New Technologies for Sustainable Commercial Finfish Culture

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LIST OF ACRYONYMS

ADU	Aquaculture Development Unit
ANOVA	Analysis of Variance
BOD	Biochemical Oxygen Demand
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DIN	Dissolved Inorganic Nitrogen
DM	Dry Matter
DMD	Dry Matter Digestibility
DOC	Dissolved Organic Carbon
DW	Dry Weight
FCR	Food Conversion Ratio
FRDC	Fisheries Research and Development Corporation
GE	Gross Energy
GSL	Great Salt Lake
HL	Hutt Lagoon
HNHS	High Nutrient, High Salinity
HNLS	High Nutrient, Low Salinity
HSD	Honestly Significant Differences
IAAS	Integrated Agri-Aquaculture Systems
ISA	Inland Saline Aquaculture
LNHS	Low Nutrient, High Salinity
LNLS	Low Nutrient, Low Salinity
LOI	Loss On Ignition
MAG	McRobert Aquaculture Group
ME	Metabolisable Energy
NOX	Nitrite + Nitrate
PQL	Practical Quantification Limit
PUFA	Polyunsaturated Fatty Acids
QDPI	Queensland Department of Primary Industries
RIRDC	Rural Industries Development Corporation
SCADA	Supervisory Control And Data Acquisition
SGR	Specific Growth Rate
SIFTS	Semi-Intensive Floating Tank System
SRP	Soluble Reactive Phosphorus
TAFE	Technical and Further Education
TAN	Total Ammonia Nitrogen
TDN	Total Dissolved Nitrogen
TDP	Total Dissolved Phosphorus
TN	Total Nitrogen
TP	Total Phosphorus

2005/213	**New technologies for sustainable finfish culture**

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OBJECTIVES:

1. Determine the effectiveness of the Semi-Intensive Floating Tank System (SIFTS) waste removal system and in-pond bioremediation in preventing boom-bust microalgal cycles.
2. Quantify the production capability of a commercial scale SIFTS.
3. Determine the efficiency of irrigated crop plants in removing nutrients, salt and other pollutants from SIFTS aquaculture effluent.

NON-TECHNICAL SUMMARY:

OUTCOMES ACHIEVED TO DATE

We have demonstrated that yields of 10 tonnes of fish per hectare cannot be sustainably achieved in static, autotrophic saline (14 ppt) ponds (i.e. ponds dominated by photosynthetic organisms) over a 3-4 month production cycle, despite the removal of settable wastes from the SIFTS. The outcome of this finding was to advise potential industry entrants of this fact and to further investigate alternative options to enable such yields to be achieved.

Our work on integrating heterotrophic pond management techniques (i.e. ponds dominated by bacterial organisms which utilize organic carbon as an energy source) with carnivorous fish production in SIFTS have demonstrated that 15 tonnes per hectare are achievable over a 100 day production cycle. Economic analysis revealed that the profitability of a stand-alone enterprise growing carnivorous fish in SIFTS within heterotrophic ponds would be marginal at 150 tonnes per year of production. The outcomes of these trials have already been used to design further trials on optimizing heterotrophic pond management at the Queensland Department of Primary Industries' Bribie Island Aquaculture Research Centre. Demonstrating that SIFTS can be integrated with heterotrophic pond management systems creates an opportunity for existing prawn farms using heterotrophic pond management to integrate barramundi farming into their existing operations with minimal changes required to their operations. The McRobert Aquaculture Group are discussing the integration of SIFTS into prawn farming ponds and settlement raceways with the prawn farming industry in Queensland.

The main outcome of our research using NyPa Forage to treat salinised and eutrophied waste is a further project funded by the RIRDC, in which NyPa Forage will be grown on a larger scale in the field and it's nutritive value determined in livestock *in vivo*. As a result of our project, NyPa forage is being investigated to fix atmospheric carbon under a carbon credit scheme. The 'Degree Celsius' project is a collaboration between Terrain Natural Resource Management and BioCarbon, a private company, who are investigating the carbon storage capability of NyPa Forage in an effort to make valuable use of salt affected lands.

Due to the lack of large-scale interception schemes and the low yields of water from saline aquifers in salt affected Western Australia, inland saline aquaculture research for finfish production in this state has focused on static water bodies. In order to facilitate economically viable production in these water bodies, a production system was developed to increase pond yields to a level which would allow profitable, stand-alone culture. The Semi-Intensive Floating Tank System (SIFTS) improves the management capability of fish in open ponds and prevents up to 5 tonnes of waste (on a dry matter basis) entering the pond on a per hectare basis per year.

Quantifying the benefits of removing this waste on the severity of yield-limiting microalgal blooms during Trial 1 revealed that the contribution of this waste to the overall pond bottom sludge load was minimal. Numerous microalgal crashes occurred and dissolved nutrients from fish excretion and remineralisation of bottom sludge led to lethal microalgal crashes. Although SIFTS enabled fish to survive during the periods of low dissolved oxygen following a microalgae crash, the

unionised ammonia toxicity caused by the high pH levels associated with the strong blooms could not be ameliorated. This first trial demonstrated that 10 tonnes of production per hectare was unachievable over a 100 day period in a static autotrophic pond and highlighted that alternative bioremediation techniques are required to limit the availability of nutrients to microalgae to achieve such yields. Additional techniques tested in this study included the use of biofiltration, heterotrophic pond management, treatment of effluent using halophytic, terrestrial crops and the cropping of microalgae using filter feeding *Artemia*.

Pond trials testing heterotrophic pond management were conducted in which the C:N:P ratios in pond water were manipulated to encourage the proliferation of heterotrophic bacteria, which incorporate nitrogen into microbial proteins. Adopting this approach enabled the production of 15 tonnes/hectare of barramundi and 10 tonnes/hectare of trout over ca. 100 days. The major advantage of this technique in the current trials was the maintenance of low and stable pH values. Although these yields represent significant improvements compared with autotrophic controls, further refinements to this management technique and its incorporation with the SIFTS technology are required to ensure it's long term sustainability. Improving pond hydrodynamics and ensuring floccules are maintained in suspension should reduce TAN and enable further improvements in yield. These floccules are also an excellent food source for potential valuable secondary crops such as prawns. The incorporation of such secondary crops not only has the potential to offset the increased operating costs associated with heterotrophic pond management, but also provides a means of exporting nutrients from the pond and subsequently enabling even greater pond yields to be achieved.

The halophytic terrestrial crop, NyPa Forage was assessed for its salt and nutrient uptake capabilities from inland saline waste. This plant showed excellent potential in this regard, removing up to 88% of total nitrogen and 95% of total phosphorous from aquaculture effluent over an 8 month period. Small amounts of sodium chloride (up to 24%) were also removed, but this was a result of a passive filtering process by the sand, which declined over time. More nitrogen and phosphorous were removed at higher nutrient levels. Higher salinity levels had a small inhibitory effect on the efficiency of phosphorous removal, but did not affect nitrogen removal. The nutritive value of NyPa Forage was greater than that of most salt-tolerant pasture plants that have been tested in Australia and these values improved significantly with both routine cropping and fertilisation with aquaculture effluent. Field trials confirmed NyPa's ability to trap nutrients from aquaculture effluent and provide a crop with nutritive value sufficient to provide a maintenance feed for grazing livestock. Yields from field trials were 2.9 tonnes DM/ha over a 4 month growing season, equivalent to that of unmanaged pasture in the south west of Western Australia.

Laboratory trials demonstrated the ability of *Artemia* to survive and grow across the wide range of salinities and temperatures experienced in static inland saline water bodies. These trials also effectively demonstrated the ability of *Artemia* to crop large quantities of microalgae. Trials transferring these findings to the field highlighted the difficulties associated with growing *Artemia* in static saline ponds. Inland saline water bodies exhibit seasonal variations in microalgal communities and we demonstrated that not all of the species which bloom in these ponds are suitable for *Artemia* production. The technology that we developed for retaining and culturing *Artemia* within SIFTS may have application for the culture of *Artemia* in hypersaline water bodies, where they have no competitors or predators and where the maintenance of monocultures of appropriate microalgal species is achievable.

Trials investigating the growth of barramundi fed live *Gambusia* revealed that growth and FCR was superior to those fed on an iso-calorific ration of a commercial barramundi diet. *Gambusia* are abundant in may inland saline water bodies and thus represent a viable supplementary food source for this species. If *Gambusia* are able to thrive on the bio-floccules created under heterotrophic pond management, then they have the ability to convert barramundi waste into barramundi food. We are continuing to investigate this potential.

KEYWORDS: Inland saline aquaculture, Semi Intensive Floating Tank System, SIFTS, barramundi, rainbow trout, heterotrophic pond management, halophytes, NyPa, *Artemia*

ACKNOWLEDGMENTS

This study was co-funded by the Wheatbelt Development Commission and Mr Stan Malinowski (Springfield Waters Aquaculture). Experiments to determine the nutritive value of NyPa Forage were partially funded by the Rural Industries Research and Development Corporation and the Queensland Department of Primary Industries contributed funds to the heterotrophic management trials.

We would like to acknowledge the support of Challenger and C.Y. O'Connor TAFEs and in particular former Manager Director of C.Y. O'Connor TAFE, Mr Peter Mahler. We would also like to thank Bruce Ginbey, hatchery manager of Challenger TAFE's Aquaculture Development Unit for his assistance in all aspects of the project, including assisting in the design and construction of the rotating *Artemia* screen.

1. BACKGROUND

1.1 Inland Saline Aquaculture

Secondary salinisation has rendered over 100 million hectares of land throughout the world, and over 5 million hectares in Australia, unsuitable for conventional agriculture. The utilization of salinised land and its associated water resources for mariculture is an adaptive approach to this environmental problem with many prospective economic, social and environmental benefits. Research undertaken both in Western Australia and nationally has proven the biological feasibility of culturing a range of marine and estuarine fish and penaeid prawn species in inland saline groundwater, however, inland mariculture is yet to develop into an industrial-scale, rural enterprise.

Having a commercially viable culture technology suitable for such regions is a key step in the development of a viable inland saline aquaculture industry in rural Australia. With an abundance of relatively cheap land in salt-affected areas, pond culture is an approach well suited for inland mariculture (Doupé et al., 2003). Indeed, Allan et al. (2001) suggested semi-intensive ponds to be the most prospective commercial production systems for inland mariculture in Australia. Ponds can be operated as either flow-through or static (i.e. no flow), depending on factors such as the yield and chemistry of the available water and the corresponding requirements of the species to be cultured. Large-scale saltwater interception schemes and evaporation basins characterize the salt-affected inland areas on the lower east coast of Australia. Here, saline water supplies are abundant and large volumes available at the confluence of these schemes (i.e. at the disposal sites). Such high water volumes make flow-through pond (or tank) culture an attractive proposition and researchers from NSW and SA are investigating the use of flow-through ponds and tanks for fish culture.

In the wheatbelt region of Western Australia, where over 70% of the country's salinized land is found, there are no major river systems or high-intensity irrigated agriculture industries and interception schemes and managed evaporation basins do not form a large part of the state's approach to dealing with salinity. In addition, the majority of the wheatbelt overlies fractured rock or granite aquifers, so bore yields are typically only low to moderate. Flow-through pond or tank culture is therefore unlikely to be possible in most of WA and efforts have subsequently focused on static ponds. In addition to enabling fish culture in areas with low groundwater yield, static ponds also enable more cost-effective potassium supplementation compared with flow through ponds and largely overcome the issues associated with the disposal of salt-laden and eutrophied waste water associated with flow-through farms. Yields from static ponds, however,

are typically much lower than flow-through ponds and are limited by the nutrient inputs into them. For example, a number of farmers in WA have successfully produced small quantities of rainbow trout in saline groundwater, however, yields less than 1 tonne/ha/yr are typical. The static ponds used in the well-established US channel catfish (*Ictalurus punctatus*) industry are operating at their maximum sustainable level of production of up to 6.7 tonnes/ha/yr (Hargreaves and Tucker, 2003), whilst yields of fish from flow-through ponds can exceed 30 tonnes/ha/yr (Gooley et al., 2000).

Based on previous economic studies (Ingram, 2002) we consider the minimum level of production required for a stand-alone farm to be approximately 50 tonnes per annum. Based on catfish industry yields of 6.7 tonnes/ha/year, 7.5 hectares of static ponds would be required for this level of production. Given the low yielding nature of saline aquifers in salt affect, many bores would not provide enough water to even account for evaporation over this pond area (Luke et al., 1987). Increasing yields from static ponds is therefore critical to allow commercial scale culture to occur.

1.2 A New Culture Technology for Fish Production

In an effort to increase the yield of fish from static ponds and to improve the management of fish grown in ponds, TAFEWA researchers, in conjunction with a private company (McRobert Aquaculture Systems) developed, patented and undertook preliminary testing of an innovative aquaculture production technology called the Semi Intensive Floating Tank System (SIFTS) in the years between 2002 and 2005. The system floats within conventional aquaculture ponds and captures more than 80% of the solid wastes created by the fish, thereby preventing it from entering the surrounding pond. SIFTS also provides superior stock management capabilities including the prevention of predation and escapes and facilitating simple harvesting. The system runs on low-pressure air and is therefore less expensive in terms of capital and operating costs than intensive fish production systems, such as recirculating aquaculture systems, and is also far less technically demanding. The use of air ensures optimum levels of dissolved oxygen can be maintained, even during microalgal blooms.

In a previous research project, we used a prototype SIFTS to successfully produce three species of fish (mulloway, rainbow trout, and barramundi) in a static, inland saline pond. We yielded 28 tonnes/ha/year from a 0.13 ha water body over a period of 10 months. The SIFTS reduced nutrient input into the pond by removing settleable wastes as a thick sludge with a dry matter content of 5 to 10%. The total quantity of dry waste removed over the culture period was

5 tonnes/ha/yr, which contained 144 kg of nitrogen and 153 kg of phosphorus. The release of soluble nutrients into the pond resulted in blooms of macro- and micro- algae which caused large and potentially lethal diurnal fluctuations in dissolved oxygen within the pond, however, comparatively stable levels of dissolved oxygen were maintained within each SIFT through the use of air lift pumps.

In addition to inland saline aquaculture, the SIFTS technology is applicable to fresh water bodies such as pond, irrigation dams, lakes and rivers and, given appropriate engineering development, may be applicable to protected ocean environments.

1.3 Managing Effluent Through Integrated Agri-Aquaculture

Inland saline aquaculture may have environmental impacts that need to be managed for sustainable industry development. A recent study by Starcevich et al. (2003) identified the disposal of saline, nutrient-enriched effluent as the major environmental problem for inland saline aquaculture. Preliminary studies in the WA Wheatbelt have shown that the effluent from the SIFTS comprise a rich organic sludge and dissolved nutrients which are utilized by algae in the ponds. Both the sludge and nutrient enriched water must be removed from the ponds to ensure long-term sustainability. SIFTS effectively removes settleable waste, but not dissolved nutrients. Biological treatment of dissolved nutrients, for example through seaweed biofilters, grazing invertebrates, hydroponics, constructed wetlands or field crops, has been used successfully to manage environmental impacts from aquaculture (Rakocy and Hargreaves, 1993; Schwartz and Boyd, 1995; D'Silva and Maughan, 1996; Neori, 1996). In an agricultural setting, application of aquaculture effluent to a crop could allow more efficient use of water resources and increase farm diversification. Most of the research undertaken on integrated agri-aquaculture thus far has, however, focused on freshwater systems (Gooley et al., 2001), Gooley and Gavine (2003). Research into saline agri-aquaculture has been limited, due to both a lack of an inland saline aquaculture industry and, more importantly, to the lack of a suitable crop which can be irrigated with saline water.

Researchers in the USA are examining several innovative treatment and disposal systems for saline aquaculture waste. The cornerstones of these systems are halophytic plants, which can be irrigated with saline water. These plants therefore provide a unique opportunity for integration with saline aquaculture. NyPa International have developed and patented several strains of halophytes (*Distichlis* spp.), which can be irrigated with saline water up to the salinity of seawater. These strains include a fodder, a turf and a gluten-free grain crop. NyPa are currently demonstrating their value in small experimental plots in the WA Wheatbelt and other

salt affected areas in southern Australia (Leake et al., 2002). Preliminary research into the NyPa forage in Western Australia has shown that its yields rival those of conventional fodder crops. Under an integrated agri-aquaculture situation where the crops are irrigated with nutrient-rich aquaculture effluent, yields are likely to be improved. These plants therefore potentially offer a significant additional income for an inland saline aquaculture industry.

2. NEED

A viable inland saline aquaculture industry will only develop if existing constraints to production are overcome and if the environmental impacts from effluent, which are an inevitable consequence of increasing production, are managed appropriately. For a complete review of these constraints and environmental issues refer to Partridge et al. (2008). The innovative SIFTS technology offers improved fish production by its ability to grow high densities of fish at lower cost than other intensive production systems, by efficiently preventing solid waste products from entering the natural environment, and by maintaining optimal oxygen levels and therefore fish growth even during algal blooms. Methods of treating and managing dissolved nutrients will also be required for SIFTS to be fully sustainable. Linking SIFTS to the irrigation of crop plants provides one option for managing dissolved nutrients and the potential for additional returns. Other potential options include 'in-pond' grazing of microalgal blooms with valuable filter feeding invertebrates, such as oysters or *Artemia.* All of these prospective approaches must be rigorously tested on a commercial scale in the field. The proving of the SIFTS technology in the small water bodies of the WA wheatbelt will make the technology immediately available to other environments such as in freshwater lakes, storage reservoirs and in coastal areas where its superior production and waste minimization capabilities will be highly regarded both by industry and the community. For inland saline areas, the innovative SIFTS technology offers to transform the existing low-intensity, cottage industry into a commercial industry.

The need for this research is reflected in both FRDC's National R&D plan for Inland Saline Aquaculture and the RIRDC R&D plan for Integrated Agri-Aquaculture Systems (IAAS). Specifically, the former R&D plan calls for the 'identification of constraints to commercial developments and methods of overcoming these', whereas the latter highlights the need for 'appropriate, in-situ industry trials to evaluate suitable system design requirements from IAAS operations'.

3. OBJECTIVES

1. Quantify the production capability of a commercial scale Semi-Intensive Floating Tank System (SIFTS).
2. Determine the effectiveness of the SIFTS waste removal system and in-pond bioremediation in preventing boom-bust microalgal cycles.
3. Determine the efficiency of irrigated crop plants in removing nutrients, salt and other pollutants from SIFTS aquaculture effluent.

4. GENERAL MATERIALS AND METHODS

4.1 Inland Saline Aquaculture Research and Demonstration Site

Two identical, 0.15ha ponds, each 3 metres deep were leased at Springfield Waters Aquaculture in Northam, Western Australia. Springfield Waters Aquaculture is an operational inland saline fish farm used for research and training by Murdoch University and the ADU of Challenger TAFE since 1999. The facility also served as WA's Inland Saline Aquaculture Demonstration site as part of FRDC project 2004/241 'Co-ordination of inland saline aquaculture R&D in Australia' (Allan et al. 2008).

Each drainable pond was lined with a 400μm high-density polyethylene liner thus eliminating the complex chemistry that occurs at the soil-water interface in unlined ponds. For each of the grow-out trials each pond was filled with 3.5ML of saline groundwater sourced from a nearby bore and pumped using a 20A, 2" submersible, electric bore pump from a depth of 35m. The bore water has a salinity of 14‰ and temperature of 22^{o}C. The potassium concentration of 70 mg/L (45% equivalence) is sufficient for both barramundi and rainbow trout (Partridge and Lymbery, 2008; Partridge et al., 2008) at this salinity. Both ponds were maintained at the same water level throughout each trial through periodic topping up to replace water lost due to evaporation.

4.2 Semi-Intensive Floating Tank System (SIFTS) Trials

The construction phase of the project was completed by December 2005. Eight $\times$ 10m^3, rotationally moulded SIFTs and 2 $\times$ 10m^3 transfer SIFTs (i.e. 'taxi' SIFTs) were constructed and installed in addition to associated equipment including blowers, walkways and the computer controlled, electronic monitoring and backup system. Four SIFTS units were deployed into each pond and connected to a floating pontoon for access (Figure 4-1a). Each of the SIFTS were fitted with two 80 mm air-water lifts capable of providing up to 330 litres/min (4 exchanges per hour) (see Figure 4-1b). Each SIFT was also fitted with MAG's patented waste arm and an air-water-lift driven swirl separator to remove settleable solids.

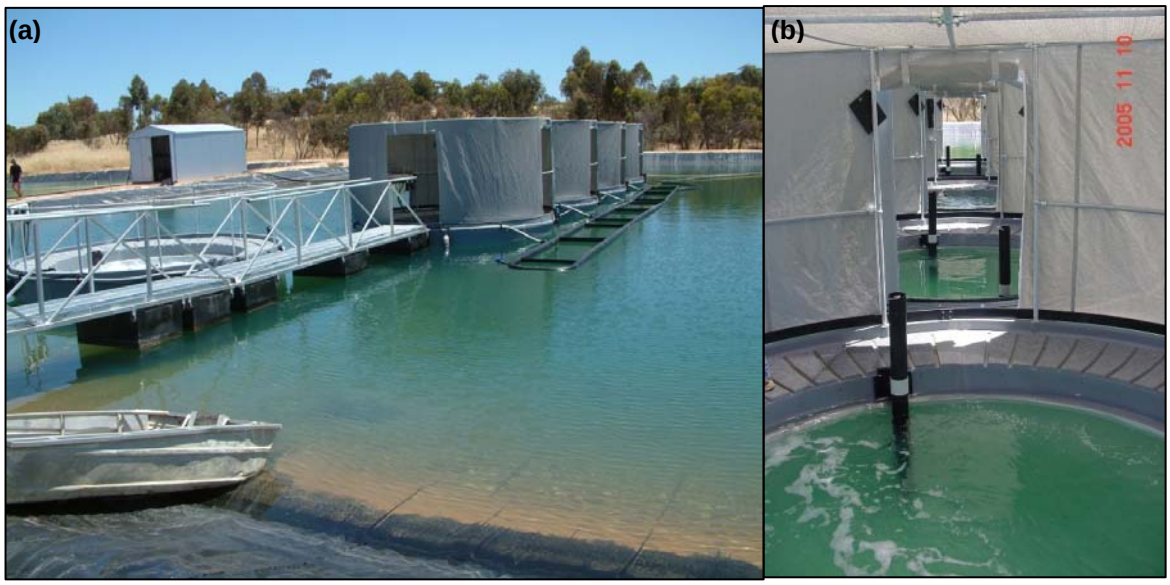


Figure 4-1: (a) Four × 10m^3, rotationally-moulded, shade-covered SIFTs and 1 × 10m^3 'taxi' SIFT (to the left of walkway) installed into a 0.15 ha lined saline pond in Northam. An identical pond setup is located in the background. (b) Internal view through the SIFTS.

Oxygen probes were placed in the outflow of each SIFTS and within both ponds. Each probe was connected to a computer running the SCADA package, LookOut^{TM}. The software was employed to monitor and log the dissolved oxygen concentration and water temperature every 5 minutes over the duration of all grow-out trials.

The system also incorporated a telephone dialer and a fail open solenoid to provide diffused, pure oxygen to each SIFTS in the event of air-blower failure or any other cause of oxygen depletion. A computer monitoring, alarming and emergency backup system operated on 24 V DC power to remove the reliance on mains power.

A summary of the fish grow-out trials undertaken during the project is provided in Table 4-1.

Table 4-1: SIFTS-based pond trials undertaken at the Northam ISA facility between December 2005 and October 2007.

	Trial Period	Treatment(s)	Test Species
Trial A	Dec 05 – April 06	Evaluation of MAG waste removal system, high/low stocking density	Barramundi *(Lates calcarifer)*
Trial B	June 06 – September 06	Vertical artificial substrates, pond destratification	Trout *(Oncorhynchus mykiss)*
Trial C	Dec 06 – March 07	Heterotrophic pond management (warm water)	Barramundi
Trial D	June 07 – September 07	Heterotrophic pond management (cold water), partial water exchange)	Trout

4.3 NyPa Forage Trials

Over the course of the project, three separate trials (Table 4-2) were undertaken to assess the efficacy of NyPa Forage in trapping nutrients from inland saline aquaculture effluent, and to determine the value of NyPa Forage, when irrigated with saline aquaculture effluent, as a livestock feed.

Laboratory trials were undertaken at the Fish Health Unit, Murdoch University. We constructed 20, 1.3 m $\times$ 0.55 m $\times$ 0.45 m elevated wetland cells with a 0.05% slope for horizontal flow (Figure 4-2). Each cell had a 0.15 $\times$ 0.15 m infiltration area comprising basalt gravel (15 mm particle size) and a discharge/collection point of outflow. The total volume of growing media added to each cell was 0.12 m^3 of washed quartz sand (1.08 mm particle size) and a preliminary test of the hydraulic properties of the wetlands showed that 12 L of distilled water was required twice weekly to achieve a consistent water flow.

Table 4-2: NyPa laboratory and field-based pond trials undertaken April 2005 and May 2008.

	Trial Period	Treatment(s)	Location
Trial A	April 05 – May 06	Effect of salinity and nutrient level on nutrient removal	Laboratory trial - Murdoch University
Trial B	Sep 06 – May 07	Effect of nutrient level and cropping rate on yield and nutritive value	Laboratory trial - Murdoch University
Trial C	Sept 07 – May 08	Field assessment of nutrient removal and nutrient value	Field trial – Springfield waters Aquaculture

(A) **(B)**

Figure 4-2: Subsurface flow wetland cells, showing cell design (A) and collection system (B).

The field trial was undertaken at Springfield waters Aquaculture. Four 100m^2 plots were established adjacent to the ponds containing the SIFTS (Figure 4-3). All plots were fenced to prevent intrusion by rabbits and planted with NyPa Forage at a spacing of one plants per 0.5 m. The plots were reticulated with a dual-source irrigation system which allowed watering of different plots with different water sources.

(A) **(B)**

Figure 4-3: Four irrigated and fenced 100 m^2 NyPa field plots adjacent to SIFTS fish ponds; when first established (A) and after several months growth of plants (B).

4.4 *Artemia* Trials

The aim of this aspect of the project was to investigate the potential of *Artemia* to crop micro-algae in static, inland saline aquaculture ponds. The specific objectives of this study were to determine:

1) If *Artemia* can grow at salinities commonly experienced in areas used for inland saline aquaculture?
2) If *Artemia* can grow at temperatures commonly experienced in areas used for inland saline aquaculture?
3) If *Artemia* can grow on the species of algae that bloom in aquaculture ponds?
4) What is the expected ingestion rate at various temperatures and algal species?
5) How many *Artemia* are required to crop microalgae to safe levels based on the answers to questions 1-4?

The study involved an iterative process of laboratory and field trials to determine the suitability of *Artemia* to control microalgal blooms (Figure 4-4, Table 4-3). Laboratory trials were undertaken at the ADU to determine the range of environmental parameters over which different strains of *Artemia* could survive and grow, and to determine the relationship between salinity, temperature, microalgal consumption and growth rate of *Artemia.* These data were used to predict the expected consumption of algae in the field using a simple spreadsheet model. The predictions of the model were tested in field trials at Springfield Waters in the ponds previously described. In order to facilitate the management of *Artemia,* these field trials were conducted in a Semi Intensive Floating Tank, which retained *Artemia* by the use of a specially designed rotating screen (Figure 4-5).

Table 4-3: Laboratory and field-based pond trials involving *Artemia* between July 2006 and December 2007.

	Trial Period	Treatment(s)	Location
Lab 1	July 06 – Aug 06	Effects of temperature and salinity on growth and survival of two *Artemia* strains	ADU
Lab 2	Nov 07 – Dec07	Effects of temperature on growth and ingestion rates of *Chaetoceros muelleri*, cultured in 15ppt water.	ADU
Field 1	Mar 07 – Apr 07	Asses the effectiveness of using *Artemia* to crop micro-algae from an inland saline aquaculture pond	Springfield waters aquaculture facility
Lab 3	Aug 07 – Sep 07	Effect of microalgal species on survival, ingestion and growth of *Artemia*	ADU
Field 2	Nov 07 – Dec 07	Asses the effectiveness of using *Artemia* to crop micro-algae from an inland saline aquaculture pond.	Springfield waters aquaculture facility

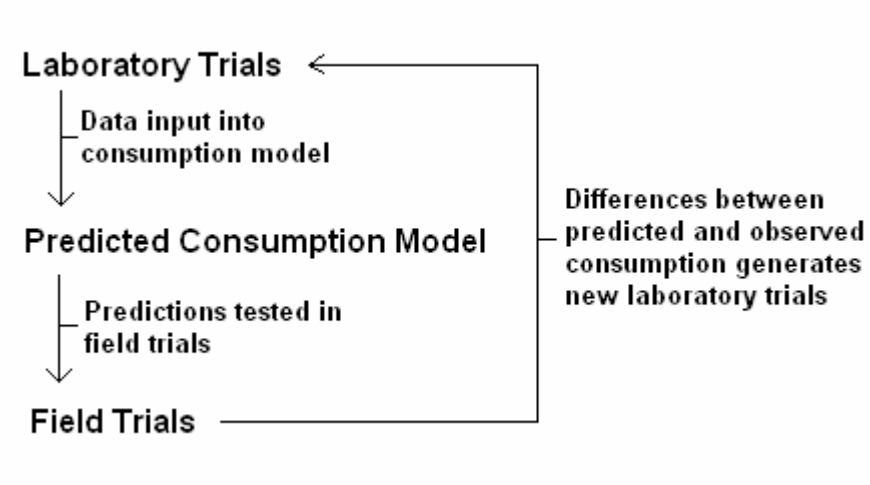


Figure 4-4: Iterative process of laboratory and field trials to determine suitability of *Artemia* to control microalgal blooms in static ponds.

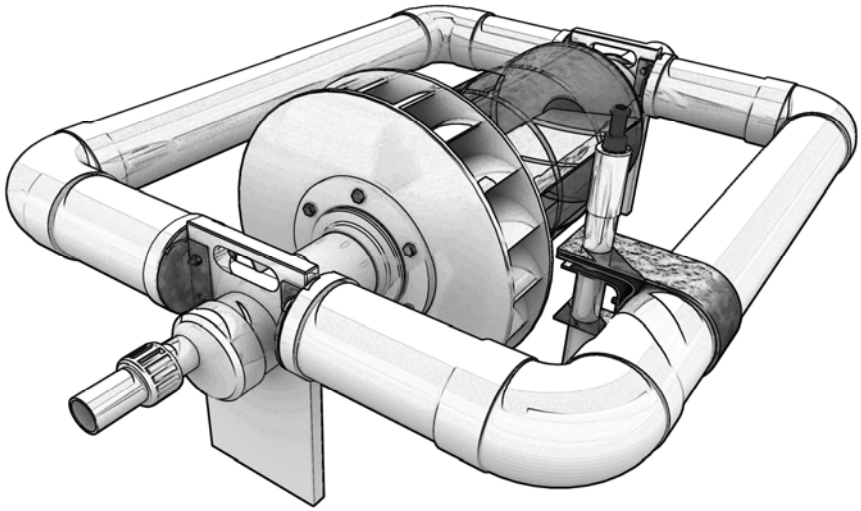


Figure 4-5: Purpose-built *Artemia* drum screen for retaining *Artemia* within SIFTS.

4.5 The Use of *Gambusia* as a Feed for Barramundi In Inland Saline Ponds

In our previous trials testing the efficiency of SIFTS we achieved excellent FCRs of barramundi, which we partially attributed to their ingestion of mosquito fish (*Gambusia holbrooki*) that were drawn into the SIFTS from the pond via the airwater lift pumps (Partridge et al., 2006). *Gambusia* is an introduced species which is abundant in inland saline water bodies in the wheatbelt. Anecdotal evidence suggests that *Gambusia* thrive as they are unpalatable to predators. In order to confirm our observations that barramundi will ingest *Gambusia* and get some nutritional benefit from them, a laboratory study was conducted at the ADU. In this study the growth of juvenile barramundi fed an isocalorific ration of either live *Gambusia*, commercial barramundi pellets or a 50:50 ratio of both diets was determined in triplicate over a two week period.

5. SIFTS PRODUCTION TRIALS

5.1 Trial A: Assessment of the MAG Waste Removal System to Minimise Pond Sediment and Microalgal Blooms.

5.1.1 Introduction

Although semi-intensive ponds have many advantages for inland saline aquaculture, a major constraint to their commercial use stems from the fact that they are prone to the boom-bust phytoplankton cycles described by Erler et al. (1999). While blooms of microalgae are inevitable in small static ponds, maintaining phytoplankton communities in a healthy (stable) state is important to maintain stable and acceptable levels of dissolved oxygen, ammonia and pH. Boom-bust cycles are triggered by the microbial remineralisation of ammonia from pond sediments, which cause blooms of microalgae. As these blooms strengthen, dissolved oxygen levels fluctuate between super-saturated and critically low levels. Fluctuations in pH are more pronounced in high density blooms, and this also influences the toxicity of ammonia to fish stock. When the blooms 'crash', oxygen is depleted and TAN is released as the dead algae settles to the bottom of the pond, where it becomes part of the sediment and the cycle thus continues.

Semi-intensive pond systems (which include in-pond cage culture) are limited in the yields they can produce, as high stocking densities of fish accelerate the build up of sediment and the severity of microalgal blooms. As pond sediments act as a major sink for ammonia, preventing their build-up will be an effective means of breaking this cycle, allowing greater densities of fish to be cultured. Hopkins et al. (1994) proved that removing sludge improves water quality and production, but Brune et al. (2003) pointed out that effective sludge removal has not been transferred to large scale systems and as such, is not widely practised in the aquaculture industry. The SIFTS technology has the ability to continuously remove settleable wastes (fish faeces and uneaten food).

The aim of this trial was to determine the effect of removing the solid wastes collected in the SIFTS waste extraction system on microalgal production and bottom sludge deposition in static ponds. A secondary objective of this experiment was to determine the effect of stocking density within SIFTS on the growth, survival and food conversion ratio of barramundi.

5.1.2 Materials and Methods

In each of the two 1500 m^2 static inland saline ponds described in Section 4.2, duplicate 10 m^3 SIFTS were stocked with either 800 or 1600 juvenile barramundi (average weight 66.0 ± 0.8 grams) on December 2nd, 2005. The water exchange rate through each SIFTS was set at 4 exchanges per hour and maintained at this rate for the duration of the trial.

Barramundi were fed twice daily on a fixed ration (Skretting Nova ME, 45% protein, 25% lipid) determined by their wet-weight from data previously obtained for this species in SIFTS (Partridge et al., 2006). Fifteen minutes after each feeding, waste extractors were emptied and the weight of any remaining pellets recorded after applying a correction factor for the water absorbed by the pellets over the fifteen minute period. All wastes and uneaten food thus collected from SIFTS in Pond 1 were taken ashore and dewatered/stored within a GeoTube™ whereas those collected from Pond 2 were released back into the pond.

In each pond, water temperature and dissolved oxygen concentrations were constantly measured and logged to a PC every 5 minutes. Dissolved oxygen concentration in the outlet water of each SIFTS was similarly measured. In analysing these data, a microalgal crash was arbitrarily defined as having occurred when the maximum daily oxygen concentration in the pond declined by at least 40% over a maximum of four consecutive days.

Samples of pond water and bottom sludge were taken four times throughout the trial for nutrient analyses. Pond water was measured for a range of parameters, namely: biological oxygen demand (BOD), chlorophyll, phaeophytin, total nitrogen (TN), total dissolved nitrogen (TDN), ammonia nitrogen (TAN), nitrite + nitrate nitrogen (NO_X), total phosphorus (TP), total dissolved phosphorus (TDP) and orthophosphate (SRP). The total nitrogen, total phosphorus, dry matter (DM) content and organic matter content (LOI; % loss on ignition at 550°C) of bottom sludge was measured from sediment collectors randomly placed within one of 12 transect grids on the floor of each pond. The total quantity of such parameters collected from the entire pond floor was extrapolated from the ratio of the surface area of the sediment collectors to that of the pond bottom. The same parameters were measured in the contents of waste collected from the waste extractors as follows. From Days 11-17, 67-73, and 116-122, the waste collected from each SIFTS was collected fifteen minutes after each of the two daily feeds as previously described. At each sampling, the volume of waste collected from each SIFTS was measured, and then thoroughly homogenised prior to taking a 200 mL subsample, which was immediately frozen. The remaining volume of waste collected from SIFTS in Pond 2 was returned to that pond. At the end of each 7 day period, the 14 subsamples per SIFTS (two frozen subsamples

per day) were thawed, pooled and thoroughly homogenised prior to taking triplicate 4 mL subsamples for determining TN, TP, DM and LOI. Each of these variables was expressed as a % of the total fed during the 7 day sampling period and these values then used to extrapolate the total quantity of each variable captured during the overall trial period. During each of these intensive sampling periods, pH was logged in each pond over a 24 hour period.

Four times throughout the trial, SIFTS liners were inverted, crowding the fish and enabling representative subsamples to be obtained for measuring growth (specific growth rate; SGR). A number of fish equaling 5% of the total fish number in each SIFTS were weighed to 0.1 gram at each sampling.

The trial was concluded on April 11th (Day 130) when all fish were counted to determine survival and 10% in each of the SIFTS weighed to determine growth. A subsample of these fish, and the feed fed throughout the trial, were also analysed for total nitrogen and phosphorus content.

5.1.3 Results and Discussion.

Survival, Growth and Yield

In the last week of the trial a considerable mortality of fish was experienced in all SIFTS in Pond 1 (average 60 ± 6%). This mortality was attributed to the combination of high pH and TAN (see *pH & TAN*, below). Survival in Pond 2 averaged 90 ± 4%, with no significant difference in survival between high and low density SIFTS (P = 0.56) (Table 5-1).

In both ponds, the rate of growth of fish in the high density SIFTS was slightly lower than that in the low density SIFTS, however, in both cases this difference was not significant (P>0.05) (Table 5-1). The final mean weight attained by the fish in the two pond treatments differed by < 0.5 g (Table 5-1).

Table 5-1: Summary of grow-out parameters for barramundi produced in the two SIFTS ponds over 130 days between December 2005 and April 2006.

	Pond 1 - waste removed		Pond 2 - waste retained	
	High density	**Low density**	**High density**	**Low density**
Mean weight (g)	316.0	345.9	310.7	351.7
Mean weight pond (g)	330.9		331.2	
Survival (%)	38 ± 4		90 ± 4	
Biomass harvested (kg)	588.7		1445.7	
Tonnes/ha (equivalent)	3.9		9.6	
SGR	1.21 ± 0.01	1.26 ± 0.03	1.20 ± 0.07	1.30 ± 0.03
FCR	1.39 ± 0.04	1.24 ± 0.01	1.48 ± 0.08	1.35 ± 0.13

The average specific growth rate of barramundi pooled across all SIFTS was 1.24 ± 0.02%, (Table 5-1) considerably lower than the rate of 1.73% reported by Partridge et al. (2006) under similar conditions. As shown in Figure 5-1, fish in the current trial did not increase in weight between Day 103 and Day 130, when the daily temperature in the two ponds averaged 19.8°C (Figure 5-3). Figure 5-1 also highlights that fish were growing at a similar rate to that described by Partridge et al. (2006), prior to this decrease in temperature i.e. prior to Day 103 (see Figure 5-3).

Similarly to growth, the food conversion ratio of fish grown in the high density SIFTS was slightly higher than that in the low density SIFTS, however, in both ponds this difference was not significant ($P>0.05$)(Table 5-1).

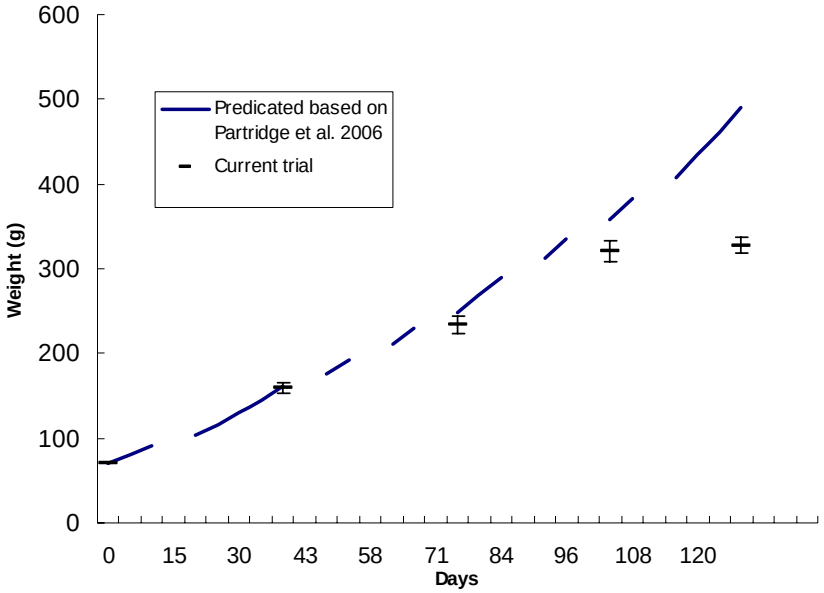


Figure 5-1: Average increase in weight of barramundi across all SIFTS and ponds.

pH and TAN

During the 7 days prior to the planned harvest, a high pH value of 8.8 (Figure 5-2) combined with a TAN of 4.0 mg/L resulted in an unionised ammonia concentration of 0.8 mg/L in Pond 1. Given that barramundi's 96 hr LC_{50} for unionised ammonia is 1.3 mg/L (Økelsrud and Pearson, 2007), we hypothesise that exposure of the barramundi in Pond 1 to this lower concentration over a longer period of time led to their mortality.

Although a similar concentration of TAN was experienced in Pond 2, pH in this pond did not rise above 7.9 (Figure 5-2), yielding a unionized ammonia concentration of only 0.1 mg/L. Prior to the mortality event in Pond 1, survival averaged 94 ± 2%, with no significant effect of density on survival (P = 0.87).

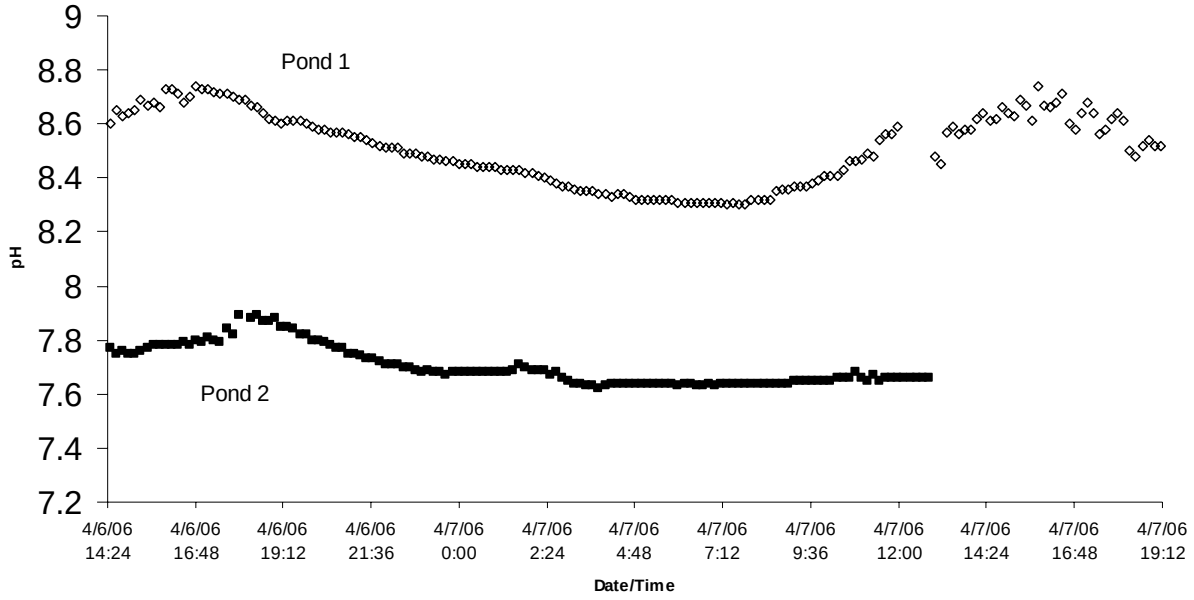


Figure 5-2: Variation in pH over a 24 hour period in Ponds 1 and 2 on Day 126 of Trial A.

As is outlined in more detail below, a strong microalgal bloom was developing in Pond 1 at the time of mortality. When blooms are sufficiently strong, insufficient carbon dioxide for photosynthesis can be obtained from the water via atmospheric diffusion and it is therefore sourced from the conversion bicarbonate to carbonate, a consequence of which is an increase in pH. At the same time, Pond 2 was experiencing a decline in microalgal density and hence pH was low. These microalgal blooms and their effects on water quality are discussed in further detail below.

Water Temperature

Water temperature ranged from 18.0 to 28.8°C and 18.7 to 32.4°C in Ponds 1 and 2, respectively and averaged 24.5°C and 23.9°C, respectively. These average temperatures were considerably lower than the 26°C experienced over a similar period in the previous year (Partridge et al., 2006) (Figure 5-3). In both ponds in the current trial, average daily water temperature declined rapidly from approximately 25°C on Day 109 to less than 20°C by Day 119 (Figure 5-3).

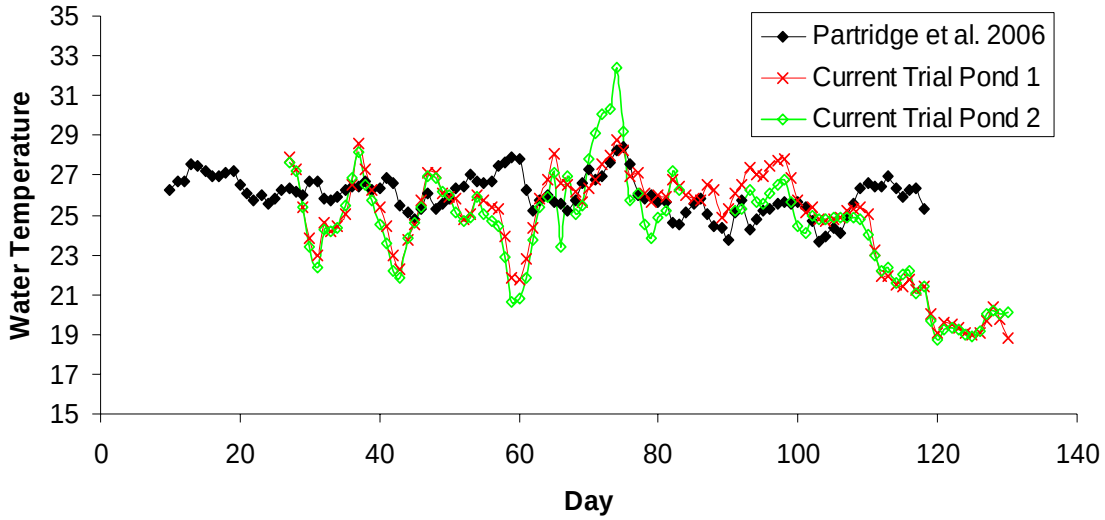


Figure 5-3: Average daily water temperature in Ponds 1 and 2 in the current trial and in a similar sized pond on the same property at a similar time of year (Partridge et al., 2006)

Dissolved Oxygen

Figure 5-4 show the daily maximum and minimum concentrations of dissolved oxygen in Ponds 1 and 2, respectively. As previously described, a microalgal crash was arbitrarily defined as having occurred when maximum daily dissolved oxygen concentrations decreased by at least 40% over a maximum of four consecutive days and such events are shown in Figure 5-4 as vertical dashed lines. This figure shows that a similar number of microalgal crashes occurred in both ponds over the course of the trial, suggesting that the 8.02 kg of nitrogen and 2.50 kg of phosphorus prevented from entering Pond 1 (Table 5-2) had little effect on the frequency of such crashes.

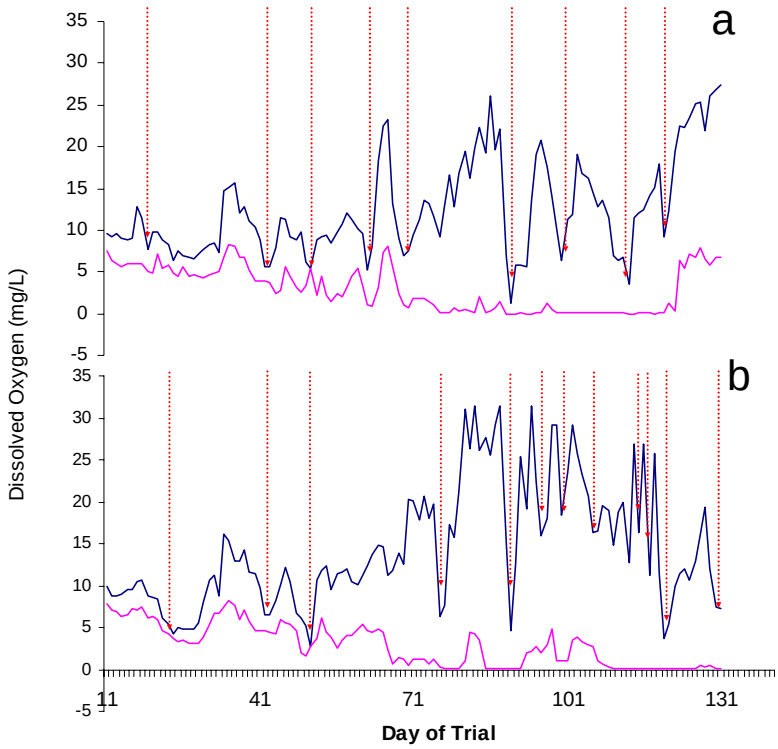


Figure 5-4: Daily maximum and minimum oxygen concentrations in Pond 1 (a) and Pond 2 (b) during Trial A. Microalgal crashes are indicated by vertical dashed lines.

Waste Collection, Bottom Sludge Deposition and Microalgal Blooms

The average quantities of nitrogen, phosphorus and dry matter collected in the waste extractors as a percentage of those fed during the three intensive sampling periods are shown in Table 5-2. Within each pond, there was no significant difference in the TN, TP or DM captured as a percentage of that fed between high and low density SIFTS. As such, for each pond these values were averaged then applied to the amount fed throughout the trial to derive the total quantity (kg) of each component either entering the pond (Pond 2) or prevented entering the pond (Pond 1).

Table 5-2: Nitrogen, phosphorus and dry matter of waste captured in waste extractors.

	Pond 1- waste removed		Pond 2- waste retained	
	Low Density	**High Density**	**Low Density**	**High Density**
N captured as % fed	6.88 ± 0.03	7.04 ± 1.37	6.01 ± 0.22	6.16 ± 1.58
Total N captured/released (kg)	8.02		6.98	
P captured as % fed	13.34 ± 0.57	13.45 ± 1.78	13.99 ± 0.28	13.06 ± 2.96
Total P captured/released (kg)	2.50		2.53	
DM captured as % fed	6.94 ± 0.27	6.93 ± 0.41	5.57 ± 0.42	5.83 ± 1.32
Total DM captured/released (kg)	87		72	

Data on the quantities of dry matter, nitrogen, phosphorus and organic matter remaining on the bottom of each pond at the end of the trial are shown in Table 5-3. Despite the waste extractors preventing considerable amounts of nutrient and dry matter entering Pond 1 the quantities present on the pond floor at the end of the trial were actually higher in Pond 1 compared to Pond 2 (Table 5-3). That the quantities of waste prevented entering Pond 1 from the sludge extractors were small compared with those found on the pond floor (see Figure 5-5) suggests there exists a far more significant source of deposition than the faecal waste and uneaten food collected by the waste extractors.

Table 5-3: Dry matter, nitrogen, phosphorus and organic matter contents of bottom sludge collected from the floor of each pond at the conclusion of the trial

	Pond 1 (waste removed)	**Pond 2 (waste retained)**
Dry matter (kg)	709.4	643.2
Total nitrogen (kg)	26.4	21.9
Total phosphorous (kg)	4.9	3.3
Organic matter (%)	49	40

Figure 5-5: Floor of Pond 1 (solid waste removed) immediately after draining. Insert: close-up of sediment deposition.

It is hypothesized that the microalgal crashes, which in both ponds occurred more frequently than anticipated, contributed the majority of dry matter and nutrients to the bottom of each pond, thereby masking the smaller contribution to nutrient reduction made by the waste extractors. Jimenez-Montealegre et al. (2005) demonstrated the significant effect that the rate of microalgal settlement has on the amount of nutrients found on the pond floor. Microalgal settlement rates have been reported from 2% of the standing biomass per day in natural lakes to 20-50% in aquaculture ponds (Jimenez-Montealegre et al., 2005), with crashes resulting in even higher instantaneous settlement rates.

The rate of nitrogen flux to the pond floor in the current study (135 and 112 mgN/m^2/day for Ponds 1 and 2, respectively), is significantly higher than the 46 mgN/m^2/day described for channel catfish ponds (Gross et al., 2000) and supports the theory that frequent microalgal crashes resulted in high concentrations of nutrients being deposited on the ponds' floors. Although daily feed rates were higher than the maximum sustainable level defined by Hargreaves and Tucker (2003) for much of the latter part of this trial (125 kg/ha/day), the frequent crashes cannot be attributed to this alone, as the daily feed intake into each pond did not exceed this value until Day 93, by which time microalgae crashes had occurred 5 – 6 times in each pond (see Figure 5-4).

A potential causative factor for such frequent crashes was the fact that both ponds were highly stratified. This stratification was caused by the diel vertical migration of *Heterocapsa* (the

predominant microalgal species in both ponds) (Figure 5-6) and the inappropriate orientation of SIFTS's air-lift intakes.

Figure 5-6: Dense, brown coloured bloom of the dinoflagellate, *Heterocapsa* sp. within the SIFTS pond as evidenced by secchi disk reading of < 100mm.

During the day *Heterocapsa* exhibited positive phototaxis, resulting in very high cell densities close to the water surface. Oxygen concentrations were therefore high at the surface, but low in the underlying water. At night, the dinoflagellate exhibited a positive geotaxis and migrated to the pond bottom. There are two potentially important consequences of this migration. Firstly, the high degree of self-shading caused by the migrating *Heterocapsa* may have contributed to the frequent microalgal crashes. Secondly, this daily migration resulted in a constantly low level of oxygen close to the pond floor and the subsequent elimination of the aerobic boundary layer at the water/sediment interface. This layer is critical for maintaining pond health through the oxidation of organic matter and by preventing the diffusion of toxic, reduced metabolites such as hydrogen sulphide and ferrous iron back into the water column. Evidence for insufficient oxygen concentrations for organic matter oxidation is provided by the high organic matter content of the bottom sludge. In both ponds the material on the pond floor contained over 40% organic matter, well in excess of the recommended maximum value of 6% described by Boyd and Bowman (1997). Anaerobic conditions also increase the solubility of phosphorus, increasing its availability to microalgae and further encouraging blooms (Boyd, 1996).

The orientation of the SIFTS air-water lift pumps exacerbated the problem of the stratification. In order to maximise the benefit of warmer surface waters for barramundi growth, the air-water lifts were set to draw water close to the surface. With the discharge water from the SIFTS being returned at the surface, the air-water lifts did little to assist in the breakdown of the stratification caused by the *Heterocapsa*. Positioning the intakes of the air-water lift pumps at the base of the pond may have broken the stratification of the water column, increasing light penetration into

the pond. Oxygenated water would also have been drawn across the sediment/water interface, increasing organic matter breakdown and maintaining phosphorus in its insoluble form (i.e. unavailable to microalgae).

That the ponds are lined with plastic may have also been a contributing factor to the frequent crashes of microalgae. In earthen ponds, clay soils effectively adsorb phosphorus, reducing its availability to microalgae and potentially limiting its growth. Indeed, during the first half of the trial the molar ratio of dissolved inorganic nitrogen to orthophosphate was less than 16, suggesting that microalgae was not phosphate limited (Burford, 1997).

Pond Water Quality and Nutrient Budgets

The nitrogen inputs and outputs for the two ponds are shown in Figure 5-7. It has been assumed that all of the nitrogen inputs to the pond were accounted for. Nitrogen fixation as a source of nitrogen input to the pond was considered to be negligible because the total nitrogen to total phosphorus ratio (TN:TP) was always greater than 13 (Gross et al., 2000). The majority of the nitrogen input to both ponds (>90%) was in the form of the feed, a figure which is typical of all semi-intensive fish ponds (Krom and Neori, 1989; Gross et al., 2000). The amount of uneaten food lost to Pond 2 (and which was otherwise captured in Pond 1) equated to 2% of the total nitrogen input.

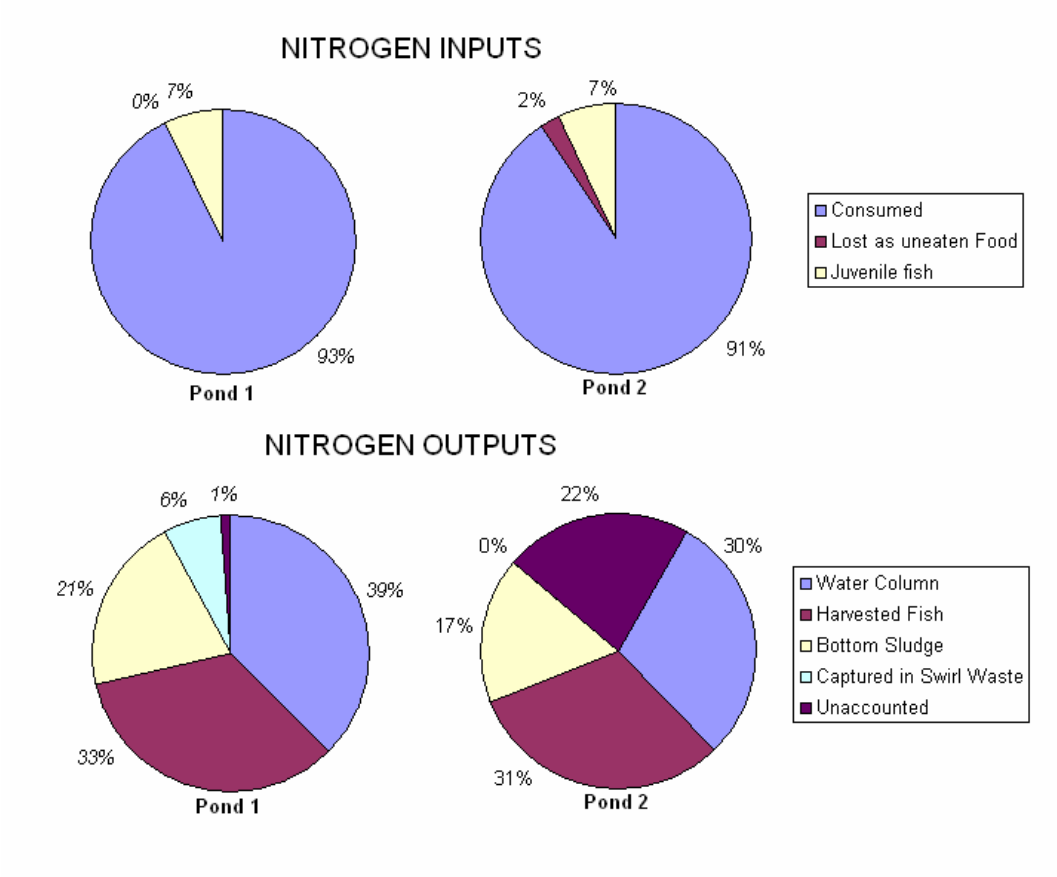


Figure 5-7: Nitrogen inputs and outputs for the two ponds used in the current trial.

Consistent with other studies on fish production in semi-intensive ponds, approximately 30% of the nitrogen removed from both ponds was within the fish biomass (Krom and Neori, 1989; Gross et al., 2000). The amount of nitrogen remaining in the water column was also similar in both ponds (39 and 30% for ponds 1 and 2, respectively) a figure which is considerably higher than that reported by Gross et al. (2000) (14%) but lower than that reported by Preston et al. (2000) (57%) and Krom and Neori (1989)(59%). Of the nitrogen found in the water column, 71% was in the particulate form in Pond 1, compared to 46% in Pond 2, which is consistent with the strong bloom of microalgae which was underway in Pond 1 at the time of final sampling. The amount of nitrogen remaining as bottom sludge (21 and 17% for Ponds 1 and 2) was similar to that described by Gross et al. (2000) (23%) and Preston et al. (2000) (14%). The waste extractors on SIFTS in Pond 1 captured 6% of the total nitrogen input into this pond. Although all of the nitrogen inputs could be accounted for in the measured outputs in Pond 1, 22% of the nitrogen inputs to Pond 2 could not be accounted for. In the nitrogen budgets conducted by Gross et al. (2000), 30% of nitrogen outputs were comprised of mechanisms not quantified in this study i.e. ammonia volatilisation (13%) and denitrification (17%). In many aquaculture ponds, however, denitrification is often not a significant sink for nitrogen because low rates of nitrification provide little nitrate for denitrification to occur (Hargreaves, 1998;

Burford and Longmore, 2001). This appears to be the case in the current study where the combined nitrite and nitrate concentration did not exceed 0.015 mg/L. If ammonia volatilisation was a significant source of nitrogen removal from Pond 2, it is unclear why this would not have also been the case in Pond 1.

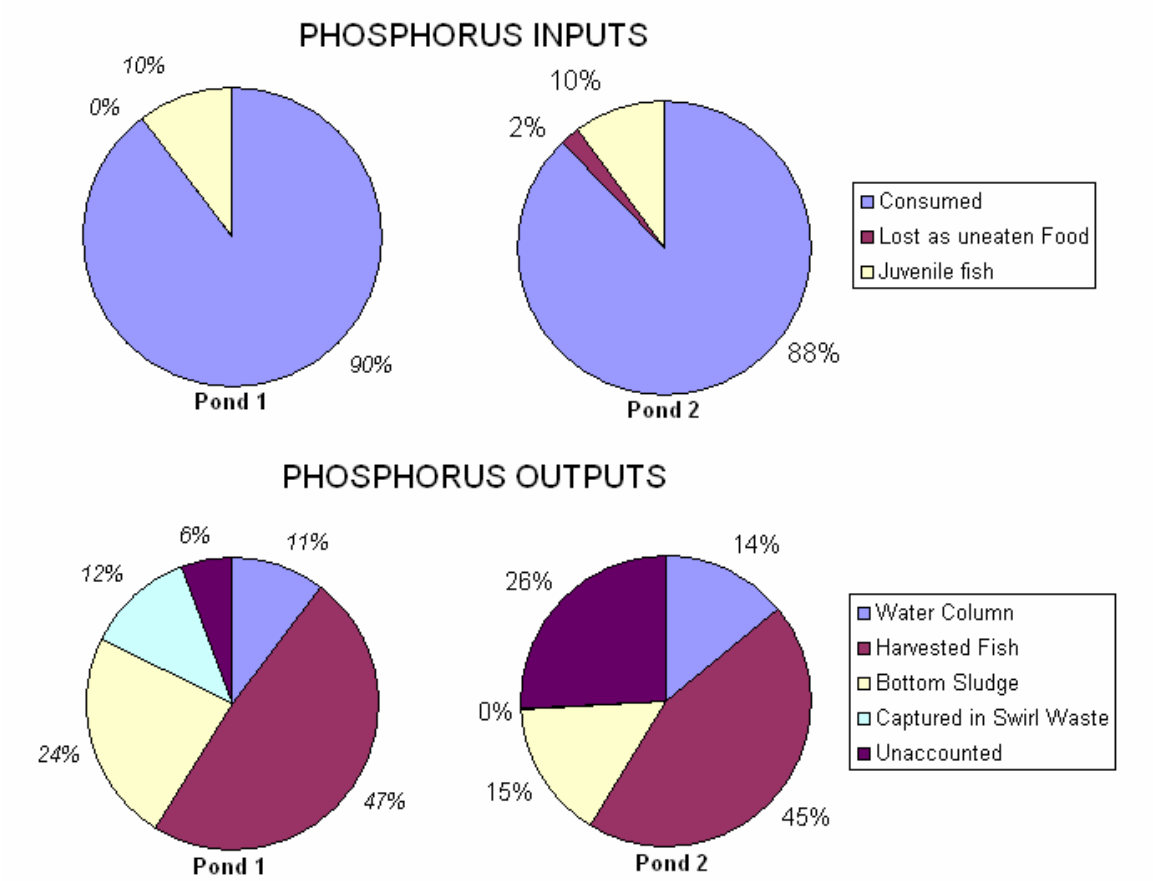


Figure 5-8: Phosphorus inputs and outputs for the two ponds used in the current trial.

The phosphorus inputs and outputs for the two ponds are shown in Figure 5-8. As with the nitrogen inputs, it has been assumed that all phosphorus inputs to the pond were accounted for. Similar to nitrogen, the majority of the phosphorus input (>88%) into both ponds was in the form of feed. Between 45 and 47% of the phosphorus output was in the harvested biomass, considerably higher than the 21% reported by Krom and Neori (1989) and the 30% by Boyd (1985). Our data showing a high proportion of retained phosphorus may indicate an optimum content of phosphorus in the diet used (1.2%) for barramundi. This is due to the fact that past a certain (species-specific) dietary inclusion level, excretion of phosphorus increases with increasing levels of phosphorus in the diet (Bureau and Cho, 1999; Rodehutscord et al., 2000). Of the total quantity of phosphorus applied to the ponds, 24% and 15% were accounted for in the bottom sludge in Ponds 1 and 2, respectively. This figure is significantly lower than the 67%

reported in earthen ponds by Masuda and Boyd (1994) and (Boyd et al., 2006) but similar to the 17% reported by Krom and Neori (1989) for plastic lined ponds; highlighting the lack of phosphorus adsorption onto bottom soil in plastic lined ponds. As a consequence of this lack of adsorption onto the pond floor, coupled with increased phosphorus solubility due to the anaerobic conditions, the quantity of phosphorus found in the water column in the two ponds (11 and 14%, respectively) was higher than reported in other studies. For example (Boyd, 1985) reported that only 7% of the total phosphorus input was found in the water column.

5.1.4 Conclusions

Strong microalgal blooms occurred in both ponds in the current trial and these crashed with greater frequency than anticipated. This resulted in significant amounts of nutrients being deposited on the floor of both ponds. Such frequent crashes were likely caused by the stratification in the ponds due to the diel vertical migration of *Heterocapsa* and the (now apparent) inappropriate location of the SIFTS water intakes. This stratification also limited the oxidation of nutrients on the floor of the pond and increased the solubility of phosphorus. In addition to the problems of nutrient deposition created by these blooms, they also resulted in high pH levels which were a significant contributing factor to the mortality event which occurred in the latter part of the trial. These data highlight the importance of controlling such microalgal blooms and subsequent trials have thus focused on a number of approaches for achieving this objective.

5.2 Trial B: Assessment of Vertical Artificial Substrates as Nitrogen Sinks in Inland Saline Aquaculture Ponds and Assessment of Destratification Methods

5.2.1 Introduction

This trial sought to determine the effectiveness of vertical artificial substrates as biofilters and their ability to convert ammonia into nitrate. We aimed to encourage nitrification in order to provide the nitrate required for denitrification; a process that would export nitrogen from the ponds in the form of nitrogen gas.

The trial was modified from that originally proposed when the rainbow trout in the ponds died within two weeks of stocking. While all measured water quality parameters (salinity, temperature, TAN, pH, dissolved oxygen) were found to be optimal during this mortality, Microtox™ analyses of the pond waters were positive, indicating a toxin was present. Examination of moribund fish appeared to confirm that a toxin had caused severe gill damage and fusion of the gill lamellae. As the research farm is located adjacent to a golf course, a turf club, farming land and a small airport, chemical spray drift was considered the likely origin of the toxin. Despite its location, this fish farm has been operating for many years and fish mortality due to spray drift has never been previously reported. In addition, of 29 pesticides and 10 herbicides which are known to be used in the local area, none were detected in water samples sent for analysis (Table 5-4).

Rapid breakdown of a pesticide or herbicide prior to analysis or failure to analyse for the correct pesticide/herbicide could not be ruled out.

Table 5-4: Results of pesticide and herbicide analysis. PQL = practical quantification limit. Black entries are pesticides; red entries are herbicides.

Analyte	PQL (µg/L)	Northam Pond µg/L	Analyte	PQL (µg/L)	Northam Pond µg/L
alpha Chlordane	<0.015	nd	Malathion	<0.05	nd
gamma Chlordane	<0.015	nd	ethyl Parathion	<0.004	nd
p,p'-DDT	<0.01	nd	PCB Congener C28	<0.02	nd
Endosulfan Sulphate	<0.01	nd	PCB Congener C52	<0.01	nd
Endrin	<0.01	nd	PCB Congener C101	<0.005	nd
Heptachlor	<0.01	nd	PCB Congener C118	<0.005	nd
Lindane	<0.07	nd	PCB Congener C138	<0.005	nd
Aldrin	<0.01	nd	PCB congeners C153	<0.005	nd
p,p'-DDE	<0.01	nd	PCB Congener C180	<0.005	nd
p,p'-DDD	<0.01	nd	2,4,5-TP (Silvex)	<0.5	nd
Dieldrin	<0.01	nd	2,4-DB	<0.5	nd
alpha Endosulfan	<0.01	nd	2,4,5-T	<0.5	nd
beta Endosulfan	<0.01	nd	2,4-D	<0.5	nd
Methoxychlor	<0.04	nd	Dicamba	<0.5	nd
Mirex	<0.01	nd	Dichlorprop	<0.5	nd
HCB	<0.01	nd	Dinoseb	<0.5	nd
Azinphos-methyl	<0.025	nd	MCPA	<0.5	nd
Chlorpyrifos	<0.01	nd	Mecoprop	<0.5	nd
Diazinon	<0.01	nd	Picloram	<1	nd
Fenitrothion	<0.2	nd			

Due to the loss of the trout, increasing quantities of inorganic nutrients were added to both ponds to mimic the excretion of nitrogen and phosphorus by fish. This approach allowed us to assess the effectiveness of the vertical artificial substrates and also to test the effectiveness of changing the SIFTS' air-lift orientation to overcome the stratification experienced in the previous trial (see section 5.1.3)

Finally, since a heterotrophic pond management trial was proposed for the next trial (see section 5.3) a short-term experiment was conducted after the vertical artificial substrates had been assessed to provide preliminary data on rate of molasses addition required to remove ammonia and stabilise pH (see section 5.3.1 for further details).

5.2.2 Materials and Methods

Nitrification by Aquamats®

Inorganic nutrients were added drop-wise daily to each SIFTS. The rate of inorganic nutrient addition (NH_4Cl, urea and superphosphate) was equivalent to that at which the barramundi excreted these dissolved nutrients over the previous trial.

In one pond, nine vertical artificial substrates (Aquamats® model 25001, Meridian Aquatic Technology, Maryland USA) were placed in the outflow of each of the 4 SIFTS in a custom made channel (Figure 5-9). This channel ensured the Aquamats® were exposed to ammonia directly after being excreted by the fish (thereby minimising microalgal uptake) and also to ensure an adequate level of dissolved oxygen was supplied to the mats, independent of the oxygen concentration in the pond itself. The number of mats was based on the manufacturer's specifications that mats can remove 12 grams N/m^2/day and on the expected rate of nitrogen excretion when the pond was stocked at maximum biomass.

Figure 5-9: (a) Individual vertical artificial Aquamat® and (b) when installed in groups into the channel at outflow of each SIFTS.

The rate of nitrification was assessed after allowing a biofilm to establish on the mats for a period of 8 weeks. Peristaltic pumps sampled water continuously from the channel inlet and outlet over 24 hour trial periods. The 20 litres of water thus sampled represented a 24-hour average ammonia concentration entering and leaving the channel, with the difference used to calculate the rate at which ammonia was being oxidised. These measurements were made on three separate occasions.

Destratification

Air-lift intakes on the four SIFTS in each pond were placed close to the bottom of the ponds to determine if this arrangement minimised or prevented the stratification in temperature and dissolved oxygen experienced during the initial trial (see section 5.1.3).

In each pond, water temperature was constantly measured and logged to a PC every 5 minutes both at the water surface and at a depth of 2.5 m which was ca. 500 mm above the pond floor. Dissolved oxygen concentration in the pond was similarly measured at the water surface and measured manually on a routine basis at 2.5 m depth using an electronic hand-held meter (YSI model 85).

Heterotrophic Pond Management

At the conclusion of the Aquamat® trial, molasses was added to one pond at 32 g/g TAN (Willett and Morrison, 2006) when the concentration of TAN was 4.1 mg/L and maximum daily pH values reached 9.8. After molasses addition, TAN, pH and dissolved oxygen concentrations were regularly monitored

5.2.3 Results and Discussion

Nitrification by Aquamats®

The mean channel influent ammonia concentration averaged 1.44 ± 0.11 mg/L between the three sampling occasions. At each sampling, the concentration of ammonia in the outflow differed by < 2% of the inflow concentration, highlighting that no ammonia was being oxidised by the mats. Although we have successfully used these vertical artificial substrates as biofilters in indoor tank cultures, we believe that their inability to convert ammonia into nitrate in the ponds was due to fouling of the mats with small particulate matter (e.g. senescent microalgae). Erler et al. (2004) also found these mats encouraged the settlement of particulate matter onto them. The high concentrations of particulate matter encourage the growth of heterotrophic bacteria which grow more rapidly and hence out-compete nitrifiers for substrate surface area.

Destratification

Over the 125 day trial period, the difference between the daily average temperature at the water surface and pond bottom averaged 0.3 and 0.1°C for the two ponds, with a maximum difference of 0.8°C in both ponds (Figure 5-10). This compares with maximum differences experienced in Trial A of 5°C. Similarly, there were no measurable differences in dissolved oxygen with depth in the current trial, yet dissolved oxygen differed by as much as 12 mg/L over a depth of 1.5 m in Trial A.

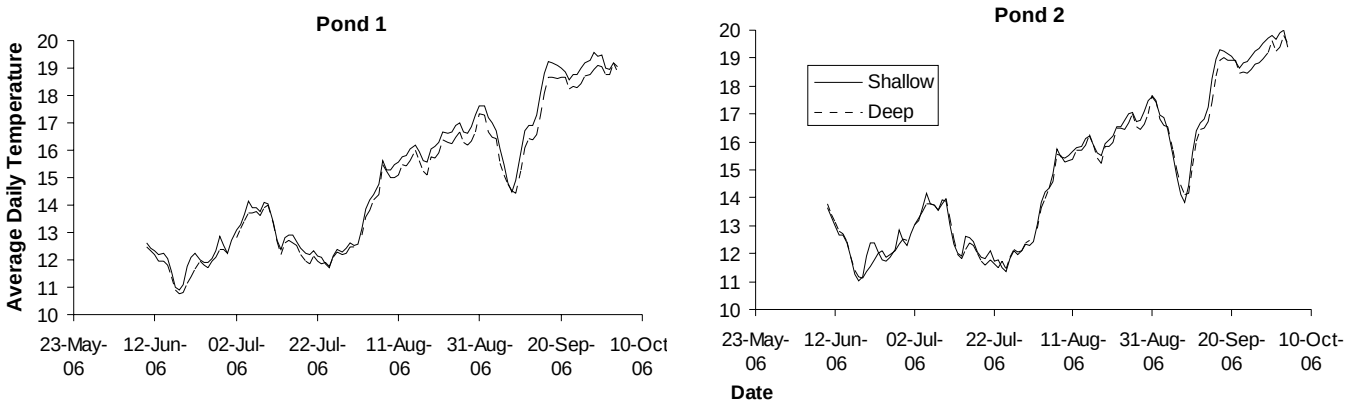


Figure 5-10: Average daily water temperature at 0.5 m (shallow) and 2.5 m (deep) depths in the two ponds during Trial B.

Although the degree of stratification in this trial was significantly less than experienced in the previous trial, it cannot be attributed alone to the change in orientation of the airlifts, as during this trial blooms of *Heterocapsa* did not occur. This species of microalgae exhibits strong diel migration and was therefore at least a contributing factor in the stratification experienced in the previous trial. As these blooms were not experienced in the current trial we remained unsure whether the changed air-lift orientation was sufficient to break any stratification caused by this migration. The air-lift configuration therefore remained unchanged for subsequent trials and stratification data collection continued.

Heterotrophic Pond Management

Within 45 hours of molasses addition, ammonia decreased from 4.1 to 2.2 mg/L but then began to increase again (Figure 5-11). After 11 days, TAN had returned to its initial concentration of 4 mg/L. This rate of initial decline is much slower than that described by Willett and Morrison (2006), who found that TAN dropped by 65% within 6 hours and is probably the result of the cooler water temperatures in the current study (i.e. 18°C), which would slow the development of

the heterotrophic bacteria. Dissolved oxygen concentration in the pond declined markedly after molasses addition due to the respiration of aerobic, heterotrophic bacteria, with minimum daily oxygen concentrations dropping from approximately 9 mg/L to 0 mg/L (Figure 5-11). Maximum daily pH values declined marginally in the days following molasses addition (Figure 5-12). Of the 108 kg of organic carbon added to this pond in the form of molasses, 52% was found deposited on the pond floor within 21 days.

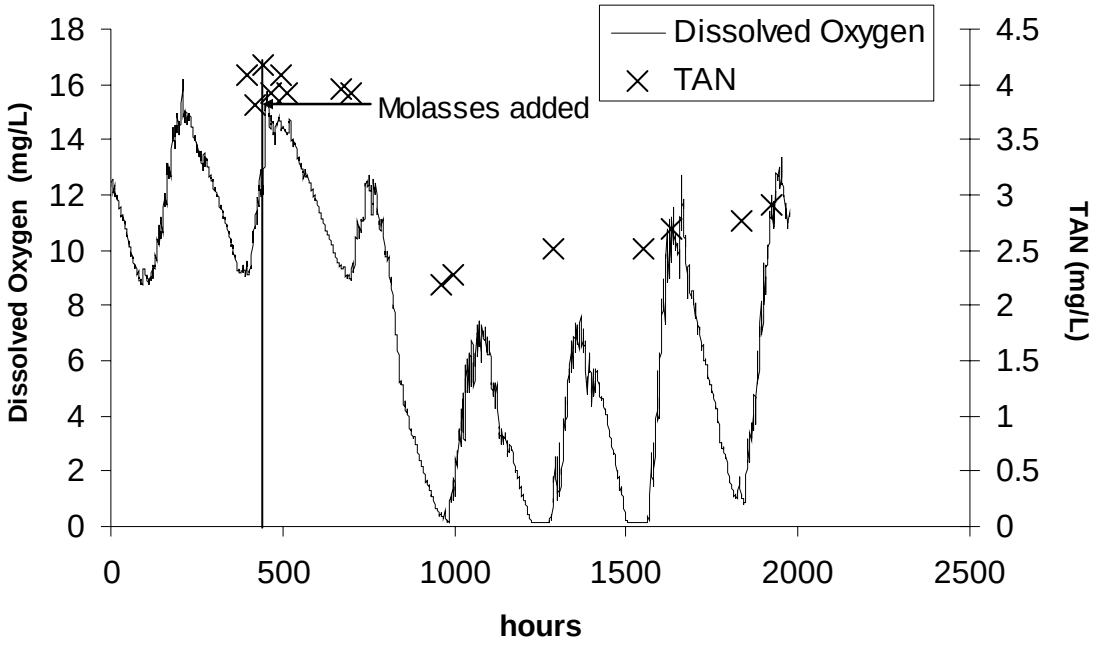


Figure 5-11: Variation in dissolved oxygen and TAN in a static pond before and after addition of molasses.

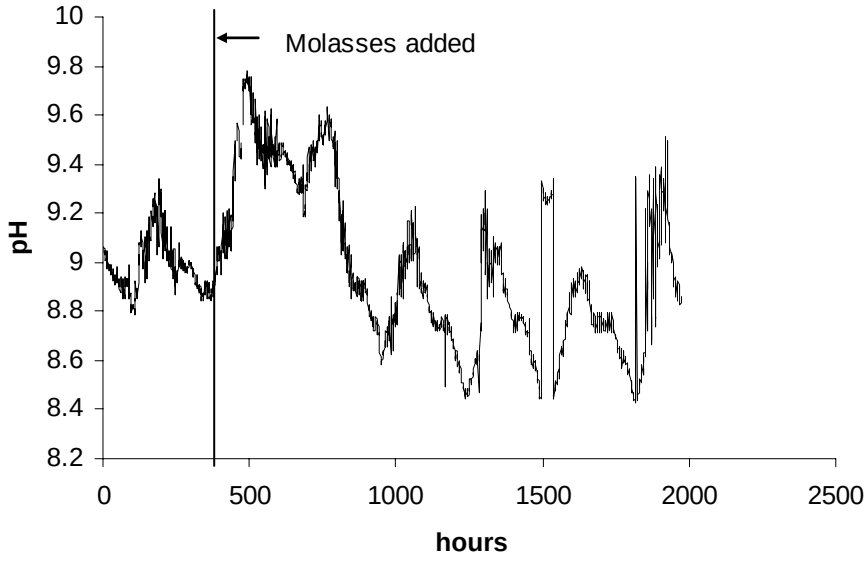


Figure 5-12: Variation in pH in a static pond before and after addition of molasses.

Collectively, these data suggest that the molasses addition used was insufficient to maintain TAN at a reduced level and that the circulation and aeration of the entire pond volume by the SIFTS air-lifts was insufficient to maintain the heterotrophic floccules in suspension. Based on these data and observations, molasses addition to the ponds on a daily basis in the next trial (Trial C) was at a higher rate. This rate was calculated on the theoretical nitrogenous excretion rate of the fish. Additional circulation/aeration was be also implemented in the following trial to maintain the heterotrophic floccules in suspension (see section 5.3.2).

5.3 Trial C: Investigating the Use of Heterotrophic Pond Management Techniques to Improve Barramundi Yields from Static Inland Saline Ponds.

5.3.1 Introduction

The original objective of this trial was to incorporate the optimum in-pond bioremediation treatment/s developed during previous experiments over an extended period of 1 year (two fish crop cycles). Due to the unexplained mortality of trout experienced during Trial B, we were yet to fully optimise in-pond bioremediation treatments at this stage and this objective was subsequently modified to enable further testing of bioremediation techniques in two separate, 6-month experiments.

It was demonstrated in Trial A above, that removal of solid wastes via SIFTS waste collectors did not sufficiently improve water quality to enable yields of 10 tonnes/ha over a 120 day cropping cycle. Fish mortality was caused by the combination of high ammonia and pH from strong microalgal blooms, a problem common in zero- or low-exchange autotrophic ponds (Burford et al., 2003; Ebeling et al., 2006). This result highlighted the need for alternative approaches to managing microalgae blooms. Heterotrophic pond management has the ability to overcome issues associated with strong microalgal blooms by manipulating the C:N ratio of the water to stimulate the uptake of ammonia and organic nitrogen into bacterial biomass. Such systems facilitate rapid removal of ammonia (Avnimelech, 1999; Crab et al., 2007) and exhibit lower and more stable pH values than autotrophic systems because they are net producers of carbon dioxide (Ebeling et al., 2006).

Staff from the Queensland Department of Primary Industries and Southern Cross University are experienced in heterotrophic pond management and collaborated on this aspect of the project. The first of these experiments tested heterotrophic pond management techniques during a crop cycle of barramundi and further investigated the issue of stratification.

5.3.2 Materials and Methods

Prior to the commencement of this trial, both ponds were completely drained, dried and the liners thoroughly cleaned (Figure 5-13), as a precautionary measure due to the mortality of trout in the previous trial.

Figure 5-13: High pressure cleaning of pond liners and the removal of floor sediment prior to the commencement of current trial.

In each of two 1500 m^2 static, inland-saline ponds, four 10 m^3 SIFTS were each stocked with 1200 juvenile barramundi (average weight 110 grams) on December 8th, 2006. In Pond 1, a heterotrophic management regime was implemented for the duration of the trial. Based on our preliminary findings on the heterotrophic system (see section 5.2.3) daily additions of molasses were made as a function of the daily feed rate. Pond 2 was managed in the same manner as described for Trial A (i.e 'autotrophically; zero-exchange') with the exception that the destratification techniques described in Trial B were tested again to determine if the pond remained destratified during *Heterocapsa* blooms.

Molasses was dosed were using a method modified from Avinmelech (1999):

$$Molasses_{add} = Feed \times N_{feed} \times \%N\ excrete \times ([C/N]_{mic}/E) \times Molasses_{carbon}$$

Where:

- $Molasses_{add}$ is the amount of molasses required per day (kg)
- Feed is the amount of feed added per day (kg).
- N_{feed} is the nitrogen content of the feed (45% protein / 6.25)
- % N excreted is the % of the N ingested which is excreted as dissolved nitrogen, taken to be 60% of N_{feed} (Porter et al., 1987; Brune et al., 2003).
- $[C/N]_{mic}$ is the C:N ratio of bacterial biomass, which was taken to be 5 (Moriarty, 1997; Hargreaves, 2006)
- *E* is the bacterial C assimilation efficiency, taken as 40% (Avnimelech, 1999)

- $Molasses_{carbon}$ is the carbon content of molasses, taken to be 40%. (Avnimelech, 1999)

Three, 1.5 kW Force 7 propeller aspirators, providing the equivalent of 30 kW/ha, operated in the heterotrophic pond to provide the mixing and aeration required to maintain the aerobic microbial proteins in suspension.

In both ponds, barramundi were fed to satiety twice daily (Nove ME, 45% protein, 25% lipid). Fifteen minutes after each feeding, waste extractors were emptied and the weight of any remaining pellets recorded after applying a correction factor for the water absorbed by the pellets over the fifteen minute period. All wastes and uneaten food thus collected from SIFTS in both ponds were taken ashore and dewatered through a Geotube™. Water temperatures in each pond were constantly measured at the depth of the water intakes (2 metres) and logged to a PC every 5 minutes. Dissolved oxygen concentration in the outlet water of each SIFTS was similarly logged. On a routine basis, manual measurements of temperature and dissolved oxygen were made at the surface (0.5 m) and depths of 1, 2 and 3 metres to determine the extent of stratification. pH was constantly measured and logged at a depth of 0.5 m in each pond.

Water quality and bottom sludge analysis for these two ponds were conducted according to previous experiments (see section 5.1.2). In addition, water quality samples were assessed for microbial floccule formation according to Avinmelech (2007). 1000 mL samples of pond water were collected in an Imhoff cone and left to settle for 15 minutes before measuring the volume of floccules. Four times throughout the trial, SIFTS liners were inverted, crowding the fish and enabling representative subsamples to be obtained for measuring growth. A number of fish equaling 5% of the total fish number in each SIFTS were weighed to 0.1 gram at each sampling. The trial was concluded on March 22nd (Day 104) when all fish were counted to determine survival and 10% weighed to determine growth.

5.3.3 Results and Discussion

Survival, Growth and Yield

Survival in the heterotrophic and autotrophic ponds averaged 101 ± 2% and 98 ± 2%, respectively (Table 5-5). Growth of fish was significantly greater in the heterotrophic pond compared with the autotrophic pond (Figure 5-14), with those in the former pond averaging 467 ± 32 g at the end of the trial (SGR = 1.38 ± 0.02 %/day), compared with 341 ± 11 grams in the

latter (SGR = 1.08 ± 0.02 %/day) (Table 5-5). Total biomass harvested from the heterotrophic pond was therefore considerably higher (2.3 tonnes; 15.2 tonnes/ha) than from the autotrophic pond (1.5 tonnes; 9.8 tonnes/ha) (Table 5-5). Stocking densities reached 51.0 ± 0.3 kg/m3 in the SIFTS within the heterotrophic pond and 40.6 ± 0.5 kg/m^3 in those within the autotrophic pond.

Table 5-5: Summary of grow-out parameters for barramundi produced in the two SIFTS ponds during Trial C.

	Heterotrophic	Autotrophic
Mean weight (g)	467 ± 32	341 ± 11
Survival (%)	101 ± 2	98 ± 2
Biomass harvested (kg)	2300	1500
Tonnes/ha (equivalent)	15.2	9.8
SGR	1.38 ± 0.02	1.08 ± 0.02
FCR	1.09 ± 0.01	1.24 ± 0.03

Total feed added to the heterotrophic pond was 1.7 tonnes and 1.3 tonnes in the autotrophic pond. Food conversion ratio of fish in the heterotrophic ponds was significantly less (1.09 ± 0.01) than the autotrophic pond (1.24 ± 0.03). Although we have largely attributed the growth difference between the two ponds to a difference in temperature (see below), the difference in FCR suggests that the differences in growth was not only due to increased food intake at the higher water temperature. We are confident that the higher FCR obtained in the autotrophic pond was not an artifact of overfeeding, as the efficiency of the waste collectors in collecting uneaten food has been previously demonstrated (Partridge et al., 2006). Although the nutritional value of the heterotrophic bacterial biomass has been demonstrated for filter feeding fish species such as tilapia (Avnimelech, 1999), it cannot be ruled out that barramundi obtained some nutritional benefit from the bacterial biomass ingested with the imbibed seawater and this theory warrants further investigation. Supporting evidence for this hypothesis is provided by co-investigator's Willett and Erler's recent study (unpublished data) which tracked the fate of isotope-labelled bioflocs in whiting (*Sillago ciliata*) culture systems. In that study biofloc protein was assimilated into the flesh of the whiting despite the fact that their dietary preferences would likely preclude them specifically targeting bioflocs. In addition, Avnimelech (2006) suggested that grazing of bioflocs can occur independently of feeding in tilapia.

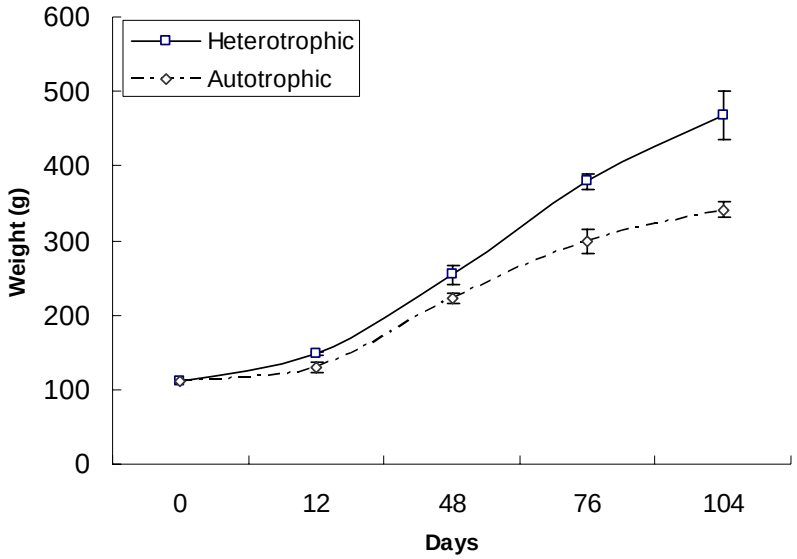


Figure 5-14: Growth rate of barramundi in the heterotrophic and autotrophic ponds

Water Temperature

As discussed above, the faster rate of growth in the heterotrophic pond was largely attributable to the higher average water temperature at the depth of the air-water-lift intakes (Figure 5-15). Over the course of the experiment, the heterotrophic pond averaged 26.7°C at this depth, compared with 25.2°C in the autotrophic pond; a difference of 1.5°C. Models of barramundi growth by Glencross et al.[II] suggest that the final weights of fish in the heterotrophic and autotrophic ponds should have been 400 and 320 grams, respectively based on these average temperatures. This compares somewhat favourably with the 467 and 341 grams actually obtained.

[II] http://www.fish.wa.gov.au/docs/pub/AquaOutputModel/index.php?0308

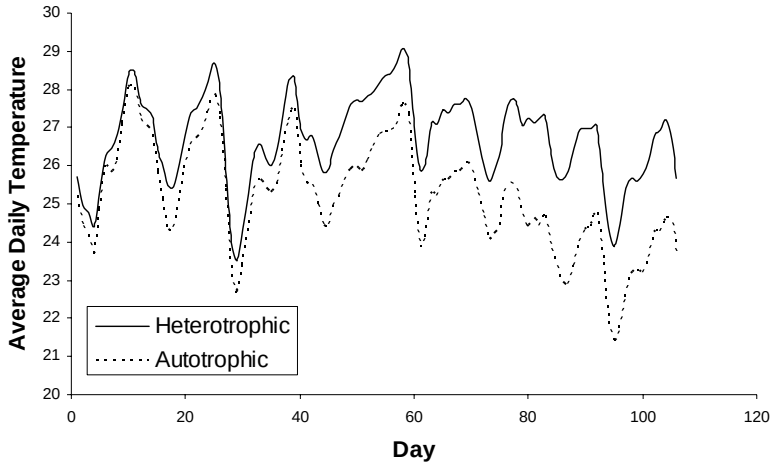


Figure 5-15: Average daily water temperatures recorded in the heterotrophic and autotrophic ponds in Trial C.

As demonstrated in Figure 5-15, the difference in water temperature between the two ponds increased over time due to the increased degree of stratification in the autotrophic pond. This resulted in warmer water at the surface and cooler water at the depth of the air-lift intakes. This stratification is clearly demonstrated in Figure 5-16, which shows a depth profile of temperature in the two ponds on Day 91. These data clearly indicate that the intake position and rate of water turnover provided by the air-water lifts on the SIFTS (1 pond exchange per day) was insufficient to prevent pond stratification in the autotrophic pond. This figure also demonstrates that the mixing provided by the three Force 7 aerators in heterotrophic pond was sufficient to prevent stratification.

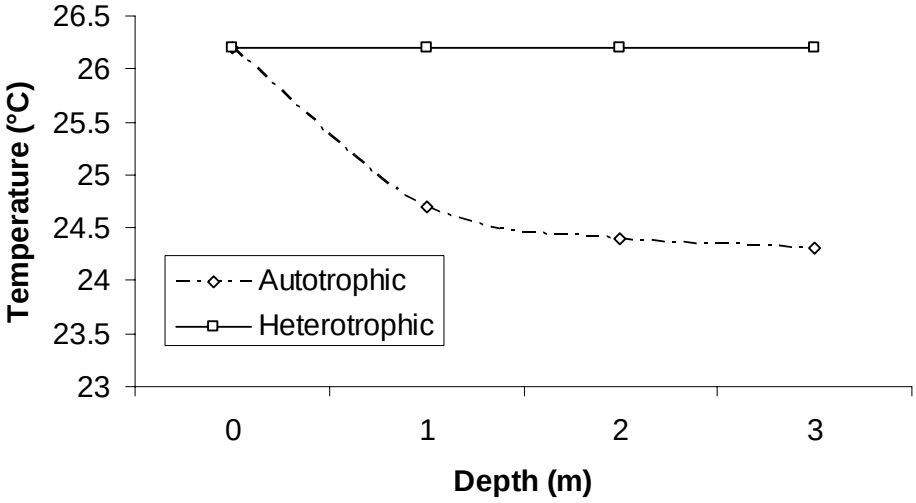


Figure 5-16: Temperature profile by depth in the heterotrophic and autotrophic ponds on Day 91 of Trial C.

pH & Total Ammonia Nitrogen (TAN)

The maximum daily pH values in the two ponds are shown in Figure 5-17. The values were similar between the two ponds for the first half of the trial before declining in the heterotrophic pond and increasing in the autotrophic pond. Declining pH values are typical of heterotrophic ponds due to the net production of CO_2 by microbial proteins (Ebeling et al., 2006). High pH values are typical of ponds with strong microalgal blooms during which insufficient carbon dioxide for photosynthesis can be obtained from the water via diffusion and it is therefore sourced from the conversion of bicarbonate to carbonate (Wurts and Durborow, 1992).

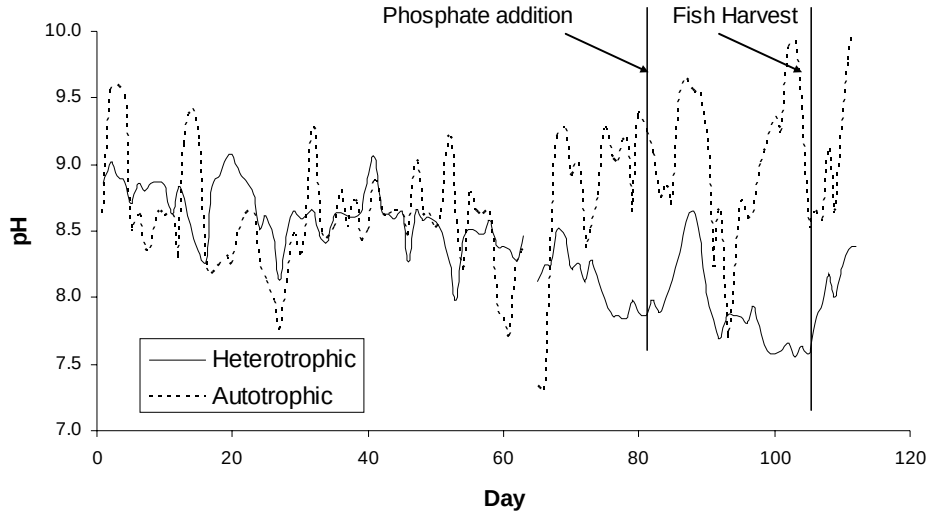


Figure 5-17: Maximum daily pH values in the heterotrophic and autotrophic ponds during Trial C.

Typical daily pH fluctuations of the two ponds in the first half of the trial (Day 28) and second half of the trial (Day 100) are presented in Figure 5-18. That the two ponds had similar pH values during the first half of the trial suggests that the microbial proteins were not dominating the heterotrophic pond during this time. We present further data below which supports this hypothesis.

For the majority of the trial, concentrations of total ammonia nitrogen (TAN) were higher in the heterotrophic pond (Figure 5-19). On Day 73, TAN in this pond rapidly increased from a stable value of 1 to 2 mg/L to 4.34 mg/L by Day 80. Analysis of the other dissolved nutrients at this time suggested that phosphate was limiting the formation of heterotrophic microbial proteins (see section on 'Chlorophyll and Nutrients' below). Following a corrective dose of superphosphate on Day 82, TAN stabilised at 4.78 mg/L for the following two days before

rapidly declining to 0.20 mg/L by Day 97. The pH of the heterotrophic pond at the height of this TAN peak was 7.8, corresponding to an unionised ammonia nitrogen (NH_3-N) concentration of 0.18 mg/L (temperature 26°C), well below the chronically lethal level of 0.75 mg/L identified in the previous trial.

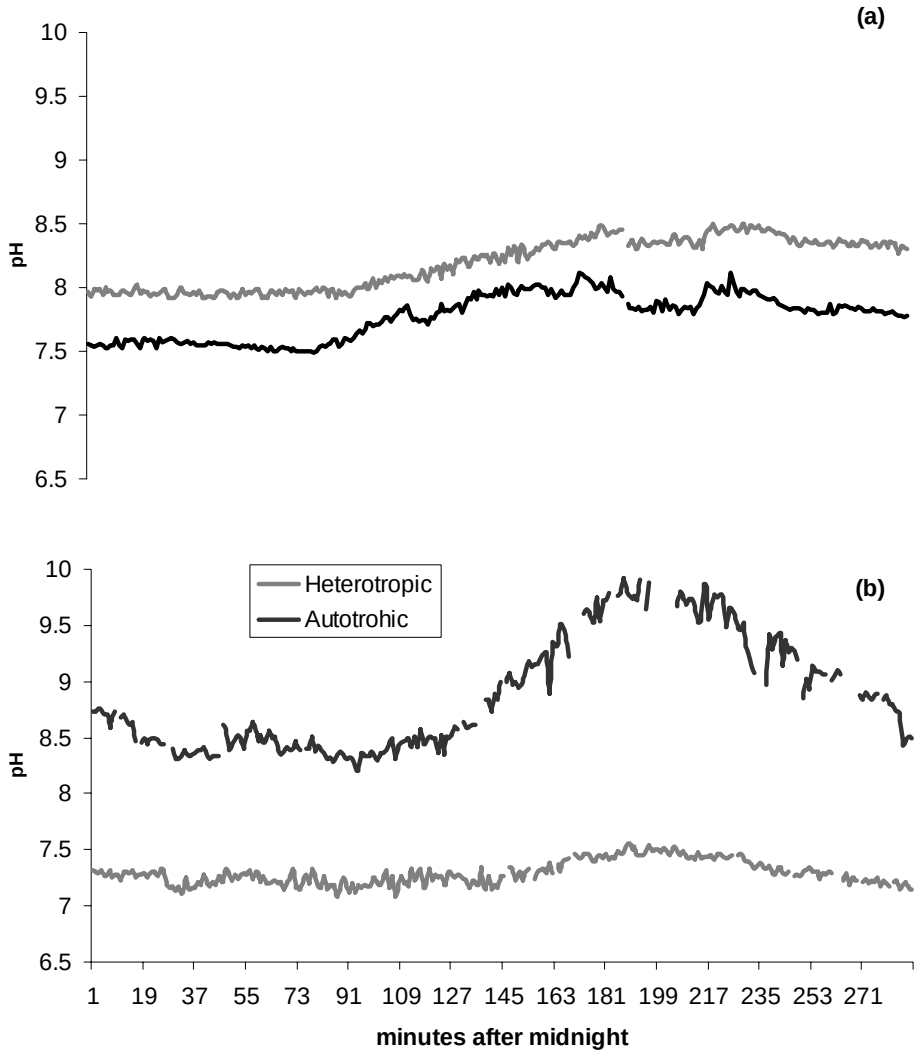


Figure 5-18: Diurnal variations in pH in the heterotrophic and autotrophic ponds on a) Day 28 and b) Day 100 of Trial C.

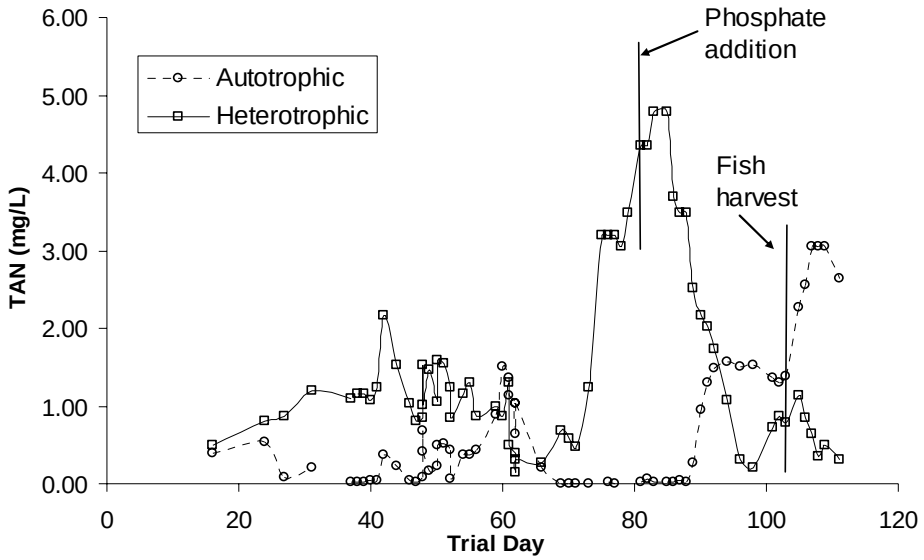


Figure 5-19: TAN concentrations in the heterotrophic and autotrophic ponds during Trial C.

Although the autotrophic pond exhibited comparatively stable TAN values throughout the trial, Figure 5-19 demonstrates that TAN was increasing at the time of harvest. As we discuss in further detail below, this increase in TAN was attributed to senescing microalgae. This is consistent with our previous experiment where high mortality of barramundi occurred prior to harvest due to a combination of high TAN and pH. With pH values in the current trial reaching 10 at the end of the trial (Figure 5-17), the measured TAN values would have been approaching the lethal limit previously experienced. Indeed shortly after the fish were harvested, TAN rapidly increased from 1.3 to 3.0 mg/L. With a concomitant pH of 10.1, this concentration of TAN equates to an unionised ammonia concentration of 2.6 mg/L (at 26°C), a concentration which would certainly have resulted in 100% mortality. These data are effective in demonstrating that only low levels of TAN can be tolerated under such pH conditions and highlight a major limitation in the use of autotrophic ponds for producing high yielding crops of fish in static ponds.

That the total quantity of feed added to the autotrophic pond (1338 kg) was similar to that described for the same treatment pond which experienced high mortality due to high pH and TAN in Trial A (1570 kg) suggests that this level of nitrogen input (640 kg N/ha) is beyond the assimilatory capacity of a zero-exchange autotrophic pond over a 100 day production cycle and therefore yields of 10 tonnes/ha are unachievable over this time-frame. Although similar yields are obtainable from static ponds with species such as silver perch (Rowland et al., 1995), these crops are produced over a much longer time frame (ca. 12 months) and the nutrient loading on

the pond is therefore less intense. Heterotrophic pond management, on the other hand, has been demonstrated to enable production rates up to 30 tonnes/ha over similar short durations (Chamberlain et al., 2001).

Dissolved Oxygen

Average daily dissolved oxygen concentrations at a depth of 2 m in each pond are shown in Figure 5-20. Despite the use of aerators at a level of 30 kW/ha, dissolved oxygen the heterotrophic pond was consistently lower than in the autotrophic pond, demonstrating the additional oxygen demand created by the addition of molasses. Despite this, the average daily dissolved oxygen did not drop below 2.7 mg/L in this pond. This figure also demonstrates the effect of senescing microalgae on dissolved oxygen, with average values in the autotrophic pond reaching 0.5 mg/L on several occasions towards the end of the crop. This is consistent with our previous trial which reported decreasing dissolved oxygen after microalgal crashes and further supports our theory that this level of feed input is unsustainable in a static pond over 100 days. Despite low pond concentrations, adequate dissolved oxygen was maintained in all SIFTS due to the action of the air-water lifts and vertical inlet manifolds.

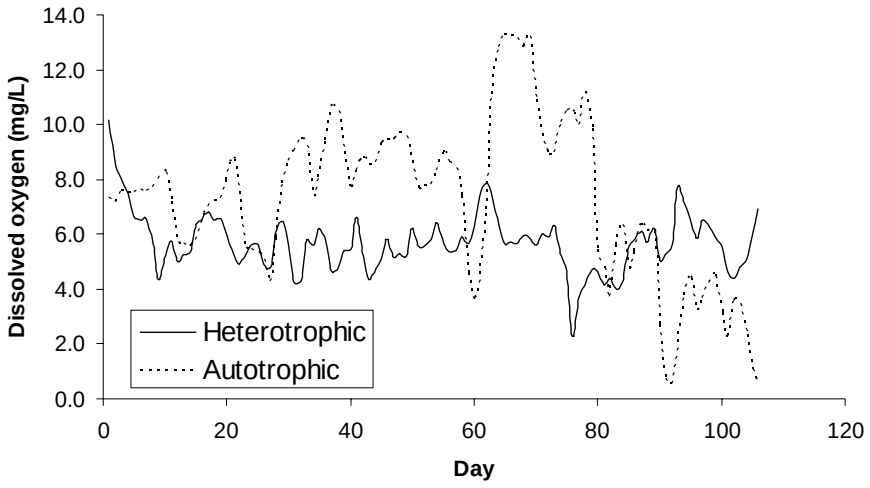


Figure 5-20: Average daily dissolved oxygen concentrations in the autotrophic and heterotrophic ponds during Trial C.

As anticipated, stratification of dissolved oxygen (Figure 5-21) followed the same pattern as temperature, with the heterotrophic pond showing uniform dissolved oxygen with depth, compared to the decreasing oxygen concentration in the autotrophic pond.

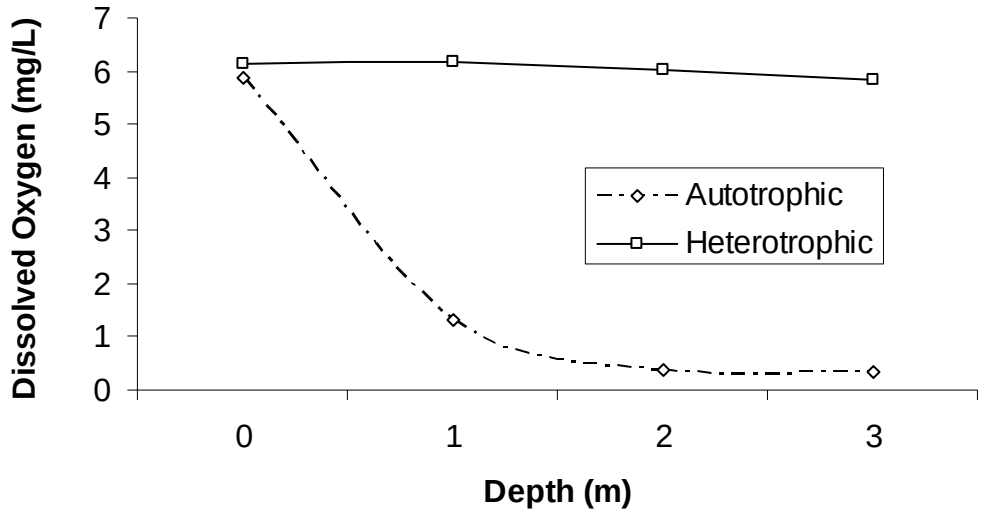


Figure 5-21: Dissolved oxygen profile by depth in the heterotrophic and autotrophic ponds on Day 91 of Trial C.

Chlorophyll and Nutrients

The chlorophyll data presented in Figure 5-22 suggests that similar blooms of microalgae occurred in both ponds. That a bloom occurred in the heterotrophic pond in the first half of the trial could be used to support the hypothesis that this pond was not dominated by microbial proteins during this time. Microalgae are, however, often found as components of agglutinated flocculant particles in heterotrophic ponds (Avnimelech, 2006) and Hari et al. (2006) found no correlation between the concentration of chlorophyll and the extent of microbial protein formation. It seems logical, however, that the release of TAN from microalgal cells senescing within this agglutinated matrix would be rapidly assimilated by the associated microbial protein. If this is the case, the fact there was a concomitant increase in TAN with the decline in chlorophyll in the heterotrophic pond (see Figure 5-19; Day 63 – 76) suggests that these microalgae were not held within microbial proteins.

The rapid decline in chlorophyll in the autotrophic pond between Day 90 and 104 was followed by the rapid rise in TAN that was observed post-harvest (see Figure 5-19).

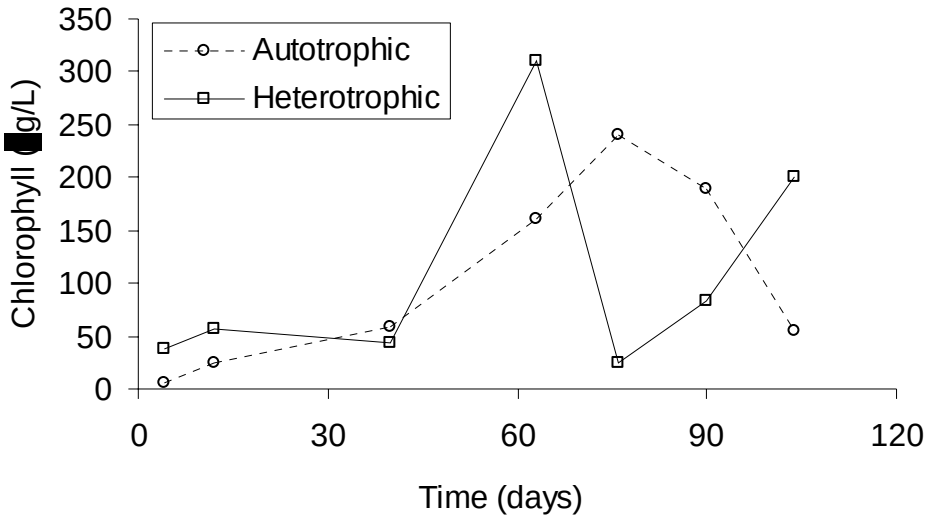


Figure 5-22: Chlorophyll A concentrations in the heterotrophic and autotrophic ponds during Trial C.

The changing concentrations of the major nutrients, DOC, TDN and SRP in both ponds throughout the trial are shown in

Figure 5-23. This figure demonstrates that all of these nutrients increased over time in both ponds. Despite no organic carbon being added to the autotrophic pond, it increased at a similar rate to that in the heterotrophic pond which received daily additions of molasses. This increase was attributed to the excretion by and senescence of microalgae, which release the organic carbon fixed from inorganic carbon during photosynthesis. This released DOC can be recycled in the system but interactions are complex so it is difficult to correlate DOC levels directly with microalgae levels over the duration of the trial.

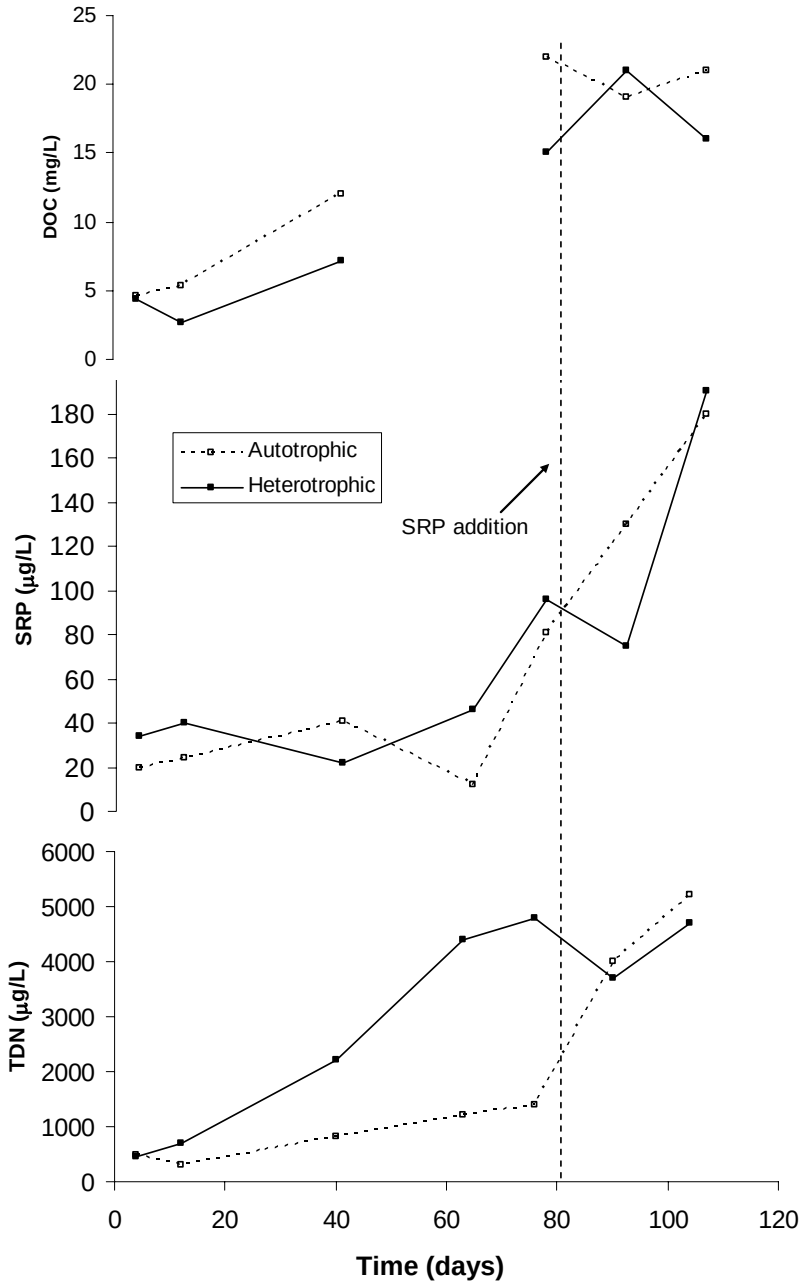


Figure 5-23: Concentrations of dissolved organic carbon (DOC), soluble reactive phosphorous (SRP) and total ammonia nitrogen (TAN) in the two ponds during Trial C. No DOC data were obtained for Day 63.

In order for microbial proteins and microalgae to efficiently process nitrogenous waste, they must not be limited by carbon or phosphorus. It has been demonstrated that the optimum C:N:P molar ratio for the formation of microbial protein is 100:10:1 (Liu and Han, 2004), whereas for microalgal production the optimum ratio is 106:16:1 (Redfield, 1958). Bacteria and microalgae, however, differ in the form of each nutrient they utilise most efficiently. Microalgae, for example, are more efficient at utilising inorganic nitrogen and carbon than the organic forms.

We have therefore calculated and presented ratios for both SRP and DOC relative to TDN for the heterotrophic pond (Figure 5-24) and SRP relative to DIN for the autotrophic pond (Figure 5-25) (we have assumed that microalgae could obtain all of their inorganic carbon requirements from alkalinity or dissolved carbon dioxide and therefore dissolved inorganic carbon would never limit microalgal blooms).

These figures demonstrate that SRP was limiting for microbial protein development but not for microalgal growth. As previously discussed, we attributed the slow development of microbial proteins to this phosphorus deficiency and on Day 82, superphosphate was added to this pond to increase the molar ratio of SRP:TDN to the optimum value of 0.10. The rapid decline in TAN which followed over the following days suggests that this addition enabled microbial proteins to develop and supports our previous hypothesis that this pond was not dominated by microbial proteins prior to this time. After the addition of superphosphate on Day 82, the concentration of SRP in the heterotrophic pond on Day 90 was lower than the previous sampling point on Day 76 (Figure 5-24), suggesting that it had been taken up within the microbial proteins. This observation is supported by Liu and Han (2004), who demonstrated that TAN removal in a heterotrophic system was significantly improved when TAN:SRP was increased from 17 to between 5 and 7 (on a weight basis, equivalent to a molar SRP:TDN of 0.09 to 0.06). Figure 5-24 also demonstrates that organic carbon was not limiting for microbial protein formation in heterotrophic pond for the majority of the trial.

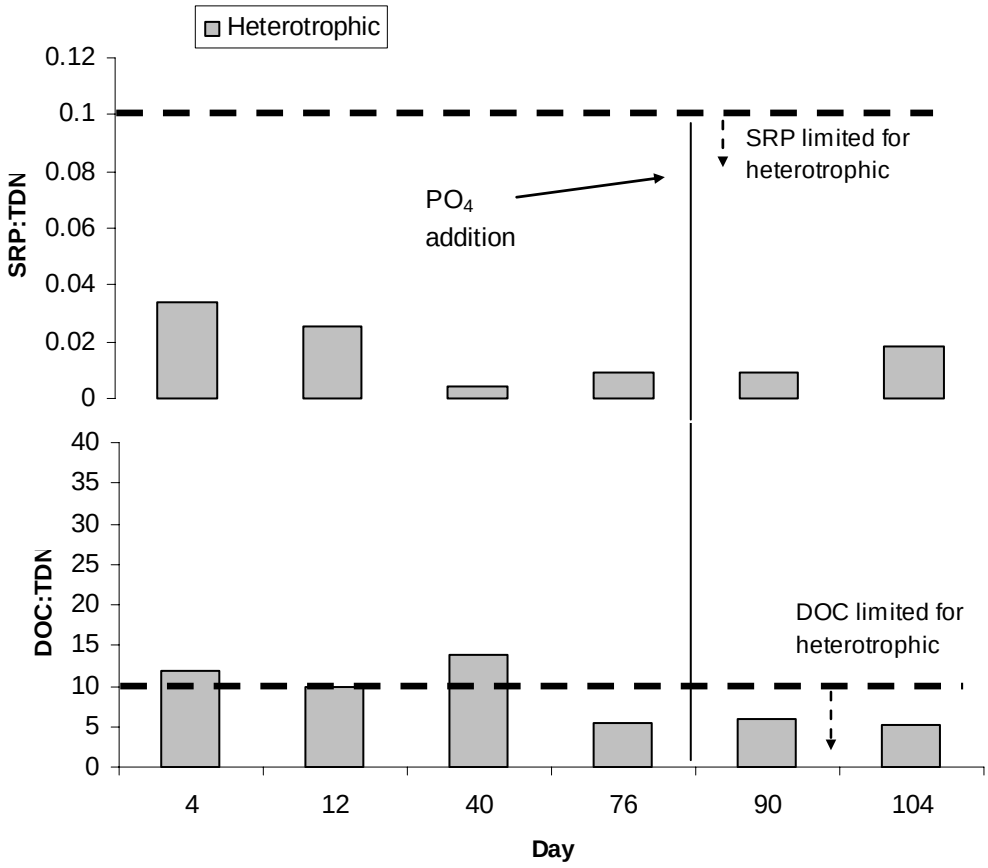


Figure 5-24: The ratios of SRP and DOC relative to TDN in the heterotrophic pond during Trial C. The horizontal dashed lines represent the optimum ratio relative to nitrogen for the formation of microbial proteins. Values below this line represent a limiting nutrient.

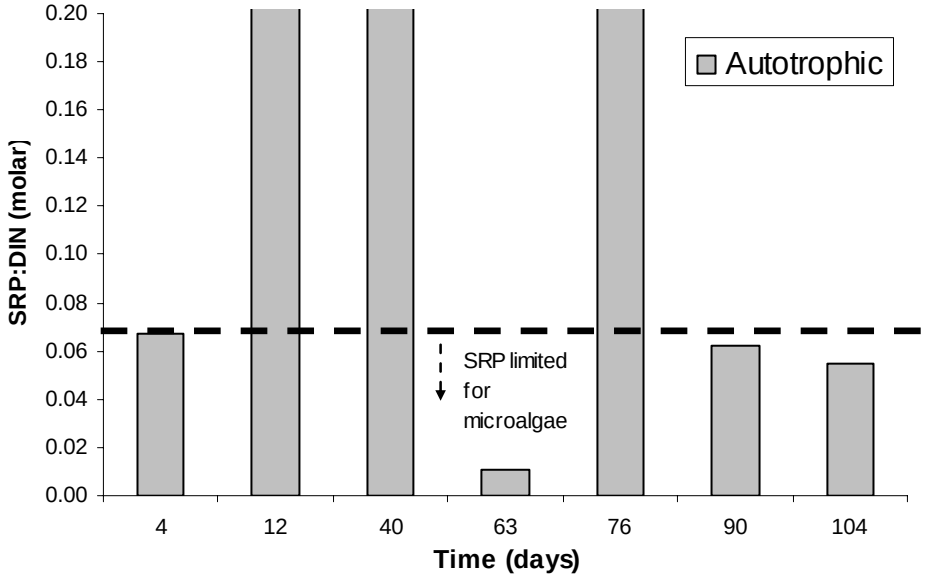


Figure 5-25: The ratio of SRP to DIN in the autotrophic pond over time during Trial C. The horizontal dashed line represents the optimum ratio for the growth of microalgae. Values below the dashed line are deficient in SRP relative to DIN.

Bottom Sludge Data and Floccule Formation

The quantities of nitrogen, phosphorus and carbon on the bottom of each pond on Day 40 and 76 is shown in Figure 5-26.

That there were greater quantities of all three nutrients on the bottom of the heterotrophic pond suggests that greater settlement occurred in this pond. High levels of aeration are required in heterotrophic ponds to maintain the negatively buoyant, agglutinated microbial proteins in suspension (Avnimelech, 2006). Although we employed a higher rate of aeration in the heterotrophic pond (30 kW/ha) than is typical for such ponds (22 kW/ha) (Chamberlain et al., 2001), we were never able to measure any microbial floccules using the Imhoff cone method. Together, these data suggest that the agglutinated particles in the heterotrophic pond were rapidly settling out of suspension before they could be detected. Given that our fish are confined within the SIFTS they contribute no bioturbation to the pond bottom or within the pond water column to assist in keeping floccules in suspension. In addition, the greater depth of our ponds (3 m) compared with more conventional aquaculture ponds may have resulted in inefficiencies in the Force 7s ability to maintain floccules in suspension.

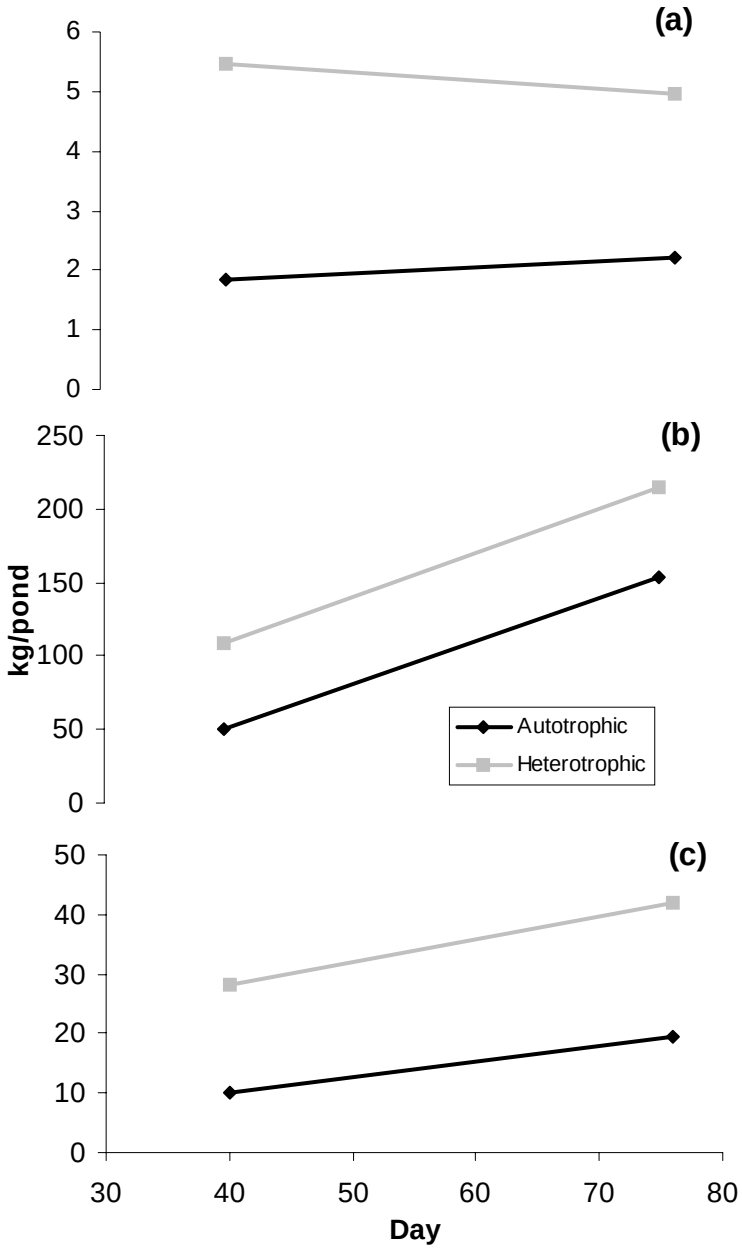


Figure 5-26: Total quantities of a) phosphorus, b) carbon and c) nitrogen on the floor of heterotrophic and autotrophic ponds in Trial C.

5.3.4 Conclusions

Results demonstrate that SIFTS can be integrated with heterotrophic management techniques to increase the yields of fish from zero-exchange, inland saline ponds. The most obvious benefit of this system in the current trial was the lower pH in the latter half of the trial, which reduced the toxicity of TAN. The data suggested that microbial proteins did not develop as expected during the first half of the trial, which we attributed to a deficiency of soluble reactive phosphorus. If microbial proteins had dominated from the onset then TAN may have remained lower throughout the trial. Further research is therefore required to optimise the ratios of carbon, phosphorus and nitrogen within inland saline ponds in order to maximise the benefit of microbial proteins. Improved methods for expediting the establishment of a bio-floccule community are also needed. Although low concentrations of dissolved oxygen are typical of heterotrophic ponds, integration with SIFTS ensures these concentrations will not cause fish mortality. Our data also suggest that despite a high level of aeration, microbial floccules were settling out of suspension. Likely explanations for this observation are the lack of bioturbation on the pond floor or water column with the target species confined within the SIFTS or the depth of ponds. Further research is required to determine the rate of aeration required in a heterotrophic SIFTS system. Once floccules can be effectively kept in suspension, pond sediments will be reduced, biofloccules will be available for filter feeding or grazing organisms and greater pond yields will be achievable.

Heterotrophic pond management is particularly well suited for the culture of filter feeders such as prawns or tilapia, as these species can ingest the microbial proteins, thereby improving feed utilisation whilst converting waste products into valuable crops. With such species, production rates from static ponds up to 30 tonnes/ha/crop are achievable. Although most species targeted for inland saline aquaculture in Australia are carnivorous, we have demonstrated that heterotrophic pond management is still a worthy management tool for such species, as it can provide at least a 50% improvement in yield compared with autotrophic ponds. Increasing yields from static ponds is an important focus for Western Australia, where groundwater yields are typically only low to moderate and no large-scale interception schemes exist to support flow-through culture industries.

Although there is an increased cost associated with heterotrophic pond management through the need for molasses and greater rates of aeration these costs may be offset by the greater yields. The integration with SIFTS also provides an opportunity for the polyculture of filter feeding species such as prawns in the surrounding pond. Ebeling et al. (2006) recently pointed

out the importance of removing excessive microbial proteins from heterotrophic ponds and such polyculture would contribute to this removal. Integrated prawn culture has the added benefit of a valuable secondary crop. Our modeling suggests that approximately 1 tonne of prawns could be cultured on the heterotrophic microbial proteins alone generated from the culture of 15 tonnes of barramundi. *Artemia* also have the ability to ingest microbial proteins and as such, the modified SIFTs described in section 7.4.1 could be utilised to grow *Artemia* and crop microbial proteins. Finally, given the excellent growth and FCR shown for barramundi fed on *Gambusia*, (see section 8), we are continuing to investigate the potential for growing *Gambusia* on heterotrophic bio-floccules.

5.4 Trial D: Further Refinements to Heterotrophic Pond Management in Static Inland Saline Water Bodies.

5.4.1 Introduction

In Trial C we demonstrated the potential benefits of heterotrophic pond management. We were unable to produce 10 tonnes of barramundi per hectare over 100 days in a static, autotrophic control pond, yet the heterotrophic treatment pond yielded 15 tonnes per hectare. The addition of organic carbon to the heterotrophic pond resulted in lower, more stable pH values, which ameliorated the effects of total ammonia nitrogen. We hypothesised that soluble reactive phosphorus limited the formation of microbial proteins and once this limitation was overcome, ammonia concentrations declined significantly. We concluded that further research was required to optimise the management of microbial proteins to maintain low levels of TAN. This trial also concluded that changing the orientation of the air-water-lift pumps on each of the four SIFTS was insufficient to overcome the effects of stratification in the static, autotrophic control pond.

In an effort to overcome the constraints identified in the previous trial relating to heterotrophic pond management, the current trial sought to improve the management of the heterotrophic ponds, including optimising nutrient ratios and determining strategies for encouraging the early establishment of microbial proteins. We continued our collaboration with QDPI and the University of Southern Cross in this regard. Mr Dan Willett and Dr Dirk Erler from these institutions advised on the best management approaches for achieving these objectives. Establishing a bio-floccule community prior to a continuous influx of nitrogen from the fish has proven beneficial in trials at QDPI. This approach ensures that microalgae do not initially dominate the pond once nitrogen influx from fish excretion begins.

Given our previous conclusions that 10 tonnes per hectare were not achievable over a 100 day cropping cycle in a static autotrophic pond, the autotrophic control pond in the current experiment was operated with a 5% daily exchange to determine if this level of flow was sufficient to achieve these yields. This level of water exchange is the maximum achievable at Springfield Waters Aquaculture, which has a high yielding bore compared with most saline bores in Western Australia.

5.4.2 Materials and Methods

In each of the two ponds, four 10 m^3 SIFTS were stocked with 1100 juvenile trout. Due to the fish having been sourced from two hatcheries, two separate size cohorts were stocked. Two of the four SIFTS in each pond were stocked with small fish (mean weight 48.3 g) while the remaining two SIFTS were stocked with the larger fish (mean weight 116.0 g).

Refer to Section 5.3.2 for a detailed methodology pertaining to the various pond management regimes employed and the data collection techniques used in this trial. In addition to these methods we made the following changes to achieve our trial objectives. To encourage the early establishment of microbial floccules, 15 kg of white flour was added to the heterotrophic pond every 3 days for two weeks prior to fish stocking. Flour has been demonstrated by scientists at QDPI to encourage establishment of bio-floccule communities due to its suitable ratio of C:N:P and because it acts as an initial substrate on which the bacteria can form. The dose of flour used yielded 0.1 mg/L of nitrogen per addition. Phosphate was also added (460 grams; NaH_2PO_4) with each flour addition to ensure the optimum C:N:P molar ratio of 100:10:1 was achieved.

To ensure that the optimum nutrient ratios were maintained throughout the trial, we routinely measured orthophosphate in the pond and added soluble inorganic phosphate (NaH_2PO_4) as necessary to ensure this nutrient was not limiting. Additions were made to maintain the optimum SRP:TDN molar ratio of 0.1, however, given we have no means of measuring TDN onsite, we estimated TDN based on TAN and on the ratio of TAN to TDN in our previous trials.

5.4.3 Results and Discussion

Survival, Growth and Yield

On Day 69 all 1100 trout in a single SIFT in the autotrophic pond died overnight. Initial investigations revealed no apparent cause, since dissolved oxygen levels were adequate in the 24 hours prior to the kill. Analysis of hydrogen sulphide (H_2S) revealed concentrations of 0.03 and 0.10 mg/L in the surface and bottom water samples, respectively. Levels of H_2S in the heterotrophic pond were below the detectable limit of 0.02 mg/L. Given that rainbow trout have 96 hour LC_{50} values to hydrogen sulphide in the low micromolar range (Volkel and Berenbrink, 2000), these data provide strong evidence that hydrogen sulphide was the cause of mortality. Hydrogen sulphide is produced by the anaerobic degradation of organic matter and its presence, combined with the stratification data presented below, demonstrates that the 5%

daily water exchange did not assist in preventing stratification and maintaining an oxidised boundary layer on the bottom of this autotrophic pond. The problem of hydrogen sulphide was exacerbated by the fact the air water lifts were drawing from close to the bottom of the pond and it was subsequently revealed that the airlifts on the SIFT in which mortality occurred were closer to the pond floor than the other three SIFTS. In order to prevent further mortalities in the remaining three SIFTS the air water lifts were modified to draw from mid-water. The fact that even surface water had detectable levels of H_2S suggested that the above measures would provide only a temporary solution to the issue it was decided to end the trial in the autotrophic pond and harvest the remaining three SIFTS on Day 86. The fact no H_2S was present in the heterotrophic pond indicated it was being adequately mixed and the trial therefore ran full term to Day 109 when all four SIFTS were harvested. Survival in all 7 remaining SIFTS was > 97% ± 2% (Table 5-6).

There were no differences in weight between the two ponds for both the large and small fish at each sub-sample up to day 78 (Figure 5-27). The higher final weights and total biomass harvested from the heterotrophic pond were thus a function of the extra 28 days of grow-out (Table 5-6).

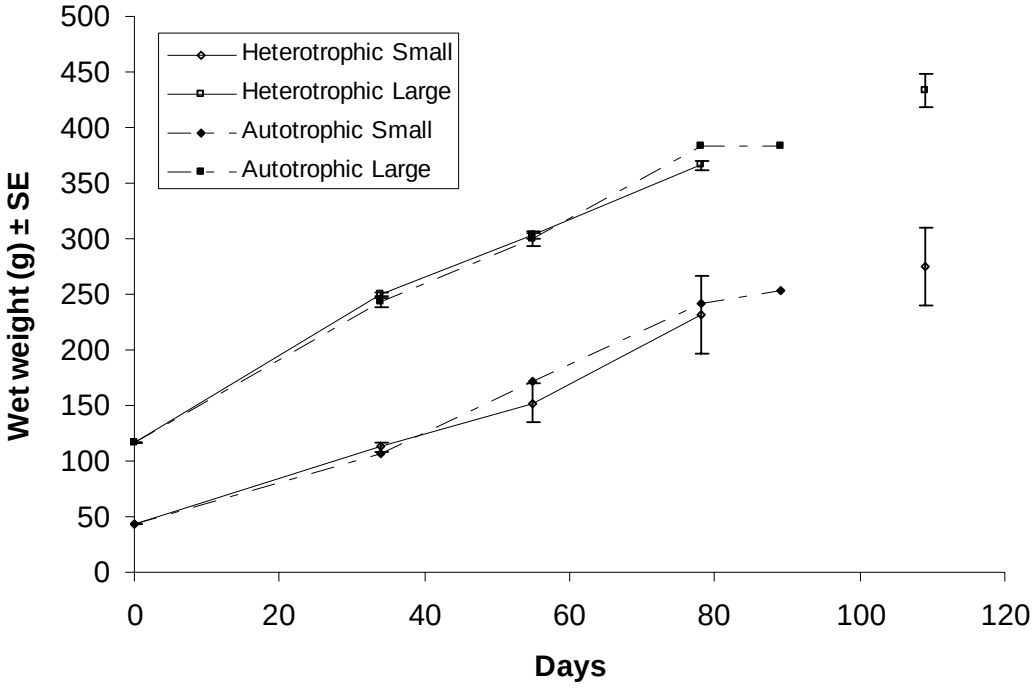


Figure 5-27: Mean weights (± S.E.) of the small and large cohorts of trout in the heterotrophic and autotrophic ponds at each sub-sampling period.

Food conversion ratios (FCR's) of the trout produced under the two pond management regimes were excellent and almost identical (Table 5-6).

Table 5-6: Summary of grow-out parameters for trout produced in the heterotrophic and autotrophic pond for Trial D. *Function of the 100% mortality of larger trout in one SIFT due to H_2S toxicity. Survival in the second SIFT of large trout in this pond at harvest was > 98%

	Heterotrophic Pond (Day 109)		Autotrophic Pond (Day 86)	
Parameter	**Small fish**	**Large fish**	**Small fish**	**Large fish**
Mean fish weight (g)	276 ± 35	434 ± 15	242.4 ± 33	363.3
Max. Stocking density (kg/m^3)	30.2	47.6	26.6	40.0
Survival (%)	97.7	99.8	99.9	49.8*
Total biomass harvested (kg)	604	950	532	800
Tonnes/ha (equivalent)	10.4		6.2	
Total feed input (kg)	1548.1		1220.2	
FCR	0.98		0.97	

Pond Stratification

By Day 55 the autotrophic pond had become highly stratified, particularly in dissolved oxygen (Figure 5-28). Temperature stratification was not as severe as previous trials (maximum 2°C between pond surface and bottom) (Figure 5-28) whereas dissolved oxygen differed by as much as 15 mg/L. Dissolved oxygen levels immediately above the pond floor never exceeded 2.0 mg/L and after Day 79 remained below 0.5 mg/L. This situation would have been a significant contributor the production of toxic H_2S from anaerobic decomposition of organics observed in this pond and a direct result of poor mixing through the water column. As was the case in the previous trial the action of the three Force 7 aerators in the heterotrophic pond prevented such stratification with dissolved oxygen levels from surface to bottom varying by less than 0.5 mg/L (Figure 5-28). Clearly the addition of 5% new water to the pond per day was insufficient to prevent pond stratification.

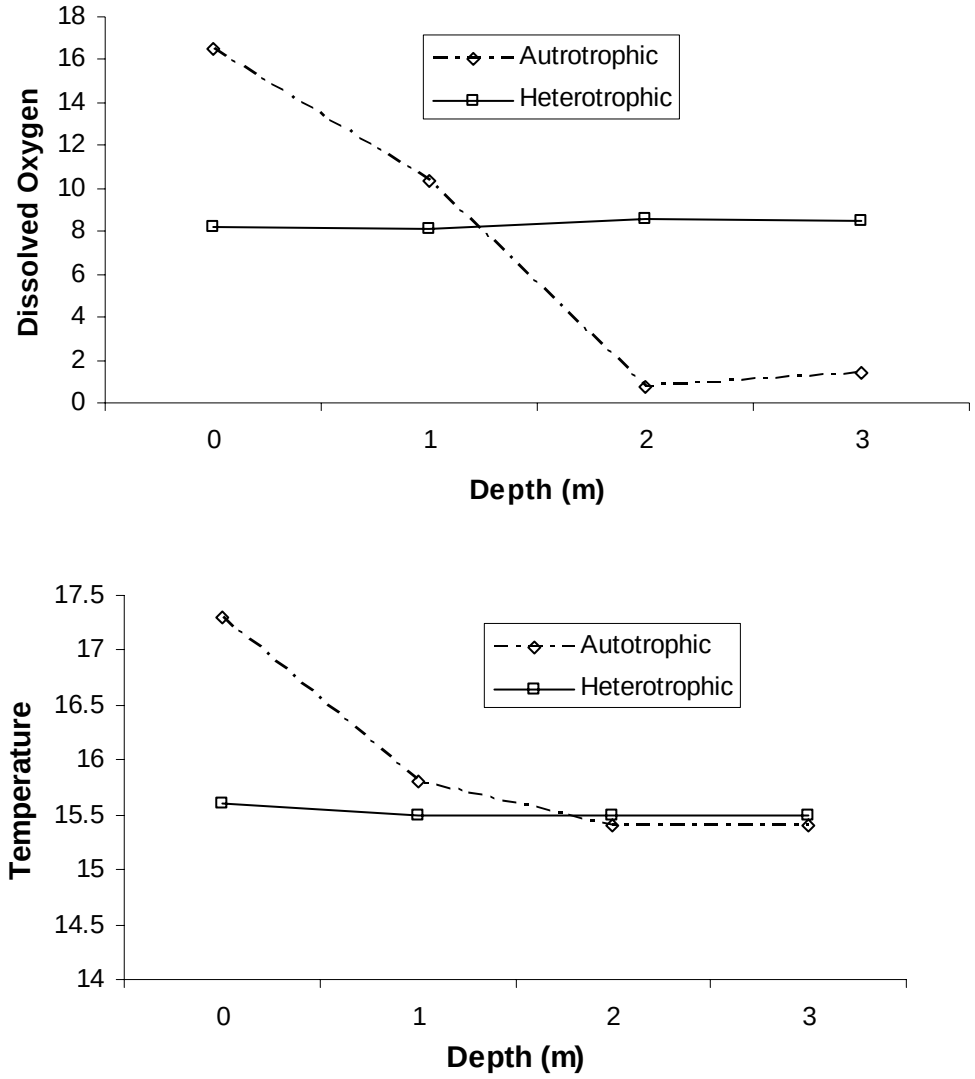


Figure 5-28: Temperature and dissolved oxygen recorded from different depths in the autotrophic and heterotrophic ponds on Day 55 of Trial D.

pH & Total Ammonia Nitrogen (TAN)

As previously reported for autotrophic ponds in Trial C, pH in the current autotrophic pond showed significant variations. Maximum daily pH routinely peaked at values over 9 and a maximum pH value of 10.6 was recorded on Day 88 (two days after the fish had been harvested). In contrast, the pH in the heterotrophic pond remained very stable throughout the entire trial with a range of 7.3 to 8.3 (Figure 5-29). The stable pH is typical of a heterotrophically managed pond system and indicates a bacterial versus micro-algal dominated water biota. This was of particular importance in the current trial given the high concentrations of TAN that were present in the pond for the majority of the trial (see below).

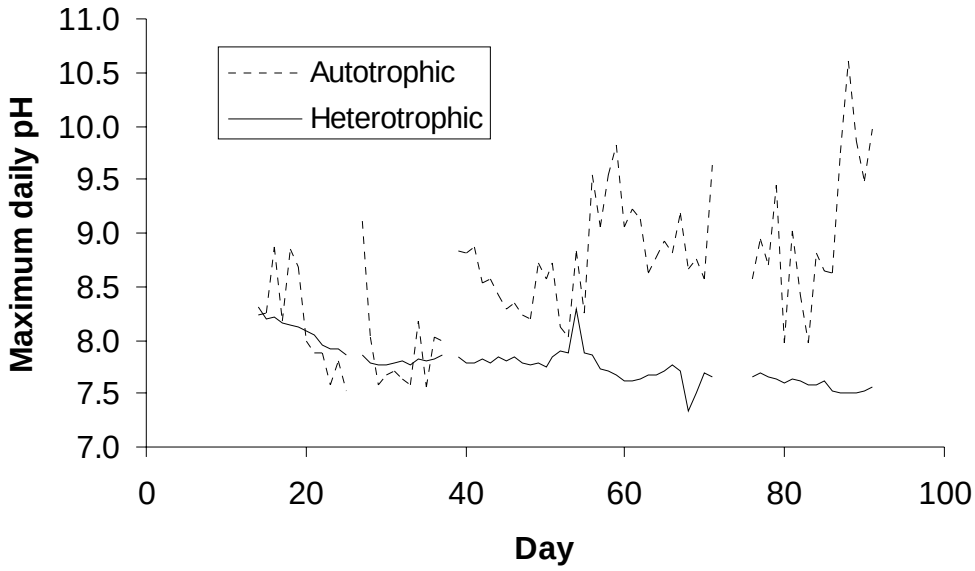


Figure 5-29: Maximum daily pH values in the heterotrophic and autotrophic ponds during Trial D.

From Day 40, concentrations of total ammonia nitrogen (TAN) were higher in the heterotrophic pond than in the autotrophic pond, which never exceeded 1.4 mg/L (Figure 5-30). By Day 64 TAN concentration in the heterotrophic pond had reached 4.8 mg/L and would have far exceeded the lethal unionised level for trout had the pH not been low and stable. In our previous milestone report we attributed the high levels of TAN in the heterotrophic barramundi pond to a low level of orthophosphate limiting the formation of the bio-floccules. In the current trial, however, we routinely measured orthophosphate and attempted to maintain an optimum C:N:P ratio (see below).

In response to the continuing rise in TAN it was decided to trial an 'emergency' dose of molasses. This 200 L dose of molasses administered on Day 66 rapidly reduced the TAN concentration by 64% to 1.68 mg/L over the following 48 hr (Figure 5-30). The benefit of this emergency dose was, however, only temporary with the concentration of TAN subsequently returning to close to its original value by Day 95. A second dose of 200 L of molasses was added on Day 96 which resulted in only a 22% reduction in TAN and within 4 days it had exceeded the pre-molasses dose level of 4.5 mg/L (Figure 5-30) and peaked at 5.5 mg/L on Day 104.

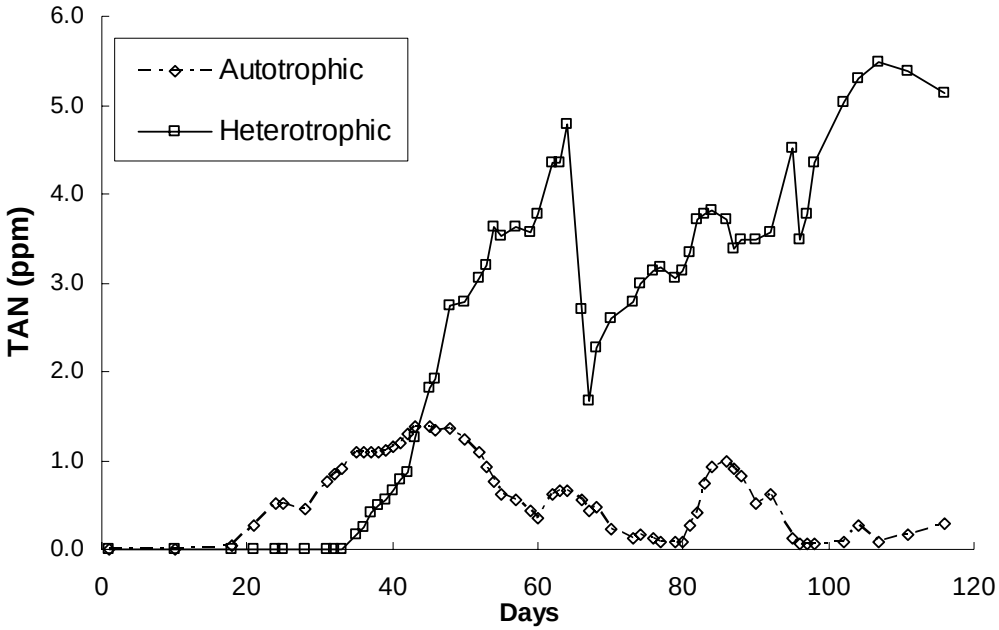


Figure 5-30: TAN concentrations in the heterotrophic and autotrophic ponds during Trial D.

As was the case in Trial C we were again unable to identify any bio-floccules in the Imhoff cone samples. Given that we routinely added soluble phosphorus in an attempt to maintain optimum C:N:P, we hypothesis that the high level of TAN experienced and the only temporary abatement of TAN after the emergency doses of molasses are related to the lack of bio-floccules in the water column. We previously identified that insufficient mixing due either to the lack of bioturbation or pond depth may be responsible for a lack of floccules within the water column. As these microbes are aerobic, insufficient mixing will result in them settling out onto the anaerobic pond floor, where the ammonia will again be remineralised into the water column.

Although TAN in the autotrophic pond was low in the current trial, we cannot attribute this only to the 5% water exchange, as the fish were harvested early and therefore food intake into the pond was lower than previous trials. The concentrations of TAN in the early parts of the trial were similar to previous autotrophic trials. Although the low level of TAN may suggest that (in combination with a 5% daily exchange) the level of food input (1220 kg or 8.1 tonnes/ha) into the pond in the current trial is sustainable over the approximately 90 day production cycle, the high values of pH obtained after the fish were harvested would have resulted in mortality (TAN = 0.83 mg/L, pH = 10.5, temperature = 17°C, $\%NH_3$ = 0.75 mg/L). This effectively highlights that pH is the primary limiting factor to sustainable yields in static ponds if TAN is present.

Dissolved Oxygen

Consistent with our previous trials, dissolved oxygen concentrations displayed greater fluctuations in the autotrophic pond compared with the heterotrophic pond, particularly toward the end of the trial (Figure 5-31).

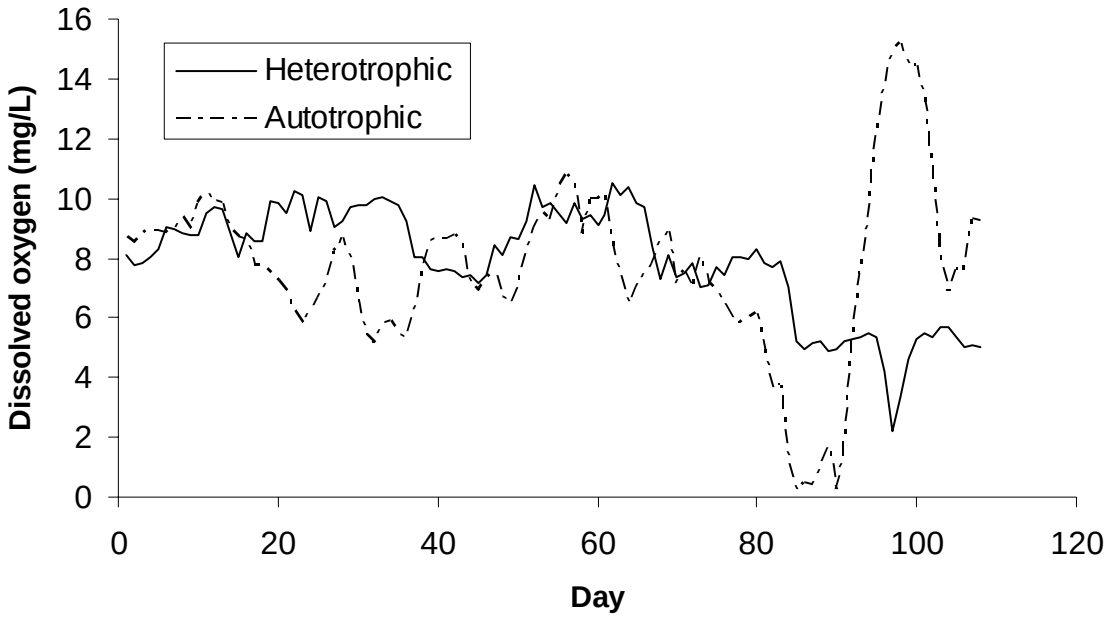


Figure 5-31: Average daily dissolved oxygen concentrations in the autotrophic and heterotrophic ponds during Trial D.

Chlorophyll, Nutrients and Bottom Sludge Data

Chlorophyll concentrations in the heterotrophic pond were considerably lower than in the previous trial, suggesting that microalgae concentrations were lower. Given there is little correlation between chlorophyll and heterotrophic floccules (see section 5.3.3) this lack of chlorophyll is not necessarily indicative of a water biota more heavily dominated by bacteria, but may be the result of cooler water temperatures compared with our previous trial under which conditions the microalgae grow more slowly.

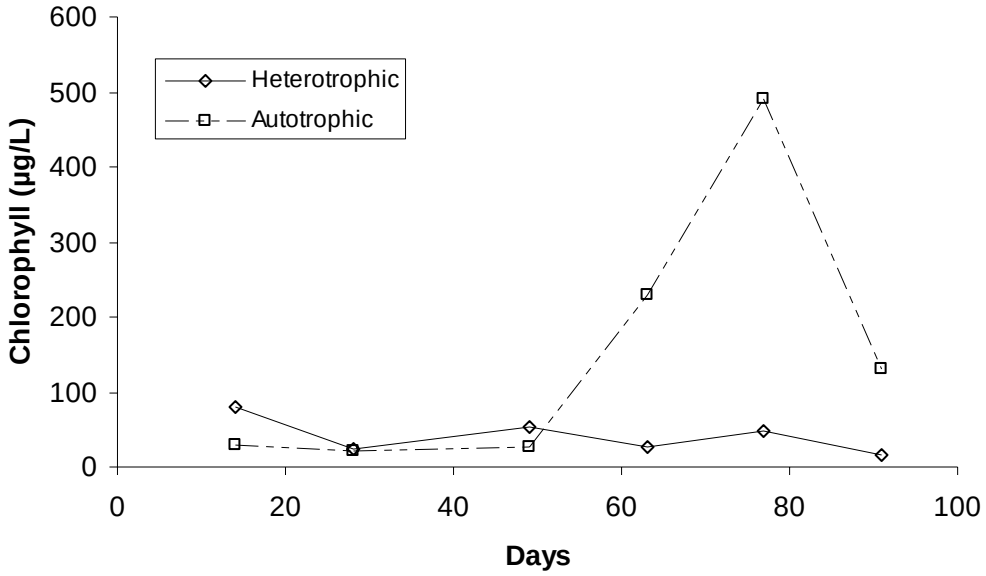


Figure 5-32: Chlorophyll A concentrations in the heterotrophic and autotrophic ponds during Trial D.

As occurred in our previous trial, the peak in chlorophyll in the autotrophic pond was shortly followed by a peak in TAN (Figure 5-30) and a low in average dissolved oxygen (Figure 5-31), indicating a microalgae crash.

The changing concentrations of the major nutrients, DOC, TDN and SRP in both ponds throughout the trial are shown in Figure 5-33. This figure demonstrates that all of these nutrients increased over time at a greater rate in the heterotrophic pond compared with the autotrophic pond.

Despite regular additions of soluble phosphorus, the molar ratio of SRP to TDN was always below the optimum value of 0.10 (Figure 5-34). As previously described, our attempts to maintain an optimum SRP to TDN ratio were based on estimates of TDN from TAN, as we had no method for measuring TDN in the field. That SRP was always limited suggests these estimates were inaccurate. In addition to SRP limiting the formation of heterotrophic bio-floccules, so too was dissolved organic carbon (Figure 5-34). Analysis revealed that on most occasions soluble reactive phosphorus was the first limiting nutrient. Given our ponds were lined with plastic, phosphorus adsorption onto clay soils cannot be responsible for the low levels of SRP. Precipitation of calcium phosphate is an alternate pathway potentially explaining this deficiency. Despite the emergency doses of molasses on Days 66, DOC remained limiting as indicated by the DOC:TDN ratio on Day 77 (Figure 5-34). SRP was not as limiting for

microalgal growth, which is consistent with our previous trial and is due to the fact that microalgae has a lower requirement for SRP than heterotrophic bacteria.

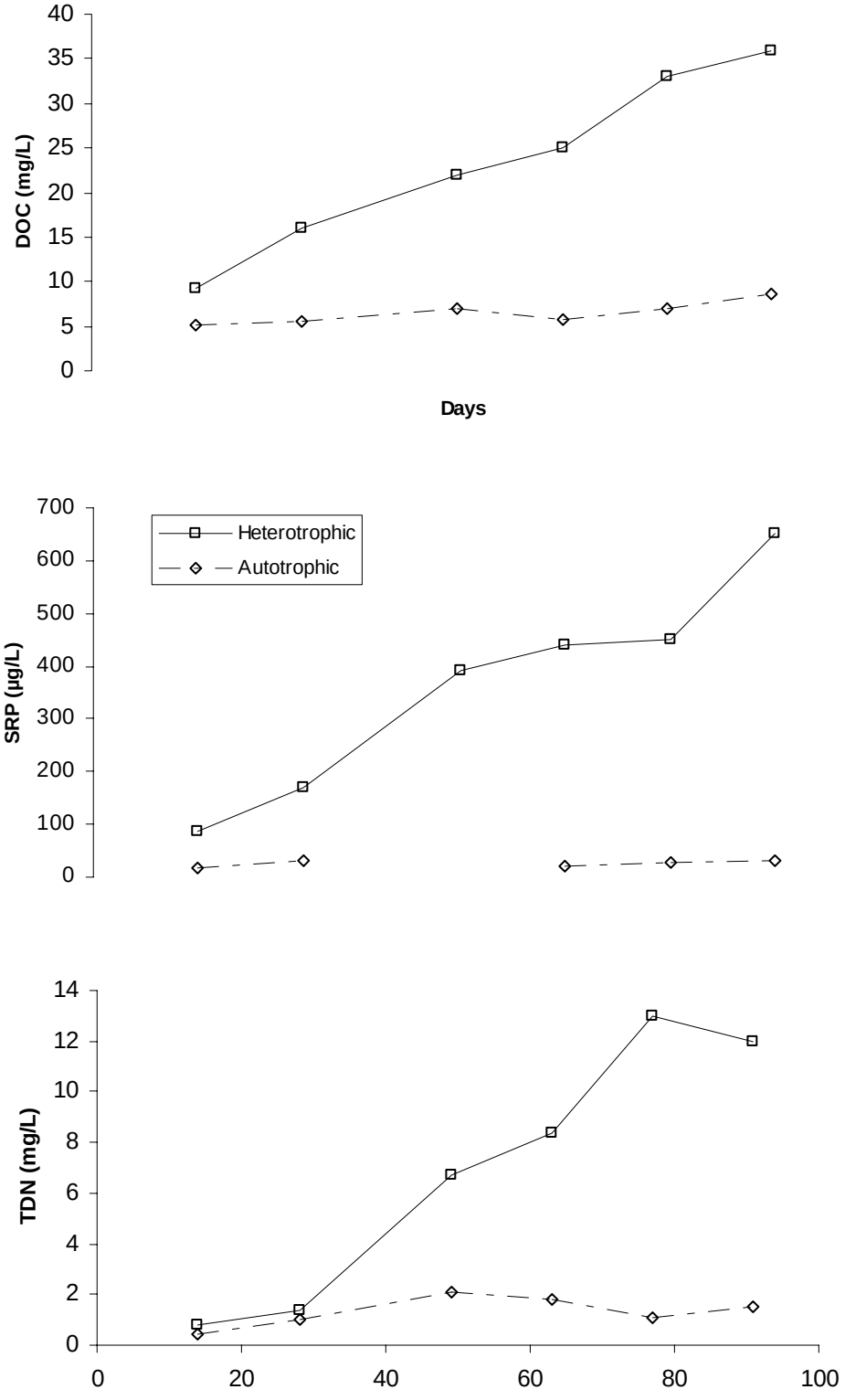


Figure 5-33: Concentrations of dissolved organic carbon (DOC), soluble reactive phosphorus (SRP) and total dissolved nitrogen (TDN) in the heterotrophic and autotrophic ponds during Trial D.

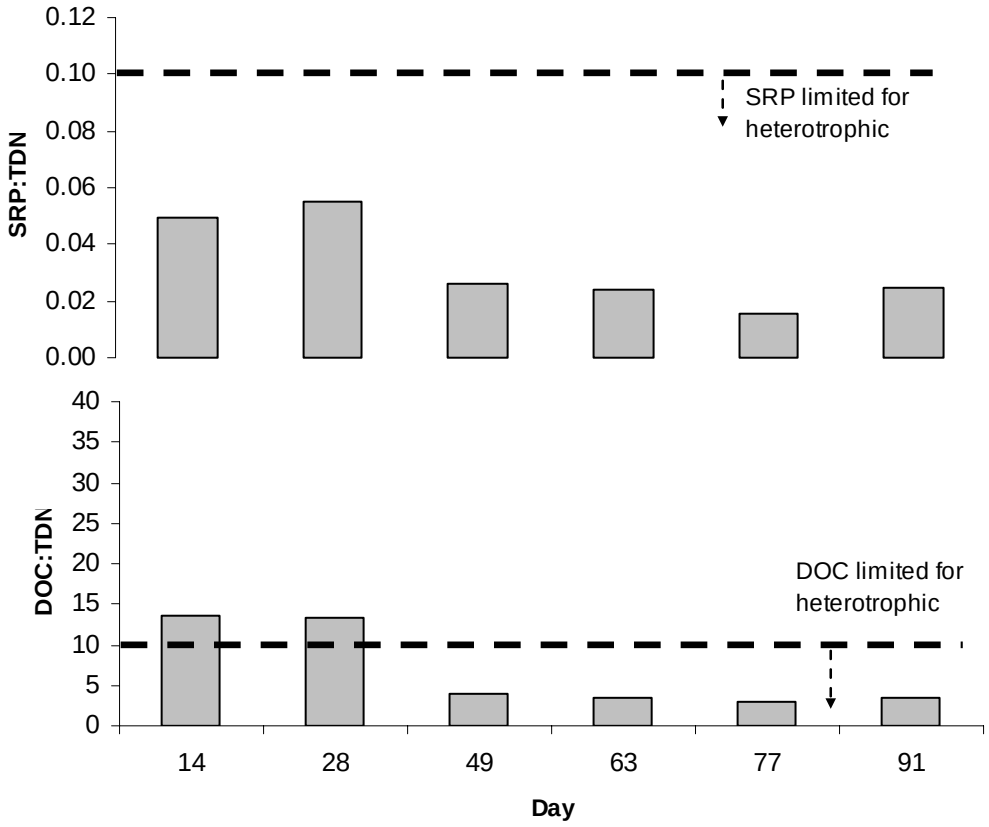


Figure 5-34: The ratios of SRP (a) and DOC (b) relative to TDN in the heterotrophic pond during Trial D. The horizontal dashed lines represent the optimum ratio relative to nitrogen for the formation of microbial proteins. Values below this line represent a limiting nutrient.

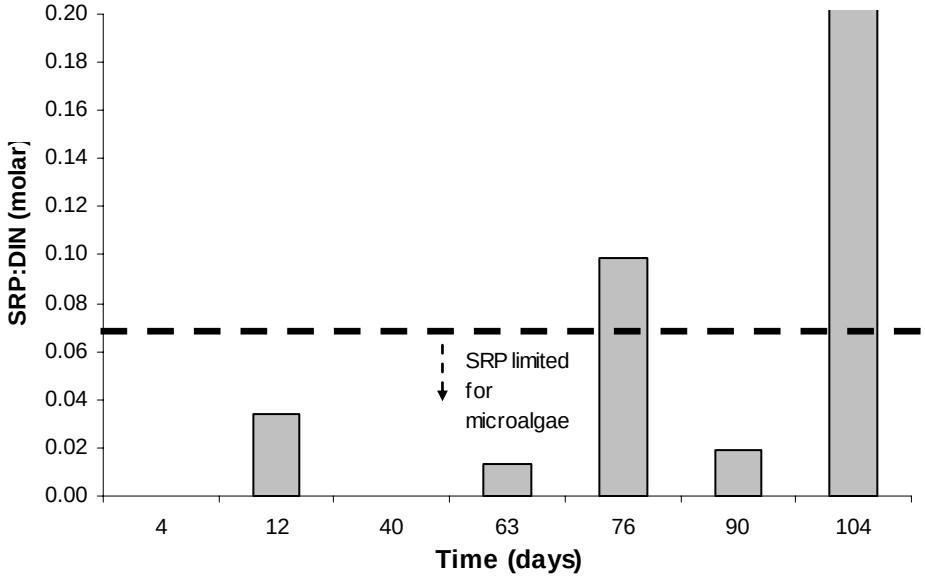


Figure 5-35: The ratio of SRP to DIN in the autotrophic pond during Trial D. The horizontal dashed line represents the optimum ratio for the growth of microalgae. Values below the dashed line are deficient in SRP relative to DIN.

Figure 5-36 shows the change in nutrient composition of the bottom sludge in the two ponds over time. Consistent with Trial C (see section 5.3.3), the quantity of nutrients was consistently higher in the heterotrophic pond compared with the autotrophic pond. This supports our previous hypothesis that despite a high level of aeration, negatively buoyant bio-floccules were settling out of suspension at a faster rate than microalgal cells.

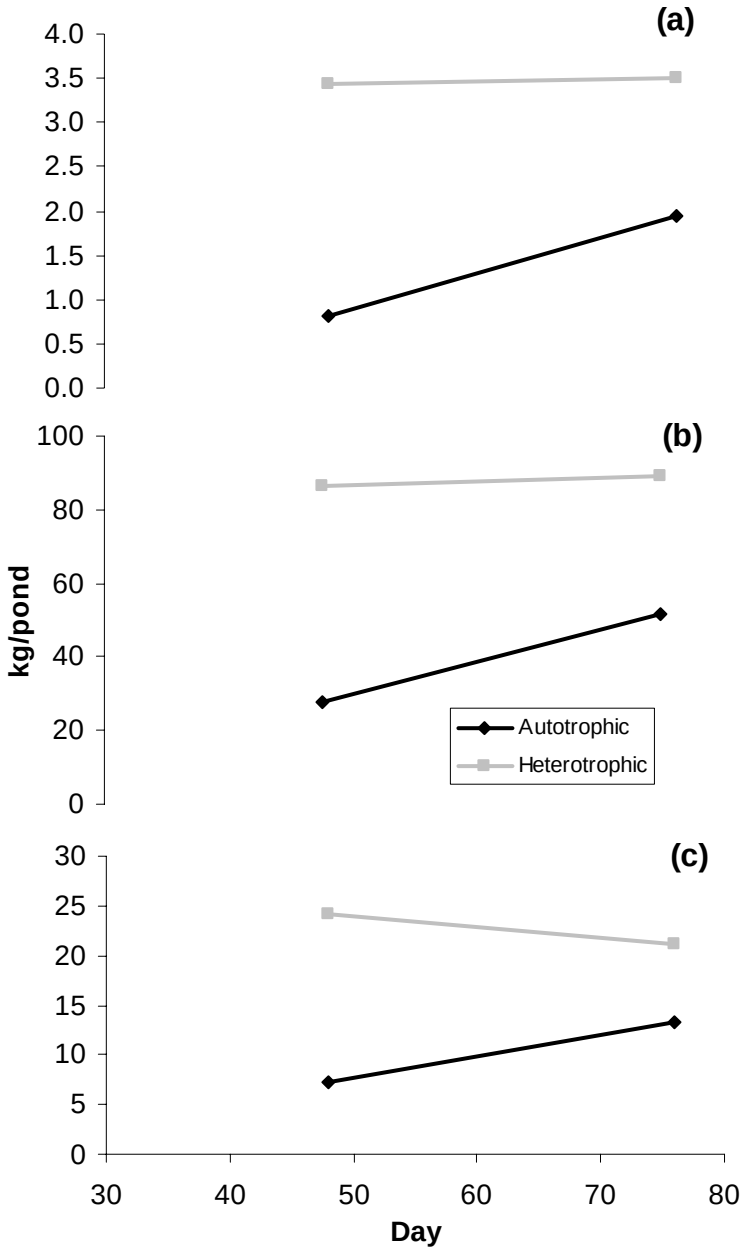


Figure 5-36: Total quantities of a) phosphorus, b) carbon and c) nitrogen on the floor of heterotrophic and autotrophic ponds in Trial D.

5.4.4 Conclusions

The results of this trial confirm the benefits of heterotrophic pond management in maintaining a low and stable pond pH, which enabled us to produce over 10 tonnes of fish per hectare over a period of 109 days. This pH data is indicative of a pond dominated by heterotrophic bacteria, however, despite the early addition of flour and attempts to maintain optimum nutrient ratios, TAN was not maintained at low concentrations. We believe that the lack of bio-floccules observed in the water column was largely responsible for the high concentration of TAN observed, as once the aerobic bio-floccules settle out of suspension; they are no longer capable of capturing TAN. As previously reported, the lack of bio-floccules in the water column may be related to a lack of bioturbation or insufficient mixing and further research is therefore required to determine the optimum level of pond mixing when using heterotrophic pond management in SIFTS ponds. Ensuring floccules are maintained in suspension should reduce TAN and enable further improvements in yield. In order to truly optimise the nutrient ratios in the pond, it would be of great benefit to have a reliable and cost-effective method of measuring total dissolved nitrogen and organic carbon in the field.

A water exchange rate of 5% was insufficient to prevent stratification in the autotrophic pond and the consequent production of hydrogen sulphide resulted in fish mortality. Although the use of 30 kW/ha of aeration with Force 7s has proven adequate to prevent stratification, lower levels may be sufficient and further research is required to determine this. Overall food input to the pond was lower than previous autotrophic pond trials and TAN remained at low levels. Based on this data, a food intake of 8.1 tonnes/ha over 90 days with a 5% water exchange may be sufficient to prevent high levels of TAN, however, microalgal blooms under this level of food input may still produce levels of pH high enough to render even low levels of TAN to be toxic.

6. NYPA FORAGE EFFLUENT TRAPPING TRIALS

6.1 Trial A: Laboratory Assessment of Nutrient Uptake from Aquaculture Effluent

6.1.1 Introduction

Previous studies on the use of constructed wetlands for treating nutrient-enriched effluent from inland saline aquaculture have found that high salinity inhibits plant growth and the long-term efficiency of nutrient uptake (Lymbery et al., 2006). This problem may be overcome by utilising a more salt-tolerant plant, such as NyPa Forage, a cultivar of *Distichlis spicata* which has been developed as a livestock fodder crop. Although NyPa Forage has been grown successfully in Australia in saline soils, and the broad ecological tolerances of the plant are well established (Leake et al., 2002), no data are available on its efficiency for treating aquaculture effluent. In this trial we wished to determine the ability of NyPa Forage to remove nitrogen, phosphorous and sodium chloride from effluent of different salinities and nutrient concentrations

6.1.2 Materials And Methods

In each of 16 arbitrarily selected wetland cells described in section 4.3, we planted four rooted shoots of a cultivated variety of the halophyte *Distichlis* sp. at equal spacing and these were established over a six-week period using distilled water. The remaining four cells had no plants. Two separate experiments were undertaken. In experiment 1 we compared the filtering efficiency of wetland cells containing sand only and cells containing sand planted with NyPa Forage, when presented with aquaculture waste containing 5,000 µg/L N, 1,000 µg/L P and 25,000 mg/L NaCl. In experiment 2 we investigated the effect of two different levels of nutrients (High: 5,000 µg N/L and 1,000 µg P/L; Low: 1,000 µg N/L and 200 µg P/L) and two different levels of salt (High: 25,000 mg NaCl/L; Low: 5,000 mg NaCl/L) on the filtering efficiency of wetland cells containing sand planted with NyPa Forage. In both experiments there were four replicate cells per treatment. Different salinity levels were created using sodium chloride) and different nutrient levels were made from a water soluble commercial fertilizer (Polyfeed^{TM} , Micros Bros, Perth Western Australia).

Wetland cells were randomly allocated to each treatment combination and watered with 12.0 L of solution twice weekly. Plants were gradually acclimated to the different salinity and nutrient levels for a further 6 week pre-experimental phase, prior to a test period of 231 days. To measure removal efficiency, we sampled inflow and outflow waters from each cell at days 10, 38, 66, 94, 164, 192 and 231 of the test period. Samples were analysed for NaCl, TN, NH_3, NO_2/NO_3, TP and SRP concentrations using standard methods adapted from the American Public Health Association (APHA, 2005). For NaCl, TN and TP, these concentrations were

converted to loads (g) so that the amount removed could be expressed as a percentage of the input load.

Percentage removal values for all elements were normalised using an arcsine transformation. The effect of planted or unplanted wetland cells on the percentage removal of Cl, TN and TP after 231 days, were analysed using a single-factor analysis of variance (ANOVA). The effects of nutrient and salinity level on the percentage removal of Cl, TN, NH3, NO_2/NO_3, TP and SRP after 231 days, were analysed using a two-factor ANOVA. Since measurements of each parameter in each wetland cell at different times were not independent, the effect of time on the percentage removal of Cl, TN, NH_3, NO_2/NO_3, TP and SRP was tested using repeated measures analysis of variance, with treatment regarded as the between-subject (i.e. wetland cell) effect and time as the within-subject effect.

6.1.3 Results And Discussion

Experiment 1

Aquaculture effluent was applied to wetland cells with and without NyPa forage plants. Figure 6-1 shows the difference, after 231 days, in percentage removal of total nitrogen (TN), total phosphorous (TP) and chloride (Cl) from planted and unplanted wetland cells. Single factor analysis of variance confirmed a significant effect of plants on the percentage removal of TN ($P < 0.0001$) and TP ($P < 0.0001$), but not Cl ($P = 0.96$) over the entire experimental period.

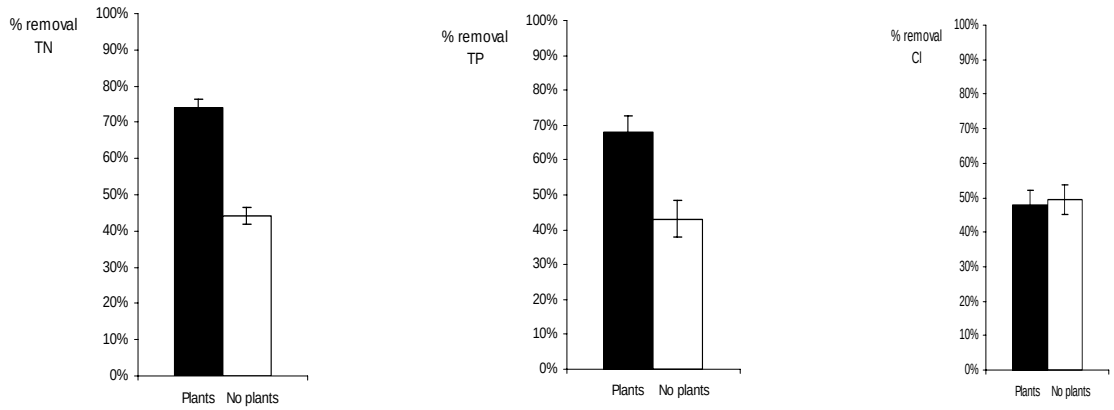


Figure 6-1: Percentage removal of (a) TN (b) TP and (c) Cl in wetland cells with (black) and without (white) plants after 231 days.

Figure 6-2, Figure 6-3 and Figure 6-4 show the change in percentage removal of TN, TP and Cl over time in the wetland cells with and without NyPa Forage plants. The unplanted cells showed an initial increase in removal efficiency of both nutrients and Cl, followed by a steady decline over time. Although the planted cells showed the same declining trend for Cl removal, they showed a steady increase in TN and TP removal to 91.2% TP and 87.7% TN at day 231. The spike in the measurements at time 2 (June) correlated to an extreme rainfall incident. Repeated measures analysis of variance confirmed that the percentage removal of TN, TP and Cl was significantly affected by time (for TN, $P < 0.01$; for TP, $P < 0.001$; for Cl, $P < 0.0001$), and that percentage removal of TN, TP but not Cl, was significantly greater in the planted than in the unplanted cells over the entire measurement period (for TN, $P < 0.0001$; for TP, $P < 0.001$; for Cl, P 0.94).

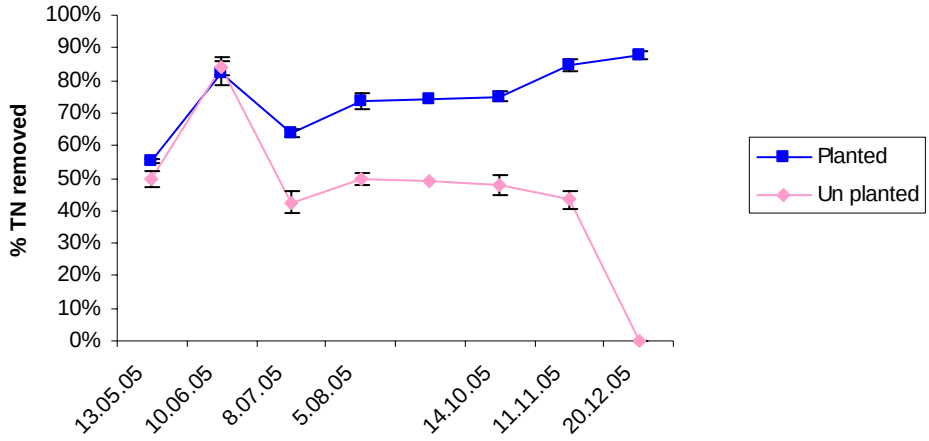


Figure 6-2: Percentage removal of TN (± SE) by planted and unplanted wetland cells receiving effluent over 8 months

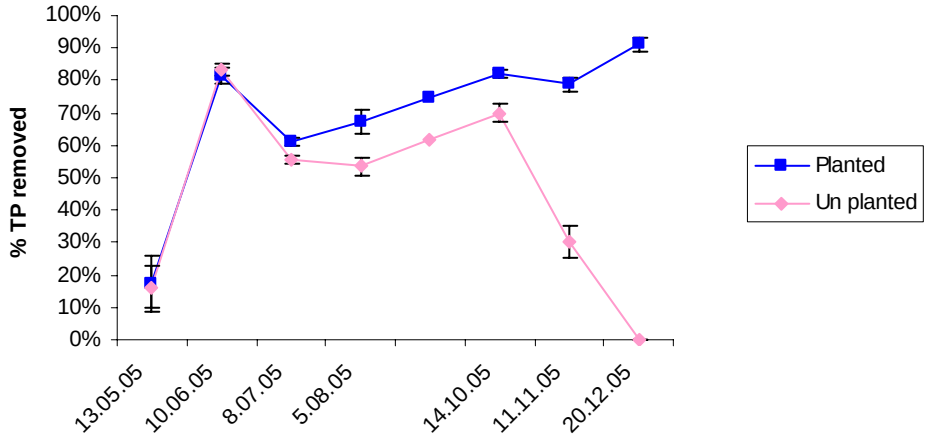


Figure 6-3: Percentage removal of TP (± SE) by planted and unplanted wetland cells receiving effluent over 8 months.

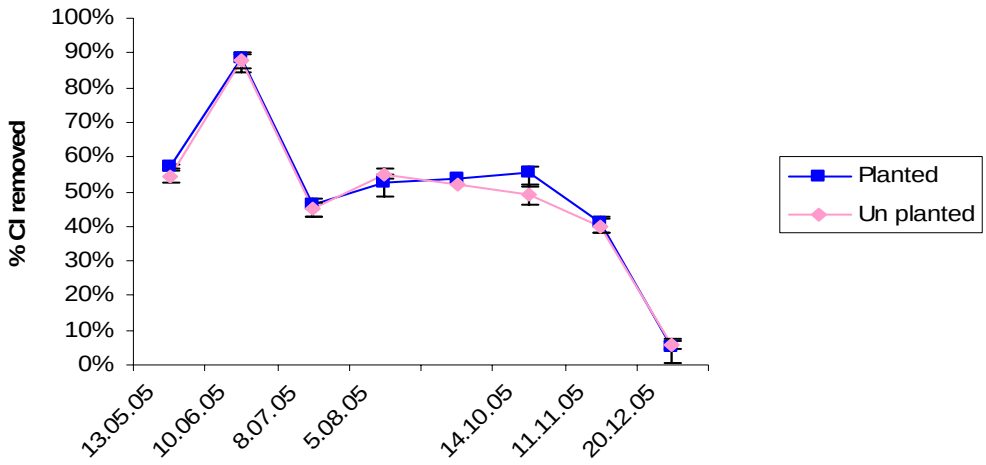


Figure 6-4: Percentage removal of Cl (± SE) by planted and unplanted wetland cells receiving effluent over 8 months.

Experiment 2

Aquaculture effluent was applied to planted wetland cells in 4 treatments: high nutrient/high salinity (HNHS); high nutrient/low salinity (HNLS); low nutrient/high salinity (LNHS); low nutrient/low salinity (LNLS). Table 6-1 shows the outflow volumes, concentrations and loads of total nitrogen (TN), ammonia (NH_3), nitrite/ nitrate ($NO_2 + NO_3$), total phosphorous (TP), orthophosphate (SRP) and chloride (Cl) in outflow water and the corresponding percentage removal rates from inflow water for all treatments at the completion of the experimental period (day 231).

Table 6-1: Mean (±SE) leaching fraction, outflow concentrations and % removal of TN, nitrogen components; TP, phosphorus components; and Cl for each treatment at the end of the trial (day 231). Treatments are high nutrient, high salt (HNHS); high nutrient, low salt (HNLS); low nutrient high salt (LNHS); and low nutrient low salt (LNLS).

		Treatment			
		HSHN	HNLS	LNHS	LNLS
Outflow volume	% of inflow	78.8	64.7	74.7	72.4
	(±SE)	0.5	1.5	2.2	1.9
TN	Out Conc (µg/L)	690	847.5	540	500
	(±SE)	63.4	103.5	68.6	123.7
	Load change (mg)	46674.3	46653.5	6759.6	7303.5
	(±SE)	588.9	687.4	619.1	954.3
	% Removal	87.7	87.9	58.3	63.0
	(±SE)	1.1	1.3	5.3	8.2
NO_2/ NO_3	Out Conc (µg/L)	197.5	125.3	121.3	40.3
	(±SE)	70	34	35.5	14.8
	% Removal	96.0	97.9	88.1	96.2
	(±SE)	1.4	0.6	3.4	1.3
NH_3	Out Conc (µg/L)	46.8	23	49.3	42.8
	(±SE)	2.1	3.6	4.3	4.7
	% Removal	91.5	96.5	57.7	64.3
	(±SE)	0.4	0.6	3.1	3.9
TP	Out Conc (µg/L)	103.8	67.3	38.5	20.3
	(±SE)	27.2	20.4	8.5	1.5
	Load change (mg)	10216	10683.9	1893.3	2064.5
	(±SE)	263.2	144.9	77.1	11.3
	% Removal	91.2	95.4	84.5	92.2
	(±SE)	2.4	1.3	3.4	0.5
SRP	Out Conc (µg/L)	104.5	12.3	37	13.3
	(±SE)	14.6	3.7	8.4	2.5
	% Removal	91.2	99.1	85.1	94.8
	(±SE)	1.3	0.3	3.5	1.1
Cl	Out Conc(mg/L)	19250	4050	20000	3875
	(±SE)	629.15	28.87	707.11	62.92
	Load change (mg)	4712.5	9883	7980	7674.75
	(±SE)	6744.21	543.12	5853.89	571.64
	% Removal	4.52	23.93	5.42	18.58
	(±SE)	1.74	1.32	2.07	1.38

Two factor analyses of variance showed that by the completion of the trial the percentage removal of TN and TP was significantly affected by nutrient level, and the percentage removal of Cl and TP was significantly affected by salinity. More nitrogen and phosphorus were removed at higher nutrient levels and more phosphorus and chloride were removed at lower salinity levels. Both nutrient and salinity level had a significant effect on the percentage removal of nitrite/nitrate, ammonia and orthophosphate, with more of these components being removed at higher nutrient levels and lower salinity levels. There were no significant interactions between nutrient and salinity levels on the percentage removal of any parameter.

The percentage removal of TN, TP and Cl in each treatment over time is shown in Figure 6-5, Figure 6-6 and Figure 6-7. While the percentage removal of both the TN and TP increased over time, there was a steady decline in the percentage removal of Cl. Repeated measures analyses of variance confirmed that the percentage removal of TN, TP and Cl all changed significantly over time, with no significant differences between wetland cells nested in the same treatment. There was a significant interaction between treatment and time for the % removal of TN, TP and Cl.

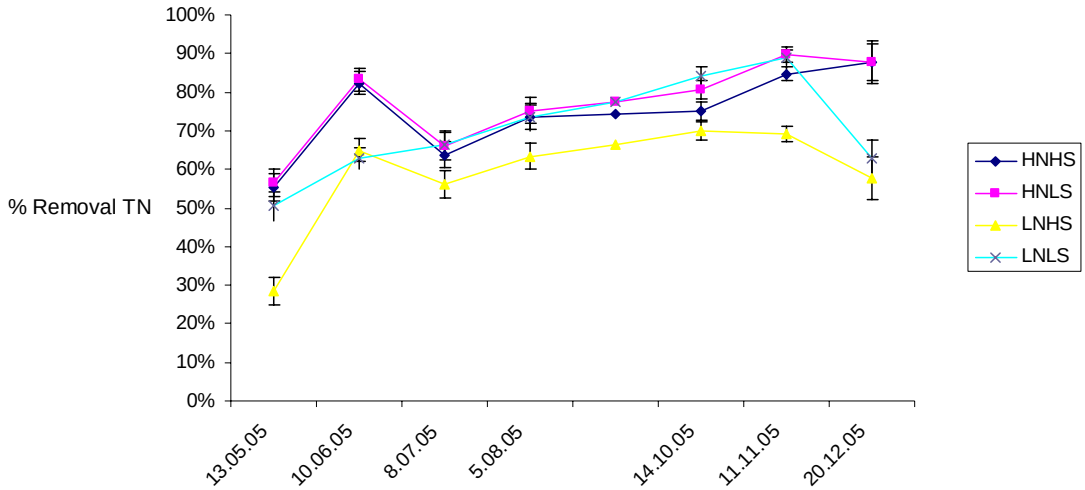


Figure 6-5: Percentage removal rates of TN over time for each treatment over 231 days. Bars represent standard errors. Treatments are high nutrient, high salt (HNHS); high nutrient, low salt (HNLS); low nutrient high salt (LNHS); and low nutrient low salt (LNLS).

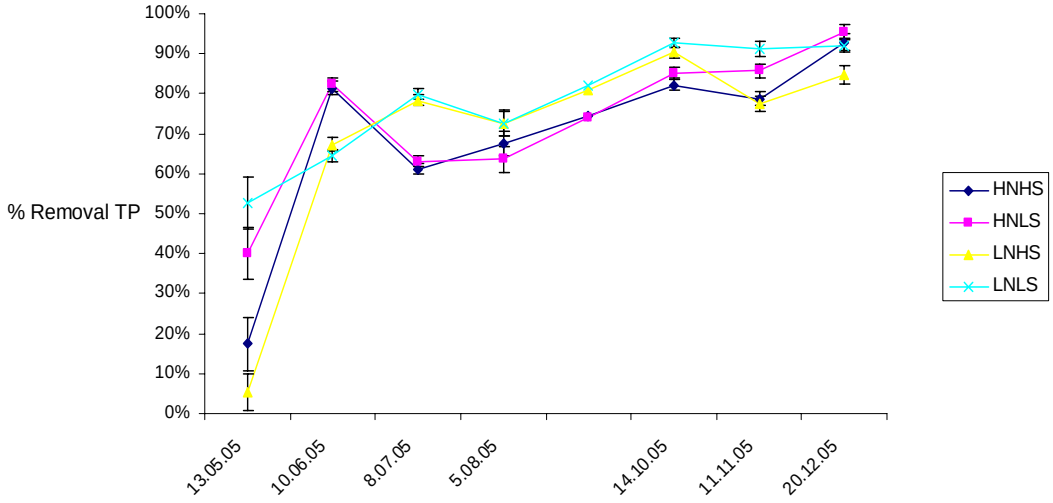


Figure 6-6: Percentage removal rates of TP over time for each treatment over 231 days. Bars represent standard errors. Treatments are high nutrient, high salt (HNHS); high nutrient, low salt (HNLS); low nutrient high salt (LNHS); and low nutrient low salt (LNLS).

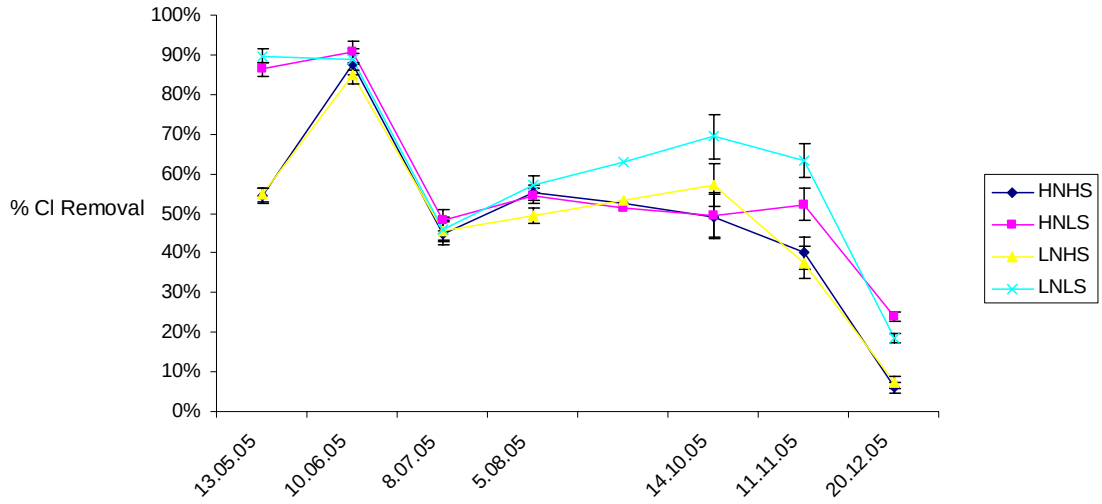


Figure 6-7: Percentage removal rates of Cl for each treatment over 231 days. Bars represent standard errors. Treatments are high nutrient, high salt (HNHS); high nutrient, low salt (HNLS); low nutrient high salt (LNHS); and low nutrient low salt (LNLS).

6.1.4 Conclusions

The wetland cells planted with NyPa Forage removed up to 88% of total nitrogen and 95% of total phosphorous from aquaculture effluent over an 8 month period. Importantly, removal efficiencies were high for all of the potentially toxic components of nitrogen such as ammonia (96%) and nitrite/ nitrate (98%). Most of this uptake was the result of active processes by the plants or by processes occurring at the plant/soil interface. Small amounts of sodium chloride (up to 24%) were also removed, but this was a result of a passive filtering process by the sand, which declined over time. More nitrogen and phosphorous were removed at higher nutrient levels. Higher salinity levels had a small inhibitory effect on the efficiency of phosphorous removal, but did not affect nitrogen removal. On the basis of these results NyPa Forage appears to be much more suitable for nutrient removal from inland saline aquaculture effluent than the estuarine sedge species *Juncus kraussii*, previously studied by Lymbery et al. (2006).

6.2 Trial B: Laboratory Assessment of Nutritive Value of NyPa Forage

6.2.1 Introduction

NyPa Forage may have additional benefits, in addition to its role in effluent treatment for an inland saline aquaculture operation. NyPa Forage has been actively selected for increased yield and nutritional value as a fodder plant. The productivity (above-ground dry biomass) of NyPa Forage is 5-10 times greater than the productivity of wild saltgrass in the United States (Yensen et al., 1985). Field trials of NyPa Forage under a range of different conditions in Australia have produced yields of 1.5-10 tonnes dry matter/ha, with a digestibility of 46-61%, crude protein concentration of 6-17% and metabolisable energy of 6-7.5 MJ/kg. Although these field trials were preliminary and largely uncontrolled, they did indicate that NyPa Forage may be a useful fodder species, and they also suggested that both yield and nutritive value were positively related to pasture fertilisation (Leake et al., 2002). Under an integrated agri-aquaculture situation where the plants are irrigated with nutrient-rich aquaculture effluent, both yields and nutritive value are likely to be improved. Successful integration of a livestock feeding enterprise with a semi-intensive saline aquaculture enterprise offers economic benefits through the utilisation of salt-affected farmland, environmental benefits by offsetting the costs of important environmental management procedures, and social benefits by assisting in the diversification of depressed rural economies.

6.2.2 Materials and Methods

Two experiments were undertaken, using the same wetland cell system described in section 6.1.2. The aim of the first experiment was to compare growth and nutritive value of NyPa Forage plants grown at different nutrient levels. Subsurface flow wetland cells, filled with washed quartz sand, were planted with NyPa Forage and nutrients were applied as an effluent sludge at a salinity of 15,000 mg/L. Five nutrient treatment levels were used: (1) 0 mg/L N: 0 mg/L P; (2) 2.5 mg/L N: 0.5 mg/L P; (3) 5 mg/L N: 1 mg/L P; (4) 7.5 mg/L N: 1.5 mg/L P; (5) 10 mg/L N: 2 mg/L P. There were four replicate cells per treatment. After 90 days, all above ground plant material was harvested from each cell and measured for wet weight, dry weight, sodium chloride, crude protein, dry matter digestibility and total ash. Ash components were in the normal range (9-12%) for pasture plants, so metabolisable energy (ME) was calculated from dry matter digestibility (DMD) as ME = 0.17 (%DMD) – 2.0 (AFRC, 1993). Differences among treatments were compared by linear regression or one-way analysis of variance, with *post hoc* comparison of means using the Tukey-Kramer HSD test.

The aim of the second experiment was to compare growth and nutritive value of NyPa Forage plants at different cropping levels. Subsurface flow wetland cells, filled with washed quartz sand, were planted with NyPa Forage and fertilised with an effluent sludge with a nutrient concentration of 10 mg/L N: 2 mg/L P and a salinity of 15,000 mg/L. Four different cropping treatments were applied: (1) no cropping; (2) growing shoots cropped every 21 days; (2) growing shoots cropped every 42 days; (3) growing shoots cropped every 63 days. There were five replicate cells per treatment. At each cropping time and at the end of the experiment (126 days), harvested plant material from each cell was measured for wet weight, dry weight, sodium chloride, crude protein, dry matter digestibility, total ash and metabolisable energy as for experiment 1. Differences among treatments were compared by one-way analysis of variance, with *post hoc* comparison of means using the Tukey-Kramer HSD test.

6.2.3 Results and Discussion

Experiment 1

There was a significant, positive linear relationship between nutrient level and dry weight of plant material harvested (Figure 6-8; $r^2 = 0.96$, $P < 0.0001$). Extrapolation from these results suggests that yields on a per hectare per day basis would range between 1.5 kg DM/ha/day (with no nutrient fertilisation) and 31.4 kgDM/ha/day (with fertilisation at a level of 10 mg/L N: 2 mg/L P).

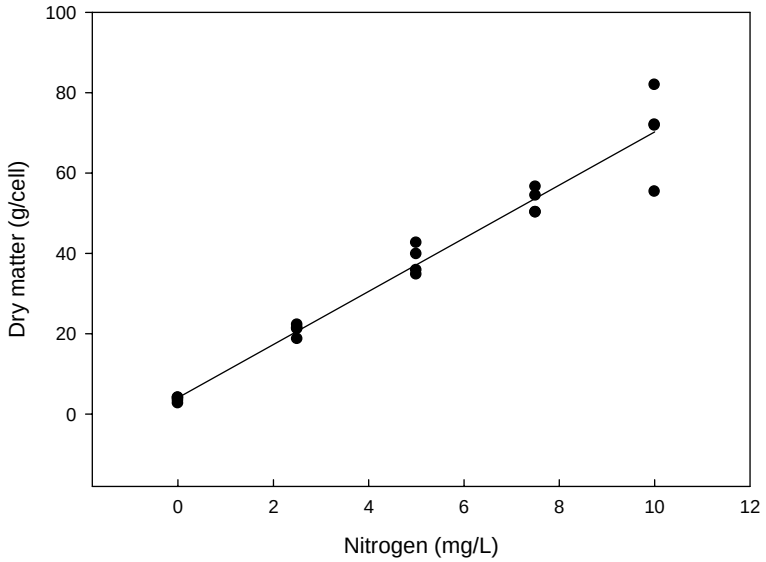


Figure 6-8: Relationship between dry matter harvested from wetland cells and nutrient content of water applied (expressed as mg/L nitrogen) after 90 days of growth.

The mean sodium chloride content of plant material was 8.2 ± 0.5 mg/g and did not differ significantly among treatments.

Crude protein level was significantly greater when the NyPa Forage was fertilised than with no fertilisation (c7.5% compared to 5.4%; P < 0.0001), but did not differ significantly among nutrient treatments (Figure 6-9). Similarly, dry matter digestibility, and therefore metabolisable energy, were significantly greater when the NyPa Forage was fertilised than with no fertilisation (for digestibility 54.7% compared to 51.6%; P = 0.005; for metabolisable energy c7.3 MJ/kg compared to 6.8 MJ/kg; P = 0.005), but did not differ significantly among nutrient treatments (Figure 6-10 and Figure 6-11).

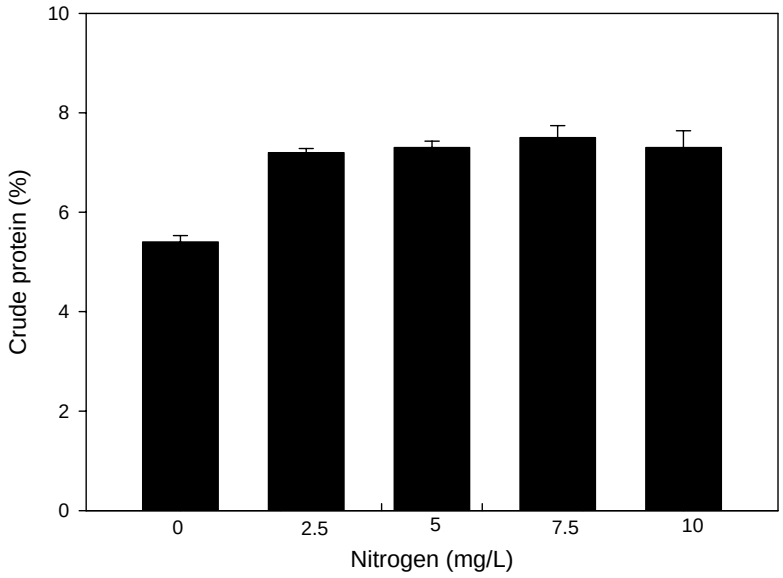


Figure 6-9: Percentage crude protein content of NyPa Forage from wetland cells fertilised with five different nutrient treatments (expressed as mg/L nitrogen).

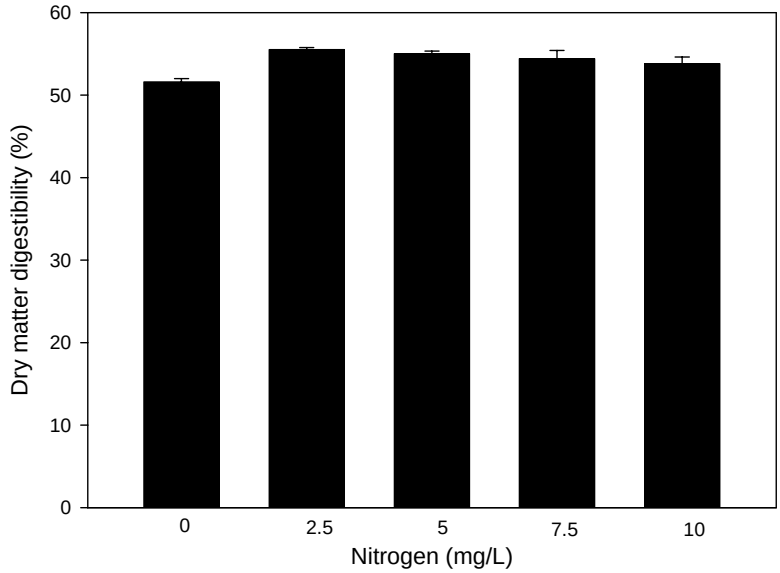


Figure 6-10: Percentage dry matter digestibility of NyPa Forage from wetland cells fertilised with five different nutrient treatments (expressed as mg/L nitrogen).

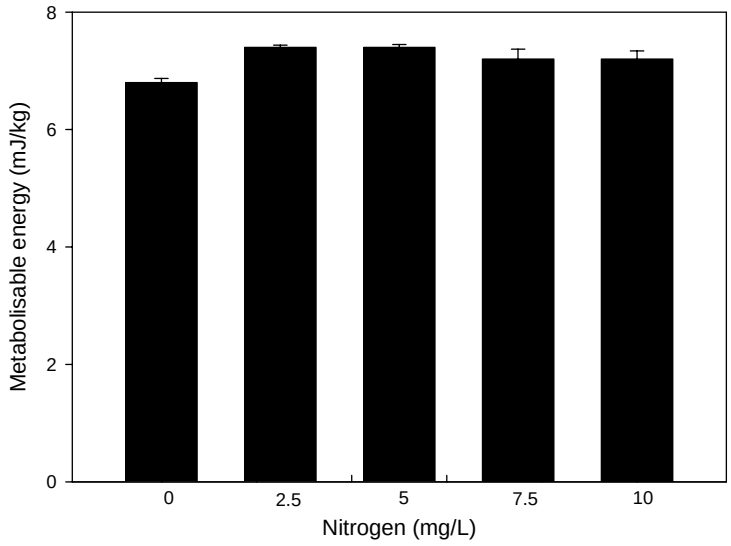


Figure 6-11: Metabolisable energy (MJ/kg) of NyPa Forage from wetland cells fertilised with five different nutrient treatments (expressed as mg/L nitrogen).

Experiment 2

Cropping rate did not significantly affect the total amount of dry matter harvested per cell after 126 days, with a mean over all treatments of 88.8 ± 4.0 g (Figure 6-12; P = 0.37). Extrapolation from these results suggest a total yield on a per hectare per day basis of 27.9 kgDM/ha/day, similar to the extrapolated yield from experiment 1.

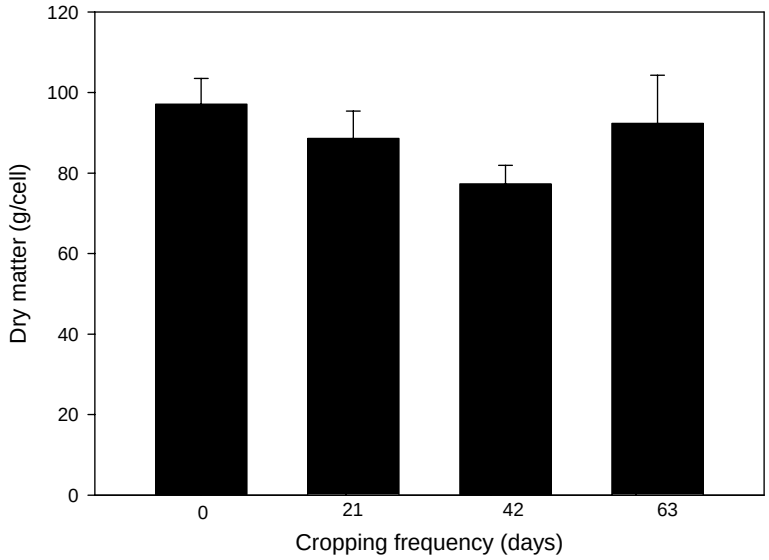


Figure 6-12: Amount of dry matter harvested from wetland cells with four different cropping frequencies.

The mean sodium chloride content of plant material was similar to that found in experiment 1; 8.1 ± 0.4 mg/g, with no significant difference among treatments.

Crude protein level differed significantly among cropping treatments ($P < 0.0001$), being greatest at 21 and 42 days, significantly lower at 63 days and least with no cropping until 126 days (Figure 6-13). Similarly, dry matter digestibility and therefore metabolisable energy differed significantly among cropping treatments (for digestibility $P < 0.0001$; for metabolisable energy $P < 0.0001$), and was again greatest at 21 and 42 days, significantly lower at 63 days and least with no cropping until 126 days (Figure 6-14 and Figure 6-15).

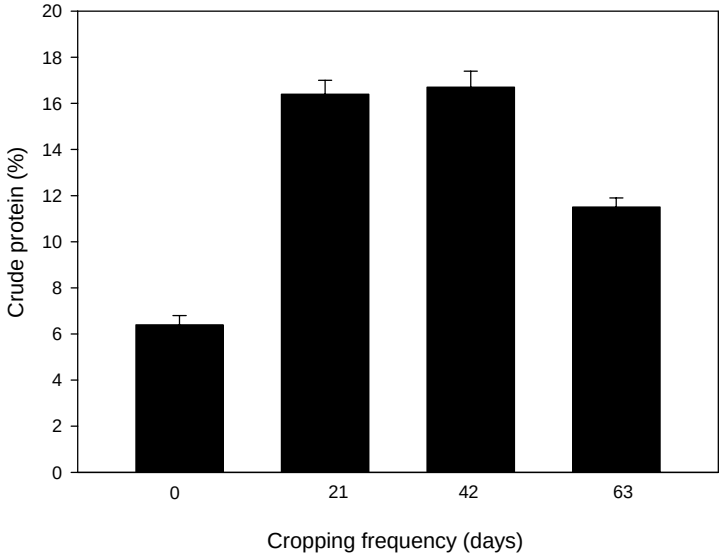


Figure 6-13: Percentage crude protein content of NyPa Forage from wetland cells with four different cropping frequencies.

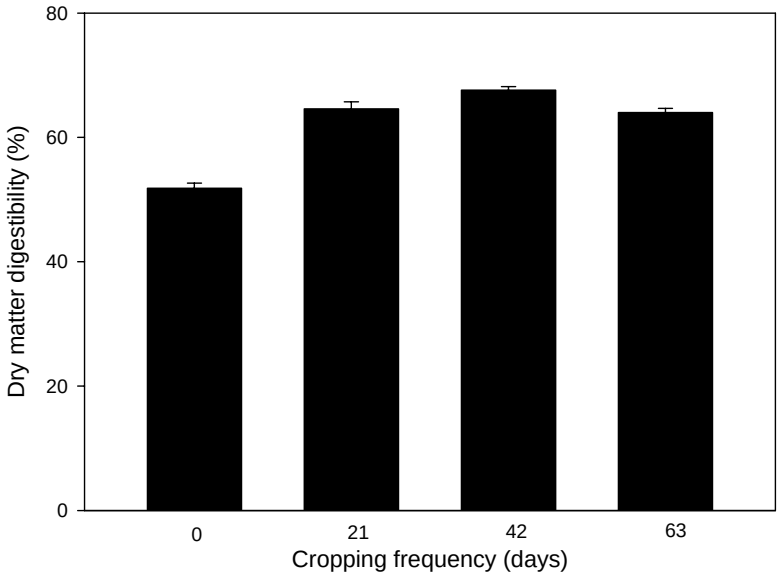


Figure 6-14: Dry matter digestibility of NyPa Forage from wetland cells with four different cropping frequencies.

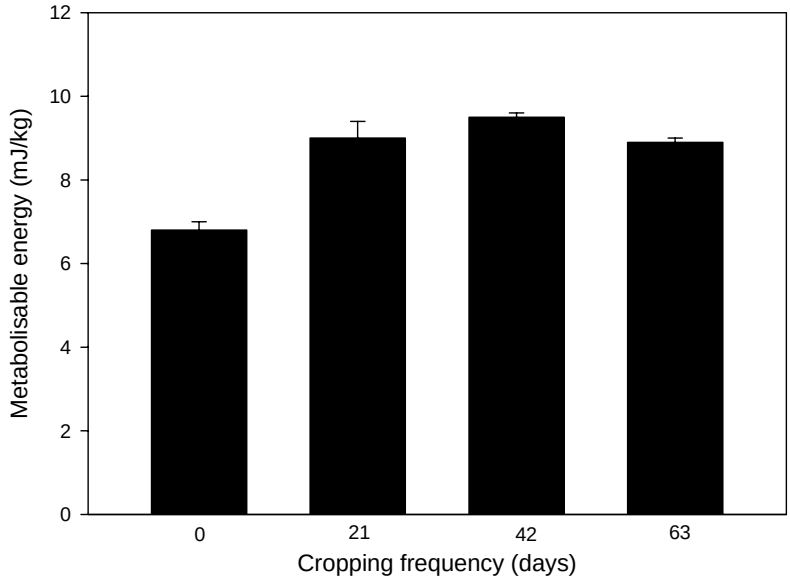


Figure 6-15: Metabolisable energy (MJ/kg) of NyPa Forage from wetland cells with four different cropping frequencies.

6.2.4 Conclusions

The nutritive value of NyPa Forage, as determined from *in vitro* laboratory analyses, was greater than that of most salt-tolerant pasture plants that have been tested in Australia (Norman et al., 2002; Thompson, 2002). As a rather general rule of thumb, liveweight maintenance of a 50 kg wether requires feed with a protein content of 8% and an energy value of more than 7.4

MJ/kg (equivalent to a dry matter digestibility of 55%) (AFRC, 1993; Freer et al., 1997). Most salt-tolerant annual and perennial grasses have less than these protein and energy requirements, while halophytic shrubs, such as saltbush, usually have sufficient crude protein, but are deficient in energy (Norman et al., 2002).

NyPa Forage, when grown in sand with no nutrient fertilisation and no cropping, had a mean crude protein content of 5.4 ± 0.1 %, a dry matter digestibility of 51.6 ± 0.1 % and an energy value of 6.8 ± 0.1 MJ/kg. All of these values were significantly improved, however, by fertilisation with aquaculture effluent and by regular cropping. The greatest values were obtained by fertilisation with effluent containing 10 mg/L N and 2 mg/L P, and with cropping every 42 days; with these treatments NyPa Forage had a mean crude protein content of 16.7 ± 0.7 %, a dry matter digestibility of 67.6 ± 0.6 % and an energy value of 9.5 ± 0.1 MJ/kg. These values are equivalent to those contained by a good quality conserved fodder, and assuming an equivalent fibre content and voluntary food intake as grass hay, would be sufficient for maintenance of dry adult sheep or cattle or moderate live-weight gain (≤ 0.5 kg/day) in growing animals (AFRC, 1993; Freer et al., 1997).

The sodium chloride concentration of NyPa Forage, irrigated with water with a salinity of 15,000 mg/L, was approximately 8 mg/g, towards the high end of the range found in most pasture plants, but well below levels often found in other halophytes (≥80 mg/g) and known to depress intake and live-weight gain in grazing livestock (Minson, 1990; CSIRO, 2000)(Minson 1990; CSIRO 2007). This relatively low sodium chloride concentration reflects the fact that, unlike many other halophytes that tolerate saline conditions by accumulating salts in their tissues, NyPa Forage actively excretes salt through salt glands (Flowers et al., 1986; Yensen, 2002).

The yield of NyPa Forage was not affected by cropping rate, but showed a direct linear response to nutrient level in the effluent with which it was watered. Estimated yield on a per hectare basis ranged from 1.5 kgDM/ha/day (with no nutrient fertilisation) and 31.4 kgDM/ha/day (with fertilisation at a level of 10 mg/L N: 2 mg/L P).

6.3 Field Assessment of Nutrient Uptake and Nutritive Value of NyPa Forage

6.3.1 Introduction

Although laboratory trials, as described above, are an essential part of determining economic potential and design criteria for irrigating NyPa Forage crops, they need to be complemented with an on-farm pilot trial to confirm production efficiency over the long-term and under the influence of extraneous environmental factors. We therefore conducted a pilot field trial to estimate the growth and nutritive value of NyPa Forage plants irrigated with aquaculture effluent.

6.3.2 Materials and Methods

The initial planting, in summer 2007, of 1600 NyPa Forage shoots was unsuccessful due to poor shoot formation when irrigated with saline water. A second attempt to plant advanced seedlings was also unsuccessful, due to extremely hot conditions within 10 days of planting. An additional 1600, salt conditioned NyPa seedlings, were then planted during September 2007, when climatic and soil conditions were more favourable. The four experimental plots at Springfield waters Aquaculture were each planted with 400 plants and initially irrigated with bore water for 6 weeks. Thereafter, two plots were switched to water sourced from the autotrophic (algal) SIFTS pond described in Trial D ("fertilised" plots) while the remaining two plots continued to receive bore water ('unfertilised" plots). Soil samples were taken from each plot prior to planting and at the end of the field trial in May 2008. Soil samples were analysed for total nitrogen, total phosphorous and sodium chloride. From February to May 2008, 1 m^2 quadrats in each plot were cropped, using a lawn mower, every 21 or 42 days. Harvested plant material was measured for wet weight, dry weight, crude protein, dry matter digestibility, total ash and metabolisable energy, as for the laboratory trials. Because of the difficulties in initially establishing the NyPa Forage plants, and subsequent reduced size of the plots, we did not have enough replicated plots for statistical comparisons among watering treatments. In essence, the field trial was used to confirm the nutrient uptake, yield and nutritive value results obtained from the laboratory trials, in a more realistic production environment.

6.3.3 Results and Discussion

There was little difference in soil parameter measurements between fertilised and unfertilised plots. Over all plots, soil total nitrogen was 0.35 ± 0.03 mg/g, total phosphorous was 0.18 ± 0.01 mg/g and sodium chloride was 3.60 ± 0.52 mg/g. Yield and nutritive value of NyPa Forage

was greater in fertilised than in unfertilised plots, and we present only the values in fertilised plots for comparison with laboratory trials. Over all quadrats, the mean dry matter yield per quadrat was 50.9 ± 6.4 g, giving an estimated yield on a per hectare per day basis of 24.2 kgDM/ha/day, slightly less than obtained in the laboratory trials. The mean crude protein level was 15.6 ± 0.7 %, the mean dry matter digestibility 64.6 ± 1.1 % and the mean metabolisable energy 8.8 ± 0.1 MJ/kg, again slightly less than the values obtained at the same cropping rates and in the laboratory trials.

6.3.4 Conclusions

The field trial confirmed that, under realistic production conditions, NyPa Forage was able to trap nutrients from aquaculture effluent and provide a crop with nutritive value sufficient to provide a maintenance feed for grazing livestock. The yield obtained in the field trial (24.2 kgDM/ha/day) provides a realistic estimate of the yield which would be expected if NyPa Forage were used in an integrated agri-aquaculture system. Assuming a 4 month growing season, this equates to a total yield over late summer/autumn of 2.9 tDM/ha, which is less than half the expected yield from well managed improved pasture, but equivalent to the expected yield from unmanaged pasture in the south west of Western Australia.

7. THE USE OF *ARTEMIA* (BRINE SHRIMP) TO CONTROL MICROALGAL BLOOMS WITHIN INLAND SALINE AQUACULTURE

7.1 General Introduction

Filter feeding organisms such as brine shrimp (*Artemia* spp.) have the potential to treat nutrient waste by cropping the microalgae which blooms in response to these nutrients. Nutrients can therefore be exported from ponds by harvesting these filter feeders before they die (Wang, 2003). *Artemia* have excellent potential to fulfill this role as they can be grown within the ponds with minimal additional infrastructure; they do not require water to leave the pond and risk salt build-up (as is the case with constructed wetlands); and they can provide a high value secondary cash crop for farmers.

The specific questions addressed in this study were:

1) Can *Artemia* grow at salinities commonly experienced in areas used for inland saline aquaculture?
2) Can *Artemia* grow at temperatures commonly experienced in areas used for inland saline aquaculture?
3) Can *Artemia* grow on the species of algae that bloom in aquaculture ponds?
4) What is the expected ingestion rate at various temperatures and algal species?
5) How many *Artemia* are required to crop microalgae to safe levels based on the answers to questions 1-4?

This study involved an iterative process of laboratory and field trials to determine the suitability of *Artemia* to control microalgal blooms within inland saline aquaculture ponds (Figure 7-1). Laboratory trials were undertaken to determine the range of environmental parameters over which different strains of *Artemia* could survive and grow, and to determine the relationship between salinity, temperature, microalgal consumption and growth rate of *Artemia*. These data were used to predict the expected consumption of algae in the field using a simple spreadsheet model. The predictions of the model were subsequently tested in field trials.

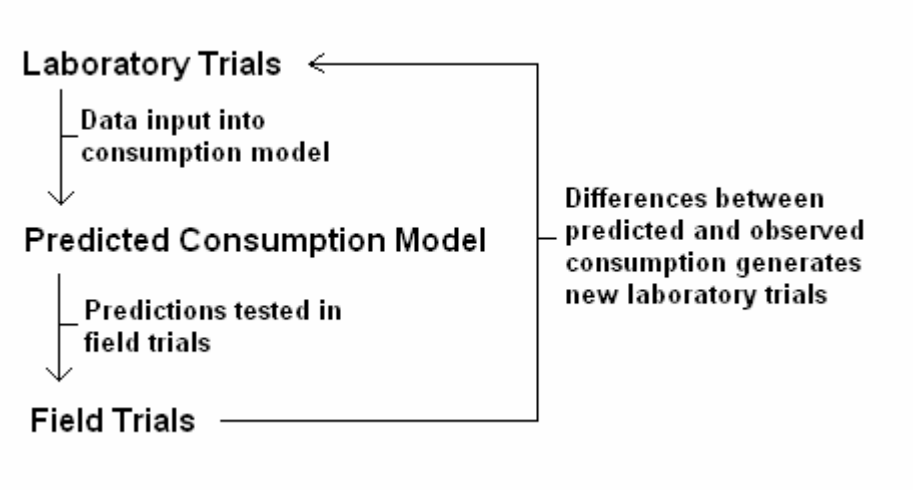


Figure 7-1: **Iterative process of laboratory and field trials to determine suitability of *Artemia* to control microalgal blooms in static ponds**.

7.2 Laboratory Trial 1: The Effects of Temperature and Salinity on Growth and Survival of Two *Artemia* Strains.

7.2.1 Introduction

Research has been conducted in Australia on intensive production of *Artemia* using inland saline groundwater (Gavine and Bretherton, 2007), however, little is known about the ability of *Artemia* to control microalgal blooms in aquaculture ponds, particularly under conditions of variable salinity and temperature. The aim of this trial was therefore to determine if *Artemia* of different origin were able to survive and grow over the range of temperatures and salinities typical of static inland saline ponds in temperate Australia. The strains of *Artemia* used were a commercially available strain sourced from the Great Salt Lakes (GSL) in the USA and a locally sourced strain from Hutt Lagoon (HL) in Western Australia. This strain has recently been recognized as having potential commercial significance and is currently being investigated by scientists from the Western Australian Department of Fisheries (Fletcher, 2007).

7.2.2 Materials and Methods

GSL and HL *Artemia* were cultured from newly hatched nauplii to day 14 in a series of 10 mL culture vessels held at one of three temperatures (10˚C, 20˚C or 30˚C) and one of two salinities (15 ppt and 35 ppt). The temperatures represent the annual extremes in water temperature experienced at Springfield Waters Aquaculture in Northam. A salinity of 15 ppt is the salinity at the Northam site, while 35 ppt (seawater) is regarded as close to optimum salinity for good growth of *Artemia*. Temperature of the culture vessels was controlled with either heated or chilled water baths. Culture water was either used directly from the ADU seawater bore (35 ppt) or diluted with fresh water (15 ppt).

Each culture vessel contained eight raceways, comprising four replicates of each of the two strains. Five individual *Artemia* were stocked into each raceway. Each raceway had a daily water exchange of 80%. Exchanged water was replaced with *Chaetoceros muelleri* cultured at either 15 ppt or 35 ppt. Culture vessels and water baths were housed on a shaking table within the temperature controlled room to ensure microalgal cells remained in suspension.

Chaetoceros muelleri was selected as the food source because it is easily cultured under controlled conditions, readily available and is of similar size and shape to *Heterocapsa* sp., the dominant microalgal species identified from regular seasonal sampling within the ponds at the

Northam field site. *Heterocapsa* sp. was unable to be used as a food source in the laboratory trials because we were unsuccessful in isolating a pure strain for culture. *C. muelleri* was supplied to each raceway at 833,000 cells/mL/day, which was calculated to be the density required to ensure that *Artemia* were not food limited. This ration was calculated based on previous studies by Naegel (1999), Evjemo and Olsen (1999) and Leonardos and Geider (2004).

There was an additional control culture vessel placed in each water bath which was used to determine if algae were settling out of the raceways. No *Artemia* were stocked into these vessels, however they still underwent the same daily 80% water exchange.

Ingestion rate of microalgal cells by *Artemia* was assessed by collecting the exchange water and counting the remaining cells using a 0.1 mm deep improved neaubauer hameocytometer, according to methods described in Partridge et al. (2003). The difference in cell density was converted to number of cells consumed from 10 mL, then converted to cells consumed per animal. Culture water was collected from each well, from each vessel on days 2, 3, 8, 9, 13 and 14 of the trial.

Survival, total length and individual dry weight of *Artemia* were assessed after the 14 day trial period. *Artemia* in each raceway were killed using a few drops of acetic acid before being placed on a 100 µm screen. They were then thoroughly rinsed with de-ionised water. A 0.5 M solution of ammonium formate was then washed over the *Artemia* to remove any remaining salts. The *Artemia* from each replicate were then laid flat on a pre-weighed glass slide. They were then photographed under a dissecting microscope and total length digitally measured using the software Motic Images 2.0.

7.2.3 Results and Discussion

There was a strong positive relationship between body length and dry weight for both strains over all temperatures and salinities used in the trial (Figure 7-2; for GSL *Artemia,* r^2=0.78, P<0.0001; for HL *Artemia,* r^2=0.67, P<0.0001). Because of the very small dry weight of the animals, there was a much greater measurement error for weight than for length, as reflected in their respective coefficients of variation (CVL=0.50; CVDW=1.56). Therefore in this and subsequent trials, only body length as a measure of animal size are reported.

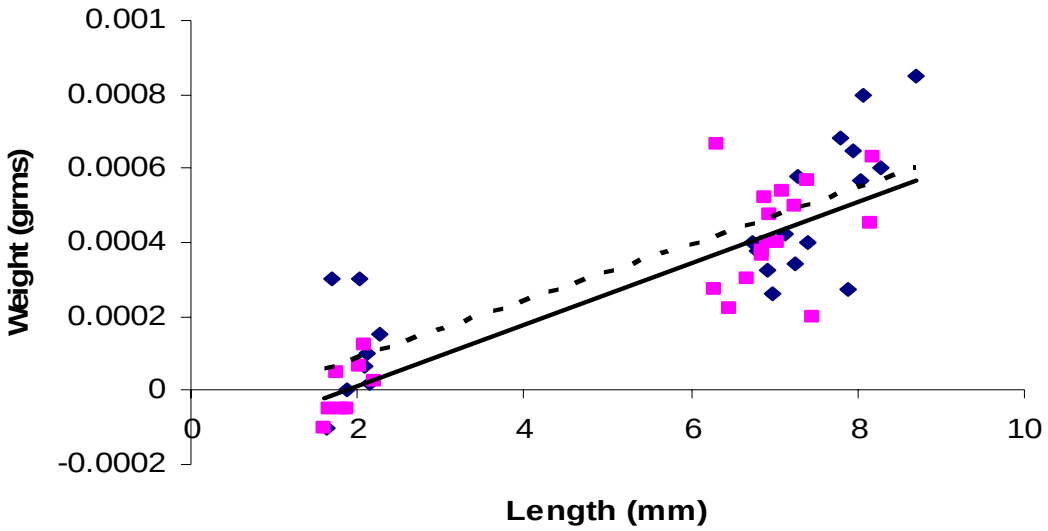


Figure 7-2: **Relationship between body length (mm) and dry weight (grams) for two strains of *Artemia,* GSL (squares, solid line) and HL (diamonds, dotted line).**

Figure 7-3 shows the mean length of *Artemia* in each treatment combination of strain (GSL, HL), temperature (10°C, 20°C, 30°C) and salinity (15 ppt, 35 ppt) after 14 days. Three-factor analysis of variance showed significant main effects of strain and temperature on length at 14 days, but no significant effects of salinity or interactions at any level. Comparison of least square means showed that after 14 days the HL strain of *Artemia* was significantly larger than the GSL *Artemia* over all salinities and temperatures (Figure 7-4a), and that length increased with increasing temperature over both strains and both salinities (Figure 7-4b), with growth being significantly different at all temperatures.

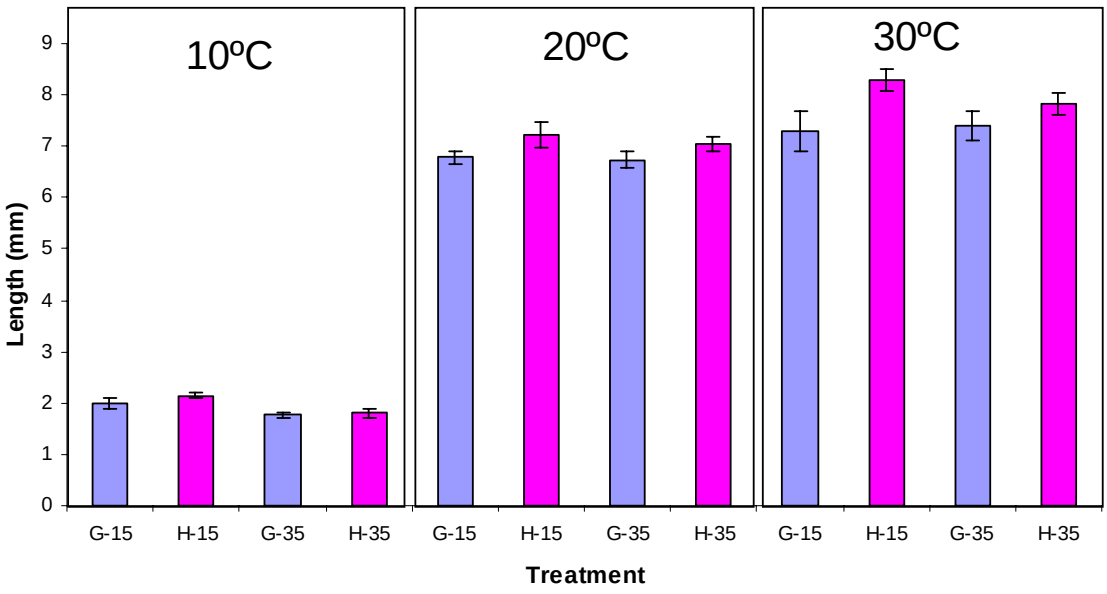


Figure 7-3: **Mean length (± SE) of *Artemia* from each treatment at the end of the 14 day trial period. Two strains, (G = GSL, H = Hutt Lagoon), two salinities, (15 ppt and 35 ppt), and three temperatures (10 ºC, 20 ºC and 30 ºC).**

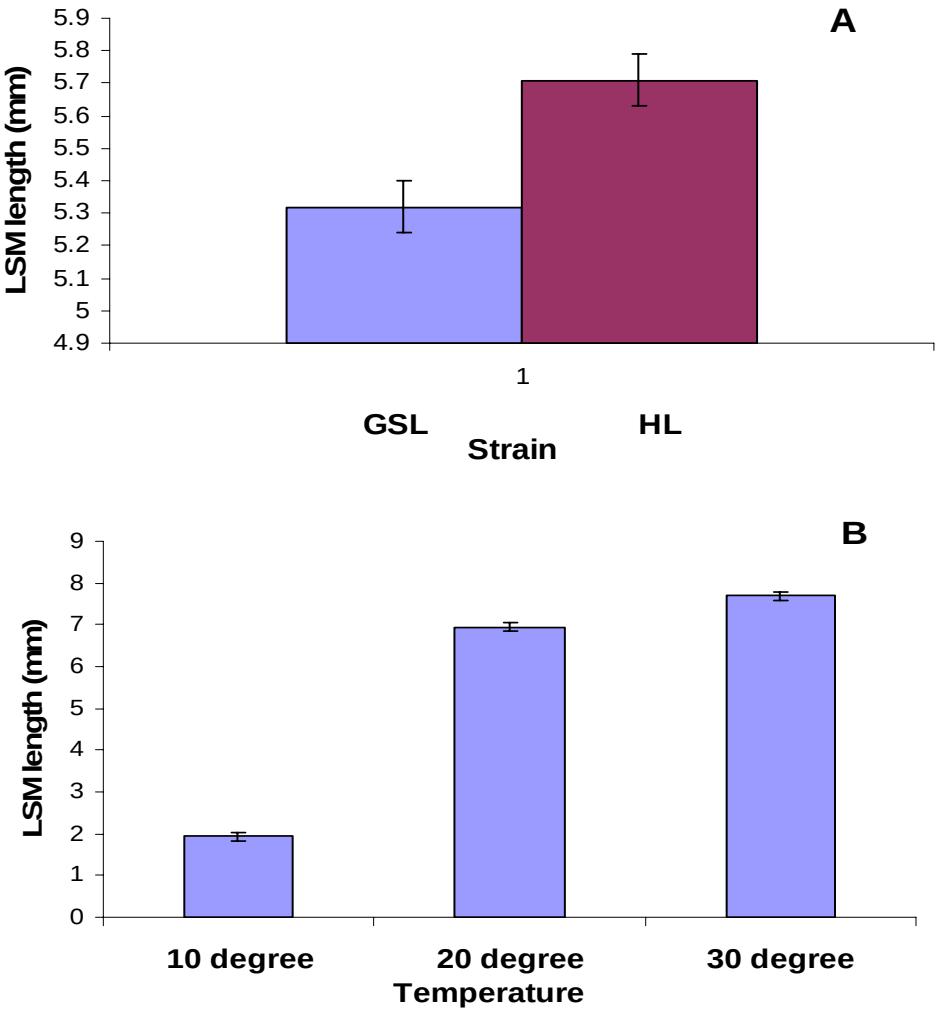


Figure 7-4: Least square mean lengths of *Artemia* (± SE) for (a) HL and GSL strains, averaged over temperatures and salinities, and (b)10°C, 20°C, and 30°C, averaged over strains and salinities.

Figure 7-5 shows the mean percentage survival of *Artemia* in each treatment combination of strain (GSL, HL), temperature (10°C, 20°C, and 30°C) and salinity (15 ppt and 35 ppt) after 14 days.

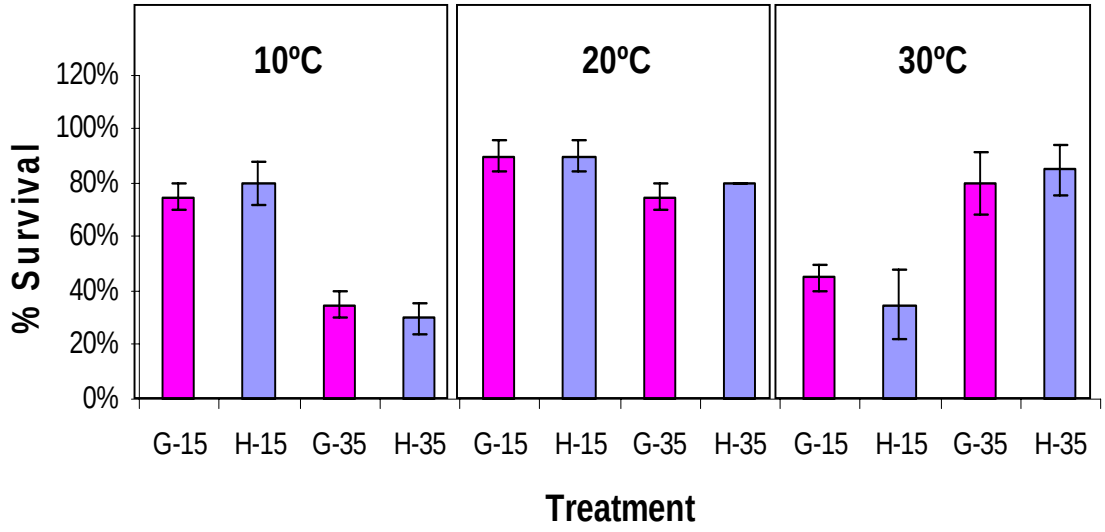


Figure 7-5: Mean survival (± SE) of *Artemia* from each treatment at the end of the 14 day trial period. Two strains, (G = GSL, H = Hutt Lagoon), two salinities, (15 ppt and 35 ppt), and three temperatures (10 ºC, 20 ºC and 30 ºC).

Three factor analyses of variance showed a significant main effect only for temperature and a significant interaction between temperature and salinity on survival at 14 days.

Comparisons of least square means showed that at 35 ppt, survival increased with increasing temperature, whereas at 15 ppt, survival increased from 10°C - 20°C, but decreased significantly at 30°C (Figure 7-6).

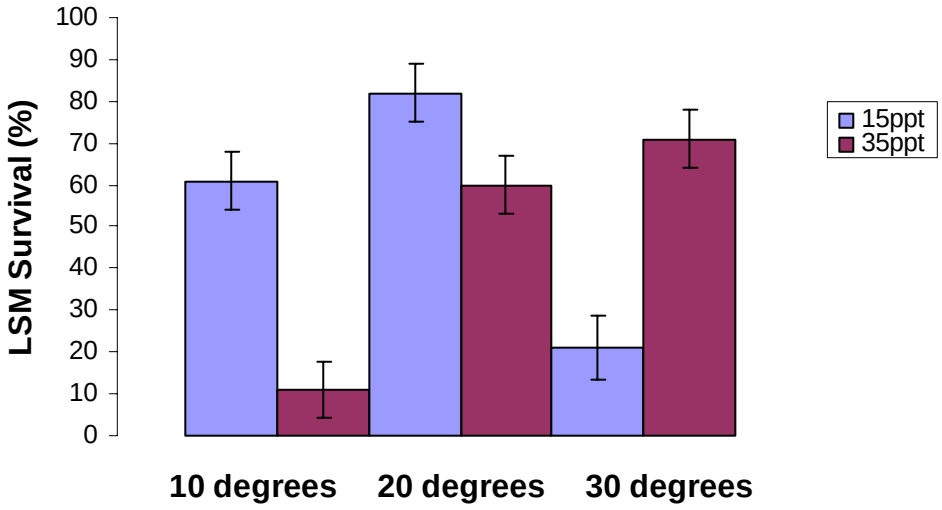


Figure 7-6: Least square mean percentage survival of *Artemia* (± SE) at 15 ppt and 35 ppt treatments at 10°C, 20°C and 30°C, averaged over strains.

These data demonstrate that growth is equivalent between 15 ppt and 35 ppt, which is encouraging for the use of *Artemia* for cropping nutrients in inland saline water. Survival, however, may be compromised during the peak of summer. Although *Artemia* are capable of surviving the winter temperatures experienced in Northam, in order to obtain good growth (and therefore be effective at cropping microalgae) the data suggests that temperature must be maintained above 20°C.

Artemia are generally considered to be a euryhaline warm water species and although it has been noted that salinity resistance of *Artemia* is high, a tolerance range from 3 ppt to 300 ppt as generalized for the genus *Artemia,* is not valid for all species of *Artemia,* particularly at lower salinities (<50ppt) (Vanhaecke et al., 1984). Data on the effects of low salinity on *Artemia* growth and survival are limited. Authors such as Gilchrist (1959); D'Agostino and Provasoli (1968); Hernandorena (1974); Cuellar (1990); and Browne and Wanigasekera (2000), all suggest that good growth and survival of *Artemia* can be achieved in 'low salinities', however these 'low salinities' were in the range of 32 ppt to 60 ppt. Although these salinities are indeed low relative to the upper salinity tolerance of *Artemia*, they are considerably higher than is typically used in static fish ponds in Australia. Partridge et al. (2008), for example, state that although groundwater salinities in Australia vary from fresh to hypersaline, those suitable for the culture of euryhaline fish average 21 ppt.

Although we intended to calculate algal consumption rates during this trial by comparing algal cell densities in the culture vessels at different times, observations of the exchange microalgal water taken on days 2, 3, 8, 9, 13 and 14 showed that there was a high rate of settlement occurring in all the culture wells (overall average of 48% ± 6% for all treatments). The experimental system was therefore modified in the following trial in order to obtain accurate ingestion data.

7.3 Laboratory Trial 2: Effect of temperature on growth and food consumption of GSL *Artemia* fed *Chaetoceros muelleri*.

7.3.1 Materials and Methods

The aim of this trial was to determine the effects of a more defined range of temperatures (10˚C, 15˚C, 20˚C, 25˚C, 30˚C and 35˚C) on the growth and ingestion rate of *Artemia*. Only GSL *Artemia* were used, and the trial was conducted only at 15 ppt salinity. Although HL *Artemia* were found to grow faster, GSL were selected due to their greater availability.

Artemia were not cultured in the 10 mL wells used in the first trial because of the high rates of algal settlement that occurred in these wells (Section 7.2.3). Instead, a population of *Artemia* was cultured in each of six 3 L flasks each held in a 30 L water bath and maintained at one of the six treatment temperatures at 15 ppt. *Artemia* cysts were de-capsulated and hatched at 28˚C for 24 hrs following a standard protocol (Partridge et al. 2003). After 24 hrs newly hatched nauplii were counted and added to the stock flask. The 3 L flasks were initially stocked with 2500 newly hatched *Artemia. Chaetoceros muelleri* was supplied at a rate that varied with size and age of the *Artemia,* based on data from Reeve (1962).

On days 1, 3, 5, 7, 9, 11 and 14, a subsample of *Artemia* were removed from the 3 L flasks and placed into six aerated experimental beakers in the same water bath in order to measure ingestion rate. On each day, different sized beakers were used and stocked with different numbers of *Artemia*, depending on their age and size (Table 7-1). This was to ensure that the decrease in microalgal density was measurable, whilst maintaining the minimum cell density required to maximize growth. *C. muelleri* were added to each beaker to create a final density of 2×10^6 cells/mL. After 24 hrs the *Artemia* were removed from each beaker and killed with acetic acid. They were placed on glass slides, photographed and measured as described in Section 7.2.2. *Artemia* consumption rate was also calculated as previously described.

Table 7-1: Volume of beakers, number of *Artemia* /beaker and density of *C. muelleri* added to beaker for measurement of ingestion rate in *Artemia* at different ages.

Age (day)	Length (mm)	Trial beaker volume	No. of *Artemia* in beaker	Density of *C. muelleri* added to beaker
13	10	100	4	2×10^6 cells/mL
11	7.5	50	4	2×10^6 cells/mL
9	5	50	10	2×10^6 cells/mL
7	2	50	20	2×10^6 cells/mL
5	1	25	50	2×10^6 cells/mL
3	0.5	25	50	2×10^6 cells/mL
1	0.2	25	100	2×10^6 cells/mL

7.3.2 Results and Discussion

Figure 7-7 shows the mean length of GSL *Artemia* after 14 days when cultured on a diet of *Chaetoceros muelleri* at six different temperatures at 15 ppt salinity. *Artemia* increased in size over the 14 days of the experiment and this increase in size was greater at higher temperatures.

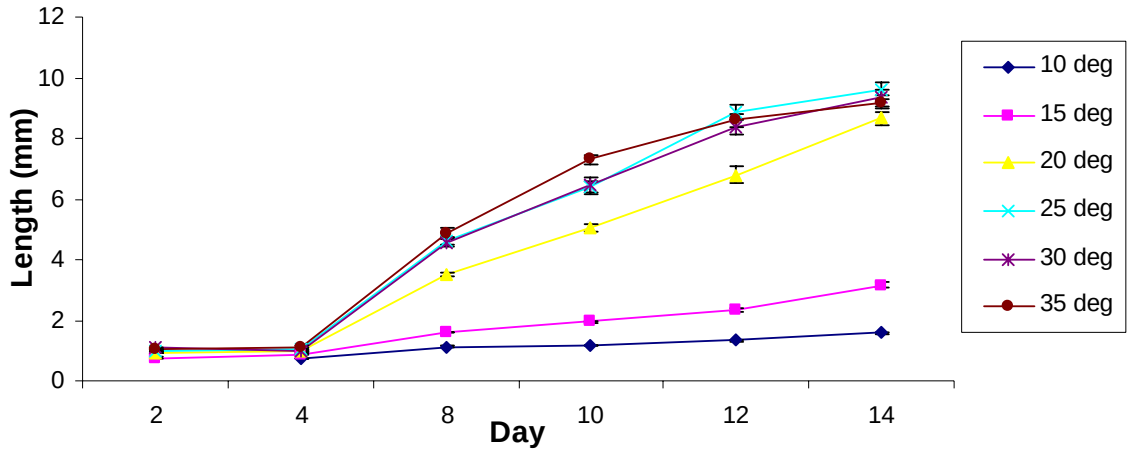


Figure 7-7: Average length (± S.E.) of GSL *Artemia* cultured at 15 ppt on *Chaetoceros muelleri* at 10°C, 15°C, 20°C, 25°C, 30°C and 35°C over 14 days.

Repeated measures ANOVA found a significant effect of both temperature and day of measurement on length, but no effect of replicates nested within temperature treatments. One way analysis of variance on mean lengths at day 14 confirmed that length was significantly

affected by temperature (P<0.0001). Post hoc comparison of means using Tukey-Kramer HSD showed that, in general, there were no significant differences between lengths at 10°C and 15°C or between lengths at 20°C, 25°C, 30°C and 35°C. However differences did occur between the lower temperatures (10°C and 15°C) and the upper temperatures (20°C, 25°C, 30°C and 35°C) (Figure 7-8). Maximum growth of *Artemia* was achieved at temperatures above 20°C with an optimum temperature range being between 25°C and 30°C. This compares well with the data of Gilchrist (1959), Johnson and Triantaphyllidis et al. (1998) which all show a similar maximum length of 8mm – 9mm being achieved after 14 days of culture. Temperatures between 25°C and 30°C have been previously identified as optimum and has been used widely in previous studies by, Gilchrist (1956; 1959); Dobbeleir et al. (1979); Claus et al. (1979); Sorgeloos et al. (1980); Nimura (1980); Johnson (1980); Browne (1980); Bossuyt and Sorgeloos (1980); Browne (1982); Brisset et al. (1982); Vanhaecke et al. (1984); Vanhaecke and Sorgeloos (1979); Abreu-Grobois (1991); Dhert et al. (1992); Nimura et al. (1994); Triantaphyllidis et al. (1995); Evejemo et al. (1997); Garcia-Ortega (1998); Evjemo and Olsen (1999); Naegel (1999); Browne and Wanigasekera (2000); Fabregras et al. (2001); Sorgeloos et al. (2001); Baxevanis et al. (2004); and Zmora and Shpigel (2006).

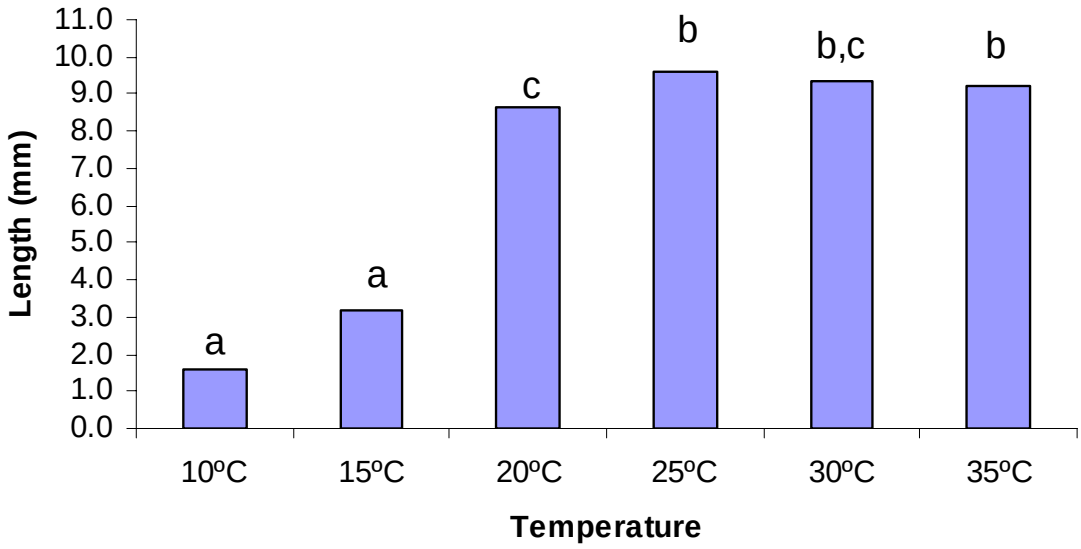


Figure 7-8: Mean lengths of *Artemia* at day 14 when cultured at six different temperatures on a diet of *Chaetoceros muelleri* at 15 ppt. Columns sharing the same letter are not significantly different from each other, using the Tukey-Kramer HSD test.

Figure 7-9 shows the average number of cells consumed per individual per day at 10°C, 15°C, 20°C, 25°C 30°C and 35°C. The figure shows that average individual consumption of cells increased over time with increasing temperature, and that the greatest increases were observed at the higher temperatures.

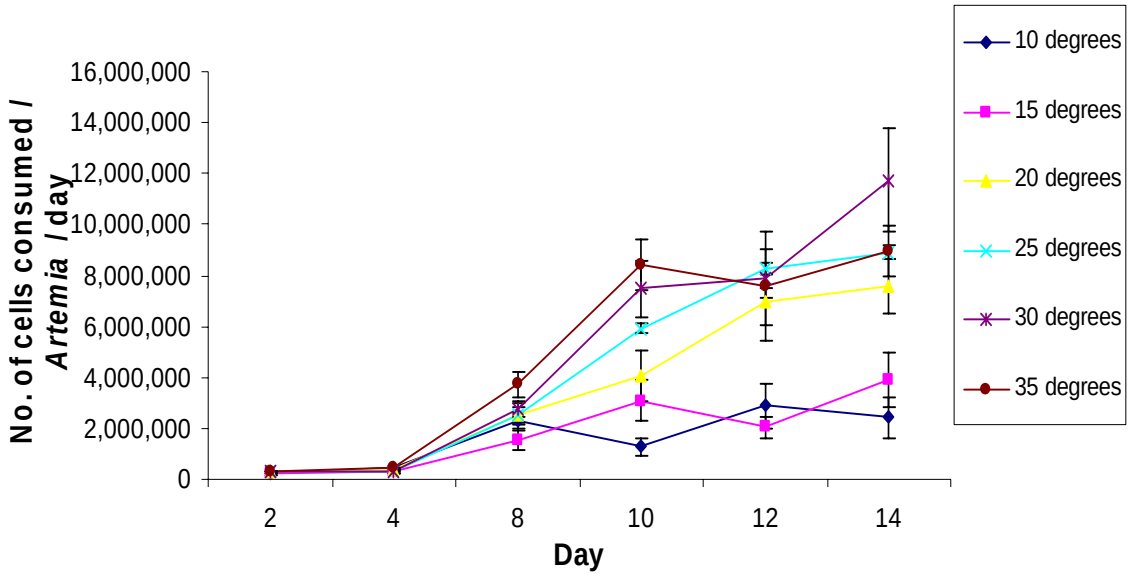


Figure 7-9: Daily average number of microalgae cells (± SE) consumed per individual GSL *Artemia* per day when grown at 10°C, 15°C, 20°C, 25°C, 30°C and 35°C and 15 ppt salinity.

Repeated measures analysis of variance found a significant effect of both temperature and day of measurement on consumption, but no effect of replicates nested within temperature treatments.

One way analysis of variance on mean individual cell consumption at day 14 confirmed that consumption rate was significantly affected by temperature (P = 0.0002). Post-hoc comparison of means using Tukey-Kramer HSD showed that, in general, there were no significant differences between consumption at 10°C and 15°C or between consumption at 20°C, 25°C, 30°C and 35°C. However differences did occur between 30°C and all other temperatures (Figure 7-10).

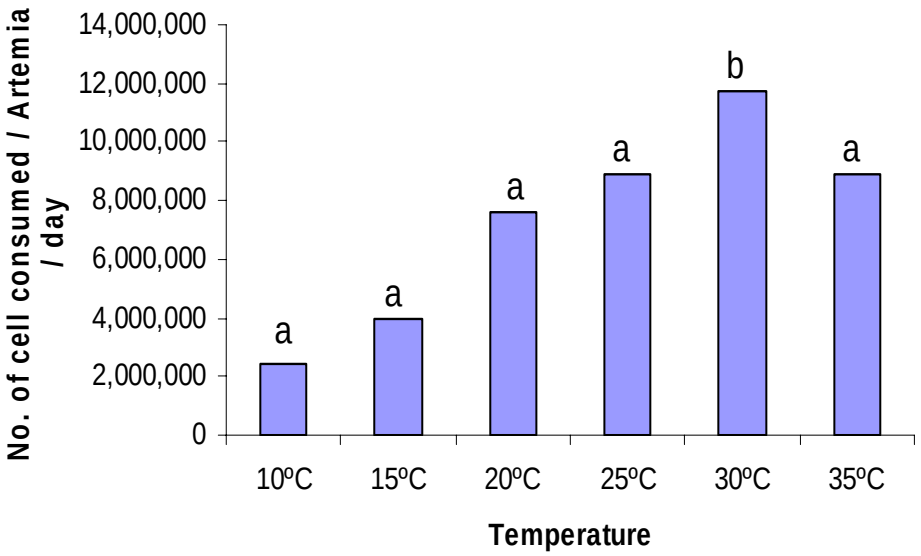


Figure 7-10: Mean individual algal cell consumption of GSL *Artemia* cultured for 14 days at 10°C, 15°C, 20°C, 25°C, 30°C, and 35°. Columns sharing the same letter are not significantly different from on another, using Tukey-Kramer HSD test.

Algal consumption data from this trial (Figure 7-9) were fitted with polynomial curves (Figure 7-11), which were then used to estimate consumption rates for *Artemia* of any age when cultured at temperatures between 10ºC and 30ºC. These values were then entered into a spreadsheet-based prediction model and a reduction in overall pond cell density calculated for a range of temperatures. An example of the outcome of such calculations for a stocking of 50 million *Artemia* into a 3 million litre pond (ie. the size of the Northam SIFTS ponds) is given in Figure 7-12.

Based on these results, at 20°C an individual *Artemia* will consume a total of 51×10^6 *Chaetoceros muelleri* cells over a 14 day period. Using the assumptions detailed in Table 7-2, we have calculated that over a typical production cycle of barramundi, where 1800 kg of food containing 45% protein is added to a static pond, approximately 2.5 tonnes of *Artemia* could be produced. Given that the retail value of *Artemia* biomass is approximately $25-$36/kg (Gavine and Bretherton, 2007), this secondary harvest of *Artemia* can contribute significantly to the value of the aquaculture venture.

Table 7-2: Parameters used to calculate the expected harvest of *Artemia* biomass from static inland saline ponds

	Parameter	Value	Reference
1	Soluble nitrogenous excretion rate of barramundi	60% of ingested N	(Almendras, 1994)
2	Protein content of barramundi food	45%	-
3	Uptake rate of soluble nitrogen by *C. muelleri*	100%	Assumed
4	Protein content of Day 14 *Artemia*	60% DW	(Sorgeloos et al., 1986)
5	Dry weight of Day 14 *Artemia*	0.33 mg	Figure 7-2
6	Total *C. muelleri* cells consumed in 14 days per *Artemia*	5.10×10^{7} cells	Figure 7-11
7	Temperature	20°C	-
8	N content of *C. muelleri* per cell	9.8×10^{-13} g/cell	(Leonardos and Geider, 2004)
9	N retention efficiency of *Artemia*	63%	Calculated based on 4, 5 & 6
10	Water content of Day 14 *Artemia*	80%	Assumed

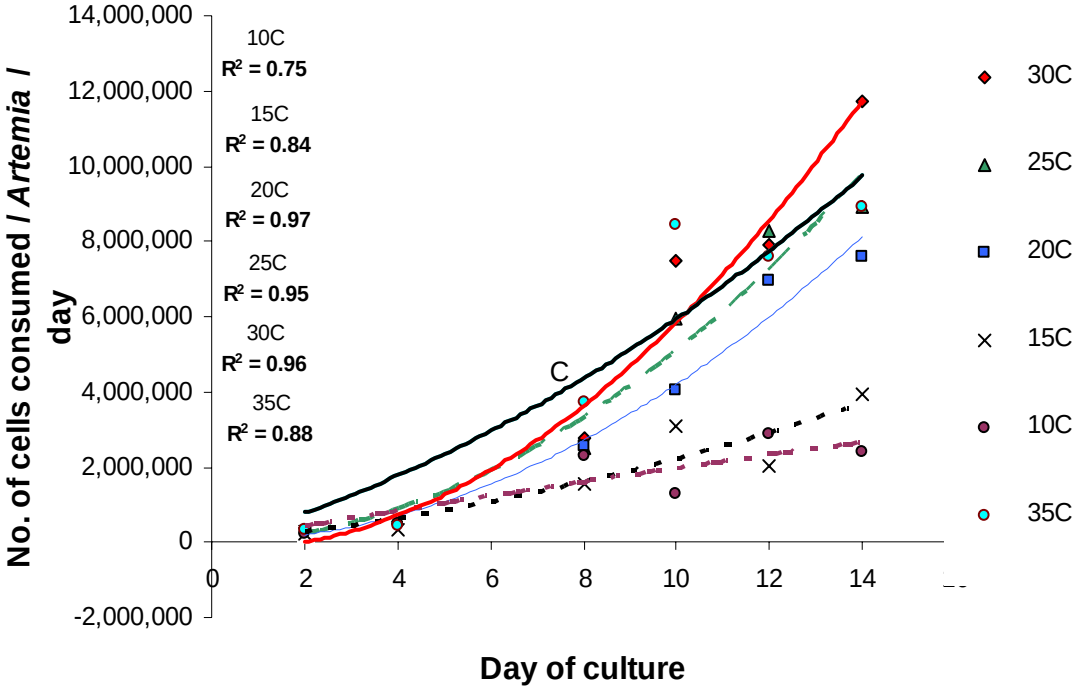


Figure 7-11: Consumption graph showing the predicted average number of cells consumed per individual per day when GSL *Artemia* are grown in one of five different water temperatures on a diet of *Chaetoceros muelleri* at 15 ppt. Curves were fitted from data obtained in laboratory experiments (Figure 7-9). r^2 values show the proportion of variation in algal consumption explained by the polynomial functions.

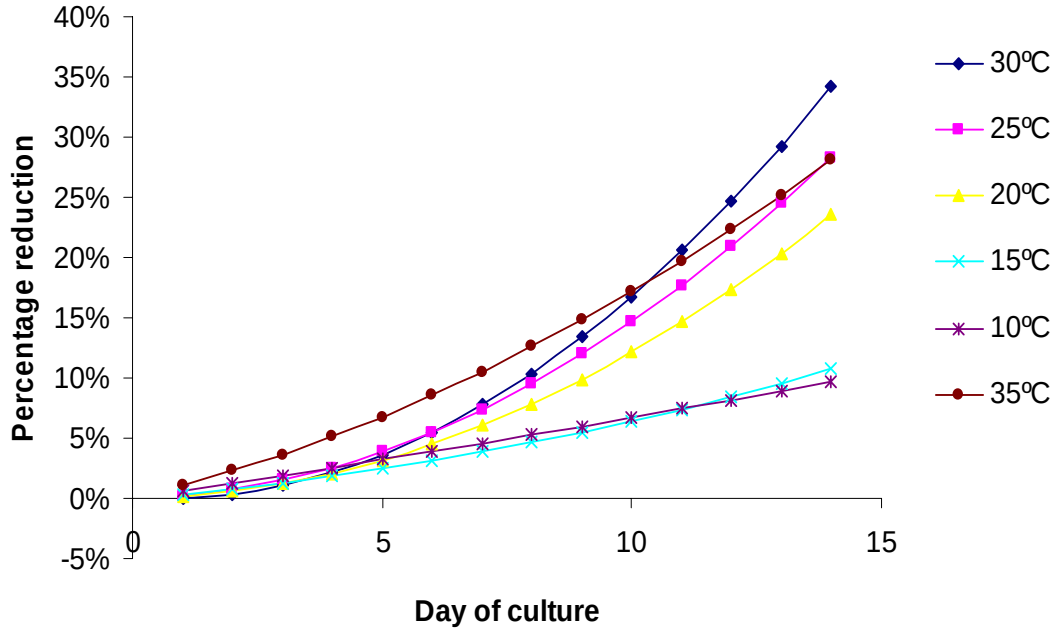
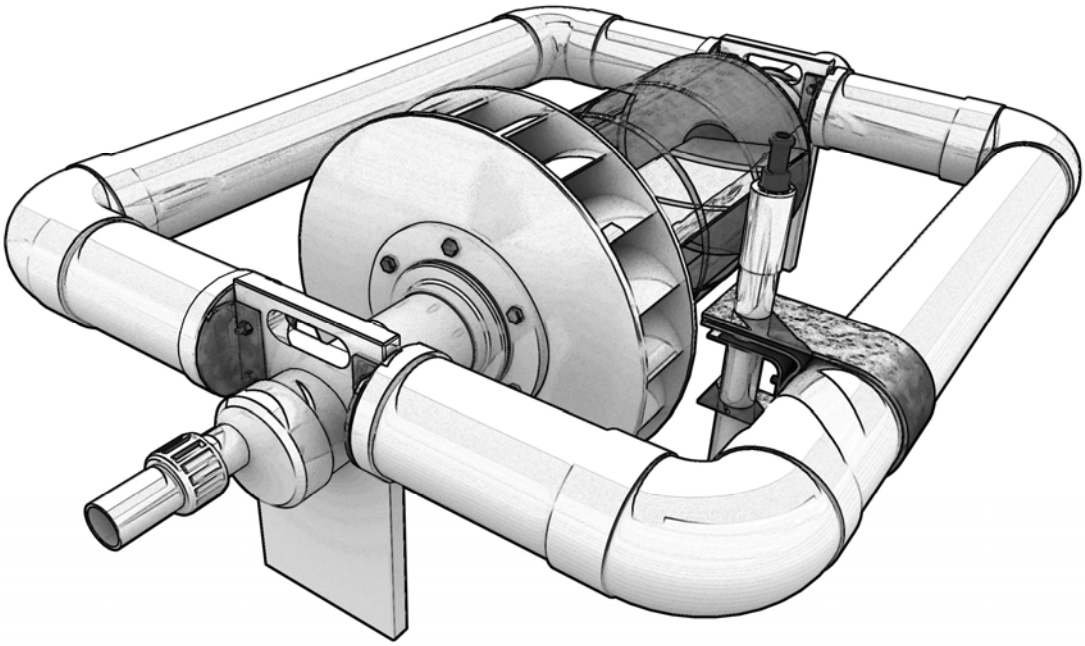


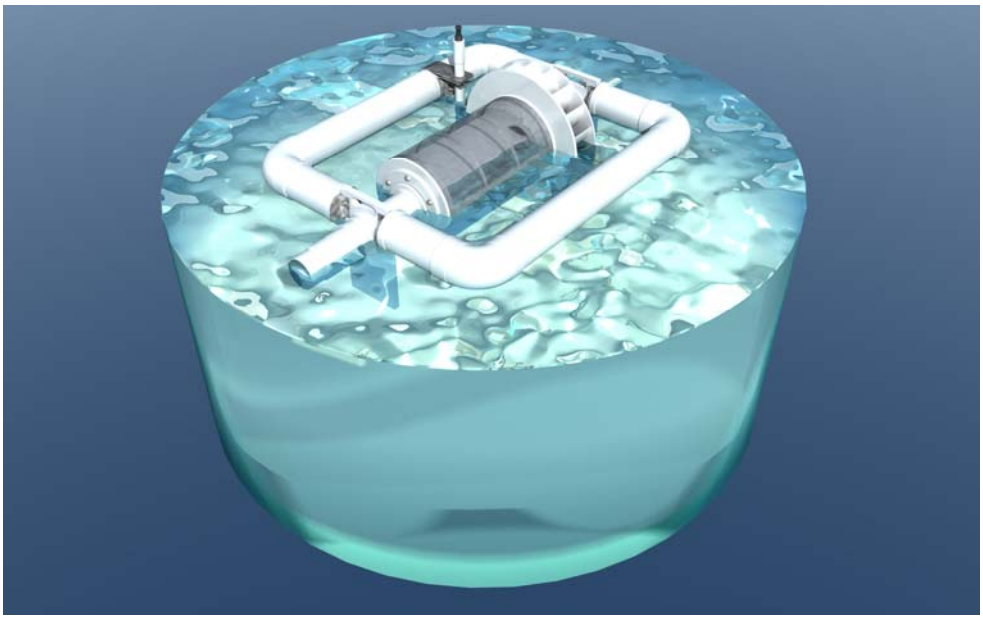
Figure 7-12: Predicted daily percentage reduction in algal cell density in a pond stocked with 50 million GSL *Artemia* at 10°C, 15°C, 20°C, 25°C, 30°C and 35°C. Calculated using predicted consumption chart (Figure 7-11) and a spreadsheet model.

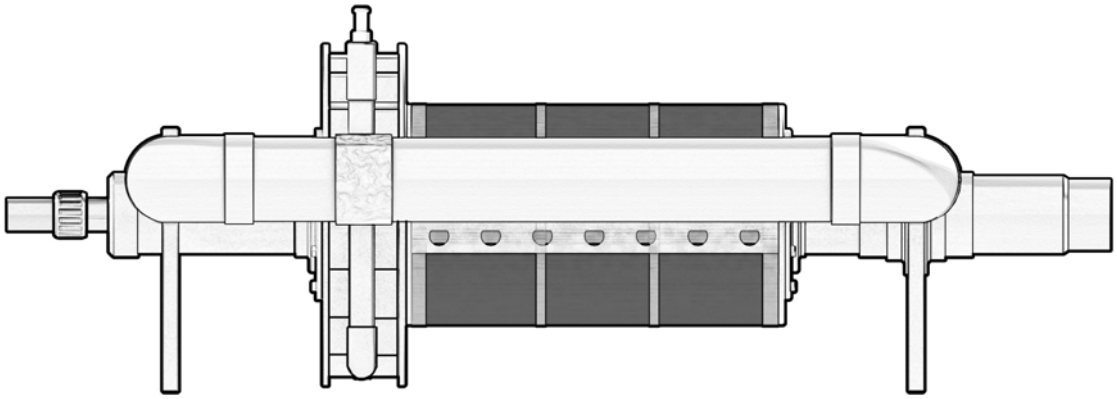
7.4 Growth and Algal Consumption of GSL *Artemia* Cultured in a Static Inland Pond, 2006/2007.

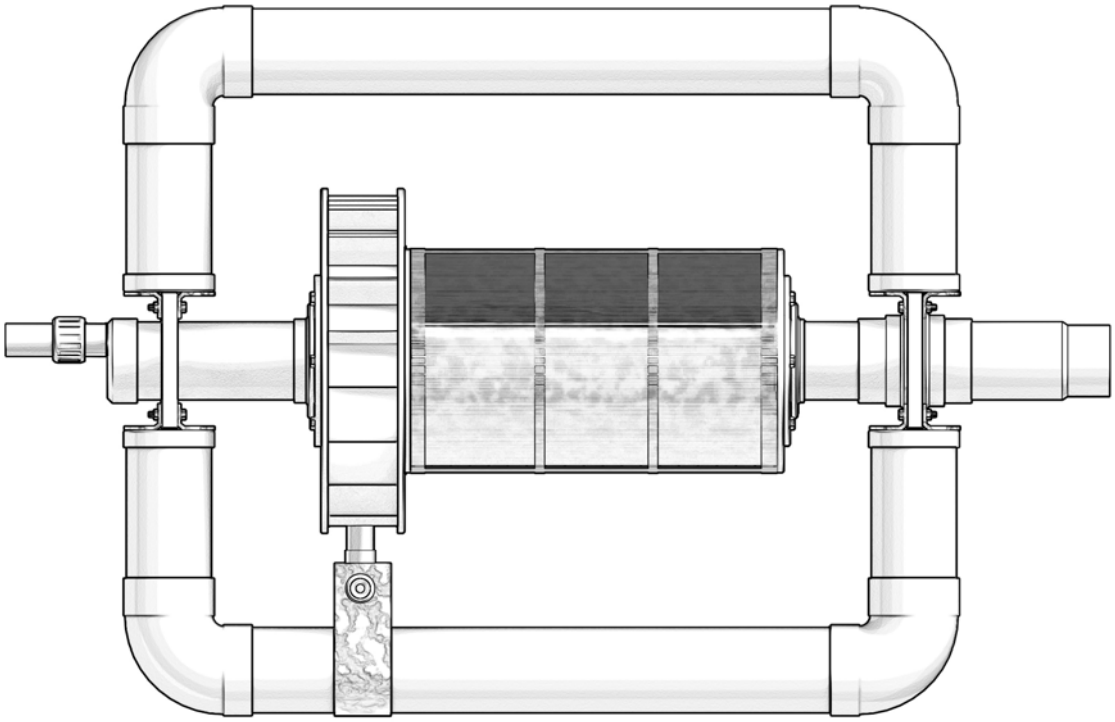
7.4.1 Methods

A purpose-built, rotating screen filter was designed and built to retain *Artemia* within a 10,000L SIFT (Figure 7-13). The air driven rotating screen was designed and constructed in collaboration with McRobert Aquaculture Group, as no commercially available screen allowed large volumes of algal rich pond water (>250L/min) to pass through the SIFT whilst retaining the *Artemia*. The screen was fitted with an internal air knife, which constantly injected a thin blade of air along the inside of the constantly rotating 80 μm stainless steel screen allowing the device to be self-cleaning. The filter was driven by a large paddle-wheel-like mechanism at one end that was driven by an air water lift pump.









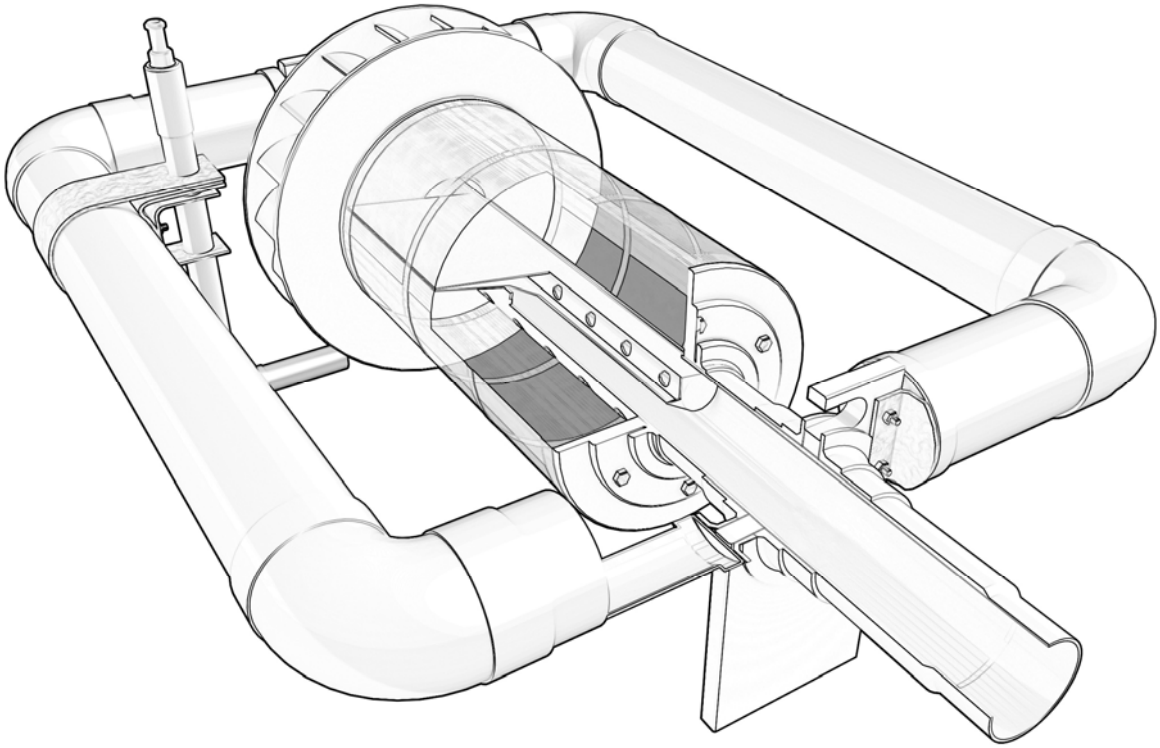


Figure 7-13: Purpose built rotating screen filter for retaining *Artemia* within modified SIFTS.

Two experiments were conducted during which this modified SIFT was stocked with newly hatched *Artemia* at a density of 5/mL (50,000,000 total). Results from the first trial were used to improve the experimental design for the second trial. In both trials, water was pumped through this SIFT at the rate of 160 L/min. Using 12 VDC peristaltic pumps, 24-hour composite water samples were collected daily from the inlet and outlet of the SIFT. The difference in microalgal density between these inlet and outlet samples, combined with the flow rate through the SIFT was used to calculate the number of cells of microalgae ingested (assuming that no cells proliferate within the SIFT).

7.4.2 Results and Discussion

Trial 1

At the end of this 14 day trial, no *Artemia* remained in the SIFT. This led us to three potential hypotheses. Firstly we hypothesised that the *Artemia* starved due to an inability to either ingest or digest the small (ca. 2 µm) green microalgal cells which dominated the pond during this trial. Secondly, we considered that the *Artemia* may have escaped or thirdly that they had been predated on, or out-competed by the other invertebrates which were concentrated within the SIFTS after being drawn into it through the unscreened inlet.

Trial 2

Based on the results from our laboratory trials it was deemed that the density of microalgae within the pond at the start of this trial was sufficient to sustain newly hatched *Artemia* for five days. We therefore filled the SIFT with this water (via an inlet screened to 100 µm) and left it

without flow for five days. This enabled us to test the hypothesis that *Artemia* were unable to survive on this species of algae without the potentially confounding effects of possible escapes or the introduction of competitors or predators.

On day 5 we observed many *Artemia* within the SIFT, demonstrating that they were capable of surviving on this species of microalgae. The flow was subsequently turned on (whilst maintaining the 100 µm screened inlet), however, we noticed the following day that there were many *Artemia* in the outflow. The flow was therefore turned off and remained off until Day 9 before being restored at this time with a screened outlet. This screen remained in place until the end of the trial on Day 14, however, no *Artemia* were collected in it between Day 9 and 14. This suggests that the remaining *Artemia* within the SIFTS had grown to a size where they were unable to escape, or that the hole in which they had previously escaped had become blocked. At the completion of the trial, the rotating screen was removed from the SIFT and a small, salt-encrusted gap between the rotating mechanism and its seal was observed and repaired.

A total of 1 million *Artemia* were harvested from the SIFT on day 14 with a wet weight biomass of 250g. Although the final survival was only 2%, we were unable to determine how many *Artemia* escaped and this figure is therefore not an accurate measure of survival.

The *Artemia* remaining in the SIFT reached an average length of 2.7 mm after 14 days. Water temperature over these 14 days averaged 23.3°C. Comparison of the growth rate of the *Artemia* from this field trial and those grown at 20°C and 25°C in the previously described laboratory trials is shown in Figure 7-14. These data clearly indicate that the growth of *Artemia* within the pond was considerably slower than in the laboratory. Figure 7-15 shows the microalgal cell density in the pond (i.e SIFT inlet) and outlet. This data shows that the microalgal density within the SIFT was high, so the *Artemia* should not have been food limited. The small difference between the inlet and outlet densities is likely to be largely due to the low numbers of *Artemia* within the SIFT following the escapes.

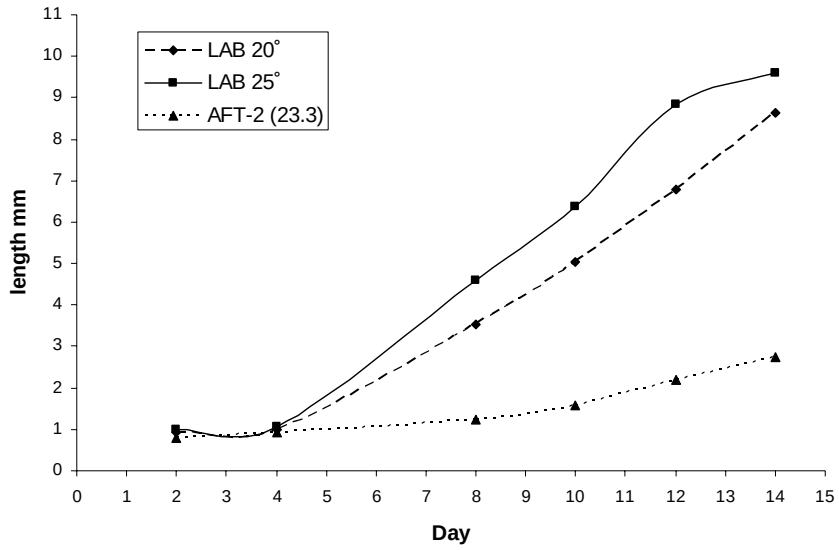


Figure 7-14: Average daily length of *Artemia* grown in the field at 23.3 °C compared to those grown in the laboratory at 20°C and 25°C.

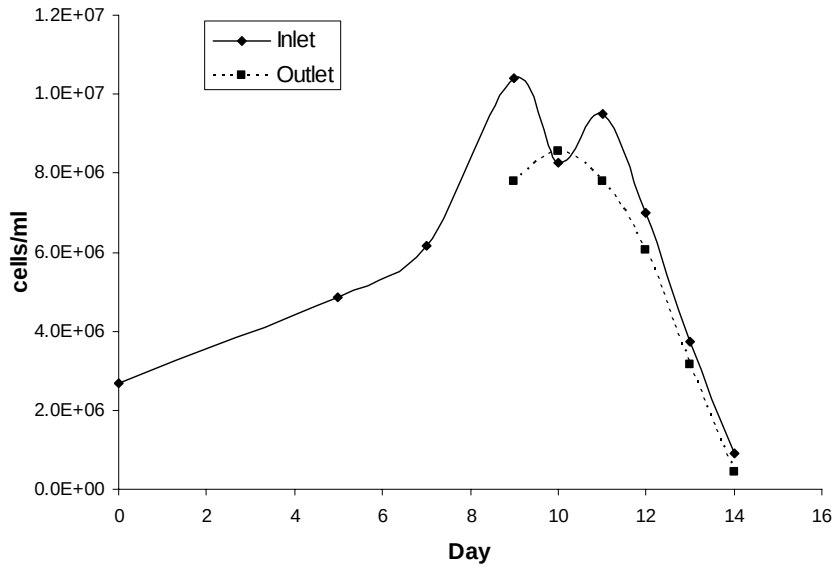


Figure 7-15: Density of algal cells within the SIFT pond water as it enters and leaves the *Artemia* SIFT during the second field trial.

Although microalgae are generally an excellent food source for *Artemia*, not all species are suitable (Lavens and Sorgeloos, 1996). d'Agostino (1968) for example showed that the thick cell wall of *Chlorella* sp makes it indigestible by *Artemia*. We therefore hypothesise that the poor growth obtained in the field trial was due to the dominant algae being either nutritionally poor or not highly digestible. Indeed, the microalgae within the pond were of similar size, shape and colour to *Chlorella*. Alternatively, the very high cell densities observed in this pond may

have impeded the *Artemia's* ability to efficiently ingest it (Nimura, 1980). These hypotheses were tested in a laboratory study prior to conducting further field trials.

7.5 Laboratory Trial 3: Effect of Temperature on Growth and Food Consumption of GSL *Artemia* Fed *Nannochloropsis oculata*.

7.5.1 Materials and Methods

In order to test the theories above, the current trial was conducted to determine the effect of *N. oculata* on the survival and growth of *Artemia* at a salinity of 15 ppt and a range of temperatures (10˚C, 15˚C, 20˚C, 25˚C, 30˚C and 35˚C). *N. oculata* was selected for this trial as it is a single celled, non-motile chlorophyte similar in size and shape to the microalgal species which dominated the inland saline aquaculture ponds in Northam during 2006 and 2007.

Artemia were cultured in 3 L flasks in six 30 L water baths, maintained at a temperature of 10, 15, 20, 25, 30 or 35˚C. An additional 20˚C water bath was established to house a control culture of *Artemia* fed only *C. muelleri*. *Artemia* cysts were de-capsulated and hatched at 28˚C for 24 hrs following a standard protocol (Partridge et al., 2003). After 24 hrs, newly hatched nauplii were counted and added to the stock flask. The 3 L flasks were initially stocked with 7,500 newly hatched *Artemia*. A density of 5×10^6 cells per mL of *N. oculata* was maintained in the 3 L stock flasks to ensure *Artemia* were not food limited for the duration of the trial. This ration was calculated by multiplying the anticipated number of *C. muelleri* cells consumed per hour (as calculated in the previous trial (Section 7.3.2) by 15, as *N. oculata* is 15 times smaller (in volume) than *C. muelleri* (Brown et al. (1989); Payne and Rippingale (2001) and because Reeve (1963) showed that cell volume is the primary determinant to ingestion rate.

On days 1, 3, 5, 7, 9, 11 and 14, *Artemia* were removed from the 3 L flasks and placed into six clean, aerated beakers in the same water bath in order to measure ingestion rate. The beakers were of different sizes and contained different numbers of *Artemia* on each day, depending on the age and size of the *Artemia* (Table 7-3). *N. oculata* were added to each beaker to create a final density of 5×10^6 cells/mL on days 1, 3, 5 and 7, 6×10^6 cells/mL on day 9 and 10×10^6 cells/mL on days 11 and 14. The additional water bath followed the same routine, however *C. muelleri* was fed to the *Artemia* at the same ration as the previous trial (2×10^6 cells/mL) to act as a control.

After 24 hrs the *Artemia* were removed from each beaker and killed with acetic acid. They were placed on glass slides, photographed and measured as described in Section 7.2.2. Algal consumption was also calculated as described in Section 7.3.1.

Table 7-3: Volume of beakers, number of *Artemial*beaker and density of *N. oculata* added to beaker for measurement of ingestion rate in *Artemia* at different ages.

Age (day)	Length (mm)	Trial beaker volume (mL)	No. of *Artemia* in beaker	Density of *N. oculata* added to beaker
14	10	100	4	10×10^6 cells/mL
11	7.5	100	4	10×10^6 cells/mL
9	5	50	6	6×10^6 cells/mL
7	2	50	25	5×10^6 cells/mL
5	1	25	25	5×10^6 cells/mL
3	0.5	25	50	5×10^6 cells/mL
1	0.2	25	50	5×10^6 cells/mL

7.5.2 Results and Discussion

Overall survival of *Artemia* fed N. oculata was poor at all temperatures with 100% mortality occurring after day four for the 20°C, 25°C, 30°C and 35°C treatments and day six for the 10°C treatment. It is speculated that starvation resulted in the high mortality rate (see below) and that the *Artemia* in the 10°C treatment were able to survive slightly longer due to the slowing effect of low temperature on their metabolism. Figure 7-16 shows the daily mean length of GSL *Artemia* when cultured on a diet of *Nannochloropsis oculata* at the five different temperatures. Consistent with previous laboratory trials, *Artemia* fed *C. muelleri* at 20°C exhibited good growth.

One way analysis of variance at day 4 showed a significant difference in lengths of *Artemia* fed *N. oculata* at the five different temperatures ($P < 0.0001$)(Figure 7-17). Post-hoc comparison of means using Tukey-Kramer HSD showed that significant differences in length occurred between the 10ºC treatment and all other temperatures. On Day 4, *Artemia* fed *C. muelleri* at 20°C were significantly larger than those fed *N. oculata* at the same temperature ($P < 0.0001$).

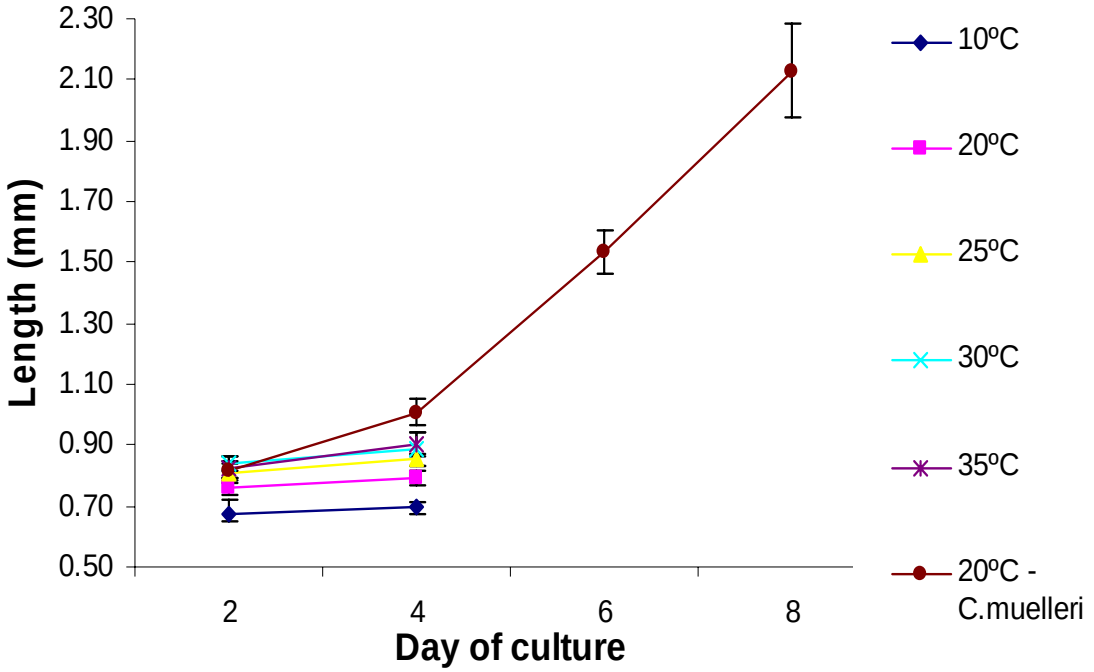


Figure 7-16: Daily mean length (with standard error) of GSL *Artemia* cultured on *N. oculata* at 10°C, 20°C, 25°C, 30°C and 35°C as well as the mean length of a control batch of GSL *Artemia* fed *C. muelleri* at 20ºC

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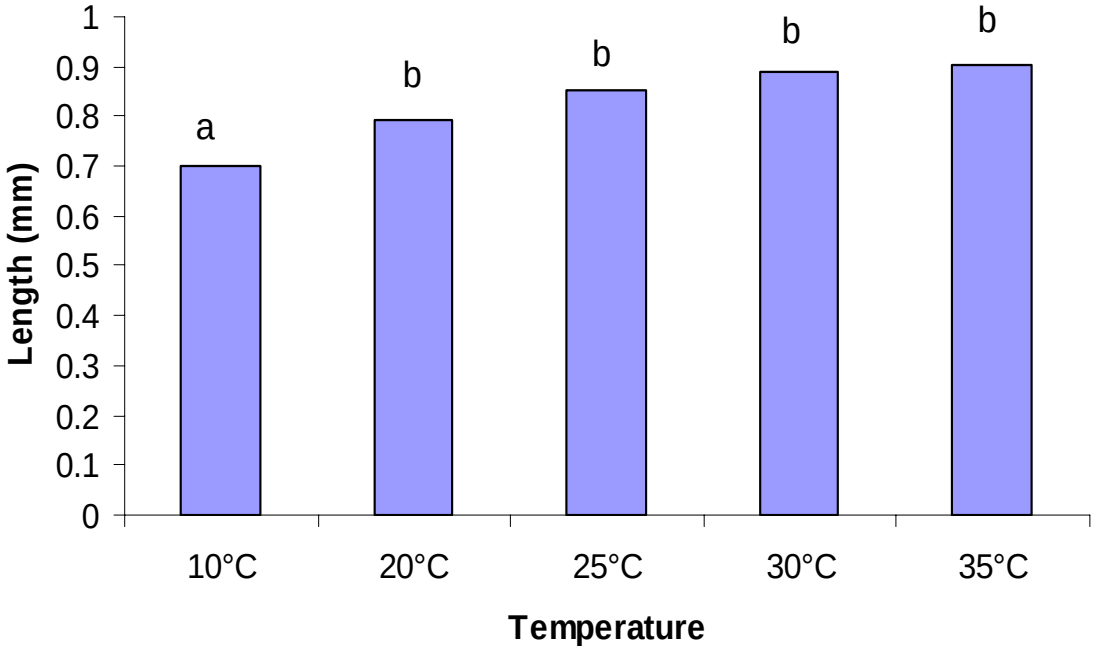

Figure 7-17: A post hoc comparison of mean individual length on Day 4 of *Artemia* cultured at 10°C, 20°C, 25°C, 30°C and 35°C using Tukey-Kramer HSD test. Columns sharing the same letter are not significantly different from one another.

Figure 7-18 shows the daily mean individual cell consumption rate of GSL *Artemia* when cultured on a diet of *Nannochloropsis oculata* at five different temperatures (10°C, 20°C, 25°C, 30°C and 35°C) and of a control batch of *Artemia* cultured at 20°C on *C. muelleri.*

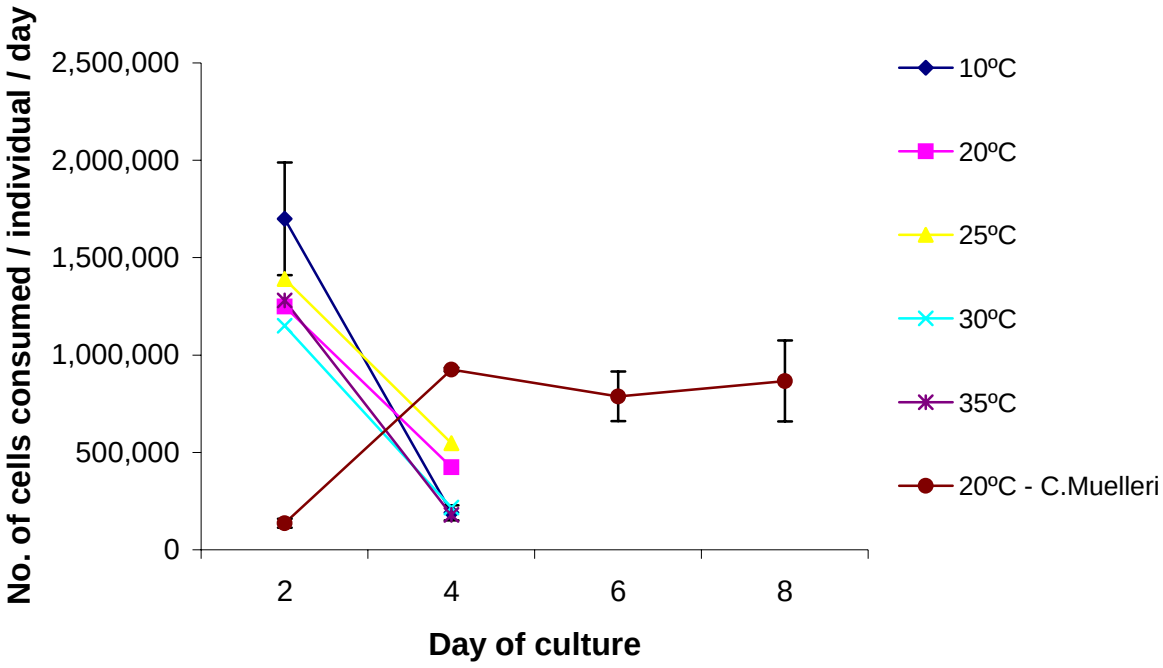

Figure 7-18: Mean number of *N. oculata* cells consumed per individual per day when grown at each of the five temperatures (10°C, 20°C, 25°C, 30°C and 35°C) and a control culture of GSL *Artemia* fed only on *C. muelleri*.

Cell consumption rapidly decreased in all the treatments fed on *N. oculata* between day two and four. The 20ºC vessel fed *C. muelleri* showed a stable consumption rate. A one-way analysis of variance showed no significant difference between algal consumption rates on Day 4 of *Artemia* fed *N. oculata* at different temperatures ($P = 0.11$), but a significant difference between algal consumption rates of *Artemia* fed *N. oculata* or *C. muelleri* at 20°C ($P < 0.0001$).

Although many species of marine microalgae have been used in mariculture operations, not all are equally successful in supporting growth and survival (Brown et al., 1989). There have been no previous studies on the survival and growth of *Artemia* on *N. oculata*, but bivalve larvae and juveniles of the species *Ostrea edulis* and *Mytilus edulis* fed pure cultures of *Nannochloropsis* resulted in poor growth and survival (Walne, 1970). The poor performance of *Artemia* fed *N. oculata* may be due to issues of digestibility. D'Agostino (1968), for example showed that the

thick cell wall of *Chlorella* sp makes it indigestible by *Artemia*. Alternatively, the issues may relate to nutritional deficiencies in the algae. Sick (1976) found that the highest rates of growth among *Artemia* occurred when they where fed microalgal cells with relatively large protein and energy reserves and Webb and Chu (1983) and Chan (1978) found that there is a significantly lower protein content in *N. oculata* cells (55fg/µm^3) than *C. muelleri* cells (129fg/µm^3). The concentration of PUFAs found in *Nannochloropsis* (<0.5fg/µm^3) are also lower than the minimum amount considered by Brown et al. (1989) to be required to achieve good growth and survival. Growth may also have been limited by the low ascorbic acid content of *N. oculata*. Ascorbic acid performs a range of important physiological and biochemical functions in plants and animals (Tobert, 1979) and is believed to be responsible for triggering moulting in crustaceans (Desjardins, 1985). Brown and Miller (1992) tested the ascorbic acid content of eleven species of microalgae commonly used in mariculture and found that *N. oculata* had a lower ascorbic acid content (0.6% dry weight) than *Chaetoceros* which had the highest (1.87% dry weight) of all eleven species tested.

7.6 Growth and Algal Consumption of GSL *Artemia* Cultured in Static Inland Ponds, 2007/2008.

7.6.1 Materials and Methods

This field trial was run in the same manner as those described in Section 7.4.1, with the exception that the SIFTS themselves remained static after filling, as regular algal sampling showed cell densities within the SIFT remained well above the minimum feed requirement levels.

7.6.2 Results and Discussion

The dominant algal species was again the green picoplankton 2 to 2.5 µm in diameter. Once the SIFTS was filled with pond water all incoming flow was turned off, as cell density within the SIFTS remained high enough to ensure an adequate food supply (average density $7.3 \times 10^6 \pm 0.6 \times 10^6$). No ingestion data were therefore collected. Daily mean pond temperature for this trial was 22.8°C ± 0.26°C, similar to the previous field trials.

Growth and survival of *Artemia* in this field were compared to predictions based on the performance of GSL *Artemia* fed on *N. oculata* in the laboratory (see Section 7.5.2). Observed survival (Figure 7-19) and growth (Figure 7-20) were slightly better than predicted.

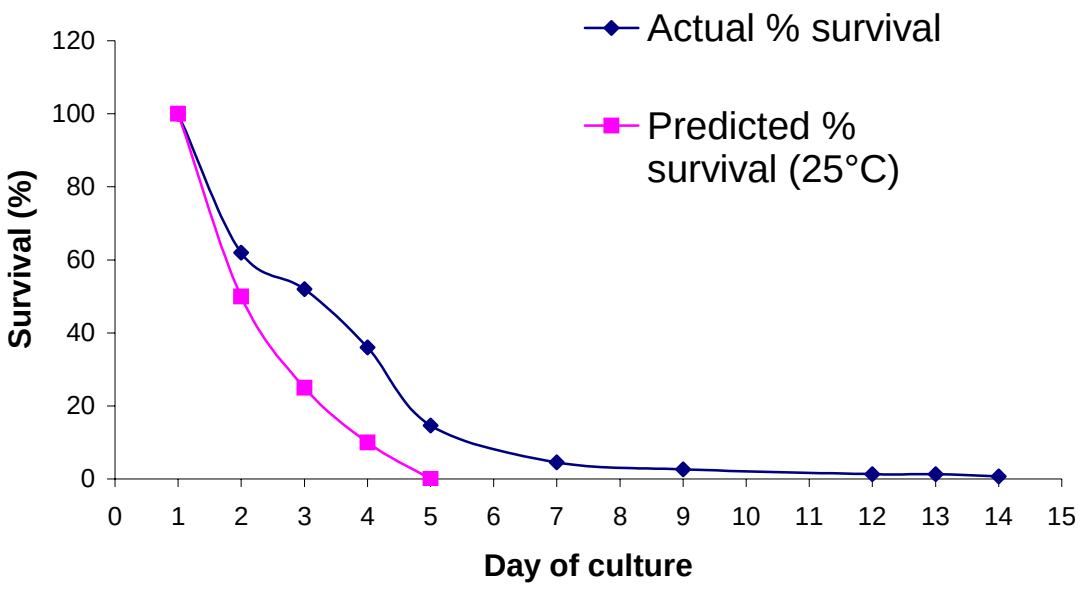

Figure 7-19: Predicted survival of GSL *Artemia* grown at 25ºC and actual survival of GSL *Artemia* in the field (mean pond temperature 22.8ºC ± 0.26ºC)

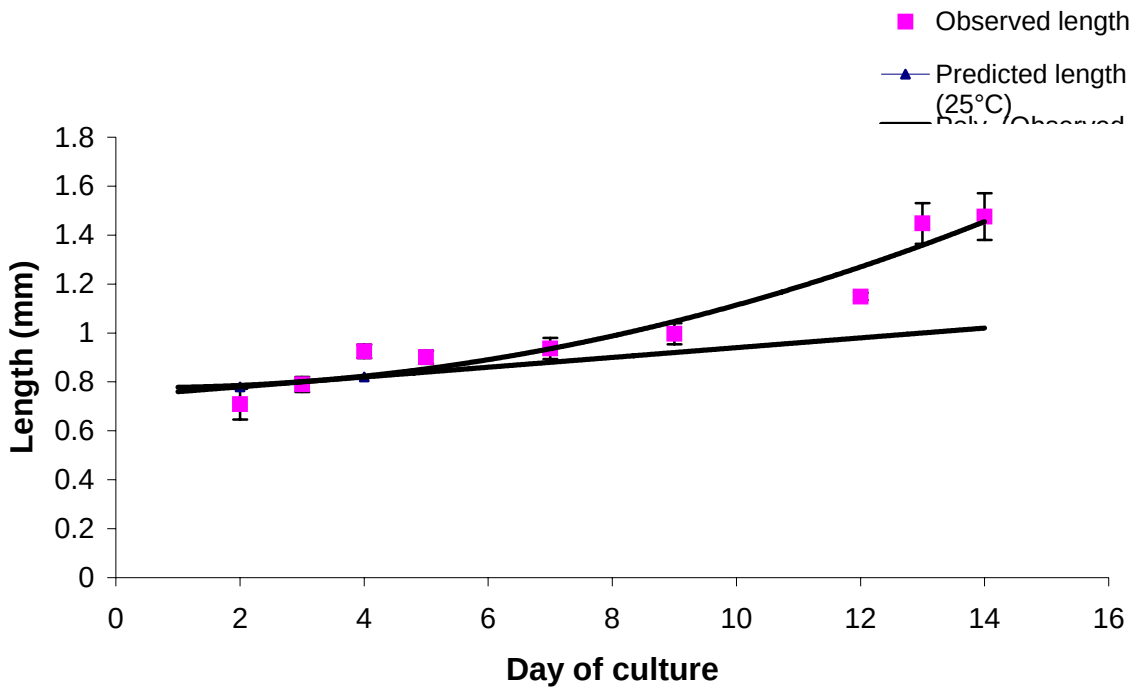

Figure 7-20: Predicted mean daily length of GSL *Artemia* grown at 25ºC and actual mean daily length (with standard error) of *Artemia* in the field (mean pond temperature 22.8ºC ± 0.26ºC)

The results of this field trial support our hypothesis that this small green flagellated algae is an unsuitable food source for *Artemia*. The slightly improved performance of *Artemia* in the field, compared with the laboratory may be due to the algae being a different species than *N. oculata*, or the additional presence of less abundant prey items in the pond for the *Artemia*. It is believed, for example, that some bacteria play a positive role on animal nutrition. Brown et al. (1989) suggests that bacteria in the animal gut could provide essential micronutrients lacking in the algae or aid in the digestion of algal components by breaking down polysaccharides and proteins, thereby liberating nutrients that might otherwise be unavailable. This may explain why *Artemia* were able to survive the full 14 days in the field (even though the nutritional value of the microalgae was low) compared to the 5 days predicted.

7.7 *Artemia* Conclusions

The laboratory trials described in this chapter highlight that *Artemia* can grow and survive at the temperatures and salinities typical of static inland saline ponds in temperate Australia. They also suggested that if *Artemia* perform as indicated in the laboratory trials and if stocked at sufficient densities, they have the capacity to ameliorate the negative effects of strong microalgal blooms which occur in static semi-intensive ponds. When stocked at such densities, the quantities of *Artemia* harvested would provide a secondary crop with significant value.

The field trials designed to confirm the results achieved in the laboratory highlighted the difficulties in achieving this goal. Variation in temperature, nutrient input and sometimes salinity leaves phytoplankton growth in these semi–intensive, outdoor systems susceptible to rapid changes in species composition and cell density and as we have shown, not all species which bloom naturally are suitable for supporting growth and survival of *Artemia*. Such unpredictability makes it difficult to produce constant supplies of adult *Artemia* and to predict microalgal consumption rates in order to help manage nutrient inputs. One way of overcoming the difficulties experienced in culturing *Artemia* in these kind of external environments, is to develop stable, micro-algal monocultures of species which are suitable as a food source for *Artemia*. Although algal monocultures are routinely produced under laboratory conditions (Partridge et al., 2003), limited literature is available on attempts to develop such monocultures in open pond systems. High salinity is used to produce monocultures of algae such as *Dunaliella salina*, however, the high salinities required to keep this species in monoculture are unsuitable for fish production. The rotating screen developed during this study was shown to be effective in retaining *Artemia* and also for concentrating natural zooplankton. Thus the production system we have developed shows potential for growing *Artemia* in hypersaline environments where microalgal species composition would be more stable and in which *Artemia* have no competitors or predators.

8. THE USE OF GAMBUSIA AS A FEED FOR BARRAMUNDI IN INLAND SALINE PONDS

8.1 Introduction

Gambusia holbrooki (mosquito fish) are native to Southern and Eastern USA, but were introduced to Australia in the 1930s to control mosquito larvae (Coy, 1979). *Gambusia* can survive in a wide array of habitats, including highly degraded waters with extremely low levels of oxygen and salinities up to at least 58 ppt and their hardiness has allowed them to flourish throughout most of south Western Australia (Morgan et al., 2004). During our preliminary assessment of SIFTS in static saline water bodies, we obtained excellent FCR with barramundi (0.90) over 138 days. The pond in which these SIFTS were located had an abundance of *Gambusia*, which we observed being drawn into the SIFTS via the airwater lifts. Despite anecdotal evidence suggesting *Gambusia* is unpalatable to other fishes, we hypothesised that the barramundi were eating and gaining at least some nutritional benefit from them. In order to test this hypothesis we conducted a laboratory trial in which the growth of barramundi was compared between treatments fed iso-calorific rations of the following diets over 12 days:

1. Live *Gambusia.*
2. Skretting, Nova ME pellets (45% protein, 20% lipid).
3. 50:50 ration of both feed types.

8.2 Materials and Methods

Gambusia

Approximately 4 kg of live *Gambusia* were harvested via seine net from a static inland saline pond at Springfield Water's Aquaculture in Northam and transferred live to the ADU. The fish were maintained in a 1,000 L tank at 20ºC and a salinity of 15 ppt. Subsamples of male and female *Gambusia* (10 fish per sample in triplicate) were taken from the tank for the determination of average wet weight, % moisture content, gross energy (bomb calorimetery), lipid, protein, ash and essential amino acid content. The same analyses were conducted on triplicate samples of the Skretting pellets.

Barramundi

Five barramundi were randomly stocked into each of nine x 180 L tanks (3 replicates of each treatment). Each flow through tank was maintained at 28ºC and received seawater at 1 litre/minute. After a 1 week acclimation period to the experimental conditions (during which time all fish were fed on Skretting Nova ME pellets), fish were weighed and feeding on the treatment diets began. The average starting weight of the barramundi was 81.2 ± 0.3 grams.

Feeding

Barramundi were fed iso-calorific rations of each treatment, based on feeding tables published by the Department of Fisheries, Western Australia[III]. To ensure all food would be consumed in all treatments, 80% of the recommended ration was offered to all treatments. The same ration was fed each day throughout the 12 day trial.

Measurement and Statistical Analysis

At the end of the trial, all fish were weighed and specific growth rate determined as follows:

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

Food conversion ratio for each treatment was calculated as the total equivalent dry food fed divided by the total biomass gain. Specific growth rates and food conversion ratios were compared between treatments using a one-way analysis of variance.

8.3 Results and Discussion

Female *Gambusia* had a significantly higher wet weight (0.48 ± 0.06 grams) than males (0.22 ± 0.01 grams) ($P<0.005$), however there were no significant differences in gross energy on a dry weight basis ($P = 0.95$) and hence males and females were pooled for feeding. Table 8-1 shows the gross energy content of each dietary treatment and the calculations made to determine the wet ration offered to each treatment.

[III] http://www.fish.wa.gov.au/docs/pub/AquaOutputModel/index.php?0308

Table 8-1: Ration calculations for feeding barramundi on an iso-calorific ration of *Gambusia* and pellets.

	80% of a full ration (g DW/fish/day)*	Gross Energy (kJ/g DW)	GE intake required to meet 80% of full ration (kJ/fish/day)	Moisture Content (%)	Daily Ration (g ww/fish/day)
Pellets	1.15	23.7 ± 0.1	27.3	10.1	1.28
Gambusia	-	21.2 ± 0.3	27.3	88.5	11.18

We suggest there was no difference in gross energy between the male and female *Gambusia*, as they were collected from Northam when the water temperature was ca. 20°C and hence the females were not gravid. Had the females been carrying eggs, we would have expected them to have a higher energy content.

Growth and FCR

Specific growth of barramundi was significantly affected by treatment (P = 0.005)(Figure 8-1). Barramundi fed live *Gambusia* had significantly faster growth (3.28 ± 0.42%/day) than those fed on pellets (1.35 ± 0.05%/day), whilst those fed a combination of *Gambusia* and pellets had an intermediate rate of growth (2.41 ± 0.14%/day), which was not different to that obtained on both other rations. These data clearly demonstrate that *Gambusia* are palatable to barramundi and not only useful as a supplementary feed, but also as a complete ration, at least for fish of this size and over this short experimental period. Further investigations will be required to confirm the long term suitability of *Gambusia* and to determine if they remain a suitable feed for larger barramundi.

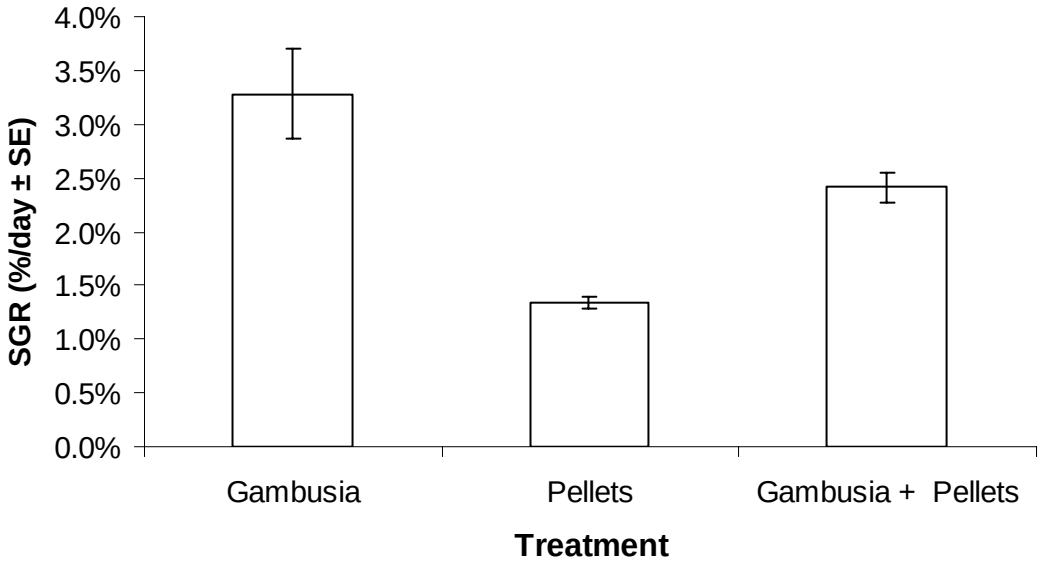

Figure 8-1: Specific growth rates (SGR) of barramundi fed on iso-calorific rations of live *Gambusia*, pellets or an equal combination of these two diets.

The food conversion ratio of barramundi was also affected by treatment (P<0.001) (Figure 8-2). In order to compare treatments on an equal basis, FCRs were calculated

on a dry weight basis. Those barramundi fed on live *Gambusia* had a significantly lower (i.e. better) FCR (0.44 ± 0.08) than those on both pellets only (1.06 ± 0.05) and the combination of pellets and *Gambusia* (0.59 ± 0.04).

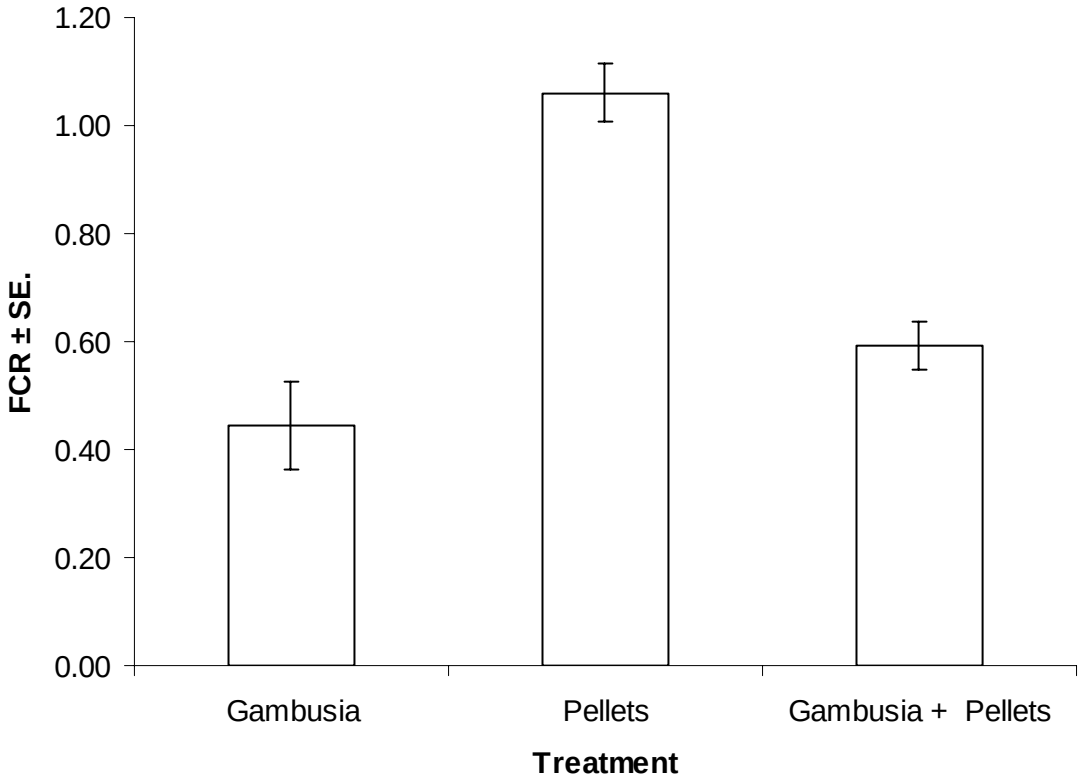

Figure 8-2: Food Conversion Ratio (FCR) of barramundi fed on iso-calorific rations of live *Gambusia*, pellets or an equal combination of these two diets.

Nutritional Profile

To determine the reason for the superior performance of barramundi fed on *Gambusia*, we measured the proximate composition of both feed types (Table 8-2). The protein content of *Gambusia* did not differ between males and females (pooled average 59.1 ± 3.1%) and was significantly (P = 0.05) higher than in the pellets (45.9 ± 0.9%). The lipid content of male *Gambusia* (19.5 ± 1.1%) was significantly higher than females (15.4 ± 0.7%) and was similar to the pellets (18.8 ± 0.8%). The percentage carbohydrate content (calculated as the difference between 100 and the sum of protein, lipid and ash) was found to be much lower in the *Gambusia* compared to pellets. That barramundi have a limited ability to digest carbohydrates (Glencross, 2006) suggests that the lower carbohydrate content of *Gambusia* may result in them more digestible than pellets.

Table 8-2: Proximate composition of *Gambusia* and Skretting Nova ME pellets.

	Gross Energy (kJ/g DW)	Moisture Content (%)	Lipid Content (%)	Protein Content (%)	Ash Content (%)	Carbohydrate Content* (%)	Protein to Energy ratio
Pellets	23.7 ± 0.1	10.1 ± 0.3	18.8 ± 0.8	45.9 ± 0.9%	8.1 ± 0.1%	27.20	19.4
Male Gambusia	21.2 ± 0.3	89.5 ± 1.1	19.5 ± 1.1	59.4 ± 4.9%	13.0 ± 0.2%	8.10	28.0
Female Gambusia	21.3 ± 0.3	82.6 ± 1.9	15.4 ± 0.7	58.8 ± 5.1%	12.7 ± 0.1%	13.1	27.6

The protein to energy ratio of *Gambusia* (28 g/MJ) was much greater than the pellets (19.4 g/MJ). Glencross (2007) determined that the theoretical optimum protein to energy ratio for a 75 gram barramundi was 32 g/MJ, which agreed closely to the experimentally determined value of 30 g/MJ by Williams and Barlow (1998). Williams et al. (2003) also found a linear increase in growth rate with protein to energy ratios up to 30 g/MJ and the optimum protein content was found to be 60% for 80 gram barramundi. Williams and Barlow (1999) showed that the FCR of barramundi fed on an artificial diet containing 28 g/MJ was considerably better than those fed diets containing 18 or 21 g/MJ. These data suggest that the higher PE ratio of *Gambusia* is likely to be the major factor for the improved growth and FCR seen with barramundi fed on *Gambusia*. Given that both Glencross (2007) and Williams et al. (2003) concur that larger barramundi (ca 200 g) require a diet containing 26 g/MJ suggest that *Gambusia* will remain a suitable food source for fish of this size. The protein content and protein to energy ratio of *Gambusia* are thus close to ideal for barramundi and higher than any commercially available barramundi pellets.

A comparison of the essential amino acid indices for the two diets against the reference of whole barramundi are shown in Figure 8-3. Essential amino acids below the line are potentially limiting and the further the distance from this line, the greater the limitation. Each diet only had one essential amino acid which could be considered limiting (>10% difference from the ideal line), methionine in the pellets and arginine in the *Gambusia*. Providing a 50:50 ration of *Gambusia* and pellets should overcome these deficiencies, however given that this 50:50 ration not outperform the *Gambusia*-only treatment, suggests that the arginine deficiency in *Gambusia* was not severe enough to limit the growth of the barramundi.

Work is continuing to determine the fatty acid profile of the *Gambusia*. Given, however, that *Gambusia* in the pond graze on detritus formed from the decomposition of marine microalgae, then it is likely they contain the highly unsaturated fatty acids required by barramundi.

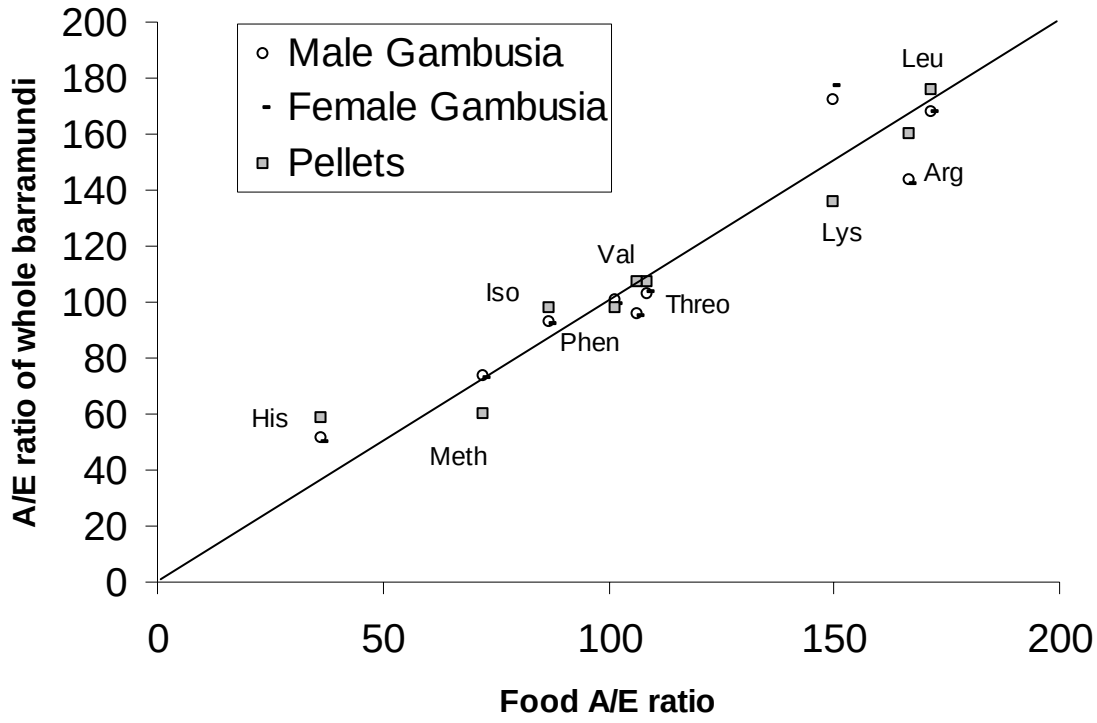

Figure 8-3: A comparison of the A/E ratio of *Gambusia* and pellets against whole barramundi flesh. Amino acids below the line are potentially limiting.

8.4 Conclusions

Barramundi fed live *Gambusia* exhibited significantly faster growth and improved FCR than those fed an iso-calorific ration of commercial barramundi pellets and a 50:50 ration of *Gambusia* and pellets. The improved performance of barramundi fed *Gambusia* was attributed to their higher protein content, higher protein to energy ratio and lower carbohydrate content, which contributes to improved digestibility. The essential amino acid indices between the two diets were similar, suggesting that amino acid imbalance was not the limiting factor to the slower growth of barramundi fed on pellets.

Given the excellent results achieved using *Gambusia* as a food source, we are continuing to investigate their potential uses in inland saline aquaculture. Of particular interest is their ability to consume heterotrophic bio-floccules. If *Gambusia* can thrive on these floccules, then excellent potential exists for converting barramundi waste into a highly efficient barramundi food.

9. ECONOMIC MODEL OF INTEGRATED AGRI-AGRICULTURAL ENTERPRISE

9.1 Introduction

Based on the SIFTS production data and operational costs determined during the grow-out trials we present two, scaled-up economic scenarios which we believe represent the most likely commercial, SIFTS-based, fin-fish enterprise(s) in an inland saline environment.

The two scenarios are:

- Seasonal production of barramundi and trout employing autotrophic (micro-algal dominant) pond management.
- Seasonal production of barramundi and trout employing heterotrophic (bacterial floc dominant) pond management with the option of prawn polyculture.

9.2 Materials and methods

The economic scenarios presented utilised the “Inland Saline Recirculation Culture” Excel spreadsheet model developed by Bill Johnston during the concurrent ISA coordination project (Allan et al. 2008; FRDC Project 2004/241). The model employs a cost benefit analysis technique with discounted cash flow as the basis.

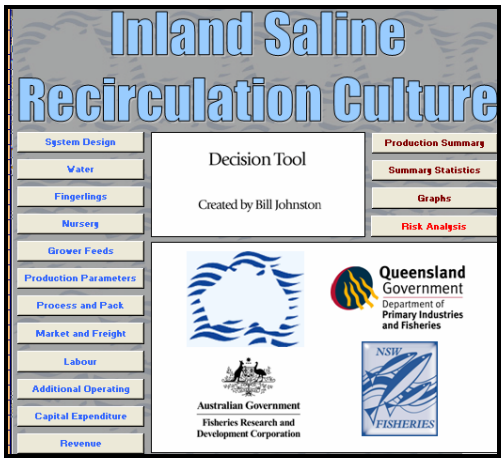

Figure 9-1: Excel-based, computer model used to generate the economic data for the two SIFTS-based aquaculture scenarios.

The Springfield Waters Research Facility was considered the hypothetical site for the two scenarios in relation to power costs, groundwater chemistry, availability and cost, evaporation data and its proximity to a major market i.e. Perth.

The over-riding assumption governing the two scenarios is that the total pond area available is limited by the volume of available water. Unlike the eastern states of Australia, the majority of salt affected land in Western Australia overlies fractured rock or granite aquifers. The yields of water from such aquifers are only low to moderate and the highest yielding bores in these areas yield approximately 300 kL/day. Given that the rate of evaporation in such inland areas is high, the maximum pond area that could be serviced by a 300 kL.day bore is approximately 5 hectares, and we have used this figure in our assumptions. It is possible that increased groundwater yields could be obtained through the use of a bore field; however, professional hydrogeological advice would be required to determine the characteristics of the local aquifer and the subsequent minimum distance between bores to ensure they do not influence the water yield from each other.

The maximum production estimates used for each fish species were based on the data collected during the experimental trials and are considered to be conservatively achievable under the specific pond management systems.

It is also assumed that each enterprise has a pre-established market for its total production of fish at a fixed cost (see Table 9-1)

Commercial pricing of the 50 tonne capacity SIFTS and ancillary infrastructure was provided by McRobert Aquaculture Systems.

The heterotrophic pond scenario includes the capital and operational costs associated with addition of molasses as the carbon source and the requirement for mechanical pond circulation/aeration.

The universal and enterprise-specific model input variables for the two scenarios are shown in Table 9-1 and Table 9-2, respectively

Table 9-1: Model input variables used for both economic scenarios.

Scenario input variables	
Market price both species	$10/kg
Feed cost $/tonne (bulk purchase)	$2000
Individual SIFT module capacity	50m^3
Total area of production ponds	5Ha
Total volume of production ponds	150,000 m^3
Maximum daily water availability (bore)	300kL/Day
Water top-up cost (5kW saline bore)	0.05c/kL
Stocking size of juveniles	100mm
Cost of juveniles	1c/mm
Feed Conversion Ratio (FCR):	1.2
Number of crops/year	2
Fish harvest size	500g
Mortality over grow-out	5%
Product form:	Bulk whole chilled

Table 9-2: Model input variables used for the specific economic, ISA aquaculture scenarios

	Scenario 1	Scenario 2
Pond management	Autotrophic	Heterotrophic
Max production (tonnes/Ha/yr)	11	30
Number SIFTS	14	27
Pond volume: SIFT volume (total)	214	112
Additional pond aeration/circulation (W/m^2)	Nil	1.5
Labour	Manger x 1, Farmhand x 1	Manger x 1, Farmhand x 2
Potential in-pond polyculture species	Nil	Prawns

9.3 Results and Discussion

The summary statistics for each ISA scenario as generated by the model are shown in Figures 2 and 3.

Summary Statistics
Species: Trout - Autotrophic pond management

Output Summary		*Economic Indicators*	
Annual production (kg)	50,691	Net present value	-$377,322
Annual gross revenue	$506,914	Annual return	-$38,431
Annual production cost	$545,345	Internal rate of return	2.88%
Production cost per kilogram	$10.76	Benefit - cost ratio	0.93
Revenue per kilogram	$10.00	Break-even production (kg)	59,741

Cost Structure Summary

	Annual Cost	Cost per Kilogram	% of Cost
Fingerlings	$112,000	$2.21	21%
Water supply and treatment	$5,588	$0.11	1%
Grower feed	$127,680	$2.52	23%
Labour	$108,096	$2.13	20%
Processing, packing, freight and marketing.	$51,959	$1.03	10%
Fuel, oil, repairs and maintenance	$5,500	$0.11	1%
Electricity	$34,000	$0.67	6%
Additional operating	$10,200	$0.20	2%
Capital	$90,323	$1.78	17%

Figure 9-2: Economic summary generated by the ISA recirculation economic model for scenario one

Summary Statistics

Species: Barramundi - Heterotrophic pond management

Output Summary	
Annual production (kg)	149,216
Annual gross revenue	$1,492,157
Annual production cost	$1,229,517
Production cost per kilogram	$8.24
Revenue per kilogram	$10.00

Economic Indicators	
Net present value	$2,578,643
Annual return	$262,640
Internal rate of return	21.72%
Benefit - cost ratio	1.21
Break-even production (kg)	97,999

Cost Structure Summary

	Annual Cost	Cost per Kilogram	% of Cost
Fingerlings	$263,747	$1.77	21%
Water supply and treatment	$5,588	$0.04	0%
Grower feed	$338,256	$2.27	28%
Labour	$151,776	$1.02	12%
Processing, packing, freight and marketing.	$124,968	$0.84	10%
Fuel, oil, repairs and maintenance	$6,000	$0.04	0%
Electricity	$117,000	$0.78	10%
Additional operating	$52,200	$0.35	4%
Capital	$169,981	$1.14	14%

Figure 9-3: Economic summary generated by the ISA recirculation economic model for scenario two.

Given the yield constraints of autotrophic ponds that we identified in this project, we have estimated that the maximum production obtainable from 5 hectares of static autotrophic ponds is approximately 50 tonnes/year. At this level of production, the economic analyses reveal that production is unprofitable, with the cost of production exceeding the farm gate price of $10/kg. Increases in yield are unlikely to be achievable without increased water availability, or significantly more research to develop techniques of overcome the yield-limiting constraints of static, autotrophic pond culture (see Section 12 for details).

The increased yields achievable using heterotrophic pond management enable a three-fold increase in production from the same 5 hectares of static ponds. Such intensification enables

some economies of scale in terms of operating costs. For example, we believe that tripling production using heterotrophic pond management only requires an increase of 50% in the requirement for labour. Heterotrophic pond management does, however, impose other significant costs on the operation, particularly in terms of power (for mixing, aeration and circulation) and the use of molasses. The economic analyses revealed that despite the increased production, the cost of production was only slightly lower than that of the autotrophic pond. This cost was, however, less than the farm gate price, and this farm therefore has the potential to be profitable. Increasing the profitability of this enterprise could be achieved by optimizing the methods of circulation and mixing, in order to save on electricity costs associated with maintaining the heterotrophic floccules in suspension. Testing alternative, cheaper sources organic carbon instead of molasses also has the potential for significant cost reductions. In addition, our economic analyses assume no added value through polyculture. Based on our previously described modeling, we believe the production of approximately 1 tonne of prawns per hectare could be cultured on the heterotrophic floccules alone (without the need for additional food). Assuming a farm gate price for prawns of $12/kg then an additional $60,000 of income could be realised, with very little additional work or investment in capital or operating costs. Given that well established heterotrophic prawn farms can produce up to 60 tonnes/ha/yr, then once heterotrophic pond management techniques are optimized for integration with SIFTS in static, inland saline water bodies, it is conceivable that such yields could also be achieved. Such yields would further improve the economies of scale and profitability.

Both scenarios presented here require advanced juveniles in order to achieve a marketable sized product within the limited growing season for each species and we have assumed that these fingerlings would be purchased from an external provider. Significant savings could be made on the price of juveniles if a small controlled environment, recirculating aquaculture system were included on the farm plan in order to take newly weaned juvenile fish to an advanced size for stocking SIFTS.

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11. BENEFITS AND ADOPTION

This project has demonstrated that commercial scale fish culture in static saline ponds in the Wheatbelt of Western Australia is a difficult task. We believe that sustainable and economically viable production at a commercial scale in static autotrophic ponds is not possible using the current species and water sources. Introduced fish species or the use of alternate water sources such as deep saline aquifers may result in commercial possibilities.

Sustainable fish production at a commercial scale in heterotrophic ponds shows greater promise. Due to the complexity of heterotrophic pond management though, it is unlikely that farmers will be able to diversify into this area without employing professionally trained scientists. Some further work is required on heterotrophic pond fish/prawn culture and this is being undertaken by QDPI staff at their Bribie Island facility. The use of heterotrophic ponds and the culture of two species utilising the SIFTS technology show considerable potential for the future for inland, salinised Australia.

Farmers and salinity managers will benefit from the work completed within this project on the use of NyPa forage. And TAFE aquaculture students will benefit from the greatly improved understanding of fish culture in static saline ponds gained during this project.

Another benefit of the project, as has been described in the FRDC project 2004/241 ‘Development of industrial scale inland saline aquaculture: Coordination and communication of R&D in Australia’, a project that the authors of this report were closely involved with is the ‘reliable science based information now available to assess the potential for aquaculture’.

Another benefit of the current project was the experience gained by researchers and the technology manufacturers, McRobert Aquaculture Group, in operating this semi-commercial scale system for an extended period of three years. These hands-on experiences enabled further improvements and refinements to be incorporated into the larger, commercial scale SIFTS, which were the focus of FRDC Project 2008/038 ‘Improvements to Semi Intensive Floating Tank System to achieve commercial readiness in marine environments' (see Appendix 15.3). The McRobert Aquaculture Group are currently in discussion with a number of flow-through pond farmers both in Australia and overseas, who are interested in integrating this technology into their existing farming operations.

12. FURTHER DEVELOPMENT

If static water pond culture is to become a viable production system for growing fish, further research is required to optimise the bioremediation processes described in the summary below. Although heterotrophic pond management has been demonstrated to enable successful yields of 15 tonnes/ha over ca. 100 days, further research is required to optimise this management strategy and reduce the operating costs associated with it. For example, it is now well established that periodic removal of bio-floccules is essential for exporting nutrients. Without the capability for such removal, the management strategy used in our current trials is unlikely to be sustainable over the longer term. A research need therefore exists to identify viable removal processes. In addition, our research demonstrated that phosphorus limitation may impede the development of bio-floccules and that the depth of the SIFTS ponds may lead to ineffective mixing and rapid settlement of the bio-floccules. Further research on optimizing bio-floccule communities in static ponds is not ISA specific and would benefit other aquaculture industries using heterotrophic pond management. We believe that production from static, heterotrophic ponds could be intensified up to 30 tonnes/ha over 90 days through improved mixing and harvesting of bio-floccules. Similar production yields are already achievable using heterotrophic pond management with prawn production (Chamberlain et al., 2001).

We have also been discussing alternative bioremediation strategies with the CSIRO and a private company, Phoslock. As previously mentioned, biofiltration was shown to be ineffective in static ponds due to the inability of nitrifying bacteria to compete with microalgae and heterotrophic bacteria for available nutrients. As both microalgae and heterotrophic bacteria have a requirement for phosphorus, the removal of this nutrient using Phoslock has the potential to minimise microalgal and heterotrophic bacterial blooms, thereby enabling nitrifying bacteria to convert ammonia into nitrate. Representatives from Phoslock having indicated their interest in testing this concept and collaborating with our group if the opportunity arises.

Our work highlighting the benefits of feeding *Gambusia* to barramundi has the potential to create value from this abundant and noxious species. We are continuing our work with *Gambusia* and aim to appoint an honours student to investigate the potential of growing *Gambusia* on heterotrophic bio-floccules. If this project is successful, it has the ability to convert barramundi waste into barramundi food and (through the continuous cropping of bio-floccules), further increase the carrying capacity of static heterotrophic ponds.

All of the outcomes of this project are of relevance to the development of an inland saline aquaculture industry in Western Australia, where volumes of saline water constrain the use of flow-through production systems, and may now also be of interest to other states in which the volume of saline water available for aquaculture is decreasing.

The outcomes of this project and the SIFTS technology are also of interest to flow-through pond farms, where water quality does not have the same impact on yields and where the efficiency of solids waste removal from SIFTS will improve the environmental outcomes of such farms. The McRobert Aquaculture Group is in discussions with several flow-through pond farmers both in Australia and overseas.

In terms of the SIFTS technology itself, further refinements have been made to the commercial scale (50 m3) units following the experiences gained with the smaller prototypes in the current trials. For example, following routine repeated liner inversions in the current trial the liner fixings began to fail. Similarly, small pin holes in the welds of the prototype SIFTS developed leaks and the units were therefore sinking by the end of the project. The newer commercial scale SIFTS (as detailed in FRDC Project Report 2008/038 – Appendix 15.3) have overcome these issues with newer and significantly stronger methods of liner fixing and by filling each module with polystyrene foam. In addition, the manufacturing process of the $50m^3$ modules results in the SIFTS being significantly stronger and easier to assemble on-site. FRDC Project 2008/038 improved certain aspects of the SIFTS to achieve commercial readiness in marine environments. Some of these aspects, including automated sludge removal and processing will be of direct relevance to Inland Saline Aquaculture.

13. PLANNED OUTCOMES

1. Documentation (including engineering, culture protocols and cost analysis) of a culture system for growing marketable marine finfish in inland saline groundwater at yields of 20 tonnes/ha/yr.

- This project has shown that it is not possible to grow marketable marine finfish in static inland saline ponds at yields of 10 tonnes/ha over 3 months using conventional autotrophic pond systems. Our research into heterotrophic pond systems for the culture of marine fish in combination with other aquaculture species that was conducted within this project is promising and is being further investigated by one of our project partners, QDPI, at the Bribie Island Aquaculture Centre in Queensland.

2. A demonstration site, for interested stakeholders, of commercial technology for the culture of fish in inland saline groundwater.

- Due to a combination of factors including the ill-health of the land-owner, new management of CY O'Connor College of TAFE at Northam, the ISA demonstration site in Northam WA has closed.

3. Demonstration that a valuable plant crop, suitable for feeding to livestock, can be grown using nutrient enriched, saline aquaculture waste water.

- This project has demonstrated that nutrient enriched, saline aquaculture waste water is suitable for growing the salt-tolerant NyPa Forage crop. This work is being further investigated in a RIRDC program, in which NyPa Forage will be grown on a large field trial and its nutritive vale determined in livestock in vivo. This forage is also now being investigated for its ability to fix carbon under a carbon-trading scheme.

4. Demonstration that commercial inland saline aquaculture can be undertaken in an environmentally sustainable manner.

- This project has demonstrated that inland saline aquaculture can be undertaken in an environmentally sustainable manner, although not using the accepted method (autotrophic pond) at the expected commercial intensity.

5. Alternative options to manage microalgal blooms and nutrient levels with a bioremediation

system.

- The results of this project clearly demonstrates that the SIFTS technology can be used in conjunction with heterotrophic pond management to increase the yields of fish from zero-exchange inland saline ponds. Our economic modeling suggests that the profitability of SIFTS-based fish culture in a heterotrophic pond may be marginal; however the integration of fish culture into existing heterotrophic prawn farms has the potential to increase the profits for such a business. We also believe that *Artemia* and *Gambusia* may be able to effectively crop heterotrophic microbial proteins with financial benefits.

14. CONCLUSIONS

Our R&D has focused on utilising innovative technologies and methods to increase yields from static ponds to a level where commercially viable, stand-alone enterprises can exist. This focus is important for Western Australia, where groundwater yields are typically only low to moderate and no large-scale interception schemes exist. Based on previous economic studies we consider the minimum level of production required for a stand-alone farm to be approximately 50 tonnes per annum. Using typical pond production figures of 10 tonnes per hectare per year, most groundwater bores in Western Australia would not provide enough water to even account for evaporation over the 5 hectares on pond area required for a commercial scale farm. Increasing yields is therefore critical to allow commercial scale culture to occur.

Despite SIFTS preventing settleable fish wastes entering the pond, we demonstrated that yields of 10 tonnes/ha are not achievable in static, autotrophic ponds over periods of ca. 100 days, due to water quality issues created by microalgal blooms. Although similar yields are obtainable from static ponds with species such as silver perch (Rowland et al., 1995), these crops are produced over a much longer time frame (ca. 12 months) and the nutrient loading on the pond is therefore less intense.

Biofiltration was demonstrated to be ineffective in ameliorating the negative effects of microalgal blooms, due to the inability of nitrifying bacteria to compete with microalgae and heterotrophic bacteria for available nutrients. By integrating the SIFTS technology with heterotrophic pond management, however, we were able to achieve yields of 15 tonnes/ha over a culture period of ca 100 days. This technique stabilises microalgal populations and provides more stable pH and dissolved oxygen values than static autotrophic ponds.

Although there is an increased cost associated with heterotrophic pond management through the need for an organic carbon source and higher rates of aeration, these costs are largely offset by the greater yields. The integration with SIFTS also provides an opportunity for the polyculture of valuable filter feeding or grazing crop such as prawns in the surrounding pond. Ebeling et al. (2006) pointed out the importance of removing excessive microbial proteins from heterotrophic ponds and polyculture facilitates this removal. Integrated prawn culture has the added benefit of a valuable secondary crop. Our modeling suggests that approximately 1 tonne of prawns could be cultured on the heterotrophic microbial proteins alone generated from the culture of 15 tonnes of barramundi. *Artemia* also have the ability to ingest microbial proteins and as such, the modified SIFTs described in section 7.4.1 could be utilised to grow *Artemia*

and crop microbial proteins. Finally, given the excellent growth and FCR exhibited by barramundi fed on *Gambusia*, (section 8.3), we are continuing to investigate the potential for growing *Gambusia* on heterotrophic bio-floccules in order to convert barramundi waste into an effective barramundi food..

We believe that production from static, heterotrophic ponds could be intensified up to 30 tonnes/ha over 90 days through improved mixing and harvesting of bio-floccules. Similar production yields are already achievable using heterotrophic pond management with prawn production (Chamberlain et al., 2001).

This project effectively demonstrated the ability of NyPa Forage to remove nutrients from aquaculture effluent and trials testing the potential of this crop as a fodder plant showed it to have a reasonable protein and energy content. Laboratory testing also has demonstrated that *Artemia* have potential for cropping microalgae from static, inland saline ponds, however field testing demonstrated that variations in microalgal species composition make reliable *Artemia* production difficult.

15. APPENDICES

15.1 APPENDIX 1 - INTELLECTUAL PROPERTY

There are no intellectual property issues associated with this project.

15.2 APPENDIX 2 - STAFF

Full-time Staff

CY O'Connor College of TAFE, Northam

Dr Gavin Sarre

Part-time Staff

Challenger TAFE, Fremantle

Dr Gavin Partridge
Mr Greg Jenkins
Mr Gavin Kay
Mr Robert Michael
Mr Bruce Ginbey

Murdoch University, Murdoch

Dr Alan Lymbery
Dr Rob Doupe
Mr Robert Michael

Casual Staff

Mr Vin Omera
Ms Jess Rodgers

Graduate Students

Gavin Kay and Robert Michael of Challenger TAFE both completed first class honours projects through Murdoch University on NyPa Forage and microalgae cropping with *Artemia*,

respectively. Challenger TAFE graduate diploma students Thomas Hoffman and Emmanual Caro completed a research project on the feeding of *Gambusia* to barramundi.

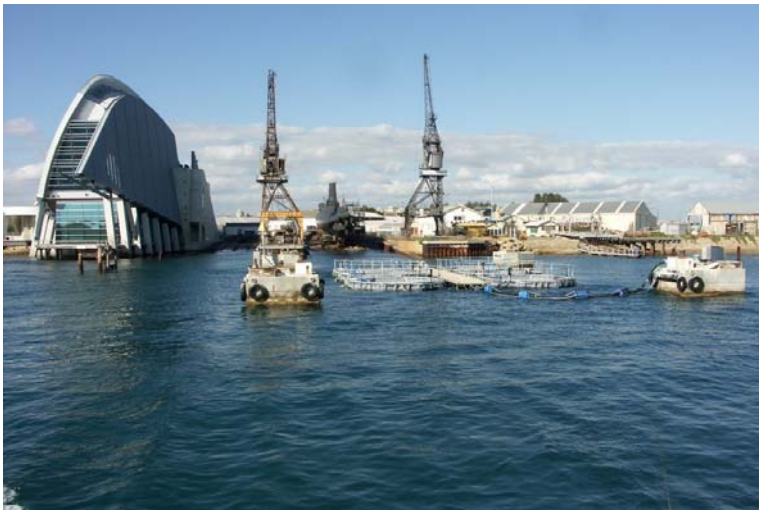
Volunteers/Work Experience Students

Dick Nuich
Lawson Callaway
Justin Fox
Chris Giles
Cody Hull

15.3 Appendix 3 - Improvements to Semi Intensive Floating Tank System to Achieve Commercial Readiness in Marine Environments

Improvements to Semi Intensive Floating Tank System to Achieve Commercial Readiness in Marine Environments

McRobert, I. R. & Partridge, G. J.



Australian Government

Fisheries Research and Development Corporation

McROBERT AQUACULTURE SYSTEMS

TAFEWA challenger

Project Number 2008/038

Fisheries Research and Development Corporation Report

FRDC project 2008/038

Improvements to Semi Intensive Floating Tank System to Achieve Commercial Readiness in Marine Environments

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The outcomes of this project are improvements to the Semi-Intensive Floating Tank System which make this technology close to being market-ready for protected marine environments. During this project we developed an underwater mortality detection and removal device, and an efficient method of transferring and processing the solid waste which the SIFTS captures. The improvements described in this report are not only relevant to the SIFTS deployment into protected ocean environments, but are also of direct relevance to the use of SIFTS in its original application of inland saline water bodies (see associated FRDC Project #2005/213 'New Technologies for Sustainable Commercial Finfish Culture') and also to those land based aquaculture systems which generate high volumes of solid wastes.

This project has enabled MAG to develop an efficient system of transferring sludge waste from the SIFTS to the shore. We have investigated a number of options for processing this waste. The most prospective of these systems is still undergoing final testing. We have also developed an effective means of finding and removing mortalities from the SIFTS using a video camera and air water lift system.

With these and other improvements to aspects such as liner fixings and moorings, the SIFTS is close to being ready for promotion to the market for protected ocean environments.

ACKNOWLEDGMENTS

We would like to thank Craig McRobert and Bruce Ginbey for their work in designing the septic tank system and all of the McRobert Contracting Services staff for their assistance in constructing and assisting in maintaining the system. Thanks also to Tim Beck, Robert Michael and Rowan Kleindienst of the ADU for their feedback during operation of the new systems.

BACKGROUND

The Semi-Intensive Floating Tank System was designed and patented by McRobert Aquaculture Group in collaboration with Challenger TAFE specifically for improving the management and yield of fish from static inland saline water bodies. Field trials testing this technology, funded by the Fisheries Research and Development Corporation and the state government of Western Australia, showed it to exhibit efficient waste capture, high fish stocking densities and excellent fish management capabilities. Following these trials it was concluded that the SIFTS has application in sensitive and protected marine environments. Therefore, MAG have funded the R&D to date ($550,000) modifying the SIFTS for a sheltered marine environment. This R&D has resulted in a prototype in Fremantle Harbour. To date, only small quantities of fish have been trialed as the system still needs to have a number of components completed before it can be put into full utilisation.

NEED

This project relates to Challenge 3 – ‘Increased Demand and Profitability’. The priority it relates to is "Develop capacity to produce more fish for consumption or for fishing experiences." Growing demand for finfish cannot be met by Australian commercial fishing sources, (particularly with cancellation of commercial licenses in WA). Aquaculture can potentially meet this growing demand. However, there is a reducing number of marine aquaculture sites in Australia due to the increased attention to the environmental impact of traditional sea cage aquaculture. This project is focused on creating an environmentally sensitive marine aquaculture production system, to increase aquaculture production, offsetting the reduction of commercial fishing licenses and help address the increasing prices of finfish in WA.

OBJECTIVES

1. To develop an efficient commercial waste process that results in an onshore waste product that can either be used commercially or disposed of economically

2. To design and commission a labour efficient system which removes sick/dead fish from SIFTS without diving and stressing the remaining fish.

1. WASTE PUMPING AND PROCESSING SYSTEMS

1.1. Methods

A number of commercially available waste pumping systems were investigated. The most elaborate of these comprised a series of vacuum and separation pumps and collection tanks which was automated via a PLC to pump the waste from the SIFTS. Although beyond the budget of the current project (ca. $60,000), this system would be appropriate for a commercial scale SIFTS farm and would integrate well with our fully automated control and monitoring system. For the current project we utilised the concept behind this pumping system to develop a low-cost option in which to prove the concept. The system comprises an air-operated Sandpiper flap-valve pump to suck the waste from the SIFTS waste collector and transfer it to a shore-based operation processing facility.

At the on-shore waste processing facility, two approaches to dewatering and processing the waste were tested. The first was a 7 m3 primary septic tank to separate the solid fish wastes and supernatant and the second a synthetic geotextile bag. Up to 600 L of waste, with a dry matter content of 5 to 10% are pumped daily from the SIFTS units.

In order to test the effectiveness of the primary septic tank, we measured the nutrient content of the waste entering this tank and the nutrient content of the overflow from this tank over a period of 4 days. We have assumed for this analysis that the entire waste processing system was in steady state. During this time, three SIFTS were in operation, two containing snapper and one containing mulloway.

1.2. Results and Discussion

The waste collection and pumping systems are shown in Figure 1. Our decision to trial the air-operated Sandpiper flap-valve pump proved to be a good choice, as it was a robust and reliable pump.

(a)

(b)

Figure 4: (a) Sandpiper flap-valve pump and (b) removal of sludge from the SIFTS waste collector.

Because of its application in a marine environment, we were required to include several modifications to the pumping system, such as a purpose made particle trap with removable screens to prevent small crustaceans, seaweed etc. blocking the pump and pipe-work (Figure 5)

Figure 5: Purpose-made pre-filter on the inlet of the Sandpiper pump.

The septic tank system trialed in this project is shown in Figure 6.

Figure 6: 7 m^3 primary septic tank.

The feed input to each SIFT during the week long intensive sampling period and the corresponding nutrients collected are summarised in Table 1.

Table 3: Total feed input and quantities of nutrients collected from three SIFTS over a 4 day period.

	Food Input kg	N input kg	P input kg	N captured g	P captured g	% of dietary N recovered	% of dietary P recovered
Mulloway	46.2	3.3	0.5	20.1	8.0	0.6%	1.7%
Snapper A	20.2	1.5	0.2	125.6	19.3	8.6%	9.5%
Snapper B	27.2	2.0	0.3	58.8	15.0	3.0%	5.5%

The efficiency of waste capture in the three SIFTS varied from 0.6% to 8.6% for nitrogen and 1.7% to 9.5% for phosphorus. Given that approximately 10% of nitrogen ingested by fish is excreted in their faeces suggests that the waste extraction system on the SIFTS containing Snapper A was operating at maximum efficiency. The lower efficiencies for SIFTS containing Mulloway and Snapper B suggest that the waste extraction systems on these tanks were not operating at their maximum efficiency.

The total nutrients collected from these three SIFTS corresponded to the total nutrient input into the septic tank. Table 4 compares the nutrient content of the inflow and outflow of the septic tank.

Table 4: Total nitrogen (grams) and total phosphorus content (grams) of water entering and leaving the septic tank.

	TN	TP
In	204.5	42.2
Out	345.5	10.1
% Reduction	-69%	76%

These data reveal that the septic tank was removing 76% of the incoming phosphorus, however, the nitrogen content of the waste leaving the septic tank was higher than that entering. This suggests that our assumption that the system is in steady state is invalid. It did appear, however, that the clarity of water leaving the septic tank was significantly better than that entering and TSS analysis is ongoing to confirm this. In order to improve the performance

of the septic tank, we believe it must be of greater capacity to efficiently process the large volume of waste it receives (up to 600 litres per day) and that secondary and perhaps tertiary settlement tanks could be implemented for this type of waste processing to be more effective.

The second approach for processing the SIFTS waste is to utilise a synthetic geotextile bag (Geotube™) for dewatering the sludge. This Geotube™ can be utilised for dewatering either the material within the primary septic tank or the waste stream directly from the SIFTS. A 1m^3 V-bag (Figure 7) has been installed onsite inside a purpose-built, sealed and vented tank (Figure 8) to eliminate the possibility of odours.

Figure 7: Operation of the 1 m3 V-tube Geotube™. Image courtesy of TenCate Australia.

Figure 8: A 1m^3 V-bag has been installed onsite inside a purpose-built, sealed and vented tank.

In order for these bags to operate at their maximise efficiency, coagulants and flocculants must be added to the waste stream prior to entering the bag. As outlined in our previous report, we had engaged the services of Mr Roger Scott from Kemira Chemicals who is experienced in optimising flocculants for various waste streams. Unfortunately Mr Scott has had two trips to Perth cancelled and as such we are yet to finalise this aspect of the project. We have since engaged the services of Nalco Australia, who have recently finalised comprehensive jar-tests on samples of sludge to determine the optimum combination of chemicals for dewatering. We are currently awaiting the report and recommendations from Nalco before we can proceed with processing the waste within the septic. We estimate that we will require another 6 to 10 weeks to complete the trials with the Geotube™ and we will report the outcomes of these to the FRDC once they have been finalised. Similarly to the septic tank experiments described above, we will analyse the waste stream both entering and leaving the Geotube™. The benefit of the Geotube™ is the final product will have a low water content and have greater potential to be sold as a by-product, whereas the septic tank will have to be pumped out on a regular basis (at a cost of approximately $400 per 7 m^3 load).

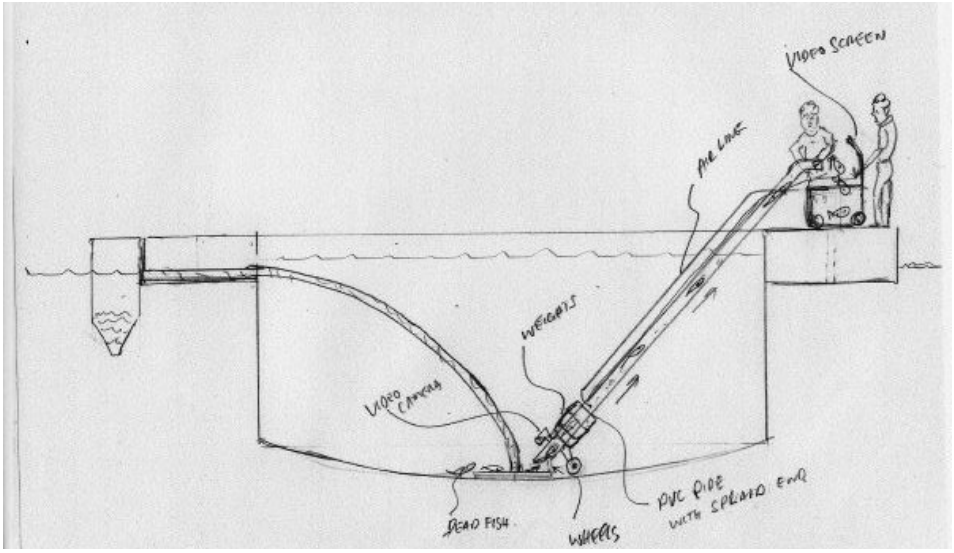
2. A LABOUR EFFICIENT SYSTEM FOR REMOVING SICK/DEAD FISH.

2.1. Methods

An air-operated vacuum system for removing sick and dead fish was developed and named the "Mort Detector-Collector". A fibre optic Rigid SeeSnake camera (www.rigid.com/Tools/SeeSnake-micro) is mounted to the front of this vacuum, which allows the operator to see any dead fish lying on the tank bottom. Once the fish has been located, the vacuum is turned on and the fish rapidly sucked up to the surface.

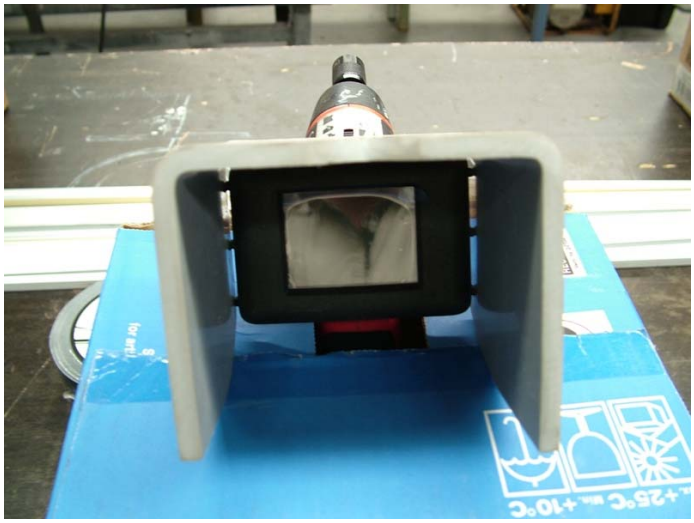
2.2. Results and Discussion

A diagram of the "Mort Detector-Collector"device is shown in Figure 9.



(a)

(b)



(c)

Figure 9: (a) Diagram showing the operation of the MAG “Mort Detector-Collector” (b) Plumbing components (c). Screen of the SeeSnake video camera.

The “Mort Detector-Collector” was found to highly efficient and was used for removal of trout mortalities shortly after the SIFTS were stocked. The device has not been used for over 6 months due to the fact we have had no mortalities in the SIFTS. The fibre optic camera is also valuable tool for routine inspection of the central waste plate. Further design modifications are underway such as video recording capabilities, improved lighting and submergence control. These modifications will improve its efficiency and manufacturing cost.

BENEFITS AND ADOPTION

The major benefit of this project was the development of a method for transferring waste from SIFTS to shore for processing. Although the septic tank had limited efficiency in reducing nutrients, it appeared to be effective in reducing total suspended solids from the waste stream. Preliminary ‘jar’ testing of the wastes from both the septic tank and the SIFTS sludge collectors investigating the effectiveness of coagulants and flocculants were very encouraging and the Nalco chemists are confident that following this process these wastes will be easily de-watereted through the Geotube™

The beneficiaries of this project will be those aquaculturists seeking an improved environmental outcome from their near-shore farming operations. MAG has received considerable interest from prawn farmers in Queensland interested in using the SIFTS technology for the polyculture of marine fish both within their prawn ponds or within their settlement channels.

In addition to the direct benefits associated with SIFTS, the methods we have developed and are continuing to develop for sludge dewatering and economic disposal will also have potential benefits for existing and future recirculation systems.

FURTHER DEVELOPMENT

Our first priority is to finalize the work with the Geotubes.

In addition to the advancements we have made in waste transfer and collection system in the current project we are continuing to make other improvements to the design and construction of the SIFTS in order to make them adequately robust for a marine environment. The Fremantle pilot site has proven ideal for such testing as despite being protected from certain weather patterns, the inner harbour experiences very little protection from the north-west, the predominate direction of the winter storms. As such, SIFTS have experienced short-period waves up to 1 metre in height. The constant pressure and stress on the liners within the SIFTS caused by these waves has resulted in failures of the liner fixings, which we have subsequently made significant improvements to.

PLANNED OUTCOMES

The planned outcomes of this project were to:

‘Enable McRobert Aquaculture Group (MAG) to complete the prototype SIFTS for sheltered marine environments. It will cover the remaining critical components of the SIFTS that needed to be modified to meet the conditions in a marine environment’

The project was successful in developing a system for detecting and removing fish mortalities and we have made excellent progress towards developing an effective system for capturing and processing the fish wastes captured by the SIFTS technology. Since the commencement of this project, several other system component have been identified as requiring further improvements to ensure the overall system will be adequately robust in the marine environment. These include:

1. Sourcing stronger liner materials and an improved method of welding the same
2. Designing a more robust mooring point system that affixes to a greater surface area of the floatation structure to increase the load bearing capacity
3. Independently buoyant swirl separator
4. Accessible airlift screens

We have made varying degrees of progress on each of these aspects and once we have finalised each of these we will be in the position to seek partners in a commercial SIFTS farm. We are in discussions with a group of Queensland prawn farmers who are keen to pursue this technology and another major fish aquaculture business has also expressed an interest in SIFTS for a protected marine nursery site in which low levels of dissolved oxygen cause routine problems.

CONCLUSION

This project has enabled MAG to develop an efficient system of transferring sludge waste from the SIFTS to the shore. We have investigated a number of options for processing this waste. The most prospective of these systems is still undergoing final testing. We have also developed an effective means of finding and removing mortalities from the SIFTS using a video camera and air water lift system.