Australian Government

Rural Industries Research and Development Corporation

Growing organic prawns —in inland saline waters—

by Steve L Slattery and Paul J Palmer

July 2008

RIRDC Publication No 08/126
RIRDC Project No DAQ-336A

ISBN 1 74151 715 X
ISSN 1440-6845

Growing organic prawns— in inland saline waters
Publication No.08/126
Project No. DAQ-336A

Researcher Contact Details

Steve L Slattery
Department of Primary Industries & Fisheries
19 Hercules Street
HAMILTON QLD 4007

Phone: 07 3406 8623
Fax: 07 3406 8698
Email: Steve.Slattery@dpi.qld.gov.au

Paul J Palmer
Department of Primary Industries & Fisheries
144 North Street Woorim
PO Box 2066
BRIBIE ISLAND QLD 4507

Phone: 07 3400 2050
Fax: 07 3408 3535
Email: Paul.Palmer@dpi.qld.gov.au

In submitting this report, the researcher has agreed to RIRDC publishing this material in its edited form.

RIRDC Contact Details

Rural Industries Research and Development Corporation
Level 2, 15 National Circuit
BARTON ACT 2600

PO Box 4776
KINGSTON ACT 2604

Phone: 02 6271 4100
Fax: 02 6271 4199
Email: rirdc@rirdc.gov.au.
Web: http://www.rirdc.gov.au

Published in July 2008
Printed by Union Offset Printing, Canberra

Foreword

There is an increasing demand world wide for organic foods. Australian production of organic foods is worth $250 million (Landline, 2002) yet currently it does not meet the level of demand. There are very few aquaculture ventures in Australia producing and or processing organic seafood. These include a mussel farm in Victoria and a silver perch farm in New South Wales which are both certified by the National Association for Sustainable Agriculture Australia Limited (NASAA).

Retail prices of A$50-60/kg for organic modified atmosphere packaging (MAP) prawns sold via the internet in the UK constitute a major premium from the current domestic wholesale price for Australian aquaculture prawns (A$16/kg). The Australian prawn aquaculture industry is currently suffering from an influx of cheaper imports so a change of product form and market will improve their viability.

Expansion of existing prawn aquaculture production is restricted by limits on new coastal sites. The Bribie Island Aquaculture Research Centre (BIARC) has already pioneered the production of prawns using saline bore water in one coastal region of Queensland. The utilisation of inland saline waters in locations more remote from the coast, where larger parcels of land are less expensive presenting opportunities to lower production costs through less intensive production systems, is the next step for industry to consider.

Another hurdle for organic prawn production is a local source of feed. The only commercially available organically certified prawn feed available has to be imported. This study focussed on assessing potentially suitable and certifiable protein sources that may become available in the future assuming current industrial development trends. In addition to this feed development work, some of the possible processing and packaging conditions were also evaluated. No trial diet was identified that matches the growth rates produced by normal conventional feed but improvements will continue to be made in future feed trials supported by the Department of Primary Industries and Fisheries (DPI&F). A new aquaculture industry partner has been identified wanting to progress to full organic certification.

The only farm trial that could be implemented in this study operated at Bauple during the 2005-06 season. However, the lack of supply of a certified organic prawn feed two months after stocking meant that a commercially available non-organic feed had to be used to ensure good growth and health of the prawns thereafter. Future commercialisation trials will be conducted near Bundaberg when a suitable feed has been developed. Finally the MAP of prawns resulted in an extended shelf life that will assist the export of this product. The successful inhibition of blackspot on the uncooked prawns in MAP is a world first. All other organic prawns currently sold in MAP are in the cooked form.

This project was jointly funded from Rural Industries Research and Development Corporation (RIRDC) Core Funds which are provided by the Australian Government and the Queensland Government through the DPI&F. This report, an addition to RIRDC's diverse range of over 1800 research publications, forms part of our Organic Produce Research and Development (R&D) program, which aims to facilitate the development of a viable organic industry through increasing adoption of sustainable organic farming systems. Most of our publications are available for viewing, downloading or purchasing online through our website:

- downloads at www.rirdc.gov.au/fullreports/index.html
- purchases at www.rirdc.gov.au/eshop

Peter O'Brien
Managing Director
Rural Industries Research and Development Corporation

Acknowledgments

The researchers would like to thank Noel Herbst and the staff of Gold Coast Marine Aquaculture for the supply of prawn juveniles for feeding trials, and Wayne and Donna Hellmuth for access to their Bauple farm for a grow-out trial. We also would like to thank Kialla Pure Foods, Queensland Sea Scallops, the Rocky Point Sugar Mill, Mr Gary Pinzone and Mr Leyland Minter for donation and supply of raw materials for prawn feeds testing.

Many thanks to Adrian Collins and Benjamin Russell and for this cooperative adjunct to the larger DPI&F inland prawn farming research initiative. Thanks also to Beverley Kelly, Michael Burke, Trevor Borchert, Michael Salini, Martyn Lee and Scott Shanks for their technical assistance at BIARC, to our biometrician Dr David Mayer for advice regarding statistical analyses, and to Peter Martin, Adam Pytko, Michael Gravel and Ian Brock of the Animal Research Institutes nutrition lab for proximate chemical, amino acid and fatty acid analyses.

Abbreviations

Arg	arginine
C	centigrade
CFU	colony forming unit
cm	centimeter
Cys	cystine
F.	*Fenneropenaeus*
G	gram
His	histidine
Iso	isoleucine
L	litre
Leu	leucine
log	logarithm scale
Lys	lysine
MAP	modified atmosphere packaging
Met	methionine
mL	millilitre
mm	millimeter
P.	*Penaeus*
Phe	phenylalanine
ppt	part per thousand
Thr	threonine
Try	tryptophan
Tyr	tyrosine
Val	valine

Contents

Tables

Figures

Executive Summary

What the report is about

This report details the results of research into organic production of prawns in Australia. This has involved activities and experiments over two years at several sites and using a multidisciplinary approach. This includes farm trials at an inland demonstration prawn farm which solely utilises saline bore water, sample collection from two commercial prawn farms in coastal regions of south-eastern Queensland, replicated feed trials at one of DPI&F's aquaculture research stations, specified feed manufacture at the laboratories of University of Queensland, and packaging and product storage trials and food analyses at two of DPI&F's food technology laboratories. This work was designed to investigate and assist in the possible adoption of organic procedures by the Australian prawn farming industry.

The import from Asia of cheaply produced prawns has forced all Australian prawn farmers to review their marketing procedures. Additionally investors are becoming increasingly concerned at the prospects for the expansion of this industry in Australia. Since the competition of seafood products in the marketplace is increasing on a global basis, alternate products are being investigated by those wishing to maintain and/or grow their market share. The premium paid for organic food products would hopefully provide an economic incentive for farmers to convert to organic production systems, with an added advantage that the standards that apply have beneficial implications also for the social and environmental practices of industry.

Who is the report targeted at?

This report will assist not only conventional prawn farmers but also farmers with access to inland saline waters and also producers of organically certified feeds. The report is targeted at these prospective growers of organic prawns, and those in government and industry who would support the structured development of this specialised industry sector.

Background

Existing prawn farming practices in Australia lend themselves to organic conversion. Since there are already stringent and suitable regulatory controls over chemicals usage and environmental issues which are central to organic standards, and since ecologically and socially acceptable values are already applied in the industry, participants in Australia could look towards mostly favourable assessments for conversion. There is a limit to the number of sites available to the aquaculture industry so expansion may have to occur at inland sites where artesian saline water is available.

There appears to be two critical issues for existing Australian prawn farmers to convert. The first is common to all organic food producers, that being the potential for contamination of their product with off-farm pollutants. Since natural waters from estuaries are sourced in an uncontrolled way, farmers would likely be required to test the stock on a regular basis for particular bio-accumulating substances before sale as organic product. The second critical issue will be in sourcing a suitably certified and appropriate feed to support acceptable growth of stock at a high enough density to be economically viable. Although prawns are commonly found in nature at ultra-high densities (eg. prawn boils), thus adding support to the notion of high density organic culture, most standards refer to low density culture systems, due to issues related to unsustainable feed supplies, energy inputs, and prevalence of disease.

The development of inland operations would potentially address several of the impediments for conversion to organic production systems mentioned above. Firstly, the artesian water source in Australia is of consistent quality and cannot be contaminated by upstream activities and events such as can occur in industrialised estuaries. Also, the greater biosecurity offered by a site remote from natural brackish-water systems may provide protection from the influx of pathogens to maintain a disease free status. And thirdly, the lower cost of land in inland Australia would allow more extensive practices to be economically viable, thus taking pressures away from the need for highly formulated stand alone feeds. Greater emphasis could be placed on converting natural feeds like plankton and biofilms into prawn biomass, with reduced reliance on high protein feed ingredients like fish meal.

Aims/objectives

The aims of the project were to:

- Investigate the organic standards that relate to low and high density grow out.
- Negotiate contract with industry partner for access to grow out site and for production procedures.
- Prepare ponds using organic procedures for introduction of seed.
- Use banana prawn seed (post larvae) for stocking ponds and use stock from these ponds for tank trial.
- Stock ponds at low density rates.
- Manage water quality and monitor prawn growth rates during the growing season.
- Concurrently conduct organic feed trials with juveniles as a contingency for poor growth rates in extensive ponds.
- If growth rate in low density ponds is too slow, apply limited supplemental feeding of the most effective organic feed identified from tank trial.
- Conduct treatment and packaging trials using a range of organic methods to identify optimum treatment.
- Drain harvest the ponds retaining water in separate reservoir for future use.

Methods used

Methodologies for preparing feeds for experimental use according to industrial methodologies, and effectively testing feeds in controlled growth trials were developed and are documented in detail for future reference.

The inability of contracted feed suppliers to reliably provide certified organic prawn feed, or large quantities of certified organic fish products for the project's use, seriously compromised farm trials that were planned. To compensate for the feed supply setback, project researchers placed greater emphasis on the formulation of organic prawn feeds using a range of certified ingredients that were available in Queensland, or using certifiable ingredients that were likely to be available in the future with reasonable rationale.

Experimental formulations were tested in replicated feed trials using commercially available prawn feeds as the benchmark for growth and survival.

Prawn packaging and storage trials focussed on modified atmosphere packaging (MAP) which uses certifiable practices and avoids the need for commonly used preservatives that would not adhere to organic principles. Modified atmosphere packaging was used to extend shelf life so that this product could compete in overseas market where a suitable premium is paid for organic seafood. The microbiological status of the product was evaluated during storage trials.

Results/key findings

The grow-out trial that was attempted showed that organic principals can be economically applied during the early part of production, and can provide savings for prawn farmers in general.

After only one feed trial for each prawn species under investigation, formulations have been identified that can produce growth rates up to 66% of that possible with locally produced commercial (though uncertified) feed. However, further development of a certified feed is required before any commercialisation of this research can occur. The feed's impact on pond conditions and the flavour of the product will be just as important as stock growth and density issues.

Significant background information on the nutritional contents of potential ingredients has been produced, and the preliminary feed trials using simple dietary formulations provide scope and offer direction for fine tuning organic feeds in the future.

Linkages have been made with several organic feed ingredient suppliers (eg. Kialla Pure Foods) and they have expressed a strong interest in supporting this development if sufficient demand for organic prawn feed can be demonstrated. The project has also succeeded in creating a critical mass of information and a collaborative experience base which can be successfully applied to organic aquaculture research and development.

Although the original intent of the project was to focus purely on new inland systems for organic prawn production, interest was also identified from existing coastal farmers. It is likely that operating prawn farmers will be better placed to successfully commercialise organic production of prawns in Australia, because they have the relevant experience in production and marketing aspects which are critical for success.

Prawn packaging experiments demonstrated an extended storage life of prawns in a chilled but unfrozen state. Regardless of organic certification, this represents a potentially new product that is not marketed anywhere yet, namely raw prawns in MAP. An organic product of this nature is likely to be more valuable to consumers than the currently produced organically certified cooked prawns in MAP, offering excellent export opportunities. Publication of this packaging work will be delayed so that DPI&F can assist Australian industry to maximise any market advantages it may offer.

Implications for relevant stakeholders

Industry: The results of this research imply that organic prawn production is feasible in Australia, but it remains to be seen if Australian businesses are willing to embrace this industrial development. If water stable organic prawn feeds were commercially available, there would certainly be marketing advantages for existing coastal prawn farmers to convert, and the development of an industry based on inland production systems could very beneficially focus on this form of high value product. However, businesses will need to fully consider this initiative and their position in the global future of cultured sea foods, since a number of countries are already moving towards these forms of quality assurances and are no doubt hoping for the competitive advantages they will provide in discerning markets like our own. There would be a strong need for a cooperative approach from all sectors involved in supply and production chains due to the need generally for economies of scale. This would be particularly important in the manufacture and reliable supply of feeds and continuous supply of products that meet consumer demands in large seafood markets.

Communities: Although organic seafood is new to Australian consumers, this report documents the increasing trends for this type of food certification in markets in Europe and many other developed countries. Any increased public uncertainly regarding the safety of food in the future will undoubtedly support the uptake of these forms of specified food certifications, and the largest food chains in the world are supporting its development.

Policy makers: Perhaps one of the most pertinent questions being asked of this direction by industry in Australia is whether the “clean and green image” which Australia generally possesses can take the place of, or compete with, the standardised organic certification of an agency. Since there are presently several certifying agencies with different standards that each refers to, there is also some scepticism regarding its authenticity and ability to deliver a better product. If industry is to fully embrace this approach, there will be a need for policies and procedures which validate product labelling so that consumers can be confident in the qualities claimed by the manufacturers.

Recommendations

- Producers who are interested in pursuing organic certification should make contact with a certifying agency to identify where their current practices fit and what practices will need to be modified for conversion. They should look towards a cooperative arrangement with the certifying agency and its auditors, expect a positive assessment for most of their activities, but also expect that some aspects of their production system will need close scrutiny since prawn aquaculture is new to this specialist industry sector and to existing auditing processes.

- Interested producers should also make themselves known to government through Industry Development representatives so that further ongoing research can be supported in critical areas (eg. feeds development), and so that supporting industries (eg. feed manufactures) can be made aware of the possible future demand for essential supplies.

- Further diet development research needs to be undertaken, so that higher densities of prawns can be grown for improved on-farm profitability. Suitable raw ingredients for these feeds will also need to be confirmed so that commercial quantities can be made available at reasonable cost.

- A packaging method exists that adheres to organic principles and could get Australian product into Europe or the US in a form which consumers are demanding in the larger supermarkets. Allowances would need to be made to incorporate this into seafood processing facilities.

- When organic feed is available in sufficient quantities a commercial trial should be conducted to allow proof of concept at an industrially relevant scale.

- Publication of the packaging work will be delayed so that DPI&F can assist Australian industry to maximise any market advantages it may offer.

Introduction

There is an increasing demand world wide for organic foods. Over the last decade, organic foods have been one of the most dynamic of the international food markets. Many countries have shown remarkable growth rates for organic foods. Denmark and Sweden, for example, have exhibited growth rates ranging from 30-40% and Switzerland and the United Kingdom, 20-30%. The international market for organic food was worth approximately US$20 billion in 2000. The largest single market was the USA with sales of around US$10 billion, followed by Europe with US$9 billion, and Japan with US$1.5 billion. Although worth only a fraction of the global conventional food market, the organic market sector is nevertheless considered an important, rapidly growing niche market, not to be ignored by marketers.

The Australian organic industry generates between $250-300 million per year, with annual growth around 20-30% (Landline, 2002), yet currently it does not meet the level of demand (IFOAM, 2003). Obviously, consumers are prepared to pay a premium for organic foods. There is a premium between 50 and 75% for organic foods in Australia (FAO 2002). A more recent review (DPI&F 2004) found premiums between 5 and 300% and very high export growth for our organic produce in 2004-2005.

There are very few aquaculture ventures in Australia producing and or processing organic seafood. There is a mussel farm in Victoria and a silver perch farm in NSW. Both are certified by The National Association for Sustainable Agriculture Australia Limited (NASAA). There are only a few prawn farms in the world producing organic prawns. Recently Lyons Seafoods in the UK became the first company in the world to retail organic prawns, after joining forces with an organic farm in Ecuador. Ready-to-Eat Organic King Prawns are now sold at selected branches of Tesco (£2.99 for a 125g pack or A$63/kg) while Waitrose sells Anchor Seafoods Freshly Cooked Organic King Prawns (£3.49 for a 125g pack or A$74/kg). These existing markets in major UK food chains offer an encouraging premium for Australian aquaculture prawns, with a retail price of at least A$60/kg.

There is also considerable commercial interest in organic prawns in the USA. One farm addressing these opportunities is The Organic Certified Texan farm “Permian Sea Shrimp Company” which uses artesian saline water. Their prawns retail at prices from US$12.50 to $14 per pound (A$39-44/kg). Another is Oceanboy Farms Inc., who also recently gained organic accreditation for their cultured shrimp. These wholesale for US$8/pd (A$26/kg) to a chain of supermarkets specialising in ‘green wise’ foods in Florida. Waddell Mariculture Center is also presently testing an organic shrimp diet designed by Advanced BioNutrition (ABN) Corp. in Maryland, while Freedom Feeds in Ohio have one currently available. Whilst the suitability of these feeds for Australian species and conditions is yet to be tested and proven, it is unlikely that this feeds could be economically imported for Australian commercial prawn production.

An article in the Guardian Unlimited newspaper in July 2003 encapsulates some of the environmental problems that organic principles could help address in this industry in developing countries the future. It was written by Felicity Lawrence and was derived from a report from the Environmental Justice Foundation. It points out that uncontrolled shrimp aquaculture has led to the loss of mangrove forests (40%) worldwide, and has also led to salination and chemical pollution of farm land and drinking water for rural communities. It claims that The World Bank has encouraged much of the development of shrimp farms in Asia, where there is a heavy reliance on antibiotics to keep diseases at bay. However, the EU has now banned prawns from China because of this antibiotic use while the United Kingdom (UK) food standards agency has found problems with shrimp from Thailand, Indonesia, Pakistan and Vietnam. Many of the production ponds in these countries now lie abandoned due to loss of productivity and disease, and future lower production is predicted. Yet by converting to organic practices and extensive growing conditions some of these farms may survive. For example, a German wholesale food company is now assisting Vietnamese marine prawn farmers to produce organically.

The Australian prawn aquaculture industry has so far managed to avoid many of the problems faced overseas. This is due to good farming practices, better planning and environmental controls, and a lower incidence of virulent diseases. Nevertheless, the Australian industry has been seeking alternate feed materials, and this project and the wider organic initiative could provide the ideal vehicle to show how prawns can be produced in a more environmental friendly way with less reliance on imported fishmeal. As these finite resources become increasingly difficult to source in a reliable fashion, and accordingly increase in price, the importance of alternatives will also rise. This project seeks to begin these investigations and bring together other feeds suppliers who may assist in these developments.

This project also offers a solution to one of the most limiting factors for the expansion of the Australian prawn industry using the current marine farm format, that being that few new marine aquaculture sites are now available. The industrially proven viability of using inland sub-artesian saline waters in Texas in the United States (US), and to a lesser degree at Bauple in Queensland, draws attention to the vast inland areas with suitable saline waters that are available in rural Australia. In addition to the direct industrial opportunities and indirect benefits for the existing prawn farmers such as favourable advertising and media campaigns focussed on environmentally friendly production methods, these developments would also benefit rural communities in many ways. For example, prawn farms require staff with a range of skills and generate significant employment and flow on effects in local economies. A general gauge of this is that every 2 hectares of prawn ponds generates one job, and for every three jobs on farms another is created in support services. Currently the site of these farms and thus jobs are on the coast, but the development of inland prawn farms would provide jobs badly needed in some rural regions.

Market trends in the US suggest organic prawns are worth pursuing. For example, the demand in the US for organic products grew by 21.2% from 1997 to 2003, and Beatrice Viertl Bujard of the Organic Support and Advisory Department identified the US market as a target for expanding organic shrimp sales (Brister, 2004). This suggests that ready markets exist in the US for Australian producers of organic prawns.

As the premium markets for organic prawns are overseas, suitable packaging needs to be developed that will ensure that consumers get a high quality product with an effective shelf life. Conventional storage treatment of prawns involves the application of chemicals such as sulphites or hexylresorcinol to prevent the endogenic enzymic formation of the dark pigment melanin, commonly called black spot. While this is only a visual defect it does impact on the attractiveness of the prawns to consumers. These chemicals would not be satisfactory for organic production. The only ones that meet the requirements are ascorbic acid and citric acid. Both are not as effective as the previous two chemicals mentioned and may need the assistance of a supporting technology. The removal of oxygen, such as occurs with vacuum and modified atmosphere packages (MAP) inhibits blackspot development. Good presentation and shelf life is attainable using MAP technology. This type of product has grown rapidly in the last decade so that it now occupies a large proportion of the supermarket sales in Europe for fresh food.

In the US, the shortfall of domestic prawn production to meet demand is worth US$4.3 billion. In Europe the import of crustaceans and molluscs is worth Euro 1.7 billion. By comparison, the value of Australian prawn exports currently is $208 million, only a small fraction of this overseas demand. The value of Australian prawn aquaculture was A$52 million in 1999-00, grew to A$57 by 2003-04 and dropped to A$50 by 2005-06 (Abare 2007). While the Australian prawn farming industry output has grown to provide almost half the amount of prawns produced in Australia, markets overseas could easily absorb a lot more of our production. In 2002-2003 the seafood sector only contributed around 5% to total Australian rural exports. The limited number of organic prawn farms in the world highlights the business opportunities for exporting this product.

Rising saline groundwater is one of the biggest environmental problems in Australia and it currently affects over 2.5 million ha of land. It is estimated that within the next 30-40 years, the affected area will grow more than fourfold. One of the key methods to ameliorate the effects of salinisation is to pump the saline groundwater into large ponds for disposal by evaporation. Inland saline aquaculture may offer a partial solution to the shortage of coastal sites for aquaculture while incorporating aquaculture into saline groundwater interception and evaporation schemes to provide an economic return to the costly business of building and operating these schemes. The National Aquaculture Council (NAC) recognises the importance of developing prawn farms that utilise inland saline waters and has been supporting DPI&F's experiments at the Bauple farm for some time. The present project seeks to add the organic certification option to this approach, and to the business plans of coastal farmers for improved profitability and environmental sustainability.

Objectives

The objectives defined in the RIRDC contract are listed below. These have been summarised into the topics listed in the Executive Summary and the major chapter headings.

Investigate the organic standards that relate to low and high density grow out

Some organic standards are more tolerant of intensive principals so a review will identify the conditions most suitable for all types of Australian prawn farms. This information will help industry determine which markets to target using the appropriate grow out conditions. The nutritional requirements for prawn larvae are known and the researchers will try to match these nutritional requirements with ingredients available from organic certified suppliers.

Negotiate contract with industry partner for access to grow out site and for production procedures

The negotiation of access has already been conducted and the grow out experiments will be conducted on the property of W & D Hellmuth at Bauple. Experimentation on prawn aquaculture using inland saline water has been underway for several seasons at Bauple.

Prepare ponds using organic procedures for introduction of seed

Where the soil requires a conditioner, such as lime, or the water chemistry requires additional minerals then these will be applied using organic sources whenever possible.

Use banana prawn seed (post larvae) for stocking ponds and use stock from these ponds for tank trial

Banana prawn (*Fenneropenaeus merguiensis*) post larvae (PL) will be obtained from either a commercial hatchery or from the hatchery unit at BIARC for stocking into the ponds. Tank trials will begin when this stock reaches a size suitable for feed trials and when the stock can be effectively captured from the ponds without causing excessive physical damage

Stock ponds at low density rates

As the grow out will mainly be extensive to match EU organic requirements, low stocking rates of PLs will be applied. For less particular markets higher densities will be used for experiments such as the prawn feeding trials.

Manage water quality and monitor prawn growth rates during the growing season

Good husbandry practices require the regular testing of pond water and monitoring of prawn growth. This requires a project participant to be present on farm throughout the grow out phase.

Concurrently conduct organic feed trials with juveniles as a contingency for poor growth rates in extensive ponds

Currently there are no locally produced organic prawn feeds. One of the industry partners, Kingfisher Enterprises, is an organic feed supplier who wants to trial his ingredients in a prawn feed formulation. A scientific evaluation of this developmental prawn feed is required, as there is little published data available on the range of ingredients and the performance of this type of diet. Experiments need to be undertaken to establish the effectiveness of this feed with respect to a number of different feeds that are commercially available.

If growth rate in low density ponds is too slow, apply limited supplemental feeding of the most effective organic feed identified from tank trial

It is recognised that once the prawns have attained a certain size they will be less able to utilise the natural feed in the ponds so some supplemental feeding will be required to ready the crop for market.

Conduct treatment and packaging trials using a range of organic methods to identify optimum treatment

The conventional blackspot treatment for prawns of sulphite dipping is not considered organic (yet organic wines are allowed this treatment). Those that are certified as organic are less effective so trials of their application in conjunction with modified atmosphere packaging will be required to assist industry to produce organic prawns with good shelf life for the most appropriate market. Modified atmosphere packaging using carbon dioxide and nitrogen gases is allowed for organic production, so storage trials will be conducted to evaluate the effectiveness of this treatment on both raw and cooked prawns. This type of packaging is ideal for producing retail packs that can be shipped as airfreight. These trials will be conducted mid way through the grow out period.

Drain harvest the ponds retaining water in separate reservoir for future use

As the grow out system is intended to have minimal waste water issues, the ponds will be drained at harvest and the water stored in adjoining ponds for the next crop. If a disease or excessive build up of contaminants occur then a suitable treatment regime will be utilised.

Methodology

Investigate the organic standards that relate to low and high density ‘grow out’

The standards that relate to certification of organic aquaculture have been investigated. The Organic Standard — version 6 (August 2003) and version 7 (December 2004) of the Biological Farmers of Australia Co-op Ltd. were followed closely. The standards were used to conduct a desktop study of the existing prawn industry’s production practices, to assess the industries organic potential, and to determine the feasibility of developing new large-scale organic prawn production in inland regional areas. Alternatives to activities that could not be certified were investigated via worldwide information searches and consultation with recognised global experts.

Identify a suitable diet for organic prawn aquaculture

Several prawn diets were tested against a standard widely used commercial feed in two experimental tank trials. Kingfisher Enterprises was to develop several prawn feeds containing certified ingredients for inclusion in the trials but unfortunately did not supply. To compensate for this setback, the first feed trial involved feeding various forms and simple diets based on wheat flour to banana prawns in support of the farm trial that was concurrently underway. The diets for the later second trial tested a wider range of potential ingredients and were prepared with the help of an animal nutritionist from DPI&F's Animal Research Instituite at Yeerongpilly, Dr John Kopinski, and in conjunction with contracted feed manufacture by the University of Queensland.

Experimental tank system

The experimental tank system used for the trials was the same as that described in previous growth and survival research with banana prawns at Bribie Island Aquaculture Research Centre (BIARC) (Palmer *et al*., 2005a). It incorporates 26 round plastic tanks (diameter of 1550 mm) with netting covers (to prevent bird predation and prawns jumping out), all housed inside a greenhouse designed to minimise diurnal temperature fluctuations. Each tank was fitted with a 50 mm standpipe for screened water to overflow to waste, giving a constant water depth of 900 mm and maximum water volume of 1700 litres. They were also fitted with a 4 mm airline, which delivered constant moderate aeration (0.5-0.8 L min^{-1}) to the centre of each tank in mid-water column. Unfiltered seawater was continuously pumped into each tank at similar rates (3 L min^{-1}) for the duration of the trial and for four days prior to stocking prawns. All tanks were initially clean and dry. The unfiltered seawater supply carried a low level of silt and naturally occurring suspended matter (eg: phytoplankton and zooplankton) from Moreton Bay. Supply lines were flushed daily and designed so that each tank received a similar suspended matter load. Debris that collected on the tank bottoms during the trials was left undisturbed for the duration of the trials.

Experimental design

Nine experimental treatments involving different feeds and control diets were applied in each trial. In each case these were assigned randomly to each tank in a completely randomised design. There were three replicates for each treatment (except where indicated in the results). Methods specific to each trial are described in the respective sections.

Water qualities were generally tested twice weekly. Prior to stocking, an average juvenile length and weight was obtained from the central pool of seed stock. Juveniles were then stocked randomly into each 2,000 L tank at moderate densities. Prawns were fed at regular intervals and each trial continued for a total of four weeks. On completion, all tanks were harvested to record length, weight, survival and growth for each treatment. Results from these trials were used to determine the performance of the diets and their relative merits in organic farming operations.

Pellet water stability assessments

All pellet diets (including the control) were qualitatively assessed for their water stability in high salinity (36 ppt.) seawater at 26-27°C. Two grams of each feed was immersed in still seawater (1.5-2 L) in transparent hemispherical fish bowls with regular observations and physical comparisons made thereafter. At 5, 10 and 20 min and 1, 3 and 6 h after submersion, each diet was mixed with a small spoon and allowed to settle. Comparisons focused on any apparent differences in the experimental diets with the commercial control diet, in terms of the texture and integrity of the pellet, and the clarity of water in each bowl. In the second feed trial, a series of photos of each bowl were also taken after the 3 h inspection.

Data collection and statistical analyses

Max/min water temperatures were recorded daily from tank 10 which was located in the middle of the tank complex. On several occasion during each trial, water pH, temperature and dissolved oxygen readings were taken from each tank to determine between-tank consistencies in water qualities. Qualitative measures of prawn activity, survival and condition were undertaken the day after stocking and every few days thereafter. Prawn bulk weights (total prawn biomass per tank) were measured before stocking and after harvest for each tank. In weighing procedures, the time prawns spent in a net draining before being placed into a tared bucket of seawater was standardised at 10 sec. The number of prawns in each bulk weight was also recorded. After harvest at the end of the first trial (10 Jan 2006), the condition of prawns in each tank was qualitatively assessed and the length of each prawn's longest antennae was measured to the nearest mm (base of eye stalk to tip of antennae). For the second trial, the condition of prawns in each tank were qualitatively assessed.

Data were analysed using GenStat® for PC/Windows XP, Eighth Edition. Analyses of variance were performed for continuous data followed by comparisons of means using least significance difference (LSD) testing with a 5% level of significance. Percentage survival data were analysed using a generalised linear model (McCullagh and Nelder, 1989) with the binomial distribution and logit link (GenStat, 2005), followed by protected t-tests to determine significant differences between the means.

Document the requirements for organic prawn 'grow-out'

The pond growout trials were conducted on the property of W & D Hellmuth at Bauple, south of Maryborough. Originally a redclaw farm, some of the ponds on this site are sponsored through the NAC and the FRDC national inland saline aquaculture communications project.

Three ponds of approximately 650 m^2 were used for the trial, each holding 350,000 litres. Each pond was approximately 1.5m at the deep end and 0.3m at the shallow end. Water was sourced from a nearby bore and stored in a holding dam where it can be treated prior to entry into production ponds. Initial testing gave a salinity of 2.9 ppt, conductivity of 5710 µS/cm and importantly a relatively low potassium level of 6 mg/l. As a result, the holding dam was treated with potash (KCl) to raise potassium levels nearer to that of a diluted seawater equivalent (40mg/l). Other ingredients were also required to ensure a healthy plankton bloom. All these components are available in organically certified forms. A 1.5 hp Force-7™ aspirator provided aeration and circulation in each pond and was used only when pond oxygen readings dictated its use.

Banana prawn post-larvae seed stock (PL's) were collected on two occasions from a commercial prawn hatchery (Tomei Prawn Farm P/L) and transported to the trial site where they were placed into one of three 2000 l tanks under a covered pond-side site. In these tanks the PL's were acclimated over a 72 hour period from 30 ppt down to 2.4 ppt at a rate of 0.3 ppt/hr using pond water, prior to pond stocking. This acclimation rate has been the basis of previous research at BIARC and has been found to be both highly successful and applicable on-farm. The PL's were fed live harvested zooplankton during the acclimation process to retain health and reduce cannibalism.

In an attempt to ascertain post-stocking survival, each pond held a sub-sample of 60 PL's placed into a Hapas net and checked 2 weeks after stocking. The first batch of banana prawns were stocked into pond B1 on 27 October, 2005 with 8228 PL's (12.6/m^2) and an estimated post-stocking survival rate of 82% (10.4/m^2). Ponds B2 and B3 were each stocked with 6568 PL's on 3 November, 2005 with survival calculated at 40% and 25% respectively. This left B2 with 2627 prawns (4/m^2) and B3 with 1642 prawns (2.5/m^2). As this stocking level was far lower then anticipated and there were excess tiger prawns available, the decision was made to stock the excess black tiger prawn PL's into B2 and B3 to ensure acceptable research and commercial returns. Accordingly 7000 tiger prawns were stocked into each pond on 6 November, 2005 with an estimated survival of 82% in B2 (5740) and 92% (6440) in B3. Final total stocking numbers of banana and tiger prawns into B2 are 8367 at 12.8/m^2 and B3 at 12.4/m^2 or 8082 PL's.

A weekly sample of approximately 50 prawns was captured from each pond to estimate average weights, prawn condition and biomass, initially using a feed tray and then when the prawns were large enough via a cast net. In pond B1 the sample was bulk weighed and counted to give an average weight and returned to their pond. In ponds B2 and B3 the prawns had to be separated into species before being bulk weighed and counted and then returned to their respective pond. Water quality was monitored and recorded twice daily (AM/PM) measuring temperature, dissolved oxygen (DO), pH and salinity. Ammonia, nitrite and potassium were measured weekly unless there was reason to believe that these parameters were likely to be outside a safe range (i.e. after heavy rainfall). Potassium was added when required to maintain a minimum level of 30mg/l. The Force-7™ aspirators were used only when DO levels dropped to an unsuitable level.

Develop modified atmosphere packaging (MAP) as an organic-acceptable procedure to extend shelf-life

Packing and storage

The feasibility of packaging sulphite-free prawns in MAP was investigated at the laboratory of Innovative Food Technologies, Hamilton Brisbane, using prawns from three sources; BIARC, Bauple and a commercial farm north of Brisbane. Banana prawns were harvested from the BIARC research pond and taken to the laboratory at Hamilton where they were packed in MAP or cooked then packed in MAP. At the Bauple and the other commercial prawn farm the prawns were packed on site.

An Emrich Practika modified atmosphere machine packed 195 mm (l) x 145 mm (w) x 30 mm (d) trays containing approximately 200g of prawns using a gas mixture of 60% CO_2 and 40% N_2 at 600 kPa for 2 seconds. Packs were stored in a cold room at 4°C.

The shelf life of prawns using this packaging treatment was determined. Microbiological analyses and some sensory analyses for consumer acceptance were incorporated into this shelf life testing regime.

Physical Analysis

The headspace of the sealed packs was evaluated after storage using a standard Shimadzu Gas Chromatograph. This identified the proportions of oxygen, carbon dioxide and nitrogen present.

Statistical analysis

Statistical analysis has been carried out using ANOVA via the packages Statistix and Genstat.

Microbiological evaluation

The microbiological quality of the prawns stored under MAP conditions was evaluated through a series of storage trials at 4°C. Test samples were prepared by aseptically sub-sampling 10 g from several prawn tails and transferring it into sterile stomacher bag. The sub-sample was diluted 1:10 with 0.1% peptone. The mixture was then homogenised for 60 seconds using a Colworth Stomacher 400.

Aerobic count

The total number of colony forming units (CFU) present per gram was determined using the surface spread method (Australian Standard, 1991b) and nutrient agar. The plates were incubated at 25°C for three to four days

Anaerobic count

Anaerobic counts were determined using the surface spread method (Australian Standard, 1991b) on reinforced Clostridial agar (Oxoid). A modified Differential Clostridial Media (**DRCM**) with the addition of polymyxin B sulphate was also utilised. This media is selective for gram +ve anaerobic rods and cocci. A further step was pasteurisation of a sample to grow the heat-activated spores of anaerobic gram +ve spore formers on this media (**Pasteurised DRCM**). The plates were incubated anaerobically at 30°C for 10 days in an anaerobic jar charged with hydrogen and carbon dioxide gases using Gas-Pak Plus (Beckton Dickinson).

H_2S positive bacterial count

Total count of dihydrogen sulphide (**H_2S**) producing organisms was determined using the pour plate method (Australian Standard, 1991a) on iron agar of Gram *et al*. (1987) and when set, the agar was overlayed with the same agar. The plates were incubated aerobically at 25°C for 3 days.

Demerit assessment

The appearance of the product in the packs before and after opening and the odour and the taste and texture were rated based on a modification of the demerit score system devised by Bremner *et al*. (1986). A number of descriptive parameters designed specifically for this product were scored on sheets at the time of sampling. Due to the large number of samples appraised each session and the limited time available the prawns were rated by just three individuals. A copy is provided in Appendix 4. The accumulated scores were calculated and these and the individual parameter scores were analysed for significant difference using analysis of variance. The smell of the prawns was appraised as soon as the layer of impermeable membrane was removed. The fact that the end point consumer may be preparing the prawns for consumption requires that a package of this type have few off odours when opened. Each trial was conducted to determine probable shelf life and safety of the product. The maximum score that could be achieved was 27 for raw prawns and 24 for cooked.

References

ABARE 2007. Australian Fisheries Statistics, Canberra, June:43-45.

IFOAM 2002. The organic guarantee system” from the Conference on International Harmonisation and Equivalence in Organic Agriculture.

Landline 2002. Farmers consider going organic. Australian Broadcasting Commission, Canberra, 7/72002.

1 Review of organic standards for aquaculture

1.1 Discussion on the Australian National Standard for Organic and Bio-Dynamic Product

The latest Australian National Standard for Organic and Bio-dynamic Product Edition 3.3 came into operation on 1 July 2007. It is available at <http://www.daffa.gov.au/__data/assets/pdf_file/0018/126261/National_Standard_Edition_3_3_1st_July_2007.pdf>. It contains a few key Australian farm standards pertinent to prawn aquaculture under a number of section headings. This standard is used by AQIS at the moment for export products. In the future it seems they will leave certification issues to the appropriate bodies for those importing countries. The Aquaculture Standards (section 3.22) are brief but specific. The relevant sections of the endorsed Australian Standard are presented in Appendix I.

As there are currently prawn farms operating overseas with organic certification they must have complied with the Standards required not only by their home country but also of the importing nation. It may be that prawns can be produced in Australia under conditions that would satisfy the importing country requirements but not meet the Australian Standards. It is not the role of this project to attempt to get new standards adopted. These have to be negotiated by those individuals wishing to attain organic certification for export from Australia. This discussion seeks to identify areas in the Standards where some interpretations need to be made on certain practices that may be essential for production of prawns in inland saline waters.

Standard 3.21 Aquaculture

General Principles

i. Aquaculture includes many forms of production in fresh, brackish and salt water. The Standard covers aquatic livestock grown from fingerlings or spat, in any form under controlled conditions. It does not cover organisms that are harvested from open waters.

ii. Organic or bio-dynamic Aquaculture is based on:
   a. high quality water entering the system, and
   b. sound management practices, and
   c. the use of appropriate stocking rates, and
   d. consideration of stock welfare, and
   e. the use of approved inputs

Response

Most of these general principles should be easy for Australian prawn producers to comply with. There are currently no hatcheries that currently produce organically certified post larvae. For an organic prawn aquaculture industry to develop all aspects of production need to be evaluated with the view for certification. The issue of commercially available post larvae which have been produced with the aid of antibiotics will have to be evaluated by an auditor. As the post larvae would fall under the title of “fingerlings or sprat” any antibiotic treatments applied to seedstock before placing them in ponds would be of lower concern due to the subsequent lengthy grow out period. In effect, any bioaccumulation would be very low due to their very low biomass when they were exposed, and the remaining culture period would be well in excess of withholding periods. Chemical leaching from the prawn tissue over this period and the many-fold increase in body size would almost certainly reduce residues to undetectable levels. If this aspect of seed production remains as an issue in the certifying body's standards, it could alternatively be carried out in an organic hatchery and/or at the organic farm, although this will likely increase the setup costs for prospective producers of organic prawns.

Standards

3.21.1 Aquatic products must be under a system of inspection for at least 12-months before any products can be labelled as organic or bio-dynamic.

Response As the grow out of prawns normally takes less than 12 months this required period would obviously have to incorporate the preparation of the ponds prior to introduction of the post larvae.

3.21.2 Breeds adapted to local conditions shall be chosen. Natural breeding behaviour, settlement and hatching are desirable traits.

Response In an inland area this cannot apply since there are no marine breeds that are local. Additionally, it would not be economically feasible to grow prawns outside of their normal environmental range in outdoor ponds. As the crop is usually harvested before they can breed this issue is also not very relevant.

3.23.3 Polyploid and genetically engineered aquatic species are not allowed.

Response Only non-genetically modified spawned seed would be suitable for organic prawn farming.

3.23.4 Provision of ample clean water.

Response This may conflict with the preferred green water culture system which lowers stress and provides significant natural feeds for prawns. Thus clean or clear water may be counter to best culture practice. This also relates to section 3.21.7b which addresses suitable feed, and may best be evaluated on a case by case basis by an auditor. In this regard, the production of plankton (phytoplankton and zooplankton) blooms could easily be conducted using organic standards.

3.23.5 The certified operator shall ensure that construction materials and production equipment shall not contain synthetic chemicals or substances, which could detrimentally affect the environment or contaminate the certified product.

Response The certified operator should be able to ensure that construction materials and production equipment shall not contain synthetic chemicals or substances, which could detrimentally affect the environment or contaminate the certified product.

3.23.6 There must be adequate room in enclosures for the stock to exhibit natural behaviour such as forming shoals.

Response This may have an influence on which species of prawn is cultured. Banana prawns are tolerant of very high densities throughout their life cycle, while others do form dense schools as they mature. In fact, a case could be presented which suggests prawns prefer to be in a school, and thus low density culture becomes more of an issue for the level of artificial feed to be applied and the levels of nutrients that are considered acceptable in the discharge waters (see 3.21.8).

3.23.7 The diet must be suitable for the species and be from any of the following sources:

a. plant and animal products produced according to this standard; and/or

b. plankton and zooplankton grown in the organic aquaculture system; and/or

c. nutrients contained within the water supply; and/or

d. disease-free processed waste from wild harvested marine organisms.

Response The diet used will be suitable for the species and can be from any of the following sources:

a. plant and animal products produced according to this standard will be incorporated by certified organic feed producers; and/or

b. it is an inherent part of prawn culture to encourage the growth of plankton and zooplankton in the aquaculture system; the preparation of the pond water for production of this plankton would be conducted using the permitted materials listed in the standards; and/or

c. nutrients contained within the water supply may not impact directly on prawns but indirectly though plankton bloom culture.; and/or

d. disease-free processed waste from wild harvested marine organisms is part of normal prawn aquaculture diets but there is currently a trend by industry to reduce the amount of marine component in feeds. The species grown will influence the amount of waste required for making feeds.

3.28.8 Minerals and vitamins used as feed supplements must be naturally sourced.

Response These would be naturally sourced from certified organic prawn feed suppliers.

3.28.9 Operators must demonstrate that water and the nutrient load leaving the system will not adversely affect the environment, natural ecology or biodiversity.

Response Discharge permits for prawn farms in Australia uphold the premise that water and the nutrient load leaving the system will not adversely affect the environment, natural ecology or have biodiversity impacts. Closed system practices are still some way of for the industry, but partial recirculation of wastewaters is currently in use. This would be another issue for case by case auditing.

3.21.10 The use of allopathic veterinary drugs is not permitted in the treatment of organic Aquaculture. Where such a substance is required, the treated pond/tank area(s) affected cannot be used for organic production for a minimum of 12 months. Treated species will lose their organic certification status.

Response The use of allopathic veterinary drugs will negate any organic certification so would only be used to treat a crop that would be later sold to non-organic markets because of the loss of organic certification. Where such a substance was required, the treated pond/tank area(s) affected obviously cannot be used for organic production for a minimum of 12 months.

3.21.11 Capture and handling techniques can stress and damage stock. Aquatic stock should be handled as little as practical and fish shall not be out of water for more than 30 seconds during any handling procedure.

Response There is no need to handle prawn stock and return those individuals to the pond after any handling procedure.

3.21.12 The use of synthetic chemical tranquillisers is not permitted.

Response Synthetic or organic chemical tranquillisers are rarely if ever used in prawn production.

3.21.13 Oil of cloves or ice slurry or carbon dioxide is permitted for the sedation of fish, for pre-slaughter or transportation purposes.

Response While other methods are permitted it is likely that only ice slurry would be used for pre-slaughter or transportation purposes.

3.21.14 Any sorting or moving of aquatic stock must be recorded.

Response Good husbandry requirements ensure the recording of any sorting or moving of aquatic stock.

3.5 Water Management

General Principles

i Water and agriculture are inextricably linked. The harvest, storage, use and fate of waters are integral components of an organic farm. Management of water will include management of vegetation, soil and drainage on the organic or bio-dynamic farm.

ii Recycling of water should be carried out as much as possible.

iii Surface water leaving an organic farm should not contain greater levels of nutrients, salts and turbidity than when the surface water entered the farm.

Response The use of inland saline waters under closed systems will meet these principles. While some surface water may be retained for culture purposes the majority of water required will come from saline bores. The recycling of water will occur because green water culturing methods allows the reuse of the water taken from the pond at harvest. As the culture water will have greater levels of nutrients, salts (eg: potassium has been added) and turbidity than when the bore water entered the farm it would be retained and not discharged, because these are valuable characteristics needed for production.

Standards

3.5.1 On-site harvest of water for agricultural use (including stock water, aquaculture and processing) must allow for maintenance of on-farm and local ecosystems that are under the immediate influence of the operator. Provisional must be made for environmental flows to maintain existing riverine health, wetlands and biodiversity.

Response This standard would be followed.

3.5.2 Where appropriate operators shall design, measure and monitor irrigation water application to minimise water loss.

Response It is in the interests of all prawn farmers that water loss would be kept to a minimum.

3.5.3 Water from off-farm sources (e.g. river, public or shared channels, bores or drainage water) requires appropriate and regular testing to determine if substances incompatible with this standard are being inadvertently applied. If testing indicates prohibited substances are entering the water system, certification status may be affected.

Response As water quality is of critical importance to aquaculture it is likely that testing to determine if substances incompatible with this standard are being inadvertently applied would be more frequent than that applied to land based agriculture. Testing should identify prohibited substances entering the water system before it is introduced into the ponds ensuring that certification status is not affected.

3.5.4 Water containing treated human and industrial effluents, and/or their treated by-products can only be used after the water has been subject to effective treatments and has re-entered a natural public waterway system.

Response It is extremely unlikely that water containing treated human and industrial effluents, and/or their treated by-products would be used on an organic prawn farm even after the water has been subject to effective treatments and has re-entered a natural public waterway system as they would have an adverse effect on production yields.

3.5.5 Partially treated human and industrial waste can only be used on timber producing wood-lots, provided such application does not contribute to ground or surface contamination. Such water sources must be used with caution as they have potential to exclude the land from future grazing and agricultural use under this Standard.

Response Partially treated human and industrial waste for timber producing wood-lots would have to be kept separate to organic prawn production system as contamination could dramatically reduce the crop.

3.5.6 Raw animal liquid waste must be from a certified organic production system and can only be applied to green manure crops or pastures and never be directly applied to edible crops for human consumption. Applications of such substances must not contaminate ground water.

Response It is unlikely that raw animal liquid waste even from a certified organic production system would be used on an organic prawn farm.

3.5.7 Adequate dams and /or drinking facilities shall be established to allow rotational grazing management. Establishment of such sites must ensure overgrazing does not occur near water sites.

Response Adequate dams would be established to allow rotational dam management.

3.5.8 Water cannot be produced or harvested and labelled as organic or bio-dynamic.

Response Water from an organic prawn farm would not be produced or harvested and labelled as organic or bio-dynamic.

1.2 Discussion on the Biological Farmers of Australia Standard for Organic and Bio-Dynamic Product

The standard developed by the Biological Farmers Association is very similar to the Australian Standard. It is available at http://www.bfa.com.au/index.asp?Sec_ID=135. Unfortunately there is not a lot of detail for potential organic aquaculturists to base their plans on.

1.3 Discussion on the NASAA Standards for Organic and Bio-Dynamic Product

This was the standard used by Organic Fish Australia to obtain their organic certification and appears to be the most appropriate for other aquaculture species such as prawns. It is available at http://www.nasaa.com.au/publications.html.

1.4 Discussion on the Naturland Standard for Organic and Bio-Dynamic Product

This standard has been developed by the German company Naturland at www.naturland.de. It is the companies intention to be more stringent than the European Union Organic Standard. This is the standard used by contracted prawn farms in Vietnam in producing organic prawns for the European market.

The standard for prawns is very precise. There is the limit of stocking rates to $15/m^3$ and directions about fertilising, feeding, husbandry, harvesting and processing. While this standard is not required by AQIS, all potential organic prawn farmers who want to export to EU countries would be well advised to meet as many of these as possible because this is what is currently used by the market suppliers.

2 Identify a suitable diet for organic prawn aquaculture

2.1 Using wheat flour in the production of organic banana prawns

By Paul J Palmer and Steve L Slattery

The banana prawn *Penaeus* (*Fenneropenaeus*) *merguiensis* is one of the most popular prawn species cultured with extensive farming practices in Southeast Asia (Boonyaratpalin, 1998; Thongrod and Boonyaratpalin, 1998). It has also been the subject of several recent studies in Australia focussing on its particularly amenable culture attributes (Hoang, 2001; Palmer *et al*., 2005ab). This trial was intended to provide information about the use of simple feeds in extensive prawn production systems, and if possible, to provide immediate feedback to managers of the related farm trials, to maximise the on-farm growth of prawns using certified-organic feeds.

Since a Certified Organic fish-meal-based prawn diet which had previously been planned for use in the research project was unexpectedly not available, the feed trial focussed on the use of supplemental organic feeds that were nevertheless in use at the farm. Certified-Organic whole wheat flour, had been in regular use in the organic farm trials since stocking prawn seed. Its regular addition to ponds was intended to lift natural pond productivity whilst also being consumed directly by the prawns. Farm operators had observed the prawns eating the flour directly when added as a powder or thick paste (dough balls placed on feed trays), and gross inspection of their digestive tracts indicated that they were feeding directly on this organic feed supplement. Certified organic molasses from sugar cane was also being applied directly to ponds to help control algal blooms. Chickens egg and polychaete worms were two other ingredients considered to be readily organically certifiable and potentially useful in prepared diets at low inclusion levels as feeding stimulants.

Wheat flour is generally used as a binder and nutrient source in many pelletised aquaculture feeds (Tacon, 1990a; 1990b; Cheng *et al*., 2002) and is a common ingredient in commercial prawn feeds used in Australia (eg: Ridley Aquafeed prawn feed; Charoen Pokphand prawn feed). It is one of the least expensive sources of carbohydrate and is generally used in prawn feeds as a dietary energy source to spare the more costly protein for growth (Shiau and Peng, 1992). Alone it could be expected to lack a balanced nutritional profile (eg: some essential amino acids and fatty acids), but in combination with the natural foods that prawns utilise in eutrophic ponds including zooplankton, algae and detritus, and at low prawn stocking densities, significant growth may be possible with this as a supplemental source of nutrition.

This trial therefore sought to assess a range of ways that wholemeal wheat flour could be used as the main organic feed ingredient. Treatments were designed to identify the most efficient way to feed wheat flour, and pellets consisting mainly of wheat flour, in terms of prawn survival, growth, and condition, assuming that prawns can gain nutritional benefits from its direct consumption, in addition to its indirect stimulation of natural feed sources. The trial diets were crude formulations based on available organically certifiable ingredients intended for application as supplemental feeds in an extensive pond grow-out situation.

Specific materials and methods

Treatments were allocated randomly as indicated in Table 1.

Table 1 Treatment allocation for tanks and starting tank bulk weights

Treatment-replicate	Tank	Prawn bulk weight
1-1	9	32.94
1-2	13	30.22
1-3	26	36.52
2-1	21	31.54
2-2	7	30.98
2-3	6	37.63
3-1	20	34.87
3-2	2	29.21
3-3	18	34.89
4-1	4	31.88
4-2	12	34.35
4-3	1	33.85
5-1	3	33.67
5-2	10	33.94
5-3	19	32.09
6-1	8	33.65
6-2	22	30.58
6-3	15	38.10
7-1	23	37.14
7-2	5	33.23
7-3	16	32.20
8-1	24	35.21
8-2	14	36.64
8-3	25	34.56
9-1	11	32.75
9-2	17	31.88

Mean biomass per tank = 33.635 g Mean prawn weight = 1.345 g

The treatments were as follows:

1. Commercial Australian prawn feed (Ridley Aquafeed Starter #2) — control treatment for maximum growth possible.
2. No feed — control treatment for minimum growth likely on low level of natural food sources present in supply water.
3. Wheat flour added as powder — simplest feed additive.
4. Wheat flour rolled into 1 dough ball — second simplest feed additive.
5. Wheat flour only pellet — consisting of 100% wheat.
6. Wheat flour + chicken egg pellet — consisting of approx. 1.5% egg as attractant.
7. Wheat flour + marine worm (lower level inclusion) pellet — consisting of approx. 1.1% worm as attractant.
8. Wheat flour + molasses pellet — consisting of approx. 4.2% molasses as attractant.
9. Wheat flour + marine worm (higher level inclusion) pellet — consisting of approx. 6.8% worm as attractant.

Experimental feed ingredients and preparation

All ingredients used in experimental feeds were either organically certified, or what was assumed to be organically certifiable with a reasonable rationale. The flour used as the base ingredient was Organic Stoneground Wholemeal plain wheat (*Triticum aestivum*) flour certified by Organic Food Chain TOFC 27 and manufactured by Kialla Pure Foods, Greenmount Qld 4359. The organic standards state that it is free from genetically modified organisms (GMO), chemicals, infestation, mould and foreign materials. The chicken egg was large fresh whole egg (without shell) marketed as Coles Organic free range (Australian Certified Organic, in conversion 101081C). The marine worms (Polychaeta: Nereididae) were small (individuals <0.5 g wet weight) cultured *Perinereis* spp. grown without any chemical additives and exclusively fed on prawn farm effluent (phytoplankton and other naturally derived organic matter). These were frozen following purging for 2 hr after harvest from constructed sand beds. Thawed worms were blended into homogenous slurry prior to feed inclusion. The molasses used was certified as organic produce from the Rocky Point Refinery at Woongoolba in Southeast Qld.

Preparation of experimental feeds involved blending ingredients to homogeneity with minimal use of reverse osmosis (RO) water to make similar consistency pastes. The amounts of egg or molasses added to the pastes (wet weights as a percentage of flour) were approximately 5%, and for the worm inclusion diets were 5% and 33%. This gave a 1.45% dry weight (DW) inclusion for the egg, a 4.15% DW inclusion for the molasses, and 1.14% and 6.81% DW inclusions for the worm diets, due to the different moisture contents of different feed additives (exact formulation given below).

Experimental feed formulation

Flour dough ball — certified organic stoneground wholemeal plain flour (2 g) mixed with 22 drops (1 mL) of tap water and immediately kneaded to a homogenous dough and rolled into a sphere.

Flour only pellet — flour (as above) (900 g) mixed with 300 mL reverse osmosis (RO) water to make extrusion paste. Dried pellet consisting of 100% wheat.

Flour + egg pellet — flour (as above) (950 g) mixed with one whole chicken's egg without the shell (48 g) and 350 mL RO water to make extrusion paste. Assuming a moisture content for flour of 12% (Kialla Pure Foods), and 74.4% for the egg (Tacon, 1990b), this final dried pellet consisted of approx. 1.45% dry weight of egg.

Flour + low worm pellet — flour (as above) (950 g) mixed with worm (50 g) slurry and 350 mL RO water to make extrusion paste. Assuming moisture content of flour as above (12%) and 80.7% for the worm slurry (DPI&F unpublished data), this final dried pellet consisted of approx. 1.14% dry weight of worm.

Flour + molasses pellet — flour (as above) (900 g) mixed with molasses (50 g) and 300 mL RO water to make extrusion paste. Assuming moisture content of flour as above (12%) and 31.4% for the molasses (pers. com. Rocky Point Refinery, 2006), this final dried pellet consisted of approx. 4.15% dry weight of molasses.

Flour + high worm pellet — flour (as above) (900 g) mixed with worm (300 g) slurry and 200 mL RO water to make extrusion paste. Assuming moisture content of flour as above (12%) and 80.7% for the worm slurry (DPI&F unpublished data), this final dried pellet consisted of approx. 6.81% dry weight of worm.

For pelletised feeds this paste was immediately extruded through a 4 mm die, steamed for 8 min, and dried overnight (20-24 hr) at 40°C. Dried strands were then crumbled/crushed and sieved to provide similar sized particles of >1 mm and < 2 mm. The commercial prawn feed control was also crushed and sieved in this manner. All experimental feeds were prepared one week before the trial commenced, and were stored in sealed plastic jars at 4°C. Daily aliquots for each tank were weighed to the nearest 0.01 g from these bulk packs each day.

Prawn acquisition, transport, handling and tank management

Juvenile prawns for the trial were obtained from one of the three organic prawn ponds at the inland farm site at Bauple in Southern Queensland (Pond B3), following clear health and histological examinations performed one week earlier. Only certified organic feeds had been used in ponds prior to this. Prawns were collected on the 12 December 2005, 39 days after stocking as PL18, from feed trays repeatedly baited with commercial prawn feed. This was undertaken from early to mid morning to minimise water temperatures, as necessary to maximise survival during handling and transport. Trays were lifted periodically and captured prawns were tipped onto wetted shade cloth to gently contain them. From there they were carefully transferred with soft nets into an 800 L fibreglass transport tank supplied with oxygen. To adjust prawn juveniles from the low salinity at the Bauple site (2 ppt.) to the high salinity at BIARC (36 ppt.), and to minimise stresses associated with pH and other water chemistry changes, the transport tank was filled with 40% BIARC water and 60% Bauple pond water, providing transport conditions of approximately 16 ppt and 27°C.

A total of 800 juvenile prawns were collected and transported by road (3 hour transit time) to BIARC. On arrival they were funnelled out of the transporter's 50 mm bottom drain, directly into a 4,000 L parabolic tank filled with seawater. They were held there overnight (without feeding) to isolate prawns damaged during the transfer, so that only healthy prawns were used to set up the experiment the following day. The trial began on the 13 December 2005, when 25 randomly collected prawns were stocked into each tank after weighing their collective bulk wet weight. Feeds were added to the tanks once per day on the opposite side of the tank to the overflow point. The daily feeding rate for each tank (2.0 g) was calculated as 6% of the overall mean prawn biomass weight across all tanks. The day after stocking, each tank was inspected for mortalities or unhealthy individuals. These would have been clearly visible on tank bottoms when the air and water supplies were turned off, due to the white discoloration of dead prawns against the green tank bottoms, or against the dark coloured silt/detritus which had previously accumulated in small areas of the tank bottoms. This silt/detritus build up on tank bottoms remained undisturbed until just before harvest and trial termination 28 days later, when it was siphoned to waste prior to prawn collection with soft nets.

Results

Pellet stability in water

All pellets reacted similarly when added to seawater. A small portion (2-5%) of the experimental diets floated, sinking with slight disturbance of the water surface. After 5 minutes submersion, all diets (including the control) had softened to a similar texture. Similarly after 20 minutes submersion, similar textures prevailed with all pellets easily able to be smeared into a paste between the fingers, but the pellet shape without disturbance remained unchanged. After one, two, three and seven hour submersion there were no apparent changes in any feed.

Feed ingredient proximal analyses

Table 2 provides some previously documented proximal analyses for feed ingredients generally used in prawn feeds (ie. wheat as % of grain; typical chicken's egg excluding shell; and various fish meals excluding bone and offal meal), for feeds used in this study (including product manufacturers specifications for the wholemeal plain flour, egg, molasses, and commercial prawn feed), and for the Polychaete worms (% wet weight) used in this study after harvest from a prawn farm wastewater remediation system (DPI&F unpublished data).

Table 2 Feed ingredient proximal analyses (%)

Feed type	Water	Crude Protein	EE	Crude Fibre	NFE	Ash	Calcium	Phosphorus
Wheat*	12.1	12.0	1.7	2.5	70.0	1.7	0.05	0.36
Wheat**	12.0	13.1	2.1	11.2 df	52.4 tc	-	-	-
Egg*	74.4	12.4	11.0	0.0	1.3	0.9	0.06	0.18
Egg**	-	12.8	10.1	-	<1.0 tc	-	-	-
Worms***	80.7	13.3	1.2	-	1.0 tc	3.8	-	-
Molasses****	21.9	0.0	0.1	0.0	74.7 tc	3.3	0.21	0.03
Molasses**	31.4	4.8#	-	-	60.8 tc!	12.2	0.71#	5.06
Fish meal*	7.0-9.1	57.0-72.7	4.2-9.3	0.8-1.0	0.8-4.4	10.1-26.0	2.04-7.86	1.42-4.21
Commercial prawn feed**	-	43.0	6.0	3.0	-	13.0	-	-

EE = lipid or ether extract, NFE = nitrogen-free extractives *given by Tacon (1990b); **Manufacturers specifications for product used in this study; ***DPI&F unpublished data; ****NutritionData.com (2006); df = dietary fibre; tc = total carbohydrate; #data from different batch of molasses produced at same mill in 2002.

The organic wheat flour used in the present work had slightly higher protein and fat levels than generally reported for whole wheat, whilst the organic eggs had a slightly lower level of total fat compared with the non-organic eggs (as described by Tacon, 1990b). The marine worms used in the present study had comparable moisture content to chicken eggs, and relatively similar protein levels to both whole wheat and eggs, but lower, and much lower, levels of fat compared with whole wheat, and eggs, respectively. On the other hand, molasses is almost entirely made up of water and soluble carbohydrates (see Table 2) such as sucrose (33.4%) and reducing sugars (15.2%), although these contents can vary by several percent yearly and between batches (pers.com. Senior Chemist J. Lal, Rocky Point Mill). Fish meal and commercial prawn feeds have much higher levels of protein than the other ingredients listed in Table 2 and have higher lipid levels than this study’s wheat-based experimental diets. The types of protein (amino acids) and lipids (fatty acids) are also quite different as discussed in more detail in later sections.

Tank trial conditions

Careful inspections conducted the day after stocking (9-11 am) did not reveal mortalities on the tank bottoms or in the clutches of live prawns (as is often the case as they are slowly consumed). Only one live prawn in tank 17 appeared to have the white-muscle syndrome typical of some banana prawns after handling, which is often unavoidable and presumably due to muscle cramp. It was not replaced. All prawns also appeared to have full gut tracts during these initial inspections. Similar silt and detritus levels were present on tank bottoms, covering approx. 20% of bottom areas. The salinity of supply and tank water remained high (35 ppt) throughout the trial. Water temperatures ranged from 26.5°C to 31°C (see Table 3) and between-tank variations of <0.2°C prevailed throughout the experimental system.

Table 3 Max/Min water temperatures (from tank 10) and feeding times

Day	Date	Min – Max Temp (°C)	Time feed added
Tues	13-12-05	29.0	5.30 pm
Wed	14-12-05	27.0–29.0	3.30 pm
Thurs	15-12-05	–	3.30 pm
Fri	16-12-05	28.5–30.0	1.00 pm
Sat	17-12-05	28.9–29.0	10.20 am
Sun	18-12-05	28.0–30.0	10.20 am
Mon	19-12-05	27.0–29.0	1.30 pm
Tues	20-12-05	26.5–29.0	4.00 pm
Wed	21-12-05	27.0–29.5	3.00 pm
Thurs	22-12-05	27.5–29.5	3.00 pm
Fri	23-12-05	27.5–29.5	1.30 pm
Sat	24-12-05	27.5–29.5	10.20 am
Sun	25-12-05	28.0–29.5	10.30 am
Mon	26-12-05	28.0–29.0	9.45 am
Tues	27-12-05	27.5–30.0	1.30 pm
Wed	28-12-05	28.5–30.0	1.00 pm
Thurs	29-12-05	28.0–30.5	2.30 pm
Fri	30-12-05	28.0–30.5	1.45 pm
Sat	31-12-05	29.5–30.5	10.30 am
Sun	1-1-06	28.5–29.5	10.30 am
Mon	2-1-06	28.0–30.0	12.15 pm
Tues	3-1-06	28.0–30.0	12.30 pm
Wed	4-1-06	28.0–30.0	2.30 pm
Thurs	5-1-06	28.0–30.0	2.00 pm
Fri	6-1-06	28.5–30.5	12.30 pm
Sat	7-1-06	28.5–30.5	10.45 am
Sun	8-1-06	27.5–31.0	10.45 am
Mon	9-1-06	27.0–28.5	3.30 pm
Tues	10-1-06	Harvest	

Similarly, pH remained stable at 8.2 (<0.04 difference between tanks), and dissolved oxygen levels remained high (6.3–6.6 mg L^{-1}). At the end of the trial, a small portion of sediments on the bottom of all tanks was in a reduced state, as evidenced by the anaerobic odour when tank bottoms were siphoned to waste prior to harvest. Despite this, the harvested prawns were vigorous and healthy, free from shell fouling and with full gut tracts.

Prawn survival and growth

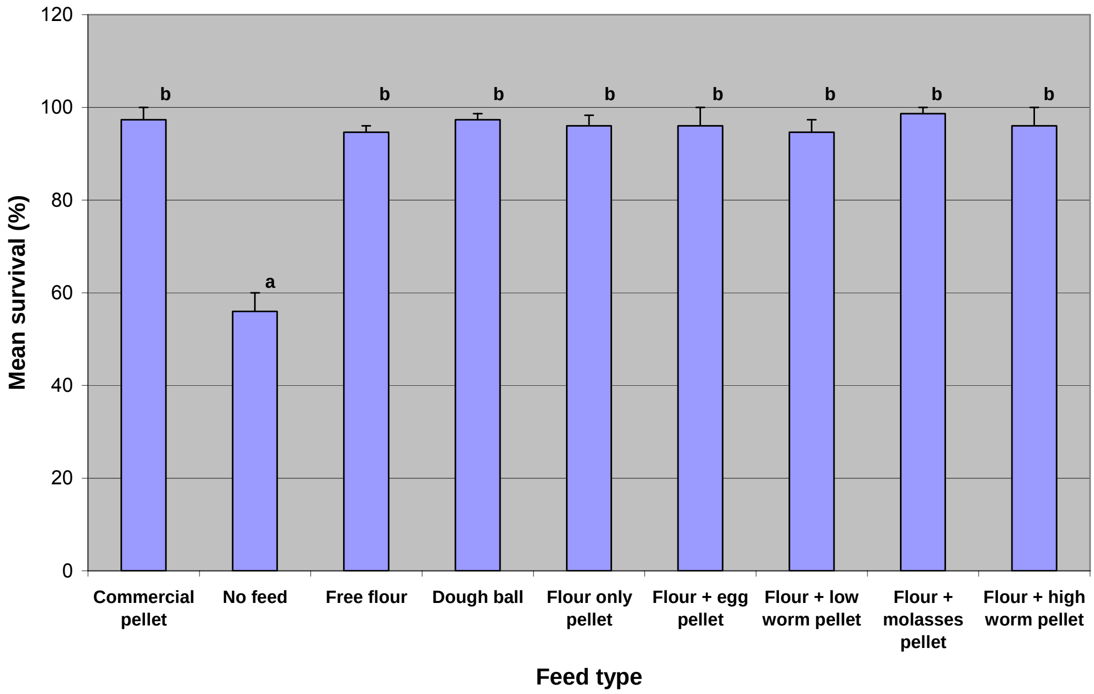
Similar (P>0.05) numbers of prawns were retrieved from all treatments except the unfed control (see Table 4 below). Unfed prawns had lower (P<0.05) survival (54%) than fed prawns and all fed treatments had a similarly (P>0.05) high survival (>94%) (see Figure 1 below).

Table 4 **Numbers of banana prawns surviving from 25 stocked into different tanks and fed different feeds over a one month period**

Feed type	Survivors in each replicate	Mean ± se. survivors
Commercial pellet	23, 25, 28*	25.3^{b} ± 1.45
No feed	13, 13, 16	14.0^{a} ± 1.00
Free flour	24, 24, 23	23.7^{b} ± 0.33
Dough ball	24, 24, 25	24.3^{b} ± 0.33
Flour only pellet	23, 24, 25	24.0^{b} ± 0.58
Flour + egg pellet	25, 22, 25	24.0^{b} ± 1.00
Flour + low worm pellet	23, 23, 25	23.7^{b} ± 0.67
Flour + molasses pellet	24, 25, 25	24.7^{b} ± 0.33
Flour + high worm pellet **	23, 25	24.0^{b} ± 1.00

*Unexplained error in initial number stocked, 100% survival assumed in later calculations; ** *n* = 2. Means with similar superscripts are not significantly different (P>0.05).

Figure 1 **Mean (± se; *n* = 3) banana prawn survival expressed as a percentage of the number stocked (25) into each tank for different feeds supplied over a one month period**



Note: Means with similar letters are not significantly different (P>0.05)

Average prawn weight at the start of the trial was 1.3 (± 0.02) g as calculated from the mean (± se; *n* = 26) prawn bulk weight across all tanks of 33.6 (± 0.46) g (Table 1). This initial biomass estimate provided the feeding rate of 2.0 g per day (6% body weight) applied to each tank for the duration of the trial. Mean (± se; *n* = 26) prawn bulk weight across all tanks at the end of the trial was 41.5 (± 2.41) g. There were no differences (P>0.05) between the mean bulk weights or mean prawn weights in different treatments at the start of the feeding trial (Table 5).

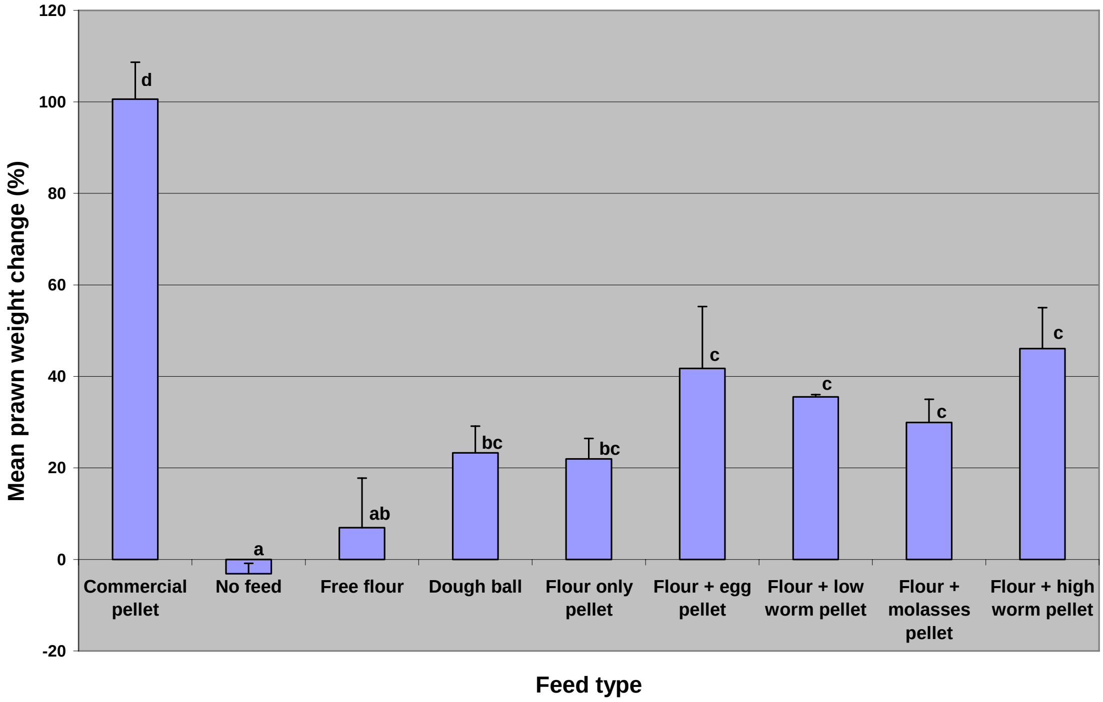
Table 5 **Mean (± se; *n* = 3) starting and finishing bulk and individual banana prawn weights in tanks fed different feeds over a one month period**

Feed type	Mean ± se start bulk weight (g)	Mean ± se start prawn weight (g)	Mean ± se finish bulk weight (g)	Mean ± se finish prawn weight (g)
Commercial pellet	$33.2^{a} \pm 1.82$	$1.3^{a} \pm 0.07$	$64.6^{e} \pm 2.31$	$2.6^{e} \pm 0.09$
No feed	$33.4^{a} \pm 2.13$	$1.3^{a} \pm 0.09$	$18.2^{a} \pm 2.07$	$1.3^{a} \pm 0.15$
Free flour	$33.0^{a} \pm 1.89$	$1.3^{a} \pm 0.08$	$33.0^{b} \pm 1.50$	$1.4^{a} \pm 0.06$
Dough ball	$33.4^{a} \pm 0.75$	$1.3^{a} \pm 0.03$	$40.0^{cf} \pm 1.04$	$1.6^{bc} \pm 0.04$
Flour only pellet	$33.2^{a} \pm 0.58$	$1.3^{a} \pm 0.02$	$38.9^{c} \pm 2.00$	$1.6^{b} \pm 0.08$
Flour + egg pellet	$34.1^{a} \pm 2.18$	$1.4^{a} \pm 0.09$	$45.8^{d} \pm 1.64$	$1.9^{d} \pm 0.07$
Flour + low worm pellet	$34.2^{a} \pm 1.50$	$1.4^{a} \pm 0.06$	$43.8^{cd} \pm 1.36$	$1.9^{d} \pm 0.06$
Flour + molasses pellet	$35.5^{a} \pm 0.61$	$1.4^{a} \pm 0.02$	$45.4^{d} \pm 0.71$	$1.8^{cd} \pm 0.03$
Flour + high worm pellet *	$32.3^{a} \pm 0.44$	$1.3^{a} \pm 0.02$	$45.2^{df} \pm 1.49$	$1.9^{d} \pm 0.06$

* *n* = 2. Within columns, means with similar superscripts are not significantly different (P>0.05).

However on completion, significant effects (P<0.05) were detected for bulk weights and prawn weights. The commercial pellet by far produced the fastest growth with an average of 101.6% weight gain (Figure 2). Prawn growth was negative in the unfed control (weight loss of 3.1%) and low in the free flour treatment (weight gain of 6.9%). Most pellets produced a higher weight gain than the free flour treatment, particularly when a feeding attractant was included (weight gains from 29.9% to 46.1%). There were no differences (P>0.05) in the growth of prawns fed pellets with different feed attractants (ie: egg, low or high worm, or molasses). Feeding the flour as a dough ball produced larger (P<0.05) mean bulk weights and prawn weights than adding it as a powder (Table 5), but when compared as a percentage weight gain this difference was not significant (P>0.05) (Figure 2).

Figure 2 **Mean (± se; *n* = 3) banana prawn weight changes expressed as a percentage of mean starting weights for different feeds supplied to 25 prawns over a one month period**

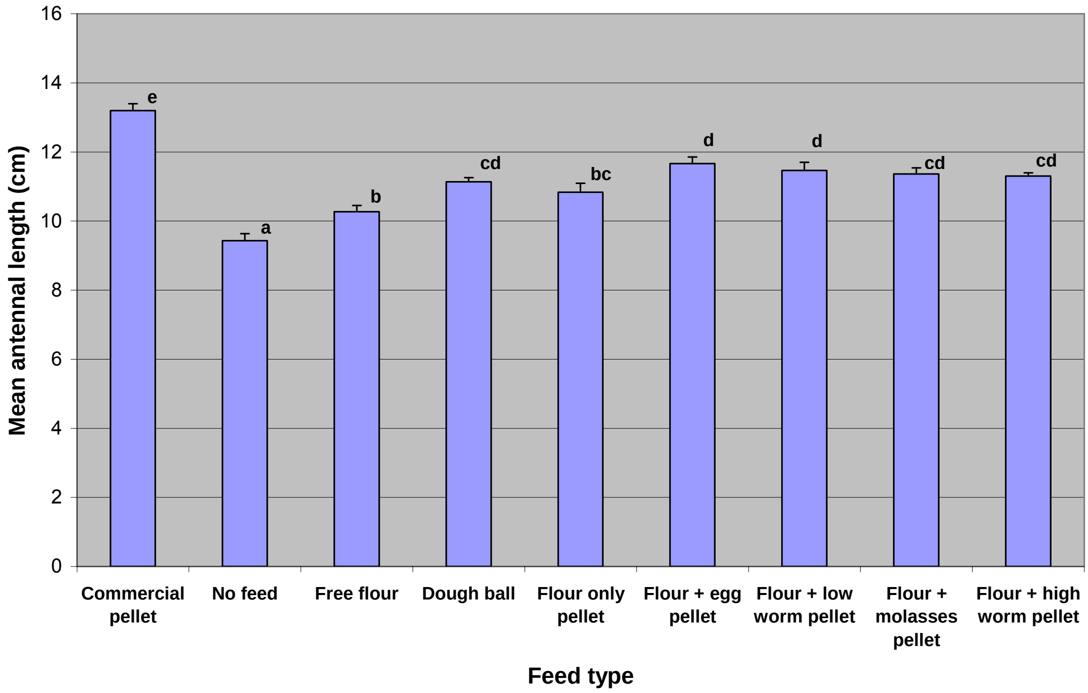


Note: Means with similar letters are not significantly different (P>0.05).

Prawn condition

Prawn condition as measured by antennal length was highest in prawns fed the commercial pellet (13.2 cm) and lowest in the unfed treatment (9.4 cm). Adding free flour to tanks produced shorter (P<0.05) antennae (10.3 cm) than adding pelletised flour with the feed attractants included (ie: egg, worms, molasses: 11.3-11.7 cm). Feeding the flour as a dough ball produced antennal lengths (11.1 cm) that were not significantly different (P>0.05) to that produced with pelletised feeds, but longer (P<0.05) than when it was added as a powder.

Figure 3 Mean (± se; *n* = 3) banana prawn antennal lengths for surviving prawns supplied with different feeds over a one month period



Means with similar letters are not significantly different (P>0.05).

Discussion

Nutrient sources and their suitability

The organic certification process for products derived from cereals and grains is well developed within Australia due to their direct and indirect consumption by humans. Cereals and grains are also some of the least expensive food sources that are available for intensive livestock, and they are used widely as energy sources and binders in manufactured feeds for fish and shrimp (Tacon, 1990b). Generally, they are low in protein (8-12% of dry matter), but high in carbohydrates (60-80%) comprised mainly of starch in the grains' endosperm (amylase 25%, amylopectin 75%). Of the protein that does exist in grains, the amino acids lysine and threonine are generally low, and the fatty acids present (eg. mainly linoleic and oleic acid) are normally unsaturated. These and other constituents (eg. fibre, vitamins, minerals) vary with different grain species, but notably, cereal grains are also considered to be a good source of phosphorus as phytates (Tacon, 1990b).

Fish meals are generally used as the main source of protein and fat in aquaculture diets. They have what is considered to be a better balance of amino acids for aquaculture feeds and a much broader range of fatty acids. Table 6 provides some previously documented amino acid contents for wheat (% of grain), for typical chicken's egg (excluding shell), for various fish meals (excluding bone and offal meal), and for whole dried *Penaeus monodon* juveniles (average wet weight of 1.5 g).

Table 6 Documented amino acid contents* (%) for two feed ingredients used in this trial, for a range of different fish meals*, and for *P. monodon***

Feed type	Arg	Cys	Met	Thr	Iso	Leu	Lys	Val	Tyr	Try	Phe	His
Wheat	0.60	0.13	0.15	0.38	0.50	1.35	0.95	0.57	0.31	0.26	0.58	0.28
Egg	0.76	0.29	0.40	0.61	0.76	1.07	0.83	0.86	0.50	0.19	0.70	0.30
Fish meals	3.25-4.62	0.4-0.76	1.46-2.14	2.31-3.25	2.41-3.5	3.81-5.3	4.04-6.6	2.8-4.37	1.86-2.29	0.56-0.8	2.16-2.92	1.3-1.88
P.monodon	4.99	-	1.50	2.69	2.65	5.96	4.40	3.04	0.80	0.66	2.97	1.18

*given by Tacon (1990b). ** given by Sarac (1994).
Arg = arginine, Cys = cystine, Met = methionine, Thr = threonine, Iso = isoleucine, Leu = leucine, Lys = lysine, Val = valine, Tyr = tyrosine, Try = tryptophan, Phe = phenylalanine, and His = histidine.

Here it can be seen that for most amino acids, wheat and eggs have comparatively similar levels whilst fish meal is several fold higher. Compared with fish meals, wheat is particularly low in methionine, arginine, threonine and tyrosine (<20% of the minimum values given in Table 6), as well as in isoleucine, lysine, valine, and histidine (<15% of the maximum values given in Table 6). Although prawns generally require lower amounts of protein in their diets as they grow larger, suboptimal growth is often due to amino acid imbalances (Akiyama and Dominy, 1989). Compared with levels in prawn juveniles (ie. *P. monodon*), wheat is also several fold lower in content for all major amino acids, and is particularly low in methionine, arginine, threonine, isoleucine, valine and phenylalanine (all <20% of the values given in Table 6).

Fish and prawns generally have a high dietary protein requirement (24-57%) and preferentially derive energy from protein over carbohydrate (Akiyama and Dominy, 1989; Tacon, 1990b). Despite this there has been considerable research over the last decade directed at the dietary substitution of carbohydrate for protein in aquaculture feeds, and particularly with regard to replacing fish meal with terrestrial based products. Starch from cereal grains is one of those most studied, even though it is not generally found in marine food sources. That of wheat has been shown to provide good digestibility in several Penaeids which have been studied, and particularly after it has been gelatinised through cooking (Cousin *et al.*, 1996). However, the digestibility of carbohydrates differs between Penaeid species (Catacutan, 1991) and there is still a paucity of relevant information and some general scepticism about carbohydrates as suitable nutrients for prawns.

Shiau (1998) provides a useful summary of carbohydrate research for various Penaeid species. Generally, simple or reducing sugars are thought to be less effective than more complex polysaccharides such as starch (Chen, 1998). For example, Shiau and Peng (1992) showed that corn starch (20-30%) provides better growth and is a better carbohydrate for protein-sparing in *Penaeus monodon* juveniles (0.5 g) than simpler carbohydrates like dextrin and glucose. Earlier work by Andrews *et al*. (1972) had also shown that for *P. setiferus* juveniles (4 g), 30% starch provided higher growth rates and reduced the effect on growth of variable protein levels. Pascual *et al.* (1983) also found that for *P. monodon* juveniles (1.8 g), sucrose (10%) provided the highest survival (56%) for a range of simple and complex carbohydrates tested, but that at 40% inclusion all carbohydrates (particularly maltose and molasses) provided very low survival.

In contrast, the present study demonstrates that for *P. merguiensis*, high survival can be achieved with very high levels of complex carbohydrates (wheat flour) over short periods (one month), but suggests that without supplemental protein growth will be suboptimal. This result may have been due to low levels of natural feeds present in the intake water supplying essential nutrients for health maintenance, but insufficient protein levels for substantial growth. Natural zooplankton diets are reportedly good sources of protein, lipids, minerals, vitamins and carotenoid pigments for fish and shrimp, although a range of factors influence their value to particular species of different ages (eg: size, stain, source) (Tacon, 1990b). Suitable natural foods in the culture system can therefore permit a reduction in the protein levels of artificial feeds (Akiyama and Dominy, 1989). Whilst better growth results could be expected for prawns exposed to higher levels of natural feeds, as would exist in an extensive earthen pond environment, to make the process more reliable and increase production levels, it is likely that a certified organic protein source with high digestibility and growth potential for prawns is needed to supplement that of the wheat flour in a prepared diet. Various forms of wheat or bread flour may be a suitable base for this organic dietary development, perhaps up to a maximum inclusion level of 35% (Catacutan, 1991; Shiau, 1998).

Feeding wheat flour clearly improved survival in the present study, and in some forms, also the growth of prawns. Feeding the flour as a dough ball also appeared to marginally improve its direct utilisation by the prawns, probably by making it more accessible at higher concentration and with lower expenditure of foraging energy. Akiyama and Dominy (1989) recommend the use of a 6:1 protein:lipid ratio for energy levels in diets for shrimp. Sedgwick (1979) found that for *P.merguiensis* juveniles (0.3–4.9 g), the energy content of the diet affected food consumption, protein conversion efficiency and growth, and that with sufficient energy supplied in the range 2.9–4.4 kcal g^{-1}, maximum growth was obtained with protein and lipid levels of 34–42% and 7.4%, respectively. This energy level is similar to that used in contemporary commercial prawn feeds such as the control diet in the present study which contained a gross energy level of 18.66 MJ kg^{-1} (4.5 kcal g^{-1}), with 43% protein and 6% lipid. In comparison, the wheat flour used in the present study had an energy content (12.59 kJ g^{-1} or 3.0 kcal g^{-1}) in the lower end of this optimal range suggested by Sedgwick (1979), as well as lower levels of protein (13.1%) and lipid (2.1%). For an energetic prawn like *P. merguiensis*, which spends a large proportion of its time swimming in the water column, the generally lower energy content of carbohydrate combined with lower inclusions of energy rich food groups (ie: lipid and protein) may also compound to cause minor energy deficiencies with the 100% wheat diet.

Molasses was added to one of the wheat pellet diets in the present work to provide an additional energy source. Levels of inclusion (4.2% DW) were well below the critically detrimental levels reported for *P. monodon* by Pascual *et al.* (1983) (discussed above), and similarly had no apparent affect on survival of *P. merguiensis*. The marginal increase in growth of *P. merguinesis* through addition of this simpler carbohydrate to the pelletised wheat flour was also not significant. Molasses used in the present work contained 15.2% reducing sugar (most likely the monosaccharides glucose and fructose), and 33.4% sucrose, a non-reducing disaccharide that has been shown to be a beneficial dietary inclusion (10%) for growth in at least two Penaeid species (ie: *P. japonicus* and *P. monodon*: see review by Shiau, 1998). On the other hand, Penaeids are thought to utilise glucose to a far lesser degree due to problems with digestive system absorption and metabolism (Shiau, 1998).

Nevertheless, molasses is also known to be a good nutritional source of potassium, and trace elements like magnesium and manganese (eg. 1.5% K, 0.2% Mg and 0.002% Mn: NutritionData.com, 2006). Since the low salinity ground waters that exist in Queensland have typically unbalanced ionic concentrations compared with seawater, and particularly with regards to low potassium levels (Collins *et al*., 2005), molasses in feeds or used to help control pond water qualities may provide an additional source of this element in inland prawn farming systems. Whole grain wheat flour is also a good source of potassium (0.4%: NutritionData.com, 2006), so it’s inclusion in the diets or in pond fertilisation regimes may further alleviate this potential ionic imbalance.

This study also sought to test for potential advantages of including feeding stimulants such as chicken’s egg and marine Polychaetes with the wheat flour. Diet palatability has recently been shown to be a factor that influenced growth of *Litopenaeus vannamei* when replacing marine animal protein with fermented grains and wheat gluten (Molina-Poveda and Morales, 2004). Free amino acids and small peptides that leach from the feed may provide attractants for shrimp (Akiyama and Dominy, 1989), and since palatable ingredients like fish meal and other seafood products were lacking in our simple diets, it was possible that such ingredients could improve palatability, feed utilisation and conversion. The inclusion levels used in the present study (1.1–6.8%) were slightly higher than those recommended by Akiyama and Dominy (1989) for fish solubles (1–5%), however, the marginal increases in weight gain were not statistically significant. Interestingly, even free wheat flour added to the tanks appeared to stimulate a strong foraging response in the experimental stock, so it is likely that wheat in itself may hold significant feeding stimulants for this Penaeid.

Marine Polychaetes are widely recognised as feeding stimulants and chickens egg has traditionally been used as a control diet in much nutrition research (Tacon (1990b). But regardless of their feeding stimuli, if protein levels in the basal diet are too low, their inclusion may not significantly improve growth even if it is improving its consumption. Additionally, any effect of these feed additives on growth could also have been due to slightly improved fatty acid and/or amino acid profiles of the composite diet. Behavioural aspects of prawns exposed to feeds with feeding attractants may be necessary to better evaluate their usefulness in the future. A recent example of such work is that by Sanchez *et al*. (2005), who tested krill and squid meal added to a wheat flour base. This could be combined with digestibility and enzyme inhibition studies of the like recently used by Lemos *et al.* (2004) to study *Farfantepenaeus paulensis.* That work used *in vitro* shrimp enzymes to screen and compare potential ingredients. They found similarly high digestibility for Brazilian fish meal, meat meal and wheat flour, intermediate digestibility for super prime Chilean and Argentinean fish meals, and least digestibility for soybean meal and blood meal, the later being impeded by inhibition of proteinases.

The commercial prawn feed used as the control diet in this study produced by far the highest prawn growth for all feeds tested. But it is more costly and a more highly processed feed, with fish meal and other ingredients that collectively may have difficulty passing organic certification standards. As discussed earlier, this better growth result can generally be attributed to it having a much higher level of protein (43%), and probably a more suitable range of amino and fatty acids, having been specifically formulated as a complete diet for Penaeid species. Its ingredients include steam dried fish meal, squid meal, squid liver meal, antioxidant–treated marine and vegetable oils, oilseed meal, wheat flour, phospholipids, vitamins and minerals. These ingredients provide many of the essential nutrients that are known to be required by Penaeids (for recent reviews see Chen, 1998; Shiau, 1998). Some of these essential nutrients like phospholipids are not found in wheat flour (NutritionData.com, 2006) and are not able to be synthesized or are synthesised too slowly by crustaeceans (Akiyama and Dominy, 1989), so must be included in their diets; for *P. merguiensis* between 1 and 2% lecithin provides sufficient phospholipid for good growth and survival (Thongrod and Boonyaratpalin, 1998). In earlier work, Aquacop (1978) suggested that a diet containing 50-55% protein, 7% fish oil, carbohydrate in the form of starch, and vitamen and mineral mixes rich in vitamen C, choline, inositol, and magnesium (3%) is optimal for *P. merguiensis* juveniles.

Species, feeds and processes for organic certification

Carnivorous fish like rainbow trout are poor digesters of crude starch due to reduced intestinal amylase activity (Spannhof and Plantikow, 1983), so their ability to digest complex carbohydrates from grains is reduced. Fish meal replacement with grain products in aquaculture diets is therefore thought to be more likely to be successful for omnivorous or herbivorous fish species like tilapia and carp (Tacon, 1990a). This may also be due to their ability to extract more nutrients from natural sources in ponds rather than due to large differences in their overall protein requirements. Banana prawns are generally considered to have a lower protein requirement and be more herbivorous than other species of cultured prawns like *Penaeus monodon* (Hoang, 2001). This is one of the reasons for choosing banana prawns in this work. They have also been shown to grow to market size in prawn farm settlement ponds without the direct application of artificial feeds (Palmer *et al*., 2005b), and thus have proven ability to adequately utilise a pond ecosystem for this extensive rearing approach. Like *P. vannamei*, which appears to digest gelatinised and natural wheat starch almost as well as many terrestrial animals (Cousin *et al*., 1996), *P. merguiensis* may hold dietary advantages for production systems based largely on grain feeds.

However other factors may also drive the selection of species for the organic systems under investigation. For example, their general salinity tolerance and changing osmoregulatory abilities and temperature tolerances as they get older may be overriding factors in extensive low salinity inland systems. In related farm trials, *P. merguiensis* postlarvae have been shown to successfully acclimatise and grow to small sizes at low salinities, but they are yet to yield adequate production levels for economic assessment. Seed stocks have also sometimes proved more problematic than *P. monodon* during low salinity acclimation, and they have generally shown lower survival as they grow. Despite its more carnivorous nature, the black tiger prawn (*P. monodon*) may therefore be a better candidate for this research direction in the future, particularly if the economic viability of extensive rearing systems can only be realised in inland regions using low salinity bore water, and in areas where high temperatures can also often prevail. This of course would be more contingent on identifying suitable and organically certifiable protein sources.

The use of low protein feeds may also be important in the future to help avoid excessive eutrophication in low-water-exchange culture ponds. In this regard the levels of digestibility and waste produced with different feeds are of high importance. Water stable feeds which do not leach excessive ingredients before ingestion, and hold their form for several hours before breaking down, are instrumental in avoiding pond bottom fouling and wasted nutrients. A high inclusion of cooked wheat flour provides this water stability, along with greater than 34% moisture content of the extrusion paste (Cheng et al., 2002). In the present study, the steaming of extruded paste provided this stabilising gelatinisation, and the moisture contents of paste ranged from 33.4% (egg pellet) through to 39.3% (high worm pellet), which appeared to provide similar and acceptable physical stability after several hours in seawater.

The economics associated with low density or extensive rearing systems requires that it is performed on a large scale. This is why the current project's focus was on the use of saline ground waters that exist at considerable scale in inland areas of Queensland (Collins *et al*., 2005). Low feed costs and labour would also be vital to minimising the costs of production, so that acceptable profit margins are achieved. The feeding rate and frequency used in this trial were therefore kept to a minimum (6% once per day), assuming that in an extensive pond natural feeds would likely make up many nutritive shortfalls. The need for these practical considerations in nutritional studies with crustaceans is highlighted by Tacon (1996), who has criticised laboratory based trials in artificial environments due to their potential to skew experimental results and provide misleading findings. Whilst the present study can be similarly criticised for differing salinities, ionic concentrations and natural feeds in the culture water compared with inland extensive pond environments for which it was intended, these factors can vary significantly between potential sites making it difficult to practically address all possible scenarios that could affect such nutrition research.

Other operational considerations for conversion

The United Kingdom Register of Organic Food Standards (UKROFS) require that organic production systems are designed to avoid the use of agricultural chemical inputs and minimise any related damage to the environment and wildlife. They seek to encourage the integration of enhanced biological cycles involving micro-organisms, plants and animals in sustainable farming systems. As far as possible, renewable resources and closed farming systems are employed with recyclable or biodegradable materials used or produced. Pollution needs to be minimised, human rights, workers satisfaction and safety aspects of the operation are maximised, and the wider social and ecological impacts are also considered. Product traceability is built into the certification and marketing of products and producers are bound to report any actual or suspected contaminations. Where organic products are being produced along-side non-organic products, separation of products must be ensured with efficient operational and record keeping activities. Genetically modified organisms and their derivatives are prohibited at all stages of production. Veterinary drugs, in-feed medications/antibiotics and pest control materials (including pyrethrum) are prohibited or severely restricted (except for approved rodent baits), and emphasis is placed on good hygiene, house keeping and exclusion. Some pesticide ingredients are allowed with prior approval from the certifying body and the use of stacked or composted manures is closely regulated. Whilst referring more to land based agricultural systems, organic seed is preferable but parent crops for seed production can be non-organic.

Cavilli *et al*. (2005) provides a description of proposed practices in Brazil where the first recognised organic aquaculture farm in the world (Primar Aquaculture) was established in December 2003. Polyculture is practiced on this farm, producing shrimp (*Litopenaeus vannamei*), oysters (*Crassostrea* *rizophorae*), crabs (*Callinectes* sp.), mullet (*Mugil* sp.) and macroalgae (*Gracilaria*). In addition to many of the UKROFS principals stated above, the animals natural behaviour patterns guide culture practices, and local resources are used wherever possible. For example, lined ponds without bottom substrate are not considered to provide adequate burrowing habitats for prawns. Uncontaminated water supplies are a prerequisite, on-farm water recirculation schemes are recommended, and artificial aeration devices are not considered appropriate. Standards comprehensively address all operational conditions, such as site selection avoiding destruction of natural habitats, ethical slaughter of animals (immediate dipping in cold water), and processing and storage for maximum product quality. They suggest that only local species should be farmed to avoid ecological impacts of escapements. It is also proposed that fish meals used in diets should only be from the waste products of fish caught for human consumption (eg: offal and fillet trimmings), and that organic ingredients suitable for human consumption should not be used in the diets. Rather, ponds should be managed with organic inputs to increase natural productivity. Interestingly, these authors suggest that underground waters should not be used, but they fail to provide justification for this. They also suggest that antibiotic usage be restricted to post-larval production or curative measures where no homeopathic method is available.

In Australia, many of these considerations are already built into best practice requirements of the overarching industry body (Australian Prawn Farmers Association) and the licensing controls of governing bodies (Departments of Primary Industries and Fisheries, Environmental Protection Agencies, Departments of Health). For example, permits to construct farms are considered in the context of acceptable environmental impacts and appropriate site selection. Safeguards such as implementing systems to prevent stock from escaping into adjacent environments are part of licence conditions. Water discharge limits are tailored to the capacities of the receiving environments to assimilate nutrient loads, and disease reporting requirements are supported by the ready availability of high quality diagnostic services. Strict chemical usage is routinely regulated through the Australian Pesticides and Veterinary Medicines Authority (APVMA) (eg: prophylactic antibiotic use is illegal).

The two organic certifying bodies in Australia are the Biological Farmers Association (BFA) and the National Association for Sustainable Agriculture Australia (NASAA). Neither body requires that prawn postlarvae for production systems are organically certified. NASAA do not allow use of fishery by-catch in feeds but the utilisation of waste streams (eg: offal and fish frames) is in line with the organic principles of recycling, reusing and composting. No chemicals for use with prawns are currently approved by these bodies. Those used in production of other organic foods (sulphites, ascorbic acid) will need to be assessed within the context of specific application. Regarding growth densities, the BFA accept that fish have a tendency to school in nature. Prawns do exist at high densities in natural environments and their behavioural needs may provide a reasonable argument for more intensive production levels, as long as environmental aspects and stressors that can cause disease are minimised. Regarding water qualities, the main focus is contaminants coming into the system. Audits take into account what is upstream. If the water source quality is poor it is likely that closed systems with water recirculation will be necessary. However, MAP is generally approved for organic use. Economic initiatives will benefit from the extended shelf life that this packaging system can provide, particularly for chilled (unfrozen) product because of delays in transport and handling before going into retail environments. Frozen MAP products will also be useful but will also compete with wild caught product and suffer from lower prices.

Future research will best be directed at developing organic diets for more intensive production systems and evaluating the economic drivers and levels of production dependant on site, operational efficiencies, labour costs and many other issues that are still to be quantified. These include the costs associated with the certification process (fees and auditing costs) and conversion from production systems that do not adhere to acceptable organic standards. Whilst the economic assessments that have been made thus far for organic prawn farms are encouraging (eg: Xie *et al.* 2005; Wainberg, 2005), they have been undertaken in other countries (China and Brazil) where different cost structures and regulatory considerations will greatly affect operating environments. Economic assessments in Australia will only come from direct applications under localised conditions so that the full production and marketing cycle can be comprehensively assessed.

Summary

The survival, growth and condition of banana prawn (*Fenneropenaeus merguiensis*) juveniles (1-2 g) were compared after feeding in tanks over one month with several simple diets based on organically certified whole wheat flour. All feeds were applied once per day at 6% of the starting body weight, and produced high survival (>94%). A commercial Australian prawn feed used as the control diet produced the highest ($P<0.05$) growth (101% weight gain) and condition measured as the length of antennae (13.2 cm). The unfed control had significantly ($P<0.05$) lower survival (56%), and produced a weight loss (3.1%) and the shortest antennae (9.4 cm). Adding free flour to tanks produced lower ($P<0.05$) growth (6.9%) and shorter ($P<0.05$) antennae (10.3 cm) than adding pelletised flour with low levels (dry weight) of additional nutritional substances and feed attractants (chicken's whole egg: 1.5%, Polychaetes: 1.1% and 6.8%, molasses: 4.2%). Feeding flour as a dough ball also appeared to marginally improve its direct utilisation by the prawns. These results are considered within the context of appropriate nutrition for Penaeids and successfully producing certified organic prawns in Australia.

Acknowledgments

The authors would like to thank Donna and Wayne Helmuth for the supply of prawn juveniles for this feed trial. Thanks also to Beverley Kelly, Michael Burke, Trevor Borchert, Michael Salini, Martin Lee and Scott Shanks for their technical assistance at BIARC, and to our biometrician Dr David Mayer for advice regarding statistical analyses.

References

Akiyama, D.M. and Dominy, W.G. (1989). Penaeid shrimp nutrition for the commercial feed industry. *In*: Texas Shrimp Farming Manual, Vol. 1: Grow-out Technology, Technical Report of Texas Agricultural Extension Service and Texas A&M University Sea Grant College Program, Corpus Christi, Texas, U.S.A.

Andrews, J. W., Sick, L. V. and Baptist, G. J. (1972). The influence of dietary protein and energy levels on growth and survival of penaeid shrimp *Aquaculture* 1:341-347.

Aquacop. (1978). Study of nutritional requirements and growth of *Penaeus merguiensis* in tanks by means of purified and artificial diets. *Proc. World Mariculture Soc.* 9: 225-234.

Boonyaratpalin, M. (1998). Nutrition of *Penaeus merguiensis* and *Penaeus indicus*. *Reviews in Fisheries Science* 6(1&2): 69-78.

Catacutan, M. R. (1991). Apparent digestibility of diets with various carbohydrate levels and the growth response of *Penaeus monodon*. *Aquaculture* 95:89-96.

Cavalli, R. O., Peixoto, S., Wasielesky W. Jr. and Poersch, L. H. (2005). Guidelines proposed for certification of organic Penaeid shrimp in Brazil. *Global Aquaculture Advocate* April 2005: 60-61.

Chen, H.-Y. (1998). Nutritional requirements of the black tiger shrimp: *Penaeus monodon*. *Reviews in Fisheries Science* 6(1&2): 79-95.

Cheng, Z. J., Behnke, K. C. and Dominy, W. G. (2002). Effect of moisture content, processing water temperature, and immersion time on water stability of pelleted shrimp diets. *Journal of Applied Aquaculture* 12(2): 79-89.

Collins, A., Russell, B., Walls, A. and Hoang, T. (2005). Inland prawn farming: Studies into the potential for inland marine prawn farming in Queensland. www2.dpi.qld.gov.au/far/17819.html

Cousin, M., Cuzon, G., Guillaume, J. and Aquacop. (1996). Digestibility of starch in *Penaeus vannamei*: In vivo and in vitro study on eight samples of various origin. *Aquaculture* 140: 361-372.

GenStat (2005) Release 8.1 for PC/Windows XP. Eight Edition, Lawes Agricultural Trust (Rothamsted Experimental Station).

Hoang, T., (2001). The banana prawn — the right species for shrimp farming? *World Aquaculture*. **Dec**: p. 40-69.

Lemos, D., Navarrete del Toro, A. N., Cordova-Murueta, J. H. and Garcia-Carreno, F. (2004). Testing feeds and feed ingredients for juvenile pink shrimp *Farfantepenaeus paulensis*: in vitro determination of protein digestibility and proteinase inhibition. *Aquaculture* 239: 307-321.

McCullagh P. and Nelder J.A. (1989). Generalised Linear Models (2nd ed.). Chapman and Hall, London.

Molina-Poveda, C. and Morales, M. E. (2004). Use of a mixture of barley-based fermented grains and wheat gluten as an alternative protein source in practical diets for *Litopenaeus vannamei* (Boone). *Aquaculture Research* 35: 1158-1165.

NutritionData.com. (2006). Nutritional summary for molasses: www.nutritiondata. com/facts-001-02s04at.html ; Nutritional summary for whole grain wheat flour: www.nutritiondata.com/facts-B00001-01c21Ue.html

Palmer, P. J., Erler, D., Morrison, C. and Rutherford, B. W. (2005a). Nutrient levels in experimental tanks supplied with prawn pond effluent: the effect of artificial substrate and different densities of the banana prawn *Penaeus merguiensis* (de Man). *In*: Wastewater remediation options for prawn farms — Aquaculture Industry Development Initiative 2002-04. (ed. by P. J. Palmer). Queensland Department of Primary Industries and Fisheries Project Report No. QO04018. ISSN 0727-6273. pp. 25-39.

Palmer, P. J., Erler, D., Burke, M. J., Lobegeiger, R., Morrison, C., Bell, G. and Knibb, W. (2005b). Growing banana prawns *Penaeus merguiensis* (de Man) in prawn farm settlement ponds to utilise and help remove waste nutrients. In: Wastewater remediation options for prawn farms — Aquaculture Industry Development Initiative 2002-04. (ed. by P. J. Palmer). Queensland Department of Primary Industries and Fisheries Project Report No. QO04018. ISSN 0727-6273. pp. 5-24.

Pascual, F. P., Coloso, R. M. and Tamse, C. T. (1983). Survival and some histological changes in *Penaeus monodon* Fabricius juveniles fed various carbohydrates *Aquaculture* 31: 169-180.

Sanchez, D. R., Fox, J. M., Lawrence, A. L., Castille, F. L. and Dunsford, B. (2005). A methodology for evaluation of dietary feeding stimulants for the pacific white shrimp, *Litopenaeus vannamei*. *Journal of the World Aquaculture Society* 36(1): 14-23.

Sarac, H. Z. (1994). Development of cost effective diets for prawn growout using local ingredients. Final Report to the Fisheries Research and Development Corporation Project No. 90/67. 57 pp.

Sedgwick, R.W. (1979). Influence of dietary protein and energy on growth, food consumption and food conversion efficiency in *Penaeus merguiensis* De Man. *Aquaculture* 16: 7-30.

Shiau, S-Y. (1998). Nutrient requirements of Penaeid shrimps. *Aquaculture* 164: 77-93.

Shiau, S. Y. and Peng, C. Y. (1992). Utilization of different carbohydrates at different dietary protein levels in grass prawn, *Penaeus monodon,* reared in seawater. *Aquaculture* 101: 241-250.

Spannhof, L. and Plantikow, H. (1983). Studies on carbohydrate digestion in rainbow trout. *Aquaculture* 30: 95-108.

Tacon, A.G.J. (1990a). The essential nutrients. *In*: Standard methods for the nutrition and feeding of farmed fish and shrimp. Volume 1. pp 1-117. Argent Laboratories Press. Redmond, Washington, USA.

Tacon, A.G.J. (1990b). Nutrient Sources and composition. *In*: Standard methods for the nutrition and feeding of farmed fish and shrimp. Volume 2. pp 1-129. Argent Laboratories Press. Redmond, Washington, USA.

Tacon, A.G.J. (1996). Nutritional studies in crustaceans and the problems of applying research findings to practical farming systems. *Aquaculture nutrition* 2(3): 165-174.

Thongrod, S. and Boonyaratpalin, M. (1998). Cholesterol and lecithin requirement of juvenile banana shrimp, *Penaeus merguiensis*. *Aquaculture* 161: 315-321.

Wainberg, A. A. (2005). Shrimp farm strives for success after organic conversion. *Global Aquaculture Advocate* April 2005: 58-59.

Xie, B., Li, J. and Wang, X. (2005). Assessing the consequences of converting to organic shrimp farming. *Aquaculture Asia Magazine* July-September 2005: 11-17.

2.2 Organic diet development for black tiger prawns

PJ Palmer, J S Kopinski and S L Slattery

Abstract

The survival and growth of black tiger prawn (*Penaeus monodon*) juveniles (~3.3 g) were compared after feeding in tanks over one month with several prepared diets based on organically certified ingredients. The extrusion process in the manufacture of pelletised experimental diets was similar to processes used in commercial plants and was closely documented. The daily feeding rate (6% of starting mean body weight) was split equally into two feeds, one in the morning and one in the afternoon. All diets tested produced high survival (97-100%).

A widely-used commercial Australian prawn feed was used as a control diet. It contained 41.2% protein with 29.5g kg^{-1} lysine, and produced the highest ($P<0.05$) growth (117% weight gain). Three of the experimental organic diets tested (namely, 1. wheat + soy, 2. pig weaner diet + soy, and 3. pig weaner diet + dried fish waste) produced moderate growth (73–77% weight gain). These contained 33%, 36% or 31% protein, respectively, and produced better ($P<0.05$) growth than diets utilising a range of other prospective ingredients (eg: wheat + dried scallop gut, wheat + fish waste, wheat + chickpea or wheat + macadamia meal, containing 23%, 25%, 29% or 24% protein, respectively). An unfed control-treatment produced the lowest ($P<0.05$) growth (4% weight gain).

The water stability of the experimental diets that produced the best growth was poorer than the commercial diet, suggesting that improvements in the manufacture of these organic feed's could result in additional performance benefits and possibly reduced feed wastage. Analyses revealed a linear relationship between diet performance (in terms of weight gains) and the protein and lysine contents of diets. About 70% of diet performance was explained by these factors. The superior performance of the commercial diet could be attributed primarily to its formulation using mainly marine proteins, as well as a range of other unknown factors (commercial in confidence). These other factors range from use of feed attractants, better knowledge of ingredient nutrient availability, different extrusion conditions and the use of other unspecified micro-nutrients not present in the experimental diets. It is unfortunate that the supplier of this feed was not interested in developing organic versions of their products, thus the development of feeds for this work had to start from a more rudimentary level.

The organic diets studied still require a degree of fine-tuning before structured commercial uptake. This would sensibly include further detailed investigations of the composition and nutrient availabilities of these and other organic dietary ingredients, and refinement of the extrusion process for formulated diets.

Introduction

The black tiger prawn, *Penaeus monodon*, has traditionally been the most commonly farmed prawn species in Australia and the world. It is a hardy species that can grow at high densities and withstand low salinities in outdoor ponds. This later attribute makes it suitable for culture in inland areas, as has been demonstrated in Thailand over the last decade. There, disease outbreaks in coastal areas have encouraged farmers to move operations inland into freshwater environments which have a lower incidence of such pathogens. It is prudent for Australia to also investigate this strategy, should diseases that have devastated prawn production in South-east Asia and other countries somehow find their way to our shores.

Although these inland practices have provided great economic benefits to Thailand, a number of environmental concerns are now forcing authorities to re-evaluate the impacts to soils and water qualities (Flaherty *et al.*, 2000). In Australia, the focus of inland prawn farming opportunities is more on expansive marginal areas of land with pre-existing soil salination problems. Thus, such operations in Australia in existing salt basins would not compromise soil quality and would provide better opportunities for containing adverse environmental impacts.

Accordingly, our previous investigations into organic prawn production focussed on extensive production systems in inland areas. It was also expected that low culture densities would be necessary to adhere to organic certification standards. We had previously assumed that larger ponds (eg: 2–10 ha) on low-cost salt affected parcels of land, low stocking densities of prawns (up to 10 m^{-2}), and management regimes which minimised the most expensive input components (labour, power and feed), would be necessary for organic prawn farming to be economically viable in Australia. Additionally, we had anticipated that the infrastructures and overheads of existing coastal prawn farms, which were geared to semi-intensive production (30-40 m^{-2}), could not accommodate the lower production capacities of an extensive approach, even with the price premiums offered by certified organic product. This caused us to initially focus our work on the more herbivorous banana prawn, *Penaeus (Fenneropenaeus) merguiensis*, and simple feed inputs which provided pond fertilisation for increased natural feeds as well as some direct feeding.

Whilst these assertions may be true to varying degrees, interest in certified organic prawn production from existing coastal farmers wishing to improve the competitiveness and marketing opportunities for their products prompted us to review several of these guiding preconceptions.

Firstly, since prawns are a schooling species in nature, a strong argument can be made for higher culture densities in organic standards. Perhaps much higher than the densities that can be sustained by natural pond productivities alone. To accommodate higher culture densities, a more balanced diet that is consumed directly would be necessary.

Secondly, the banana prawn was more problematic than black tiger prawns in our low-salinity farm trials. Several physical and biological factors are thought to be responsible for these difficulties, including:

- the tolerances of these species for very low salinities and various ionic imbalances that can exist in the source ground waters
- the tolerances of these species for high temperatures which prevail during the growing season in such areas, and
- changes to these tolerances in aging prawns.

A greater focus on the more tolerant black tiger prawn was therefore recommended. These directional changes also increased the relevance of our current work to the existing Australian industry, namely coastal farmers interested in diversifying their products with organic certification.

Consequently the present study has:

- focused on developing an improved organic diet for *P. monodon* cultured at moderate densities (~ 20 m^{-2})
- examined a number of new potential organic feed ingredients which could potentially be supplied in sufficient quantities for commercial use
- used a similar feed testing environment to that previously applied to banana prawns, and
- manufactured the experimental feeds using a documented extrusion process not unlike those used by commercial feed mills.

Materials and methods

Experimental tank system

The experimental tank system used for the trial was the same as that described in previous growth and survival research with banana prawns at BIARC (Palmer *et al*., 2005). It incorporated 26 round plastic tanks (diameter of 1550 mm) with netting covers (to prevent bird predation and prawns jumping out), all housed inside a greenhouse designed to minimise diurnal temperature fluctuations. Each tank was fitted with a 50 mm standpipe for screened water to overflow to waste, giving a constant water depth of 900 mm and maximum water volume of 1700 L. Each tank was also fitted with a 4 mm airline, which delivered constant aeration (~ 6 L min^{-1}) to the centre of each tank in mid-water column.

All tanks were initially clean and dry. Unfiltered seawater was continuously pumped into each tank at similar rates (3 L min^{-1}) for the duration of the trial and for four days prior to stocking prawns. As is the norm for the unfiltered seawater supply at BIARC, it carried a low level of silt and naturally occurring suspended matter (eg: phytoplankton and zooplankton) from Moreton Bay. Supply lines were flushed weekly and designed so that each tank received a similar load of suspended matter. Debris that collected on the tank bottoms during the trial was left undisturbed for the duration of the trial.

Experimental design

Nine experimental treatments (T) included:

T1 an unfed control used to demonstrate the minimum growth likely from the low level of natural food sources present in supply water and tanks.

T2 a commercial reference diet, namely Ridley Aquafeed Prawn Starter #2, used to demonstrate the maximum growth possible in the testing system.

T3-9 seven different feed formulations (diets 3–9 as displayed in Table 7) based on preliminary calculations made for ingredient moisture and protein contents. Dietary markers were also added for future analyses not reported here.

Table 7 Experimental diet composition—ingredient amounts (g) estimated to yield > 1 kg dry matter (DM) of each diet

Treatment	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9
Name	Wheat fish	Wheat scallop	Wheat soy	Wheat chickpea	Wheat macadamia	Weaner fish	Weaner soy
Ingredients							
Wheat flour	950	950	550	550	550	0	0
Weaner pig diet	0	0	0	0	0	950	550
Fish frames	190	10	190	190	190	190	190
Scallop gut	10	190	10	10	10	10	10
Soy flour	0	0	400	0	0	0	400
Chickpea flour	0	0	0	400	0	0	0
Macadamia meal	0	0	0	0	400	0	0
Molasses	50	50	50	50	50	50	50
Casein	50	50	50	50	50	50	50
Kelp meal	10	10	10	10	10	10	10
Markers*	8.5	8.5	8.5	8.5	8.5	8.5	8.5
TOTAL	1268.5	1268.5	1268.5	1268.5	1268.5	1268.5	1268.5
~DM yield	1152	1152	1175	1171	1152	1152	1175
~Protein %	32.2	32.2	41.1	34.8	31.6	38.8	44.9

* Titanium dioxide 3.33 g, barium hydroxide 3.33 g and celite 10 g

Treatments were assigned randomly to each tank (Appendix 5) in a completely randomised design. There were three replicates for all treatments except treatment one which had two (due to the availability of only 26 tanks for the experiment).

Experimental feed ingredients and diet preparation

All ingredients used in experimental feeds were either organically certified, or what was assumed to be organically certifiable with a reasonable rationale. Table 8 provides a list of the feed ingredients, the respective product description, the supplier, the price paid for the product for the trial, and the expected cost of bulk supplies produced in industrial quantities.

The manufacture of extruded diets based on the above formulations and raw materials was contracted to Dr Peter Torley, School of Land and Food Sciences at The University of Queensland. This was conducted to ensure a controlled and documented extrusion process was undertaken for each of the diets. The processing was similar in nature to that which could be used in an industrial plant. The report detailing extrusion conditions that were applied is available in Appendix 6 of this report. Since this process did not provide a completely dry product, the extruded material was dried overnight in an oven at <60ºC before being crushed with a corn cracking machine and sieved to retain the 1–2 mm fraction. All experimental feeds were prepared one week before the trial commenced, and were stored in sealed plastic jars at 4°C. Daily aliquots for each tank were weighed to the nearest 0.01 g from these bulk packs each day.

Pellet water stability assessments

All pellet diets (including the control) were qualitatively assessed for their water stability in high salinity (36 ppt.) seawater at 26°C. Two grams of each feed was immersed in still seawater (2 L) in transparent hemispherical fish bowls with regular observations and physical comparisons made thereafter. At 5, 10 and 20 min and 1, 3 and 6 h after submersion, each diet was mixed with a small spoon and allowed to settle. Comparisons focused on any apparent differences in the experimental diets with the commercial control diet, in terms of the texture and integrity of the pellet, and the clarity of water in each bowl. A series of photos of each bowl were also taken after the 3 h inspection.

Table 8 Experimental feed ingredients, actual costs for the project, and expected costs with an industrial bulk supply

Ingredient	Product description	Supplier, location	Project cost (price per kg)	Anticipated industrial costs*
Wheat flour	Organic high protein white flour (HPWUB25)	Kialla Pure Foods Pty Ltd, Greenmount, Qld 4359	$38.27 / 25 kg ($1.53 / kg)	$473/tonne
Weaner pig diet	Organic pig weaner mash (FFPWM25)	Kialla Pure Foods Pty Ltd, Greenmount, Qld 4359	$750 / tonne ($0.75 / kg)	$750/tonne
Fish frames	Diver whiting frozen waste — heads, frames and gut	Pinzonne and Sons, Warana, Qld 4575	Free sample	$1200/tonne**
Scallop gut	Saucer scallop waste — gut and gonad	Queensland Sea Scallop Ltd, Burnett Heads, Qld 4670	Free sample	$1000/tonne**
Soy flour	Organic soya flour (SF25)	Kialla Pure Foods Pty Ltd, Greenmount, Qld 4359	$53.82 / 25 kg ($2.15 / kg)	$900/tonne
Chickpea flour	Organic besan flour (BES25)	Kialla Pure Foods Pty Ltd, Greenmount, Qld 4359	$52.35 / 25 kg ($2.09 / kg)	$450/tonne
Macadamia meal	Dried macadamia by-product after oil extraction	Mr L. Minter, Ewingsdale, NSW 0481	Free sample	$500/tonne
Molasses	Stockfeed grade organic certified molasses	Rocky Point Sugar Mill, Woongoolba, Qld 4207	$220 / 200 L ($1.10 / L)	$1,100/tonne
Casein	2nd grade Floor sweepings	Total Food tech	$5/kg	$10,000/tonne
Kelp meal	Imported kelp from sustainable harvest	Mr L. Minter, Ewingsdale, NSW 0481	Free sample	$2,500/tonne
Prawn feed	Commercial prawn diet manufactured in Australia	Ridley Aquafeed, Narangba, Qld	-	$1,700-$2,000/tonne

*Based on recent market price of raw ingredient as of 25 June 2007 and allowing a 50% price premium for organic production. Comparative figures for non-organic pig weaner are $475/t, with non-organic wheat $315/t (drought price), non-organic soy meal $448/t, non-organic full fat soy $600/t and non-organic chickpea gradings $300/t and also allowing $35/t diet mixing/processing costs. **Non-organic imported fishmeal is $1200/t assume equivalent cost for fish processing waste after drying

Prawn acquisition, transport, handling and tank management

Juvenile prawns for the trial were obtained from one commercial prawn pond at Gold Coast Marine Aquaculture Pty Ltd, Woongoolba, Qld. following clear health and histological examinations performed one week earlier. They were collected on the 17 Nov 2006 from feed trays repeatedly baited with commercial prawn feed. This was undertaken from early to mid morning to minimise water temperatures, as necessary to maximise survival during handling and transport. Trays were lifted periodically and captured prawns were tipped into bins filled with pond water. They were gently counted into smaller buckets and then carefully released into an 800 L fibreglass transport tank supplied with oxygen that was previously ¼ filled with the same pond water. The transport tank was topped up (50%) with clean filtered seawater before transport to BIARC.

On arrival at BIARC the prawns whilst still in the transport tank were given a slow (1 hr) 100% water exchange using unfiltered seawater from the same supply that was supplying the tanks in the experimental unit. They were then funnelled out of the transport tank's 50 mm bottom drain, directly into a 4,000 L parabolic tank filled with seawater, where they were held overnight to isolate prawns damaged during the transfer. During this time they were fed once with commercial prawn feed at approx. 6% of body weight. Unlike the banana prawns in the previous trial, which had not been exposed to commercial diets prior to the feed trial, these tiger prawns had come from a commercial farm that was following normal culture routines, and thus were accustomed to the availability of artificial diets.

Only healthy prawns were used to set up the experiment the following day. The trial began on the 18 Nov 2006, when 20 randomly collected prawns were stocked into each tank after weighing their collective bulk wet weight. Feeds were added to the tanks twice per day on the opposite side of the tank to the overflow point. The selected feeding rate of 6% of body weight per day followed conventional feeding charts and was derived from the overall mean prawn biomass weight across all tanks at the start of the trial. This was calculated to be 4 g daily, which was equally split into a morning and afternoon feed (2 g per tank).

The day after stocking, each tank was inspected for mortalities or unhealthy individuals during a short period without air or water supply. This yielded 1 dead prawn in tank 1 and one dead prawn in tank 9. These were weighed and replaced with two healthy weighed spare prawns that were left in the parabolic tank from allocations the day before. Starting weights for each of these tanks were adjusted accordingly. From this point no further mortalities were detected in the experimental tanks. As with previous trials, silt and detrital build up on tank bottoms remained undisturbed until just before harvest and trial termination 28 days later, when it was siphoned to waste prior to prawn collection with soft nets.

Data collection and statistical analyses

Max/min water temperatures were recorded daily from tank 10 which was located in the middle of the tank complex. On several occasions during the trial, water pH, temperature and dissolved oxygen readings were taken from each tank to determine between-tank consistencies in water qualities. Qualitative measures of prawn activity, survival and condition were undertaken the day after stocking and every few days thereafter. Prawn bulk weights (total prawn biomass per tank) were measured before stocking and after harvest for each tank. In weighing procedures, the time prawns spent in a net draining before being placed into a tared bucket of seawater was standardised at 10 sec. The number of prawns in each bulk weight was also recorded. The general condition of prawns in each tank was also qualitatively assessed.

Data were analysed using GenStat® for PC/Windows XP, Eighth Edition. Analyses of variance were performed for continuous data followed by comparisons of means using least significance difference (LSD) testing with a 5% level of significance. Percentage survival data were analysed using a generalised linear model (McCullagh and Nelder, 1989) with the binomial distribution and logit link (GenStat, 2005), followed by protected t-tests to determine significant differences between the means.

Results

Pellet stability in water

All pellets sank to the bottom of the water column in a similar fashion. The pellet form of the commercial reference diet remained stable throughout the trial period and it did not have any effect on water clarity. Experimental diets 3, 4, 6 and 8 also displayed acceptable stability for the first 20 min. Of these, diets 3, 4 and 6 remained stable after 6 h, however diet 8 began partial disintegration at 1 h, and although it did not affect the clarity of water its pellet form was fully compromised after 6 h.

On the other hand, diets 5, 7 and 9 began leaching material soon after submersion causing water clouding. Diets 5 and 7 had a particularly pronounced effect on water clarity by the 5 min mark. The pellet form of all three of these later diets was markedly compromised after 1 h in seawater.

Photos taken of the bowls containing each diet at the 3 h interval are provided in Appendix 6.

Feed ingredient proximal analyses

Table 9 provides some previously documented proximal analyses for similar feed ingredients, and information that could be supplied by the manufactures of feeds and ingredients used in the study. Table 10 provides the analysed proximate and amino acid results (DM basis) of all major ingredients used in the experimental diets, and Table 11 provides their fatty acid compositions. These analyses were performed by DPIF laboratories at the Animal Research Institute, Yeerongpilly Qld. Table 12 provides an estimated of the nutrient contents of the experimental diets as calculated from ingredients. The commercial prawn feed bag label indicated the diet as having an energy level of 18.66 MJ kg^{-1}, and was made from ingredients that include steam dried fish meal, squid meal, squid liver meal, antioxidant–treated marine and vegetable oils, oilseed meal, wheat flour, phospholipids, vitamins and minerals. The organic pig weaner diet that was used as the base in two of the experimental diets was certified by the Organic Food Chain 0451. It had a list of ingredients that included wheat flour, corn, wheat, fishmeal, meat meal, soybean meal, sunflower or safflower meal, and a mineral mix. Since it is normally produced in a coarse mash for piglets, prior to inclusion in our diets it was ground to a powder in a hammermill.

Table 9 Feed ingredient—manufacturer's information and other sources (% air dry basis)

Feed type	Source data	Moisture	Crude protein	Ether extract	Crude fibre	Nitrogen free extract	Ash	Calcium	Phos-phorus	Energy
Wheat feed flour	(Tacon, 1990)	12.0	11.7	1.2	1.3	73.3	0.5	0.03	0.18	-
High protein white plain wheat flour	Manufacturer spec	13.0	13.5	1.2	3.8*	70.6**	-	-	-	15.04
Dried catfish processing waste	(Tacon, 1990)	-	42.0	35.0	-	-	16.0	5.40	2.80	-
Dried whiting processing waste ^	DPI — see Table 10									
Dried scallop processing waste^^	DPI — see Table 10									
Mill feed soy flour	(Tacon, 1990)	10.3	12.9	1.7	32.5	37.9	4.7	0.41	0.18	-
Soya flour	Manufacturer spec	10.0	43.0	20.7	12.7*	22.4**	-	-	-	18.51
Brown chick pea flour	Manufacturer spec	13.0	22.4	6.7	10.8 *	48.0**	-	-	-	15.31
Macadamia meal	DPI									
Weaner pig mash	Manufacturer spec	-	21.9	-	3.29	-	-	-	-	14.2
Molasses	Nutr.Data.com (2006)	21.9	0.0	0.1	0.0	74.7**	3.3	0.21	0.03	-
Molasses	Manufacturer spec	31.4	4.8#	-	-	60.8**	12.2	0.71#	5.06	-
Casein	DPI — see Table 10									
Kelp	DPI — see Table 10									
Commercial prawn feed	Manufacturer spec	-	43.0	6.0	3.0	-	13.0		-	-

*dietary fibre; **total carbohydrate; #data from different batch of molasses produced at same mill in 2002; ^ Original material DM 21.5%; ^^ Original material DM 13.4%.

Table 10 Proximate chemistry and amino acid composition of organic prawn diet ingredients (dried matter (DM) basis)

Sample Components	Units	fish frames oven dry	macadamia meal	scallop gut oven dry	wheat flour	soy flour	casein	Besan chickpea flour	weaner diet	molasses old	kelp	fish frame freeze-dry	scallop gut freeze-dry	molasses new
Total DM	%	19.7	90.1	12.4	87.1	90.5	89.0	89.9	92.1	75.8	82.3	19.7	12.4	75.6
DM	%	97.4	90.1	97.2	87.1	90.5	89.0	89.9	92.1	75.8	82.3	94.4	93.9	75.6
Ash	%	29.6	1.7	35.6	0.7	5.0	3.9	2.3	8.8	-	28.6	32.7	34.7	-
N	%	10.21	2.11	8.32	2.68	6.79	14.96	4.57	3.78	-	1.06	9.95	8.74	-
Protein	%	63.81	13.19	52.0	16.75	42.44	93.5	28.56	23.63	0.0	6.63	62.19	54.63	0.0
Fat	%	5.1	13.9	2.9	1.6	22.2	0.1	5.7	6.5	-	2.9	5.0	4.2	-
Crude fibre	%	0.2	37.1	8.3	7.0	4.3	1.0	0.6	3.7	-	-	-	-	-
NDF	%	-	47.0	-	1.7	6.6	1.3	3.1	13.8	-	-	-	-	-
ADF	%	-	39.2	-	0.3	5.4	1.6	1.1	5.6	-	-	-	-	-
GE	MJ	16.58	22.53	13.97	18.55	23.62	23.71	19.87	18.76	-	-	-	-	-
Ca	%	10.36	<0.1	3.95	0.03	0.21	<0.1	<0.1	1.79	-	-	-	-	-
P	%	3.79	0.24	0.61	0.16	0.63	0.34	0.25	1.05	-	0.12	4.01	0.64	-
Amino acids														
Aspartic Acid	g/kg	72.09	14.32	58.63	6.57	62.28	73.69	32.69	14.53	-	7.83	73.74	63.29	-
Threonine	g/kg	22.70	3.96	15.99	4.00	14.30	35.39	8.60	7.48	-	2.17	21.34	17.37	-
Serine	g/kg	21.23	4.78	16.95	6.91	18.17	48.32	12.91	9.12	-	2.07	20.84	18.00	-
Glutamic acid	g/kg	106.43	28.96	77.01	59.46	96.14	233.48	46.89	40.08	-	12.60	105.31	85.63	-
Proline	g/kg	26.71	4.42	16.56	15.34	18.42	90.88	9.97	14.66	-	2.30	27.30	18.94	-
Glycine	g/kg	39.95	4.80	46.02	5.15	13.56	14.19	8.99	13.29	-	2.30	39.75	49.26	-
Alanine	g/kg	43.49	5.19	23.09	4.32	18.20	27.17	9.90	10.57	-	3.31	43.49	26.49	-
Valine	g/kg	23.16	4.34	15.33	5.32	15.97	51.13	9.75	8.28	-	2.42	23.09	17.09	-
isoLeucine	g/kg	20.62	3.57	14.72	4.99	16.85	42.71	10.52	7.48	-	2.04	20.43	16.36	-
Leucine	g/kg	36.26	6.62	24.12	9.55	27.85	78.84	17.80	13.93	-	3.41	36.53	27.06	-
Tyrosine	g/kg	12.72	3.12	9.72	4.30	9.94	43.56	6.65	5.42	-	0.92	12.43	10.52	-
Phenylalanine	g/kg	16.52	3.18	11.65	7.03	15.37	41.77	15.47	8.37	-	1.96	16.60	12.73	-
Lysine	g/kg	55.25	5.49	30.54	2.95	30.33	73.30	15.29	11.23	-	2.85	51.36	33.46	-
Histidine	g/kg	9.43	2.11	6.69	3.08	7.97	22.39	6.62	4.94	-	0.59	9.94	8.39	-
Arginine	g/kg	24.33	11.96	24.54	5.81	24.97	31.30	28.50	13.87	-	1.99	31.84	28.16	-
Tryptophan	g/kg	5.00	1.27	4.47	2.00	5.73	12.51	2.41	1.96	-	0.49	5.49	4.78	-
Cystine	g/kg	6.46	4.33	7.09	4.16	8.94	3.79	4.42	4.53	-	1.32	6.47	7.45	-
Methionine	g/kg	20.22	2.46	11.76	3.02	8.59	36.20	4.64	5.43	-	1.54	18.18	11.90	-

GE = Gross energy MJ = megajoules

Table 11 Fatty acid (mg g^{-1} of dry sample) composition of organic prawn diet ingredients

Sample Fatty acid	whiting fish frames freeze dried	macadamia meal	scallop gut freeze dried	organic wheat flour	organic soy flour	casein	organic chickpea flour	organic weaner meal	molasses	organic kelp meal	fish frames oven dried	scallop gut oven dried
14	1.0	0.8	0.5	-	20.9	1.1	-	0.5	-	1.4	1.2	0.5
14:1n-5	-	-	-	-	-	0.1	-	-	-	-	-	-
15	0.3	-	0.1	-	-	0.1	-	0.1	-	0.1	0.4	0.1
16	6.2	10.9	3.1	2.5	-	3.3	5.9	10.0	0.3	2.2	6.1	3.4
16:1n-7	1.4	21.5	0.5	-	-	0.1	-	0.4	-	0.2	1.4	0.5
17	0.5	-	0.3	-	-	0.1	-	0.3	-	0.0	0.6	0.4
17:1n-8	-	-	-	-	-	-	-	-	-	-	-	-
18	3.8	4.6	1.8	0.2	8.4	1.4	0.7	4.8	0.0	0.2	4.1	1.9
18:1n-9	3.6	69.0	0.6	2.1	52.6	2.5	12.0	20.8	0.2	8.3	3.4	0.7
18:1n-7	1.5	4.8	0.4	0.1	2.8	0.1	0.6	0.8	-	0.1	1.5	0.5
18:2n-6	0.4	2.7	0.2	8.3	106.8	0.3	30.2	24.3	0.5	1.5	0.2	0.4
19	-	-	-	-	-	-	-	-	-	-	-	-
18:3n-3	0.1	0.2	0.1	0.4	13.0	0.1	1.5	2.3	0.1	0.4	-	0.2
18:4n-3	0.1	-	0.3	-	-	-	-	-	-	0.2	-	0.4
20	0.2	3.6	-	-	0.7	-	0.3	-	-	0.1	0.3	0.3
20:1n-11	0.7	-	0.3	-	-	-	-	-	-	0.0	0.8	0.3
20:1n-9	0.2	3.1	0.2	0.1	0.4	-	0.2	0.2	-	0.0	0.2	0.2
20:1n-7	0.2	0.2	-	-	-	-	-	-	-	-	0.1	-
20:2n-6	0.2	-	-	-	-	-	-	-	-	0.2	-	-
20:3n-6	-	-	-	-	-	-	-	-	-	-	-	-
20:4n-6	2.7	-	0.5	-	-	-	-	-	-	0.9	0.8	1.1
20:3n-3	-	-	-	-	-	-	-	-	-	0.0	-	-
20:4n-3	-	-	-	-	-	-	-	-	-	-	-	-
20:5n-3	2.8	-	0.9	-	-	-	-	-	-	0.4	0.5	1.3
22	0.2	1.1	-	-	0.9	-	0.2	0.3	-	0.0	0.3	-
22:1n-11	-	-	-	-	-	-	-	-	-	-	-	-
22:1n-9	-	0.3	-	-	-	-	-	-	-	-	-	-
22:1n-7	-	-	-	-	-	-	-	-	-	-	-	-
22:4n-6	0.6	-	-	-	-	-	-	-	-	-	0.2	0.2
22:3n-3	-	-	-	-	-	-	-	-	-	-	-	-
22:5n-6	0.5	-	0.2	-	-	-	-	-	-	-	-	0.5
24	0.4	0.6	-	-	0.3	-	-	-	-	0.0	0.3	-
22:5n-3	1.0	-	-	-	-	-	-	-	-	-	0.2	0.1
22:6n-3	5.8	-	2.4	-	-	-	-	0.3	-	-	1.0	4.5
24:1n-9	0.3	-	-	-	-	-	-	-	-	-	0.4	-

Table 12 Calculated organic prawn diet nutrient content

Sample Components	Units	wheat fish	wheat scallop	wheat chickpea	wheat macadamia	wheat soy	weaner soy	weaner fish	Commercial Diet
Total DM									
DM	%								
Ash	%	5.63	6.49	6.11	5.93	6.92	10.33	11.51	7.33
N	%	4.02	3.74	4.6	3.88	5.28	5.8	4.92	6.6
Protein	%	25.12	23.39	28.78	24.22	32.99	36.25	30.74	41.23
Fat	%	1.92	1.6	3.15	5.6	8.1	10.19	5.53	5.88
GE	MJ	16.3	15.9	16.9	17.7	18.0	22.4	19.7	18.29
Ca	%	1.65	0.72	1.66	1.66	1.71	2.96	3.54	1.23
P	%	0.71	0.25	0.74	0.73	0.85	1.23	1.36	0.99
Amino acid	g/kg								
Aspartic Acid	g/kg	18.75	16.78	26.58	21.12	35.49	38.98	24.77	49.16
Threonine	g/kg	7.65	6.67	9.06	7.68	10.78	12.33	10.32	13.42
Serine	g/kg	9.9	9.27	11.75	9.33	13.35	14.43	11.77	14.56
Glutamic acid	g/kg	66.23	61.94	63.04	57.74	77.87	71.11	54.55	97.14
Proline	g/kg	18.07	16.59	16.61	14.96	19.16	19.22	18.18	19.99
Glycine	g/kg	10.54	11.4	11.73	10.49	13.11	16.64	16.63	16.05
Alanine	g/kg	10.81	7.85	12.51	11.12	15.01	17.73	15.5	22.35
Valine	g/kg	9.2	8.06	10.57	8.96	12.45	13.81	11.55	16.01
isoLeucine	g/kg	8.27	7.41	9.96	7.9	11.87	13.03	10.26	15.09
Leucine	g/kg	15.19	13.43	17.73	14.41	20.77	22.82	18.73	25.82
Tyrosine	g/kg	6.57	6.13	7.31	6.26	8.31	8.88	7.55	8.79
Phenylalanine	g/kg	8.98	8.27	11.55	7.9	11.55	12.27	10.22	12.91
Lysine	g/kg	13.43	9.84	17.12	14.22	21.65	25.19	19.53	29.49
Histidine	g/kg	4.43	4.03	5.51	4.17	5.93	6.77	5.9	8.59
Arginine	g/kg	9.05	9.08	15.85	10.95	14.85	18.36	15.11	19.74
Tryptophan	g/kg	2.63	2.55	2.77	2.43	3.77	3.8	2.68	4.06
Cystine	g/kg	4.03	4.12	4.15	4.13	5.51	5.76	4.47	6.18
Methionine	g/kg	6.59	5.36	7.1	6.46	8.29	9.37	8.45	10.38

GE = Gross energy MJ = megajoules

Tank trial conditions

Careful inspections conducted the day after juveniles were stocked into the experimental tanks revealed 1 dead prawn in each of tanks 1 and 9. These were weighed and replaced as discussed previously. All remaining prawns in tanks appeared active and healthy and faeces were apparent on the bottoms of all tanks. Over the next few days, there were variable amounts of uneaten feeds on the tank bottoms, but due to the pellet stability issues, depth of tank and a degree of water turbidity it was not possible to assess this in a meaningful way for feed comparison. As with the previous trial with banana prawns, similar silt and detritus levels were apparent on tank bottoms, covering 10-20% of bottom areas.

The salinity of supply and tank water remained high (36 ppt) throughout the trial. Water temperatures ranged from 22.5ºC at the beginning of the trial up to 28.0ºC towards the end (see Appendix 5). This pattern of slow temperature increase during the trial and daily variations of only 3-4ºC provided ideal conditions that should have been conducive to high survival, and which mirrored conditions that would likely occur in outdoor ponds. Between-tank variations of <0.3°C were observed within the experimental system. Similarly, pH values in tanks were stable (8.1-8.2) and dissolved oxygen levels remained high (6.3–7.1 mg L^{-1}). Towards the end of the trial reduced (anoxic) sediments were present on the bottom of all tanks, and again these were siphoned to waste prior to harvest.

Prawn survival, growth and condition

Survival was very high in the trial and no significant differences were detected between treatments (Table 13). The average prawn weight at the start of the trial was 3.3 ± 0.06 g as calculated from the mean (± se; *n* = 26) prawn bulk weight across all tanks of 65.6 ± 1.22 g (Appendix 5). This initial biomass estimate provided the feeding rate of approx. 4 g per day (6% of tank biomass average = 3.94 g) that was applied to each tank for the duration of the trial (split into 2 x 2.0 g feeds daily).

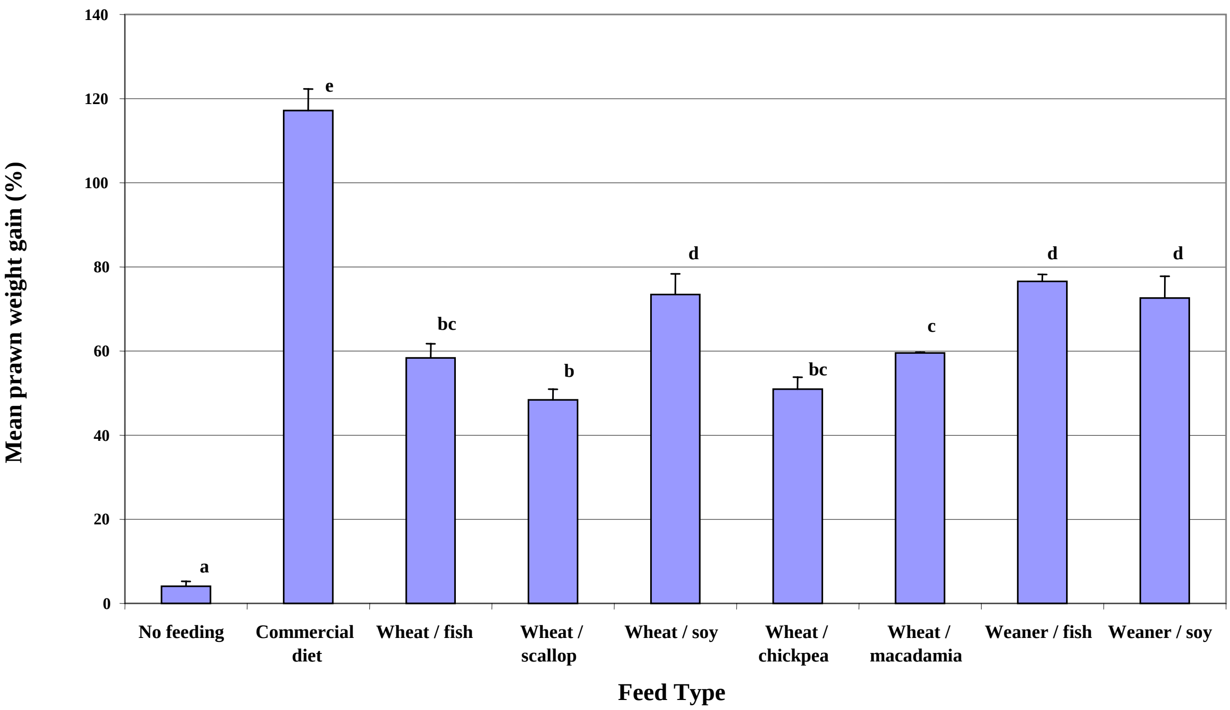
Table 13 Numbers and percentages of black tiger prawns surviving from 20 stocked into different tanks and fed different feeds over a one month period

Feed type	Number surviving in each replicate	Mean ± se. % survival
No feeding*	15, 20	87.5^a ± 12.50
Commercial diet 2	20, 20, 21**	100.0^a ± 0.00
Wheat fish diet 3	20, 20, 20	100.0^a ± 0.00
Wheat Scallop diet 4	20, 20, 19	98.3^a ± 1.67
Wheat Soy diet 5	20, 20, 21**	100.0^a ± 0.00
Wheat Chickpea diet 6	20, 20, 20	100.0^a ± 0.00
Wheat Macadamia diet 7	20, 20, 20	100.0^a ± 0.00
Weaner Fish diet 8	20, 20, 20	100.0^a ± 0.00
Weaner Soy diet 9	20, 20, 18	96.7^a ± 3.33

Note: Means with similar superscripts are not significantly different (P>0.05).
* *n* = 2.
**Unexplained error in initial number stocked with 100% survival assumed in later calculations.

The mean (± se; *n* = 26) prawn bulk weight across all tanks at the end of the trial was 106.4 ± 4.26 g. Figure 4 provides an assessment of the mean percentage weight increases for different feeds over the trial period. The commercial reference diet produced the highest (P<0.05) mean weight gain (117.2%). This was followed by the wheat/soy (73.5%), weaner/fish (76.6%) and the weaner/soy diets (72.6%). All other diets produced significantly (P<0.05) lower weight gain. The unfed prawns produced only a slight weight gain (4.1%) and were fouled with epibiotic algae and other organisms, indicating that they had probably not moulted during the trial period. All prawns from the other fed treatments were free from fouling, had long antennae, were without obvious tail bites, and were vigorous and healthy when harvested.

Figure 4 Mean (± se; *n* = 3) percentage of black tiger prawn weight gain for different feeds supplied to 20 prawns over a one month period



Note: Means with similar letters are not significantly different (P>0.05).

Observed prawn performance response and relationship to diets

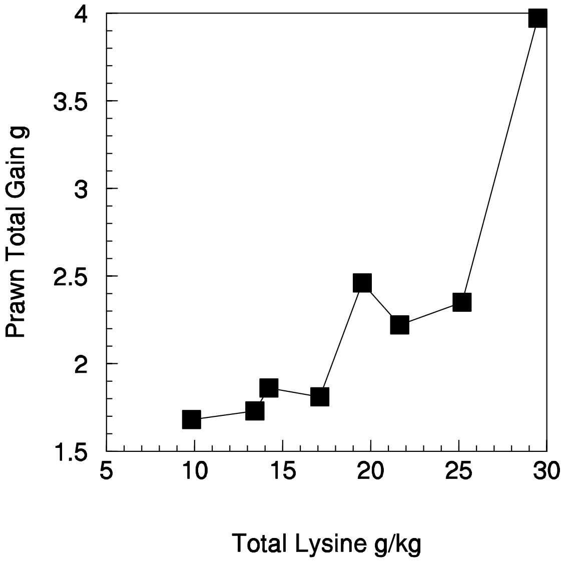
Further examination of the composition of diets and the total prawn weight gains for each diet are provided in Figures 5 and 6. Plots revealed a positive linear trend in weight gains for increasing total lysine content (Figure 5) and increasing protein content (Figure 6) for all diets. The control (commercial) diet which was not based on organic ingredients also showed a similar response to increases in both the protein and lysine contents although the overall prawn total gain response observed was much greater that that for the experimental organic diets. This indicates that other parameters associated with the commercial diet, of which we have little information (it being confidential) result in the better performance of prawns when fed this diet.

Based on literature information the superiority of this commercial diet could be due in part to:

- The better long term water stability of the commercial diet pellets. Our observations on pellet stability did indicate that the commercial diet displayed superior pellet water stability (see Appendix 5). This could be due to differences in extrusion or due to post-extrusion coating with marine or vegetable oils. The net result of such pellet stability is that the pellet remains intact longer and consequently there is less nutrient loss into the water from pellet dissolution and also reduced non-consumption and feed wastage by the prawn as a result of pellet disintegration.

- The use of highly digestible ingredients that include steam dried fish meal, squid meal, squid liver meal, oilseed meals and wheat flour. These ingredients were not certified-organic, are generally expensive and some of the fishery sourced products could be considered unsustainable in the long term. The marine based products are likely to be more nutrient rich and highly digestible for prawns. Additionally, although our organic diet ingredients have been analysed for the total content of nutrients we have no information about the relative availability of these nutrients, whilst the commercial diet formulation is likely to have taken into account the availability/digestibility of essential nutrients in the ingredients utilised.

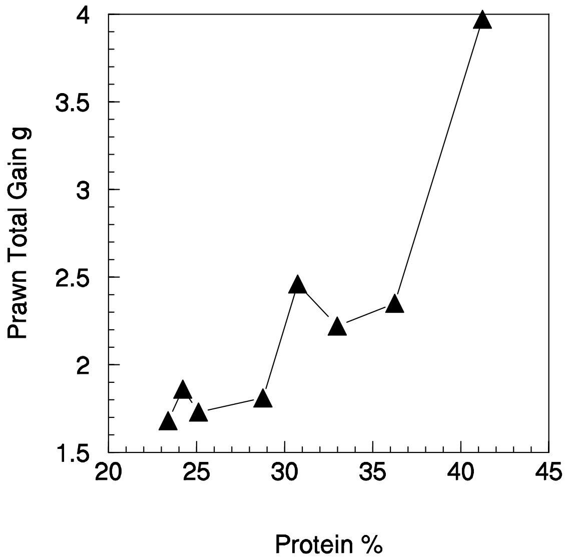
Figure 5 Plot of prawn total gain against total lysine content of diets



- A greater overall intake of the commercial diet, which could have been a result of the squid meal generally used in commercial prawn feeds as an attractant. Squid meal is used in commercial prawn feeds because of its reported growth promoting and feed attractant properties at levels as low as 1.5%. This is reportedly a result of it containing low molecular weight peptides, free amino acids and betaine (Meyers, 1989; Takaoka *et al.*, 1995). Unfortunately the level of feed consumption could not be ascertained under the tank conditions of this trial. Ideally in a performance study, any variation in the resultant weight gain of an experimental animal can usually be attributed correctly by knowledge of both the actual feed consumption of the animal and determination of the nutritive value of that feed.

- The addition of antioxidant–treated marine and vegetable oils, phospholipids, vitamins and minerals (non-organic micronutrients) which could be deficient in our experimental organic diets and hence may have affected the growth response.

Figure 6 Plot of prawn total gain against protein content of diets



Discussion

Organic market analysis

Organic agriculture is an increasingly important food production sector. At the beginning of 2005 it involved over 550,000 certified farms covering more than 26 million hectares in 108 countries. The Australian organic market is currently valued at $400 million (BFA, 2006). Demand for organic products greatly exceeds supply in Australia, as in most other developed economies, and it is the fastest growing food and beverage category worldwide (McKinna, 2006). The global organic seafood market is estimated to be worth US$600 million (Anon, 2006b). However there are very few aquaculture ventures in Australia producing and/or processing organic seafood.

The Queensland prawn aquaculture industry (including black tiger, banana and kuruma prawns) is under considerable competitive pressure from cheaper imports. Its value decreased by 14% from $53.3 million to $45.9 million in 2004–05, and total farm ponded area decreased from 846 hectares in 2003–04 to 794 hectares in 2004–05. The number of producing farms has declined for the first time in the last five years from 37 farms in 2003–04 down to 32 farms in 2004–05 (Lobegeiger & Wingfield, 2006)). There are a number of farms that have decreased production and are for sale as a result of import price pressures on Australian markets and lower overseas market prices. Retail prices for Australian product during this period ranged from $9.50 to $17.50 per kg with the average price at $14.60 per kg, which is almost the same as farms received in 2003–04. Despite this the cost of feed and labour has risen.

These point-of-sale and cost of production pressures have created interest within the industry in alternative products and markets such as organic prawns. More efficient production methods that utilise lower stocking densities and reduced power and feed inputs may be a viable way for local farmers to compete effectively with cheaper imports. These are the basic principles of organic production, along with avoidance of chemicals and sustainability issues which are highly favoured by discerning consumers.

The Ecuadorian Organic Support and Advisory Department has identified the US as a major target for expanding organic shrimp sales and recommended increases in production of organic shrimp from 40 to 400 tonnes per month. One of the two organic farms in Ecuador, Biocentinela, has annual sales of organic shrimp into the European market worth US $1.14 million. The main partners are the retail chains Tesco, Marks & Spencer and Waitrose of the UK, Biofach of Germany and Coop of

Switzerland. The company's expectations for the future were optimistic with production increasing from 7 tonnes per month in 2002 to 15 tonnes per month in 2004.

China is also moving towards this style of aquaculture production, with a 100,000 tons per annum organic extrusion mill for aquatic feeds currently under construction in Hainan (Anon, 2007). Dragon Feeds of Aberavon are also looking towards production of organic feeds for aquaculture by incorporating farmed Polychaete worms in the diets. This company is looking towards completion of a 500,000 tonnes per annum feed mill in Wales by the end of 2007 (Anon, 2006a, 2006b). This shows a world wide trend towards organic aquaculture products, which Australia must address if it is to compete in future markets. Despite such increases in worldwide "Organic aquaculture" there remains a large untapped potential for Organic aquaculture produce from Australia, particular as Australian production for many animal and plant industries is frequently viewed as being "Clean and Green"

A recent on-line price (02/10/2006) for cooked and peeled organic prawns in MAP from Waitrose in the UK quoted a price equivalent to A$73 per kg. With a realistic wholesale value of A$36.50 for this product (ie: nominally 50% of retail value), it shows plenty of potential for attaining double the current Queensland farm gate price. When the product offered could be larger sized uncooked organic prawns, currently not sold in this form but proven by recent MAP storage trials at DPI&F's Innovative Food Technologies Centre (also in studies associated with the present project), there is opportunity for even larger premiums. If only 5% of the current crop was converted to organic and exported the industry could grow by A$4.6 million rather than decrease under the current trend.

Nutrient requirements of Penaeus monodon and the ability of organic feed stuff to meet these needs

The change in this study with a focus on a more intensive culture regime, and on the use of the black tiger prawn thought to be less herbivorous than the previously studied banana prawn, necessitated a greater focus on the development of a diet supplying a higher protein level in organic prawn diets. When comparing low density extensive systems to more intensive systems with higher stocking densities, there is a general need to provide better nutrition for the later through more detailed knowledge of nutrient requirements of the species in question (Shiau, 1998 and Chen, 1998).

Protein and energy requirement

Proteins are indispensable nutrients for prawns, with the level required varying between 30-57%. This requirement is influenced by the protein quality, physiological state of the prawn, its age and the environment in which prawns are grown. For example, Shiau (1998) suggests that the optimum dietary protein level is lower in juvenile *P. monodon* when reared in seawater (40%) than when reared in brackish water (in 16 ppt. they need 44% protein). This author suggests that in low salinity water prawns use protein and not lipid as an energy source. Additionally, Shiau (1998) suggested that salinity affects protein digestibility for sources such as soy but not for fish meal or casein. Thus, there may be benefits in developing specific diets based on protein levels and sources for different environments.

Amino acids

It has been reported that prawns have a nutrient requirement for 10 amino acids, although the incapability of juvenile or adult prawns to utilise dietary crystalline amino acids has made it difficult to quantify specific essential amino acid requirements. The nutrient requirement for various amino acids in *P. monodon* has been investigated for methionine (Millamena *et al.*, 1996), threonine (Millamena *et al.*, 1997), lysine and arginine (Millamena *et al.*, 1998), histidine, isoleucine, leucine, phenylalanine and tryptophan (Millamena *et al.*, 1999).

Energy components

Fish and prawns generally have a high dietary protein requirement (24-57%) and preferentially derive energy from protein over carbohydrate (Akiyama & Dominy, 1989; Tacon, 1990). However, there is a clear need in the future to move away from the use of aquatic feed ingredients such as fish meal to those based on cheaper and more sustainable terrestrial based feed stuffs. Terrestrial sources generally have lower protein content and higher starch and hence there is much research needed into how prawn energy need can be met from starch or processed starch in these feeds. Generally, simple or reducing sugars are thought to be less effective in meeting prawn nutritive needs than more complex polysaccharides such as starch (Chen, 1998). For example, Shiau & Peng (1992) showed that corn starch (20-30%) provides better growth and is a better carbohydrate for protein-sparing in *Penaeus monodon* juveniles (0.5 g) than simpler carbohydrates like dextrin and glucose.

Organic ingredients

Akiyama & Dominy (1989) recommend the use of a 6:1 protein: lipid ratio for energy levels in diets for shrimp. Generally the sources of protein and lipids used in prawn diets has traditionally been from marine sources, however these are becoming increasingly more expensive and are limited in supply, a problem caused by over fishing and failing fisheries. In general terms there is a need to explore alternative terrestrial based sources of ingredients for use in aquaculture diets in the future, and the potential exists for these to also be certified organic products to add value to the end product

Wheat

We continued to use wheat as the basal ingredient in the formulations for black tiger prawns, because it is a commonly used ingredient in commercial prawn feeds as energy and nutrient sources and binders. Additionally, it is believed that provision of starch components of long chain carbohydrates can spare some of the use of amino acids in prawn diets as a source of energy. The use of wheat in aquafeeds and prawn feeds is reviewed in our previous work with banana prawns (1st feed trial for this project in Section 2)

Hari *et al.,* (2004) also showed that in extensive systems additional carbohydrate in the water column helped control the C/N ratio and through increased heterotrophic bacterial growth the bacterial protein produced could augment the shrimp requirement.

Soy flour

Soy products are also often used in aqua feeds because they are a rich source of protein which can significantly boost the total protein levels in feeds. The adequate boosting of protein levels may be one of the biggest challenges to organic diet development. However, soy flour is also high in fat (22% — Table 10), and its high inclusion levels in feeds could contribute to excessive levels for prawns and possible problems. When compared with the whiting waste, the soy flour used in this study was particularly high in the 18:2n-6 (107 mg g^{-1}) and 18:1n-9 (53 mg g^{-1}) classes of fatty acids but it did not contain any 22:6n-3 which is represented in relatively high proportions in the fish waste. The soy flour was also comparatively low when compared to fish in the amino acids threonine, glycine, alanine, lysine, and low in ash and phosphorus.

Anti-nutritional factors, such as trypsin-inhibitors, are also present in some soy products and they can present a problem especially if they are inadequately heat processed to eliminate the inhibitor. This has implications in protein digestion as these trypsin-inhibiting compounds reduce the activities of trypsin and chymotrypsin which are pancreatic enzymes involved in protein digestion and hence protein utilization. Additionally with soy, over-heating can pose a problem in that this will result in reduced amino acid availability.

Following the conclusion of the trial enquires to the supplier of the soy flour revealed that the organic full fat soy flour, which was primarily produced for human use, as supplied and used in this study had undergone no heat treatment to remove any anti-nutritional factors. Although the extrusion process used to produce the prawn feed pellets in this study may itself have reduced somewhat the level of trypsin inhibitor anti-nutrients remaining in the diet. The impact of no treatment for soy anti-nutrients may have significantly affected the overall digestibility of diets based on soy and the resultant prawn growth performance recorded.

Nevertheless, both experimental diets that had high soy inclusion levels (wheat-soy and weaner-soy) were among the best performing experimental diets in terms of prawn growth, though still at a lower level compared with the commercial diet. An apparent leaching of ingredients from these high-soy diets suggested either that other forms of soy which are less soluble in water, or further pellet stabilisation fine-tuning procedures, could provide additional benefits for the use of this ingredient.

Macadamia meal

Macadamia meal was a new stockfeed ingredient trialled in this study although Balogun & Fagbenro (1995) previously reported the use of a similar product in tilapia diets. It was considered because it can be supplied in large quantities in Australia as an organic by-product of oil extraction activities. It had a relatively high level of fat (14%) compared with the fish waste (5%), and was found to be particularly high in the 18:1n-9 (69 mg g^{-1}) and 16:1n-7 (22 mg g^{-1}) fatty acid classes which are normally found at much lower levels in marine based products (eg: 1-3 mg g^{-1} in the whiting waste; see Table 11). It also had much lower levels of protein than was hoped for (13% compared with 64% in the fish waste), and consequently was much lower in most amino acids except cystine.

The previous macadamia meal study of Balogun & Fagbenro (1995) reported a substantially higher crude protein content of 39.5% and crude fibre content of only 3.0%. By comparison, the macadamia meal used in this study had a higher crude fibre value of 37.1% which indicates that it was not a de-hulled material or that the meal had a higher than anticipated hull content. Hence, the protein value of (13%) was lower due in part to dilution of the meal with hull fibre.

Chickpea flour

Chickpeas are used as feed for many terrestrial animals (eg. pigs and poultry) and can currently be sourced readily in Australia as a certified organic product. Compared with the fish waste, with 28.6% protein it had less than half the protein level, and was lower in ash and phosphorus content. Whilst it had similar total fat levels to the fish waste, this was made up with a limited range of fatty acids though with heavy representation of the 18:2n-6 and 18:1n-9 classes. The limited availability of polyunsaturated fatty acids that are essential for marine fishes and crustaceans (eg: n3-C22, n3-C20) in this feed ingredient may limit its usefulness in aqua-feeds. This fatty acid availability may partially explain the poorer performance observed in prawns fed the diet containing chickpea flour. Additionally the presence of anti-nutritional factors may also play a role in the observed poorer performance.

Most varieties of chickpea do contain anti-nutritional factors and the reported levels present for Trypsin inhibitor activity (TIA) and Chymotrypsin inhibitor (CI) activity in chickpea are 4.79 (range 1.4-7.03) and 7.72 mg/g (range 3.71-13.19) respectively (Petterson & Mackintosh, 1994). Both these anti-nutritional factors can impair the utilisation of nutrients by animals. Studies by (Mustafa *et al.*, 2000) showed that heat treatment reduced the rumen escapes values of chickpeas by inactivating the TIA and CI, while Batterham *et al.*, (1993) found that older pigs could tolerate dietary levels of 4.7mg/g TIA and 4.5 mg/g CI.

Following the conclusion of the trial, enquires to the supplier of the chickpea Besan flour (dhal flour — probably Desi chickpea) revealed that the organic chickpea flour, which was primarily produced for human use, as supplied and used in this study had undergone no heat treatment to remove any anti-nutritional factors. Thus it is unclear whether there was any significant impact on prawn growth from anti-nutrients present in the chickpea, and also whether the extrusion process to form the prawn pellet may have possibly reduced this factor.

Whiting fish frames (processing waste)

The whiting processing waste was chosen because there is current production of this from wild harvested whiting (stout whiting — *Sillago robusta*). Unlike traditional fish meals, which are primarily produced from fish harvested for fishmeal production, these fish are harvested primarily for human use and the by-product or residue could be used for prawn feeding. Under the organic definition and certification process this use of fish is allowed.

Whiting were also chosen because they are a small species which have relatively soft bones allowing them to be more easily pulverised into meals suitable for feed plants. Additionally, there may also be a significant potential in the future to farm whiting (sand whiting — *Sillago ciliata*) for the butterfly fillet market. If these whiting were also being cultured according to organic principles, it would create a substantial organic by-product feed resource (approx. 50% of biomass produced would be heads, guts and frames) that could be utilised as a feed ingredient in the formulation of organic diets for prawns. Considering the pursuit of processed organic prawns involving deheading and peeling as is the trend in European markets, this processing waste would also be of use in the formulation of diets for organic whiting, creating the potential for maximum nutrient use efficiencies in by-product utilisation.

Scallop gut waste

The scallop gut waste ingredient was also included in the study because there is potential for the availability of this material to increase substantially in the future due to scallop restocking efforts in the Hervey Bay region of Queensland for the Saucer scallop *Amusium balloti*. The commercial company which supplied this ingredient for our trial is also directly involved in these restocking endeavours.

Although it has been shown that scallop gut waste can contain high levels of cadmium, dietary inclusion at a low level in prawn diets is not likely to cause any problematic levels of this heavy metal, neither for the health of the prawns nor for the organic certification process. Another potential problem in the use of this waste stream is the potential degenerative action of the proteolytic enzymes that are present in this product, which would necessitate rapid processing (freezing and/or drying) to limit or inhibit this activity and help maintain ingredient stability to enable its use and to be classed as a reliable high quality feed ingredient.

The primary role of this ingredient's inclusion in all our experimental diets was as a prawn feeding attractant. Its value as a protein source was also examined in one dietary treatment group (Wheat/scallop diet 4). Since the higher inclusion level of the scallop gut did not appear to perform as well as some of the other protein meal formulations in these initial studies, its actions in this regard may be marginal. However further analyses may be required to assess the availability of the nutrients present in scallop gut and whether there were some other nutrient shortfalls especially as there is little information on this potential feed ingredient.

Kelp meal

Kelp meal was added to all experimental diets to add further feeding stimulants, provide additional trace elements and potentially assist in the binding of the diets. Particularly as seaweed meal from *Kappaphyycud* and *Gracilaria* have been shown to be useful as binders in diets for juvenile *P. monodon* (Dy Penaflorida & Golez, 1996). Since it is a marine product, it was expected to also provide some additional unknown nutritional factors that may have been lacking in the diets based more on terrestrial based inputs. Analyses revealed that it did indeed contain several fatty acids that were not present in the terrestrial products. But kelp meal lacked many of the C20, C22 and C24 fatty acid groups present in the fish frames and was particularly high in ash.

There may be other species of seaweed that could provide similar advantages to those sought in the kelp meal for formulated aqua feeds, and which could be more conveniently produced within aquaculture premises. These include *Enteromorpha* and *Ulva* sp. which can be produced in specialised facilities on waste nutrients made available from pond discharge waters (DPI&F unpublished data). In our preliminary analyses these other seaweed products have been shown to contain higher protein content (22.8-25.9%) than that found in kelp and consequently higher amino acid content (for example Ulva having a lysine content of 8.54 g/kg vs. only 2.85 g lysine /kg in kelp) 0.6-0.85% calcium, 0.3-0.4% phosphorus and 11 MJ/kg gross energy. Unfortunately the level of ash appears to be higher at 34-42% compared to 28% for kelp.

Molasses

Molasses was added within the water addition at the time of extrusion to all the pellet diets (except the commercial diet) to provide an additional energy source. Molasses is almost entirely made up of water and soluble carbohydrates (see Table 9) such as sucrose (33.4%) and reducing sugars (15.2%). Pascual *et al.*, (1983) found that for *P. monodon* juveniles (1.8 g), sucrose (10%) provided the highest survival (56%) for a range of simple and complex carbohydrates tested, but that at 40% inclusion all carbohydrates (particularly maltose and molasses) provided very low survival. As extrusion progressed the final levels of inclusion were much less then the critically detrimental levels reported for *P. monodon* by Pascual *et al.* (1983). The primary reason for dietary inclusion was reported protein sparing effects and to assist in pellet binding (McDonald *et al.*, 1998).

Prawn feed water stability

Better water stability appears to be an important aspect to the development of better organic prawn diet formulations in the future. Future work in this area is particularly important since we observed that some of the diets which provided the best weight gains also demonstrated a level of stability that is less than optimal. Optimum feed usage and minimisations of feed wastage are two important considerations in both the profitability and sustainability of any prawn production industry whether organic or not. Since prawns are fed every four to six hours, the pellets need to hold their integrity for at least this length of time, and preferably longer to avoid water and pond bottom fouling. Although with the high carbohydrate of these diets there could be some benefit to the C/N ratio in extensive systems through heterotrophic bacterial protein production as shown by Hari *et al.*, (2004).

Starch is a very useful ingredient for achieving increased pellet stability especially following gelatinisation of the starch with diet extrusion. However for these trials we could not identify any current organically certified sources of starch. Alternatively, pellets are also often sprayed with oil to improve water stability, but again, the fish oils that are normally used in commercial feeds would not adhere to the main organic principles, and it is uncertain how palatable organic vegetable oils would be for prawns, and what effect they may have on nutrient digestion. Sourcing and testing approved starches for increased protein and pellet stability, and testing alternative organic-oil coatings are likely to be the next steps in any further organic feeds development for prawns.

Identified future research needs

This work and our previous recent studies of prawn diets focussed on organic formulations that could be used to primarily supplement natural feed sources in ponds. Correspondingly this came with the underlining assumptions that rearing densities would generally be low and that the range of vitamins and micronutrients that are available in the natural pond biota would balance the nutritional content of the simple supplemental feeds. However, higher culture densities may arguably be allowable in organic standards, which would then place greater emphasis on the provision of supplemental diets to feed prawns adequately, and with this there will correspondingly be a need to make these diets more complete.

There are several paths following on from our work that can be recommended for further fine-tuning and developing more complete organic diets for prawns. These are:

1 Better pellet stability may increase the feed utilisation to improve the performance of our current diets. This will have the advantage of reducing feed loss from leaching and pellet breakdown. From a production viewpoint this means that the unit prawn production is maximised for each unit of feed. Additionally it reduces the fouling of pond water by uneaten feed which is particularly nutrient rich. This better pellet stability could be achieved by a number of techniques, such as:

- increasing or manipulating the starch content of diet,
- adding other ingredients with binding properties (eg. seaweed with alginates),
- by varying the extrusion parameters, and/or by
- coating pellets with a water resistant barrier such as the fish oil used in current commercial diets.

Of course these additional products would need to comply with organic standards.

2 Suitable future terrestrial protein sources for organic diets are a particular concern for this work. Two of the main concerns are their availability in commercial quantities and their nutrient digestibility values for prawns.

3 Optimising the use of diets based on these terrestrial protein sources,

- either through adding better attractants ensuring that the majority of food is being consumed, or
- investigating protein treatment methods that may improve their digestibility by aquatic species. One method that is being increasingly used to treat and stabilise seafood waste for future use as aquaculture feed is hydrolysis using endogenous proteases (Lian et al. 2005). This process increases digestibility by increasing the availability of peptides and free amino acids for uptake, and since it uses the enzymes that naturally occur in the waste products it is organically certifiable. However, for this approach to be a viable pursuit, seafood processing plants would need to implement new systems, equipment and procedures to treat wastes in a more controlled manner. They would need to be willing to foster a new product line rather than dispose of the waste in the most convenient way. A ready organic market for such improved products could drive the implementation of this waste utilisation strategy through economics.

Acknowledgements

This work was funded in part by the Rural Industries Research and Development Corporation through project No DAQ336A entitled “Growing organic prawns in inland saline systems”. The authors would like to thank Mr Noel Herbst from Gold Coast Marine Aquaculture Pty Ltd for the supply of prawn juveniles for the feed trial, and Kialla Pure Foods Pty Ltd, Mr Gary Pinzonne and Sons, Mr Rob Dean from Queensland Sea Scallop Ltd, Mr Leyland Minter, and the Rocky Point Sugar Mill from the Heck Group for the supply of feed ingredients. Many thanks to Michael Burke, Trevor Borchert, Martin Lee, Michael Salini and other staff at BIARC for their assistance with technical work. Thanks also to Peter Martin, Adam Pytko, Michael Gravel, Ian Brock of the ARI nutrition lab for proximate chemical, amino acid and fatty acid analyses.

References

Akiyama, D. M. & Dominy, W. G. (1989). Penaeid shrimp nutrition for the commercial feed industry. In *Texas Shrimp Farming Manual, Vol. 1: Grow-out Technology,*. Technical Report of Texas Agricultural Extension Service and Texas A&M University Sea Grant College Program,, Corpus Christi, Texas, U.S.A.

Anon. (2006a). Worms replace meal. Year in Review. In *Fish Farming International (*vol. 33, pp. 35.

Anon. (2006b). Polychaetes as alternative feed. Equipment & Supplies. In *INFOFISH International*, vol. 6, pp. 67.

Anon. (2006c). Seafood International.

Anon. (2007). China builds an organic feed mill. News/Meal Report. In *Fish Farming International*, vol. 34(pp. 4.

Balogun, A. M. & Fagbenro, O. A. (1995). Use of macadamia presscake as a protein feedstuff in practical diets for tilapia, *Oreochromis niloticus* (L). *Aquaculture Research* **26,** 371-377.

Batterham, E. S., Saini, H. S., Andersen, L. M. & Baigent, R. D. (1993). Tolerance of growing pigs to trypsin and chymotrypsin inhibitors in chickpeas (Cicer arietinum) and pigeonpeas (Cajanus cajan). *Journal of the Science of Food and Agriculture* **61,** 211-216.

BFA. (2006). Organic farming — from humble beginnings to big business! Biological Farmers of Australia. In *The 2nd Queensland Organic Conference 1-2 September, 2006,* Beerwah, Qld.

Chen, H.-Y. (1998). Nutritional requirements of the black tiger shrimp: Penaeus monodon. *Reviews in Fisheries Science* **6,** 79-95.

Dy Penaflorida, V. & Golez, N. V. (1996). Use of seaweed meals from Kappaphycus alvarezii and Gracilaria heteroclada as binders in diets for juvenile shrimp Penaeus monodon. *Aquaculture* **143,** 393-401.

Flaherty, M., Szuster, B. & Miller, P. (2000). Low salinity inland shrimp farming in Thailand. *Ambio* **29,** 174-179.

GenStat (2005) Release 8.1 for PC/Windows XP. Eight Edition, Lawes Agricultural Trust (Rothamsted Experimental Station).

Hari, B., Madhusoodana Kurup, B., Varghese, J. T., Schrama, J. W. & Verdegem, M. C. J. (2004). Effects of carbohydrate addition on production in extensive shrimp culture systems. *Aquaculture* **241,** 179-194.

Lobegeiger, R. & Wingfield, M. (2006). Report to farmers — Aquaculture production survey Queensland 2004-2005., pp. 42. Department of Primary Industries and Fisheries, Queensland. QI 06044. ISSN 0727-6273.

McCullagh P. and Nelder J.A. (1989). Generalised Linear Models (2nd ed.). Chapman and Hall, London.

McDonald, Edwarsd, R. A., Greenhalgh, J. F. D. & Margan, C. A. (1998). Roots, tubers and related crops. In *Animal Nutrition*, pp. 481-488. Addison Wesley Longman Ltd, Harlow, Essex, UK.

McKinna, D. (2006). Export potential for organics — opportunities and barriers., pp. 86. RIRDC Publication No 06/061. ISSN 1440-6845. ISBN 1 74151 324 3.

Meyers, S. P. (1989). Squid protein and soy lecithin as beneficial additives in shrimp diets. In *INFOFISH*, vol. 2, pp. 22.

Millamena, O. M., Bautista, M. N., Reyes, O. S. & Kanazawa, A. (1997). Threonine requirement of juvenile marine shrimp Penaeus monodon. *Aquaculture* **151,** 9-14.

Millamena, O. M., Bautista-Teruel, M. N. & Kanazawa, A. (1996). Methionine requirement of juvenile tiger shrimp Penaeus monodon Fabricius. *Aquaculture* **143,** 403-410.

Millamena, O. M., Bautista-Teruel, M. N., Reyes, O. S. & Kanazawa, A. (1998). Requirements of juvenile marine shrimp, Penaeus monodon (Fabricius) for lysine and arginine. *Aquaculture* **164,** 95-104.

Millamena, O. M., Teruel, M. B., Kanazawa, A. & Teshima, S. (1999). Quantitative dietary requirements of postlarval tiger shrimp, Penaeus monodon, for histidine, isoleucine, leucine, phenylalanine and tryptophan. *Aquaculture* **179,** 169-179.

Mustafa, A. F., Thacker, P. A., McKinnon, J. J., Christensen, D. A. & Racz, V. J. (2000). Nutritional value of feed grade chickpeas for ruminants and pigs. *Journal of the Science of Food and Agriculture* **80,** 1581-1588.

NutritionData.com. (2006). Nutritional summary for molasses: www.nutritiondata. com/facts-001-02s04at.html ; Nutritional summary for whole grain wheat flour: www.nutritiondata.com/facts-B00001-01c21Ue.html

Pascual, F. P., Coloso, R. M. & Tamse, C. T. (1983). Survival and some histological changes in Penaeus monodon Fabricius juveniles fed various carbohydrates. *Aquaculture* **31,** 169-180.

Petterson, D. S. & Mackintosh, J. B. (1994). *The chemical composition and nutritive value of Australian grain legumes*. GRDC, Canberra ACT.

Shiau, S.-Y. (1998). Nutrient requirements of penaeid shrimps. *Aquaculture* **164,** 77-93.

Shiau, S.-Y. & Peng, C.-Y. (1992). Utilization of different carbohydrates at different dietary protein levels in grass prawn, Penaeus monodon, reared in seawater. *Aquaculture* **101,** 241-250.

Tacon, A. G. J. (1990). *Nutrient Sources and composition. In: Standard methods for the nutrition and feeding of farmed fish and shrimp. Volume 2.* Argent Laboratories Press., Redmond, Washington, USA.

Takaoka, O., Takii, K., Nakamura, M., Kumai, H. & Takeda, M. (1995). Identification of feeding stimulants for tiger puffer. *Fisheries Science* **61,** 833-836.

3 Document the requirements for organic prawn 'grow out'

3.1 Production of organics prawns—commercial farm trial at Bauple

By Scott Shanks, Ben Russell and Michael Burke

Results

Table 14 shows the inputs for the experimental ponds.

Table 14 All non-feed pond inputs at Bauple experimental organic ponds

Pond	Pelletised chicken manure	Organic molasses	Lime	KCl
B1	13kg	25.5L	60kg	43kg
B2	7.5kg	49.5L	45kg	28kg
B3	21kg	44L	40kg	53kg

Table 15 shows the numbers of prawns stocked into each pond.

Table 15 Numbers of prawns initially stocked into organic ponds and surviving stocking

	Numbers stocked (date)		Numbers surviving stocking		Final
Pond identity	***merguiensis***	***monodon***	***merguiensis***	***monodon***	**numbers**
B1	8228 (27/10/2005)		6747	-	6747
B2	6568 (3/11/2005)	7000 (6/11/2005)	2627	5740	8367
B3	6568 (3/11/2005)	7000 (6/11/2005)	1642	6440	8082
Non-organic pond average	0	11150	-	9656	9656

All ponds performed well during the early stages of production with prawns in the organic ponds growing at a similar rate as those in non-organic ponds fed with commercial feed. Extracts from the trial diary are provided below.

***Day 1** (27 Oct 2005, the first PL's were stocked):* PL's counted and stocked into B1, with a sample of 60 into a Hapas net where stocking survival will be estimated in 2 weeks.

***Day 8** (3 Nov 2005):* Survival tested on B1 (see Table 16 below). Ponds B2 and B3 were stocked with banana prawns, once again 60 animals were stocked into Hapas net. Unfortunately during counting and transport of these prawns from the tank to the pond they became extremely stressed and it is estimated 50% of the prawns were lost. These ponds are located on the other side of the farm from the acclimation tanks.

***Day 11** (6 Nov 2005):* Additional Tiger Prawn PL's stocked into B2 and B3 to make up for losses from the previous stocking event.

***Day 14** (9 Nov 2005):* Hapas nets from B2 and B3 removed and PL's counted from both species.

Day 28 *(23 Nov 2005):* Weigh and measure

Day 35 *(30 Nov 2005):* Weigh and measure

Day 40 *(5 Dec 2005):* Weigh and measure

Day 43 *(8 Dec 2005):* In all organic ponds the prawns are continuously roaming the pond edge, apparently looking for food. Resultantly feeding with organic flour initiated.

Day 47 *(12 Dec 2005):* 800 banana prawns removed from B3 for feed trial.

Day 49 *(14 Dec 2005):* Weigh and measure. Prawns still roaming in organic ponds, some tail damage becoming evident.

Day 55 *(20 Dec 2005):* Weigh and measure.

Day 57 *(22 Dec 2005):* Based on all the evidence to date including the lack of a suitable organic diet, and after lengthy consultation with our commercial partner, the decision was made to end the organic trial. Feeding with a commercial non-organic diet was initiated in an attempt to salvage some financial returns for the farm and to demonstrate renewed growth of prawns which would help confirm that it was the nutritional low value of pond productivity and organic flour which was suppressing growth.

Day 63 *(28 Dec 2005):* Weigh and measure. Due to a high level of stratification in all ponds leading to lower DO levels on pond bottoms, the aerators were left on 24hrs a day.

Day 70 *(4 Jan 2006):* Weigh and measure. Difficulty catching enough prawns from B1 for sample so a further inspection was carried out. This revealed a large number of prawns dead in the sediment pile of the pond that were assumed to be 1-3days old. Very high afternoon temperatures above 33°C have been recorded over the past 4 days and with the aerator left on in this pond, as opposed to B2 and B3, no stratification developed to allow escape into cooler waters. Dissolved oxygen was low in the morning (4.1ppm) but rose to 20 by the afternoon. Neither of the other 2 ponds had substantial losses at this time due possibly to the stratification that would normally develop when their aerators were turned off during the day.

Day 77 *(11 Jan 2006),* ***84*** *(18 Jan 2006).* ***91*** *(25 Jan 2006).* ***98*** *(1 Feb 2006):* Weigh and measure

Day 106 *(9 Feb 2006),* ***112*** *(15 Feb 2006),* ***119*** *(22 Feb 2006),* ***126*** *(1 Mar 2006):* Weigh and measure

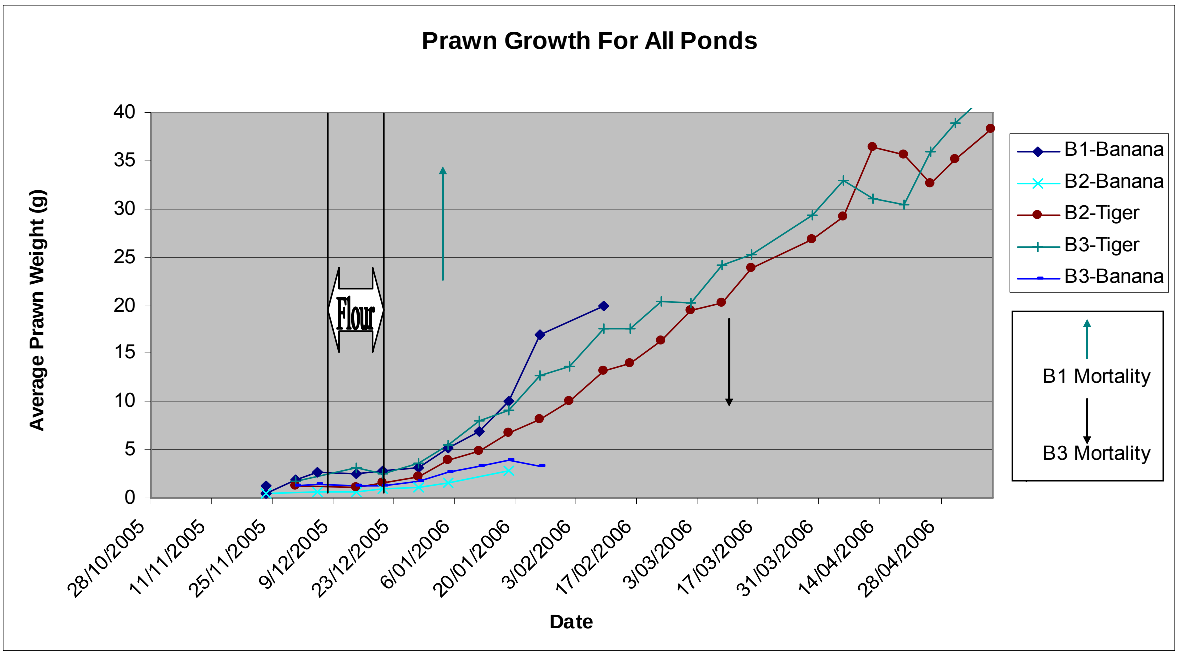
Day 133 *(8 Mar 2006):* Weigh and measure

Day 135 *(10 Mar 2006):* Aerator failure in B3, result — loss of 25.6kg prawns at 12g = 2133 prawns

Day 140 *(15 Mar 2006),* ***148*** *(23 Mar 2006),* ***154*** *29 Mar 2006),* ***161*** *(5 April 2006),* ***168*** *(12 Apr 2006),* ***175*** *(19 Apr 2006),* ***181*** *(25 Apr 2006),* ***187*** *(1 May 2006), and* ***195*** *(9 May 2006):* Weigh and measure

Water quality measurements taken over the whole period of production at the Bauple farm were collated for each organic and non-organic pond. These results are provided in Appendix 3, (Figures 23-30). Growth rates shown in Figure 7 below indicates all ponds grew well to day 40 when their growth appeared to plateau for a period of about 2 weeks. Individual charts for each pond can be seen in Figures 8 to 10.

Figure 7 Combined water quality, additions and growth data for all organic ponds



At this time it became evident that the prawns were stressed in all three organic ponds as they were constantly roaming the edge of ponds night and day. This combined with the appearance of antennae and tail damage led those involved to agree that the prawns were beginning to suffer from a lack of available food. At this time, the organic alternative was initiated in the form of flour which was added to the ponds daily for the following two week period. After such time very little increase in growth, or change in behaviour was witnessed so a decision was made to introduce a more substantial feed to circumvent mortality and increased cannibalism. In the interests of continued growth of prawns and the commercial partner a joint decision was made to introduce non-organic feed on day 57 and end the organic compliance.

Soon after this the decision was made to introduce a commercial non-organic prawn feed an increase in growth was witnessed in B1 banana prawns, B2 and B3 tiger prawns, but not with B2 and B3 banana prawns. This is difficult to explain though one possible reason is that the tiger prawns were far better equipped to handle pond conditions and compete more effectively for available feed. Any size disparity that ensued would be further exacerbated by increased cannibalism by the larger black tiger prawns on the smaller banana prawns. It is evident from the following graphs that both the organic and non-organic ponds grew at a similar rate for the first 40 days, after this time growth appeared to deteriorate in B2 and B3.

Figure 8 Water quality, pond additions and growth data for pond B1

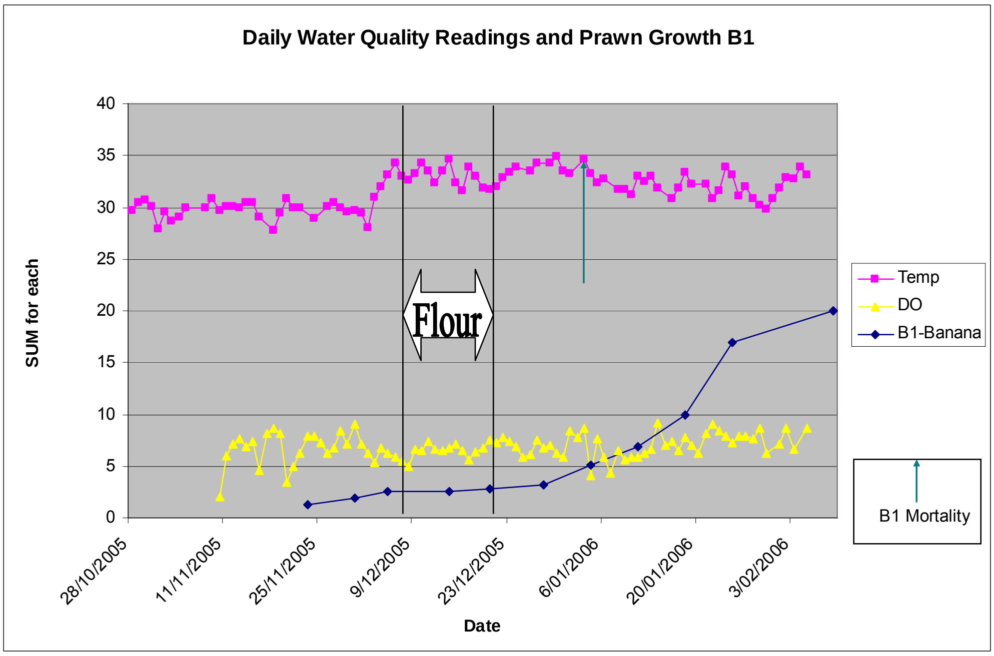


Figure 9 Water quality, pond additions and growth data for pond B2

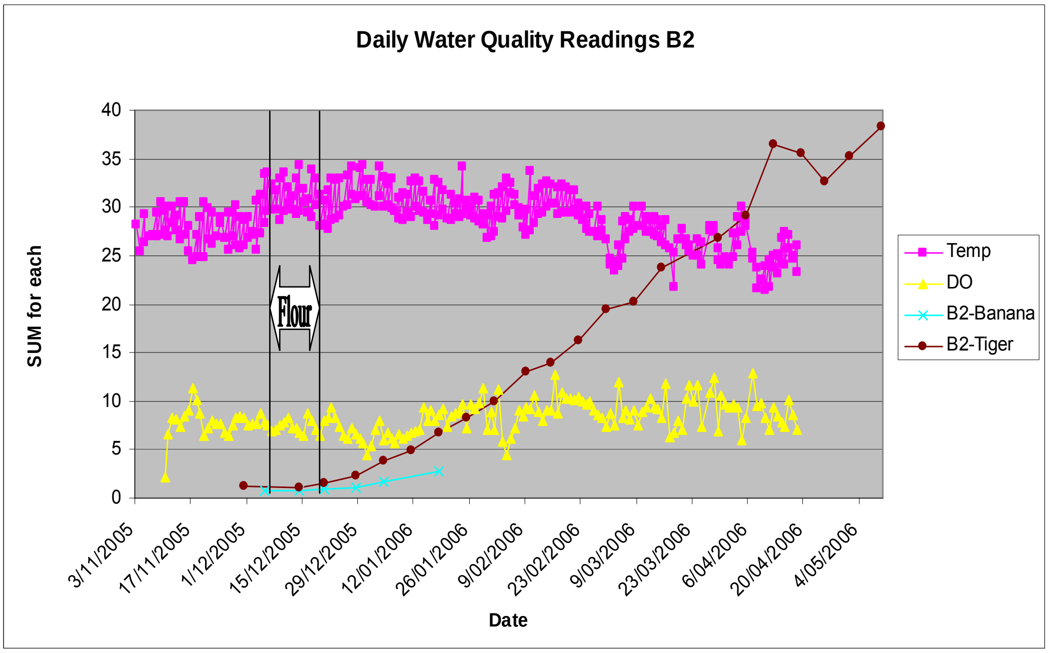
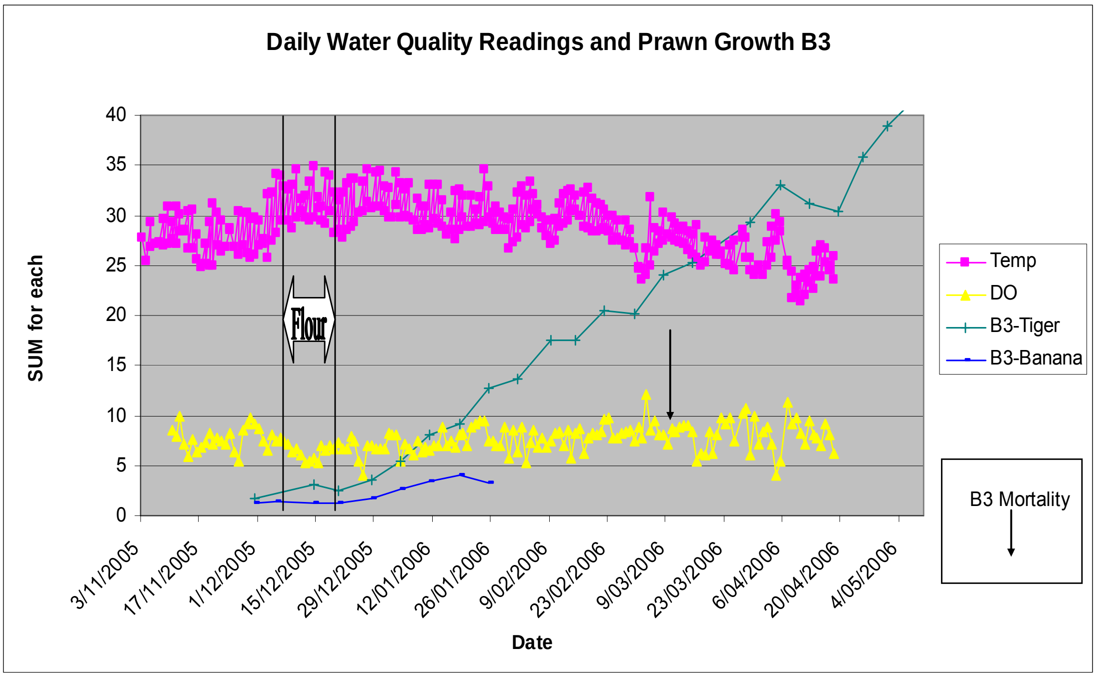


Figure 10 Water quality, pond additions and growth data for pond B3



Prawns were harvested irregularly from the ponds to meet customer needs. This makes things a bit complex to analyse for growth rates. As prawns were removed food availability increased due to reduced competition which would have led to better growth rates. The amount of feed input, total weight of prawns harvested and feed conversion ratios are present in Table 16 below.

Table 16 Feed inputs and conversion ratios

Pond identity	Feed input		Weight of prawns removed from ponds (kg)	Feed conversion ratios	
	organic flour (kg)	commercial prawn diet (kg)		commercial diet to prawn produced	all feed to prawn produced
B1	6.7	49.4	6.8	7.32:1	9.3:1
B2	6.7	282.3	108.7	2.60:1	2.72:1
B3	6.7	266.9	70.2	3.75:1	3.94:1
Non-organic pond average & (range)	1	458.8 (415.6-521.1)	77.2 (32.2-139.3)	7.46:1 (3.83-13.2)	7.49:1 (3.84-13.25)

The prawns in the organic ponds were fed on organic flour for longer than the non-organic ponds, leading to much lower amounts of commercial prawn feeds being applied to produce the crop in the later stage. Poor feed conversion is apparent for B1 due to the major mortality event that occurred early (4 January) in the trial and so should be disregarded as an indicator of efficiency. The other two organic ponds, similar to the non-organic ponds, were still producing prawns in May and June. While the overall harvest was lower in B3 because of mortality even in early March both of these ponds produced amounts of prawns equivalent to the non-organic ponds. The food conversion ratios for the prawns started in the organic ponds were much better than that achieved by the rest of the farm. The overall cost of production would have been much less for the organic ponds.

Discussion

The first two months of growth can be achieved using extensive husbandry practices and only limited organic feed inputs. After this time it is assumed that higher levels of protein will be required, dependent upon stocking density. This farm trial however did not demonstrate when this point was reached and thus was unable to confirm the assertion. Experience in managing ponds using organic fertilisation will extend the period before higher protein diets are needed. It was demonstrated that marketable sized prawns could be produced from prawns grown earlier on organic feeds then finished using commercial diet. Correspondingly, this trial found that non-organic farms could use cheaper starter feeds in their operation which will provide savings for all prawn farmers.

Implications and recommendations

The production of organically certified prawns in Australia is feasible. The availability of a cost effective certified prawn feed with appropriate growth rates in Australia will determine the adoption of this research by industry. Certainly it is difficult to formulate a palatable and digestible diet with high enough protein to be useful without using fish meal. Research around the world has sought to replace fish meal with terrestrial food sources with fairly low success rate. This apart from the need for the ingredients to also be certified organic presents us with a sizeable equation. Waste fish (head and frames) were trialled but it remains to be seen whether this will be good enough to produce a commercially viable crop, since it is far less convenient for feed companies to use and has excessive amounts of water.

The considerable work that has been conducted by experts in the fish meal replacement area, without commercial success, may be sufficient argument to lighten the burden of trying to achieve unreasonable standards. The other issue is the efficiency of feed conversion. If it is much more than 2:1 it is not very efficient and leads to pollution. Feeds that are not well assimilated will cause more problems than they are worth, and fish meal provides the most effective and efficient nutrition.

Another issue that may need to be looked at for aquaculture is the stocking density. Many schooling fish and prawns exist in nature at high density. It is the high density that makes them feel at home and leads to low stress levels. If the organic feeds that we are developing are good enough, they may be able to support high density culture. If the environmental systems that are put in place can appropriately address the nutrient discharge from intensive operations, then why should prawn aquaculture be restricted to low densities? Recently, an article published in the prominent Fish Farmer International journal (August 2006:3) draws attention to this issue.

The other aspect is that current prawn farmers are the ones most likely to adopt organic principals but because of the limits in available EPA approved pondage they want to maximise their stocking densities at levels which may be at odds with overseas practices. It appears that this issue along with feeds development for optimal feed conversion will be crucial for organic aquaculture to be viable and sustainable in this country.

The organic standards that will apply to Australian organic prawn production should reflect the dietary requirement for protein of the local species being cultured. This standard should also apply pond densities that are acceptable to the local species and which are also commercially viable.

Feedback from prawn farming industry participants was that they would not adopt organic practices until a proven certified feed is available. As the formulations in this report only achieved 66% growth when compared to a commercially produced non-organic feed, further development work is required to increase the feed conversion ratio of an organic feed.

Another issue associated with farm operations according to organic principles, which could not be tested in this farm experiment, is the treatment of wastewater discharged at harvest. As previously stated in Section 1.1 of this report (page 11) organic standards require the fate of discharge waters to be built into the operations of the farm. They require that recycling is carried out as much as possible, and that surface water leaving an organic farm should not contain greater levels of nutrients, salts and turbidity than when the surface water entered the farm. Whilst water was recycled routinely in the farm trial, it was not retained at harvest for another crop.

Since the Environmental Protection Agencies in Australia manage discharge permits so that there is no environmental harm caused by the operation, these considerations are already built into the existing regulations of Australian producers. This includes the final discharge of water to harvest the product and empty ponds for drying to prevent disease. The option of storing this final harvest water for use in refilling new ponds is not generally practiced in Australia, and although it may provide one solution to its slightly higher turbidity, this practice would require careful further research to avoid problems associated with build-up of waste products and potential pathogens.

References

D. Gruenberg 2006. Fish Farmer International:3

4 Develop modified atmosphere packaging (MAP) as an organic-acceptable procedure to extend shelf-life

By Steve L Slattery and Paul J Palmer

4.1 BIARC packaging trial

Pack headspace analysis and drip loss within prawn packs at opening

Table 17 shows the content of the headspace gases in the unopened pack and the amount of drip loss from the prawns present in the pack.

Table 17 Levels of carbon dioxide, oxygen and nitrogen and drip loss in prawn packs flushed with a mixed atmosphere of 60% carbon dioxide and 40% nitrogen

Storage time (days)	Condition	% CO^2	% O^2	% N^2	% of product lost as drip
1	raw	54.5	<0.1	45.5	0
1	raw	m	m	m	0
6	raw	45.1	<0.1	54.9	1.59
6	raw	46.8	<0.1	53.1	2.28
12	raw	46.4	<0.1	53.6	4.21
12	raw	43.9	<0.1	56	3.82
18	raw	46.7	<0.1	53.2	8.95
18	raw	50	<0.1	49.9	7.36
1	cooked	53.6	<0.1	46.3	0
1	cooked	m	m	m	0
6	cooked	36	<0.7	63.9	1.35
6	cooked	35.4	<0.8	64.5	1.19
12	cooked	35.4	0.1	64.5	1.34
12	cooked	39.4	0.2	60.4	0.93
18	cooked	35.6	0.1	64.3	1.52
18	cooked	37.4	<0.1	62.5	2.22

m = sample not analysed

The packs were stored at 4°C for up to 18 days with samples taken regularly over that period. No blackspot was recorded on any prawns over that period. All packs tested remained intact for the duration of storage. One aspect noticeable is that, after storage, the proportion of carbon dioxide is much lower in cooked prawn packs than that present in the headspace of uncooked prawn packs. Cooking alters cell wall integrity resulting in a higher capacity to absorb this gas than with raw prawns.

Demerit assessment of prawns within packs at opening

The scores are averaged from those recorded by two different staff. Table 18 shows the mean scores recorded for raw prawns.

The demerit scores increased as storage progressed for both raw and cooked prawns. The raw prawns received higher scores overall. Drip was not apparent in the cooked prawns in MAP while it only became noticeable at the end of storage for raw prawns in MAP. The odour at unpacking was also very acceptable up until the end of the trial with only minor off odours developing in both product types. The appearance of the cephalothorax (head) deteriorated considerably on the raw prawns during storage and could be an issue when purchasing this product. The leakage from the hepatopancreas is only an issue for raw prawns as this contains proteolytic enzymes that can lower quality such as staining and head loss. These enzymes are destroyed when the cooking method used is efficient.

The carbon dioxide component of the modified atmosphere can lead to etching of the shell, which was present by the end of storage of both products. Cooking also denatures the muscle protein and endogenous enzymes so that changes in flesh appearance only occur in the raw prawn tail. The colour of the tail meat changes from transparent to opaque also due to these enzymes. As the visual and odour aspects of modified atmosphere packaged prawns were acceptable for nearly all of storage the microbiological quality should be used to determine what could be anticipated as the shelf life for these products.

Table 18 Visual and odour demerit scores of raw prawns stored in MAP at 4°C

Storage time (days)	**State**	**Total****	***Drip*****	**Odour****	**Head Appearance****	**Hepatopancreas Leak****	**Staining of first segment**	**Shell Texture****	**Flesh Appearance****	**Flesh Colour****
1	raw	0.13[c]	0[b]	0[c]	0[c]	0.13[b]	0	0[b]	0[b]	0[d]
6	raw	2.06[c]	0[b]	0.73[bc]	0[c]	0.75[b]	0	0[b]	0[b]	0.59[c]
12	raw	7.6[b]	0.16[b]	1.63[ab]	1.23[b]	2[a]	0.55	0.5[b]	0.06[b]	1.48[b]
18	raw	14.25[a]	0.73[a]	1.95[a]	2.6[a]	2.38[a]	0.5	1.95[a]	1.88[a]	2.28[a]
Storage time (days)	**State**	**Total****	***Drip***	**Odour***	**Head Appearance****	**Hepatopancreas Leak**	**Staining of first segment**	**Shell Texture****	**Flesh Appearance**	**Flesh Colour****
1	cooked	1.5[c]	0	0[b]	0[b]	1.5	-	0[b]	0	0[b]
6	cooked	4.35[b]	0	1[a]	0.35[a]	3	-	0[b]	0	0[b]
12	cooked	4.64[b]	0	0.69[ab]	0[b]	3	-	0.7[b]	0	0.25[b]
18	cooked	8.55[a]	0.18	1.13[a]	0[b]	3	-	2.8[a]	0.45	1[a]

Means followed by a different letter are significantly different to those in the same column. * Indicates the differences are significant at the 5% level (P<0.05). ** Indicates the differences are significant at the 1% level (P<0.01).

Table 19 shows two bacterial counts for raw and cooked prawns during storage.

Table 19 Microbiological population counts from prawns stored in MAP at 4°C

Storage time (days)	State	Total (log cfu/g)	Psychrotroph** (log cfu/g)
1	raw	2.53	1.15[b]
6	raw	2.48	1.15[b]
12	raw	2.32	2.49[a]
18	raw	2.48	2.17[ab]
Storage time (days)	**State**	**Total** (log cfu/g)**	**Psychrotroph** (log cfu/g)**
1	cooked	2.18[b]	1.75[b]
6	cooked	2.42[b]	2.68[ab]
12	cooked	3.67[ab]	3.93[ab]
18	cooked	4.95[a]	5.03[a]

Means followed by a different letter are significantly different to those in the same column.
* Indicates the differences are significant at the 5% level.
** Indicates the differences are significant at the 1% level.

As these prawns came from lined ponds with restrictions to any outside contamination only the basic microbiological tests were undertaken. The raw prawn bacterial counts did not differ much during storage. Those of the cooked prawns however were higher than the raw prawns by the end of the trial. On the last day only one pack of cooked prawns had counts which would be considered not acceptable for consumers. Cooking can activate spores produced by some bacteria so this and the wash water used to cool after cooking are two sources of contamination of what would normally be considered a pasteurised product. Tasting identified that the cooked prawns retained good flavour until the last day.

This trial bodes well for the packaging experiment planned at harvest at the industry partner's farm but it cannot be taken as the most likely outcome. Because the success of this type of packaging is highly dependent on the mix of bacteria present on the product at the time of packaging, the producer can only rely on predictive shelf life based on trials using their own product.

4.2 Bauple packaging trial

As there was no designated processing area, packaging had to be carried out exposed to the elements. Good handling practices were however applied during packaging. Due to mortalities there were limited numbers of banana prawns available, so only one batch was processed uncooked for modified atmosphere packaging. The data from this pack was compared at each sampling time with two packs containing raw black tiger prawns. Whenever possible the differences between the two species were analysed statistically. The packs containing cooked black tiger prawns were prepared in triplicate

The packs were placed into insulated coolers with an addition of ice on top and returned to the Brisbane laboratory for evaluation. Temperatures remained low during transport. Because of the 5 hour drive the first opportunity to evaluate the product was the following day.

Raw prawns

Pack headspace analysis and drip loss within raw prawn packs at opening

Table 20 shows the content of the headspace gases in the unopened pack and the amount of drip loss from the prawns present in the pack.

Table 20 Levels of carbon dioxide, oxygen and nitrogen and drip loss in uncooked prawn packs flushed with a mixed atmosphere of 60% carbon dioxide and 40% nitrogen

Storage time (days)	Prawn species	% CO^2	% O^2	% N^2	% of product lost as drip
1	tiger	42.2	0.1	57.7	3.16
1	tiger	42.1	0.1	57.8	5.03
1	banana	19.1	12.5	68.4	3.66
7	tiger	41.7	0.1	58.2	4.44
7	tiger	49.2	0.1	50.7	2.83
7	banana	49.2	0.1	50.7	3.70
11	tiger	44	0.1	56	5.15
11	tiger	46.4	0.1	53.6	4.79
11	banana	50.2	0.1	49.7	3.11
14	tiger	45.7	0.1	54.2	6.86
14	tiger	45.5	0.1	54.4	7.09
14	banana	48.4	0.1	51.5	5.75
18	tiger	44.1	0.1	55.8	10.03
18	tiger	44.2	<0.1	55.8	11.06
18	banana	49.8	0.1	50.2	m

m = sample not analysed

Only one pack containing banana prawns tested on day one had a gas content indicating the seal had leaked prior to opening. Such a short storage time would have led to little differences from the other packs of prawns sampled on the same day so this data was left in all of the analyses.

The raw prawns lost significantly more ($P<0.05$) moisture during storage in MAP than the cooked prawns. This occurred because cooking had already removed a large amount of moisture so there was less to be lost as drip from the flesh.

There was no significant difference between the two species for drip loss from raw MAP prawns. The amount of drip loss increased significantly ($P<0.01$) as storage time in MAP increased. After 18 days the amount of drip loss was quite large and will be an important factor to consider when commercially packing prawns in MAP. This is the highest amount of drip loss seen for any species of seafood packed in MAP by the researcher so far.

Demerit assessment of raw prawns within packs at opening

As the two species of prawns packed in modified atmosphere can appear quite different, their demerit scores were compared. The scores are averaged from those recorded by three different individuals. Table 21 shows the mean scores recorded.

Table 21 Visual and odour demerit scores of raw prawns stored in MAP at 4°C

Storage time (days)	**Total****	***Drip****	**Odour****	**Head Appearance***	**Hepatopancreas Leak****	**Staining of first segment****	**Shell Texture****	**Flesh Appearance****	**Flesh Colour****
1	0.28[c]	0[c]	0.13[c]	0.16[b]	0[d]	0[c]	0[b]	0[b]	0[c]
7	1.57[c]	0.08[bc]	0.66[b]	0.34[b]	0.21[cd]	0.08[bc]	0.02[b]	0.02[b]	0.16[c]
11	5.03[b]	0.23[ab]	1.01[b]	0.9[a]	0.59[bc]	0.41[b]	0.14[b]	0.42[b]	1.32[b]
14	5.99[b]	0[c]	1.6[a]	1.02[a]	0.91[b]	0.09[bc]	0.22[ab]	0.54[ab]	1.6[ab]
18	10.28[a]	0.33[a]	1.87[a]	1.29[a]	2.17[a]	0.92[a]	0.56[a]	1.1[a]	2.04[a]

Means followed by a different letter are significantly different to those in the same column. * Indicates the differences are significant at the 5% level. ** Indicates the differences are significant at the 1% level.

There was no difference between the scores of the two species of uncooked prawn for the various attributes rated so these have been combined for the analysis of storage effects. The accumulated demerit points increased significantly as storage time progressed with a major increase after day 14. Better quality had been present up till then. The total scores were better this time than those recorded during the previous trial. This does not mean that this product was particularly better than the previously produced packs as the prawns used for this trial were much smaller and black tiger prawns are of a darker appearance.

The scores for the amount of drip visible were all quite low indicating the absorbent pads were effective in removing this defect. The prawns within the packs had lost their fresh odour by day 11 and this was quickly followed by the development of off-odours. These became strong enough to cause a halt to the trial after 18 days. Active enzymes have caused the head of the prawns to become loose by day 11 but did not progress much further by day 18. These enzymes reside in the hepatopancreas and they had leaked away at significantly high levels by day 18. This is a common visual indicator used by consumers to select fresher product. The staining, also caused by this leakage, had increased markedly at the end of the trial. The shell texture, flesh appearance and flesh colour deteriorated progressively during storage, although these attributes played a lesser role in loss of quality.

While the score for odour gives some insight into the loss of fresh odour, the type of odours that develop can vary considerably due to the mixture of bacteria present. For this reason comments were recorded for the odours detected by each individual rating the packs. These are presented in Table 22 below.

Table 22 Odour comments for raw prawns stored in MAP

Storage time (days)	Pack Number	Species	Raw odour at unpacking
	8	Black tiger	meaty/musty, fresh muddy/earthy
1	6	Black tiger	meaty, meaty/musty, muddy/earthy
	1	Banana	sl meaty, no fresh odour
	4	Black tiger	Cooked crab, sweet
7	4	Black tiger	Fruity, loss of odour
		Banana	Meaty/broth, v. sl. H_2S
	3	Black tiger	Less fresh, sl. sweet
11	16	Black tiger	Fruity, musty, lacking, earthy
	6	Banana	Fruity, musty, old, earthy, sl. H_2S
	18	Black tiger	Sweaty, fruity, old socks
14	23	Black tiger	Sweaty, fruity, sl. old socks, sl. earthy
	8	Banana	Sweet, sweaty, sl muddy, old socks, dirty
	10	Black tiger	Sweaty, old, sl. H_2S, dirty
18	11	Black tiger	Fruity, old, dirty
	4	Banana	Fruity, old

There was a muddy, earthy odour present from the start of storage. This is due to the prawns being raised in unlined ponds. Bacteria present in the soil produce two characteristic odours known as geosmin and isoborneol. The former has a muddy odour and flavour while the latter is typically musty in nature. These two compounds are common to freshwater fish and can have a sever impact on perceived quality by consumers. Sensitivity to these compounds vary within the population with some individuals not able to detect them while others perceive them to be extreme defects. The presence of these compounds at high concentration can result in financial losses if the crop is harvested. Many farms test for their presence before any harvesting can occur.

The odour comments show that one or both of these compounds are present in the water of the ponds the prawns were grown in. The farmer is obviously concerned with issue as the majority of the other ponds at this farm are lined.

As storage progresses several off odours, described as fruity and sweaty, become detectable. By day 18 the prawns odours, while not excessive, were disagreeable enough to halt any further storage testing.

Microbiological assessment of raw prawns within packs at opening

The various microbiological counts of the MAP prawns are presented in Table 23 below.

Banana prawns had significantly lower total counts ($P<0.05$), psychrotrophs ($P<0.01$) and dihydrogen sulphide producers ($P<0.01$) than the black tiger prawns. As there was only one pack of banana prawns available for testing per storage day and the difference is due to the prawns coming from different ponds on the same farm, the results from the two species have been combined for analysis of storage time effects. The different microbiological counts were low throughout storage.

The major concern is the presence of large numbers of anaerobic bacteria from the start. The heat treated and ethanol treated anaerobic counts are used to identify potential pathogens that may make the product unsafe for consumers. This is the first trial this researcher has ever identified with moderate counts present at the start of storage.

The bacteria that grow under these conditions are mainly of the genus *Clostridium* and the most important pathogen species is *botulinum*. There were moderate numbers of this group present throughout storage. Studies of spiked MAP products have found that food starting with a two log count of this pathogen can develop enough toxin within 14 days to be a serious threat to consumers. It is not known if a one log count can be dangerous but this result is of serious concern for the prawn farmer.

It became known after the completion of this storage trial that chicken manure was used at the start of the growing season for all the ponds at the farm. This fertilizer is known to harbour another human pathogen, *Salmonella*, for which there are strict limits in the Food Standards for prawns. Also a peacock and dog were noted to have access to the ponds while uphill and up wind of the ponds provided for the organic trial there was a pigsty and fowl run.

The majority of bacteria present were dihydrogen sulphide producers. As this gas is also called rotten egg gas these would be likely to play a major role in the off odour of the prawn packs at opening. As discussed earlier this trial was finished after 18 days due to adverse sensory attribute.

Table 23 Microbiological population counts from raw prawns stored in MAP at 4°C

Storage time (days)	Total* (log cfu/g)	Psychrotroph (log cfu/g)	H_2S producer (iron method) (log cfu/g)	H_2S producer (sulphite method) (log cfu/g)	Total anaerobe** (log cfu/g)	Heat treated anaerobe (log cfu/g)	EtOH treated anaerobe (log cfu/g)
1	4.55[a]	2.49	4.51	2.90	2.57[a]	0.98	1
7	3.61[abc]	2.80	3.21	2.06	1.88[ab]	0.75	0.85
11	3.42[bc]	1.99	3.26	2.71	1.05[b]	0.85	0.95
14	4.05[ab]	3.11	3.77	2.93	1.91[ab]	0.85	0.95
18	3.02[c]	1.99	3.08	2.41	1.45[b]	0.85	0.90

Means followed by a different letter are significantly different to those in the same column. * Indicates the differences are significant at the 5% level. ** Indicates the differences are significant at the 1% level.

Cooked prawns

Pack headspace analysis and drip loss within cooked prawn packs at opening

Table 24 contains the head space gas composition and drip loss data for the cooked prawns.

Table 24 Levels of carbon dioxide, oxygen and nitrogen and drip loss in cooked prawn packs flushed with a mixed atmosphere of 60% carbon dioxide and 40% nitrogen

Storage time (days)	Prawn species	% CO^2	% O^2	% N^2	% of product lost as drip
1	tiger	36.8	0.2	63	3.19
1	tiger	34.8	0.1	65	1.53
1	tiger	36.7	0.1	63.2	2.11
7	tiger	29.3	0.2	70.5	3.78
7	tiger	29.6	0.2	70.3	m
7	tiger	30.7	0.2	69.2	2.70
11	tiger	28.4	0.2	71.4	2.93
11	tiger	26.9	0.2	72.9	3.04
11	tiger	28.3	0.2	71.5	4.04
14	tiger	28.1	0.2	71.7	2.97
14	tiger	27.1	0.5	72.4	4.85
14	tiger	25.6	0.2	74.2	m
18	tiger	27.9	0.2	71.9	3.26
18	tiger	26.3	0.3	73.5	3.90
18	tiger	24.7	0.2	75.1	4.93
22	tiger	26	0.2	73.8	3.31
22	tiger	25.5	0.3	74.2	5.17
22	tiger	25.7	1	73.3	5.54

m = sample not analysed

With the limit to the number of banana prawns available this species was not cooked prior to packaging. None of the packs containing cooked tiger prawns exhibited leakage. Again it appeared that the cooked prawns absorbed more carbon dioxide than raw prawns. While the average pack drip loss increased numerically with storage time there were no significant differences ($P>0.05$) between the various storage times.

Demerit assessment of cooked prawns within packs at opening

Table 25 shows the averaged scores for cooked prawns stored in MAP.

The accumulated demerit points increased significantly as storage time progressed but the final scores were quite low for the cooked prawns. As cooking removes many of the enzymes present in the head, the attribute of staining was not scored. The total scores were much lower than those recorded for the raw prawns so the storage trial for this product was continued for 22 days. The scores for the amount of drip visible in the cooked prawn packs were all quite low, especially as the weighed drip loss was lower for the cooked prawns.

The cooked prawns within the packs had lost their fresh odour by day 14 and while this was quickly followed by the development of off-odours they did not appear as strong on day 22. The proteolytic enzymes have been removed by cooking so that the head of the prawns only showed slight loosening by day 11 which did not progress much further by day 18. The contents of the hepatopancreas had leaked away but not in any consistent manner. At day 18 and 22 the scores were much lower than those scored for raw prawns. The shell texture, flesh appearance and flesh colour deteriorated progressively during storage but at much lower levels than the raw prawns.

Table 25 Visual and odour demerit scores of cooked prawns stored in MAP at 4°C

Storage time (days)	Total**	*Drip*	Odour**	Head Appearance*	Hepatopancreas Leak**	Shell Texture*	Flesh Appearance**	Flesh Colour**
1	0.75^{e}	0.13	0.07^{d}	0^{b}	0.55^{cd}	0^{c}	0^{b}	0^{b}
7	1.47^{de}	0.04	0.68^{c}	0^{b}	0.4^{d}	0.26^{abc}	0^{b}	0.09^{b}
11	2.66^{c}	0.26	0.8^{bc}	0.02^{b}	1.04^{ab}	0.11^{bc}	0.02^{b}	0.4^{b}
14	2.07^{cd}	0.02	0.91^{bc}	0^{b}	0.6^{bcd}	0.16^{bc}	0.13^{ab}	0.24^{b}
18	5.16^{a}	0.02	1.84^{a}	0^{b}	1.33^{a}	0.49^{a}	0.39^{a}	1.08^{a}
22	3.94^{b}	0.31	1.12^{b}	0.11^{a}	1^{abc}	0.33^{ab}	0.17^{ab}	0.9^{a}

Means followed by a different letter are significantly different to those in the same column. * Indicates the differences are significant at the 5% level. ** Indicates the differences are significant at the 1% level.

As the microbiological quality of the cooked prawns was good at the start of the storage trial it was decided that tasting of the prawns could be included in this assessment. The odour and flavour comments recorded by the evaluation team are presented in Table 26 below.

Table 26 Odour comments for cooked prawns stored in MAP

Storage time (days)	Pack Number	Species	Cooked odour at unpacking	Cooked flavour at unpacking
	1	Black tiger	Meaty, sl burnt smell	Sl. peppery, v. sweet
1	15	Black tiger	Sl meaty, sl burnt smell	V. sl. muddy, v. sweet
	5	Black tiger	Meaty, fresh	Sharp
	6	Black tiger	Meaty, sl sweet, dirty	Sweet, v. sl. muddy, sharpness
7	8	Black tiger	Sweet, v. sl. Muddy, lacks odour	Sweet, v. sl. muddy, some sweetness
	24	Black tiger	Sl. sweaty, sl. burnt smell	good
	4	Black tiger	Sl fruity, sweet, lacks odour	Sweet, sl. fresh, sl. flavour loss
11	13	Black tiger	Sweet, good, meaty, lacks odour	Sweet, good, meaty
	21	Black tiger	Meaty, sl. sweet, lacks odour	Meaty, sl. sweet
	9	Black tiger	V sl. sweaty, lacks odour, none	Sweat, meaty
14	20	Black tiger	Sweet, v. sl. Musty, v. sl. metal, fruity	Sweat, v. sl. musty, v. sl. earth, lacks sweetness
	12	Black tiger	Sl. sweaty, fruity	Loss of sweetness, more musty, v. sl. metals
	10	Black tiger	Sweaty, old, dirty	Lacks prawn flavour, slight aftertaste, not fresh, dirty socks
18	14	Black tiger	Fruity, old, dirty	Lacks prawn flavour, sl. fruit
	3	Black tiger	Fruity, old	Sl. sweaty, musty, lacks prawn flavour, strong apple
	7	Black tiger	V. sl. sweaty, fruity	Lost flavour, muddiness
22	11	Black tiger	Sweaty, lacks fresh odour, fruity	Lost taste, quity muddy, dirty
	19	Black tiger	Dirty, fruity, no smell	Lost flavour, geosmin taint, muddy

The process of cooking and washing in ice slurry afterwards has been able to remove some of the muddy and/or musty characteristics present in the raw prawns. A burnt smell was present only in the early stages of storage and probably did not appear to be rated as a defect. The stale and sweaty odours started earlier than the raw prawns but these were not as strong when the odour demerit scores are compared. As the prawns started to lose odour and flavour the muddy/musty taints became more obvious. The low initial bacterial counts and resulting odours allowed the cooked prawns to be evaluated after 22 days of storage.

Microbiological assessment of cooked prawns within packs at opening

The various microbiological counts of the MAP prawns are presented in Table 27 below. While the total bacterial and psychrotroph counts did not grow to levels that would result in product failure based on general principals, the presence of moderate number of species which grow happily in anaerobic conditions are a major concern. While the anaerobic count was low they consisted of types that could be dangerous to consumers, as indicated by the treated anaerobe counts. These bacteria may have been *Clostridium botulinum* (a spore forming bacterium which requires heat treatment for the spores to germinate). If the anaerobic bacteria did not derive from the cooling wash bore water after cooking then they were already present, as suggested by the raw prawn counts, in the pond environment. If the implications discussed within this report had been clear from the start of the MAP storage trial the researchers would certainly not have done any taste testing. As the farm was only selling fresh prawns this issue was not important for their existing style of production.

Table 27 Microbiological population counts from cooked prawns stored in MAP

Storage time (days)	Total* (log cfu/g)	Psychrotroph (log cfu/g)	H_2S producer (iron method) (log cfu/g)	H_2S producer** (sulphite method) (cfu/g)	Total anaerobe (log cfu/g)	Heat treated anaerobe (log cfu/g)	EtOH treated anaerobe (log cfu/g)
1	2.07[b]	0.80[c]	1.69[b]	0.70[c]	0.70	0.75	0.80
7	2.21[b]	0.70[c]	2.06[b]	0.70[c]	0.85	0.80	0.70
11	2.49[b]	1.51[c]	2.23[b]	0.70[c]	0.70	0.75	0.80
14	2.90[b]	2.87[b]	1.86[b]	0.80[bc]	0.75	0.75	0.75
18	5.89[a]	5.89[a]	4.97[a]	1.77[ab]	1.07	0.80	0.75
22	5.83[a]	5.78[a]	4.71[a]	2.13[a]	1.46	0.80	0.70

Means followed by a different letter are significantly different to those in the same column. * Indicates the differences are significant at the 5% level. ** Indicates the differences are significant at the 1% level.

4.3 Supplemental packaging trial

To provide further evaluations of this method of packaging, a third storage trial was conducted. Since the farm at Bauple did not produce prawns in the 2006-07 season, more prawns were purchased from a coastal prawn farm operating in Queensland. These prawns were grown under normal operating conditions and so could not be considered as organic. Both raw and cooked black tiger prawns were packed at the farm and then returned to the laboratory for storage at 4°C.

Headspace analysis and drip loss within prawn packs at opening

Table 28 shows the content of the headspace gases in the unopened pack and the amount of drip loss from the prawns present in the pack. The packs were stored at 4°C for up to 16 days with samples taken regularly over that period. All packs tested remained intact for the duration of storage. It appears that oxygen was higher for this trial but this was due to changes in sensitivity of the analysis equipment. No blackspot was recorded on any prawns over that period. Again the proportion of carbon dioxide was much lower in cooked prawn packs than that present in the headspace of uncooked prawn packs due to increased permeability of the cooked tissue.

Table 28 Levels of carbon dioxide, oxygen and nitrogen and drip loss in prawn packs flushed with a mixed atmosphere of 60% carbon dioxide and 40% nitrogen

Storage time (days)	Condition	% CO^2	% O^2	% N^2	% of product lost as drip
1	raw	44	0.1	55.9	0.94
1	raw	42.7	0.1	57.2	3.02
1	raw	41.8	0.1	58.1	0.06
5	raw	46	0.1	53.9	1.11
5	raw	43.6	0.1	56.3	2.87
5	raw	44.5	0.1	55.4	3.85
9	raw	47.7	0.1	52.2	3.23
9	raw	45.1	0.1	54.8	1.12
9	raw	47.6	0.1	52.3	4.34
16	raw	48.4	0.1	51.5	5.84
16	raw	50.8	0.1	49.1	4.81
16	raw	47.6	0.1	52.3	4.49
1	cooked	40.4	0.1	59.5	0.44
1	cooked	38.8	0.1	61.1	0.89
1	cooked	41.3	0.1	58.6	0.12
5	cooked	36.4	0.2	63.4	1.01
5	cooked	37.1	0.2	62.7	0.19
5	cooked	37.7	0.2	62.1	2.45
9	cooked	37.5	0	62.5	1.19
9	cooked	35.8	0.2	64	0.96
9	cooked	35	0.1	64.9	1.20
16	cooked	37.5	0.1	62.4	1.72
16	cooked	37.9	0.1	62.0	0.85
16	cooked	36.0	0.1	63.9	1.66

m = sample not analysed

Raw prawns

Demerit assessment of prawns within packs at opening

The scores are averaged from those recorded by two different staff. Table 29 shows the mean scores recorded for raw prawns. The demerit scores increased as storage progressed for raw prawns. Drip was not apparent in the raw prawns in MAP. The odour at unpacking was also very acceptable up to day 9 but excessive off odours developed after this time. The appearance of the cephalothorax (head) deteriorated considerably in the raw prawns during storage and led to staining of the first abdominal segment.

Table 29 Visual and odour demerit scores of raw prawns stored in MAP at 4°C

Storage time (days)	Total**	*Drip*	Odour	Head Appearance**	Hepatopancreas Leak**	Staining of first segment**	Shell Texture	Flesh Appearance**	Flesh Colour**
1	0.17[c]	0	0[c]	0.17[b]	0[c]	0[c]	0	0[b]	0[c]
5	3.75[b]	0	0.75[b]	0.72[b]	0.86[b]	0.94[b]	0.11	0.06[b]	0.5[b]
9	2.75[b]	0	0.19[c]	0.67[b]	0.5[bc]	0.3[c]	0.17	0.22[b]	0.7[b]
16	10.36[a]	0	2.7[a]	1.78[a]	1.5[a]	1.5[a]	0.28	0.78[a]	1.82[a]

Means followed by a different letter are significantly different to those in the same column. * Indicates the differences are significant at the 5% level. ** Indicates the differences are significant at the 1% level.

Table 30 Microbiological population counts from raw prawns stored in MAP

Storage time (days)	Total** (log cfu/g)	Psychrotroph** (log cfu/g)	H_2S producer (iron method) (log cfu/g)	Total anaerobe** (log cfu/g)	Heat treated anaerobe* (log cfu/g)	EtOH treated anaerobe (log cfu/g)
1	4.35[b]	3.71[c]	1.47	2.91[c]	0.32[a]	0
5	3.97[c]	3.62[c]	1.17	2.79[c]	0[b]	0
9	4.49[b]	4.52[b]	1.21	4.25[b]	0[b]	0
16	6.05[a]	6.32[a]	0.86	6.01[a]	0[b]	0

Means followed by a different letter are significantly different to those in the same column. * Indicates the differences are significant at the 5% level. ** Indicates the differences are significant at the 1% level.

Table 31 Visual and odour demerit scores of cooked prawns stored in MAP at 4°C

Storage time (days)	Total**	*Drip*	Odour**	Head Appearance	Hepatopancreas Leak**	Shell Texture**	Flesh Appearance	Flesh Colour**
1	0^{c}	0	0^{b}	0	0^{b}	0^{b}	0	0^{b}
5	0.73^{bc}	0	0.23^{b}	0	0.33^{ab}	0.06^{b}	0	0.11^{b}
9	1.26^{b}	0.11	0.34^{b}	0	0.02^{b}	0.06^{b}	0	0.72^{a}
16	3.44^{a}	0	1.83^{a}	0.33	0.67^{a}	0.54^{a}	0.17	0.44^{ab}

Means followed by a different letter are significantly different to those in the same column. * Indicates the differences are significant at the 5% level. ** Indicates the differences are significant at the 1% level.

Table 32 Microbiological population counts from cooked prawns stored in MAP

Storage time (days)	Total** (log cfu/g)	Psychrotroph** (log cfu/g)	H_2S producer (iron method) (log cfu/g)	Total anaerobe** (log cfu/g)	Heat treated anaerobe (log cfu/g)	EtOH treated anaerobe (log cfu/g)*
1	4.15^{bc}	4.06^{c}	0.51	3.11^{c}	0.17	0.4^{a}
5	3.88^{c}	3.79^{c}	0	2.68^{d}	0	0^{b}
9	4.30^{b}	4.80^{b}	0	4.29^{b}	0	0^{b}
16	4.86^{a}	7.16^{a}	0.25	6.64^{a}	0	0^{b}

Means followed by a different letter are significantly different to those in the same column. * Indicates the differences are significant at the 5% level. ** Indicates the differences are significant at the 1% level.

This could be an issue when purchasing this product. Carbon dioxide etching of the shell was evident by the end of storage. Changes in flesh appearance occurred in the raw prawn tail meat significantly by day 16. As the odour of these modified atmosphere packaged prawns was unacceptable on day 16 this parameter and the microbiological quality was used to determine end of the raw prawn shelf life.

Microbiological assessment of raw prawns within packs at opening

Table 30 shows the bacterial counts for raw prawns during storage. The raw prawn total aerobic and anaerobic counts increased significantly after five days storage. By day 16 the counts were just over the acceptability limit of 10^6. The lack of any *clostridial* species indicated by the absence and in one case low numbers of selectively treated anaerobes shows the safety of this product is assured. It is likely that this product would have been acceptable after 14 days of storage.

Cooked prawns

Demerit assessment of prawns within packs at opening

Table 31 below shows the scores recorded on the demerit sheets for cooked prawns after storage in MAP at 4°C. There was a progressive loss of quality during storage of the cooked prawns but not to the same extent as the raw prawns. Cooking does destroy degrading enzymes. For most of the parameters the scores were significantly worse on day 16. Only the odour had deteriorated enough to cause concerns for consumers.

Microbiological assessment of cooked prawns within packs at opening

Table 32 shows the bacterial counts for cooked prawns after storage in MAP at 4°C. The starting total count would be considered too high for normal MAP products even though the prawns were washed well before packing. This led to the limited shelf life attained, but mostly due to the psychrotrophs and anaerobes that proliferated after 16 days storage. While there was a high anaerobe count at the end of the trial and unlike the previous trial, the safety of the food was assured as indicated by the absence of counts for the two anaerobe treatments. Surprisingly while the odour was the key parameter that designated unacceptability this was not caused by any large numbers of dihydrogen sulphite producers but most likely due to other types of anaerobe.

4.4 Effect of fig extract on black spot inhibition

Another experiment pertinent to this project but not identified in the objectives section was the treatment of prawns with a fig extract. While blackspot is prevented from developing in the oxygen free atmosphere of MAP, once the packs are opened this pigment will rapidly appear. A treatment that is organic and of food grade quality will enhance the appearance of organic MAP prawns may be needed to improve marketing of this product.

The extraction of latex from ornamental weeping figs was conducted using method of filtration which is compatible with organic principals. 290g of weeping fig stems were washed with 400mL of RO water. This mixture was shaken for 1.5 hours and filtered. This solution was then concentrated using a 10,000 dalton membrane. HPLC analysis of the extract could not identify any 4-hexylresorcinol, the active compound known to prevent blackspot. When prawns obtained from BIARC were treated with this extract there was no discernable inhibition of blackspot on the prawns. There were no further experiments of this type within this project.

4.5 Conclusion

These trials show the variability of microbiological flora present in prawn farms and indicate that anyone intending to produce MAP prawns should gain an understanding of their production environment first to be able to ensure that the best possible product can be produced. It is feasible that export packs could be produced at the farms but some major improvements to the processing conditions present will be needed to meet importing country requirements before any certification could be achieved.

4.6 References

Australian Standard AS-1766.1.3, 1991a. General procedures and techniques (Colony count) Pour plate method. Standards Association of Australia.

Australian Standard AS-1766.1.4, 1991b. General procedures and techniques (Colony count) Surface spread method. Standards Association of Australia.

Bremner, H.A., Olley, J. and Vail, A.M.A. 1986. Estimating time-temperature effects by a rapid systematic sensory method. In Seafood Quality Determination, eds D.E. Kramer and J.

Campden and Chorleywood Food Research Association 1995. Manual of microbiological methods for the food and drinks industry. Technical Manual No. 43 (aerobic and anaerobic spores)

FSANZ 2007. Standard 1.6.1 — Microbiological Limits for Food , Commonwealth of Australia, Canberra.

Gram, L., Troll, G. and Huss, H. H., 1987. Detection of specific spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures. International Journal of Food Science 4, pp: 2197-2203.

Appendix 1 Second feed trial at BIARC

Table 33 Treatment allocation for tanks, primary prawn starting bulk weights, and adjusted prawn starting bulk weights after replacement of morbid individuals

Treatment-replicate	Tank	Primary prawn starting bulk weight	Adjusted prawn starting bulk weights
1-1	8	68.33	68.33
1-2	14	68.57	68.57
2-1	4	69.92	69.92
2-2	11	74.43	74.43*
2-3	26	62.91	62.91
3-1	3	56.14	56.14
3-2	19	63.90	63.90
3-3	23	56.25	56.25
4-1	1	61.99	60.59
4-2	9	75.88	75.52
4-3	22	72.70	72.70
5-1	7	53.94	53.94*
5-2	16	61.46	61.46
5-3	20	70.83	70.83
6-1	2	75.73	75.73
6-2	13	76.26	76.26
6-3	25	61.80	61.80
7-1	5	66.09	66.09
7-2	21	60.14	60.14
7-3	24	61.64	61.64
8-1	12	63.62	63.62
8-2	15	66.01	66.01
8-3	18	63.38	63.38
9-1	6	67.43	67.43
9-2	10	67.22	67.22
9-3	17	60.31	60.31

*21 rather than 20 prawns stocked
Mean biomass per tank after adjustment = 65.58 g
Mean prawn weight after adjustment = 3.27 g

The following are pictures of the different diets investigated in the feed trial after 3 hours submersion in seawater.

Figure 11 Commercial diet 2

Figure 12 Wheat Fish diet 3

Figure 13 Wheat Scallop diet 4

Figure 14 Wheat Soy diet 5

Figure 15 Wheat Chickpea diet 6

Figure 16 Wheat Macadamia diet 7

Figure 17 Weaner Fish diet 8

Figure 18 Weaner Soy diet 9

Table 34 Max/Min water temperatures (from tank 10) and feeding times from 2nd feed trial

Day	Date	Min – Max Temp (°C)	Times feed added	
Sat	18-11-06	24.5 when stocked	Not fed	4.00 pm
Sun	19-11-06	22.5–25.0	9.00 am	5.25 pm
Mon	20-11-06	22.5–26.0	9.30 am	4.00 pm
Tues	21-11-06	23.0–26.0	Not fed	4.30 pm
Wed	22-11-06	23.5–26.5	9.00 am	4.40 pm
Thurs	23-11-06	23.5–26.5	9.00 am	5.30 pm
Fri	24-11-06	24.0–26.5	9.00 am	5.00 pm
Sat	25-11-06	24.5–27.0	9.00 am	2.30 pm
Sun	26-11-06	24.5–27.0	9.00 am	5.30 pm
Mon	27-11-06	25.0–27.5	9.15 am	4.40 pm
Tues	28-11-06	25.5–28.0	9.00 am	5.00 pm
Wed	29-11-06	25.5–28.0	8.30 am	3.45 pm
Thurs	30-11-06	25.5–28.0	8.30 am	5.30 pm
Fri	1-12-06	24.5–26.0	9.30 am	3.00 pm
Sat	2-12-06	25.0–27.5	9.40 am	3.00 pm
Sun	3-12-06	26.0–28.0	10.30 am	4.50 pm
Mon	4-12-06	25.0–27.5	9.00 am	4.00 pm
Tues	5-12-06	24.0–26.5	9.00 am	4.00 pm
Wed	6-12-06	24.0–27.0	8.40 am	5.00 pm
Thurs	7-12-06	24.0–26.5	9.00 am	4.00 pm
Fri	8-12-06	24.5–26.5	9.00 am	4.00 pm
Sat	9-12-06	24.5–26.5	8.45 am	5.00 pm
Sun	10-12-06	24.5–27.0	8.30 am	4.00 pm
Mon	11-12-06	24.5–27.0	9.30 am	3.30 pm
Tues	12-12-06	24.0–27.0	9.00 am	3.30 pm
Wed	13-12-06	24.5–27.0	9.00 am	5.00 pm
Thurs	14-12-06	25.0–27.5	9.30 am	5.00 pm
Fri	15-12-06	25.0–28.0	Harvest 9 am – 1 pm	

Appendix 2 University of Queensland report for prism extruder conditions in the manufacture of experimental diets

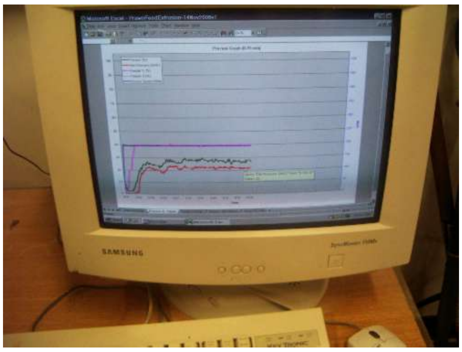
The following are pictures of the extrusion of organic prawn diets.

Figure 19 Prawn diet exiting extruder diet.

Figure 20 Extruded organic wheat/fish diet

Figure 21 Lab scale Prism extruder

Figure 22 Monitoring extruding parameters



UniQuest Pty Limited
Consulting & Research
(A.B.N. 19 010 529 898)

Cumbrae-Stewart Building
Research Road
The University of Queensland
Queensland 4072

Postal Address:
PO Box 6069
St. Lucia
Queensland 4067

Telephone: (61-7) 3365 4037
Facsimile: (61-7) 3365 7115

UniQuest Project No.	**14639**
Report Prepared for:	**Dr John Kopinski** **Department of Primary Industries and Fisheries** **665 Fairfield Road** **Yeerongpilly Qld 4105**
Subject:	**Extrusion of Prawn Diets**
Date:	**22 November 2006**
Report Prepared By:	**Dr Peter Torley** **Ms Margaret Brownjohn**

Signed for and on behalf of UniQuest Pty Limited

Mr Gary Heyden

Introduction

The Queensland Department of Primary Industries and Fisheries (QDPI&F) is interested in developing feed materials for farmed prawns. QDPI&F has identified extrusion as a technology that they believe will be useful in converting the raw materials into a cohesive pellet that will be suitable for feeding to prawns.

Background

Developing an extrusion process requires balancing of different operating parameters to give the desired product characteristics, while ensuring that the extruder is operating safely (eg die pressure below safe limits; no jamming by hard pieces in the feed material) and stably (ie minimal variation in die pressure and motor torque).

Due to limitations on the machine and also the characteristics of the raw material, it is not possible to simply programme in the desired set of operating conditions and get the extruder to produce the preferred product. It is generally necessary to evaluate a range of extruder operating conditions (eg barrel temperature profile, screw speed, moisture content, dry feed rate) in order to achieve the desired product. In some instances it will not be possible to achieve the desired product, with some compromises in product characteristics necessary.

As a result, the project will be divided into two stages. The first stage will develop a set of extrusion conditions that produce a prawn pellet. The second stage will use that set of conditions to produce seven extruded prawn feeds.

Prawn Feed Diet Formulations

Dr Kopinski of DPI&F, Queensland designed a set of prawn feed diets (Table 1).

Table 1 Proposed organic prawn diet composition — ingredient amounts (g) to yield ~1 kg dry matter of each diet.

	Wheat Flour Fish	Wheat Flour Scallop	Wheat Flour Soy	Wheat Flour Chickpea	Wheat Flour Macadamia	Weaner Fish	Weaner Soy
Wheat flour	950	950	550	550	550	-	-
Weaner pig diet	-	-	-	-	-	950	550
Fish frames	190	10	190	190	190	190	190
Scallop gut	10	190	10	10	10	10	10
Soy flour	-	-	400	-	-	-	400
Chickpea flour	-	-	-	400	-	-	-
Macadamia meal	-	-	-	-	400	-	-
Molasses	50	50	50	50	50	50	50
Casein	50	50	50	50	50	50	50
Kelp meal	10	10	10	10	10	10	10
Markers*	8.5	8.5	8.5	8.5	8.5	8.5	8.5
TOTAL	1268.5	1268.5	1268.5	1268.5	1268.5	1268.5	1268.5
DM yield	1152	1152	1175	1171	1152	1152	1175
Protein %	32.2	32.2	41.1	34.8	31.6	38.8	44.9

* Titanium dioxide 1.665 g, barium hydroxide 1.665 g and celite 5 g

Extrusion trials

DPI&F, Queensland wishes to undertake a preliminary evaluation of extruded prawn feeds, examining a variety of ingredients.

As this is a preliminary evaluation, QDPI&F does not wish to fully develop the extrusion process to optimise product characteristics. Rather QDPI&F prefers that the University of Queensland rapidly develops a process based on the formulations QDPI&F will supply and the types of operating conditions described in technical articles supplied by QDPI&F.

The preliminary trials to develop the extrusion process (Stage 1) were limited to one day of extrusion. The manufacturing trials (Stage 2) were limited to two days extrusion.

Stage 1 — Preliminary Trials to Develop Suitable Operating Conditions

Some preliminary trials were performed on 26 October 2006 to determine operating conditions suitable for the prawn feed formulations supplied by QDPI&F.

Two formulations were supplied by QDPI&F:

1. Weaner fish diet
2. Wheat flour fish diet

Dr John Kopinski of QDPI&F was present during these trials.

Extruder Description

The trials were carried out on a Prism Eurolab KX16 co-rotating twin screw extruder (Thermo Prism, Staffordshire, UK) (Fig. 1). The screw diameter was 16 mm and the extruder barrel was 640 mm long, giving a length to diameter (L/D) ratio of 40:1. The extruder barrel was divided into ten sections, the first of which (containing the dry feed port) was cooled by circulating tap water. The other nine barrel zones and the die block were electrically heated and their temperature could be individually controlled. The die had two openings each 2 mm in diameter. Melt pressure was measured with a pressure transducer fitted to the die block (Terwin, Nottinghamshire, UK).

Figure 1. Prism Eurolab KX16 twin screw extruder used for prawn meal diet extrusion trials.

Dry feed was fed through a single screw volumetric feeder (KX16 powder feeder, Brabender Technologie, Duisburg, Germany). Water was injected through a port 150 mm from the start of the barrel using a peristaltic pump (Masterflex L/S 7523, Cole-Parmer Instrument Company, Illinois, USA) fitted with Tygon Lab tubing (0.8 mm internal diameter; Masterflex 13, Cole-Parmer Instrument Company, Illinois, USA).

Dry Feed Calibration

The flow rate of the weaner fish diet formulation was through the KX16 powder feed (Brabender Technologie, Duisburg, Germany). This feeder is a single screw, volumetric powder feeder. A volumetric powder feeder operates by turning a feed screw at a set rate, and so the actual feed rate is affected by the properties of the material (eg density, tendency to lump) and the level of material in the hopper. The powder flow rate was calibrated for a single feed material (weaner fish diet), and so the calibration curve is only valid for that feed material. However, it does provide a general guide to the flow rate of the prawn feed diets.

The actual flow rate will be determined for each feed material by measuring the weight of extruded prawn feed produced. The peristaltic pump used in this study gives a very consistent flow rate. So by calculating the molasses solution flow rate at the pump speed used, and subtracting this from the total extruded prawn feed flow rate, it is possible to determine the actual dry feed flow rate.

The dry feed flow rate was determined at various speed settings of the Brabender powder feeder (fitted to the Prism Extruder) by measuring the weight of feed material collected (Fig. 2).

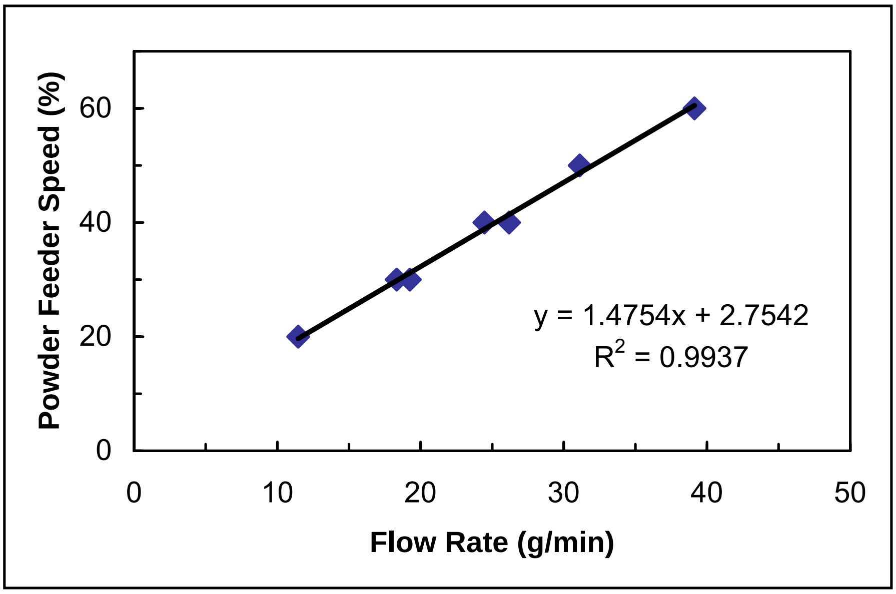


Figure 2. The flow rate of weaner fish diet through a Brabender Technologie single screw volumetric powder feeder.

Pump Calibration

Molasses was fed into the extruder using a peristaltic pump (Masterflex L/S 7523, Cole-Parmer Instrument Company, Illinois, USA) fitted with Tygon Lab tubing (0.8 mm internal diameter; Masterflex 13, Cole-Parmer Instrument Company, Illinois, USA).

To calibrate the pump, a standard molasses solution was prepared by dissolving 300 g of molasses in 2283 g of tap water. The molasses solution was held at room temperature. The flow rate at various pump speeds was determined by measuring the weight of molasses solution collected in a given period of time (Fig. 3). This calibration curve was used to calculate the molasses feed rate in all trials.

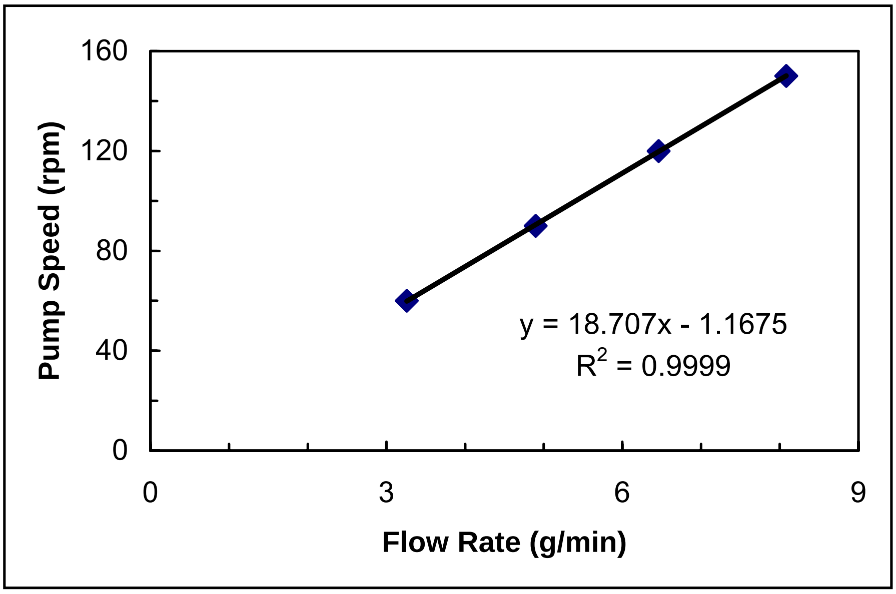


Figure 3. The flow rate of 11.6% molasses solution through a Masterflex L/S 7523 peristaltic pump fitted with Masterflex 13 (0.8 mm internal diameter) tubing.

Operate Prism Extruder

The initial extrusion trials were performed with weaner fish diet (Table 2).

The weaner fish diet could be extruded, however there were some problems with surging (fluctuations in die pressure and motor torque) which mean that the extruder is not producing a consistent product (weaner fish A). The dry feed rate was progressively increased (weaner fish diet B and C) which ultimately reduced the surging problem.

There were some problems caused by pieces of grain husk in the weaner feed. These accumulated in the die block and caused die blockage problems. This will be minimised by extruding the weaner feed diets after the wheat flour diets.

The wheat fish diet was also successfully extruded, however there were some initial problems with surging, which was reduced by turning down the extruder screw speed. Unlike the weaner feed diet, the wheat fish diet dry feed rate was not increased to reduce the surging problem as there was some material build-up in the dry feed port. Build up of material in the feed port can ultimately block feed to the extruder. A second problem was the extrudate was expanding as it left the die. The problem was eliminated by reducing the barrel temperature (Table 2). Subsequent trials by Dr Kopinski showed that after drying and grinding, both the expanded and non-expanded prawn diets sank, so in the manufacturing trials extrusion could be done using the higher barrel temperature profile.

Further Processing of Extruded Diets

The extruded prawn diets were collected as long strands. The strands were dried and ground into pieces then sieve (target size of 1 to 2 mm) at QDPI&F, after which they were evaluated by Dr Kopinski.

When the extruded, dried and ground pellets were placed in water they were found to sink and have an adequate life in the water before they started to fall apart.

Table 2 Extruder operating conditions during extrusion of weaner fish diet and wheat flour fish diet

Diet	Barrel Temperature (°C)											Screw Speed (rpm)	Feed Rate (%)	Pump Speed (rpm)	Motor Torque (%)	Die Pressure (bar)	Measured Total Flow Rate (g/min)	Calculated Molasses Flow Rate (g/min)	Calculated Dry Feed Rate (g/min)
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Die**								
Weaner Fish — A	- [a]	50	75 [b]	100	120	120	110	100	90	90	- [c]	196	40	120	24	22	-	6.5	-
Weaner Fish — B	- [a]	50	75 [b]	100	120	120	110	100	90	90	- [c]	196	45	120	31	39	-	6.5	-
Weaner Fish — C	- [a]	50	75 [b]	100	120	120	110	100	90	90	- [c]	196	50	140	32	31	53	7.5	45.5
Wheat Flour Fish	- [a]	50	75 [b]	100	120	120	110	100	90	90	- [c]	196	50	160	-	-	-	-	-
Wheat Flour Fish — D	- [a]	50	75 [b]	100	120	120	110	100	90	90	- [c]	172	50	160	45	43	-	-	-
Wheat Flour Fish — E	- [a]	50	75 [b]	100	100	100	100	90	90	90	- [c]	171	50	160	44	49	47.4	8.6	38.8
Wheat Flour Fish — F	- [a]	50	75 [b]	100	100	100	100	90	90	90	- [c]	171	50	160	46	43	-	-	-

[a] — Dry feed port. Cooled by circulating tap water (about 25°C).
[b] — Liquid feed port.
[c] — Die block temperature not set. The die was heated by conduction from barrel and from material passing through. Stabilised at 91 to 94°C.

Stage 2 — Extrusion of Seven Prawn Feeds

On 13 and 14 November 2006 we used the extrusion conditions selected after Stage 1 to produce seven prawn feeds.

Fresh molasses solutions (300 g of molasses, 2283 g of tap water) were prepared on each day of extrusion.

Dr John Kopinski of QDPI&F was present during these trials.

Extrusion Results

It was necessary to modify the operating conditions (e.g. feed rate, screw speed, pump speed) to ensure that the extruder operated stably.

The motor torque and die pressure varied considerable between formulations (Table 3). These differences in motor torque and die pressure are probably largely due to differences in the composition of the diet (e.g. oil, protein and polysaccharide concentration) and the thermal and rheological properties of the ingredients (e.g. amylose and amylopectin content, protein type).

The formulation that caused the greatest problem was the wheat flour soy diet, which caused the extruder to become very unstable. Two unsuccessful attempts were made on 13 November to run this material. The following day, the same formulation was successfully run with a slightly lower barrel temperature profile. The wheat flour chickpea diet was run under the same conditions as it was quite similar to the wheat flour soy diet, and we were concerned that similar problems would occur.

Extruded Feed Moisture Content

The moisture content of the extruded feed was determined with an infra-red moisture balance (MA-30, Sartorius) operating at 105°C. The moisture content of the extruded materials varied (Table 4) with the variation reflecting variation in the dry feed flow rate between the different diets (Fig. 4), with differences in the initial moisture content of the different dry feed formulations also contributing to the differences in the final moisture content.

Table 3 Extruder operating conditions during extrusion of seven prawn diets.

Diet	**Barrel Temperature (°C)**											**Screw Speed (rpm)**	**Dry Feed Rate (%)**	**Pump Speed (rpm)**	**Motor Torque (%)**	**Die Pressure (bar)**	**Measured Total Flow Rate (g/min)** [e]	**Calculated Molasses Flow Rate (g/min)**	**Calculated Dry Feed Flow Rate (g/min)**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Die**								
13 November																			
Wheat Flour Fish	- [a]	50	75 [b]	100	120	120	110	100	90	90	- [c]	173	40	130	48	37	39.1	7.0	32.1
Wheat Flour Scallop	-	50	75 [b]	100	120	120	110	100	90	90	- [c]	172	40	130	49	47	43.7	7.0	36.7
Wheat Flour Macadamia	-	50	75 [b]	100	120	120	110	100	90	90	- [c]	195	40	130	13	16	36.3	7.0	29.3
Wheat Flour Soy [d]	-	50	75 [b]	90	100	100	100	95	90	90	- [c]	-	40	130	-	-	-	7.0	-
Weaner Fish	-	50	75 [b]	100	120	120	110	100	90	90	- [c]	171	40	120	27	27	43.0	6.5	36.5
Weaner Soy Fish	-	50	75 [b]	100	120	120	110	100	90	90	- [c]	170	40	120	20	21	40.5	6.5	34.0
14 November																			
Wheat Flour Chickpea	-	50	75 [b]	90	100	100	100	95	90	90	- [c]	197	40	120	28	22	37.0	6.5	30.5
Wheat Flour Soy	-	50	75 [b]	90	100	100	100	95	90	90	- [c]	195	40	120	18	13	37.1	6.5	30.6

[a] — Dry feed port. Cooled by circulating tap water (about 25°C).
[b] — Liquid feed port.
[c] — Die block temperature not set. The die was heated by conduction from barrel and from material passing through. Stabilised at 91 to 94°C.
[d] — There was severe surging of the extruder with this set of conditions that caused the extruder to automatically shut-down. Formulation repeated the following day with slightly modified operating conditions. No significant surging problems occurred during the second trial.
[e] — Average of at least three measurements.

Table 4 Dry, molasses solution and total flow rates and measured moisture content of extruded prawn diets.

	Measured Total Flow Rate (g/min)	Calculated Molasses Flow Rate (g/min)	Calculated Dry Feed Flow Rate (g/min)	Measured Moisture Content (%)
13th of November				
Wheat Flour Fish	39.1	7.0	32.1	18.1
Wheat Flour Scallop	43.7	7.0	36.7	14.6
Wheat Flour Macadamia	36.3	7.0	29.3	22.9
Weaner Fish	43.0	6.5	36.5	18.1
Weaner Soy Fish	40.5	6.5	34.0	17.8
14th of November				
Wheat Flour Chickpea	37.0	6.5	30.5	18.9
Wheat Flour Soy	37.1	6.5	30.6	21.6

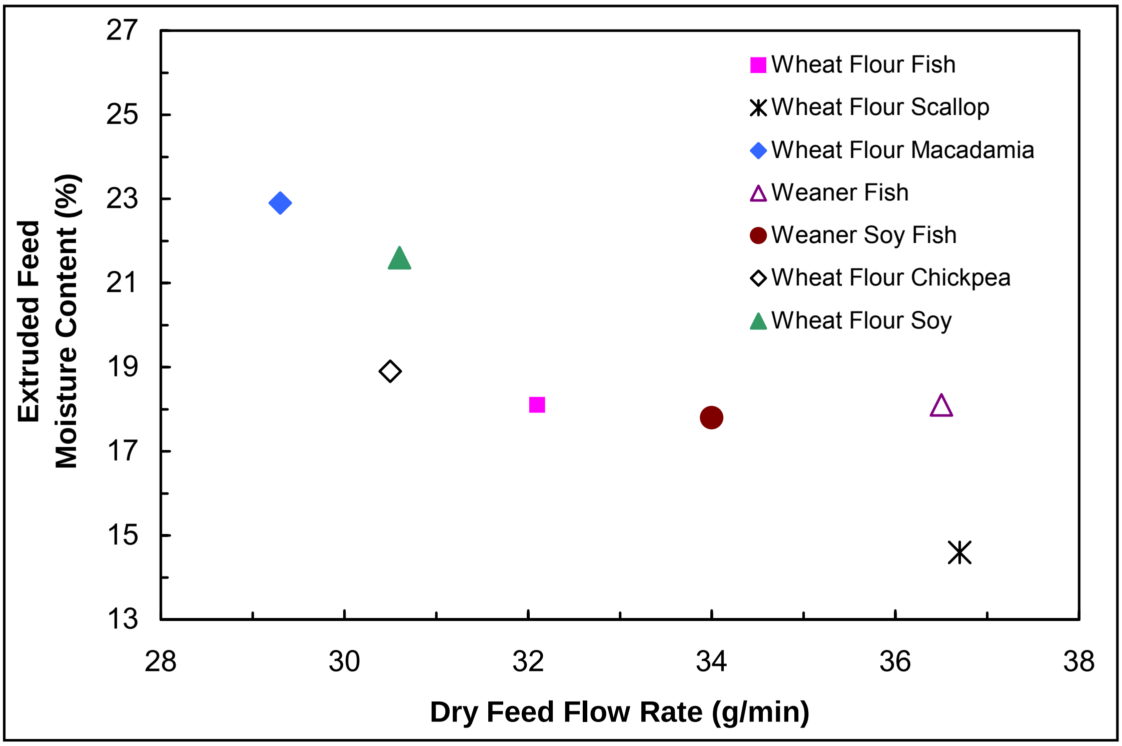


Figure 4. Variation in extruded prawn feed moisture content with the dry feed flow rate.

The prawn diets supplied for formulation were generally easy to extrude with few problems such as high motor torques or die pressures or feed port blockage.

There were some problems with blockage of the extruder die. In some cases the origin of this could be identified (eg husk in the weaner feed), but in other cases no obvious cause was found. With fine tuning of the extrusion process, it would probably be possible to overcome this problem.

Different extrudates exhibited variations physical properties such as sinking, material binding strength and water stability. Further feed formulation optimisation, drying and fine tuning of extrusion conditions may produce more desirable extrudate and pellet characteristics.

TERMS OF REPORT

UniQuest Pty Limited employees and University of Queensland staff and consultants operating with UniQuest will make all reasonable efforts to ensure an accurate understanding of client requirements. The information in reports is based on that understanding, and UniQuest strives to be accurate in its advice and to engage suitably qualified consultants with requisite skills of the highest order.

While all reasonable care will be taken in the preparation of reports, all information, assumptions, and recommendations therein are published, given, made, or expressed on the basis that:

(a) Any liability of any nature which would otherwise exist or arise in any circumstances by reference to any part or any omission from this report is excluded to the maximum extent permitted by law;

(b) Any liability which is unable to be excluded is limited to the minimum sum permitted by law;

(c) These provisions bind any person who refers to, or relies upon, all or any part of a report; and

(d) These provisions apply in favour of UniQuest and its directors, employees, servants, agents and consultants.

The client shall indemnify UniQuest and its directors, employees, servants, agents, consultants, successors in title and assigns against any claim made against any or all of them by third parties arising out of the disclosure of reports, whether directly or indirectly, to a third party.

A laboratory certificate, statement, or report may not be published except in full, unless permission for publication of an approved abstract has been obtained, in writing from the Managing Director of UniQuest.

Samples will be destroyed within 30 days unless collected by the client, or alternative arrangements have been agreed to by UniQuest.

Appendix 3 Bauple water quality

Figure 23 Total Phosphorous at Bauple

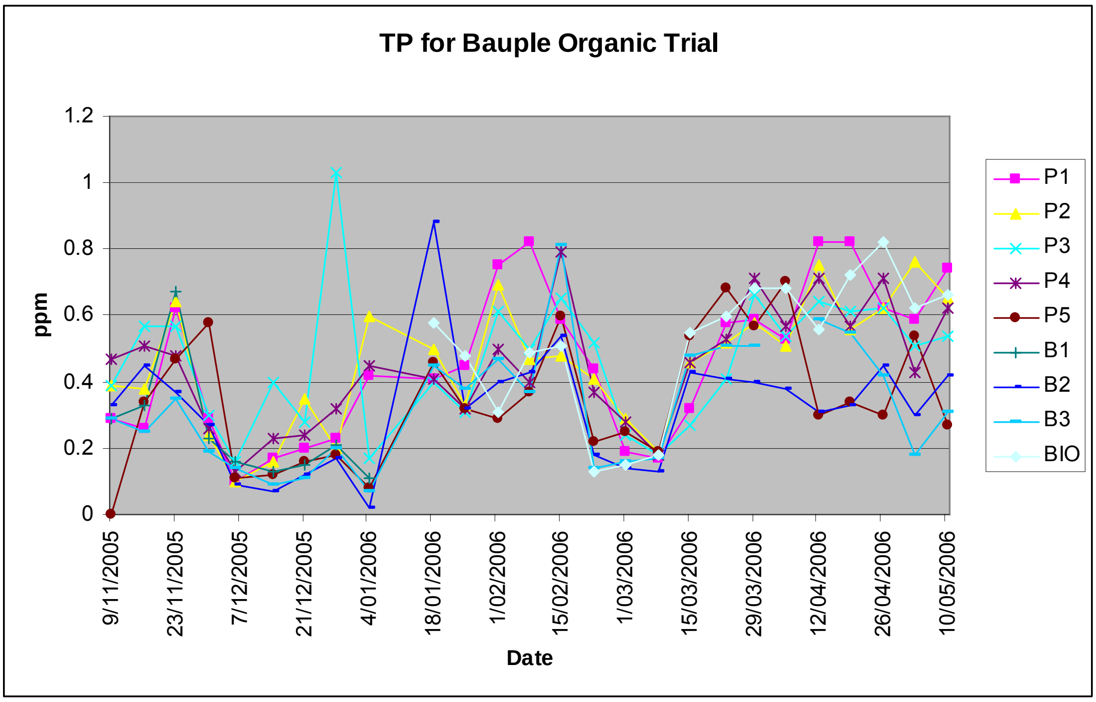


Figure 24 Total Nitrogen at Bauple

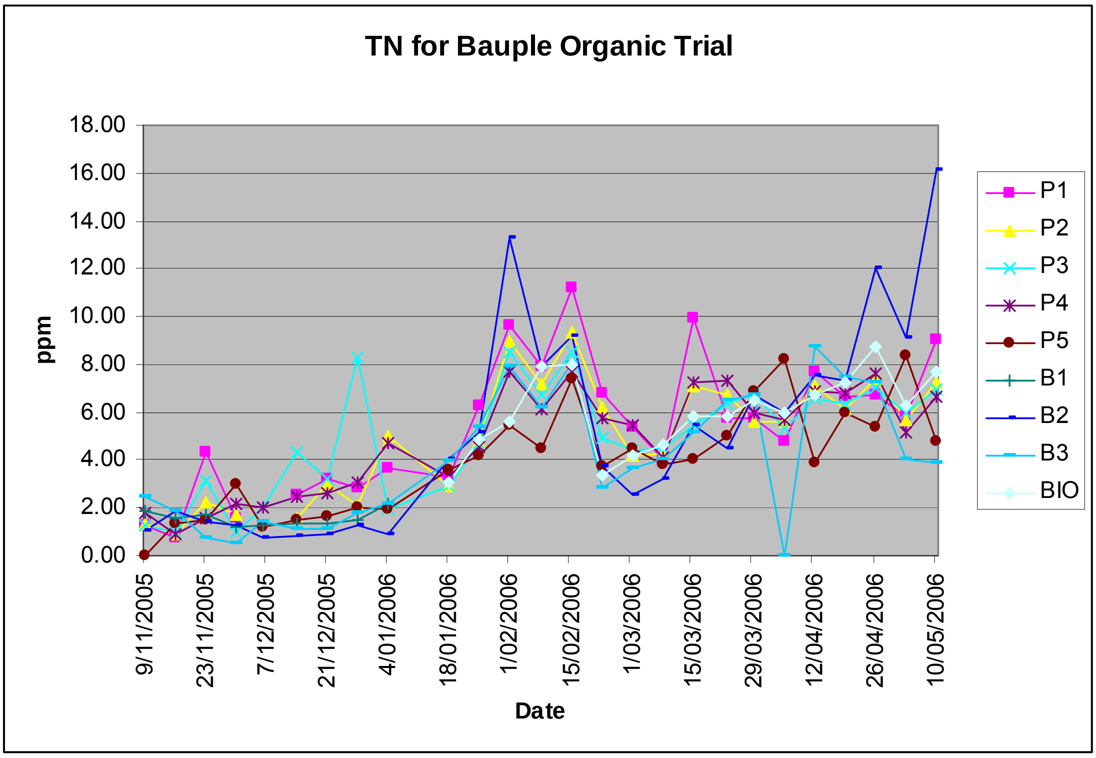


Figure 25 Chlorophyll A at Bauple

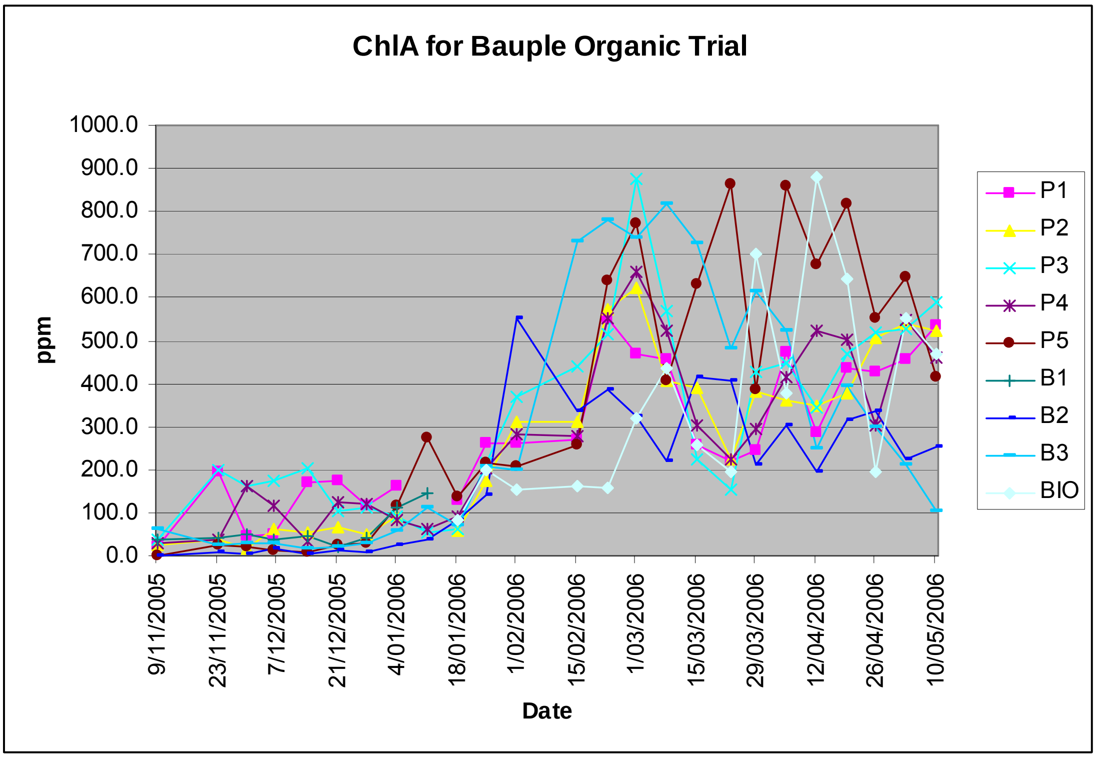


Figure 26 Total Ammonia Nitrogen at Bauple

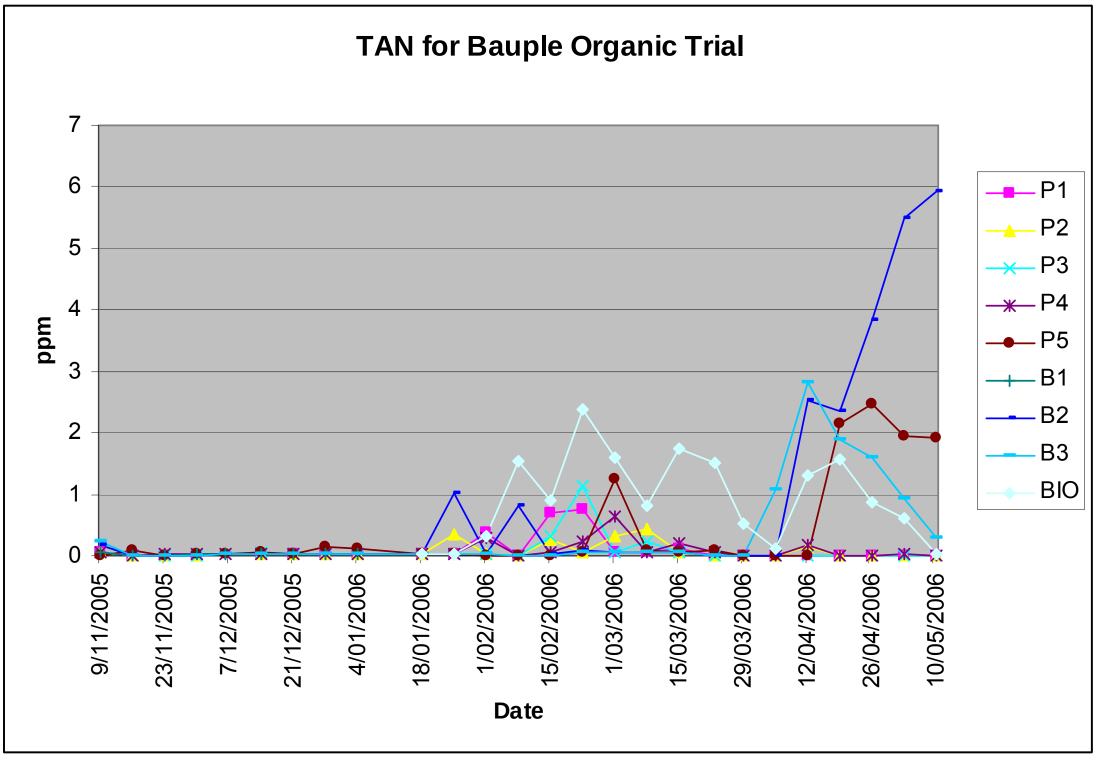


Figure 27 Ortho-phosphate at Bauple

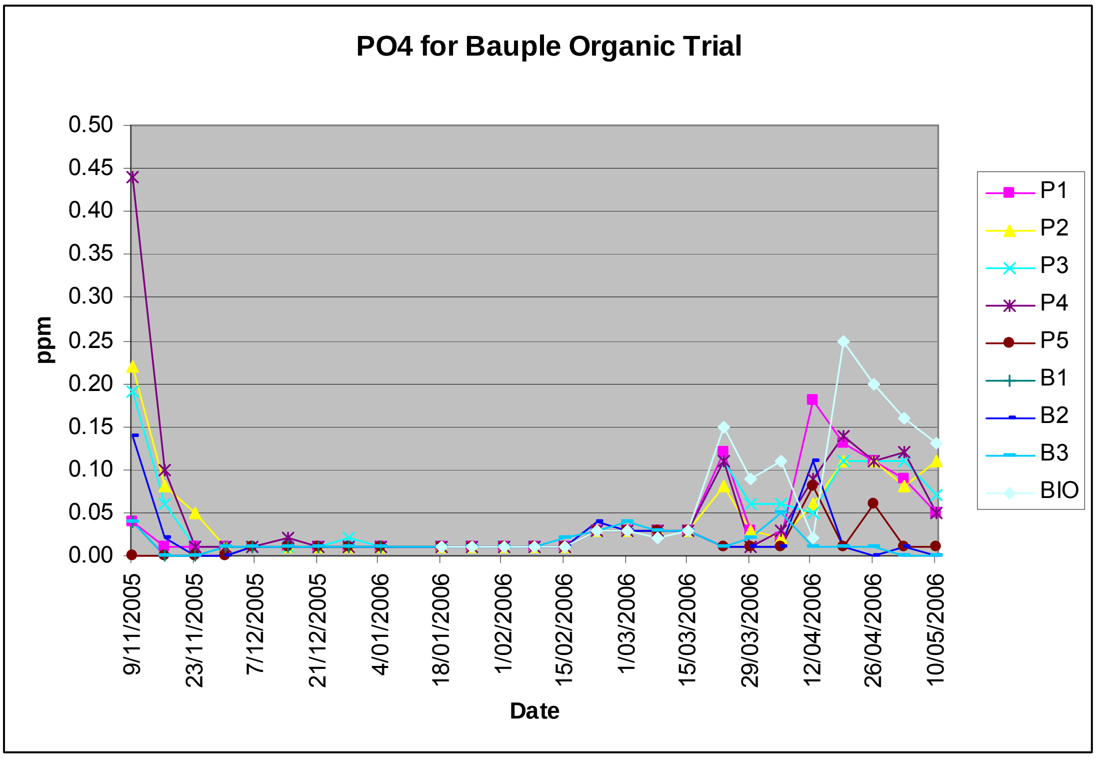


Figure 28 Oxides of Nitrogen (inorganic) at Bauple

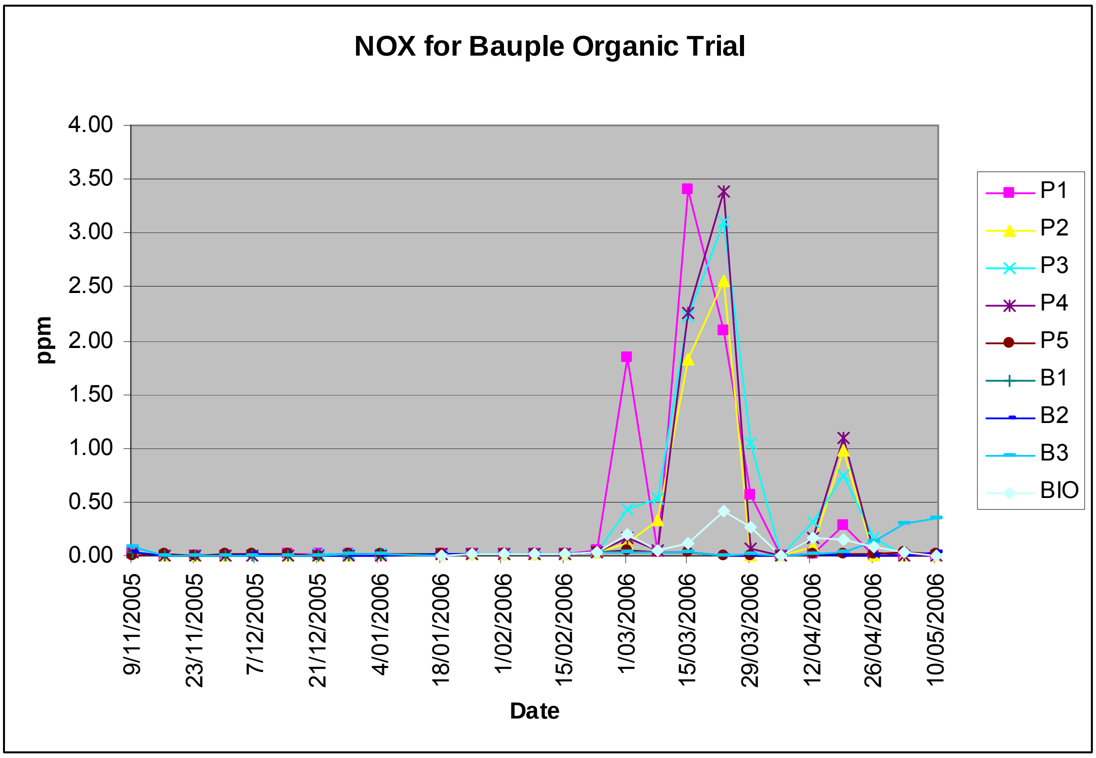


Figure 29 Total Suspended Solids at Bauple

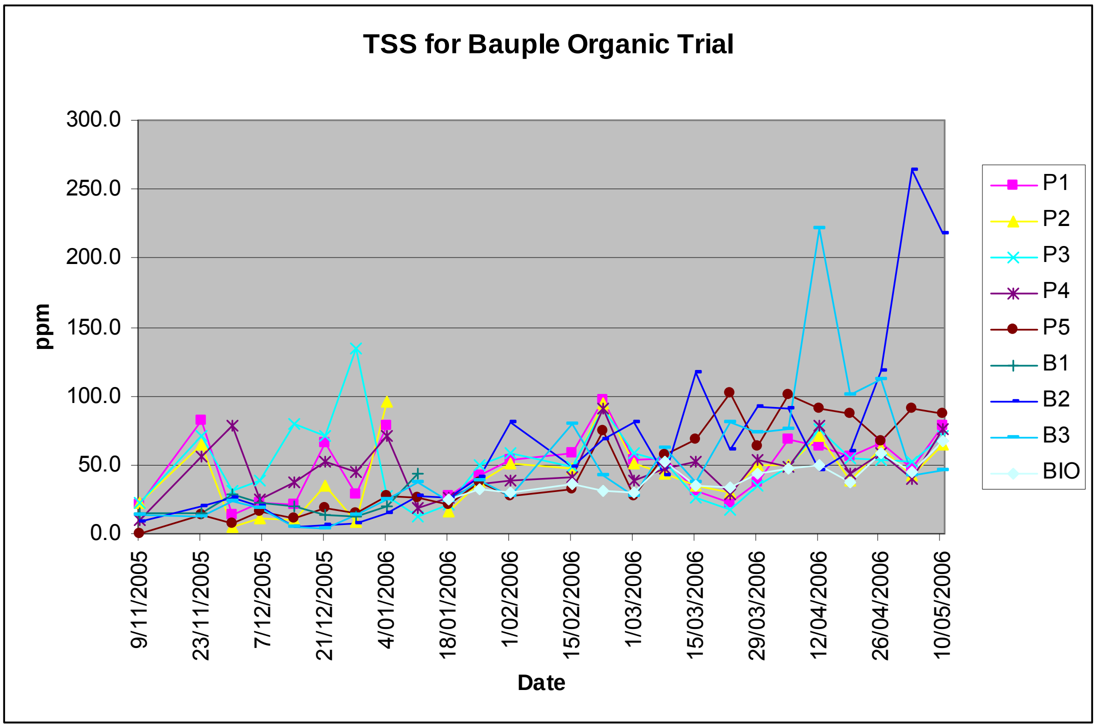
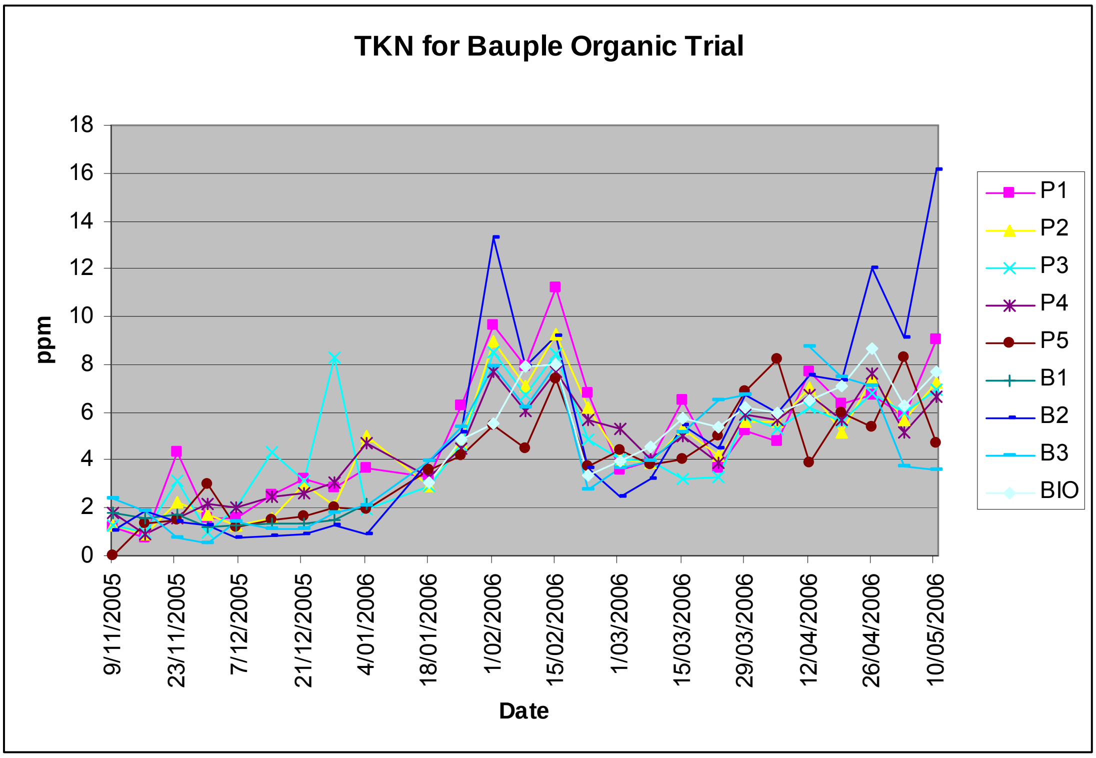


Figure 30 Total Kheldale Nitrogen (organic) at Bauple



Appendix 4 Demerit assessment score sheets

COOKED TIGER PRAWNS STORED AT 4°C IN MAP

SAMPLE A Tray Number

TIME IN CO2 PACK TIME IN AIR DATE SAMPLED

BLACKSPOT Absent / Slight / Moderate / Extensive

0 1 2 3

DRIP None / Slight / Moderate/ Excessive

0 1 2 3

COOKED ODOUR Fresh cooked/No off odours/Slight/Excess Off Odour

0 1 2 3

Description ....

HEAD APPEARANCE Firmly attached / Sl loose / drooping / lost

0 1 2 3

HEPATOPANCREAS LEAKAGE Unseen/Sl burn/ Mod blown / Fully blown

0 1 2 3

TEXTURE OF SHELL ON TAIL Smooth/Sl Etched/ Course / Gritty

0 1 2 3

FLESH APPEARANCE Firm / Sl soft / soft / mushy

0 1 2 3

FLESH COLOUR V.Bright white/White/ Dull white / Grey

0 1 2 3

Full Weight ....…………………

Empty weight ....

SAMPLE B	Tray Number
TIME IN CO2 PACK	TIME IN AIR DATE SAMPLED

BLACKSPOT	Absent / Slight / Moderate / Extensive
	0 1 2 3

DRIP	None / Slight / Moderate/ Excessive
	0 1 2 3

COOKED ODOUR	Fresh cooked/No off odours/Slight/Excess Off Odour
	0 1 2 3
	Description ....

HEAD APPEARANCE	Firmly attached / Sl loose / drooping / lost
	0 1 2 3
HEPATOPANCREAS LEAKAGE	Unseen/Sl burn/ Mod blown / Fully blown
	0 1 2 3

TEXTURE OF SHELL ON TAIL	Smooth/Sl Etched/ Course / Gritty
	0 1 2 3
FLESH APPEARANCE	Firm / Sl soft / soft / mushy
	0 1 2 3
FLESH COLOUR	V.Bright white/White/ Dull white / Grey
	0 1 2 3

Full Weight	..........................…………………
Empty weight	....

RAW BANANA PRAWNS STORED AT 4°C IN MAP

SAMPLE A Tray Number

TIME IN CO_2 PACK TIME IN AIR DATE SAMPLED

BLACKSPOT	Absent / Slight / Moderate / Extensive
	0 1 2 3

DRIP	None / Slight / Moderate/ Excessive
	0 1 2 3

RAW ODOUR	Fresh/No off odours/Slight/Excess Off Odour
	0 1 2 3
	Description ....

HEAD APPEARANCE	Firmly attached / Sl loose / drooping / lost
	0 1 2 3
HEPATOPANCREAS LEAKAGE	Unseen/Sl burn/ Mod blown / Fully blown
	0 1 2 3
STAINING Head end of body	No stain/Sl stain/Moderate stain/V. Stained
	0 1 2 3

Texture of Shell on Tail	Smooth/Sl Etched/ Course / Gritty
	0 1 2 3
FLESH APPEARANCE	Intact / Sl gaping / soft / mushy
	0 1 2 3
FLESH COLOUR	Translucent/Dull/ Sl opaque/ Opaque
	0 1 2 3

Full Weight	...........................…………………
Empty weight	....

SAMPLE B	Tray Number	
TIME IN CO2 PACK	TIME IN AIR	DATE SAMPLED

BLACKSPOT	Absent / Slight / Moderate / Extensive
	0 1 2 3

DRIP	None/Slight/Moderate/Excessive
	0 1 2 3

RAW ODOUR	Fresh/No off odours/Slight/Excess Off Odour
	0 1 2 3
	Description ....

HEAD APPEARANCE	Firmly attached / Sl loose / drooping / lost
	0 1 2 3
HEPATOPANCREAS LEAKAGE	Unseen/Sl burn/ Mod blown / Fully blown
	0 1 2 3
STAINING Head end of body	No stain/Sl stain/Moderate stain/V. Stained
	0 1 2 3

Texture of Shell on Tail	Smooth/Sl Etched/Course/Gritty
	0 1 2 3
FLESH APPEARANCE	Intact / Sl gaping / soft / mushy
	0 1 2 3
FLESH COLOUR	Translucent/Dull/ Sl opaque/ Opaque
	0 1 2 3

Full Weight	........................………………….
Empty weight	....

RAW TIGER PRAWNS STORED AT 4°C IN MAP

SAMPLE A Tray Number

TIME IN CO_2 PACK TIME IN AIR DATE SAMPLED

BLACKSPOT	Absent / Slight / Moderate / Extensive	
	0 1 2 3	
DRIP	None / Slight / Moderate/ Excessive	
	0 1 2 3	
RAW ODOUR	Fresh/No off odours/Slight/Excess Off Odour	
	0 1 2 3	
	Description	....
HEAD APPEARANCE	Firmly attached / Sl loose / drooping / lost	
	0 1 2 3	
HEPATOPANCREAS LEAKAGE	Unseen/Sl burn/ Mod blown / Fully blown	
	0 1 2 3	
STAINING Head end of body	No stain/Sl stain/Moderate stain/V. Stained	
	0 1 2 3	
Texture of Shell on Tail	Smooth/Sl Etched/ Course / Gritty	
	0 1 2 3	
FLESH APPEARANCE	Intact / Sl gaping / soft / mushy	
	0 1 2 3	
FLESH COLOUR	Translucent/Dull/ Sl opaque/ Opaque	
	0 1 2 3	
Full Weight	..........................…………………	
Empty weight	....	

SAMPLE B	Tray Number
TIME IN CO2 PACK	TIME IN AIR DATE SAMPLED

BLACKSPOT	Absent / Slight / Moderate / Extensive
	0 1 2 3

DRIP	None / Slight / Moderate/ Excessive
	0 1 2 3

RAW ODOUR	Fresh/No off odours/Slight/Excess Off Odour
	0 1 2 3
	Description ....

HEAD APPEARANCE	Firmly attached / Sl loose / drooping / lost
	0 1 2 3
HEPATOPANCREAS LEAKAGE	Unseen/Sl burn/ Mod blown / Fully blown
	0 1 2 3
STAINING Head end of body	No stain/Sl stain/Moderate stain/V. Stained
	0 1 2 3

Texture of Shell on Tail	Smooth/Sl Etched/ Course / Gritty
	0 1 2 3
FLESH APPEARANCE	Intact / Sl gaping / soft / mushy
	0 1 2 3
FLESH COLOUR	Translucent/Dull/ Sl opaque/ Opaque
	0 1 2 3

Full Weight......................	Empty weight ....