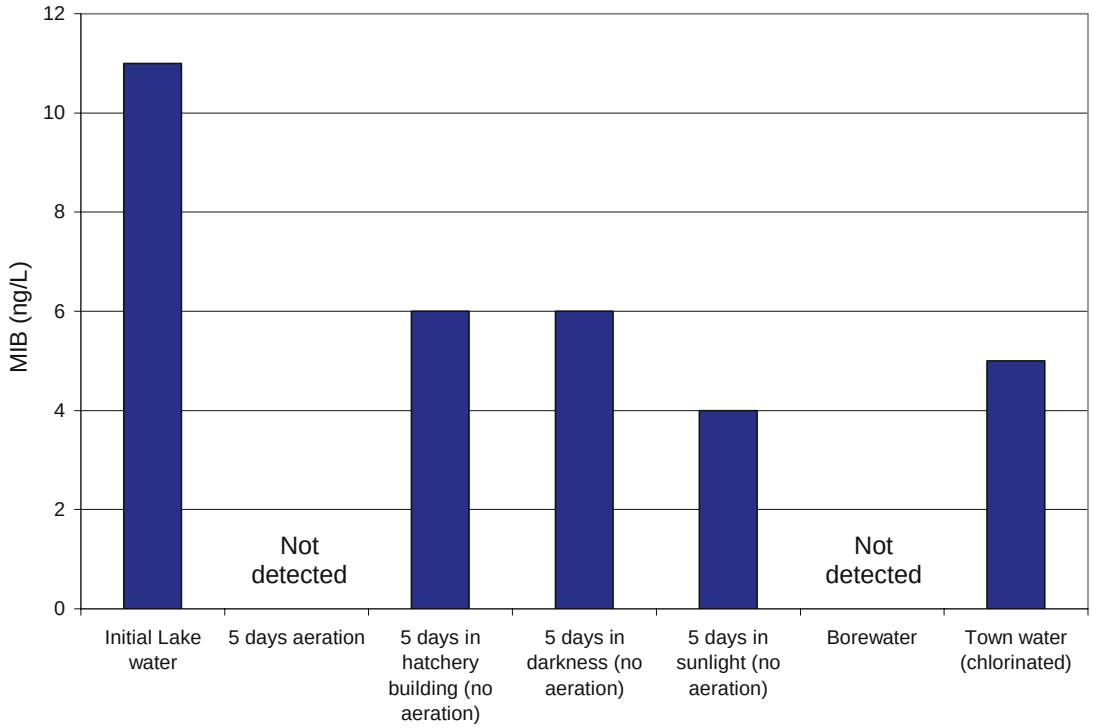

Figure 4.8 Level of 2-methylisoborneol in lake water following treatment for five days using aeration and darkness alone or in combination. (Note – there was no detectable levels of geosmin in lake water at the time of the trial).

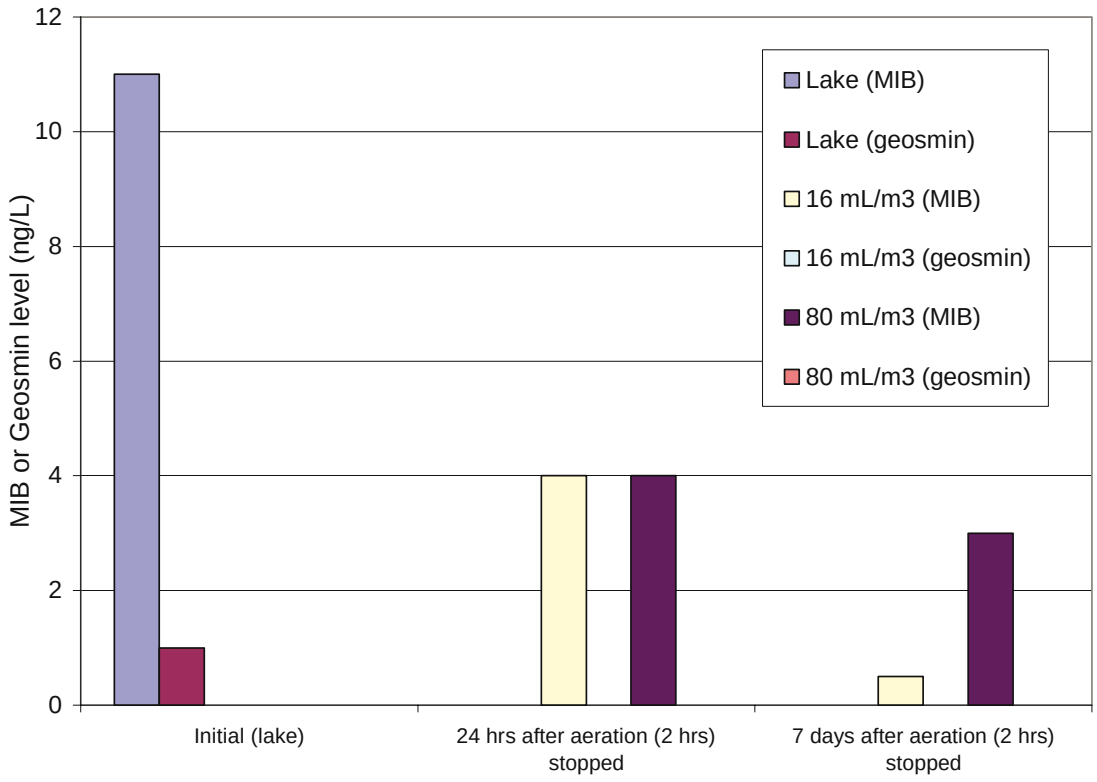

Figure 4.9 Concentrations of 2-methylisoborneol and geosmin in lake water following treatment with PAC at 16 ml/m^3 and 80 mls/m^3.

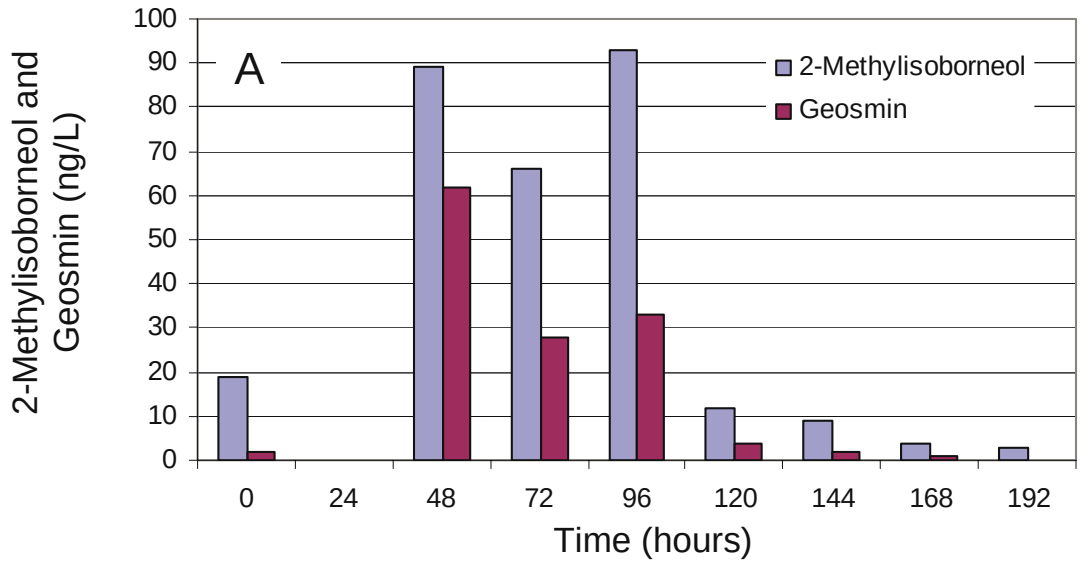

Figure 4.10a Levels of 2-methylisoborneol and geosmin in 6m x 5m x 2m liners over time with aeration only. The liners were spiked with 2-methylisoborneol and geosmin on day 2.

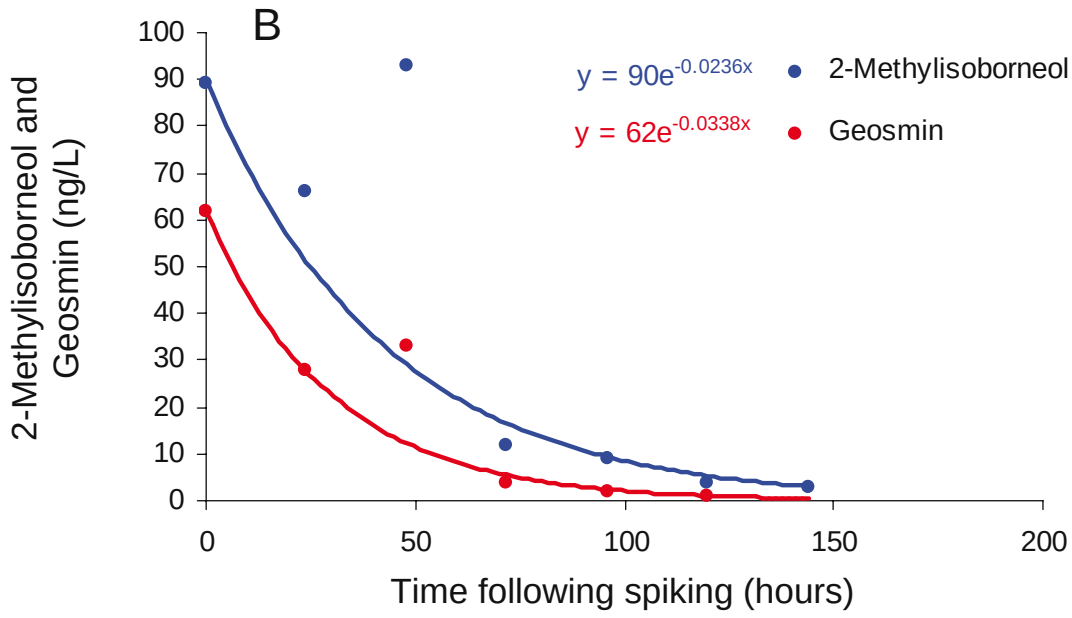

Figure 4.10b Exponential functions of the loss of 2-methylisoborneol and geosmin from the 6m x 5m x 2m liners over time with aeration. Notable is the faster rate of loss of geosmin as indicated by the higher exponent value (-0.0338) than that observed for the rate of loss of 2-methylisoborneol (-0.0236).

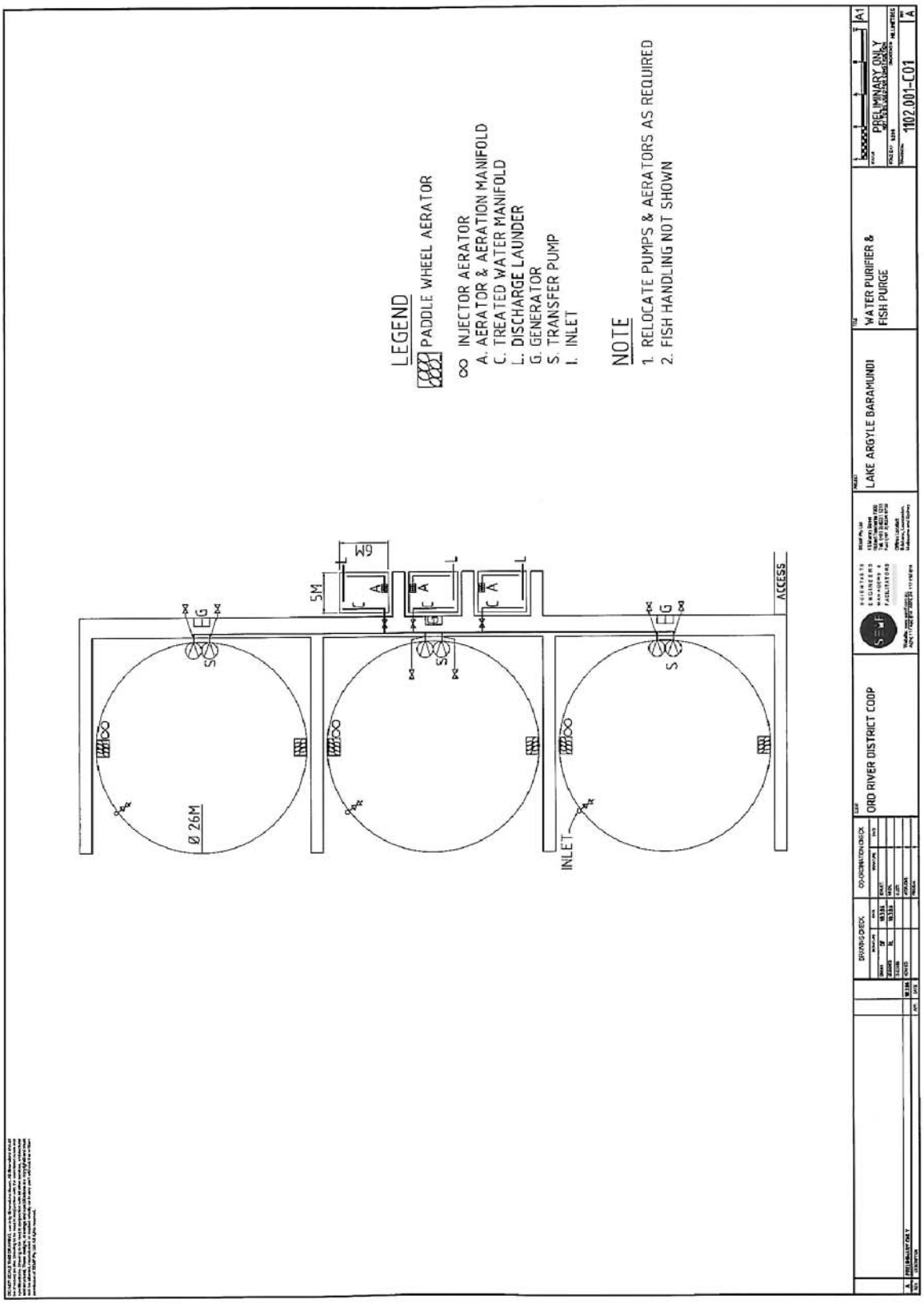

Figure 4.11 Design of a commercial scale depuration system designed to remove 2-methylisoboreneol and geosmin from lake water.

5.0 Maintaining and quarantining barramundi stocks to prevent the spread of *Streptococcus iniae*

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5.1 Introduction

A bacterium, *Streptococcus iniae*, caused significant mortalities in grow-out fish in an aquaculture facility at Lake Argyle in January 2004. Almost 120,000 barramundi weighing up to 3 kg died from the disease over 2 weeks. The outbreak followed heavy rain in which large amounts of silt had washed into the lake near the aquaculture cages. The initial outbreak was followed by smaller outbreaks over several months that required careful management.

Streptococcus iniae is a Gram positive coccus bacteria with strains showing variations in beta haemolysis from strong to weak. There are both commensal and virulent strains (Fuller et al., 2001) and several serotypes that are determined by the antigenic properties of the polysaccharide capsule (Barnes et al., 2003). The bacterium occurs worldwide and has been reported in more than 20 species of marine and freshwater fish. It is a significant pathogen in warm water aquaculture of both freshwater and marine species and cumulative mortalities can be as high as 80%. The disease also affects cold water species, and several outbreaks have been reported in rainbow trout in Australia, Israel and South Africa (Carson et al., 1993; Bachrach et al., 2001). The disease can result in significant economic loss because larger fish nearing marketable size can be severely affected. Most published reports of the disease originate from North America, the Mediterranean and Australia but Intervet has isolated the bacterium from fish in several Asian countries (Newsletter 2003).

The epidemiology of the disease is not well understood, however, large numbers of bacteria are present in diseased fish (Lahav et al., 2004). Outbreaks have occurred in wild fish in the Caribbean, Bahrain and Kuwait in warm water containing large amounts of organic matter (Ferguson et al., 2000) and (http://www.unep.org/geo/geo3/english/480.htm).

The disease is zoonotic and humans can become infected through cuts or puncture wounds when they handle infected fish. In notable incidents in 1995 and 1996 in Ontario, Canada, several people were diagnosed with bacteraemia and cellulitis following the sale of live tilapia from the USA (Weinstein, 1996). One person was more seriously affected, having endocarditis, meningitis and probable septic arthritis.

One of the aims of the current project was to develop a management plan to minimise the effects of *Streptococcus iniae* in caged fish in Lake Argyle. Developing a management plan requires an understanding of the epidemiology of the disease in Lake Argyle and identifying risk factors that can contribute to outbreaks of the disease. An investigation of the feasibility of using vaccination to reduce overt disease was also part of the original aim for the project but was not undertaken because the Ord River District Cooperative Pty Ltd scaled down its operation before vaccination trials could begin.

Factors that increased the likelihood of outbreaks of overt disease and fish mortality were identified and various management strategies were used in the face of continuing outbreaks of disease and their degree of success is outlined in the results section 5.3.

5.2 Materials and Methods

The strain of *S. iniae* isolated from barramundi in the Lake Argyle aquaculture facility was characterised using standard phenotypic and biochemical tests, the API Rapid ID32 system (bioMérieux) as well as polymerase chain reaction (PCR) (Mata et al., 2004) and capsular serotyping in an attempt to trace the source of infection.

5.3 Results

5.3.1 Characterisation of the bacterium responsible for the Lake Argyle outbreak

Streptococcus iniae was identified by its growth characteristics, Gram staining and haemolytic properties together with biochemical tests. The identity of the isolate was confirmed using polymerase chain reaction (PCR) analysis. The bacterium responsible for the Lake Argyle outbreaks had a distinct capsular serotype that did not cross react with isolates from other outbreaks of disease in Australian barramundi and ornamental fish (A. Barnes, pers. comm.). Biochemically the isolate was similar to other strains found in trout and barramundi in Australia (see Table 5.1) but differences were seen for alanine-phenylalanine-proline arylamidase and glycyl-tryptophane arylamidase using the API Rapid ID 32 kit when compared with other strains which were causing outbreaks of disease in barramundi in Western Australia at the same time.

5.3.2 Management strategies

The two issues involved with managing barramundi farms at Lake Argyle in the presence of *S. iniae* are:

(1) To limit the impact of clinical disease should it occur and;

(2) To limit exposure of farmed fish to *S. iniae.*

5.3.2.1 When clinical disease occurs:

Management of acute outbreaks of disease at Lake Argyle involved the following:

- *Instigate 'in feed' antibiotic treatment*

Erythromycin was initially the antibiotic of choice but was expensive as well as unpalatable to the fish when coated on the external surface of pelleted feed. Sensitivity testing of the bacterial isolate indicated that it was susceptible to oxytetracycline and this antibiotic was then used and was more palatable. It was important that antibiotic therapy commenced early in outbreaks before a large number of fish had ceased to feed.

- *Reduce stocking density in cages*

Reducing stocking density was found to be a key factor in limiting the impact of the disease. Initially fish were stocked at up to 220 kg/m^3. The disease was controlled when stocking density was reduced to less than 50 kg/m^3.

- *Remove dead and dying fish from cages as soon as possible*

Dead fish sink to the bottom of the cages and then float to the surface. It was important to remove fish as soon as possible to minimise opportunities for cannibalism and reduce the number of bacteria present in the water.

- *Move cages of affected fish to an area with better water quality*

When muddy water ran into the lake after heavy rainfall and caused stress on the fish it contributed to outbreaks of overt disease.

- *Isolate cages of diseased fish from cages of unaffected fish*

On days when there was little wind, there was a fatty slick on the water that might have spread disease. Sites were spatially separated to prevent disease spread by water currents or surface slicks.

5.3.2.2 To limit farm exposure to *S. iniae*.

The following were identified as measures, which could reduce the potential for fish at Lake Argyle to develop *S. iniae*.

- *Biosecuity*

It was recommended that:

- Equipment should be replicated at each cage sites so that no cross contamination occurs.
- Disinfection facilities for equipment after use should be provided and foot baths and hand wash stations be provided for entry and exit to the site.
- Preferably, vessels should be allocated to one site. If this was not possible the order for attending sites was to be changed so that the most susceptible to the disease (fingerlings) should be visited before the larger fish.
- Farms should import only high-health *Streptococcus*-free juvenile fish
- Increase the distance between cages as much as practicable.

- *Regular net cleaning*

Maintaining nets free of organic matter was an important step in reducing the number of bacteria, including *S. iniae*, in the vicinity of the fish.

- *Maintaining equipment*

Regular maintenance of fish sorting machinery was carried out to minimise superficial skin trauma that may allow the entry of environmental bacteria, including *S. iniae.*

- *Reduce stocking density*

Outbreaks were controlled once stocking densities were maintained at below 50 kg/m^3.

- *Minimise the impact of water inversions*

Water inversions in which colder, less well oxygenated water rises to the surface is a regular event in Lake Argyle. When this occurred the fish became stressed by the low oxygen content and temperature of the water. Several methods of minimising the effects of these events were identified:

- Select sites that are less subject to inversion;
- Cage water can be oxygenated when an inversion occurs;
- Cages should be deep to allow the fish to move to deeper, unaffected water. Cages should ideally be 4 to 5 metres deep.

- *Minimise exposure to muddy water*

Adverse water quality frequently followed heavy rainfall. The resultant large amount of suspended solids and reduced oxygenation was very stressful for the fish and was often followed by disease outbreaks. External protozoa such as *Chilodonella* sp. that cause gill damage and respiratory distress had caused health problems in caged fish at Lake Argyle following heavy rain in previous years, but in 2004 the main impact of periods of muddy water was an outbreak of streptococcosis. It was important to either keep fish away from areas that are prone to heavy, muddy runoff or to move cages during heavy rain.

- *Report problems immediately*

Staff were provided with a simple decision tree to assist them in determining the correct course of action following mortalities. The decision tree is provided in Figure 3.1.

- *Consider improving immune status of fish*

Both the use of vaccines and immunostimulants may help protect fish from outbreaks of *S. iniae*. These are discussed in more detail below.

5.4 Discussion

5.4.1 The pathogen

The origin of *S. iniae*, the pattern of emergence of new strains and the impact of fish translocation on spread of *S. iniae* throughout the world are poorly understood. In Israel several virulent strains of the bacterium have been identified. Serotype I occurs in freshwater and brackish water aquaculture in trout and tilapia and Serotype II, which are arginine dihydrolase negative and very virulent have appeared since the widespread introduction of vaccination in 1995. There is little molecular similarity between strains of tilapia in the USA and Israel (Kvitt and Colorni 2004), where marine fish such as red drum and sea bass are infected with strains that are arginine dihydrolase variable but have a different molecular profile to the isolates from trout and tilapia in Israel (Colorni et al., 2002; Kvitt and Colorni 2004). It is possible that *S. iniae* was introduced to marine waters around Israel with imported red drum after 1995 (Colorni et al., 2002). The epidemiology of the outbreak in Caribbean reef fish, but not pelagic fish, in 1999 (Ferguson et al., 2000) is not well understood but the strain responsible had molecular similarities to the strains found in marine fish in Israel (Kvitt and Colorni 2004). The outbreak may have been the appearance of a new disease in a naïve population or caused by the emergence of a very virulent strain (Ferguson et al., 2000).

The virulence and origin of the Lake Argyle strain of *Streptococcus iniae* remains uncertain. There is considerable strain variation in *S. iniae*, and some evidence that virulent strains (Fuller et al., 2001) have genes that determine the capsule polysaccharide structures that are important in determining virulence (Miller and Neely 2005). Some strains are primary pathogens with the ability to enter and multiply within host cells and cause apoptosis (cell death) of macrophages (Zlotkin et al., 2003; Lahav et al., 2004). These virulent strains, including the arginine dihydrolase negative serotype II strains, reduce the immune and inflammatory responses of infected fish and cause less necrosis than commensal strains and are, therefore, likely to cause higher mortality (Taylor et al., 2001; Zlotkin et al., 2003). It must be noted that the detection of arginine dihydrolase is dependent on the type of method used, and in some biochemical identification schemes a heavy inoculum with an incubation

of up to four days is required to detect a positive result. A strain may be positive by one method yet negative by another.

The bacteria penetrate the blood-brain barrier and also enter and remain viable within host cells. This enables fish to become asymptomatic carriers of the disease. In common with most pathogens, species vary in their susceptibility to the disease (Fuller et al., 2001). Some species such as red drum and channel catfish were resistant to disease in the USA but tilapia, and hybrid striped bass were highly susceptible (Perera et al., 1997). There have, however, been outbreaks of the disease in red drum in Israel (Colorni et al., 2002).

Streptococcus iniae can be spread by fish to fish transfer between farms at least 2 miles apart (Colorni et al., 2002). Wild fish in the vicinity of a fish farm are therefore a potential reservoir of infection making it impossible to eradicate the disease from caged fish (Zlotkin et al., 1998; Colorni et al., 2002).

Bacteria remain viable in mud and water for considerable periods of time and also can be a significant reservoir of infection in ponds and sea cages (Kitao and Iwata 1979; Perera et al., 1997). Cannibalism and the faecal-oral route of infection are thought to be the most important routes of infection for barramundi with as few as 100 ingested bacteria resulting in death of fish following experimental infection (Bromage and Owens 2002). Cohabitation, immersion, high stocking density, low dissolved oxygen and high nitrite were also found to increase mortality from *S. iniae* (Shoemaker et al., 2000; Zlotkin et al., 2003). Vaccination reduces mortality from the disease but cannot be used to eradication of the disease (Bachrach et al., 2001).

Certain environmental conditions favour the replication of *S. iniae* and sometimes also increase fish stress, which pre-disposes them to outbreaks of disease. Water temperature is probably important to the onset of clinical disease because bacteria have an optimal temperature for growth. Outbreaks are more frequent in Queensland barramundi in summer months (Bromage et al., 1999; Bromage and Owens 2002). Perera et al. (1997) found that the *S. iniae* grows best in warm water and outbreaks are more common above 20°C. They suggest that the bacterium is likely to have evolved from mammalian origins, possibly dolphins since it was first isolated from a dolphin but, Streptococci are often found associated with soil and faeces and perhaps *S. iniae* originated from a terrestrial source. Once *S. iniae* has been introduced to an area it has not been possible to eradicate it. The disease can now be considered to be endemic in Lake Argyle.

5.4.2 Management of the farm

In all aquaculture enterprises, management practices aim to maximize profit. An important aspect of achieving profitably is maximizing growth, feed conversion ratios and stocking density. Optimal stocking density will vary, depending on the behaviour of the species and management factors such as water quality. Stress induced by high stocking density and less than optimal water quality may result in fish developing a high prevalence of subclinical or overt disease. When susceptible species are infected with *S. iniae*, outbreaks of clinical disease often follow stressful events such as handling or a natural event that impairs water quality.

Prevention and control strategies must be carefully developed and a disease management plan implemented to minimise production losses from the disease. Both subclinical and clinical disease can have a large economic impact on the enterprise and a good plan should include strategies for identifying conditions that might pre-dispose to disease caused by *S. iniae*.

One way to do this is to employ methods such as the Hazard Analysis and Critical Control Point (HACCP) plan used in the food industry to identify risks and management strategies to minimise the impact of significant risks (see Table 3.1). Another tool could be to benchmark feed intake, growth rates and feed conversion ratios against a known optimal standard. As soon as monitored parameters fall outside a pre-designated standard an investigation can be made to identify factors that might be contributing to the decline in performance. An Emergency Management Plan should involve notifying relevant authorities (since early diagnosis is critical), quarantining the affected cage(s) and reducing the stocking density in the affected cages. Staff need to be trained in what to do. A simple 'decision tree' for staff is shown in Figure 5.1. A veterinarian and a source of antibiotics should be identified before an emergency occurs. Treatment using antibiotics is only available under veterinary supervision and is generally only an option for non-food fish (due to the with-holding periods required). Antibiotics are effective at stopping mortalities, but are expensive and result in subclinically infected or "carrier" fish.

5.4.3 Improving the immune status of fish

5.4.3.1 Use of vaccines to protect fish.

Vaccination is potentially one of the most useful methods for preventing disease outbreaks. Several vaccines based on formalin killed whole *S. iniae* cells have been developed overseas and recently two autogenous vaccines have been approved by the Australian Pesticides and Veterinary Medicines Authority (APVMA) for use in barramundi in Australia. These vaccines were designed to prevent disease caused by specific endemic strains. A small batch is prepared using a strain from a farm that has a problem infection and the vaccine is authorised for use on that site only and for that particular season. The reason for this procedure is that the dominant protective *S .iniae* antigens recognised by fish are part of the polysaccharide capsule and are variable, therefore a vaccine prepared from one isolate from one farm is unlikely to work well on another. One permit (PER8797) is for use in South Australia. The other, (PER8406) (see Fig. 5.2a-d), is also an autogenous vaccine and it is not known whether either would be useful in protecting fish against the *S. iniae* strain that is present in Lake Argyle. The vaccine production company, Intervet, is improving its commercial vaccine and is seeking Australian registration. In future this might offer protection to a much larger number of strains, including the Lake Argyle strains. Alternatively an autogenous vaccine could be developed specifically for treating fish in Lake Argyle and a Minor Use Permit obtained from the APVMA for its use.

5.4.3.2 Immunostimulants and probiotics

Immunostimulants are often used in aquaculture to improve the nonspecific immune responses of fish, thereby increasing weight gain and reducing losses from disease. Feeding 2 to 4% yeast, *Saccharomyces cerevisiae*, to hybrid striped bass continuously for up to 16 weeks reduced mortality following experimental infection with *S. iniae* (Li and Gatlin 2003). 1,3 β glucans derived from *S. cerevisiae* are recognised immunostimulants that are sometimes added to aquaculture feed. Their use is an option for reducing the impact of the disease in barramundi. One brand available in Australia is Aquagard®, although it is not registered by the APVMA as an immunostimulant but can be used as a feed.

Probiotics have also been investigated as a potential method of controlling *S. iniae*. *Aeromonas sobria* reduced mortality after intraperitoneal injection of *S. iniae*, especially when 10^6-10^8 cells were added to each gram of feed for trout and carp (Brunt and Austin 2005).

5.4.3.3 Alternative therapies

A student at James Cook University is exploring the use of phage therapy against *Streptococcus iniae* under Dr Leigh Owens. This promising technique uses naturally occurring viral bacteriophages to attack the bacteria. It has the advantage that bacteria are unable to develop immunity to the virus. Phage therapy has been used in the former Soviet Union but has generally been neglected by western medicine (Bull et al., 2002).

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5.6 Tables and Figures

Table 5.1 Comparison of characterisitics of *S. iniae* from barramundi in Lake Argyle with those from other species in Australia and overseas.

Test		Barramundi Lake Argyle Strain	Barramundi Bromage et al. (1999)	Trout Carson et al. (1993)	Marine fish Colorni et al. (2002)
Arginine dihydrolase		+	+	+	Variable
Acid from	Glucose	+	+	+	+
	Lactose	-	-	-	-
	Maltose	+	Nt	+ 91%	+
	Mannitol	+	+	+ 91%	+
	Raffinose	-	-	-	-
	Salicin	+	+	+	+
	Sucrose	+	+	-	+
	Sorbitol	-	-	-	-
	Trehalose	+	+	+	+
	Inulin	-	-	-	-
	L-arabinose	-	-	-	-
	Fructose	nt	+	+	+
	Xylose	-	-	-	-

Note: The test methods used in the different references are often not quoted and this can influence the results obtained from certain tests.

Table 5.2 A HACCP-style table for identifying risks factors for *S. iniae* outbreaks and their control or the method used to alleviate to risk factor.

Risk factor	Control
Muddy water	Select low run-off sites Tow cages to unaffected areas
Overstocking	Reduce fish density to below 50 kg/m3
Water inversion	Maintain dissolved oxygen by aeration
(low dissolved oxygen and temperature)	Use cages that are 4 to 5 m deep Select sites that are not prone to water inversion
Purchase of infected stock	Purchase stock that have been certified free of the disease
Cross contamination	Disinfect equipment and personnel between cages Minimise the entry of wild fish to cages Reduce net fouling Increase the distance between cages
Fish stress	Reduce stocking rate Minimise and improve handling Monitor and regulate water quality parameters Ensure good quality feed and optimal intake Feed immunostimulants Vaccination

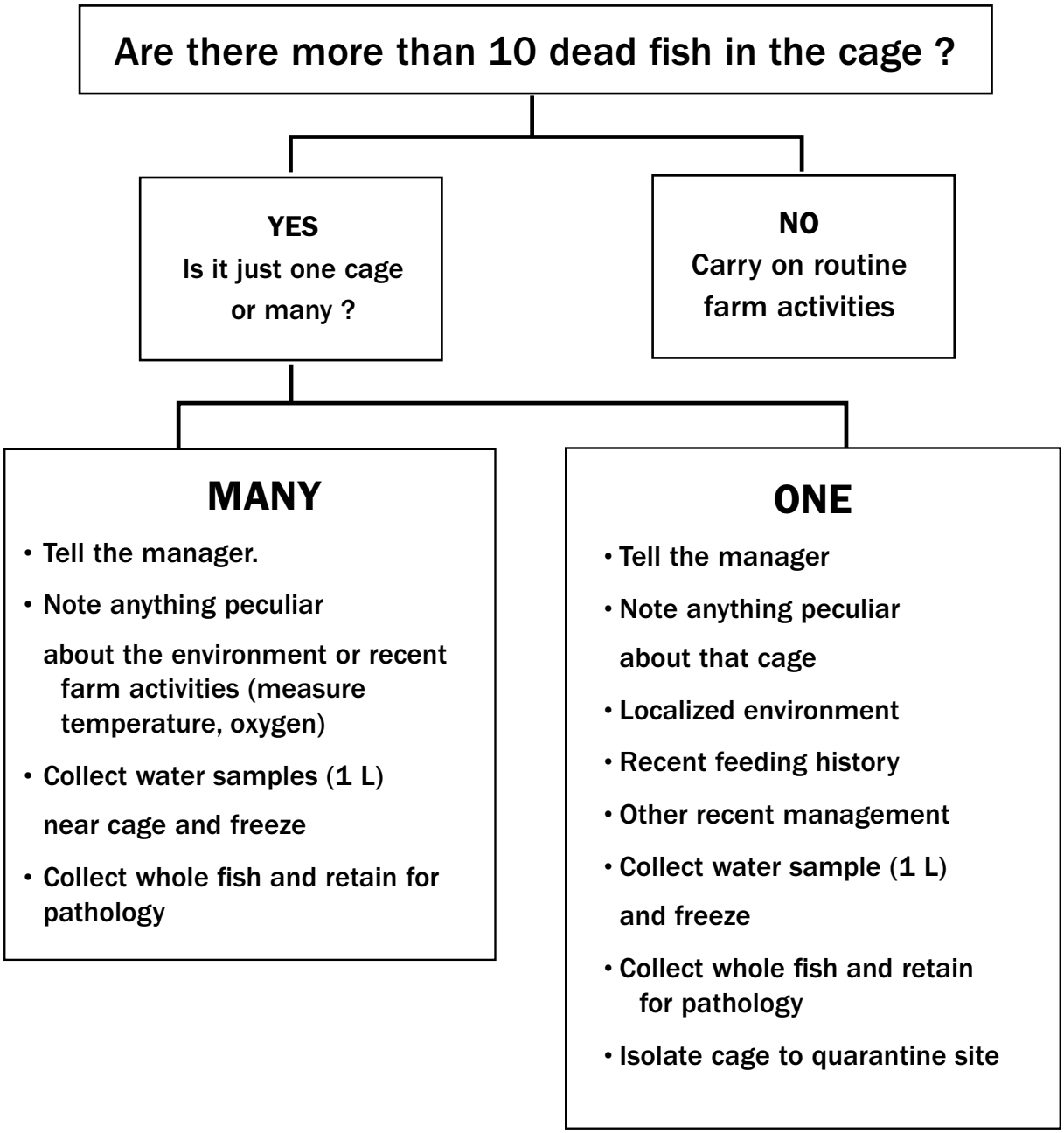
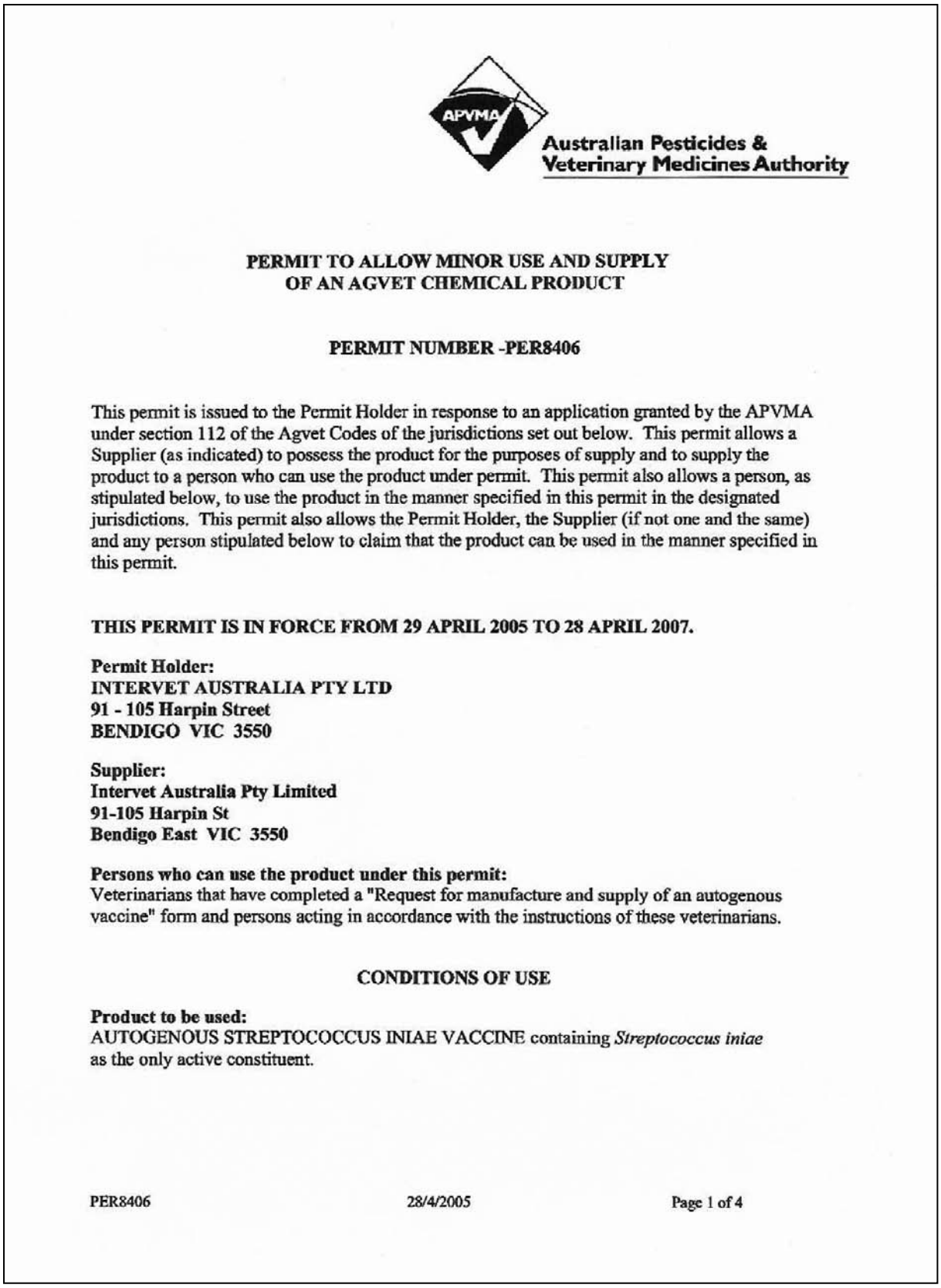


Figure 5.1 Decision tree used by farm staff when determining actions following a possible disease outbreak at Lake Argyle.



Australian Pesticides & Veterinary Medicines Authority

PERMIT TO ALLOW MINOR USE AND SUPPLY OF AN AGVET CHEMICAL PRODUCT

PERMIT NUMBER -PER8406

This permit is issued to the Permit Holder in response to an application granted by the APVMA under section 112 of the Agvet Codes of the jurisdictions set out below. This permit allows a Supplier (as indicated) to possess the product for the purposes of supply and to supply the product to a person who can use the product under permit. This permit also allows a person, as stipulated below, to use the product in the manner specified in this permit in the designated jurisdictions. This permit also allows the Permit Holder, the Supplier (if not one and the same) and any person stipulated below to claim that the product can be used in the manner specified in this permit.

THIS PERMIT IS IN FORCE FROM 29 APRIL 2005 TO 28 APRIL 2007.

Permit Holder:
INTERVET AUSTRALIA PTY LTD
91 - 105 Harpin Street
BENDIGO VIC 3550

Supplier:
Intervet Australia Pty Limited
91-105 Harpin St
Bendigo East VIC 3550

Persons who can use the product under this permit:
Veterinarians that have completed a "Request for manufacture and supply of an autogenous vaccine" form and persons acting in accordance with the instructions of these veterinarians.

CONDITIONS OF USE

Product to be used:
AUTOGENOUS STREPTOCOCCUS INIAE VACCINE containing *Streptococcus iniae* as the only active constituent.

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Figure 5.2a Minor use permit for application of *Streptoccocus iniae* vaccine use in Australia.

Directions for Use:

Animal	Purpose	Dose
FISH	STREPTOCOCCUS INIAE	Dosage and administration Preferred method for vaccination is by intraperitoneal injection from 20g and over (0.1ml at ventral side of fish). If early protection is needed fish can be vaccinated by dip immersion from 3-5g and over at 1 litre per 9 litres of clean water. Dip-immersion vaccination should be followed by intraperitoneal injection at 20gm or over

Withholding Period:
NIL

Jurisdiction:
SA, NT

General conditions

1. Manufacturing requirements
   a) The autogenous vaccine must be prepared in a GMP Category 1 (immunobiologicals) licensed facility.
   b) Preparation is restricted to cultures of microorganisms which have been inactivated and are non-toxic.
   c) In-process control testing for purity, completeness of inactivation and/or detoxification and level of formalin (BP Vet 1993) shall be carried out.

2.Seed requirements
   a) The microorganism used to prepare the autogenous vaccine must be isolated only from sick or dead fish and administered only to the herd of origin.
   b) Microorganisms used to prepare the vaccine must be identified to the level of species for bacteria and to family.
   c) Isolates used for production of autogenous vaccines should not be older than 24 months from the date of isolation. The use of such isolates or their cultures older than 24 months must be recorded and records available for audit purposes.

3. Final product requirements
   a) Tests for sterility and/or purity must be carried out according to 9CFR113.26 or BP, USP or EP testing standards.
   b) The fish shall be given 2X the recommended dose by the recommended route of administration and observed for a minimum of 7 days for any adverse reactions.

4. Labelling requirements
   a) Each label must bear the name of the microorganism, the farm and abbreviated address, the company's name, batch and expiry date, APVMA permit number, directions for use (dosage and restraints), target species and storage conditions.

5. Other conditions:
   b) Vaccines prepared from microorganisms isolated from one herd shall not be used on another herd without justification and approval from the APVMA.
   c) Shelf life must not exceed 18 months from the date of harvest.

Figure 5.2b Minor use permit for application of *Streptoccocus iniae* vaccine use in Australia.

d) Samples of each batch of vaccine prepared must be retained and stored at the recommended storage temperature for at least one month past the nominal expiry date. In the event of unexpected adverse reactions arising from the use of a particular batch of vaccine, such retention samples can be tested to determine the cause.

e) Details of manufacture, isolates, disease identified and treated, quantity prepared, vaccine components (quantitative details), dosage, amount and species treated and labels must be presented to the APVMA at the time of renewal of permit. In addition, efficacy of the product and justification for its continual use must be presented.

Issued by

Delegated Officer
Dr John Owusu
Manager Vaccines & Antibiotics Team
Veterinary Medicines Program
Australian Pesticides & Veterinary Medicine Authority

Figure 5.2c Minor use permit for application of *Streptoccocus iniae* vaccine use in Australia.

Intervet Australia Pty Ltd Streptococcus iniae Vaccine 250 mL and 500 mL Pack

FOR ANIMAL TREATMENT ONLY
Streptococcus iniae vaccine
Containing inactivated *Streptococcus iniae*.
THIS IS NOT A REGISTERED PRODUCT. APPROVED UNDER APVMA PERMIT for use at
[Farm / location]……..
A formalin inactivated bacterin to aid in the control of *Streptococcus iniae* infection in fish.
READ THE PERMIT BEFORE USING THIS PRODUCT
Contents: Net **250 mL**
DIRECTIONS FOR USE:
USE ALL PRODUCT IMMEDIATELY AFTER OPENING.
Shake well before use.
DOSAGE AND ADMINISTRATION:
Preferred method for vaccination is by intraperitoneal injection from 20g and over (0.1mL at ventral side if fish).
If early protection is needed, fish can be vaccinated by dip immersion from 3g and over at a rate of 1 litre of vaccine per 9 litres of clean
water for 30 seconds. Dip-immersion vaccination should be followed by intraperitoneal injection at 20g or over.
WITHHOLDING PERIOD: NIL
FIRST AID: If poisoning occurs, contact a doctor or Poisons Information Centre. Phone Australia 131126.
USER FIRST AID AND SAFETY DIRECTIONS:
Swallowing: Seek medical attention.
Surface spillage: Wash off with copious amounts of clean water.
Eye: Irrigate with eye-wash. Seek medical advice should discomfort or redness persist.
Accidental injection: Seek immediate medical advice.
INTERVET AUSTRALIA PTY LIMITED, 91-105 Harpin Street, BENDIGO EAST VIC 3550. Phone: (03) 5440 9888
DISPOSAL: Dispose of empty container by wrapping with paper and putting in garbage.
Discarded needles and sharps should be immediately placed in a designated and appropriately labelled 'sharps' container.
STORAGE: Store between 2°C and 8°C. (Refrigerate. DO NOT FREEZE). Protect from light.
Batch No.: Expiry Date:

Intervet Australia Pty Ltd Streptococcus iniae Vaccine 250 mL and 500 mL Pack

FOR ANIMAL TREATMENT ONLY
Streptococcus iniae vaccine
Containing inactivated *Streptococcus iniae*.
THIS IS NOT A REGISTERED PRODUCT. APPROVED UNDER APVMA PERMIT for use at
[Farm / location]……..
A formalin inactivated bacterin to aid in the control of *Streptococcus iniae* infection in fish.
READ THE PERMIT BEFORE USING THIS PRODUCT
Contents: Net **500 mL**
DIRECTIONS FOR USE:
USE ALL PRODUCT IMMEDIATELY AFTER OPENING.
Shake well before use.
DOSAGE AND ADMINISTRATION:
Preferred method for vaccination is by intraperitoneal injection from 20g and over (0.1mL at ventral side if fish).
If early protection is needed, fish can be vaccinated by dip immersion from 3g and over at a rate of 1 litre of vaccine per 9 litres of clean
water for 30 seconds. Dip-immersion vaccination should be followed by intraperitoneal injection at 20g or over.
WITHHOLDING PERIOD: NIL
FIRST AID: If poisoning occurs, contact a doctor or Poisons Information Centre. Phone Australia 131126.
USER FIRST AID AND SAFETY DIRECTIONS:
Swallowing: Seek medical attention.
Surface spillage: Wash off with copious amounts of clean water.
Eye: Irrigate with eye-wash. Seek medical advice should discomfort or redness persist.
Accidental injection: Seek immediate medical advice.
INTERVET AUSTRALIA PTY LIMITED, 91-105 Harpin Street, BENDIGO EAST VIC 3550. Phone: (03) 5440 9888
DISPOSAL: Dispose of empty container by wrapping with paper and putting in garbage.
Discarded needles and sharps should be immediately placed in a designated and appropriately labelled 'sharps' container.
STORAGE: Store between 2°C and 8°C. (Refrigerate. DO NOT FREEZE). Protect from light.
Batch No.: Expiry Date:

Figure 5.2d Minor use permit for application of *Streptoccocus iniae* vaccine use in Australia.

6.0 Modelling the growth performance and feed utilisation of barramundi

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6.1 Introduction

The use of models to examine feed use and growth in fish is not a new concept (Ursin, 1967; Cuenco et al., 1985; Machiels and Henken, 1986; Cacho, 1990). Many of these models have been based on describing flows of energy and nutrients as governed by fundamental thermodynamic and energetic principles. The recent revival of these models for use in aquaculture has seen their rapid adoption by the scientific and industrial community as their value for use in research and fish production management becomes more apparent (Kazmierczak and Caffey, 1995; Cho and Bureau, 1998; Lupatsch and Kissil, 1998). The nature of some of these models can be quite complex, though the added complexity does not necessarily result in an improved model for practical purposes, but they certainly become more theoretically correct. These simple discrepancies highlight some of the present limitations to these models, but certainly do not diminish their value.

One of the more recently promoted models is the Cho and Bureau model, Fish-PrFEQ (Cho and Bureau, 1998). This model is a derivation of earlier work by this group (Cho et al., 1991) where the food allocation (theoretical feed requirement: TFR) is based on defining energetic demands of the fish. The key to estimating the energetic demands of the fish is the defining of parameters of growth response to temperature and the composition of weight gain at any particular point in the growth cycle. In addition, the influence of water temperature on maintenance energy requirements (heat production of fasting animal: HEf) is also required to account for non tissue-deposition (non-somatic) energy demands. The Cho and Bureau model also includes a term for the heat increment of feeding (HiE) which in effect is the heat or energy lost through feeding activity. Allocations were also made for energy losses encountered through incomplete metabolism of nutrients (branchial and urinary energy losses). The overall determination of energy requirements in the Cho and Bureau model then being the sum of somatic requirements + maintenance requirements + heat increment of feeding + energy loss through incomplete metabolism. These workers then proposed that the TFR was equivalent to the total energy demand divided by the digestible energy density of the feed being fed. A further correction factor was then applied to make the feed allocation more similar to those actually determined from empirical trials.

The factorial model (Shearer, 1995; Lupatsch and Kissil, 1998; Lupatsch et al., 2001) is more simplistic in its overall design than the Cho and Bureau model, in that it compartmentalizes the energy flows into either somatic or non-somatic components only. The non-somatic components accounting for maintenance (routine metabolism), heat loss and activity. The somatic energy component of the factorial model though is more detailed than that used in the Cho and Bureau model in that it uses a species-specific equation for estimating tissue energy density as a function of fish size. The Cho and Bureau model, in contrast, uses a set value not a variable one. It can be generally summarised as:

$$\text{Energy Demand (kJ/fish/d)} = M \times \text{Liveweight (kg)}^{b} + G \times \text{Energy gain (kJ)}$$

Where M and G are constants describing the efficiency of energy utilisation for maintenance and growth, respectively. And *b* is the metabolic weight exponent of the animal.

However, the simplification undertaken in the factorial model makes for a more workable model in that errors in parameter assessment become less critical to the overall identified energy requirements. In contrast to the Cho and Bureau model, the factorial model does not use a correction factor to define TFR, but designs a feed table / feeding regime that needs to be adhered to for the model to work as predicted. However there are a range of factors that the factorial model assumes which need to be tested. Issues such as the assumption of constancy in energy utilisation efficiency with varying fish size is one such example.

Fundamental to the construction of such models is the development of a response equation that predicts growth rate as a function of animal size and water temperature. Earlier iterations of such a growth model for barramundi based on data from Williams and Barlow (1998) or Lupatsch and Kissil (2003), have not been very robust outside some critical constraints such as fish sizes > 200 g for the Williams and Barlow data and water temperatures > 27°C for the Lupatsch and Kissil data. To counter these problems the construction of a model from farm-derived data in Australia, along with some laboratory-based data, was required.

This chapter of the study reports on the development of an advanced growth and energetic model for barramundi based on Australian laboratory and farm production data. This includes:

- The development of a multifactorial equation to predict growth response of barramundi
- The development of integrated multifactorial model of energy and protein use by barramundi
- Reassessment of the assumption of constancy among fish size variation in energy utilisation efficiency by barramundi
- The development of feeding tables based on an integrated factorial model of energy use by barramundi

These outputs are then compared to and discussed in terms of other studies on similar areas in the literature. The outputs are also discussed in terms of the implications these findings have for the Australian barramundi industry.

However, like all models, the one presented has the potential to be useful, but is still far from optimal. Therefore caution must be applied when using features of the model or applications derived from it.

6.2 Materials and Methods

6.2.1 Testing the assumption of energy utilisation variability with fish size

6.2.1.1 Experiment concept and fish management

An experiment was conducted to compare the energy utilisation efficiency of two different sizes of barramundi. Fish were sourced from the Darwin Aquaculture Centre at different times and on-grown to 14.7 ± 0.5 g and 411.3 ± 4.3 g in preparation for the experiment. The fish were weighed on an electronic top-loading balance to 0.1 g accuracy and 25 small and 10 large fish allocated to 12 tanks each. After a one-week acclimation period, where all fish were

fed to satiety once daily, the fish were re-weighed and the stocking density reduced to 20 per small fish treatment and 6 per large fish treatment. Because the information sought is based on a regressed function it was decided to increase the number of treatments at the expense of the number of replicates to increase the strength of any regressed functions determined. The experiment was conducted at the West Australian Fisheries and Marine Research Laboratories in a flow-through, heated water, 24-tank array. Water temperature was maintained at 30.2 ± 1.15°C (mean ± S.D.) for the 28-day duration of the experiment.

6.2.1.2 Diet preparation and management

A reference diet formulation prepared as part of the Australian Aquaculture Feed Grains Program for use with salmonids was used in this study (Table 6.1). The diet was formulated to provide protein at about 500 g/kg at a gross energy level of 22.0 MJ/kg. The diet was prepared using extrusion technology, with pellets being prepared at 5mm and 3 mm diameters. The pellets were prepared at the Australian Experimental Stockfeed Extrusion Centre and Curtin University respectively.

A series of six ration levels was allocated amongst the two size blocks of the experiment. These ranged from fish being completely starved to fed once daily to apparent satiety. All feed fed and uneaten was accounted for to accurately determine feed intake by each tank of fish (Helland et al., 1996). A correction factor was applied to recovered uneaten pellets to account for soluble losses incurred on the pellet between feeding and collection to make a more accurate feed intake assessment.

6.2.1.3 Diet digestibility assessment

At the end of the 28 d growth period faecal samples were collected from the satietal fed fish using stripping techniques following anaesthesia (Austreng, 1978). Samples were collected from each fish within a tank and pooled to create enough sample for subsequent analysis. Samples from each tank were kept separate. Samples were freeze-dried prior to analysis for yttrium, dry matter, energy, fat and nitrogen. Details of analytical methods used are provided in section 6.2.4. Differences in the ratios of the parameters of dry matter, protein or gross energy to yttrium, in the feed and faeces in each treatment were calculated to determine the apparent digestibility coefficient (ADC_{diet}) for each of the nutritional parameters examined in each diet based on the following formula (Maynard and Loosli, 1979):

$$ADC_{diet} = 1 - \left(\frac{Y_{diet} \times Parameter_{faeces}}{Y_{faeces} \times Parameter_{diet}} \right)$$

where Y_{diet} and Y_{faeces} represent the yttrium content of the diet and faeces respectively, and $Parameter_{diet}$ and $Parameter_{faeces}$ represent the nutritional parameter of concern (dry matter, protein or energy) content of the diet and faeces respectively. Digestible nutrient and energy values for each diet are presented in Table 6.2.

6.2.1.4 Sample preparation and analysis

At the beginning and end of the experiment three whole fish were euthanized from each replicate by immersion in iced-seawater before being minced using a commercial meat mincer (Reber, Gambugliano, Italy). Samples were then collected and their moisture content determined by oven drying at 100°C for 24 h and a second sample freeze-dried for chemical analysis. Freeze-dried samples were analysed for dry matter, ash, fat, nitrogen, phosphorus, amino acid and

gross energy content. Dry matter was calculated by gravimetric analysis following oven drying at 100°C for 24 h. Phosphorus and yttrium concentrations were determined using Inductively Coupled Plasma – Atomic Emission Spectroscopy (ICP-AES) (McQuaker et al., 1979). Protein levels were calculated from the determination of total nitrogen by LECO auto-analyser, based on N x 6.25. Amino acid analysis involved the samples being hydrolysed at 110°C for 24hr in 6M HCl with 0.05% Phenol. Cysteine and cystine are derivatized during hydrolysis by the addition of 0.05% 3,3'-dithiodipropoinic acid by the method of Barkholt and Jensen (1989). The acid hydrolysis destroyed tryptophan making it unable to be determined. Separation was by HPLC on a Hypersil AA-ODS 5µm column using an 1100 series Hewlett Packard HPLC system. Crude fat content of the diets was determined gravimetrically following extraction of the lipids according to the method of Folch et al. (1953). Gross ash content was determined gravimetrically following the loss of mass after combustion of a sample in a muffle furnace at 550°C for 12 h. Gross energy was determined by adiabatic bomb calorimetry.

6.2.1.5 Nutrient and energy retention

Protein (N), Fat and Amino acid and Energy (E) retentions were determined based on the weight gain over the course of the experiment, against their respective consumption. Values were calculated according to the following formula (Maynard and Loosli, 1969):

$$Nitrogen\ Retention = \left(\frac{Nt - Ni}{Nc} \right) \times 100$$

Where *Nt* is the nitrogen content of the fish in a specific replicate at time *t* and *Ni* is the initial nitrogen content of the fish from the beginning of the study (n=2 replicates of 3 representative fish). *Nc* is the amount of nitrogen consumed by the fish from the time of initial assessment to time t. Determination of retention of other parameters was achieved the same way, but with the substitution of the relevant parameter where the corresponding nitrogen criteria are indicated in the equation.

To provide some independence of size effects, modelling of the nutrient and energy retention efficiency data was initially done with respect to general energy and protein body-weight exponents for fish of $x^{0.8}$ and $x^{0.7}$ respectively (Brett and Groves, 1979; Lupatsch et al., 2003). The energy exponent was used for lipid retention analysis, while the protein exponent was used for the amino acid retention analysis. [Analysis of linear regression of energy retention efficiency data allowed for subsequent determination of actual metabolic weight exponents based on solving of simultaneous equations for maintenance energy demand for the two fish size classes. This was done on the premise that the purpose of the exponent is to provide for a transformation to provide a size independent function for the determination of maintenance energy demand].

6.2.1.6 Statistical analysis of size effect data

All figures are mean ± SEM unless otherwise specified. Data were analysed for homogeneity of variances using Cochrans test. Effects of fish size were examined by ANOVA using the software package Statistica (Statsoft®, Tulsa, OA, USA). Levels of significance were determined using an LSD planned comparisons test, with critical limits being set at $P < 0.05$. Effects of fish size on key performance parameters were also examined by linear regression modelling, also using the software package Statistica. Statistical analysis of the regression constants and coefficients was made using a Kimura Likelihood Ratio test (Haddon, 2001), with critical limits also being set at $P < 0.05$.

6.2.2 Development of a predictive growth and metabolism equations

Using farm and laboratory data, factorial equations of weight gain per day (g/fish/d) were determined with respect to geometric mean weight and mean water temperatures over the assessment periods. This equation was defined using the regression function of the tools package within Microsoft Excel XP version. The equation takes the form of:

$$\text{Gain (g/fish/d)} = (K + xT + yT^2) * (\text{weight})^{(k + aT + bT\text{^}2)}$$

Where, K, and k are constants, and x, y, a and b are coefficients of the functional equation. T is temperature and weight is the geometric mean weight of the fish in grams.

Using laboratory and literature derived data; factorial equations of maintenance energy per day (kJ/fish/d) were determined with respect to geometric mean weight of the fish and the mean water temperatures. This equation was defined using the regression function of the tools package within Microsoft Excel XP version. The equation takes the form of:

$$\text{Maintenance energy demand (kJ/fish/d)} = (K + xT + yT^2 + zT^3) * (\text{weight})^{0.80}$$

Where, K, is a constant and x, y and z are coefficients of the functional equation. T is temperature and weight is the geometric mean weight of the fish in grams. The exponent of 0.80 is a general metabolic weight exponent of fish (Lupatsch et al., 2002), though this has also been suggested to vary from 0.6 to 1.0 (Booth et al., 2004; Withers, 1992).

Combined these two factorial equations underpin the functional basis of a predictive growth and therefore, feed utilisation model.

6.2.3 Feed utilisation modelling

The amount of feed required to be fed was determined based on the total energy demand (TED) as derived from a combination of parameters determined in this study:

$$\text{Total energy demand (kJ/fish/d)} = \text{Maintenance energy demand} + \text{Somatic energy demand} * EUE^{-1}$$

Where EUE is the energy utilisation efficiency determined from the regression of digestible energy intake against gross energy gain (section 6.2.1). Following the determination of total energy demand, the feed requirement is determined based on the TED divided by the digestible energy density of the feed being fed.

From the prescription of a certain dietary digestible energy density not only can the feed ration be determined, but also from this the dietary concentration of digestible protein required in a specific dietary digestible energy density can also be defined. The digestible protein concentration being defined by :

$$\text{Digestible Protein (g/fish/d)} = \text{Maintenance Protein} + \text{Somatic Protein Demand} * PUE^{-1} * TED^{-1} * (\text{Diet Digestible Energy Content})^{-1}$$

Where PUE is the protein utilisation efficiency determined from the regression of digestible protein intake against crude protein gain (section 6.2.1).

6.3 Results

The results from this chapter stem primarily from those derived from the empirical determinations of the assessment of the assumption of energy utilisation variability with fish size, the assessment of growth and fish composition from farm and laboratory derived data. In addition, some of the empirically determined parameters from these studies were used to refine an existing bio-energetic model. This model has been significantly modified based on some of the data defined in this study. The modified model has then used to examine the consequences of changes of fish size and water temperature on feed intake at several digestible energy densities and also the influence of fish size the optimal diet energy and protein composition.

6.3.1 Validation of the assumption of energy utilisation efficiency

6.3.1.1 Diet digestibility

Numerical, but no significant differences between the digestibilities of the two diet sizes were determined when fed to the two fish sizes (Tables 6.2 and 6.3). Protein, amino acid and energy digestibilities were similar between the two fish sizes. Total lipid digestibilities were more divergent, but still not significantly ($P>0.05$) different.

6.3.1.2 Fish growth and feed utilisation

Significant differences for a range of growth and feed utilisation parameters were noted within each of the size classes. With increasing feed intake a significant increase in final weight (and weight gain) was observed with both fish size classes (Table 6.4). Starved fish lost weight in both size classes. The food conversion ratio (FCR) was poorest at the lowest feed ration level above starvation for both size classes. As ration size increased FCR improved (got smaller). The lowest FCR for the small fish size class was at the second highest ration level, not at the satiety feeding level. This contrasted the large fish size class, which had the best FCR at the satiety feed intake level (Table 6.4).

6.3.1.3 Energy utilisation

The efficiencies of energy gain, over the digestible energy intake levels, observed in this study were examined with respect to both the generally accepted body-weight exponent of 0.80 and also one determined from this study, whereby an exponent of 0.86 transformed the maintenance energy demand (i.e. y=0) to be equal for both size classes (Figure 6.1). Irrespective of body-weight exponent used to transform the data, the efficiencies of energy gain, over the digestible energy intake levels, observed in this study were linear (Exp 0.80: $R^2 = 0.973$ and 0.952 for small and large fish respectively; and Exp 0.86: $R^2 = 0.972$ and 0.951 for small and large fish respectively). However, significant differences ($P<0.05$) between the fish size classes were observed with respect to the utilisation of dietary digestible energy (Figure 6.1a and b). Using the 0.86 exponent data, the large (410 g) fish had an energy utilisation efficiency that was described by the linear equation of: $y = 0.765x – 36.749$, $R^2 = 0.952$. For the small (15 g) fish the energy utilisation efficiency was described by the linear equation of: $y = 0.6015x – 29.459$, $R^2 = 0.972$. Irrespective of exponent transformation, there was a clear significant effect of differing gain efficiencies between size classes.

Calculation of the maintenance digestible energy intake for each fish size was undertaken for both exponent transformations using linear regression. The maintenance digestible energy intake, with the generic exponent of 0.80, was 45.5 kJ/ $kg^{0.80}$/d for the large fish and 35.1 kJ/ $kg^{0.80}$/d for the small fish. Altering the metabolic weight exponent to 0.86 resulted in equal

maintenance digestible energy intake levels (48.0 kJ/ $kg^{0.86}$/d) for both size classes. But, again it is important to reinforce that altering the metabolic weight exponent did not alter the effect of differing gain efficiencies between size classes.

6.3.1.4 Protein utilisation

Efficiency of protein gain over the lower digestible protein intake levels for both fish size classes was linear, but over the full range was better described by a non-linear function (Figure 6.2). Over the full data range the protein gain efficiency for the large fish was described by the quadratic equation of: $y = -0.090x^2 + 0.983x - 0.334$, $R^2 = 0.989$. Over the full data range the protein gain efficiency for the small fish was described by the quadratic equation of: $y = -0.075x^2 + 1.009x - 0.335$, $R^2 = 0.986$. Over the lower range (first four ration levels) of digestible protein intake the protein gain efficiency for both the small and large fish classes was not different (Figure 6.2). Accordingly, the combined response of both size classes was described by the linear equation of: $y = 0.822x – 0.370$, $R^2 = 0.967$. Maintenance digestible protein intake for both fish size class was calculated using this linear regression assessment, as being at 0.45 g/ $kg^{0.70}$/d.

Based on the non-linear regression assessment of the protein gain efficiency, the large fish had a lower maximal protein gain capacity per unit body weight than the smaller fish. This is reflected in the lower asymptote of the regression curve for the large fish size class compared to the small fish size class (Figure 6.2). However, at these upper feed intake levels, linear regression of the upper two feed ration levels gave similar utilisation coefficients of 0.484, irrespective of the gain capacity difference of the two fish size classes.

6.3.2 Development of an improved growth and metabolic response model

The model presented in this report assumes that both the growth and maintenance energy demand equations do not assume exponential responses with temperature, but include thermal response maxima. The thermal limits in the present model are based on data derived from farm production data, laboratory data as well as other data from the literature (Katersky and Carter, 2006). The growth function for barramundi from 15°C to 35°C is given by:

Gain (g/fish/d) = $(K + xT + yT^2) * (\text{live-weight})^{(k + aT + bT\text{^}2)}$ (Figure 6.4)

Where; T = temperature (C°)

K = -1.140
k = -0.290
x = 0.100
y = -0.001
a = 0.050
b = -0.001

All values rounded to 3 decimal places.

A 3rd-order polynomial was used as the coefficient base for the maintenance energy demand model based on observations from the published data and maintenance energy demands determined in the present study (Glencross and Felsing, 2006). In the earlier published work an exponent of 0.73 was identified at the 30°C to 31°C range. In the present study the determined exponent was 0.86. However, it was decided because the average of these two studies (exponent of 0.79) was consistent with the generally accepted value of 0.80, that it was difficult to justify

using a different exponent for the maintenance energy demand model. Therefore, based on these considerations the maintenance energy demand function for barramundi from 20°C to 36°C is given by:

Maintenance Energy Demand (kJ/fish/d) = $(K + xT + yT^2 + zT^3) * (\text{live-weight})^{0.80}$
(Figure 6.5)

Where; T = temperature (C°)
K = 0.4462
x = -0.0848
y = 0.0048
z = -0.000075

Much of the somatic energy demands of the fish depend on the variability in nutrient and energy density of the fish as it increases in size. This data was derived from Glencross et al. (2002), with validation of data from the present study to ensure it was consistent with these earlier determined values. In barramundi no significant variability in nitrogen/protein composition of the fish was observed with increasing fish size. Accordingly, the protein content of barramundi was best defined as y = 0.166*live-weight. However, a significant increase in fat content of the fish was observed over the test animal size range (51 to 918g). This change in fat content could be described by the equation:

$y = 3.214*\text{live-weight}^{0.144}$, $R^2 = 0.7994$ (Glencross et al., 2002). This change in fat content was reciprocated by a change in water content of the fish with increasing fish size. This change in water content of the whole-fish could be described by the equation: $y = 14.777*\text{live-weight}^{0.130}$, $R^2 = 0.7768$. The changes in fat and water content also coincided with a significant change in energy density of the fish with increasing fish size. The change in energy density could be described by the equation:

y (MJ/kg) = $3.273*\text{live-weight}^{0.143}$, $R^2 = 0.799$ (Glencross et al., 2002) (Figure 6.7).

6.3.3 Feed design modelling

As the energy density of the fish increased, the response of the feed design model was to reduce the required ration, but to maintain the nutrient demand. Therefore as diet energy density increases, the required nutrient concentration also increases. For example, the optimal predicted protein concentration for each diet, within each fish size, increased with increasing energy density of the diet (Table 6.5).

However, as fish size increased there was a declining demand for digestible protein (DP) in terms of the g/MJ consumed. This relationship is independent of dietary digestible energy (DE) density and can be described by the equation:

$$y = 69.48*\text{live-weight}^{-0.186}, R^2 = 0.9989$$

Based on this a 10 g fish will require 45 g DP/MJ DE, a 100 g fish will require 30 g DP/MJ DE and a 1000 g fish will require 19 g DP/MJ DE.

6.3.4 Feed utilisation modelling

Based on the modelled energy demands, the amount of feed to be fed varies with the digestible energy density of the feed. In this model, as feed digestible energy density increases, the amount required to be fed to the fish declines (Tables 6.5, 6.6 and 6.7). As a function of fish size, while

there is a proportional decline in feed demand as the fish get larger (i.e. smaller fish will eat a higher percent of their bodyweight per day), the overall amount required increases substantially as fish increase in size. This increase in feed demand follows an exponential relationship (i.e. $a.x^b$, where b is the fish size effect). Similarly an increase in temperature also increases feed demand, but the increase follows a similar relationship to the 3rd order polynomial function used to describe the maintenance energy coefficient described previously (i.e. $a.x^b$, where *a* is a 3rd order polynomial function describing the temperature effect).

6.4 Discussion

6.4.1 The effect of fish size on determining bio-energetic constraints

The advantage of the modelling approach presented in this study is that it assists significantly in the processes of decision making on a range of feed management issues including, feed formulation selection, feed ration determination and fish production expectations. However, in arriving at the model presented in the present study a complex array of studies and data have been compiled to produce an integrated factorial model. Many of the earlier models have made some key assumptions and part of the present study was to examine in detail their validity. However, like all models, the one presented has the potential to be useful, but is still far from optimal. Therefore caution must be applied when using features of the model or applications derived from it.

6.4.1.1 Size effects on digestibilities

The differences, or lack thereof, between the digestibilities observed between the two diets and fish sizes is generally consistent with other observations of fish over the size ranges studied (Tables 6.2 and 6.3). The exception to this was the work of Windell et al. (1978), who noted a small effect of fish size on dry matter, protein, lipid, carbohydrate or energy digestibility of a diet fed to rainbow trout of three size classes (19 g, 207 g, and 585 g), albeit only between the smallest and largest fish. The extremes of this size range are consistent with the present study, although the limited number of replicates (n=2) in the present study probably limited any chance of a significant effect being determined between the digestibility parameters, given that only the satietal fed fish were used to collect faecal samples. Had a greater level of experimental power been inherent in this aspect of the study then significant differences in lipid, energy and dry matter digestibilities (Table 6.2). However, that the determined values were used in any subsequent calculations makes the effects of these differences inclusive in the study.

6.4.1.2 Fish growth and feed utilisation

As expected, with increasing feed intake a significant increase in final weight (and weight gain) was observed with both fish size classes. The starved fish also lost weight in both size classes (Table 6.4). With the increasing feed ration, significant changes were also observed in the food conversion ratio (FCR). FCR was poorest at the lowest feed ration level above starvation for both size classes. As ration size increased FCR improved (got smaller). The lowest FCR for the small fish size class was at the second highest ration level, not at the satiety feeding level. This contrasted the large fish size class, which had the best FCR at the satiety feed intake level (Table 6.4). These findings are largely consistent with well known effects of feed ration on FCR and growth, where above a critical growth rate efficiency, then the FCR begins to deteriorate again (Brett et al., 1979). This effect has also been reported in more recent studies where the effects have been even more pronounced (Rowland et al., 2005). Whilst the effect in

question was observed in the present study in the small fish, it was not observed in the larger fish. We suspect this was because the larger fish were still not being fed to absolute capacity and therefore were further down the effective ration intake level than that achieved for the smaller fish. Because the fish were only fed once daily, additional feeding sessions may have provided further exacerbation of this effect by increasing the total feed intake ration level for the satiety fed fish.

6.4.1.3 Energy and protein utilisation

The efficiencies of energy gain, over the digestible energy intake levels, observed in this study were examined with respect to both the generally accepted body-weight exponent of 0.80 and also one determined from this study, whereby an exponent of 0.86 transformed the maintenance energy demand to be equal (i.e. y=0) for both size classes (Figure 6.1). Irrespective of the use of either of the different exponent values, the efficiencies of energy gain observed in this study were linear for both fish size classes. However, the key important observation was that a significant difference between the fish size classes were observed with respect the utilisation of dietary digestible energy (Figure 6.1a and b). Albeit to observe this effect a large difference in fish sizes had to be used (15g cf. 410g) and this probably explains why such effects have not been observed or considered in other studies where the fish sizes used in experiments have generally been much closer (Lupatsch et al., 2001b; 2003). The energy utilisation efficiencies observed in this study were 0.61*live-weight (LW) for small fish and 0.76*LW for the large fish and were not significantly influenced by the alternative exponential transformations. The data range for each size class did influence the regression coefficient. When the upper energy intake data are removed from the small-fish data set no significant differences are observed between the two fish sizes and an average of 0.685*LW is applicable for both sizes under this scenario. Interestingly, this average (0.685) is very similar to that determined for a range (0.65 – 0.69) of other fish species (Lupatsch et al., 2003). This difference in energy utilisation efficiency, but similarities in the protein utilisation efficiency can be explained by the highly significant differences in lipid utilisation efficiency (Figure 6.3), where the gradient for the large fish in excess of 1.0 indicates that there is clear lipid synthesis (energy deposition) from nutrients other than lipid. It is suspected that this shows some differential capacity of the larger fish to gain some energetic value from the carbohydrate content of their diet.

Calculation of the maintenance digestible energy intake for each fish size was undertaken for both exponent transformations using linear regression. The maintenance digestible energy intake, with the generic exponent of 0.80, was 45.5 kJ/ $kg^{0.80}$/d for the large fish and 35.1 kJ/ $kg^{0.80}$/d for the small fish. Altering the metabolic weight exponent to 0.86 resulted in equal maintenance digestible energy intake levels (48.0 kJ/ $kg^{0.86}$/d) for both size classes. This maintenance energy demand is consistent with other data for this species (Lupatsch and Kissil, 2003; Glencross, 2006), and also remarkably similar to that of other fish species when studied at their optimal thermal response temperature where ME values from ~40 to 50 kJ/ $kg^{0.80}$/d, have been determined (Cho and Bureau, 1998; Lupatsch et al., 2003). Altering the metabolic weight exponent did not alter the effect of differing gain efficiencies between size classes.

The efficiency of protein gain over the lower digestible protein intake levels for both fish size classes was linear, but over the full range was better described by a non-linear function (Figure 6.2). This effect is consistent with data from *Sparus aurata* (Lupatsch et al., 2003) and *Oncorhynchus mykiss* (Glencross et al., 2007), but differs from that of some other species (Lupatsch et al., 2003). Notably, in the present study, over the lower range (first four ration levels) of digestible protein intake the protein gain efficiency for both the small and large fish

classes was the same. Accordingly, the response of both size classes was described by the coefficient of 0.82*$LW^{0.7}$ over this range of digestible protein intake. From this linear region of the relationship it was possible to determine the maintenance digestible protein intake determined for both fish size classes at 0.45 g/ $kg^{0.7}$/d. Interestingly, this is also similar to values determined for other fish species, where this value has ranged from 0.22 to 0.66 g/ $kg^{0.7}$/ d (Kielanowski, 1965; Klein and Hoffman, 1989; Lupatsch et al., 2001a; Lupatsch et al., 2003; Lupatsch and Kissil, 2005). Much of this variability in the literature is probably attributable to variations in protein maintenance demand varying with temperature and it is important to note that direct comparisons between species should only be considered from studies undertaken at the respective species thermal optima, as in the case of the present study.

Based on the non-linear regression assessment of the protein gain efficiency, the large fish had a lower maximal protein gain capacity per unit body weight than the smaller fish. This is reflected in the lower asymptote of the regression curve for the large fish size class compared to the small fish size class (Figure 6.2). This observation is interesting in that it is also consistent with a known decline in protein to energy ratio required as fish increase in size (Shearer, 1995). However even with the curvilinear nature of the protein utilisation, irrespective of the upper asymptote (capacity), at the upper feed intake levels, a linear regression of the upper ration levels yields similar utilisation coefficients of 0.484. This coefficient of 0.484 becomes important in that it differs substantially from the utilisation coefficient of 0.822 determined at the lower feed intake ration levels. Therefore, for application of this data to a feed model intended to manage feed design and management at practical feed intake levels, the coefficient of 0.484 becomes the more practical coefficient to use. Notably, this value is also closer to protein utilisation coefficients determined in similar studies on other species (Lupatsch et al., 1998; 2001b; 2005).

6.4.2 Development of an improved growth and metabolic response model

Several different aspects of earlier bioenergetics models were evaluated and adapted to create a revised one for barramundi. The growth equation was redefined based on farm and laboratory growth data, to provide a better fit over the typical animal size and water temperature range seen in the Australian industry, than that reported in earlier models (Lupatsch and Kissil, 2003).

The model presented in this report is a significant advance on previous models published in the literature (Cho and Bureua, 1998; Lupatsch et al., 2001; 2002; Lupatsch and Kissil, 2003). This is because both the growth and maintenance energy demand equations in the present revised model do not assume either linear or dual exponential responses with temperature, but include thermal response maxima. These previous models assumed that as temperature increased so too did the rate of growth and maintenance energy demands – with no upper thermal constraints. The need to include thermal constraints on such models is well recognised since the rate of growth of these fish has also been observed to have thermal limits (Katersky and Carter, 2006). The growth function presented in this study includes these thermal limits (both upper and lower) for this species, based on data derived from a range of sources, including laboratory, farm and published data (Williams and Barlow, 1998; Katersky and Carter, 2006; Glencross, 2006).

The evidence for thermal constraints on maintenance energy demands was recognised by Glencross and Felsing (2006), who noted that above 30°C that there was a decline in the rate of oxygen consumption. In this work, these authors used a 2nd-order polynomial as the coefficient base for their oxygen demand model. However, reworking of the data from this

study shows that a better representation of the data is obtained using a 3rd-order polynomial as the coefficient base instead. In the work by Glencross and Felsing (2006), the metabolic body weight exponent of 0.73 was determined at 30°C to 31°C. Given that the present study determined and exponent of 0.86, it was decided the average of these two studies (exponent of 0.79), was consistent with the generally accepted value of 0.80.

Most of the composition constraints of barramundi were similar to those reported for a range of other fish species (Lupatsch et al., 2003), with protein content in particular being highly consistent among the different fish species. The observation of an increase in lipid and energy density with size increase is also highly consistent with other observations of this and other fish species (Williams and Barlow, 1998; Glencross et al 2002; Lupatsch et al., 2003).

6.4.3 Managing feed for more efficient production

A good feed can be compromised by poor feed management practices and it is difficult to get good performance from a poor feed, even with good feed management. Because of this dilema, the careful selection and management of feed is critical to best practice management of all intensive aquaculture.

From the modelled energy demands determined using the revised bio-energetic model the amount of feed to be fed to achieve the predicted growth can be defined based on the digestible energy density of the feed being fed. From the present model as feed digestible energy density increases, the amount required to be fed to the fish declines (Tables 6.6 and 6.7) and of course there are significant FCR and environmental benefits to be derived from this improved efficiency.

As with other models and feed table, as function of fish size, while there is a proportional decline in feed demand as the fish get larger (i.e. smaller fish will eat a higher percent of their bodyweight per day), the overall amount required increases as the size of the fish increases (Williams and Barlow, 1998). However, in contrast to most other models as an increase in temperature occurs there is also increases in feed demand, but the increase follows 3rd order polynomial function which factors in the thermal constraints that are deficient in many of the other models, or in some cases where the equations used exacerbate their inaccuracies above certain thermal ranges.

6.4.4 Comparison of iteratively determined protein requirements with empirical data

One of the outcomes of the development of this model is the capacity to determine the protein and nutrient demands, in terms of a function of diet digestible energy density (Table 6.5). However, it should be noted that this assessment does not consider any potential effects of temperature variation on diet composition, and is based on growth occurring at the thermal optimal region, not sub- or supra-optimal temperature ranges. The table demonstrates that as diet digestible energy density increases, so too does the protein concentration required to achieve the projected growth. Concomitant with this, as the size of a fish increases its requirement for protein (g/MJ) decrease, resulting in a lower digestible protein concentration required for larger fish when fed diets of similar digestible energy density.

Based on the equation: $y = 69.48x^{-0.186}$, $R^2 = 0.999$, a range of protein to energy requirements (y) can be determined iteratively from the fish's liveweight (x). When these values are compared to empirically determined data the values can be seen to be very close. For a 75 g

fish the iteratively determined requirements were estimated to be optimal (maximal) at 31 g DP/MJ DE compared to empirically determined requirements for similar sized fish (76 g) at 29.5 g/MJ (Williams and Barlow, 1999). In an additional study with larger fish Williams et al. (2003) empirically determined that the optimal digestible protein to digestible energy ratio for 230 g barramundi was 26.8 g/MJ. This compares with the model predicted value of 25.5 g/MJ. These two independent assessments support that the iterative model is highly consistent with empirically determined data and therefore is likely to be reasonably robust at other fish sizes. However, clearly an assessment of the DP:DE values for larger fish (>1000g) would consolidate this assessment.

It was also shown that potential production efficiencies can be made through the tailoring feed type to specific production phase. This concept is best depicted by Table 6.5 and Figures 6.8 and 6.9. Ideally the fish should be fed with the most relevant, balanced diet for its particular growth phase. Excesses or deficiencies during this management process cost financially either in terms of lost nutrients or loss growth respectively. It could also be argued that excesses also contribute an environmental cost. In a practical sense it can be understood that some slight "over-catering" in nutrient specifications is likely because of the relative costs of lost growth are far more than those of lost nutrients. Despite this there are still clear gains to be made in better tailoring feed specifications and feed ration sizes according to specific fish sizes (Glencross, 2006).

During later stages of the production cycle, when the fish become more energy dependent and comparatively less nutrient dependent for growth, the only way such an energy density can be maintained is with increasing levels of dietary fat (39.5 kJ/g) or maintaining high dietary protein (23.6 kJ/g) levels. Some carbohydrate (starch: 17.3 kJ/g) can be used, but it is generally poorly utilised by fish such as barramundi and also has less energetic value that the other two options. While a similar scenario has developed within the salmonid farming industry where some diets have fat levels exceeding 35%, it is not known if barramundi can tolerate and utilize diets with fat levels in excess of 22% (Johnsen and Wandsvik, 1991; Williams and Barlow, 1998). The evidence presented in this study suggests that if they can well utilize fat above this level then it may be worthwhile exploring the potential of examining the utilization of higher fat levels, especially for larger (>1000g) fish. However, it should be kept mindful that using and storing high-fat diets in a hot tropical environment will also require better storage and handling protocols that are normally used in this industry.

6.4.5 Limitations to the application of modelling

Like all models, the one presented has the potential to be useful, but is still far from optimal. Therefore caution must be applied when using features of the model or applications derived from it. Much of the recent progress in aquaculture excretion modelling has come from an improved understanding of the dynamics of energy and nutrient utilization in fish. Clearly the use of nutrient and energy flow models, such as those by Cho and Bureau (1998) and Lupatsch and Kissil (1998) and more recently Glencross et al (2006), has improved our ability to understand these processes and developed empirical models based on a series of empirically determined parameters. However, like most models of biological systems, the present models are incomplete in their assessment of growth and nutrient utilization processes. Some of the limitations are considered below:

The present model assumes that growth is only time, initial weight and temperature dependent. It is generally assumed that such responses are genetically determined and variability among

and within species may account for the subtle and not-so subtle variations seen in the published data (Azevedo et al., 1998; Rodehutscord et al., 2001; Lupatsch et al., 2003; Glencross and Felsing 2006). Clearly, there is capacity to develop functions that recognize the growth limiting aspects of critical nutrient limitations. An add-in such as this would allow the theoretical assessment of diet formulations and predict performance based on both environmental and nutritional constraints. Further development of this capacity of the model will also allow a clear mechanism for hypothesis testing and development for nutrition R&D. It would also refine the model to be a more mechanistic rather than empirical in nature as has been the case to date.

The discrepancies in the values of metabolic weight coefficients and exponents determined in the present study and that of others demonstrates the potential variability in such models. Apparently small differences in things like the weight exponent from 0.80 to 0.86 have a significant effect on the determination of maintenance energy demands, especially if the coefficient to the metabolic demand equation is not adjusted each time an exponent varies.

A further additional function of oxygen demand required also has the potential to be built into the model. Oxygen consumption is highly dependent on metabolic rate and energy consumption and because this is known it should be feasible to provide an estimate of daily requirement. By establishing the efficiency of oxygen extraction from the water and the efficiency of transfer in barramundi it should also be feasible to determine the critical water oxygen saturation levels required. This component could be further accentuated by examining the relationship between food consumption and oxygen consumption. Recent data has identified that oxygen debt does not limit protein or energy utilisation efficiency, but has a feedback mechanism to limit feed (energy and/or protein) intake (Glencross, unpublished). Notably it is also known that the rate of oxygen consumption is not constant, but rather demand increases during periods of digestion (Jobling and Davis, 1980; Jobling, 1981). Being able to gain an enhanced understanding of this facet of fish metabolism and incorporating it into a model would have considerable merit for aquaculturists operating at potentially oxygen sensitive regimes, such as low water dissolved oxygen and high stocking densities.

Despite the advancement of such models, with better-defined empirical equations and the addition of more parameters, there still remains a need to move towards mechanistic models, rather than relying on current empirical models. While the empirical models like the one presented here provide a reasonably reliable way of predicting production, feed management and feed design issues, they do not biologically describe any specific process in terms of molecular function and/or rate limiting processes that fundamentally have to underpin the biology being observed. The Fish Pr-FEQ model (Cho and Bureau, 1998) was somewhat closer to a mechanistic model in the sense that it categorised energy partitioning more succinctly, but it still required a correction factor to provide a sensible output. This single fact identifies a key limitation with that model. What is required is further analysis of what specific molecular or biochemical reaction, pathway or series or reactions and then to relate that to the specific model parameters so these parameters can be used to describe a specific reaction or series of reactions, rather than some “guestimated”, overarching function that relates to no specific thing other than an empirically determined parameter. Such work will require a close integration of nutritional, physiological, biochemical and molecular studies.

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6.7 Tables and Figures

Table 6.1 Experiment diet formulation and chemical composition.

	Small (3mm)	Large (5mm)
Formulation (g/kg)		
Fish meal a	700	700
Fish oil a	150	150
Pre-mix vitamins b	5	5
Wheat a	144	144
Yttrium Oxide c	1	1
Composition (g/kg DM unless otherwise detailed)		
Dry Matter (g/kg)	903	928
Crude Protein	524	539
Total Lipid	233	206
Ash	123	124
Phosphorus	21	19
Energy (MJ/kg DM)	24.0	23.7
Sum of Amino acids	522	522
Alanine	35	34
Arginine	26	26
Asparagine	52	51
Cysteine	7	7
Glutamine	78	78
Glycine	31	31
Histidine	15	15
Isoleucine	23	25
Leucine	43	44
Lysine	42	42
Methionine	18	18
Phenylalanine	22	22
Proline	32	32
Serine	24	24
Taurine	3	3
Threnine	28	28
Tyrosine	17	16
Valine	24	24

[a] Sourced from Skretting Australia, Cambridge, Tasmania, Australia. [b] Vitamin and mineral premix sourced from DSM Nutrition, Goodna, Queensland, Australia: includes (IU/kg or g/kg of premix): Vitamin A, 2.5MIU; Vitamin D3, 0.25 MIU; Vitamin E, 16.7 g; Vitamin K,3, 1.7 g; Vitamin B1, 2.5 g; Vitamin B2, 4.2 g; Vitamin B3, 25 g; Vitamin B5, 8.3; Vitamin B6, 2.0 g; Vitamin B9, 0.8; Vitamin B12, 0.005 g; Biotin, 0.17 g; Vitamin C, 75 g; Choline, 166.7 g; Inositol, 58.3 g; Ethoxyquin, 20.8 g; Copper, 2.5 g; Ferrous iron, 10.0 g; Magnesium, 16.6 g; Manganese, 15.0 g; Zinc, 25.0 g. [c] Sourced from Stanford Materials, Aliso Viejo, USA.

Table 6.2 Apparent digestibility of the same diet formulation when fed to fish of two size classes.

	SMALL (~15 g/fish)	LARGE (~400 g/fish)
Dry matter	0.888	0.937
Lipids	0.950	0.999
Protein	0.956	0.966
Energy	0.940	0.975
Sum of Amino Acids	0.983	0.976
Alanine	0.996	0.983
Arginine	1.000	0.992
Asparagine	0.958	0.957
Cysteine	0.953	0.935
Glutamine	0.995	0.985
Glycine	0.969	0.968
Histidine	1.000	0.994
Isoleucine	1.000	0.979
Leucine	0.998	0.977
Lysine	0.997	0.985
Methionine	1.000	0.987
Phenylalanine	1.010	0.985
Proline	0.862	0.916
Serine	1.000	0.984
Taurine	1.000	0.986
Threnine	0.963	0.967
Tyrosine	1.000	0.993
Valine	1.000	0.988

Tryptophan not determined

Table 6.3 ANOVA Table of digestibility effects of fish size and digestibility parameter.

	SS	Degrees of Freedom	MS	F	p
Intercept	128.2667	1	128.2667	150902.3	0.000000
Parameter	0.1826	22	0.0083	9.8	0.000000
Size	0.0006	1	0.0006	0.7	0.398057
Parameter x Size	0.0310	22	0.0014	1.7	0.050416
Error	0.0773	91	0.0008		

Table 6.4 Growth and feed utilisation in fish of two size classes.

	Small fish treatments						Pooled
	s0	**s1**	**s2**	**s3**	**s4**	**s5**	**SEM**
Initial weight (g/fish)	14.6	14.9	14.5	14.5	14.5	15.0	0.05
Final weight (g/fish)	12.3 [a]	17.6 [ab]	22.6 [bc]	26.8 [cd]	31.9 [d]	49.5 [e]	3.64
Gain (g/fish)	-2.3 [a]	2.7 [ab]	8.1 [bc]	12.3 [cd]	17.3 [d]	34.4 [e]	3.61
Growth rate (g/fish/d)	-0.09 [a]	0.10 [ab]	0.30 [bc]	0.45 [cd]	0.64 [d]	1.28 [e]	0.13
FCR (g feed/g gain)	0.00 [a]	0.96 [b]	0.64 [c]	0.63 [c]	0.60 [c]	0.63 [c]	0.09
Intake (g/fish)	0.0 [a]	2.6 [b]	5.2 [bc]	7.7 [cd]	10.3 [d]	21.5 [e]	2.12

	Large fish treatments						Pooled
	L0	**L1**	**L2**	**L3**	**L4**	**L5**	**SEM**
Initial weight (g/fish)	411.9	414.1	410.4	410.8	412	408.7	2.96
Final weight (g/fish)	381.2 [a]	428.2 [ab]	466.1 [bc]	502 [cd]	525.7 [de]	567.7 [e]	18.80
Gain (g/fish)	-30.7 [a]	14.1 [ab]	55.7 [bc]	91.2 [cd]	113.7 [de]	158.9 [e]	19.06
Growth rate (g/fish/d)	-1.14 [a]	0.52 [ab]	2.06 [bc]	3.38 [cd]	4.21 [de]	5.89 [e]	0.71
FCR (g feed/g gain)	0.00 [a]	2.21 [b]	0.91 [c]	0.82 [c]	0.85 [c]	0.77 [c]	0.23
Intake (g/fish)	0 [a]	25.3 [b]	50.5 [bc]	74.9 [cd]	95.6 [d]	122.9 [e]	12.53

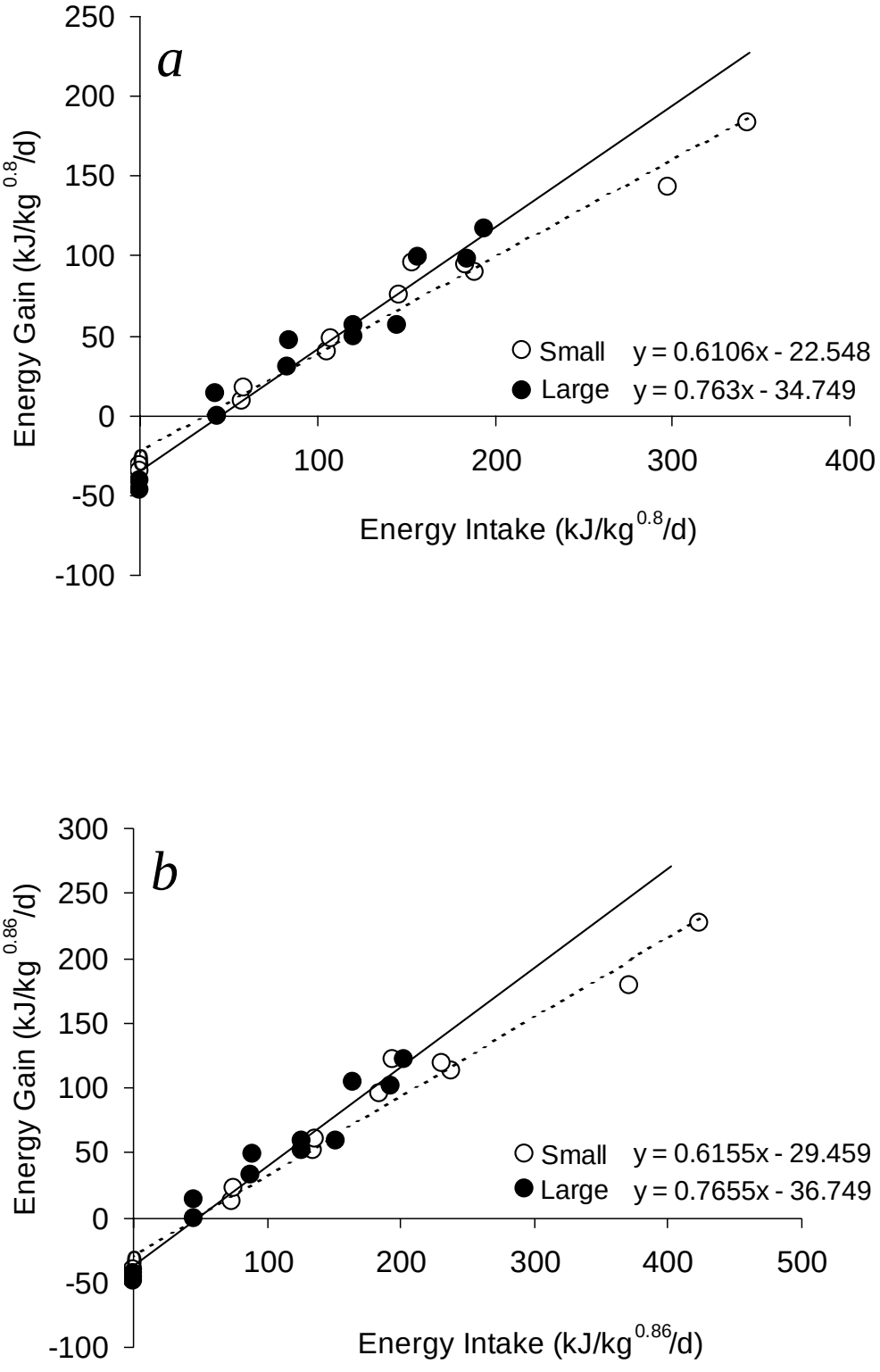

Figure 6.1 Energy gain with varying digestible energy intake by barramundi of two different size classes. Figure a shows response modelled using an exponent of 0.80. Figure b shows response modelled using an exponent of 0.86.

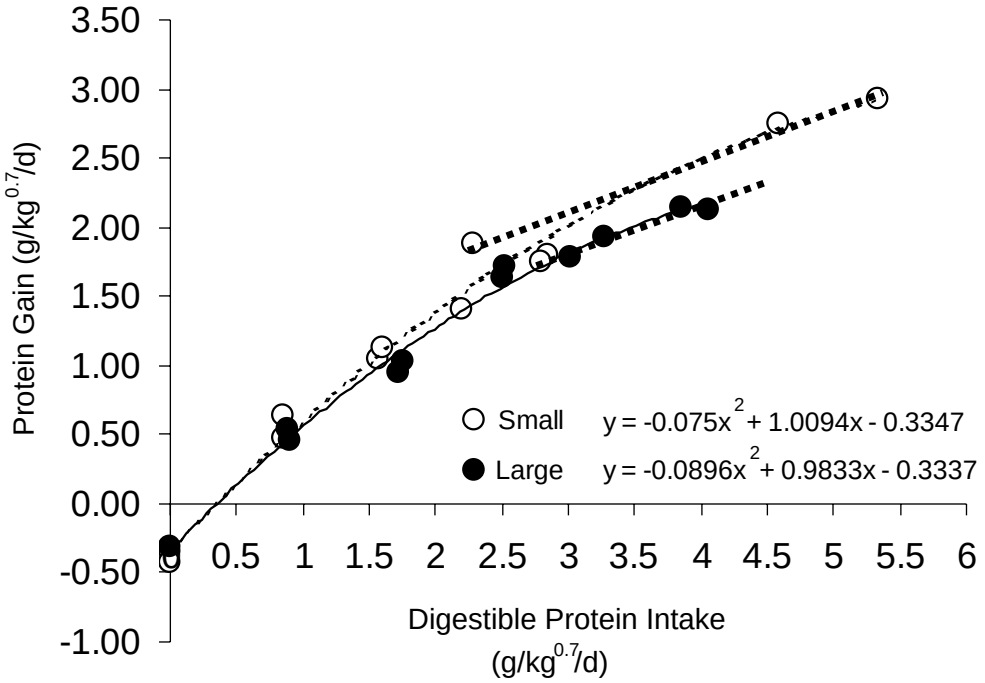

Figure 6.2 Protein gain with varying digestible protein intake levels by barramundi of two different size classes. A slope coefficient of y = 0.48*x* was determined at the highest protein intake levels consistent with the featured partial regressed lines.

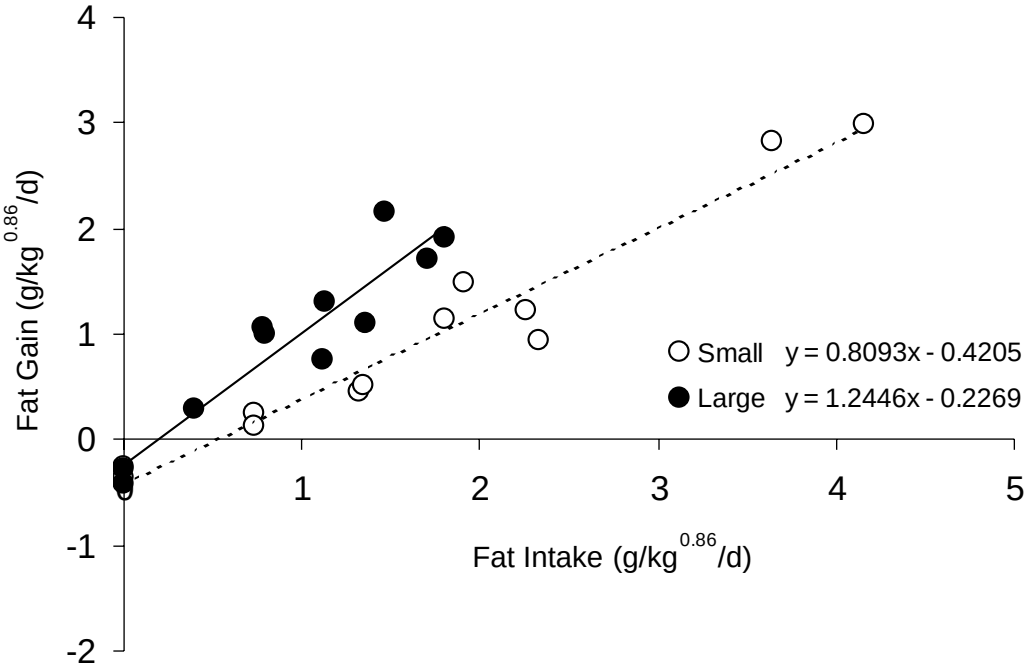

Figure 6.3 Fat gain with varying digestible fat intake levels by barramundi of two different size classes.

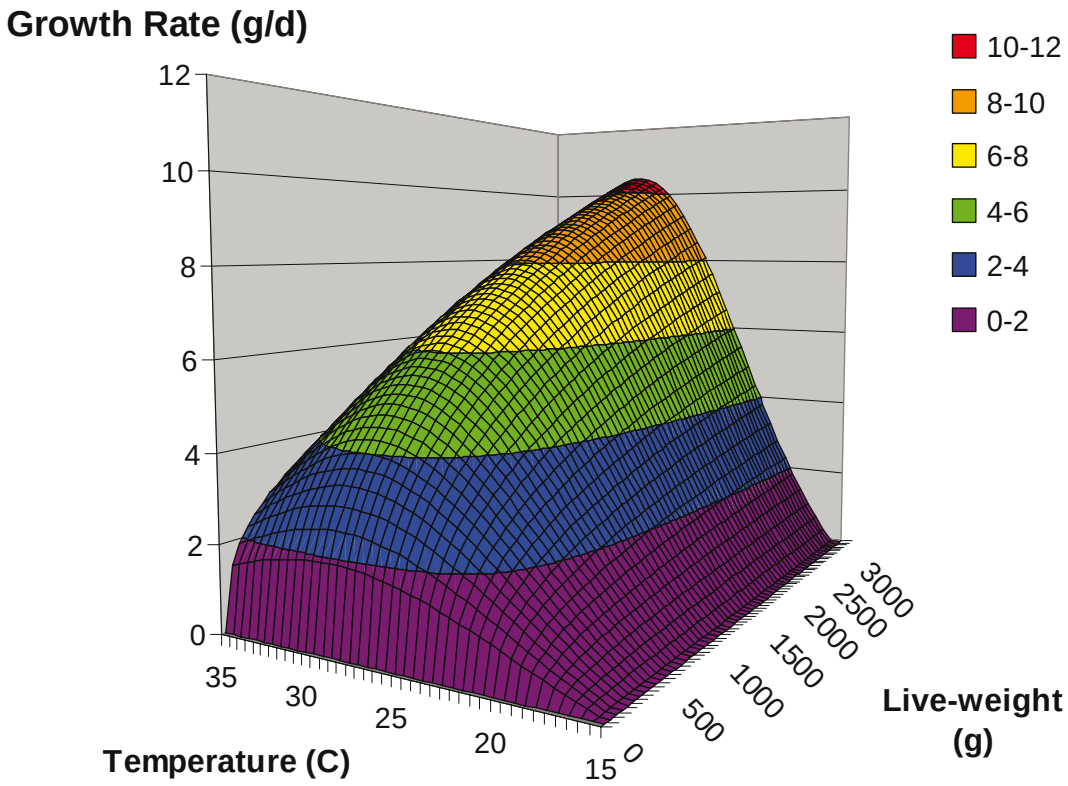

Figure 6.4 Growth model for barramundi growth (g/d) from 15°C to 35°C and up to fish of 3000 g in weight.

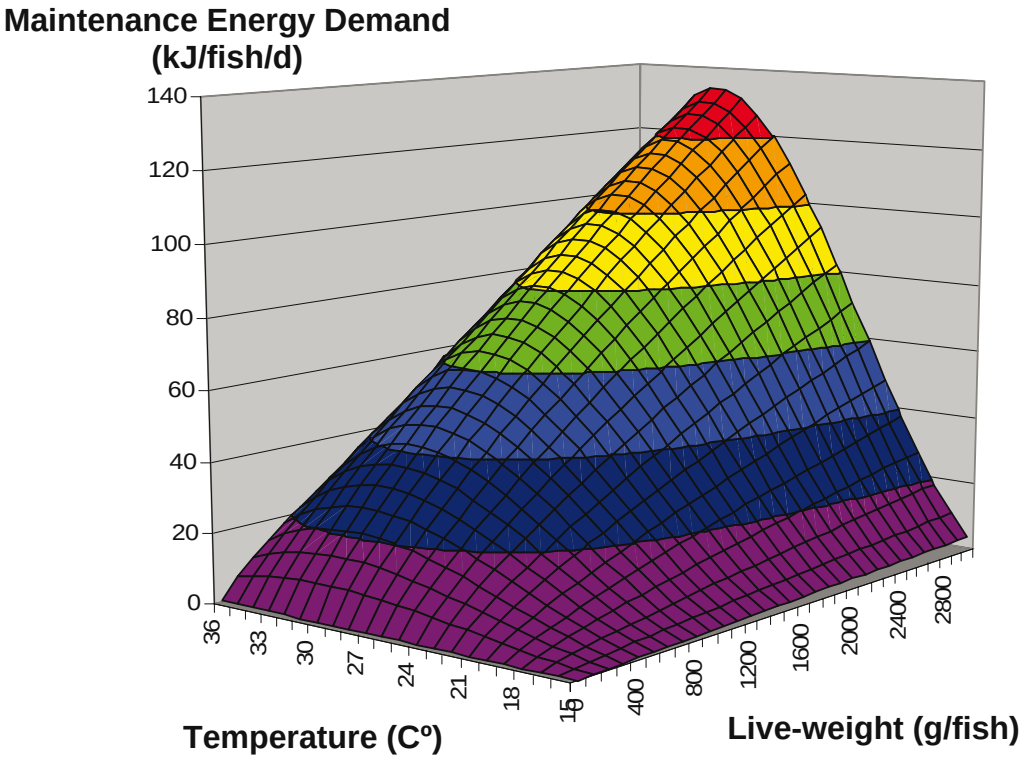

Figure 6.5 Maintenance energy demand model for barramundi (kJ/d) from 15°C to 36°C and up to fish of 3000 g in weight.

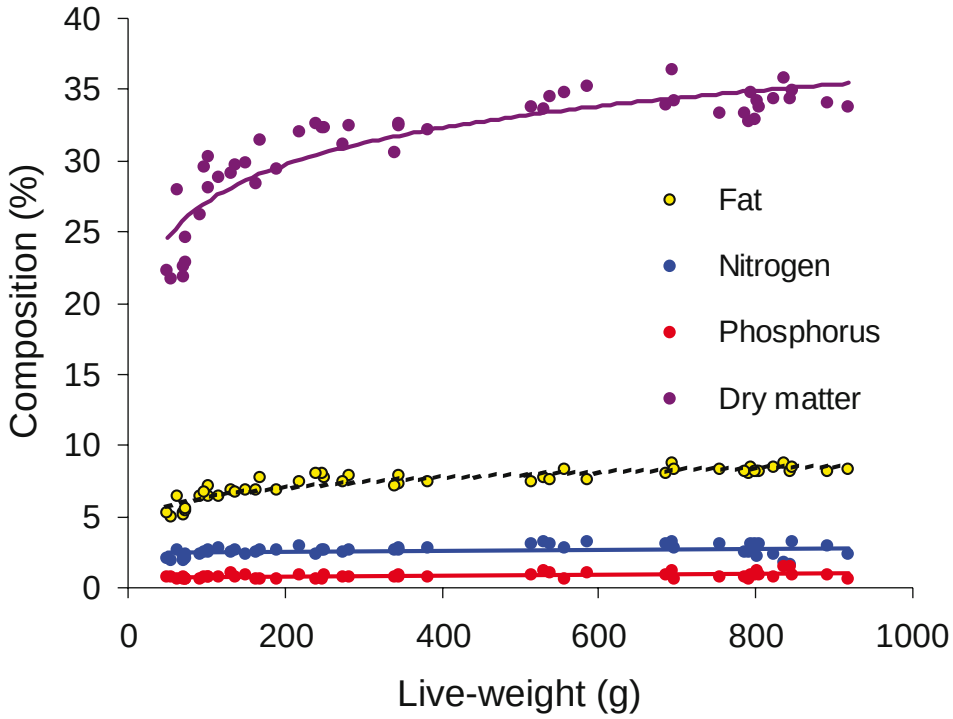

Figure 6.6 Variation in barramundi composition of dry matter (1/moisture), fat, nitrogen (protein /6.25) and phosphorus, with fish size.

Table 6.5 Iteratively determined diet specifications for barramundi growth at 30°C based on redefined growth and utilisation parameters. Circled are the typical commercial diet specifications presently being used in the Australian barramundi industry. Shown is the basis for the calculations of the diet compositions as well as expected FCR for each formulation and the amount of feed required daily at each energy density to achieve the modelled growth. In each iterative diet option is indicated the digestible protein demand. Metabolic and Protein body weight exponents were kept at accepted values of 0.80 and 0.70 respectively because the differences observed in the present study were not of significant enough magnitude to warrant disputing these generic values.

Fish weight (g)	**10**	**75**	**100**	**500**	**1000**	**2000**
Growth (g/d)	1.96	3.66	4.00	6.59	8.17	10.13
Energy requirement						
Metabolic BW (kg)^0.80	0.03	0.13	0.16	0.57	1.00	1.74
DEmaint (kJ/fish/d)	1.39	6.98	8.79	31.86	55.48	96.59
Energy gain (kJ/fish/d)	8.91	22.21	25.31	52.48	71.85	98.37
DEgrowth (kJ/fish/d)	13.11	32.67	37.21	77.18	105.66	144.66
DEtotal (kJ/fish/d)	14.50	39.65	46.01	109.04	161.14	241.25
Protein requirement						
Protein BW (kg)^0.70	0.040	0.163	0.200	0.616	1.000	1.625
DNmaint (g/fish/d)	0.02	0.07	0.09	0.28	0.45	0.73
Protein gain (g/fish/d)	0.33	0.61	0.67	1.10	1.36	1.68
DNgrowth (g/fish/d)	0.68	1.26	1.38	2.27	2.82	3.49
DNtotal (g/fish/d)	0.69	1.34	1.47	2.55	3.27	4.22
15 MJ DE diet - FCR	0.49	0.72	0.77	1.10	1.31	1.59
%BW intake	9.7%	3.5%	3.1%	1.5%	1.1%	0.8%
Feed intake (g/d)	0.97	2.64	3.07	7.27	10.74	16.08
Protein (%)	0.72	0.51	0.48	0.35	0.30	0.26
16 MJ DE diet - FCR	0.46	0.68	0.72	1.03	1.23	1.49
%BW intake	9.1%	3.3%	2.9%	1.4%	1.0%	0.8%
Feed intake (g/d)	0.91	2.48	2.88	6.81	10.07	15.08
Protein (%)	0.77	0.54	0.51	0.37	0.32	0.28
17 MJ DE diet - FCR	0.44	0.64	0.68	0.97	1.16	1.40
%BW intake	8.5%	3.1%	2.7%	1.3%	0.9%	0.7%
Feed intake (g/d)	0.85	2.33	2.71	6.41	9.48	14.19
Protein (%)	0.81	0.57	0.54	0.40	0.34	0.30
18 MJ DE diet - FCR	0.41	0.60	0.64	0.92	1.10	1.32
%BW intake	8.1%	2.9%	2.6%	1.2%	0.9%	0.7%
Feed intake (g/d)	0.81	2.20	2.56	6.06	8.95	13.40
Protein (%)	0.86	0.61	0.58	0.42	0.37	0.32
19 MJ DE diet - FCR	0.39	0.57	0.61	0.87	1.04	1.25
%BW intake	7.6%	2.8%	2.4%	1.1%	0.8%	0.6%
Feed intake (g/d)	0.76	2.09	2.42	5.74	8.48	12.70
Protein (%)	0.91	0.64	0.61	0.44	0.39	0.33
20 MJ DE diet - FCR	0.37	0.54	0.57	0.83	0.99	1.19
%BW intake	7.3%	2.6%	2.3%	1.1%	0.8%	0.6%
Feed intake (g/d)	0.73	1.98	2.30	5.45	8.06	12.06
Protein (%)	0.96	0.67	0.64	0.47	0.41	0.35

Table 6.6 Daily feeding ration table for a 16 MJ/kg DE diet fed to fish of various sizes at various temperatures.

	TEMPERATURE			
16 MJDE	**20**	**24**	**28**	**32**
Weight	**g/fish**			
10	0.48	0.70	0.86	0.92
30	0.86	1.25	1.51	1.55
100	1.66	2.40	2.83	2.80
300	3.16	4.45	5.15	4.92
500	4.30	5.98	6.85	6.48
1000	6.63	9.02	10.20	9.51
1500	8.60	11.54	12.94	11.98
2000	10.37	13.77	15.35	14.17
3000	13.56	17.73	19.62	18.03
Weight	**%BW**			
10	4.80	7.00	8.60	9.20
30	2.87	4.17	5.03	5.17
100	1.66	2.40	2.83	2.80
300	1.05	1.48	1.72	1.64
500	0.86	1.20	1.37	1.30
1000	0.66	0.90	1.02	0.95
1500	0.57	0.77	0.86	0.80
2000	0.52	0.69	0.77	0.71
3000	0.45	0.59	0.65	0.60

Table 6.7 Feeding ration table for an 18 MJ/kg DE diet fed to fish of various sizes at various temperatures.

	TEMPERATURE			
18 MJDE	**20**	**24**	**28**	**32**
Weight	**g/fish**			
10	0.43	0.63	0.77	0.82
30	0.76	1.11	1.34	1.38
100	1.48	2.13	2.53	2.49
300	2.81	3.96	4.58	4.38
500	3.82	5.32	6.09	5.76
1000	5.89	8.02	9.06	8.45
1500	7.64	10.25	11.50	10.65
2000	9.22	12.24	13.65	12.59
3000	12.05	15.76	17.44	16.02
Weight	**%BW**			
10	4.30	6.30	7.70	8.20
30	2.53	3.70	4.47	4.60
100	1.48	2.13	2.53	2.49
300	0.94	1.32	1.53	1.46
500	0.76	1.06	1.22	1.15
1000	0.59	0.80	0.91	0.85
1500	0.51	0.68	0.77	0.71
2000	0.46	0.61	0.68	0.63
3000	0.40	0.53	0.58	0.53

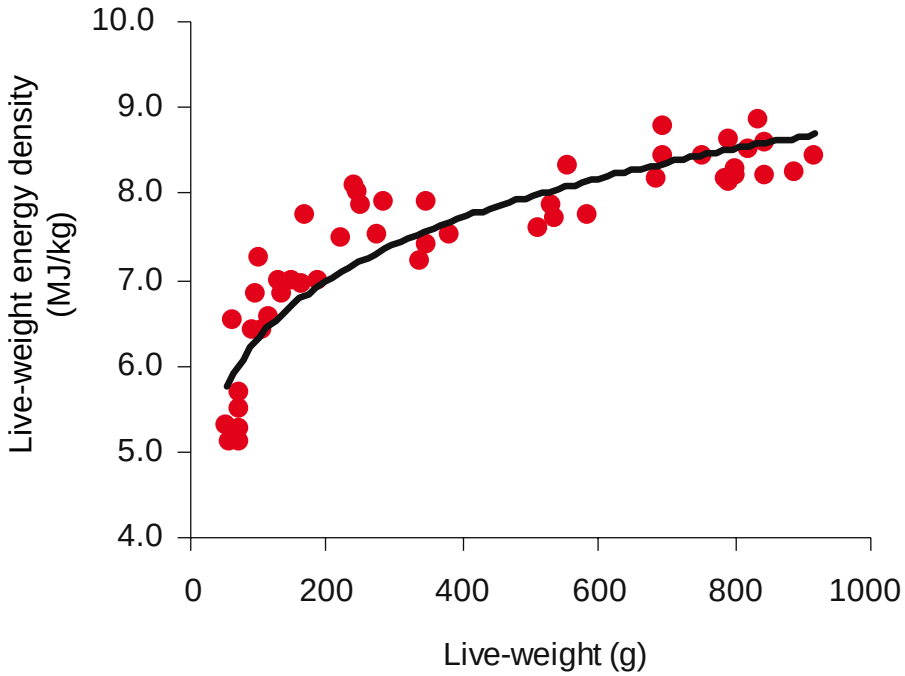

Figure 6.7 Variation in barramundi energy density with fish size.

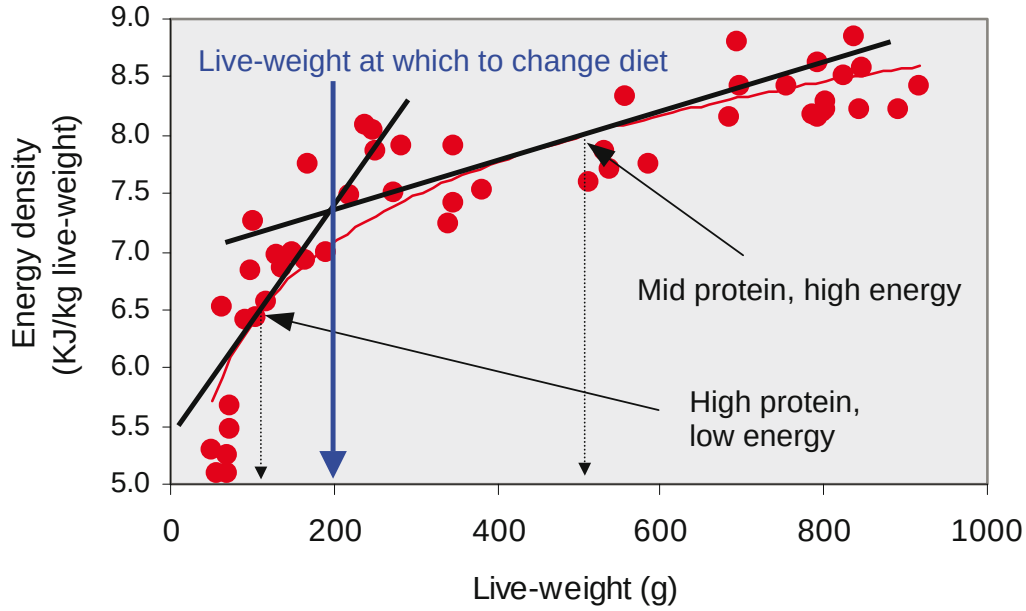

Figure 6.8 Practical assignment of diets based on energetic requirements of barramundi over the growth phase of 50 g to 900 g. Indicated (blue arrow) is the live-weight at which to change-over to different diet specifications.

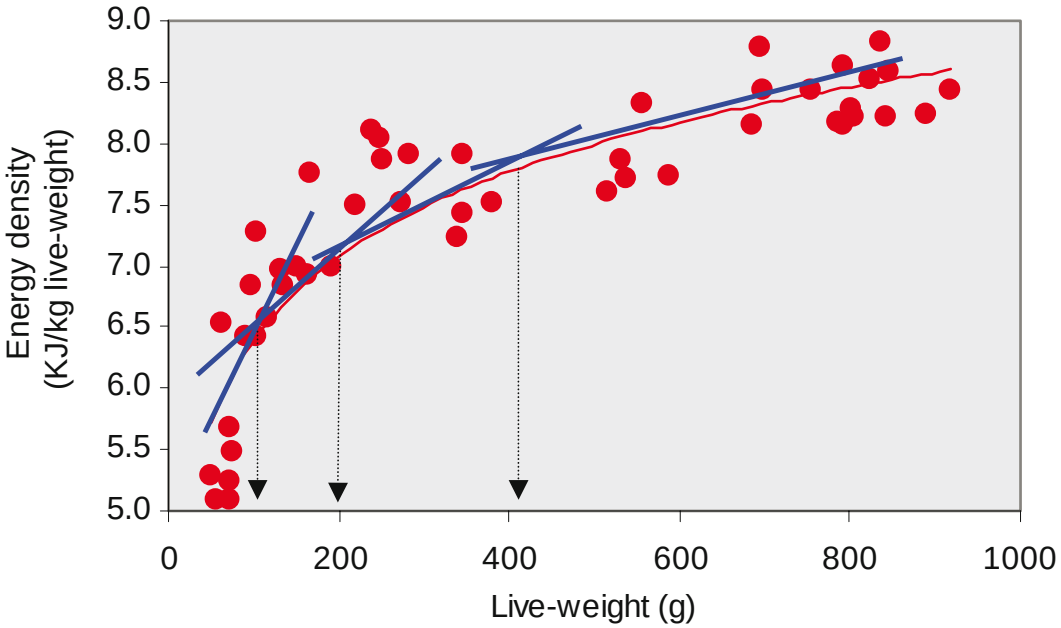

Figure 6.9 Theoretical assignment of diets based on energetic requirements of barramundi over the growth phase of 50 g to 900 g.

7. General Discussion

7.1 Introduction

This project was undertaken to define and resolve a range of issues affecting the viability of the barramundi cage aquaculture industry at Lake Argyle, in Western Australia's Kimberley region. Through a close working relationship with the industry a range of issues that were limiting viability and efficiency were identified. These included resolving problems associated with flavour quality management (muddy-flavour taint) of the fish that was affecting market viability, the management of a disease outbreak and in particular the occurrence of *Streptococcus iniae* within the farm, the long-term environmental impact and better management of feed use to improve production efficiency. As part of the project, a review point was included to justify the undertaking of the long-term environmental studies. The lack of any industry expansion by July 2005 triggered this clause and as a result of this the long-term environmental work was not initiated. However, the other three key objectives of the project were undertaken. Progress was made against all objectives undertaken and the findings of this project have numerous implications for improving barramundi aquaculture management throughout Australia and are not just limited to operations in Lake Argyle.

7.2 Outcomes and Implications

7.2.1 Flavour quality management

The first part of this aspect of the project was to verify the reports that had been passed back to the industry partner about the flavour quality of their fish. Initially this was based on an on-site sensory evaluation by farm staff. However, it was decided to conduct a more thorough independent study to confirm the presence of the problem and to also benchmark the farm product against some key competitor products.

The initial independent sensory evaluation found that it was possible to detect a significant muddy taint flavour in unpurged Lake Argyle fish, but that if the fish were purged using either of the two regimes tested then the presence of a muddy-flavour was indistinguishable from that in wild or farmed saltwater barramundi (Chapter 2). The unpurged Lake fish were also perceived to be less sweet than the other treatments (purged and saltwater farmed, and wild fish).

The study also demonstrated, that from a sensory perspective, that it was difficult to distinguish between wild and farmed barramundi (Chapter 2). While there were no discernable flavour or odour differences, some differences in texture and colour of the fillet were noted. The fillets of farmed barramundi had a distinctly greater level of greyness, while the wild fish fillet was perceived as being whiter. It is suspected that this effect is a degree of melaninisation of the muscle in farmed fish, though this remains to be confirmed (see Figure 7.1). Whether the effect occurs as a consequence of dietary or environmental factors is also still to be resolved (Glencross, 2006). The farmed fish were also perceived as less chewy and dry than the wild fish, but was also considered oilier and slightly mushier than the wild fish fillets. These findings dispel any notion that wild barramundi has superior sensory characteristics to that of farmed barramundi, from either freshwater or salt water.

From the initial independent sensory study, unpurged fish harvested from the lake were noted to be considerably darker in pigmentation than those fish that were brought to an indoor

facility, placed in 2000L tanks with a pale background for purging. While the purging per se is unlikely to have induced the skin colour change, the influence of background and change in ambient lighting is known to influence similar such parameters with other fish species (Booth et al., 2004). While the colour of the fish was not raised as a key marketing drawback, this problem has however been raised with other species as an issue and may be an issue to resolve to improve marketability of farmed barramundi in the future.

Following the confirmation of the presence of a muddy-taint flavour problem, it was decided to commission the development of a trained sensory panel at the Australian Centre for Food Technology (CFT; Hamilton, Brisbane, QLD). This was done to provide a further degree of independence, but also to utilise the specialist expertise and facilities available at CFT (Chapters 2 and 3).

In a condensed repeat of the initial independent study, purged and unpurged fish were compared against each other. In the CFT evaluation by a trained sensory panel a list of seven aromas, seven primary flavours and three aftertaste flavours were evaluated. This work confirmed unequivocally that a muddy-flavour problem existed with fish from Lake Argyle and therefore necessitated further development work to identify specific causes, predisposing factors and potential remedies. Based on the findings from other studies with similar problems it was speculated that the cause of the muddy-flavour problem was attributable to the presence of geosmin and 2-methylisoborneol in the lake water (Lovell, 1983; Lovell and Broce, 1985; Howgate, 2004; Robertson et al., 2006; Robin et al., 2006).

The threshold levels of geosmin (GSM) and 2-methylisoborneol (MIB) in ambient water, for sensory detection of muddy-flavour taint, were both assessed and found to be similar to those reported for other species (Yaprayoon and Noonhorm, 2000; Howgate, 2004; Robertson et al., 2005). While there were significant correlations with both compounds and the muddy-flavour taint, the relationship with MIB was considerably stronger, in part due to the higher levels of this compound in the water. Based on this it was proposed that MIB was the likely compound causing the muddy-flavour taint in barramundi from Lake Argyle. However, this still remains only a strongly supported hypothesis and needs to be directly confirmed if these results are to be applied more broadly to other industry sites throughout Australia.

The sensory comparison of large (~2000g) and small (500g) fish was also undertaken to determine whether fish size was a predisposing factor to the muddy-flavour taint. It was confirmed that larger fish are more susceptible to this problem. This finding could mean that the farming of small, plate-size fish is a more viable proposition in its own right in places, like Lake Argyle, that are subject to geosmin and MIB taint in the water. The farming of the smaller fish size would reduce risk associated with flavour taint, but is unlikely to eliminate it.

Within the fillet of large (~2000g) fish some variation in flavour attributes were also noted. This study was undertaken to allow some standardisation of the sensory evaluation process. The fillet was divided into three sections: belly, dorsal and tail sections. The muddy-flavour taint was significantly greater in the belly section of the fish. This study was also mirrored by a small, untrained panel at the Department of Fisheries (WA) that also arrived at the same result. The fillet samples that were assessed at the Department of Fisheries were also sampled for total lipids analysis. Distinct differences in lipid content of the various sections of the fillet were observed and the higher lipid levels of some sections correlated with greater muddy-flavour taint.

The uptake of muddy-flavour taint by purged fish placed in GSM and MIB tainted water was consistent with typical first-order rate-kinetic mediated transfer of the compounds, where the

concentration of the compound being transported is the primary determinant of the rate at which it is transported (Withers, 1992). Uptake of the muddy-flavour taint was rapid, with a plateau reached within three hours of the fish being placed in the tainted water. The deterioration in "fresh" flavour was essentially the inverse of that observed of the muddy-taint flavour.

The reciprocal of this study was the rate of depuration study. In this study fish from the lake were placed in GSM and MIB free water and fish sampled every 24 hours to assess their level of muddy-flavour taint by sensory evaluation. Essentially, this study showed that the rate of depuration was substantially longer than that of uptake. The decline in muddy-flavour taint was also consistent with rate-turnover kinetics (and exponential function) and showed that the taint halved approximately every 36 hours. In retrospect inclusion of a long-term purged fish as a control/reference in this study would have allowed some indication of how close the fish were after a defined period of purging, to a muddy-flavour taint-free fish.

7.2.2 Water quality management in purging systems

Because of the necessity to hold fish in a confined water volume for the purging process, accessory studies on water quality during the purging experiments were also undertaken. Oxygen concentration was initially found to be low and with time rose to around 6.0 mg/L and stabilised. This effect was independent of flow-rate of the purging tank. The initial lower concentration is suspected to be attributed to higher oxygen consumption of the fish immediately after handling, as other studies have shown that at least 5 to 6 hours is required for the fish to return to a basal oxygen consumption rate (Neill and Bryan, 1991; Glencross and Felsing, 2006). Ammonia build-up was negligible, although initially higher at the lower flow rate, over time it also showed no difference. This is consistent with what is widely known of unfed fish, in that post-feeding ammonia excretion increases during the 6 to 12 hour period following feeding, but is otherwise maintained at a basal level (Hepher, 1988; Russo and Thurston, 1991).

In subsequent trials the build up of ammonia in the purging tanks was observed to be flow-rate dependent. A two-stage build up of ammonia was also observed, with the first suspected to be a higher level due to excretion of ammonia by the fish following feeding, and the second stage as a consequence of the build up of faecal matter in the tank. Oxygen levels in the water were also somewhat flow-rate dependent, but this was not as pronounced as the ammonia effect.

As was generally anticipated, the ammonia concentrations in the purging systems were highly dependent on the stocking density, with higher stocking densities producing higher water ammonia concentrations (Russo and Thurston, 1991). The variability in ammonia concentrations over time was also similar to that observed in the second study, which given that the fish were unfed whilst in the purging system, is again likely to be a build-up of residual excretion from prior feeding and subsequent faecal loading (Phillips et al., 1991). Dissolved oxygen (DO) concentrations were also dependent on stocking densities and were generally consistent with the biomass demand for oxygen (Glencross and Felsing, 2006). The high dissolved carbon dioxide (CO_2) concentrations at the commencement of the study are indicative of a high level of energy expenditure by the fish post-handling, probably associated with the stress of handling (Schreck and Li. 1991). Consistent with that observed for oxygen consumption, with increasing stocking density, there was a corresponding increase in CO_2 concentrations.

7.2.3 Water preparation for purging systems

Understanding the variability inherent in GSM and MIB levels in the Lake Argyle system is also a key aspect of managing the problem. This affects not only the severity of the taint uptake by the fish, but also the requirements for preparing lake water for use in any purging regimes.

Rainfall and subsequent lake inflow is highly variable in the Lake Argyle system. Peak rain periods occur from November to April each year, with inflows occurring up to a month or more later. These inflow events have also been shown to be correlated with algal bloom events and increases in total phytoplankton and cyanobacteria levels. An increase in the levels of these organisms is generally concomitant with an increase in the muddy-flavour problem due to the excretion of the terpenoid metabolites GSM and MIB from the phytoplankton and cyanobacteria (Howgate, 2004). Elevated water temperatures have also been reported to exacerbate GSM and MIB production by phytoplankton and cyanobacteria (Dionigi and Ingram, 1994). This may provide some explanation for the elevated GSM and MIB levels at various times of the year, though other effects such as increase water and nutrient inflow into the Lake Argyle system cannot be discounted.

Limited spatial variability in MIB and especially GSM was observed throughout the lake. Generally, variability was generally more pronounced as a function of sample depth than location at which it was collected, with lower levels in deeper waters almost always. The more intensive assessment of MIB and GSM undertaken near the main barramundi production site showed that concentrations of MIB were consistently higher than those of geosmin throughout the survey period. Peaks in MIB greater than 12 ng/L were observed during several periods throughout the survey period (May 2004, September 2004 and March 2005), though there was limited variation in GSM levels observed throughout the study period.

Surveys of phytoplankton in Lake Argyle in the vicinity of the production site showed the presence of over 120 freshwater algae and cyanobacteria species. Almost half of the observed phytoplankton species identified are known GSM and/or MIB producers (Izguirre et al., 1982; Hallegraff, 1992). About 40% of the cyanobacteria species identified are known GSM and/or MIB producers (Rashash et al., 1995; Oliva et al., 2001). This suggests that the presence of these compounds in the Lake Argyle system is widely endemic and that broad scale water treatment is probably unviable.

Rainfall preceding the occurrence of the sensory problems encountered in Lake Argyle in late 2003, early 2004 highlighted the environmental influences on the presence of GSM and MIB (Chapter 4). The high rainfall event recorded in late 2003/early 2004 preceded a clear incidence of phytoplankton and cyanobacteria blooms that occurred a few months later. These blooms also corresponded with elevated levels of MIB, but no changes in the levels of GSM. The relationship to the rainfall event is based on one of an exacerbation of conditions predisposing the lake to algal blooms. It is suspected that the high rainfall and subsequent inflow events dramatically increase the nutrient flux into the Lake Argyle system and as a consequence the blooms resulted. In light of this, future farm operations should be mindful of the potential impacts of heavy rainfall events and the consequences they bring. In this case there appeared to be a delay in bloom and muddy-taint by a period of two to three months. This delay is suggestive of the time course taken for inflow from some distance upstream in the catchment before it reaches the embayment within Lake Argyle in which barramundi farming is based. Clearly further studies are required to gain a better understanding of the nature of the nutrient and water discharge flux within Lake Argyle to better manage this issue and long-term environmental sustainability of any expanding barramundi production industry.

To resolve the problem of muddy-flavour taint the fish need to be purged in a source of geosmin and MIB free water. Clearly the ability to obtain such water as close as possible to the fish production site is most advantageous. Algicides have been reported to reduce the productivity of certain GSM and MIB producing phytoplankton in other studies (Dionigi, 1995; Schrader et al., 1998; Schrader et al. 2004; Schrader et al., 2005). Therefore the effectiveness of a range of treatments in reducing MIB /GSM levels were tested. These included aeration, a flocculant and algaecides (Cupricide and Coptrol – both registered for use in potable water sources). However, despite Polyaluminium chloride (the flocculant), Cupricide and Coptrol being registered for use in potable water sources, these additives did not result in substantially better outcomes than just aeration alone.

Further studies investigated the effectiveness of a flocculating agent to reduce the level of MIB and GSM. Polyaluminium chloride (Al_2O_3) (PAC) was further evaluated in a second study that tested the effectiveness of a higher concentration of PAC to reduce the levels of MIB and GSM in lake water samples. Results showed that addition of PAC at 80 mL/m^3 did not provide any advantage over addition of 16 mL/m^3, which previously did not provide any additional reduction in MIB and GSM than that achieved through aeration alone. From these studies it can be concluded that there is little point tin the use of PAC to reduce the levels of MIB in the lake water for depuration.

The pilot comparison showed that the use of aeration was the most cost effective strategy in depurating lake water. Although the algaecides had some effects on water MIB levels, their effect on geosmin was somewhat limited by the inherent low levels of geosmin present at the time of the study. Notably, in all treatments the levels of MIB were reduced most substantially within the first 24 hrs, with further reductions occurring more slowly, consistent with an exponential depuration/turnover process. Therefore, based on the findings of the studies in this report, it is suggested that using persistent aeration of the water for 24 hours or longer is the preferred option to depurate the water for use in purging of barramundi in Lake Argyle.

The aeration studies also showed that use of both diffusion and mushroom sprayer systems resulted in a faster decrease in MIB than use of just a diffusion system alone. However it remains to be determined if this effect was actually an effect of the mushroom sprayer per se or just a higher level of aeration of the water allowing a greater rate of depuration.

In the purging procedure proposed by the consulting engineers (SEMP Pty Ltd), the fish are to be purged for two days in a two-stage process. In this two-stage process the fish are initially purged in water depurated for a shorter period, before being further purged in water that has been depurated for longer. This was proposed to enable faster turn-around and water preparation. Based on a one-week production operation, the proposed procedure is:

a. Day 1, 8am – Commence filling three 1 ML storages.

b. Day 2, 8am – Continue filling. Commence aeration of all three water storages.

c. Day 3, 8am – Dose water storages with an algaecide if required. Continue aeration.

d. Day 4, 8am – Continue aeration.

e. Day 5, 8am – Continue aeration. Prepare purge liners 1, 2 and 3 and begin in-liner aeration. Transfer graded fish (for example plate-size, banquet and fillet) into each of three purge liners.

f. Day 6, 8am – Commence purging fish within the purge liners.

g. Day 7, 8am – Continue purging fish in purge liners with water form storage 2 and 3, commence refill of storage 1 and start water depuration (aeration) again.

h. Day 8, 8am – Harvest fish from the purge liners, refill water storage 2 and 3 and commence aeration (repeat cycle using shorted aerated storage first for purging the purge liner. This means that the last storage used will have received maximum aeration.

Based on this suggested operating procedure a seven-day cycle can be practically implemented to enable a weekly rotating harvest system. A range of system options were proposed in the engineering report (see appendix 8.3) that considered the more detailed design, system operations and constraints and provisional costings for system construction.

Fundamentally the work in the flavour quality management component of the project has identified the specific causes and highlighted some of the predisposing factors to the muddy flavour problem. In addition to this some potential remedies to reduce the incidence of the problem were proposed, tested and commercial scale applications designed based on the studies.

7.2.4 Pathogen and disease characteristics

The virulence and origin of the Lake Argyle strain of *Streptococcus iniae* remains uncertain. There is considerable strain variation in *S. iniae* and it cannot be discounted that it may be a site-specific strain present at Lake Argyle (Miller and Neely 2005). Notably some strains are primary pathogens with the ability to enter and multiply within host cells and cause apoptosis of macrophages (Zlotkin et al., 2003; Lahav et al., 2004). These virulent strains act by reducing the immune and inflammatory responses of infected fish and are more likely to cause higher mortality than commensal strains (Taylor et al., 2001; Zlotkin et al., 2003). In common with most pathogens, species vary in their susceptibility to the *S. iniae* (Fuller et al. 2001). Some species such as red drum and channel catfish were resistant but tilapia, and hybrid striped bass have been shown to be highly susceptible (Perera et al. 1997). The case study at Lake Argyle shows that barramundi are susceptible to *S. iniae*, this is consistent with other studies that have shown that as few as 100 ingested bacteria resulting in death of barramundi following an experimental infection (Bromage and Owens 2002).

Streptococcus iniae can be spread by fish to fish transfer between farms at least 2 miles apart (Colorni et al. 2002). Wild fish in the vicinity of a fish farm are a key potential reservoir and vector of infection making it impossible to eradicate the disease from caged fish (Zlotkin et al., 1998; Colorni et al., 2002). This is an important aspect of *S. iniae* management that needs careful consideration in the development and implementation of site-specific disease management plans. It also highlights the need to consider vaccination as key disease management strategy. However, despite the benefits from vaccination reducing mortality from the disease it cannot be used to completely eradicate it (Bachrach et al., 2001).

Streptococcus iniae can remain viable in mud and water for considerable periods of time and also can be a significant reservoir of infection in ponds and sea cages (Kitao and Iwata 1979; Perera et al. 1997). Cannibalism and the faecal-oral route of infection are thought to be the most important routes of infection for barramundi (Bromage and Owens 2002). A range of factors have been found to increase mortality from *S. iniae*, including; cohabitation, immersion, high stocking density, low dissolved oxygen and high nitrite concentrations (Shoemaker et al., 2000; Zlotkin et al., 2003).

Certain environmental conditions favour replication of *S. iniae* and sometimes can also increase fish stress, which then makes them more susceptible to outbreaks of disease. Water temperature

is likely to be important to the onset of clinical disease because *S. iniae* grow best in warmer conditions when water temperatures are above 20°C (Perera et al., 1997). Importantly, this means that most barramundi production occurs at temperatures conducive to growth of the bacteria. Once *S. iniae* has been introduced to an area it has not been possible to eradicate it. The disease can now be considered to be endemic in Lake Argyle.

7.2.5 Management of the farm to minimise disease outbreaks

One of the key farm management strategies to manage disease is to ensure that optimal stocking densities are not exceeded. Stress induced by high stocking densities and less than optimal water quality may result in fish developing a high prevalence of subclinical or overt disease. When susceptible species are infected with *S. iniae*, outbreaks of clinical disease often follow stressful events such as handling or a natural event that impairs water quality. Such an event was suspected in causing the initial outbreak of *S. iniae* at Lake Argyle in 2004, after a large flooding event in December 2003 / January 2004 caused an unseasonal lake turnover event and caused widespread resuspension of lake sediments.

The development of a disease management plan must be based on both prevention and control strategies. Such a plan must be carefully developed and implemented to minimise production losses from a range of potential diseases. A good plan should include strategies for identifying conditions that might pre-dispose to disease caused by *S. iniae*. Use of the Hazard Analysis and Critical Control Point (HACCP) plan, as used in the food industry, to identify risks and management strategies could prove useful to minimise the impact of significant risks (see Table 3.1).

Benchmarking feed intake, growth rates and feed conversion ratios of individual cages against a known optimal standard is another useful method to identify fish that are susceptible or showing otherwise non-clinical signs of disease. Use of the growth model (Chapter 6) would be advantageous in this regard as it would allow for an easy method of comparing observed growth and feed utilisation against expected. As soon as monitored parameters fall outside a pre-designated standard an investigation could be made to identify factors that might be contributing to the decline in performance.

An emergency management plan for each farm also needs to be developed. Such as plan should involve a range of features including: notifying relevant authorities (since early diagnosis is critical), quarantining the affected cage(s) and reducing the stocking density in the affected cages. In particular farm staff also need to be trained in what to do, including what things to note, record and who to communicate the information to. Treatment using antibiotics is only available under veterinary supervision and is generally only an option for non-food fish (due to the with-holding periods required). Although antibiotics are effective at stopping mortalities, they are expensive and can result in subclinically infected or “carrier” fish.

7.2.6 Feed management to optimise production

Feed management is one of the most important facets of modern fish culture. For a good feed to realize its potential it must be fed properly, and good feed management can even make a poor feed perform better, while bad feed management can struggle to get good performance from an excellent feed. The basis by which feed has traditionally been managed is one of feeding to apparent satiety or excess. While this ensures, in most cases, that growth potential is met, it usually also results in considerable excess nutrient inputs into the system and can also be a cost inefficient process (Glencross, 2006).

Like most biological processes feed utilization has various constraints, efficiencies and nuances, that once understood, hold to several describable functions. These functions can for most cases be described by a series of mathematical equations – which is the basis of the factorial bio-energetic model (Ursin, 1967; Cuenco et al., 1985; Machiels and Henken, 1986; Shearer, 1995; Lupatsch et al., 2001; Lupatsch and Kissil, 2003). The basis of this model is that it is prescriptive in defining the amount of energy, and subsequently nutrient inputs required to get optimal production from a given production system. By holding to the prescribed feed regimes defined by this model, it should be possible to substantially improve the production efficiency of barramundi production. While the outcomes of this study clearly provide a basis to demonstrate that potential, a defined study whereby the fish are fed strictly to the bio-energetic models constraints, should be undertaken to fully confirm the models applicability. It would even be of more value to undertake this on a fully operational production facility (Cho and Bureau, 1998).

The results of this study also demonstrate that there is considerable potential to manipulate feed type or design for better production and environmental outcomes. Clear gains should be achievable by better tailoring the energy density of the diet used to that of the fish's energetic demands (Glencross, 2006). Essentially, this means that for bigger fish the diet energy density should be higher than for smaller fish. A careful balance also needs to be maintained between the ratio of digestible energy intake and digestible protein intake (Williams and Barlow, 1998; Lupatsch and Kissil, 2005). While the available commercial diets can easily satisfy this over the normal production range, there are opportunities to further refine those diets available to better suit the production dynamics of barramundi aquaculture.

The feed ration given to the fish throughout their production cycle is one of the central points of improved feed management (Brett and Groves, 1979; Cho et al., 1991). As has been shown, the amount of feed required depends primarily on the energetic demand of the fish and the energy density of the diet. What this implies is that for the higher energy dense diets, less should be fed. Usually demand by the fish will also marginally diminish, but careful assessment needs to be taken that feed is still not fed excess to requirement. Notably this requirement will be influenced by the energetic demand of the fish, with this later component being strongly influenced by fish size and of course water temperature (Azevedo et al., 1998).

From a practical perspective immediate benefits to barramundi production efficiency could be realized through improvements to feed management (Glencross, 2006). A comparison of the production efficiencies of using the bio-energetic feed management approach should be tested on-farm. Such a study could also benefit from associated localized water quality monitoring to examine the potential environmental benefits from improved feed management (Cho et al., 1991).

Improvements to feed design can also be made, whether it is through an alteration in the pellet size being produced by some manufacturers to make certain feeds more manageable at larger fish sizes or alterations to the protein energy balance being used at the various production stages. An assessment of the prospective production efficiency gain of using a greater range of specifically tailored diets over the production cycle needs to be undertaken. While the present use of two distinct diet types may be warranted at low production levels, considerable improvements may be possible with industry expansion and these subtle refinements.

7.3 Recommendations

Despite the considerable progress made in each of the facets of this project that were undertaken, there still remain several avenues of research that could be undertaken to further improve outcomes in each area. Notably each of these areas has a considerable economic potential to improving barramundi production throughout Australia.

7.3.1 Further initiatives for flavour quality management

The key recommendation from this report with respect to flavour management of barramundi is relatively straightforward. Barramundi farmers should endeavour to identify whether they have species of phytoplankton and/or cyanobacteria that are known GSM and/or MIB producers. This knowledge will provide them with some indication of the potential for problems within their own production system. In addition to this, it would be prudent for farmers to undertake pre-harvest sensory assessment of their own fish (Howgate, 2004). To maximise the power of such assessment the farmers should target the belly-region of the larger fish for taste-testing. If possible, using female tasters will also strengthen the assessment. If, from such an on farm assessment a muddy flavour is identified, then purging from depurated water for 48 hours or longer will most likely resolve the problem, but this will also be dependent on the level of GSM and/or MIB in the fish flesh.

The main objective of the flavour quality management work was to identify the cause, predisposing factors and potential remedies for the muddy-flavour taint problem. However, in the process of researching these issues other aspects of product quality and other opportunities to investigate were noted.

The skin colour of barramundi from cages exposed to sunlight in Lake Argyle were noted to be significantly darker in appearance than those fish that were relocated to an indoor facility for purging. Anecdotal reports indicate that lighter, silvery barramundi are better received at market, although no specific value difference has necessarily been ascribed to the difference. However, whether the effect is a result of the reduction in light (all spectrums) or a change in the colour of the background needs to be further investigated (Booth et al., 2004).

There was also a significant difference between farmed and wild fish noted in terms of the flesh colour. In particular a distinctly greater level of “greyness” was noted in the dorsal muscle groups of the fillet (Figure 7.1). The specific cause of this is unknown, but it was observed in all farmed fish from both freshwater and saltwater production systems. Fish purged indoors and with a reduced pigmentation of the skin still had significantly greyer flesh than wild-fish. Given this commonality among the farmed fish, despite a range of production systems, and the difference to wild-fish, it is highly likely that the condition is related to the feed used in barramundi production (Glencross, 2006).

One key impediment to more fully understanding the nature of the muddy-flavour taint has been the development of a viable instrumental assay to quantitatively measure either GSM and/or MIB. Most studies reported that have used an instrumental based analysis report very low and variable recoveries (Lovell et al., 1985; Grimm et al., 2004; Robertson et al., 2005).

If possible a study examining the effect of different MIB and GSM concentrations on the uptake rate and also the effect it has on the accumulation and subsequent depuration process would also be of value. Such a study may allow for easier assessment of the likely thresholds and the time periods required for subsequent depuration. An uptake study along these lines was

conducted in this report, although it only examined the sensory characteristics of the fish after a single time point. The depuration study presented in this report did not examine the effects of variable GSM and/or MIB taint levels on the rate of depuration either. It is suspected, based on the observations made on other studies presented in this report, that the process is a first-order rate kinetic process, therefore at higher concentrations the uptake will be quicker and over the similar time periods accumulate at greater concentrations in the fish flesh than lower concentrations. This is likely to increase the time taken to depurate the fish when returned to MIB and GSM free water, but remains to be validated.

There would also be some value in assessing the extent of the muddy-flavour taint issue in locations other than just Lake Argyle. While the known GSM and MIB producing phytoplankton and cyanobacteria are likely to be ubiquitous throughout the distribution of production areas in Australia, the severity of any associated muddy-flavour taint is unknown. However, that the species of phytoplankton and cyanobacteria are essentially only freshwater species, it is unlikely that any muddy-flavour taint issue will be found from production systems based in saltwater (Howgate, 2004).

From a commercial perspective, there would be some value in monitoring GSM and/or MIB levels in Lake Argyle on an ongoing basis. The development of a data history would allow for a better understanding of the environmental causes of changes in phytoplankton and cyanobacteria in the lake. Such an improved understanding could be used to guide optimal harvest times and/or times when not to harvest at all. The alternative of course is to ignore the levels of GSM and/or MIB and as a quality assurance system adopt a process of purging all fish produced within the lake. However this may still result in variable quality outputs if times of higher-taint are not accounted for in any purging process established.

Routine use of purging systems is consistent with the operations used in the US channel catfish industry (Lovell, 1983; Lovell et al., 1985; Zimba and Grimm, 2003). There has also been some effort to instigate a similar such practice with trout production in Europe (Robertson et al., 2005; 2006; Robin et al., 2006). Irrespective of whether or not purging is used in maintenance of fish quality it is still important to consider the use of routine sensory evaluation of fish before and after purging (Howgate, 2004; Robertson et al., 2005; 2006). With barramundi, a prudent strategy would be to:

- Have an ongoing assessment system for MIB and GSM in the lake water.
- To harvest a larger barramundi prior to purging and undertake taste-testing of its belly-region meat for muddy-taint. This may provide some indication of the severity of the problem at a given point in time.
- Irrespective of sensory outcome, purging should be considered mandatory to ensure quality control.
- Following purging, again harvest a larger barramundi and undertake taste-testing of its belly-region meat for muddy-taint. If any incidence of muddy taint is detected then further purging is required.

7.3.2 Further initiatives for disease management

Vaccination remains potentially one of the most useful methods for preventing disease outbreaks. Several vaccines based on formalin killed whole *S. iniae* cells have been developed overseas and recently two autogenous vaccines have been approved for use in barramundi in Australia and are already being used by some producers. These vaccines were designed to

prevent disease caused by specific endemic strains. A small batch is prepared using a strain from a farm that has a problem infection and the vaccine is authorised for use on that site only and for that particular season. The reason for this procedure is that the dominant protective *S .iniae* antigens recognised by fish are part of the polysaccharide capsule and are variable. Therefore a vaccine prepared from one isolate from one farm is unlikely to work well on another. It is not known whether either of the vaccines already produced to date would be useful in protecting fish against the *S. iniae* strain that is present in Lake Argyle or other Kimberley barramundi production operations.

Immunostimulants are often used in aquaculture to improve the non-specific immune responses of fish, thereby increasing weight gain and reducing losses from disease (Li and Gatlin, 2003). 1,3 β glucans and nucleotides derived from yeasts and some other products are recognised immunostimulants that are sometimes added to aquaculture feed (Li and Gatlin, 2005). Some major feed suppliers in Australia are already including it in their product range under the trade names of MacroGuard® and Boost®. Generally these products have been developed based on salmonids and the extent of their efficacy is yet to be defined in barramundi. Their use is an option for reducing the impact of the disease in barramundi. Probiotics have also been investigated as a potential method of controlling *S. iniae* and would benefit from further evaluation with barramundi (Brunt and Austen, 2005).

However, key disease management options that need to be considered must be based on a carefully developed disease management plan implemented to minimise production losses from a range of potential diseases. A good plan should include strategies for identifying conditions that might pre-dispose to specific diseases, such as that caused by *S. iniae* (Bromage and Thomas, 1999; Bromage and Owens, 2002). Development and implementation of a Hazard Analysis and Critical Control Point (HACCP) plan to identify risks and management strategies to minimise the impact of significant risks. Another tool could be to benchmark feed intake, growth rates and feed conversion ratios against a known optimal standard and development of the growth and feed utilization model will progress such possibilities.

Development of farm specific emergency management plans, involving notification of relevant authorities, quarantining the affected cage(s) and reducing the stocking density in the affected cages all need to be considered. A veterinarian and a source of antibiotics should be identified before an emergency occurs. And most importantly the staff need to be trained in what to do and who to contact.

7.3.3 Further initiatives for improving the barramundi model

There is a range of potential prospective problems with the existing barramundi bio-energetic model. Like all models, they are only mathematical representations depending on the accuracy of the data obtained. Therefore the more data that can be obtained and the more parameters can be included, the more accurate the model should become. However, in its current format this model is simply an empirical model that describes a series of inter-related factors through a series of mathematical equations. To progress on a biological format the model needs to become a mechanistic model that uses a series of inter-related mathematical equations that specifically define certain physiological parameters affecting the growth of the fish and its utilisation of nutrients and energy (Shearer, 1995). Presently no such model exists for fish, though the Fish Pr-FEQ model is the closest such thing presently available (Cho and Bureau, 1998).

The influence of temperature extremes (both upper and lower limits) on the growth performance and energy utilization efficiencies is one factor that requires further attention. The existing model

is defined primarily on data derived from fish grown at 30°C, but uses other data sources for its growth parameters (20 – 38°C) and metabolic demands (26 – 33°C). Furthermore, whether the problem at higher temperatures is really one of temperature shock or of oxygen deficiency also remains to be resolved (Katersky and Carter, 2006). Trials involving respirometry at a range of temperatures may resolve this issue. Development of molecular assays, such as 2-dimensional polyacrylamide gel electrophoresis (2D-PAGE), Enzyme-Linked Immuno-Sorbent Assays (ELISA's), real-time quantitative polymerase chain reaction assays (RT-qPCR) or mRNA Northern blotting techniques, for determination of heat-shock protein production may also shed some light on this issue (Melamed et al., 2002; Fuentes et al., 2005).

From a production perspective, a greater database of environmental and fish performance parameters will not only assist the management of barramundi production, but also the refinement of the empirical-model. This is likely to require comprehensive monitoring for several simple parameters notably, fish weights, time of measurements, water temperatures, photoperiod and dissolved oxygen among others. Further gains to efficiency in barramundi production are there to be made. However, these gains will depend on better management of feeding ration, feed type allocation and feed design. These gains in efficiency will also translate to better environmental outcomes, with lower nutrient inputs and better designed and managed feeds resulting in lower nutrient losses to the environment.

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Figure 7.1 Difference in pigmentation between dorsal and ventral muscle groups within the fillet of a farmed barramundi. Notable is the significantly greyer dorsal section of the fillet.

8.0 Appendices

8.1. Intellectual property and/or valuable information arising from this project

No specific intellectual property is anticipated from this project.

Facets of the knowledge gained from the purging work are valuable in determining optimal purging conditions for barramundi, as well as the identification of some of the critical constraints to this process. These details are provided and discussed more fully in chapters 2, 3 and 4.

Details on the commercialisation strategy for the development of an industrial scale purging system are provided in appendix 8.5.

The factorial model revised in this project is one that was declared as pre-owned intellectual property by the host agency and remains their intellectual property. Through this report several of the details underpinning this model, and some of the functional equations and parameters are made available for others to develop as the need arises.

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8.3 Potential of VIS-NIRS spectroscopy to predict sensory properties of food (CFT abstract)

Exploring the potential of VIS-NIR spectroscopy to predict sensory properties of foods

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Sensory analysis of food involves the measurement, interpretation and understanding of human responses to the properties of food perceived by the senses such as sight, smell, and taste (Cozzolino et al. 2005). It is important to have a quantitative means for assessing sensory properties in a reasonable way, to enable the food industry to rapidly respond to the changing demands of both consumers and the market. Aroma and flavour are among the most important properties for the consumer and numerous studies have been performed in attempts to find correlations between sensory qualities and objective instrumental measurements. Rapid, non-destructive instrumental methods such as near infrared spectroscopy (NIR) might be advantageous to predict quality of food and agricultural products due to the speed of analysis, minimum sample preparation and low cost. The advantages of such technologies are not only to assess chemical structures but also to build a spectrum, characteristic of the sample, which behaves as a “finger print”.

At the Department of Primary Industries and Fisheries, Queensland, recent research efforts have involved including VIS-NIR spectroscopy in broader sensory trials to explore the potential of this spectroscopic technique to predict sensory properties of foods. This work has been conducted through ongoing collaboration with Dr. Daniel Cozzolino of the Australian Wine Research Institute, South Australia. Examples of recent trials conducted in Brisbane include exploring the ability of VIS-NIR spectroscopy to predict trained sensory panel scores for 'muddiness' in barramundi and 'toughness' in saddletail snapper. In the future, sensory and NIR investigations will be extended to horticultural products in support of breeding programs.

Barramundi trial

Barramundi (*Lates calcarifer*) is an Australian fish that is popular among consumers due to its deliciously strong, gamey and distinctive flavour. Not surprisingly, barramundi farming and wild capture of barramundi are now profitable industries in Australia. A problem that the barramundi industry faces is the occurrence of a muddy taint, which can occur in both farm and wild environments, that has given barramundi an unfortunate reputation among consumers.

The compounds responsible for the taint in the fish flesh are geosmin and 2-methylisoborneol (Figure 1), which are metabolites of certain algae and bacteria (Tucker 2000). These compounds are present naturally in water at very low concentrations. Under certain environmental conditions they can build up in the water and cause high levels to accumulate in the fish flesh, which translates to a muddy or earthy aroma and flavour. It has been reported that these compounds cause consumer rejection in fish flesh at the parts per billion range (Persson 1980).

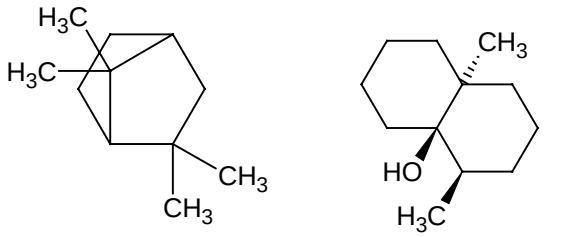

Figure 1. Compounds responsible for muddy taint in barramundi.

Measuring geosmin and 2-methylisoborneol in water is relatively straightforward; however, measuring these compounds in fish flesh is extremely difficult, due to the high fat levels in fish flesh, making extraction for chemical analysis almost impossible and not suitable for quality control. Routine sensory evaluation of fillets for quality control is also not feasible due to sensory adaptation, increased sensory fatigue and flavour carry-over between samples that is associated with tasting muddy-tainted fish (Johnsen and Bett 1996).

NIR spectroscopy is used widely in many industries for quality control as it offers rapid, non-destructive and in-line measurement of product compositional parameters that relate to product quality. The objective of this preliminary study was to investigate the potential of NIR to predict sensory perception of muddy taint in farmed barramundi for future use in quality control.

Barramundi samples used in this study were from a larger project conducted in collaboration with the Australian Veterinary Association and Lake Argyle Industries. Fish samples from Lake Argyle, Western Australia, which were known to exhibit a broad range of the muddy taint, were sent to the DPI&F, Innovative Food Technologies, in Brisbane for sensory evaluation from

March to June 2005. A total of 74 fish samples were assessed using a panel of ten trained tasters using descriptive analysis techniques. Through vocabulary development, the panel selected a number of sensory attributes for aroma, flavour and aftertaste which were used to score the cooked barramundi samples on structured linear scales from 0 – 100, anchored from none to high.

The raw samples, often the second fillet of the same fish used for sensory evaluation, were scanned, in triplicate, in reflectance mode by VIS-NIR 400 – 2500 nm (FossNIRSystems 6500) using a rectangular cell. To reduce the mess of scanning raw fillets, the samples were placed in a HDPE plastic bag prior to scanning. The second derivative of the VIS-NIR spectra is shown in Figure 2. Spectral features include water peaks (950, 1400, 1900 nm), CH stretch overtones due to lipids (1200, 1750, 1730 nm), CH combination tones from lipids and fatty acids (2200 – 2400 nm).

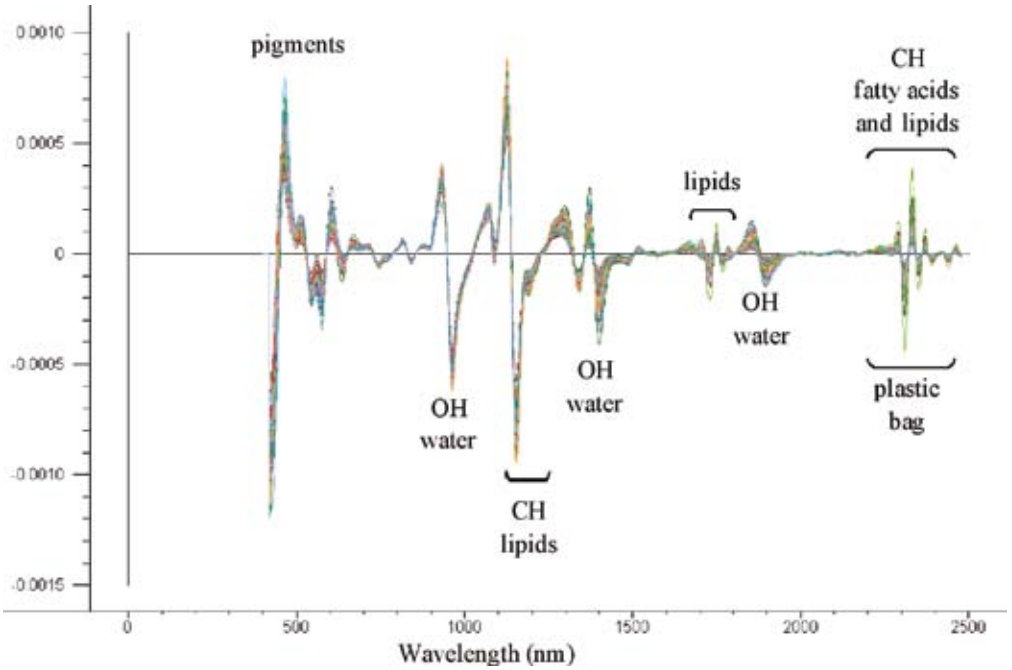

Figure 2. Second derivative VIS-NIR spectra of barramundi fillets (n = 222).

The data from sensory and VIS-NIR analysis were combined in The Unscrambler (version 7.8, CAMO ASA, Oslo, Norway) for analysis. Partial least squares (PLS1) regression was used to develop predictive equations for the scores of sensory attributes *muddy/earthy aroma*, *muddy / earthy flavour*, *fresh flavour* and *muddy aftertaste*, using the second derivative of the VIS-NIR spectra.

The results from the predictive models developed using full cross validation, and limited to 10 components, are shown in Table 1. The models were evaluated by comparing the coefficient of correlation (r_{cal}) and the root mean square standard error in cross validation ($RMSECV_{cal}$) in calibration.

Table 1. Results of prediction models for sensory properties using VIS-NIR.

	2D VIS-NIR region	n	Rcal	RMSECVcal	Copt
muddy / earthy aroma	400-2500	222	0.54	3.2	10
fresh flavour	400-2500	222	0.73	4.7	10
muddy / earthy flavour	400-2500	222	0.73	5.0	10
muddy aftertaste	400-2500	222	0.60	3.7	10

The results show that VIS-NIR shows good potential to predict *muddy / earthy flavour* and *fresh flavour* in barramundi with reasonable correlation coefficients (r_{cal} 0.73) and relatively low standard error ($RMSECV_{cal}$ 4.7 - 5.0). The results from these predictions are quite promising given the limited number of samples and the subjective nature of the sensory scores.

According to the loadings on the VIS-NIR data, it was clear that the regressions did not rely on one or two specific regions of particular importance. Rather, the regressions used a combination of information from across the entire spectrum to build the best predictive model for these sensory properties. This indicates that the VIS-NIR is not simply picking up on the unique vibrations in the bonds of geosmin and 2-methylisoborneol responsible for the muddy taint. We have observed this phenomenon in previous studies and similar results have been reported by other authors for the prediction of sensory and organoleptic properties of foods and beverages using NIR spectra (Cozzolino et al. 2005).

Further work must be conducted to further investigate these preliminary findings before this technique can be developed for use in the barramundi industry. In particular, a broader range of samples from across Australia and from a number of seasons must be evaluated, and the predictions tested using an external validation set of samples. Should this rapid assessment technique be developed further it could provide a far cheaper option to the difficult and expensive chemical analysis of geosmin and 2-methylisoborneol in fish flesh, and time consuming and problematic sensory analysis. This technique could potentially be used by the industry to improve the quality of their product and to rectify the unfortunate reputation of muddy barramundi among consumers.

8.4 Acknowledgements

The authors wish to acknowledge Dr. Brett Glencross, WA Department of Fisheries, for the valuable background work he conducted with barramundi at Lake Argyle and for his support of this project. The authors also acknowledge the support and continuing involvement of Dr. John Guthrie (Rockhampton) of Emerging Technologies, DPI&F.

8.5 References

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8.6 Engineering report for design of a commercial scale purging system

REPORT ISSUE AUTHORISATION

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Date	Purpose of Issue/Nature of Revision	Rev	Reviewed by	Issue Authorised by
29 Jul. 05	Draft for review	Draft		R S Locke
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10 Mar 06	Report issued	0		R S Locke

LIMITATION STATEMENT

This Engineering Report has been prepared in accordance with the scope of services agreed upon between SEMF Holdings Pty Ltd (SEMF) and the Ord River District Coop (the Client). To the best of SEMF's knowledge, the report presented herein represents the Client's intentions at the time of printing of the report. However, the passage of time, manifestation of latent conditions or impacts of future events may result in the actual project and its impact differing from that described in this report. In preparing this report SEMF has relied upon data, surveys, analysis, designs, plans and other information provided by the client, and other individuals and organisations referenced herein. Except as otherwise stated in this report, SEMF has not verified the accuracy or completeness of such data, surveys, analysis, designs, plans and other information.

No responsibility is accepted for use of any part of this report in any other context or for any other purpose by third parties.

This report does not purport to provide legal advice. Readers should engage professional legal advisers for this purpose.

SEMF does not act as a professional Quantity Surveyor and as such, whilst we have used our best endeavours to produce an estimate that is consistent with the aims of the report, the reader should engage the services of a professional Quantity Surveyor if the estimates are to be used for the purposes of further investment or expenditure.

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CONTENTS

1. SCOPE OF REPORT

1.1 General

SEMF have been engaged to assist in establishing parameters for a commercial size barramundi purging system for farmed fish on Lake Argyle. Purging is necessary to reduce the quantity of off-flavour compounds in the fish which otherwise spoil the taste.

1.2 Reference Documentation

SEMF have been provided with the results of pilot tests which indicate that the off-flavour compound content of the lake water, can be reduced to acceptable levels through aeration of the water in lined storages.

2. AERATION

The results show that the reduction over 5 days provides water suitable for purging, whether the water is lake water, or contained water spiked with off-flavour compounds. For initial purging, it is likely that 3 day aerated water will be effective.

Dosing with algicide may also be required.

Tests were undertaken in a liner 6 metres by 5 metres by 2 metres deep, a total of 60 cubic metres. This investigation looks at the requirements for treatment of a 1,000 cubic metre container; this could be a square of about 18 metres, 3 metres deep, or a 26 metre diameter circle 2.5 metres deep.

The test liner was aerated by a 550 watt Futi splash aerator which is likely to have been transferring 13.8 grams of oxygen per cubic metre hour. The dissolved air saturation rate is of the order of 10 grams per cubic metre (10mg/L) at 20 degrees, and less at the temperatures of Lake Argyle, and so it is unlikely that all the transferred oxygen was being used. A lower aeration rate is likely to achieve the same result. The actual oxygen demand of the process has not been calculated.

A transfer rate of 5 mg/Lh would restore saturation levels over less than a two hour period, and is likely to maintain the reduction rate of the compounds. This can be achieved with about 3.5 kW of aerator power with published transfer rates of 1.5 kg of oxygen per kilowatt hour. This level of aeration could be provided by two paddle wheel aerators driven by a 5 kVA portable generator. A 750 kW injector aerator set to aerate the deeper levels of the liner would avoid any tendency for deeper levels to avoid mixing and thus aeration.

In actual operation, the dissolved oxygen content of the water should be measured and compared to off-flavour compound dissipation rates, so that aeration can be maintained at an oxygen consumption level to reduce aeration costs. For example, all three aerators may operate for the first day or two if water is heavily contaminated, reducing to two and finally the small injector to maintain air saturation in a holding situation.

Aerators discussed above, weigh in the order of 120 kg each, and so a small gantry or lifting arm would be necessary to transfer them in and out of treatment liners. This could be positioned on a pontoon between the liners. Alternatively, aeration and pumping equipment may be moved with the same lifting equipment used for liners, nets and other farming equipment.

3. FILLING LINERS

Water can be drawn from the lake by gravity using suitably designed liners. The rate of filling will depend on the pipe diameter, and placement of the equipment.

A 1,000 cubic metre storage could be filled in 48 hours at a flow rate of 5.8 litres per second. This can be achieved through a 150 mm diameter pipe with 30 to 40 mm head loss for suction from 10 to 20 metres below the surface of the lake.

This means that the outlet of the pipe must be positioned about 50 mm below the surface of the lake, and the base of the liner must be below the pipe, that is about 200 mm below the lake surface. This is the optimum pipe diameter for this filling rate, because a 100 mm diameter pipe has a head loss in the order of 150 to 210 mm, requiring the liner to be lower in the water.

The base of the liner could be weighted to provide the necessary clearance for filling. It is not necessary for the whole base area to sink to the bottom of the inlet pipe, providing the whole base has negative buoyancy equivalent to the head loss, and there is clearance around the inlet to allow filling to commence. Thus the whole of the base needs to be weighted to 50 to 60 kg/square metre, submerged weight, and the area around the inlet needs to be additionally weighted to 200 kg per square metre, or mechanically kept clear of the pipe.

Sides of the liner will need to resist lateral pressure due to submergence.

If the valve to the inlet pipe is left open, the water level inside the storage will eventually reach lake level, but at an ever decreasing rate. To reduce filling time, the last 50 mm could be pumped into the storage, or the level could be left at the 50 mm below lake level. It may be possible to leave the inlet valve open during the first day or two of aeration, to complete filling.

4. TRANSFER TO PURGE

Transfer of treated water from storages into purging liners, could be accomplished by gravity into liners with weighted bases, or by pumping.

Transfer of 100 cubic metres of water through a 100 mm diameter line in 24 hours would require 15 to 20 mm of submergence of the liner base, below the water level in the storage. This is a transfer rate of 1.2 L/s, and so insufficient for the purpose.

Alternatively, a small pump with 550 watt motor, is likely to transfer at 4 to 4.5 L/s. A low head floating pump (250 W), is rated at 10 cubic metres per hour. A similar 550 watt pump is rated at 20 cubic metres per hour and 1.1 kW at 30 cubic metres per hour.

A flow of 10 litres per minute through a 2,000 litre test tank was required to keep the carbon dioxide and ammonia content of water at acceptable levels for fish comfort. This is equivalent to a movement of 33 cubic metres per hour through the 100,000 litre purging liner.

A flow of 9.3 litres per second is required for this, which is not feasible for gravity flow, even if the 100,000 litres is divided into three liners. Thus pumping from the storage liner is likely to be the preferred method. The size and number of pumps will depend on the number of purging liners.

5. RECYCLE OR AERATION

A report by G Westbrook suggests that aeration can be carried out in the purge liner if stocking density is 40 kg/m^3 or less. This would allow for the water to be used in the purge liner for a longer period, before being replaced and without the need for recycling.

In order to provide 10 tonnes per week capacity, there needs to be three purge liners in use each week, for a size of say 6 metres by 5 metres by 3.5 metres deep. This would allow for pens of fish of differing size grades.

If it is assumed that fish are introduced into the purge liner together with raw lake water, purified water could be pumped into a manifold on one side of the purge liner, and air introduced through a manifold on the other side.

Spent water would be drained off by a launder at the top of the liner. Water quality would improve as the process continues, and raw water is either driven off or improved by aeration.

Under this scenario, total flow through the purge liner would be say 20 cubic metres per hour, or 5.6 L/s, and one 1 ML store of treated water would serve one purge liner for 2 days or three purge liners for 16 hours.

This could be achieved with 2 pumps rated at 30 cubic metres per hour pumping into a manifold. One pump would be moved over as the liner began to empty, and the other when the liner was pumped to the lowest level. A spare pump would provide backup, and could also be used in change over situations.

Throttling valves could be used to balance flow into the purge liners.

The alternative is to recycle water through the purge liners to keep carbon dioxide and ammonia levels under control. This would require the same degree of aeration, additional pumping and liners for the aeration to occur. Low stocking rates to allow in-liner aeration appears to be the preferred option.

6. OPERATING PROCEDURE

The intended procedure is that fish are purged for two days and that the quality of water for the first purge need not be as good as that in the final purge.

Allowing for a 5 hour turnover, a total of three 1 megalitre stores of treated water will be required for each two day purge of the three 100 kilolitre fish purge liners.

If this is sufficient for a one week production, the procedure would be.

Day 1, 8 am	Commence filling of three 1 ML storages
Day 2, 8 am	Continue filling. Commence aeration of three storages
Day 3, 8 am	Dose storages with algicide if required. Continue aeration
Day 4, 8 am	Continue aeration
Day 5, 8 am	Continue aeration. Prepare purge liners 1, 2 and 3 and begin in-liner aeration. Transfer graded fish (say plate-size, banquet and large) into three purge liners.
Day 6, 8 am	Commence purging purge liners with storage 1.
Day 7	Continue purging purge liners with storage 2 and 3 Refill storage 1 and start aeration.
Day 8, 8 am	Harvest purge liners Refill storage 2 and 3 and start aeration (Repeat Cycle using shortest aerated storage first for purging purge liner – this means last storage used will have received maximum aeration)

From this procedure, a seven day cycle can be developed for aeration, purging and harvesting, with a seven day start up before harvesting commences. Aeration varying from a 3 day minimum to a 5 day maximum is provided.

In order to achieve this, aerators will be required for each liner and each treated water storage, together with pumps for each. A full spare set of aerators and pumps, and one spare liner is recommended. A system could be installed with a large generator set serving the entire operation, or individual sets serving say each of the aeration liners and another for site lighting and pumping. At detailed design time, a comparison of cost options for the two alternatives should be undertaken.

If individual sets are installed, soft starting will be required to reduce the instantaneous load on the generator. Individual sets are likely to provide the most economical running, but will cost more initially.

The layout is described in the drawing 1102.001-C01. An A4 sized copy of the drawing is attached as Appendix A.

7. PRELIMINARY COSTS

A preliminary estimate of costs associated with the proposal is shown in Table 1.

Table 1.

Item	Description	Quantity	Unit	Rate	Amount
1	Purge liner 6m x 5m x 3.5 m	4	No	3,000	12,000
2	Aeration storage 26m diameter x 2.5 m	4	No	12,000	48,000
3	Structure and access, liner support	1	allow	100,000	100,000
4	Inlet system and liner weighting	3	No.	5,000	15,000
5	Treated water aerators, surface, 1.5 kW	7	No	1,500	10,500
6	injector, 750W	4	No	1,000	4,000
7	Transfer pumps, 1.1kW	3	No	2,000	6,000
8	Purge liner aerators, 1 kW	3	No	1,200	3,600
9	Purge pipework	3	No	800	2,400
10	Transfer pump pipework and manifold	3	No	1,200	3,600
11	Discharge launder and pipework	3	No	800	2,400
12	Power supplies, 4.2 kW	6	No	12,000	72,000
13	Wiring and installation	3	No	4,000	12,000
14	Delivery to site		allow		20,000
	Detailed design and documentation		allow 15%		46,000
	Provisional amount,		allow 15%		46,000
					403,500
	GST				40,350
	Total including GST				443,850
	Allow also for fish handling, chemical dosing, testing of water.				

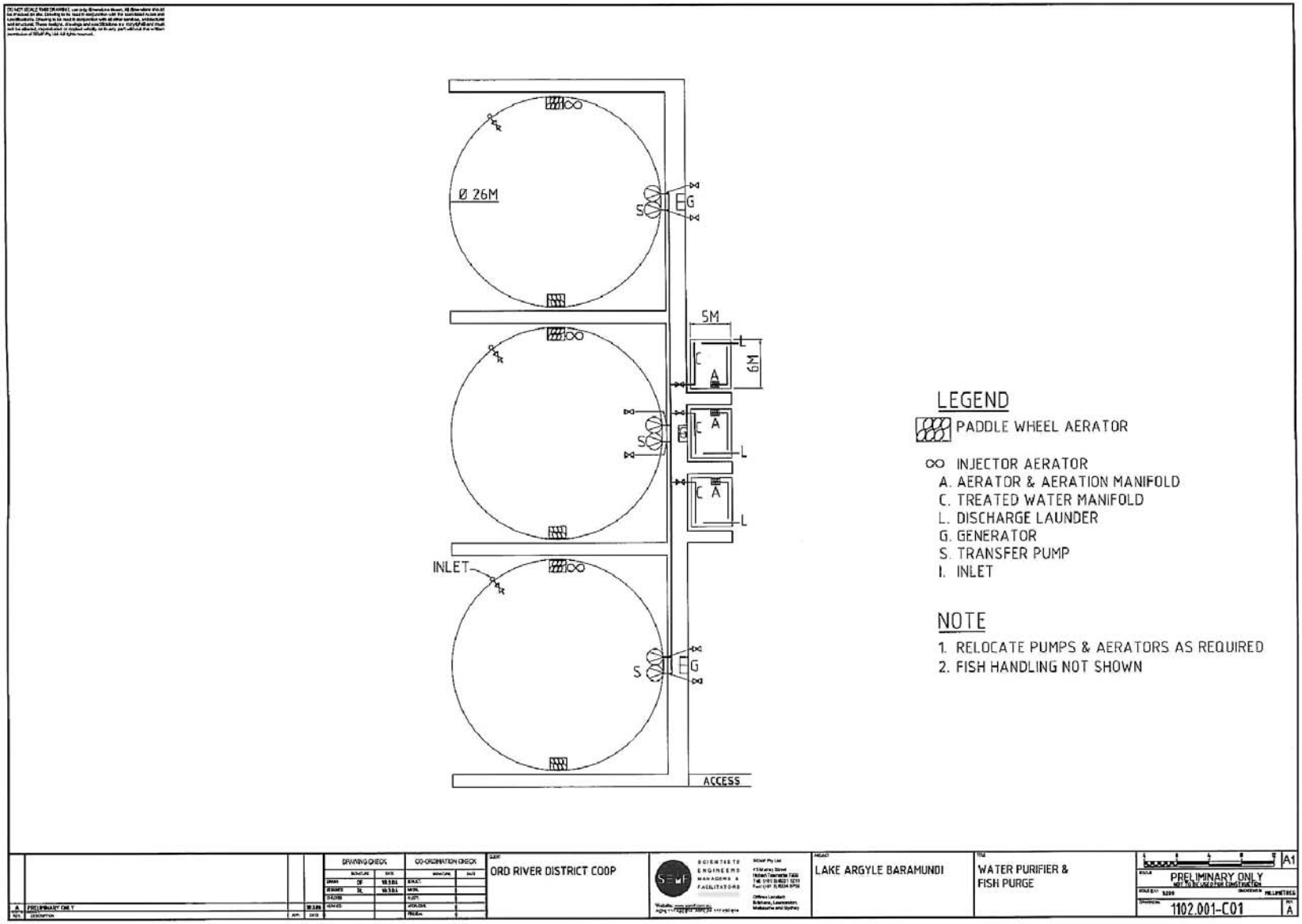
The inclusion of four significant figures in the table is the result of adding numbers of different sizes, and is not an indication of this level of accuracy. The estimate should be considered to be indicative and an order of cost only. For example, no structure has been considered, and handling of equipment may require fixed or mobile lifting equipment, not included.

Operating costs will depend on the current price of fuel for portable generators and current local prices should be considered, together with labour and handling costs for the equipment.

Other costs will include any dosing chemicals required, together with handling and storage costs.

8. CALCULATIONS

Tables summarising calculations are attached.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PADDLE WHEEL AERATOR
INJECTOR AERATOR
A. AERATOR & AERATION MANIFOLD
C. TREATED WATER MANIFOLD
L. DISCHARGE LAUNDER
G. GENERATOR
S. TRANSFER PUMP
I. INLET
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2. FISH HANDLING NOT SHOWN
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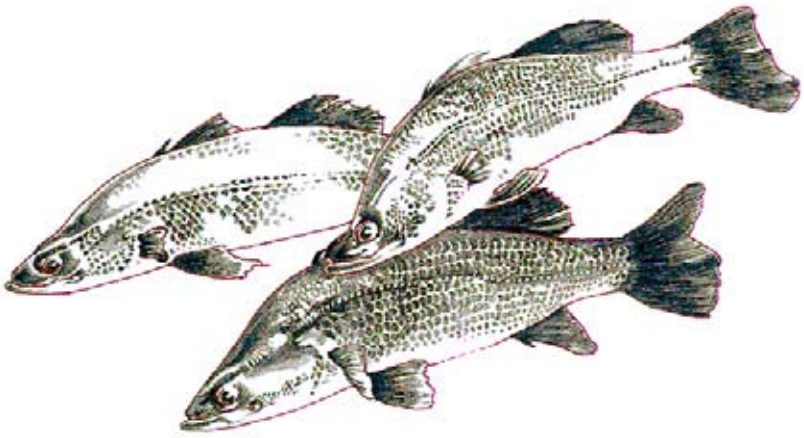
8.7 Sensory evaluation report provided by the Centre for Food Technology

Queensland **the Smart State**

Argyle Barramundi geosmin & MIB trials

Results of sensory analysis

June 2005



Trials:

Uptake of geosmin and 2-methylisoborneol (MIB)

Comparison of off flavour within the fillet

Comparison of off flavour within the population

Purging trial

Geosmin threshold trial

2-Methylisoborneol (MIB) threshold trial

Queensland Government
Department of Primary Industries and Fisheries

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Background

A Western Australian Fisheries (WA Fisheries) and Fisheries Research and Development Corporation (FRDC) project aimed at examining the prevalence of musty/muddy taints in barramundi fish exposed to geosmin and 2-methylisoborneol (MIB) tainted water was conducted at Lake Argyle, Western Australia. A number of experiments were designed and conducted by Argyle Barramundi together with Steve Percival of the Australian Veterinary Association (AVA), which involved exposing barramundi fish to geosmin and MIB at a range of levels in water. Additional trials involving purging barramundi fish that had been exposed to geosmin and MIB were also designed and conducted at Lake Argyle, WA. Fish from these trials were sent to the Department of Primary Industries and Fisheries (DPI&F) for sensory analysis to assess and measure the musty/muddy 'off flavour' caused by the presence of geosmin and MIB in the flesh of the fish.

Prior to formal sensory assessment of samples from the trials, a panel of experienced judges were trained, in accordance to the methodology of sensory descriptive analysis, to identify and rate the sensory properties of barramundi fish exhibiting the 'off flavour'.

After training was complete, formal sensory assessment of barramundi from a range of trials took place. The trials included:

- Uptake of geosmin and 2-methylisoborneol (MIB)
- Comparison of off flavour within the fillet
- Comparison of off flavour within the population
- Purging trial
- Geosmin threshold trial
- 2-Methylisoborneol (MIB) threshold trial

The data from these sensory trials were analysed statistically and the results are presented in this report, together with the methodology used for the sensory analysis.

Panel training

The panel consisted of eleven female panellists, aged between 30 and 61 years (average age of 49 years), who were experienced with sensory descriptive analysis of foods and beverages. Although eleven panellists were trained only ten panellists were engaged to complete formal assessments of barramundi samples and the remaining panellist was retained as a reserve panellist. Samples from all trials in this study were assessed by the same ten panellists with the exception of the two threshold trials, where the reserve panellist replaced one of the original ten panellists (due to availability difficulties).

The samples used for panel training comprised samples of lake fish, purged fish and large barramundi fish that were supplied by Argyle Barramundi. The large fish supplied were fish that had been stored long term in a Brisbane cold storage facility by Argyle Barramundi. These samples, in the form of frozen fillets, together with samples of Lake Argyle water, were received by the DPI&F on the 22nd and 23rd of March, 2005. The Lake Argyle water samples were not used during panel training as the taint associated with geosmin and MIB content was not evident in these water samples.

The panel were trained over four sessions, each of approximately two hours. The four training sessions took place on March 29th, 31st, and April 4th and 7th, 2005. At no point throughout the study were the panel given information about the nature of the study or told the identity of the samples other than that they were barramundi fish samples. Samples that were presented to the panel were always numbered with random 3-digit blinding codes.

In the first training session, the panel were asked to assess a number of barramundi samples, and to brainstorm terms that best described the appearance, aroma, texture, flavour and aftertaste properties of the samples. The samples assessed included all three of the samples provided for training. After brainstorming a list of terms, the panel were asked to identify the most important terms which best described the sensory differences within the set of samples assessed. In subsequent training sessions, training samples were re-assessed and the list of sensory terms refined and reduced to give a short-list of terms that could be used in the formal sessions to rate barramundi samples from the various trials. During training, sensory reference standards were presented to the panel to assist in identification of sensory properties and to aid in panel agreement over descriptors that were used to describe the samples. These sensory reference standards were developed during training, by the panel leader, to the satisfaction of the panel and were used during the formal assessment of samples from the various trials.

The attributes that were chosen by the panel to rate the barramundi samples, together with the identity of the sensory reference standard or definition of each term, are given in Table 1. An 'other' attribute for aroma, flavour and aftertaste was also included for the panel to rate if they thought they could detect a sensory property which was not covered by the chosen list of terms.

In the last two training sessions, the panel were asked to practice rating the barramundi training samples in the format that would be used during formal sessions. The data from practice sessions were assessed to ensure that the panel were suitably trained to consistently rate the attributes of interest to the geosmin and MIB trials. The results from the final training session involving the assessment of lake and purged barramundi fish are given in Table 2. Attributes for which statistically significant differences were observed between samples during training panel evaluation sessions included *salty sea breeze aroma*, *fresh aroma*, *milky flavour*, *fresh flavour*, *muddy / earthy flavour* and *muddy aftertaste*.

Further training would have resulted in a more refined list of descriptors and may have improved the sensitivity of the panel scores obtained for samples from the various trials. Nevertheless, the results from the training panel evaluation session indicated that the panel were sufficiently trained to assess the 'off flavour' of interest to this study.

Table 1 Sensory attributes chosen by the trained panel to rate the barramundi fillets and composition of sensory reference standards or definition of terms

attribute	Composition or definition of the reference standard
aroma	
milky aroma	20 mL of a hot solution of boiled whole milk (1 cup) and water (2 cups), served in a small covered glass vessel
steamed aroma	A strip (~3 x 1 x 1 cm) of hot freshly steamed chicken breast fillet (milk/water boiled above was used to steam the chicken), served in a small covered glass vessel
salty sea breeze aroma	A mixture of sand (5 g), shell grit (5 g) and sea weed (1 g) sourced from Wynnum beach, served in a small covered plastic cup
fresh aroma	No standard. Defined as the smell of recently cooked fresh white-fleshed fish
fishy aroma	20 mL of a 5 g/L mackerel fillet in brine solution, served in a small covered plastic cup
muddy / earthy aroma	20 g of moist mud sourced from the grounds of the DPI&F, Hamilton, after a shower of rain, served in a small covered plastic cup
'other' aroma	Defined by individual panellists if used
flavour	
sweetness	No standard. Defined as the sweet flavour experienced when the sample is in the mouth
milky flavour	No standard. Defined as the flavour of diluted warm milk experienced when the sample is in the mouth
fresh flavour	No standard. Defined as the fresh flavour of white-fleshed fish experienced when the sample is in the mouth
fishy flavour	No standard. Defined as the fishy flavour of old white-fleshed fish experienced when the sample is in the mouth
muddy / earthy flavour	No standard. Defined as the flavour of mud / potting mix / earth experienced when the sample is in the mouth
metallic flavour	No standard. Defined as the tingly metallic sensation / flavour that might be caused by a metal spoon in the mouth experienced when the sample is in the mouth
'other' flavour	Defined by individual panellists if used
aftertaste	
muddy aftertaste	No standard. Defined as the lingering muddy / potting mix flavour after the sample has been expectorated or swallowed
fishy aftertaste	No standard. Defined as the lingering flavour of old white-fleshed fish after the sample has been expectorated or swallowed
'other' aftertaste	Defined by individual panellists if used

Table 2 Mean sensory scores of aroma, flavour and aftertaste attributes for practice session involving training barramundi samples

Sample	*milky aroma*	*steamed aroma*	*salty sea breeze aroma***	*fresh aroma**	*fishy aroma*	*muddy / earthy aroma*	*sweetness*	*milky flavour**	*fresh flavour***	*fishy flavour*	*muddy / earthy flavour****	*metallic flavour*	*muddy aftertaste****	*fishy aftertaste*
purged fish	18	41	13^{a}	38^{ab}	21	4	21	16^{a}	34^{a}	21	7^{b}	5	3^{b}	12
purged fish	20	37	12^{a}	43^{a}	13	3	20	15^{a}	33^{ab}	26	4^{b}	7	1^{b}	20
lake fish	12	35	10^{ab}	26^{b}	18	5	9	6^{b}	10^{c}	24	47^{a}	15	31^{a}	15
lake fish	14	31	3^{b}	25^{b}	12	11	11	9^{ab}	14^{bc}	20	33^{a}	13	20^{ab}	11

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; $^{a\ b\ c}$ different letters within a column signify significant differences between treatments; mean scores calculated over 10 judges

Sample preparation, presentation and sensory assessment

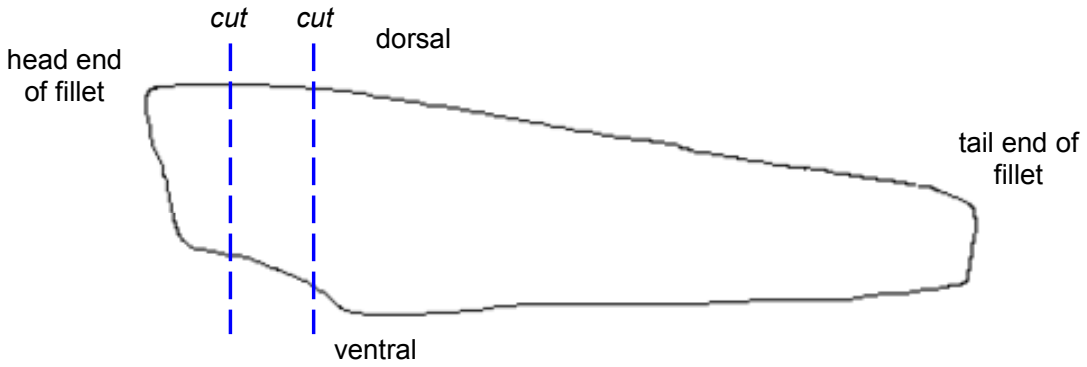
Preparation and assessment of samples took place in the sensory laboratory at the DPI&F at Hamilton, Brisbane (shown in Figure 1). The individual sensory booths were equipped with computers to collect panellist responses using the software Compusense five, version 4.0, Compusense Inc., Guelph, Ontario, Canada.

Figure 1 Sensory laboratory booth lay-out and rear servery

Samples for training sessions and formal assessments were prepared in the same manner and as described in this section.

Frozen samples were thawed overnight at 2°C prior to preparation for assessment. Slices of barramundi fillet (no skin) were cut from dorsal to ventral to give a 20 g (+/- 2 g) portion of fish (as shown in Figure 2). Samples were cut starting from the head end, such that any unused fillet always remained at the tail end of the fish fillet. Where there was sufficient flesh from one fish to serve the whole panel, one fish (of two fillets) was treated as one individual sample.

Figure 2 Preparation and cutting of fish samples



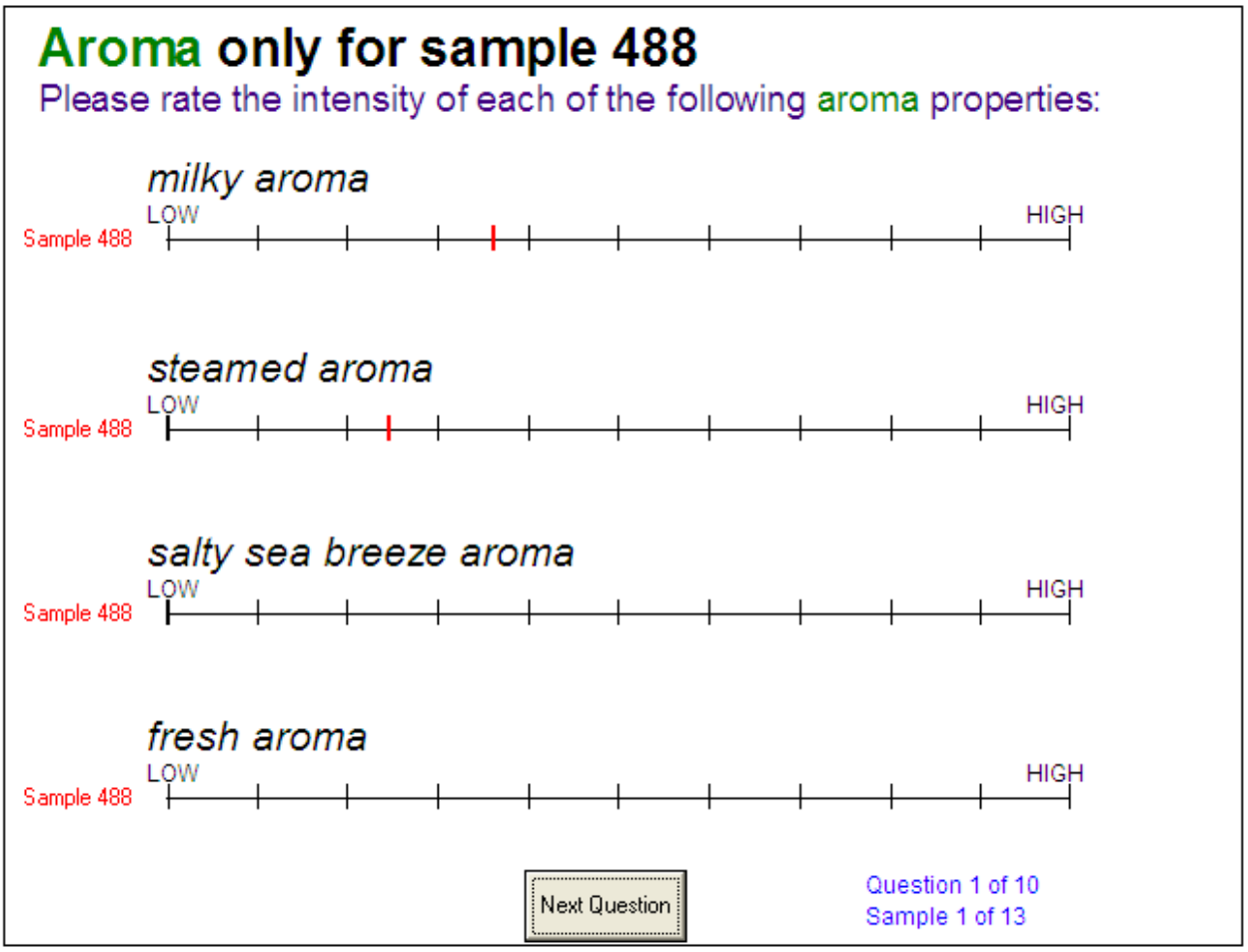
Samples were weighed into aluminium foil dishes and covered with aluminium foil sheets (shiny side down) that were pre-numbered with the applicable blinding code (shown in Figure 3). Samples were prepared up to 1 hour ahead of time and kept chilled in a refrigerator at 2 - 4°C prior to cooking. Samples were cooked no more than 30 minutes prior to serving. Samples were cooked on an oven tray, in a pre-heated fan-forced oven, at 200°C for 6 - 7 minutes. After cooking, samples were transferred to a warming oven at ~80°C until served.

Figure 3 Preparation, cooking and presentation of samples

Samples were presented to the panel in randomised order. Only three samples were presented to each panellist at a time so that all the samples would still be hot for sensory assessment. The temperature of the samples when served was between 70 and 85°C.

Plastic forks were used to taste the samples and a new fork was used for every sample assessed. It should be noted that metal forks were not used for the formal assessments as panellists described an increase in the metallic taste of the fish samples when tasted with metal forks. Panellists were also provided with small white ceramic plates on which to assess each sample individually (shown in Figure 3).

Figure 4 Computer screen lay-out for rating assessed barramundi samples



Panellists were asked to first evaluate the aroma of the sample and then to taste the sample and assess flavour, and finally aftertaste. For each sample, panellists were asked to rate the intensity of each of the attributes listed in Table 1 on a scale of 0 to 100, anchored from low to high. An

example of a typical computer screen questionnaire, using the software Compusense five, is given in Figure 4.

An ‘other’ term was also provided for panellists to rate for aroma, flavour and aftertaste. When an ‘other’ term was rated for a sample by a panellist, provision was made for the panellist to record the nature of the ‘other’ term that they rated. In addition, provision was made for panellists to record any general comments they may have had for each sample. These comments, together with any ‘other’ terms used to rate samples, are tabulated in the Appendix.

To avoid fatigue and the sensory carry-over effect that can occur with samples of this nature, panellists were forced to wait 60 seconds between samples, and were asked to leave the booths after every set of three samples to take a 5 to 10 minute extended break. Each judge was provided with purified water, plain water crackers and thin slices of Granny Smith apple to use for palate cleansing between samples.

Statistical analysis of data

Data generated by panellists were collected by the software Compusense five and then exported to Microsoft Excel for further statistical analysis. Analysis of variance (ANOVA) was conducted using the software GenStat Seventh Edition, Lawes Agricultural Trust. Where a significant ($p < 0.05$) sample F-ratio was found, pair-wise comparisons using Fishers least significant difference procedure were completed.

Uptake of geosmin and 2-methylisoborneol (MIB)

Samples

The barramundi samples for the uptake trial were supplied by Argyle Barramundi for sensory analysis as frozen barramundi fish fillets which had been exposed to a constant level of geosmin / MIB for a number of different time intervals (0, 1, 3, 6, 12, 24, 48 or 72 hours). A total of 16 fish (~2 kg in size) from the trial were supplied for sensory assessment in fillet form. Individually bagged frozen fillets were received on the 12th April, 2005. The fillets consisted of the anterior / dorsal section which yielded approximately 500 g of flesh from each fish for sensory evaluation. Further details of each sample are given in Table 3, including the different varying times of exposure to the geosmin / MIB tainted water. As shown in Table 3, two fish (each consisting of two fillet portions) from each treatment were supplied for sensory analysis.

Table 3 Sample details

Sample	Replicate	Date and time harvested		Fish	Size	Fillet Portion	Blinding code
Uptake 0 hours	1	2/04/2005	11:00	A	2 kg	anterior / dorsal	321
Uptake 0 hours	2	2/04/2005	11:00	B	2 kg	anterior / dorsal	239
Uptake 1 hour	1	2/04/2005	12:00	C	2 kg	anterior / dorsal	193
Uptake 1 hour	2	2/04/2005	12:00	D	2 kg	anterior / dorsal	932
Uptake 3 hours	1	2/04/2005	14:00	E	2 kg	anterior / dorsal	492
Uptake 3 hours	2	2/04/2005	14:00	F	2 kg	anterior / dorsal	380
Uptake 6 hours	1	2/04/2005	17:00	G	2 kg	anterior / dorsal	474
Uptake 6 hours	2	2/04/2005	17:00	H	2 kg	anterior / dorsal	430
Uptake 12 hours	1	2/04/2005	23:00	I	2 kg	anterior / dorsal	977
Uptake 12 hours	2	2/04/2005	23:00	J	2 kg	anterior / dorsal	297
Uptake 24 hours	1	3/04/2005	11:00	K	2 kg	anterior / dorsal	657
Uptake 24 hours	2	3/04/2005	11:00	L	2 kg	anterior / dorsal	357
Uptake 48 hours	1	4/04/2005	11:00	M	2 kg	anterior / dorsal	110
Uptake 48 hours	2	4/04/2005	11:00	N	2 kg	anterior / dorsal	894
Uptake 72 hours	1	5/04/2005	11:00	O	2 kg	anterior / dorsal	292
Uptake 72 hours	2	5/04/2005	11:00	P	2 kg	anterior / dorsal	130

On the 12th April 2005, sixteen samples (8 treatments by 2 replicates) from the uptake trial were assessed by the trained panel in the sensory laboratory of the DPI&F, Hamilton.

Results and discussion

The mean sensory scores for each of the aroma, flavour and aftertaste attributes rated for each fish are given in Table 4. Mean scores for each fish sample were calculated as an average of 10 judge scores on a 100 point scale.

The mean scores for each treatment (mean of 2 replicates and 10 judge scores) are given in Table 5. A one-way analysis of variance (ANOVA), blocking for Judge effect, was conducted for each sensory attribute rated, to determine if there were significant differences between treatments (refer to Table 5). The ANOVA results showed than there were significant differences between samples for aroma attributes *milky aroma*, *steamed aroma*, *salty sea breeze aroma*, and for the flavour attribute *muddy / earthy flavour*. Differences between treatments are indicated in Table 5 by different letters within a column (i.e. $^{a\ b\ c}$). The mean sensory scores of each attribute for each treatment (uptake time) are also plotted in Figure 5.

The results showed that with increasing uptake time, the *muddy / earthy* 'off' *flavour* perceived in the barramundi flesh increased to a maximum level at around 48 hours.

The scores obtained in the uptake trial for the attribute *muddy / earthy flavour*, were compared to the scores for this attribute for the lake and purged fish rated during panel training. The scores of this flavour attribute were much lower in the uptake trial samples (highest mean score of 25 for treatment 48 hours) than the lake fish used in training (mean score of 40). This indicates that these samples may not have been as high in the *muddy / earthy* character, related to MIB / geosmin uptake, in comparison to the lake fish used during panel training.

Table 4 Mean sensory scores of aroma, flavour and aftertaste attributes for each fish

Sample	Fish	*milky aroma*	*steamed aroma*	*salty sea breeze aroma*	*fresh aroma*	*fishy aroma*	*muddy / earthy aroma*	*sweetness*	*milky flavour*	*fresh flavour*	*fishy flavour*	*muddy / earthy flavour*	*metallic flavour*	*muddy aftertaste*	*fishy aftertaste*
Uptake 0 hours	A	16	35	7	29	16	4	13	11	30	25	5	11	3	14
Uptake 0 hours	B	13	32	12	29	13	7	17	8	33	28	5	5	2	13
Uptake 1 hour	C	10	27	6	32	15	4	13	10	28	30	9	7	4	16
Uptake 1 hour	D	19	27	7	32	18	5	8	7	20	24	13	7	8	14
Uptake 3 hours	E	7	26	11	20	22	5	14	6	25	17	17	5	12	13
Uptake 3 hours	F	12	29	7	25	13	6	14	10	22	23	18	8	6	18
Uptake 6 hours	G	6	26	7	17	22	20	12	8	21	22	24	13	13	12
Uptake 6 hours	H	6	22	11	21	27	6	17	9	23	25	14	2	9	15
Uptake 12 hours	I	6	30	10	22	18	12	12	8	22	22	18	4	8	16
Uptake 12 hours	J	12	29	8	23	19	7	19	7	26	27	19	6	7	19
Uptake 24 hours	K	14	37	8	37	22	5	15	7	27	14	18	7	8	12
Uptake 24 hours	L	11	26	11	28	26	6	11	4	14	25	22	9	10	16
Uptake 48 hours	M	14	39	5	28	13	6	11	6	15	27	29	12	11	18
Uptake 48 hours	N	9	32	7	28	25	8	11	3	21	19	21	8	10	13
Uptake 72 hours	O	14	36	11	26	23	4	11	6	25	16	18	7	11	15
Uptake 72 hours	P	19	38	8	27	16	5	11	7	18	23	17	8	10	15

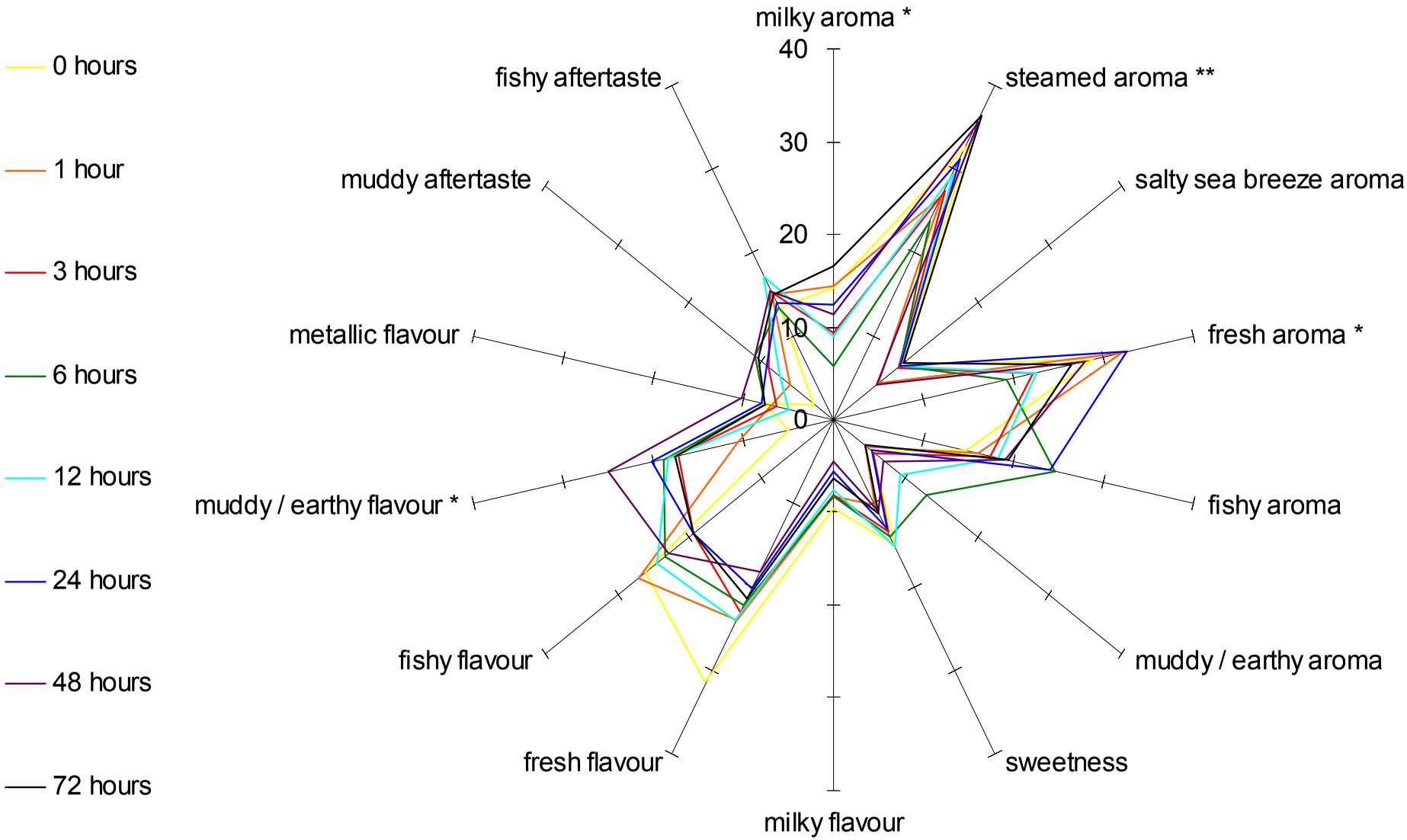
Mean scores calculated over 10 judges

Table 5 Mean sensory scores of aroma, flavour and aftertaste attributes for each uptake time treatment

Sample	*milky aroma* *	*steamed aroma* **	*salty sea breeze aroma*	*fresh aroma**	*fishy aroma*	*muddy / earthy aroma*	*sweetness*	*milky flavour*	*fresh flavour*	*fishy flavour*	*muddy / earthy flavour* *	*metallic flavour*	*muddy aftertaste*	*fishy aftertaste*
Uptake 0 hrs	14^{bc}	33^{bc}	10	29^{bc}	15	5	15	10	32	26	5^{a}	8	3	13
Uptake 1 hr	14^{bc}	27^{ab}	6	32^{c}	16	5	10	8	24	27	11^{ab}	7	6	15
Uptake 3 hrs	9^{ab}	27^{ab}	9	22^{ab}	17	6	14	8	23	20	17^{bc}	6	9	16
Uptake 6 hrs	6^{a}	24^{a}	9	19^{a}	25	13	14	8	22	23	19^{bc}	8	11	13
Uptake 12 hrs	9^{ab}	30^{abc}	9	22^{ab}	18	9	15	8	24	25	18^{bc}	5	7	17
Uptake 24 hrs	13^{abc}	31^{bc}	9	33^{c}	24	5	13	6	20	19	20^{bc}	8	9	14
Uptake 48 hrs	11^{abc}	36^{c}	6	28^{bc}	19	7	11	5	18	23	25^{c}	10	11	16
Uptake 72 hrs	17^{c}	37^{c}	10	26^{abc}	20	4	11	6	21	20	18^{bc}	8	10	15

* $p < 0.05$, ** $p < 0.01$; $^{a\ b\ c}$ different letters within a column signify significant differences between treatments; mean scores calculated over 10 judges and 2 replicates

Figure 5 Mean sensory scores of each attribute rated for samples from each treatment in the uptake trial



Attribute scores are calculated as the mean value for each treatment (mean of 10 judges and 2 replicate fish). Attributes for which statistically significant differences were observed between samples, as determined by ANOVA, are indicated by * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$).

Comparison of off flavour within the fillet

Samples

The barramundi samples for the comparison of off flavour within the fillet trial were supplied by Argyle Barramundi for sensory analysis as frozen barramundi fish fillets. The fillets consisted of anterior / dorsal, belly flap and tail section portions. Individually bagged frozen fillets were received on the 12th April, 2005. Portions from a total of 15 fish (fish ~2 kg in size) which had been exposed to a constant level of geosmin / MIB in water were provided. Each fish consisted of approximately 500 g of anterior / dorsal flesh, 250 g of tail section flesh and 100 g of belly flap flesh for sensory analysis. As the panel of ten judges required at least 200 g of fillet (20 g per person) for sensory analysis, one whole fish was not able to be used as one replicate due to the limiting amount of belly flap flesh (100 g only). Samples were therefore organised such that two fish were used as one replicate and the panel of ten was split into two so that the same five panellists assessed the three different sections from the same fish.

Further details of each sample are given in Table 6, including the section of fish and the replicate for which each fish was used. As shown in Table 6, sections of flesh from two fish were used for each single replicate. For example, flesh from different sections of fish AY and from fish BD were used for replicate one. Five of the ten panellists assessed all three portions of flesh from fish AY and the other five judges assessed all three sections of flesh from fish BD.

Table 6 Sample details

Sample / fillet portion	Replicate	Fish	Size	Blinding code
anterior / dorsal	1	AY	2 kg	990
tail flesh	1	AY	2 kg	424
belly flap	1	AY	2 kg	487
anterior / dorsal	2	AZ	2 kg	651
tail flesh	2	AZ	2 kg	188
belly flap	2	AZ	2 kg	968
anterior / dorsal	3	BA	2 kg	640
tail flesh	3	BA	2 kg	295
belly flap	3	BA	2 kg	478
anterior / dorsal	4	BB	2 kg	492
tail flesh	4	BB	2 kg	307
belly flap	4	BB	2 kg	556
anterior / dorsal	5	BC	2 kg	104
tail flesh	5	BC	2 kg	348
belly flap	5	BC	2 kg	801
anterior / dorsal	1	BD	2 kg	919
tail flesh	1	BD	2 kg	304
belly flap	1	BD	2 kg	542
anterior / dorsal	2	BE	2 kg	899
tail flesh	2	BE	2 kg	255
belly flap	2	BE	2 kg	965
anterior / dorsal	3	BF	2 kg	974
tail flesh	3	BF	2 kg	886
belly flap	3	BF	2 kg	401
anterior / dorsal	4	BG	2 kg	642
tail flesh	4	BG	2 kg	982

Sample / fillet portion	Replicate	Fish	Size	Blinding code
belly flap	4	BG	2 kg	879
anterior / dorsal	5	BH	2 kg	717
tail flesh	5	BH	2 kg	353
belly flap	5	BH	2 kg	920
anterior / dorsal	not used	BI	2 kg	-
tail flesh	not used	BI	2 kg	-
belly flap	not used	BI	2 kg	-
anterior / dorsal	not used	BJ	2 kg	-
tail flesh	not used	BJ	2 kg	-
belly flap	not used	BJ	2 kg	
anterior / dorsal	not used	BK	2 kg	-
tail flesh	not used	BK	2 kg	-
belly flap	not used	BK	2 kg	-
anterior / dorsal	not used	BL	2 kg	-
tail flesh	not used	BL	2 kg	-
belly flap	not used	BL	2 kg	-
anterior / dorsal	not used	BM	2 kg	-
tail flesh	not used	BM	2 kg	-
belly flap	not used	BM	2 kg	-

On the 18th April 2005, fifteen samples (3 treatments by 5 replicates, each replicate consisting of 2 fish) from the comparison of off flavour within the fillet trial were assessed by the trained panel in the sensory laboratory of the DPI&F, Hamilton.

Results and discussion

The mean sensory scores for each of the aroma, flavour and aftertaste attributes rated for each replicate (incorporating two fish) are given in Table 7. Mean scores for each replicate were calculated as an average of 10 judge scores on a 100 point scale.

The mean sensory scores for each of the three sections of fish are given in Table 8. The mean sensory scores of each attribute for each fillet section are plotted in Figure 6. A one-way analysis of variance (ANOVA), blocking for the effect of fish, was conducted on the raw data for each sensory attribute rated, to determine if there were significant differences between fillet sections. The ANOVA results showed than there were significant differences between samples for the aroma attribute *muddy / earthy aroma*, and flavour attributes *sweetness*, *milky flavour*, and *fresh flavour*. Differences between fillet sections are indicated in Table 8 by different letters within a column (i.e. $^{a\,b}$).

Although there was no significant differences observed between the different sections of barramundi fillet for the *muddy / earthy flavour* attribute, there were significant differences observed between sections for the *muddy / earthy aroma* property. The anterior / dorsal and belly flap sections were scored significantly higher in the *muddy / earthy aroma* than the tail section of the fish, however, on average all the samples were scored very low for this attribute on the scale of 0 - 100.

Table 7 Mean sensory scores of aroma, flavour and aftertaste attributes for each replicate

Sample	Fish		Rep	*milky aroma*	*steamed aroma*	*salty sea breeze aroma*	*fresh aroma*	*fishy aroma*	*muddy / earthy aroma*	*sweetness*	*milky flavour*	*fresh flavour*	*fishy flavour*	*muddy / earthy flavour*	*metallic flavour*	*muddy aftertaste*	*fishy aftertaste*
anterior / dorsal	AY	BD	1	11	32	10	28	27	6	8	7	14	30	14	9	5	23
belly flap	AY	BD	1	6	30	12	21	26	5	14	13	25	26	14	11	5	18
tail flesh	AY	BD	1	16	30	12	31	19	4	9	4	14	24	21	12	12	19
anterior / dorsal	AZ	BE	2	11	25	9	23	22	10	10	6	13	21	19	16	7	16
belly flap	AZ	BE	2	6	33	12	32	27	12	20	9	25	25	17	7	11	13
tail flesh	AZ	BE	2	9	19	11	24	27	3	10	5	12	27	20	9	15	23
anterior / dorsal	BA	BF	3	8	28	9	20	24	7	10	6	12	30	20	16	12	25
belly flap	BA	BF	3	7	34	10	27	24	12	17	12	22	28	16	12	6	16
tail flesh	BA	BF	3	15	31	11	30	22	2	13	5	17	27	19	7	7	21
anterior / dorsal	BB	BG	4	8	36	9	23	19	8	13	8	20	22	9	6	5	22
belly flap	BB	BG	4	10	29	7	25	23	1	10	7	21	35	8	6	4	25
tail flesh	BB	BG	4	11	37	9	31	18	6	11	5	18	20	13	14	8	16
anterior / dorsal	BC	BH	5	10	31	13	31	24	4	6	3	16	16	23	10	16	14
belly flap	BC	BH	5	12	38	12	29	25	8	12	9	22	23	22	10	15	22
tail flesh	BC	BH	5	14	30	9	28	27	1	11	8	21	26	10	8	4	19

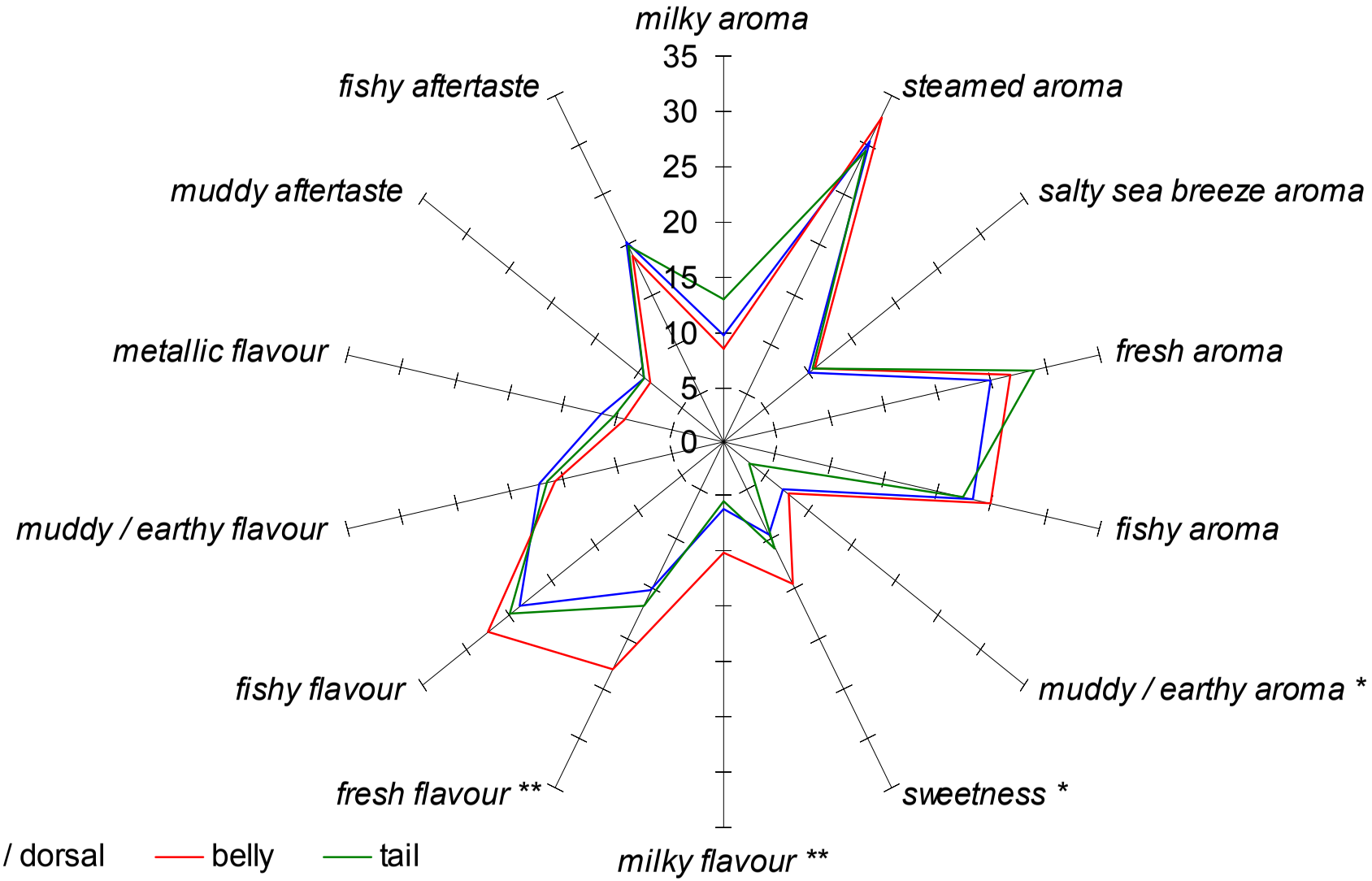
Mean scores calculated over 10 judges and 2 fish

Table 8 Mean sensory scores of aroma, flavour and aftertaste attributes for each section of fish

Sample	*milky aroma*	*steamed aroma*	*salty sea breeze aroma*	*fresh aroma*	*fishy aroma*	*muddy / earthy aroma**	*sweetness**	*milky flavour***	*fresh flavour***	*fishy flavour*	*muddy / earthy flavour*	*metallic flavour*	*muddy aftertaste*	*fishy aftertaste*
anterior / dorsal	10	30	10	25	23	7^{a}	9^{b}	6^{a}	15^{a}	24	17	11	9	20
belly flap	8	33	11	27	25	8^{a}	14^{a}	10^{b}	23^{b}	28	16	9	8	19
tail section	13	29	10	29	22	3^{b}	11^{ab}	5^{a}	16^{a}	25	16	10	9	20

* $p < 0.05$, ** $p < 0.01$; $^{a\ b}$ different letters within a column signify significant differences between treatments; mean scores calculated over 10 judges, 5 replicates and 10 fish

Figure 6 Mean sensory scores of each attribute rated for samples from each section



Attribute scores are calculated as the mean value for each section (mean of 10 judges and 5 replicates). Attributes for which statistically significant differences were observed between samples, as determined by ANOVA, are indicated by * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$).

Comparison of off flavour within the population

Samples

The barramundi samples for the comparison of off flavour within the population trial were supplied by Argyle Barramundi for sensory analysis as frozen barramundi fish fillets. The fillets were from fish of different sizes that had been exposed to a constant level of geosmin / MIB. Individually bagged frozen fillets were received on the 12th April, 2005. The fillets consisted of anterior / dorsal sections from 6 large fish (~2 kg in size) and 18 small fish (~800 g in size). Large fish yielded approximately 850 g of flesh and small fish gave around 150 g of flesh for sensory assessment. As the panel of 10 judges required at least 200 g of fillet (20 g per person) for sensory analysis, one whole small fish was not sufficient to be used as one replicate. Samples were organised such that two small fish were used as one replicate with half the panel tasting one fish and the other half tasting the second fish. On the other hand, the large fish yielded enough flesh to serve the entire panel and one fish could be used as a single replicate.

Further details of each sample are given in Table 9, including the size of the fish and the replicate for which each fish was used.

Table 9 Sample details for the comparison of off flavour within the population trial

Sample	Replicate	Fish	Size	Fillet portion	Blinding code
large	1 7	AA	2 kg	anterior / dorsal	634 575
large	2 7	AB	2 kg	anterior / dorsal	493 175
large	3	AC	2 kg	anterior / dorsal	330
large	4	AD	2 kg	anterior / dorsal	256
large	5	AE	2 kg	anterior / dorsal	463
large	6	AF	2 kg	anterior / dorsal	938
small	1	AG	800 g	anterior / dorsal	583
small	1	AH	800 g	anterior / dorsal	272
small	2	AI	800 g	anterior / dorsal	904
small	2	AJ	800 g	anterior / dorsal	864
small	3	AK	800 g	anterior / dorsal	281
small	3	AL	800 g	anterior / dorsal	143
small	4	AM	800 g	anterior / dorsal	589
small	4	AN	800 g	anterior / dorsal	715
small	5	AO	800 g	anterior / dorsal	801
small	5	AP	800 g	anterior / dorsal	922
small	6	AQ	800 g	anterior / dorsal	439
small	6	AR	800 g	anterior / dorsal	136
small	7	AS	800 g	anterior / dorsal	835
small	7	AT	800 g	anterior / dorsal	466
small	not used	AU	800 g	anterior / dorsal	-
small	not used	AV	800 g	anterior / dorsal	-
small	not used	AW	800 g	anterior / dorsal	-
small	not used	AX	800 g	anterior / dorsal	-

As shown in Table 9, flesh from two small fish was used for each single replicate. For example, fish AG and AH were used as one replicate where half the panel (5 panellists) assessed fish AG and the other half assessed fish AH. Although there were only 6 large fish provided, there was sufficient flesh remaining from the large fish to make an additional replicate sample. The unused

flesh from fish AA and AB was used to give a seventh replicate sample for large fish where half the panel assessed fish AA and the other half assessed fish AB.

On the 19th April 2005, fourteen samples (2 treatments by 7 replicates) from the comparison of off flavour within the population trial were assessed by the trained panel in the sensory laboratory of the DPI&F, Hamilton.

Results and discussion

The mean sensory scores for each of the aroma, flavour and aftertaste attributes rated for each replicate are given in Table 10. Mean scores for each replicate were calculated as an average of 10 judge scores on a 100 point scale.

The mean sensory scores for the treatments large and small fish are given in Table 11. The mean sensory scores of each attribute for each treatment are plotted in Figure 7. A one-way analysis of variance (ANOVA), blocking for judge effect, was conducted on the raw data for each sensory attribute rated, to determine if there were significant differences between treatments. The ANOVA results showed than there were significant differences between samples for the attributes *sweetness*, *fresh flavour, muddy / earthy flavour and muddy aftertaste*. Differences between treatments are indicated in Table 11 by different letters within a column (i.e. $^{a\ b}$).

Highly significant differences were observed between large and small fish for the attribute *muddy / earthy flavour* with large fish being scored significantly higher in this flavour attribute than the small fish. Similarly, the attribute *muddy aftertaste* was scored significant higher in the larger fish. On the other hand, the attributes *sweetness* and *fresh flavour* were scored significantly higher in the smaller fish.

Table 10 Mean sensory scores of aroma, flavour and aftertaste attributes for each replicate

Sample	Fish	Rep	*milky aroma*	*steamed aroma*	*salty sea breeze aroma*	*fresh aroma*	*fishy aroma*	*muddy / earthy aroma*	*sweetness*	*milky flavour*	*fresh flavour*	*fishy flavour*	*muddy / earthy flavour*	*metallic flavour*	*muddy aftertaste*	*fishy aftertaste*
large	AA	1	5	24	5	25	27	9	8	6	19	25	20	7	7	17
large	AB	2	10	33	11	26	20	6	6	4	18	25	20	6	10	14
large	AC	3	8	30	10	32	18	1	9	5	21	16	15	8	7	15
large	AD	4	7	31	12	27	27	6	6	5	17	27	14	7	10	18
large	AE	5	9	33	6	27	15	2	5	6	18	19	19	6	8	17
large	AF	6	11	36	12	26	22	8	17	7	27	20	14	7	8	17
large	AA AB	7	4	20	11	28	28	2	5	4	17	27	28	9	15	23
small	AI AJ	1	6	30	17	34	28	2	3	4	14	25	10	10	2	21
small	AK AL	2	7	29	14	30	24	6	23	11	34	21	12	3	8	13
small	AM AN	3	9	27	12	37	29	3	9	1	24	27	15	6	5	15
small	AO AP	4	5	30	10	24	31	5	17	10	27	25	12	2	9	16
small	AQ AR	5	8	38	3	28	16	4	13	7	26	22	7	4	5	16
small	AS AT	6	16	37	3	31	18	6	20	13	31	20	3	3	2	14
small	AG AH	7	6	31	2	23	20	6	12	6	18	24	15	15	11	20

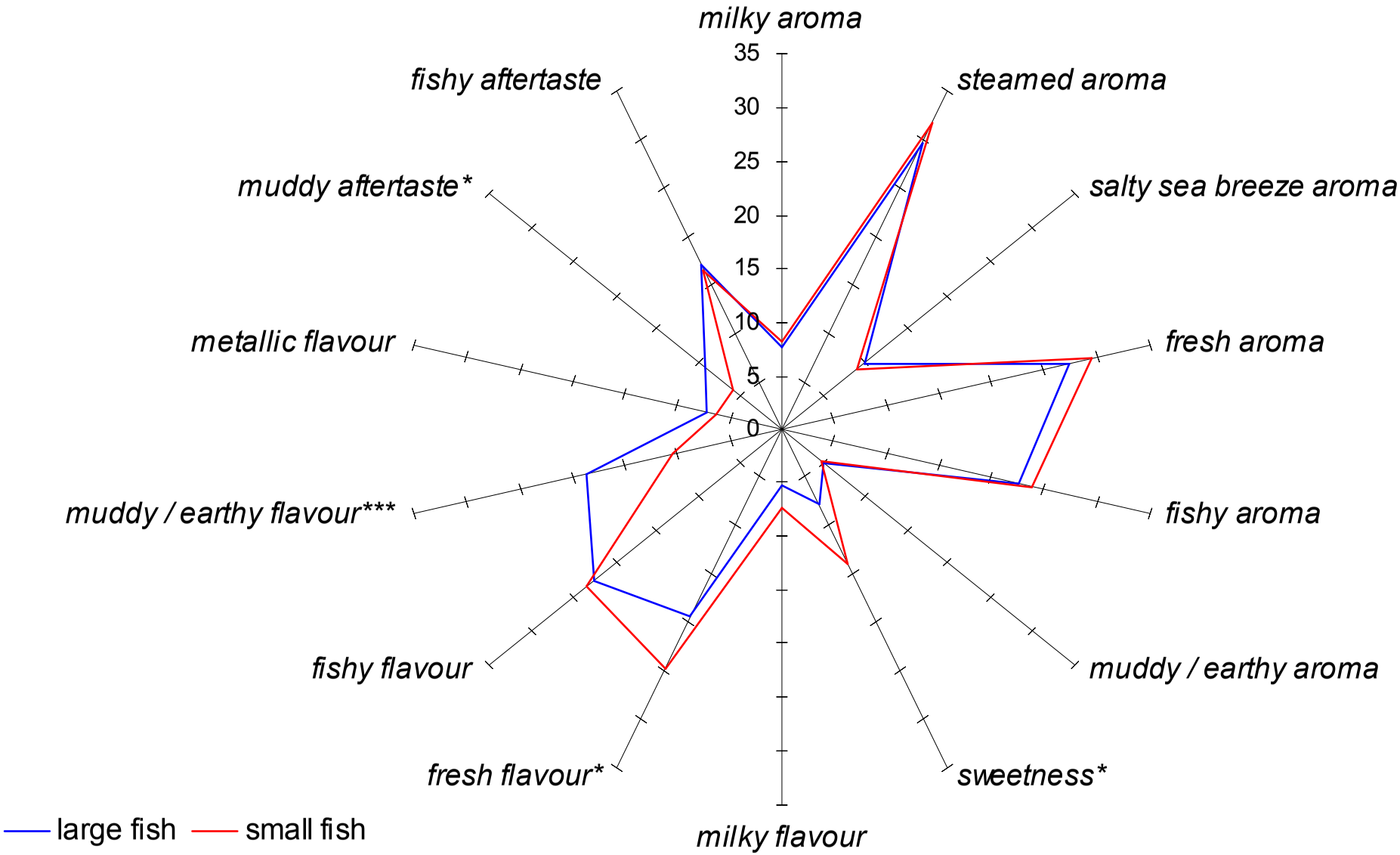
Mean scores calculated over 10 judges

Table 11 Mean sensory scores of aroma, flavour and aftertaste attributes for each fish size

Sample	*milky aroma*	*steamed aroma*	*salty sea breeze aroma*	*fresh aroma*	*fishy aroma*	*muddy / earthy aroma*	*sweetness**	*milky flavour*	*fresh flavour**	*fishy flavour*	*muddy / earthy flavour****	*metallic flavour*	*muddy aftertaste**	*fishy aftertaste*
large	8	30	10	27	22	5	8[a]	5	19[a]	22	19[a]	7	9[a]	17
small	8	32	9	29	24	5	14[b]	7	25[b]	23	10[b]	6	6[b]	16

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; [a b] different letters within a column signify significant differences between treatments; mean scores calculated over 10 judges and 7 replicates

Figure 7 Mean sensory scores of each attribute rated for samples of each fish size



Attribute scores are calculated as the mean value for each fish size (mean of 10 judges and 7 replicates). Attributes for which statistically significant differences were observed between samples, as determined by ANOVA, are indicated by * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$).

Purging trial

Samples

The barramundi samples for the purging trial were supplied by Argyle Barramundi for sensory analysis as frozen barramundi fish fillets from geosmin / MIB tainted fish that had undergone differing purging treatments. Two different flow rates were used for purging (high and low) and fish were purged under each flow rate for differing periods of time (0, 1, 2, 3, 4 and 5 days). The flesh (anterior / dorsal section) of two fish (fish ~2 kg in size) from each time-point and treatment were supplied and sufficient flesh was available such that one fish could be used as one replicate in the sensory study. Samples were received as individually bagged frozen barramundi fillets on the 9th of May, 2005.

Further details of each sample are given in Table 12, including the flow rate and the length of purging time. Tank 1 and tank 4 were replicates of the same flow time. Similarly, tank 2 and tank 3 were replicates of the same flow time. The samples labelled 'threshold start' were samples from the first threshold trial (received on the 29th April, 2005, these threshold samples otherwise not used) and were included in the purging trial on advice from the client. The 'threshold start' samples were fish that had been purged and kept in 'clean' water.

Table 12 Sample details for the purging trial

Sample	Replicate	Date harvested	Purge duration (days)	Flow rate	Blinding code
purge start	1	26/04/2005	0		634
purge start	2	26/04/2005	0		334
threshold start	1	16/04/2005			922
threshold start	2	16/04/2005			852
purge tank 1	1	27/04/2005	1	low	330
purge tank 1	1	28/04/2005	2	low	256
purge tank 1	1	29/04/2005	3	low	463
purge tank 1	1	30/04/2005	4	low	938
purge tank 1	1	1/05/2005	5	low	583
purge tank 4	2	27/04/2005	1	low	602
purge tank 4	2	28/04/2005	2	low	488
purge tank 4	2	29/04/2005	3	low	961
purge tank 4	2	30/04/2005	4	low	614
purge tank 4	2	1/05/2005	5	low	467
purge tank 2	2	27/04/2005	1	high	688
purge tank 2	1	28/04/2005	2	high	254
purge tank 2	1	29/04/2005	3	high	731
purge tank 2	1	30/04/2005	4	high	416
purge tank 2	1	1/05/2005	5	high	406
purge tank 3	2	27/04/2005	1	high	589
purge tank 3	2	28/04/2005	2	high	715
purge tank 3	2	29/04/2005	3	high	801
purge tank 3	2	30/04/2005	4	high	922
purge tank 3	2	1/05/2005	5	high	439

The 24 samples (12 treatments by 2 replicates) were assessed by the trained panel over two days on the 23rd and the 30th June 2005 at the sensory laboratory of the DPI&F, Hamilton.

Results and discussion

The mean sensory scores for each of the aroma, flavour and aftertaste attributes rated for each replicate are given in Table 13. Mean scores for each replicate were calculated as an average of 10 judge scores on a 100 point scale.

The mean sensory scores for the samples from each of the different purging treatments are given in Table 14. The mean sensory scores of each attribute for each treatment are also plotted in Figure 8. A one-way analysis of variance (ANOVA), blocking for judge effect, was conducted on the raw data for each sensory attribute rated, to determine if there were significant differences in sensory scores between purging treatments. The ANOVA results showed than there were significant differences between samples for the sensory attributes *muddy / earthy aroma*, *muddy / earthy flavour* and *muddy aftertaste*. Differences between treatments are indicated in Table 14 by different letters within the same column (i.e. $^{a\ b}$).

The results showed that over time both purging flows reduced the perceived 'off flavour' in the barramundi fish flesh. There was no significant difference between high and low flow rates in the reduction of the 'off flavour'.

Table 13 Mean sensory scores of aroma, flavour and aftertaste attributes for each fish

Sample	Day	Rep	*milky aroma*	*steamed aroma*	*salty sea breeze aroma*	*fresh aroma*	*fishy aroma*	*muddy / earthy aroma*	*sweetness*	*milky flavour*	*fresh flavour*	*fishy flavour*	*muddy / earthy flavour*	*metallic flavour*	*muddy aftertaste*	*fishy aftertaste*
purge start	0	1	8	32	14	24	19	11	12	11	14	25	27	10	21	17
purge start	0	2	6	21	10	25	17	17	12	10	20	18	29	5	19	13
threshold start		1	8	30	12	24	17	5	14	7	20	25	4	5	2	19
threshold start		2	17	36	14	31	20	1	13	11	14	17	2	3	2	13
low flow (T1)	1	1	10	25	15	23	25	10	13	9	23	26	21	12	13	15
low flow (T1)	2	1	8	25	12	21	25	11	18	11	23	30	16	8	10	21
low flow (T1)	3	1	6	22	14	24	23	7	15	16	25	24	15	17	6	21
low flow (T1)	4	1	13	31	8	27	23	4	12	7	18	37	12	12	8	22
low flow (T1)	5	1	11	30	13	25	26	8	11	7	22	29	5	18	3	23
low flow (T4)	1	2	10	27	11	24	22	14	18	12	28	29	12	6	5	22
low flow (T4)	2	2	12	25	14	34	17	1	20	8	37	23	4	6	2	16
low flow (T4)	3	2	10	24	12	25	22	4	20	11	33	19	4	4	2	17
low flow (T4)	4	2	16	31	11	38	12	1	16	8	33	24	5	2	1	15
low flow (T4)	5	2	8	27	14	28	23	4	20	13	32	22	4	6	4	19
high flow (T2)	1	2	10	31	12	19	22	10	21	9	28	19	13	8	10	13
high flow (T2)	2	1	11	30	15	26	23	2	10	9	20	23	8	8	5	15
high flow (T2)	3	1	10	27	10	26	29	7	17	12	26	30	4	9	4	24
high flow (T2)	4	1	12	32	12	32	19	3	17	7	29	19	7	3	3	15
high flow (T2)	5	1	9	30	12	27	20	5	17	8	24	33	6	12	6	23
high flow (T3)	1	2	9	24	15	27	28	8	13	7	23	24	20	8	13	18
high flow (T3)	2	2	15	28	11	29	28	12	14	9	28	29	16	13	8	22
high flow (T3)	3	2	13	29	11	32	20	4	12	8	21	29	11	11	4	20
high flow (T3)	4	2	11	35	16	32	21	4	18	10	30	23	8	13	5	14
high flow (T3)	5	2	8	30	10	27	20	6	18	9	25	18	8	4	6	15

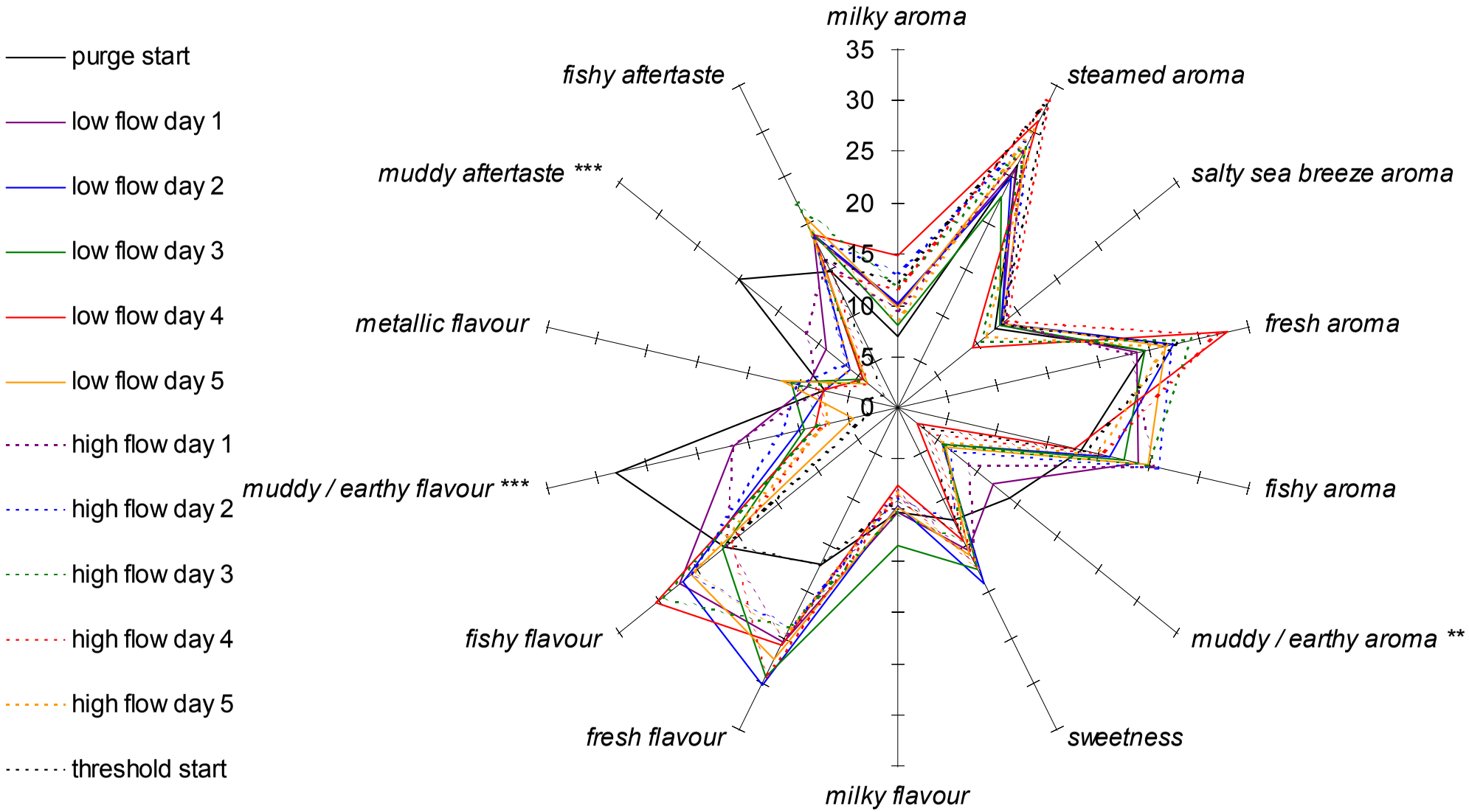
Mean scores calculated over 10 judges

Table 14 Mean sensory scores of aroma, flavour and aftertaste attributes for each purging treatment

Sample	Day	*milky aroma*	*steamed aroma*	*salty sea breeze aroma*	*fresh aroma*	*fishy aroma*	*muddy / earthy aroma***	*sweetness*	*milky flavour*	*fresh flavour*	*fishy flavour*	*muddy / earthy flavour****	*metallic flavour*	*muddy aftertaste****	*fishy aftertaste*
purge start	0	7	26	12	25	18	14^{d}	12	10	17	22	28^{d}	7	20^{d}	15
threshold start		12	33	13	28	19	3^{ab}	14	9	17	21	3^{a}	4	2^{a}	16
low flow	1	10	26	13	24	24	12^{cd}	15	10	25	27	16^{c}	9	9^{bc}	18
low flow	2	10	25	13	28	21	6^{ab}	19	10	30	27	10^{abc}	7	6^{abc}	19
low flow	3	8	23	13	25	23	6^{ab}	17	13	29	22	9^{abc}	11	4^{ab}	19
low flow	4	15	31	9	33	18	2^{a}	14	8	26	30	8^{ab}	7	4^{ab}	19
low flow	5	10	28	13	27	25	6^{abc}	16	10	27	26	4^{ab}	12	4^{ab}	21
high flow	1	9	28	13	23	25	9^{bcd}	17	8	25	21	16^{c}	8	11^{c}	16
high flow	2	13	29	13	27	26	7^{abc}	12	9	24	26	12^{bc}	10	7^{abc}	18
high flow	3	12	28	10	29	25	5^{ab}	14	10	24	29	8^{ab}	10	4^{ab}	22
high flow	4	12	33	14	32	20	4^{ab}	17	9	29	21	7^{ab}	8	4^{ab}	15
high flow	5	9	30	11	27	20	5^{ab}	18	8	25	26	7^{ab}	8	6^{abc}	19

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; $^{a\ b}$ different letters within a column signify significant differences between treatments; mean scores calculated over 10 judges and 2 replicates

Figure 8 Mean sensory scores of each attribute rated for samples from different purging treatments



Attribute scores are calculated as the mean value for each purging treatment (mean of 10 judges and 2 replicates). Attributes for which statistically significant differences were observed between samples, as determined by ANOVA, are indicated by * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$).

Geosmin threshold trial

Samples

The barramundi samples for the geosmin threshold trial were supplied by Argyle Barramundi for sensory analysis as frozen barramundi fish fillets from fish that had undergone exposure to varying levels of geosmin tainted water. All fish from this trial underwent exposure to the varying degrees of geosmin tainted water for the same period of time. The flesh (anterior / dorsal portion) of two fish (fish ~2 kg in size) from each treatment was supplied and sufficient flesh was available such that one fish could be used as one replicate in the sensory study. Samples were received as frozen barramundi fillets on the 30th May, 2005.

Further details of each sample are given in Table 15, including the indicative water geosmin level. The 14 samples (7 treatments by 2 replicates) were assessed by the trained panel on the 2nd June 2005 at the sensory laboratory of the DPI&F, Hamilton.

Table 15 Sample details for the geosmin threshold trial

Sample	Replicate	Date and time harvested		Blinding code
threshold start	1	19/05/2005	20:00	128
threshold start	2	19/05/2005	20:00	360
geosmin 0%	1	20/05/2005	10:00	738
geosmin 0%	2	20/05/2005	10:00	332
geosmin 20%	1	20/05/2005	10:00	849
geosmin 20%	2	20/05/2005	10:00	560
geosmin 40%	1	20/05/2005	10:00	458
geosmin 40%	2	20/05/2005	10:00	307
geosmin 60%	1	20/05/2005	10:00	176
geosmin 60%	2	20/05/2005	10:00	206
geosmin 80%	1	20/05/2005	10:00	364
geosmin 80%	2	20/05/2005	10:00	194
geosmin 100%	1	20/05/2005	10:00	799
geosmin 100%	2	20/05/2005	10:00	583

Results and discussion

The mean sensory scores for each of the aroma, flavour and aftertaste attributes rated for each replicate are given in Table 16. Mean scores for each replicate were calculated as an average of 10 judge scores on a 100 point scale.

The mean sensory scores for the samples from each of the different geosmin treatments are given in Table 17. The mean sensory scores of each attribute for each treatment are also plotted in Figure 9. A one-way analysis of variance (ANOVA), blocking for judge effect, was conducted on the raw data for each sensory attribute rated, to determine if there were significant differences in sensory scores between treatments. The ANOVA results showed that there were significant differences between samples for the sensory attribute *fishy flavour.* Differences between treatments are indicated in Table 17 by different letters within a column (i.e. $^{a\ b}$). The differences in the *fishy flavour* attribute did not appear to relate to the geosmin level in the water. The attributes associated with the 'off flavour' were all scored very low, indicating that no perceivable 'off flavour' was present in the fish from this trial.

It should be noted, that the panellists frequently used terms such as ‘chemical’ and ‘artificial’ to describe the fish from this trial. A summary of these comments for fish from each treatment is given in the Appendix in Table 25.

Table 16 Mean sensory scores of aroma, flavour and aftertaste attributes for each fish

Sample	Rep	*milky aroma*	*steamed aroma*	*salty sea breeze aroma*	*fresh aroma*	*fishy aroma*	*muddy / earthy aroma*	*sweetness*	*milky flavour*	*fresh flavour*	*fishy flavour*	*muddy / earthy flavour*	*metallic flavour*	*muddy aftertaste*	*fishy aftertaste*
threshold start	1	2	34	14	34	20	1	17	6	30	26	2	7	1	17
threshold start	2	10	28	12	33	23	0	16	13	30	28	2	5	2	15
geosmin 0%	1	5	33	14	29	15	1	22	15	38	14	0	6	1	11
geosmin 0%	2	10	29	12	32	16	2	21	8	34	17	3	5	2	14
geosmin 20%	1	6	32	7	26	18	3	10	8	23	18	5	5	2	15
geosmin 20%	2	10	33	14	31	16	2	20	8	35	16	3	5	2	10
geosmin 40%	1	10	33	9	28	20	4	17	15	27	19	4	2	1	13
geosmin 40%	2	13	33	8	27	13	5	11	9	26	21	6	4	3	17
geosmin 60%	1	10	29	13	29	15	1	14	6	34	27	4	2	2	13
geosmin 60%	2	4	29	12	26	23	3	11	13	31	20	6	4	2	15
geosmin 80%	1	5	33	11	35	16	6	16	10	31	14	8	5	5	11
geosmin 80%	2	6	29	12	29	27	5	17	10	30	21	2	5	1	17
geosmin 100%	1	3	30	17	35	22	3	9	9	30	17	7	6	2	7
geosmin 100%	2	13	32	10	29	18	1	13	9	24	24	2	3	1	21

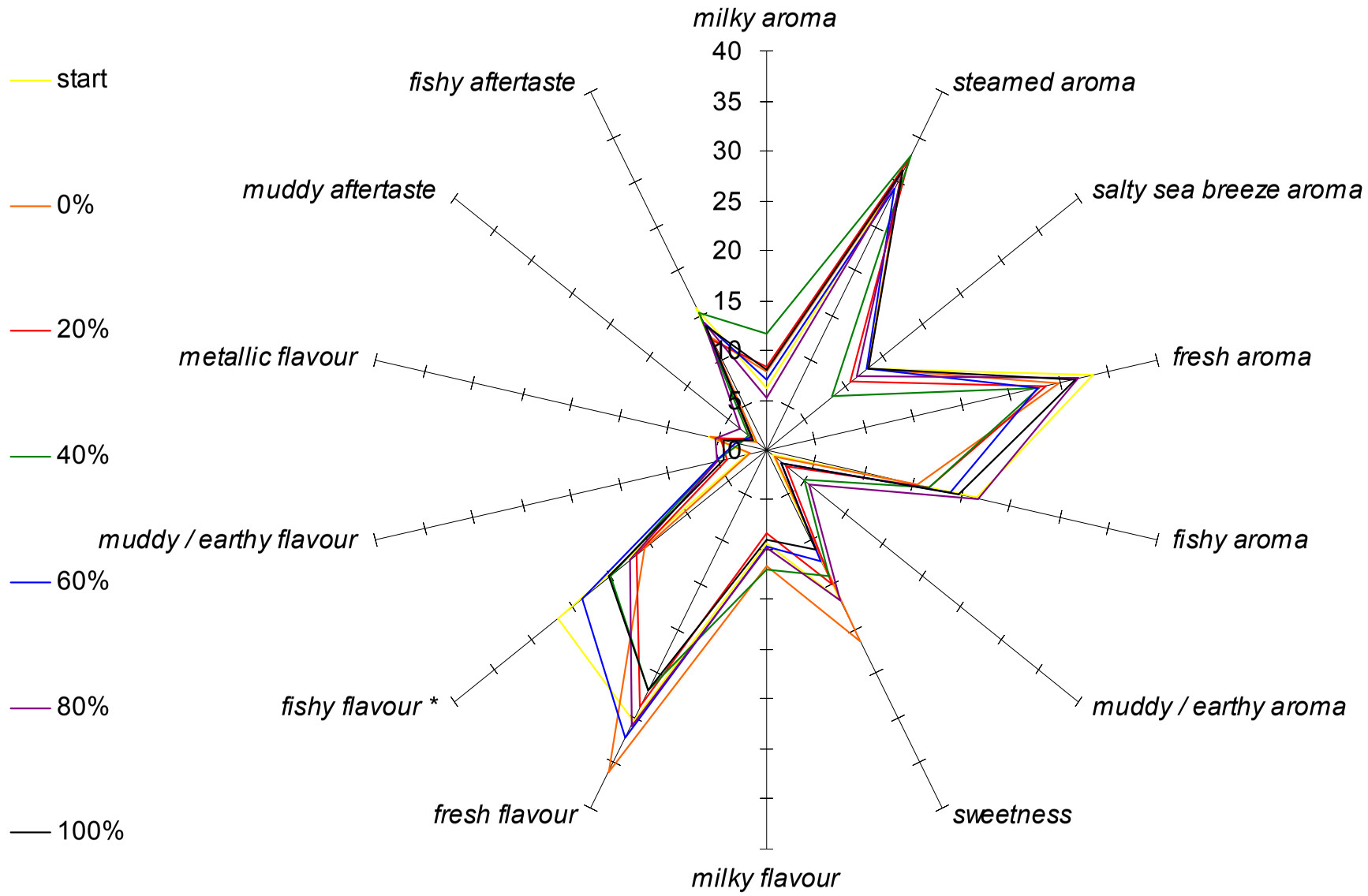
Mean scores calculated over 10 judges

Table 17 Mean sensory scores of aroma, flavour and aftertaste attributes for each geosmin treatment

Sample	*milky aroma*	*steamed aroma*	*salty sea breeze aroma*	*fresh aroma*	*fishy aroma*	*muddy / earthy aroma*	*sweetness*	*milky flavour*	*fresh flavour*	*fishy flavour**	*muddy / earthy flavour*	*metallic flavour*	*muddy aftertaste*	*fishy aftertaste*
threshold start	6	31	13	33	22	1	17	9	30	27^{c}	2	6	1	16
geosmin 0%	8	31	13	30	15	1	21	12	36	16^{a}	2	6	1	13
geosmin 20%	8	32	11	29	17	3	15	8	29	17^{ab}	4	5	2	12
geosmin 40%	12	33	9	28	17	5	14	12	27	20^{ab}	5	3	2	15
geosmin 60%	7	29	13	28	19	2	12	10	32	24^{bc}	5	3	2	14
geosmin 80%	5	31	12	32	22	5	17	10	31	18^{ab}	5	5	3	14
geosmin 100%	8	31	13	32	20	2	11	9	27	20^{abc}	4	5	2	14

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; $^{a\ b}$ different letters within a column signify significant differences between treatments; mean scores calculated over 10 judges and 2 replicates

Figure 9 Mean sensory scores of each attribute rated for samples from barramundi exposed to different geosmin water levels



Attribute scores are calculated as the mean value for each treatment (mean of 10 judges and 2 replicates). Attributes for which statistically significant differences were observed between samples, as determined by ANOVA, are indicated by * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$).

2-Methylisoborneol (MIB) threshold trial

Samples

The barramundi samples for the 2-methylisoborneol (MIB) threshold trial were supplied by Argyle Barramundi for sensory analysis as frozen barramundi fish fillets from fish that had undergone exposure to varying levels of MIB tainted water. Fish from this trial underwent exposure to the varying degrees of MIB tainted water for the same period of time. The flesh (anterior / dorsal portion) of two fish (fish ~2 kg in size) from each treatment was supplied and sufficient flesh was available such that one fish could be used as one replicate in the sensory study. Samples were received as frozen barramundi fillets on the 30th May, 2005.

Further details of each sample are given in Table 18, including the indicative level of MIB in the water. The 14 samples (7 treatments by 2 replicates) were assessed by the trained panel on the 7th June 2005 at the sensory laboratory of the DPI&F, Hamilton.

Table 18 Sample details for the MIB threshold trial

Sample	Replicate	Date and time harvested		Blinding code
threshold start	1	18/05/2005	20:00	301
threshold start	2	18/05/2005	20:00	461
MIB 0%	1	19/05/2005	11:00	596
MIB 0%	2	19/05/2005	11:00	978
MIB 20%	1	19/05/2005	11:00	160
MIB 20%	2	19/05/2005	11:00	398
MIB 40%	1	19/05/2005	11:00	241
MIB 40%	2	19/05/2005	11:00	564
MIB 60%	1	19/05/2005	11:00	356
MIB 60%	2	19/05/2005	11:00	554
MIB 80%	1	19/05/2005	11:00	194
MIB 80%	2	19/05/2005	11:00	816
MIB 100%	1	19/05/2005	11:00	244
MIB 100%	2	19/05/2005	11:00	790

Results and discussion

The mean sensory scores for each of the aroma, flavour and aftertaste attributes rated for each replicate are given in Table 19. Mean scores for each replicate were calculated as an average of 10 judge scores on a 100 point scale.

The mean sensory scores for the samples from each of the different MIB treatments are given in Table 20. The mean sensory scores of each attribute for each treatment are also plotted in Figure 10. A one-way analysis of variance (ANOVA), blocking for judge effect, was conducted on the raw data for each sensory attribute rated, to determine if there were significant differences in sensory scores between treatments. The ANOVA results showed that there were significant differences between samples in different treatments for the sensory attributes *steamed aroma*, *fresh aroma*, *other aroma*, *muddy / earthy flavour* and *muddy aftertaste*. Differences between treatments are indicated in Table 20 by different letters within columns (i.e. $^{a\,b}$).

The results demonstrated that increasing MIB concentration related to an increase in the perceived 'off flavour' reaching a maximum at 60% and 80% MIB. Interestingly, fish from the 100% MIB level in water were not scored the highest for the muddy / earthy 'off flavour'.

Samples from the MIB threshold trial were also found to show significant differences in the sensory score for the *other aroma* attribute (refer to Table 20). The 'other' attribute was included for panellists to rate if they thought they could detect a sensory property that was not otherwise covered by the formal attribute list. The MIB 40% samples was rated significantly higher for *other aroma* than the other samples in the trial. Comments recorded from the panellists who described the nature of the *other aroma* property for both replicates of this sample included: chemical (x3); stale; dog poo (x2); off / manure; fish oil; a touch of sewage; almost floral. All other comments made by the panel for this set of samples are given in Table 26, Appendix.

Table 19 Mean sensory scores of aroma, flavour and aftertaste attributes for each fish

Sample	Rep	*milky aroma*	*steamed aroma*	*salty sea breeze aroma*	*fresh aroma*	*fishy aroma*	*muddy / earthy aroma*	*sweetness*	*milky flavour*	*fresh flavour*	*fishy flavour*	*muddy / earthy flavour*	*metallic flavour*	*muddy aftertaste*	*fishy aftertaste*
threshold start	1	8	32	10	32	16	0	14	9	31	28	3	3	1	18
threshold start	2	10	29	13	41	13	1	14	9	38	20	2	2	1	10
MIB 0%	1	13	32	10	32	17	1	12	10	31	22	2	1	0	17
MIB 0%	2	8	36	12	32	15	2	12	9	30	26	2	2	2	16
MIB 20%	1	12	34	14	32	18	1	12	8	29	27	2	5	2	16
MIB 20%	2	5	31	11	30	19	1	17	6	27	24	2	4	2	16
MIB 40%	1	6	19	10	15	18	0	12	5	24	24	3	5	2	19
MIB 40%	2	5	30	12	29	15	1	16	8	28	21	3	1	3	14
MIB 60%	1	4	34	13	31	19	2	12	5	24	26	15	4	5	17
MIB 60%	2	10	32	12	36	15	2	10	5	31	19	9	5	5	15
MIB 80%	1	6	33	13	29	18	3	12	8	27	19	10	6	7	14
MIB 80%	2	14	34	14	37	19	2	15	9	29	23	12	2	8	18
MIB 100%	1	6	34	13	29	17	1	19	9	33	20	7	3	3	14
MIB 100%	2	8	33	13	29	18	2	14	11	30	19	8	5	6	14

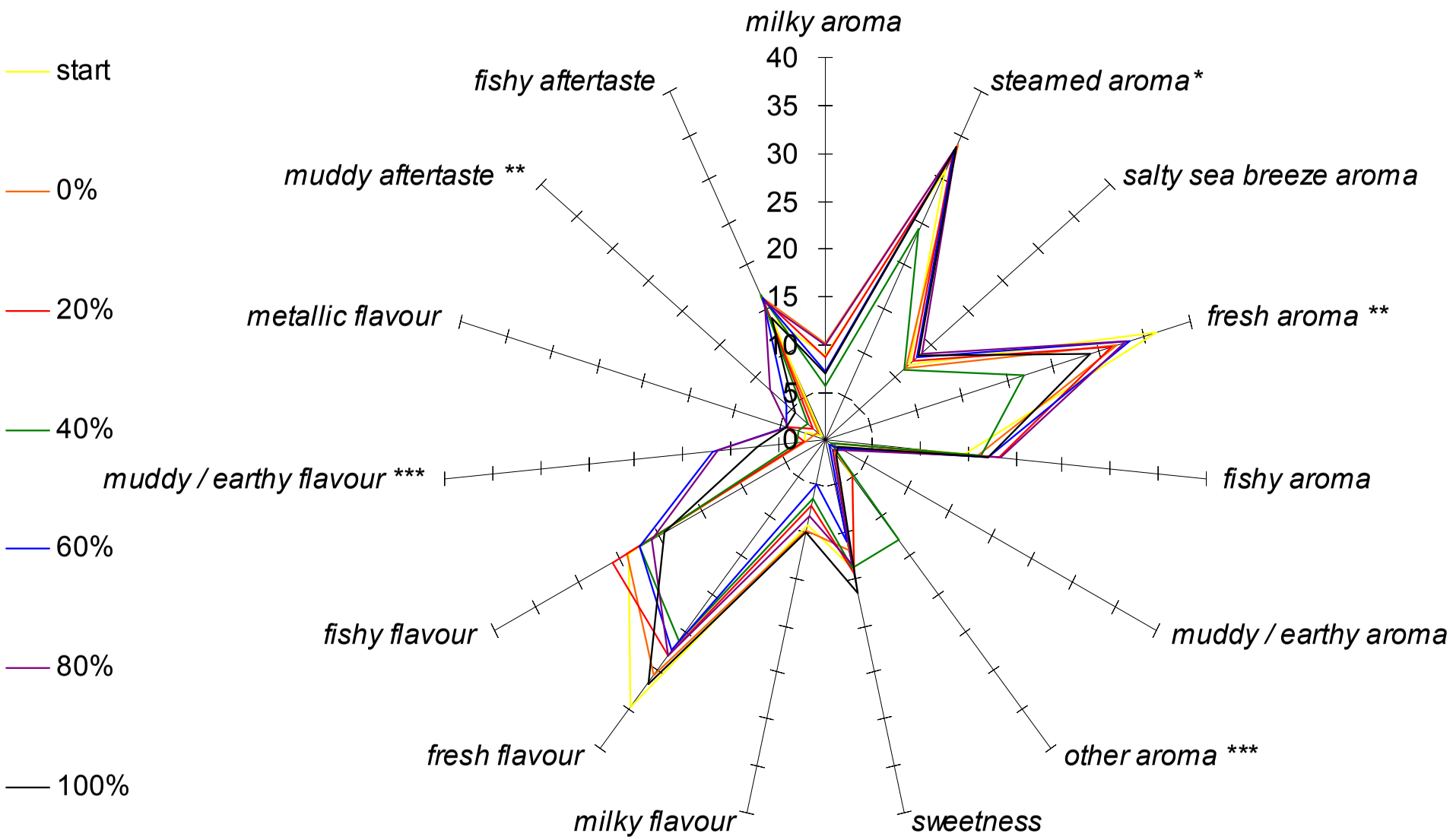
Mean scores calculated over 10 judges

Table 20 Mean sensory scores of aroma, flavour and aftertaste attributes for each MIB treatment

Sample	*milky aroma*	*steamed aroma**	*salty sea breeze aroma*	*fresh aroma***	*fishy aroma*	*muddy / earthy aroma*	*other aroma****	*sweetness*	*milky flavour*	*fresh flavour*	*fishy flavour*	*muddy / earthy flavour****	*metallic flavour*	*muddy aftertaste***	*fishy aftertaste*
threshold start	9	31^{b}	12	36^{b}	15	0	5^{a}	14	9	35	24	2^{a}	2	1^{a}	14
MIB 0%	10	34^{b}	11	32^{b}	16	1	1^{a}	12	10	31	24	2^{a}	1	1^{a}	16
MIB 20%	9	33^{b}	12	31^{b}	18	1	5^{a}	14	7	28	26	2^{a}	4	2^{ab}	16
MIB 40%	6	24^{a}	11	22^{a}	16	1	13^{c}	14	6	26	22	3^{ab}	3	3^{ab}	17
MIB 60%	7	33^{b}	13	33^{b}	17	2	1^{a}	11	5	27	22	12^{c}	4	5^{bc}	16
MIB 80%	10	33^{b}	14	33^{b}	19	2	1^{a}	13	8	28	21	11^{c}	4	8^{c}	16
MIB 100%	7	33^{b}	13	29^{b}	17	1	2^{a}	16	10	32	19	7^{bc}	4	4^{abc}	14

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; $^{a\,b\,c}$ different letters within a column signify significant differences between treatments; mean scores calculated over 10 judges and 2 replicates

Figure 10 Mean sensory scores of each attribute rated for samples from barramundi exposed to different MIB water levels



Attribute scores are calculated as the mean value for each treatment (mean of 10 judges and 2 replicates). Attributes for which statistically significant differences were observed between samples, as determined by ANOVA, are indicated by * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$).

Appendix - Summary of panellist comments recorded for individual samples

Table 21 Uptake trial samples – panellists comments

Sample	*other aroma* comments	*other flavour* comments	*other aftertaste* comments	general comments
Uptake 0 hrs	like smoke fish. May be because there is a trace of dried fish juice ('burnt' fish juice) on the brim of the tray.	Metallic; metallic, astringent; floury....in texture as well; metallic; astringent aftertaste; metallic; Astringent; corn	lot of liquid	Something like smoke fish. May be because there is a trace of dried fish juice ('burnt' fish juice) on the brim of the tray.
Uptake 1 hrs	Metallic; tangy almost citrusy aroma; sweet soya sauce smell; sardine like; metallic	Metallic; astringent; sardine like flav; oily aftertaste; Metallic; corn; small amount of the same flavour in the aftertaste; aniseed; metallic; astringent	this fish sample was extremely hot and I don't know if that is why I felt it was fresher tasting; totally inedible -smells bad, tastes bad, bad aftertaste	Metallic; tangy almost citrusy aroma; sweet soya sauce smell; sardine like; metallic
Uptake 3 hrs	Aniseed; sweet soya sauce smell; smells very sweet like fairy floss	astringent and bitter aftertaste; oily taste; metallic; astringent; bitter; metallic; metallic aftertaste; oily	chewy texture	Aniseed; sweet soya sauce smell; smells very sweet like fairy floss
Uptake 6 hrs	metallic	astringent aftertaste; astringent; metallic, astringent	Inedible; once i put the skin part of the fish in my mouth, it was very muddy, earthy tasting, however, the middle white section of the fish on its own was no where near as bad; inedible, smells bad, tastes bad, bad aftertaste	metallic
Uptake 12 hrs	sweet soya sauce smell; peppery	slight metallic aftertaste; astringent/tingling sensation on tongue; metallic aftertaste; metallic; metallic, astringent; metallic; metallic+ slight bitter; astringent; little metallic aftertaste; astringent - very dry mouth	inedible	sweet soya sauce smell; peppery
Uptake 24 hrs	bitter/metallic; sweet	chemical/bitter; The muddy aftertaste didn't overtake the fishy aftertaste; metallic, astringent; bitter; sweet; metallic, astringent; bitter	typical barra flavours overpowered by offensive artificial/chemical taste; very hot & bland	bitter/metallic; sweet
Uptake 48 hrs	sweet smell	bitter + metallic; sardine like .slightly oily; metallic; astringent; slight metallic/astringent; chemical aftertaste; astringent; metallic	not at all typical of barramundi ;inedible; not a lot of moisture	sweet smell
Uptake 72 hrs		Bitter; astringent; astringent; Chemical; metallic; metallic, astringent	very chewy with a powder like texture left in my mouth	

Individual panellist comments are separated by a semicolon.

Table 22 Comparison of off flavour within the fillet – panellist comments

Sample	*other aroma* comments	*other flavour* comments	*other aftertaste* comments	general comments
anterior / dorsal	sweet	artificial/chemical + bitter; astringent; bitter/astringent; chemical/bitter; bitter; bitter; slightly oily.; bit of a chemical taste; stale; mullet; astringent	metallic/bitter/chemical; astringent; dry; metallic + bitter; metallic/bitter; metallic/slightly bitter; metallic; metallic; slightly metallic; astringent; oily after taste; metallic aftertaste ;chemical aftertaste only a touch; astringent, metallic; astringent; astringency; metallic; stale and floury in texture; metallic / mullet; metallic; astringent	very tasty fish; very dry and chewy; bland; overpowering fishiness in flavour & aftertaste; overall quite bland; sweet for initial chewing then bland and muddy taste - chews to a powdery texture in mouth
belly flap	Oil; seawater/sewerage-sorry; sweet	very oily almost fatty at the end. the rest of the fish was very flavoursome; oily; oily; artificial/chemical taste + bitterness; bitter; bitter/chemical; fine film on top of the fish which had an oil taste to it - slimy feel; oily -; oily. My sample taste as if it is the stomach part of the fish. It also smell so when I eat it.; oily. sample is stomach part.; oily. sample is stomach part.; oily. sample is stomach part.; peppery flavour and oily; oily flavour; vary oily; very meaty in texture; astringent	oily taste and feel in the mouth; oily; chemical + bitter; metallic + slight bitterness; bitter +slight astringent; metallic; metallic/chemical/slightly bitter; slight oily aftertaste; oily aftertaste, slimy feel in the mouth from the top of the skin; astringent aftertaste; astringent and metallic; slightly sweet milky; metallic; oily.; oily.; slightly oily.; oily.; metallic aftertaste; metallic; astringent; very slight astringency; metallic, astringent; metallic; metallic, astringent; metallic; astringent	Bones; yuk; Bones; has 'gristle' on it; very clean appearance deceptive-sample inedible; very much a dirt taste rather than muddy; it did not taste as muddy as it smelled when received after re-cooking; almost had no flavour
tail section	acidic aroma; oily; smells oily ie fish oil; chemical + slight petrol; chicken smell not steamed smell, but chicken; an off/old smell	Oily; oil; Dry; slight bitterness; artificial/chemical + slight bitterness; greasy petrol taste; oily.; oily in mouth; first bite was sweet but then it had a chemical taste; astringent	Oily; oil; Dry; bitter; metallic + bitter; metallic + slightly bitter; metallic; metallic; metallic aftertaste; slightly sweet; astringent aftertaste; metallic/bitter; astringent, metallic; metallic, astringent; astringent; Astringent; slight astringency; metallic; astringent	

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Table 23 Comparison of off flavour within the population – panellist comments

Sample	*other aroma* comments	*other flavour* comments	*other aftertaste* comments	general comments
large	Baked; at first a sweet smell; slight sweet smell; oily slightly like sardine.	Dry; bitter; Had a bitterness; bitter; slightly oily.; metallic; Baked; off taste-muddy but not typical of muddy reference; oily flavour and feel; sea; off fishy taste-totally inedible-gagging effect; astringent; oily; bland; bitter; astringent; slight chemical flavour; astringent; dry; slight bitterness; astringent; Oily; dry & old tasting; astringent	Bitter; bitter metallic; oily taste in mouth; astringent; metallic; oily aftertaste; metallic/astringent; metallic; metallic; metallic; metallic; metallic; oily aftertaste; slightly astringent; astringent; astringent, metallic; metallic; slightly bitter; astringent; chemical aftertaste; astringent; astringent; bitter; dryness in mouth; metallic/bitter/astringent; astringent; metallic; metallic	very bland both aroma and taste; fairly bland; not good; A watery flavour; not good; very dry and chewy; inedible; worst sample tasted to date; smells great but dirt taste as you chew into the fish.; Started off sweet then became muddy when chewed; not good; overall bland; not good; nicest fish so far; overall fairly bland; the more you ate the more a dirt taste came out; The longer this sample is chewed the greater the muddy flavour becomes however its not strong in the aftertaste; not good; a bit chewy but not a bad taste; not good
small	sweet smell; sweet; baked; sweet smell; stale; stale; stale	Yuk; bitter; bland; dry; bitter; bitter; Dry; bitter; astringent; baked; nice piece; astringent; alkaline; astringent; virtually had no flavour; watery; watery; very bitter; astringent	Bitter; astringent; metallic; metallic/bitter; sweet; slightly metallic/bitter; nice aftertaste; slight metallic aftertaste like alfoil on teeth; slight bitterness; astringent; slightly bitter; a very pleasant aftertaste, not too fishy; astringent/bitter; astringent; alkaline; metallic; astringent; astringent; watery; metallic; little sweet aftertaste; astringent; astringent; metallic; sour, dry; astringent	Nice tasting fish; a bit dry tasting which brought out more of the fish taste; very nice fish; best sample so far today; nice fish; The sweetness only lasts for about 10 secs. initially and then has a sweeter/fishy aftertaste; overall unpleasant stale fishy taste; very nice piece of fish; A bit watery; nice tasting fish; White flesh better then the brown flesh; not good; almost no flavour at all; not good; completely inedible, bad smell, bad taste, bad aftertaste

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Table 24 Purging trial – panellist comments

Sample	*other aroma* comments	*other flavour* comments	*other aftertaste* comments	general comments
purge start, day 0	Sample had cooled down considerably therefore the aromas were low	slight bitterness - didn't seem fresh because the sample was very dry; very dry taste; fish oil; bitter; watery flavour	metallic aftertaste a little; dry mouth; metallic; dry; slightly astringent	Dry; my fish had a greeny shiny layer on top and it was quite dry tasting fish.; when first chewing, the flesh seemed to release an unpleasant gas into the mouth which seemed to intensify the muddy flavour; odour and aftertaste were distinctly muddy - flavour in mouth wasn't so obvious; very moist and melt in mouth but extremely muddy; almost inedible, very strong fishy taste; dry; some o f the flesh had a green hue; initial odour = sea breeze - then become fishy and muddy - flavour in mouth is very muddy - the most intense sample so far - aftertaste is predominated by muddy but less than the flavour in mouth
threshold start	soggy bread; very sweet	oily soggy bread old; creamy; an off taste; very dry no moister; dry tasting; old tasting and dry; bitter	Metallic; milky aftertaste; sweet/milky; metallic; dry taste in mouth; astringent; astringent	nice flavour but dry and chewy; dry and chewy; looked sad tasted sad old and unappealing; yuk sad old thing looks like poaching went wrong; flesh very dry in appearance as well as in eating; the fish was very unusual - smooth kind like texture; very dry and chewy; A little bit chewy; awful texture, taste & appearance; chewy, not a lot of flavour; chewy; this sample didn't look very appealing however tasted better than it looked; flesh was quite dry; old fishy flavour and aftertaste - very low muddy as fishy predominates
low flow, day 1	bitter/metallic/chemical	fish oil; astringent	Dry; alkaline feel; metallic, dryness; metallic; fish oil; slight metallic; sweet/milky aftertaste like from an artificial sweetener; metallic; slight astringency; astringent	dry flesh; stringy and slightly moist but chewy; A pleasant tasting fish however the sweetness disappears quickly; some parts of the flesh had a 'peppermint-green' tinge; sample was generally low in flavour overall but muddy and fishy did predominate; Dry; some of the flesh had a greenish hue. After sample was in mouth for a while, my mouth filled up with a kind of 'gas'....not pleasant taste or sensation; muddy flavour and aftertaste - odour was very bland and sea breezy - not fresh odour but not fishy either
low flow, day 2	cod liver oil; chemical/artificial; sweet smell; Metallic odour however the sample had cooled down	fish oil; leathery and dry; watery; very moist	fish oil; metallic; metallic; bitter/metallic; astringent; sweet	beautiful fresh fish; the sample had a 'neon-green' hue over the surface.....not an appealing look!; dry and chewy; Part of the sample was very mild but centre had strong fishy taste; this was a little bit more moist rather than dry, still a bit chewy; The texture was very chewy and not pleasant; sample was generally low in flavour and aftertaste - odour was sea breezy - flavour also quite sweet in comparison to other samples
low flow, day 3	sweet	bitter/sour; alkaline; slightly bitter; chemical flavour	metallic/bitter; milky sweet aftertaste; astringent, dry mouth; metallic; very metallic; Bitter; sweet/milky; astringent; sweet	Very fresh melt in mouth; chewy and dry sort of fish. it also had a green tinge to it; Very nice flavour, texture and appearance; fishy, muddy and metallic; Chewy; dry and a bit chewy; Unpleasant chewy texture; predominately fresh odours and flavours - was starting to turn towards fishy though - no muddy really
low flow, day 4		overpowering fishy; alkaline	alkaline feel; metallic; astringent, dry mouth, metallic; metallic; Metallic; alkaline aftertaste; stale taste; astringent; sweet	Slight green tinge to my fish, dry; A stronger fish flavour; nice flavour but very dry and chewy; green tinge on surface of flesh off-putting; a little bit chewy; texture is chewy; chewy, tasteless; odour was very steamed - like chicken. Flav in mouth very fishy and so was aftertaste - not much muddy though

Sample	*other aroma* comments	*other flavour* comments	*other aftertaste* comments	general comments
low flow, day 5	sweet smell; stale	artificial/chemical taste; astringent; Oil; watery; Stale; astringent	artificial/chemical + slight metallic; metallic, dryness; astringent; other aftertaste was metallic - very high and overpowering; very slight bitterness; alkaline aftertaste; astringent	extremely chewy fish; yum; the fish was slightly stringy and dry tasting; metallic predominated ion mouth and afterwards - still some fishy and muddy though; beautiful melt in mouth moist; mild tasting- nicest sample to date; very nice tasting fish; nice texture but flavour is watered down
high flow, day 1	slight chemical smell; not much smell at all; sweet smell	Dry; chemical taste-bit like petrol; alkaline; chemical taste; chemical taste; bitter	Dry; slight bitterness; alkaline aftertaste; sweet; artificial flavour/chemical; alkaline; metallic, mouth dryness; oily	Dry; nice flavour but very dry; soft and moist but not a lot of flavour; the flesh had a slight green hue; very muddy flavour in mouth and aftertaste - very strong; dry and slightly chewy; lots of liquid; the fish had a slight dry stringy feel taste; fishy and muddy mainly
high flow, day 2	fish oil	Sour; astringent; bitter	Dry; metallic/bitter; astringent; metallic + bitter; sweet milky/slightly alkaline; metallic; astringent; metallic	a little dry; almost inedible-overpowering fishy; The sweetness only detectable with first taste; flavour initially sweet and milky - then became fishy; bland aftertaste; Nice flavour and texture
high flow, day 3	slight sweet smell; metal	old tasting fish; bitter; Dry; chemical; leather & dry; bitter	Metallic; oily; Dry; alkaline feel/metallic; Leather; dryness, metallic; bitter; metallic aftertaste	dry and chewy flavour ok; nice texture; quite dry piece of fish; A little bit chewy; odour was initially old fish and then muddy earthy came through; flav in mouth was very bland with no obvious fishy/muddy - was more sweet and milky with fishy as well; aftertaste was fishy and very slight muddy; dry and slightly chewy; overall very bland; the skin part has a very slight metallic taste to It; very old fishy aroma and flavour
high flow, day 4	nice sweet smell	chemical/artificial; slightly biter-initially sweet but taste changed quickly to metallic/bitter; moist taste; a leather taste	Chemical; alkaline aftertaste; metallic, astringent; astringent; Bitter; alkaline; metallic	Yummy. Creamy texture. bliss; greenish tinge on flesh; moist and juicy, nice tasting; very mild flavours; Nice flavour and texture; sample didn't have a muddy odour but muddy in mouth and muddy aftertaste - the sample was muddy combined with fishy - not fresh to me
high flow, day 5		Astringent; bitter +sour; dry tasteless flavour; astringent	slight tangy aftertaste at back of the tongue; metallic; astringent; astringent; slightly metallic/bitter; dry aftertaste; slight dryness in mouth; astringent	beautiful fresh melt in mouth fish; overpowering fishy taste-inedible; juicy and far more tender than the others; flavour was fishy and muddy in mouth but this was quick to disappear in the aftertaste; again extremely fresh tasting fish; Not much flavour and very dry texture

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Table 25 Geosmin threshold trial – panellist comments

Sample	*other aroma* comments	*other flavour* comments	*other aftertaste* comments	general comments
threshold start	ammonia; fishy at first; stale; ammonia/fishy smell	tastes old and leathery; cardboard; tastes old; artificial/chemical;	leathery, chemical, metallic, metallic, astringent, oily, chemical	overall very bland - dryness affects freshness in taste; nice soft texture with a mild watery flavour; slightly dry; very nice and moist piece of fish; not too fishy - just right. enough moisture etc; flavour and aftertaste weren't as bad as the odour - a bit fishy and a bit muddy (more fishy though); texture:- the moisture went out of the flesh very quickly leaving the flesh dry and flaky; mild flavour; beautiful flavour slightly dry and chewy; nice and moist but a little bit chewy; the falv/aftertaste weren't fresh but they weren't fishy either - on their way to fishy though - very very sl muddy in mouth;
geosmin 0%		I don't know how to describe the 'other' flavour but it was foul; chemical / stale	Disgusting; oily; bitter; bland aftertaste; metallic; stale, chemical; slightly bitter aftertaste	nice texture; totally inedible; overall bland; lovely texture bit bland; chewy; chewy flesh, not good; overall very mild odour & taste - similar to 364; Very little flavour and chewy texture; a little bit chewy but not a lot; very bland sample - milky aroma then no fishy/muddy flav or texture
geosmin 20%	dirty, stale; chemical/artificial is dominant odour; roasted/caramelized odour; sweet	a bit chewy; dirty, chemical; astringent; artificial sweetness; bit of a creamy flavour; chemical	Metallic; astringent; Oily; metallic; a slight creamy aftertaste - smooth feeling on your palate; metallic; metallic/bitter	Chewy; very bland; Chewy; sample was sweet, milky and fresh; not good; very mild and very soft texture; very bland - no flavour at all except slight metallic tingle; a bit chewy but moist; very sweet flavour initially
geosmin 40%	slight chemical odour; slight ammonia = coconut/milky aroma	not sure, probably chemical but not as intense as some of the others; was unnatural; probably cardboard; slightly bitter	Chemical; oily; slightly metallic; bland nothing taste; metallic aftertaste; astringent	a bit on the dry side; dry and bland; mild pleasant flavour; same bland tingly metallic taste; not good; odour disappeared very quickly, overall bland, dry; very watery little flavour; very nice just a slight dry taste to it; slight muddy flav which is less as an aftertaste;
geosmin 60%	artificial/chemical; had a sweet odour although I know odours cant be sweet...; first smell was fishy that was quickly gone; off/ metallic; sweet; an ammonia type odour - quite pungent - along with fishy	Stale; artificial; astringent; sour, chemical; alkaline	stale fish; oily; metallic + chemical; astringent; chemical; alkaline; metallic; bland, nothing aftertaste	Chewy; most unpalatable; Chewy; overall very bland - dryness suggests not as fresh as other samples; creamy but mild & metallic; quite nice a tiny bit dry but nice; odour was very strong like ammonia and fishy but the flav and aftertaste were not like this - very bland

Sample	*other aroma* comments	*other flavour* comments	*other aftertaste* comments	general comments
geosmin 80%	mild ammonia type smell; metallic; ammonia - very strong!!!	Cardboard; watery taste; sorry can't quite say what the 'other' flavour is but it is something you don't want; difficult to distinguish but was a 'no-flavour' flavour; artificial/chemical taste; ammonia but not as strong as odour	very dry mouth; perhaps if the fish was 'off' it would leave this type of aftertaste; chemical aftertaste; ammonia	dry & chewy; overall very mild odour & taste; very nice flavour and texture; bland again slight metallic tingle; not much flavour; was sweet initially when chewed but then developed a more nuddy flavour - very mild muddy aftertaste; dry; not good; texture:- the moisture cane out of the flesh very quickly...then the flesh went 'ball-like' and dry; overall bland; very mild and watery with a soft texture; beautiful flavour but slightly dry and chewy
geosmin 100%	Stale; fish oil; sweet; ammonia again; stale	chemical taste; stale; chemical taste; chemical; chemical/fish oil	chemical metallic aftertaste; chemical; metallic; nothing kind of aftertaste – bland; chemical aftertaste; astringent; oily; bitter/chemical; artificial sweetener taste	Dry; flesh went dry and flaky very quickly; very bland - initially very strong odour of fish oil; A mild watery taste with no sweetness; very juicy and moist but no flavour; wasn't fresh but wasn't fishy - more fishy though but not overpowering. Flavour and aftertaste not as strong as odour; Chewy; no flavour; inedible; bland with metallic tingle; quite dry tasting fish; aroma wasn't fresh but it wasn't fishy either. Flavour was initially sweet then muddy developed. Very low muddy aftertaste

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Table 26 MIB threshold trial – panellist comments

Sample	*other aroma* comments	*other flavour* comments	*other aftertaste* comments	general comments
threshold start	baked/roasted caramelized odour; sweet; stale; very slight chemical; stale; slight ammonia type smell	fish oil/bitter/chemical; astringent flavour; taste like mullet; artificial/chemical, slightly bitter; moist; astringent; wasn't fresh but wasn't fishy	artificial/bitter; oily; chemical; sweet/milky; astringent; astringent	nice flavour , melt in mouth texture, moist; inedible; this piece was a bit chewier than the other 2, still moist; nice flavour however the colour is a bit grey and the aroma meaty; sweet flavour, no aftertaste; a little chewy; yum; very nice tasting fish - nice and moist and not over powerful fishy smell and taste. Just right; very nice texture and taste
MIB 0%	chemical unnatural odour; like a burnt sort of smell	bitter +artificial; chemical; Steamed chicken flavour; a bit leathery; could not identify 'other flavour' however did not enhance the overall flavour; strange chemical flav	Bitter; dry mouth; Oily; astringent; Alkaline; chemical aftertaste	meaty texture nice flavour; unappealing appearance poached & sad; quite watery; a dry fish; very bland aftertaste; overall bland/dry; a bit dry; strange acidic type aftertaste
MIB 20%	steamed socks; stale; other was like the grey underbelly flesh 0 more fish gamey??; steamed socks; chemical; stale	Chemical; moist; Astringent; moist on the outside but dry and flaky on the inside; low muddy but there was another gamey silty flavour there - not your typical muddy though; Chemical; old tasting; astringent	metallic/chemical; metallic; oily; other = strange gamey - quite a bit of muddy but not the classic muddy we have had previously; astringent; alkaline	tasted better than it looks; unpalatable; this piece was far more fishy tasting but still nice and moist; a gamy old barra type sample; bit dry and chewy; looked yuk tasted yum; very dry-appears to be stale; a bit dry and also has an old taste to it; a stronger fish flavour; odour was fresh and steamy! but the flavour and aftertaste were definitely fishy and old
MIB 40%	chemical smell not sure what it is; stale; dog poo; off / manure type smell; fish oil; funny smell - not quite sure what - some sort of chemical; chemical and a touch of sewage; dog poo; almost a floral aroma	bitter taste; hard to identify however was astringent-like.....this 'other flavour' dissipated quickly; same slight manure taste; bitter; chemical; astringent	bitter aftertaste; dry mouth; sweet aftertaste; bitter/metallic; sweet muddy; astringent; astringent	a tad bit chewy; unpleasant bitter/muddy aftertaste; quite bland; chewy; not very moist and a bit chewy; chewy texture; a bit chewy; bland aftertaste
MIB 60%	fishy and fresh at same time but fishy predominates	had old flavour; chemical and old	Bitter; metallic; astringent; oily; astringent; very dry mouth; unnatural chemical	bit too soft, disintegrated in mouth tasted like and old fish; texture very granular; very fishy; muddy fishy flavour in mouth which didn't linger as aftertastes to a large extent; chewy; good texture; a very unusual sample - fishy odour developed
MIB 80%	fishy odour first then it smelt fresher; chemical	dusty/dirty rather than muddy; dry and chewy; muddy chemically flavour; muddy; muddy gamey	bitter/dirty; metallic; slightly astringent; slight metallic; slight dryness	Very slight earthy taste; unpalatable; A dry texture; the fish is dry; muddy flavour in mouth predominates; bit dry and chewy; flesh very dry; on the dry side; odour was fishy, flav and aftertaste were muddy!!
MIB 100%	chemical/artificial; muddy combined with gamey smell	Poached; unfortunately could not pinpoint a name for the 'other flavour'; slight bitterness +artificial flavour; gamey muddy flavour	Metallic; dry mouth; dry mouth; gamey	Beautiful texture, moist melt in mouth. very slight earthy taste.; flesh dry; bit chewy; bland nothing aftertaste; mild flavour; slightly dry; this fish is bit dry; muddy/gamey flavour and aftertaste were the standouts from this sample

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