Increasing the profitability of *Penaeus monodon* farms via the use of low water exchange, microbial floc production systems at Australian Prawn Farms

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OBJECTIVES:

1. Determine the effects of variations in nutrient concentrations, carbon source and water exchange on microbial floc formation, composition and persistence in commercial *P. monodon* ponds.
2. Evaluate the effectiveness of different carbon sources for microbial floc formation in commercial ponds.
3. Determine the nutritional benefits of microbial flocs to *P. monodon* reared in ponds and quantify the associated reduction in feed costs.
4. Quantify the effects of low water exchange, microbial floc production system on the reduction in farm nutrient discharge and overall effects on farm profitability.

NON TECHNICAL SUMMARY:

The economic viability of the Australian prawn farming industry is under threat from increased production of farmed prawns in south-east Asia and the substantial decrease in global prawn prices. The Australian industry is experiencing difficulty competing with imports; prawns can be landed from China and Thailand for about $8 per kg, compared with the current cost of production in Australia of about $10 per kg. The industry is also under increasing pressure from environmental regulators to reduce the scale and impacts of sediment and nutrient discharges from prawn farms. There is a critical need to develop production technology for black tiger prawns (*Penaeus monodon*) that enables Australian prawn farmers to gain the same improvements in production efficiency that have been achieved overseas with Pacific white shrimp (*Litopenaeus vannamei*), with high intensity, low discharge systems. Australian Prawn Farms Pty Ltd (APF) investigated the production technology used for *L. vannamei* and concluded that it was not suitable for Australian farming conditions and for the culture of *P. monodon*. As a result, APF did some preliminary trials using their own ideas for a low water exchange system that was more suitable for *P. monodon*, with promising results. However, to make further progress, there was a need for a broader and more extensive research effort than APF could do on its own without some external funding and scientific support.

Low water exchange or discharge systems rely on microalgae, bacteria and other microbial organisms to recycle the nutrients, particularly ammonia, from the waste that is generated in a pond. The microbial community can aggregate to form small clumps commonly termed bioflocs. The formation of bioflocs tends to reduce variability in the concentration of nutrients

and enhances the stability of pond nutrient and chemical dynamics. Prawns thrive in the biofloc environment, exhibiting increased survival and growth rates.

The first objective of the project was to gain an understanding of the key nutrients involved in the development of the biofloc in a prawn pond, and to determine the type and quantity of nutrients required to bring about and maintain a biofloc in the pond. The results demonstrated that dense blooms of large diatoms are a critical prerequisite for establishing a good biofloc. Once a diatom bloom has been established biofloc will generally form within a week. The results demonstrated that the concentration of ammonia in the ponds needs to peak at above 0.9 mg/L for a diatom bloom to proliferate. The results also indicate that the concentration of reactive silica should be at least 0.2 mg/L and the concentration of reactive phosphorus at least 0.1 mg/L shortly after the concentration of ammonia has risen above 0.9 mg/L.

The second objective was to evaluate the effectiveness of different carbon sources for microbial floc formation. The microorganisms that convert excess ammonia in the pond into cellular materials also need other nutrients including a source of carbon. Some use carbon dioxide or bicarbonate from the water, whereas others use organic carbon sources in the pond. The results of the study demonstrated that molasses is a very practical and effective organic carbon source. APF has established guidelines for the use of molasses in ponds because the addition of molasses to a pond can reduce the subsequent early morning oxygen levels. When deciding on the amount of molasses to add to a pond, consideration should be given to the amount of ammonia in the water, the species and state of the microalgal bloom, the previous day's morning and afternoon pH and oxygen levels, the amount of sunshine in previous days and the forecast amount of sunshine over the following few days, and the amount of feed going into the pond.

The third objective of the project was to determine the nutritional benefits of microbial flocs to *P. monodon* reared in ponds and to quantify the associated reduction in feed costs. The low water exchange, biofloc system at APF resulted in a significant improvement in feed conversion efficiency. When compared to a conventional flow-through pond management system, the APF system achieved a saving of about $10,000 or about 30% of the total feed cost for the production of 10 tonnes of prawns. Using APF production data for 2007/08, the results showed that the presence of a dense biofloc in a pond, compared to a weak biofloc, resulted in a saving of 3 tonnes of feed; or about $6,000 per pond; or about 20% of the total feed cost.

The fourth and final objective was to determine the effect of the low water-exchange system on the amount of nutrients that are discharged to the coastal environment over the whole production season. The results demonstrated that the low water-exchange microbial floc production system at APF reduced water exchange by 70% and nitrogen discharge by 77% compared to conventional flow-through ponds.

Overall the greatest benefit from using the production system developed at APF was the significant increase in the harvest yields of prawns. APF estimate that if they had still been using a conventional flow-through system in 2008/09, they would have produced about 8 t of prawns per 1 ha pond. Using the low water exchange, biofloc system, APF's average production in 2008/09 in ponds in which a floc was established was 12 t of prawns per 1 ha pond. This increased production of 4 t per ha has a value of $60,000. Taking all the additional costs and savings into consideration, the biofloc system can increase the production from each pond by an estimated $65,000.

On the basis of these results and the system management experience at APF, two other farms are using the low water-exchange system in the 2010/11 season and at least two others are adopting techniques and learning skills in preparation for changing to this system in the future. In May 2010, Sunshine Coast TAFE ran a strategic planning workshop for farm owners and managers to equip them to make decisions about adopting this type of production system using the information generated by this project

This project has achieved all of its objectives. The results of the study have significantly improved our understanding of the nutrient requirements for establishing the biofloc in the ponds. However, further work needs to be done to improve our understanding of the factors that initially trigger the formation of the biofloc.

OUTCOMES ACHIEVED TO DATE

The low water-exchange microbial floc production system, developed and deployed at Australian Prawn Farms (APF), increased the harvest yields of black tiger prawns (*Penaeus monodon*) by 50% and the value of production per hectare by $65,000 compared to production from conventional flow-through ponds.

The low water-exchange microbial floc production system at APF reduced water exchange by 70% and nitrogen discharge by 77% compared to conventional flow-through ponds.

An effective system of stimulating the growth and dominance of diatoms in ponds by adding sodium silicate was developed and implemented at APF.

Two other Australian prawn farms (Pacific Reef and Eimeo) have adopted the production system developed at APF. Two other prawn farms are currently developing the skills required to adopt the APF system.

A strategic planning workshop was run by Sunshine Coast TAFE for farm owners and managers to equip them to make decisions about adopting the APF system using the information generated by this project.

KEYWORDS: Prawn farming, aquaculture, biofloc, low water exchange.

2. INTRODUCTION

During late 2005 Australian Prawn Farms (APF) carried out a trial to test whether black tiger prawns, *Penaeus monodon*, could be grown using a zero or minimal water exchange culture system. The approach used was based on Matt West's own ideas about how the system should run. The system is similar to the biofloc technology (BFT) pond management system that evolved independently in Israel and in the USA in the mid-1990's (Avnimelech, 2009), but operates at a lower concentration of biofloc. The BFT system had previously been applied for the commercial production of the Pacific white shrimp, *Litopenaeus vannamei,* by Belize Aquaculture Ltd. in Central America and was widely known as the "Belize" system. The concept behind the BFT system is that in a closed system, any nutrients that are added to the system (generally through the feed) that are not retained as prawn biomass, are recycled through microbial processes and form a flocculated microbial biomass which provides an additional nutrient source for the prawns. In this way, nutrients that would have been lost or discharged from the system in the traditional, open pond, system are recycled. The BFT system can result in much better feed utilization and consequently, a reduction of nutrients discharged to the environment (Avnimelech, 209). The BFT system has been successfully applied for the culture of *L. vannamei* in South America and in China, Indonesia and Thailand. However, it has not been evaluated with the most widely-grown prawn species in Australia, *P. monodon*. In the 2005/06 season, Australian Prawn Farms (AFP) successfully reared and harvested a crop of *P. monodon* from six zero water exchange ponds. In some cases, they were able to increase the productivity of the ponds. However, they were unable to explain how they achieved this and why it was successful, and were uncertain if they would be able to repeat the success in the following season. Extensive literature searches and discussions with researchers demonstrated the lack of understanding of the processes that control the production and maintenance of the microbial floc.

CSIRO Marine and Atmospheric Research (CMAR) worked *in-situ* with Belize Aquaculture Ltd. to examine the nutrient dynamics in this novel system (Burford *et al*. 2003). Furthermore, CMAR had on-going research activities that aligned closely with the interests of the Australian prawn farming industry in exploring the potential of using low water-exchange, microbial floc production systems to enhance the production efficiency of *P. monodon* farms.

In 2009, a book, "Biofloc Technology" by Yoran Avnimelech, was published by the World Aquaculture Society (Avnimelech, 2009). This book provides a guide to biofloc technology along the lines of the system used by Belize Aquaculture Ltd. system. However, due to the economics of prawn production in Australia and the business model adopted by APF, the approach used at APF was quite different from that of the "Belize" system. It was far less intensive than the "Belize" system, with lower stocking rates (30 to 60 prawns/ m^2 compared with 120 prawns/ m^2) and consequently lower feed and nutrient inputs, and the biofloc density was much lower < 1 mL/L c.f. 10 mL/L) (Avnimelech, 2009). Hence, a direct transfer of the technology used at Belize Aquaculture was not possible.

This project addressed the key issues stated in Challenge 1 of the FRDC Research and Development Plan (2005-2010). The objective of the project was to address this challenge by improving our understanding of the pond water chemistry and microbiology in order to:

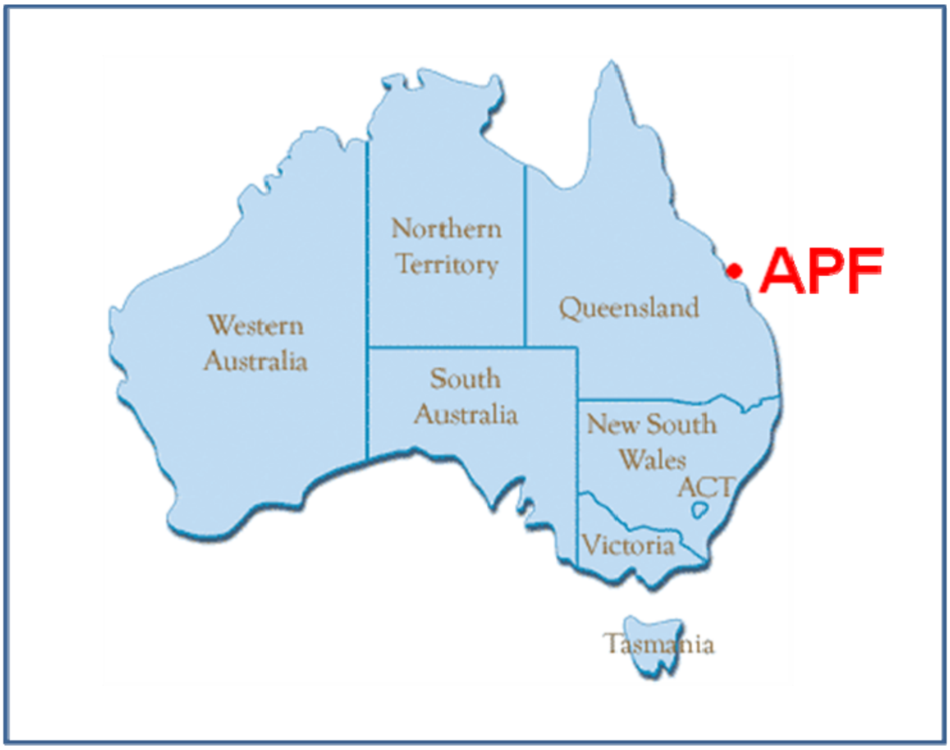
Reduce:

a. unnecessary farm costs such as excess pumping and chemical usage;
b. variation in between-pond productivity, and between grow-out seasons;
c. the concentration of nutrients discharged to the local estuarine environment and therefore "remediate" water in situ;
d. overfeeding and therefore greatly reduce farm expenses; and,
e. weather effects (manage negative effects of storm events to decrease loss of yield).

Increase:

a. the ability to compete with highly productive international producers;
b. the likelihood of a chemically balanced pond environment;
c. environmental management and to ensure EPA compliance;
d. chances of prawn survival by reducing water quality fluctuation; and,
e. biosecurity of prawns by halting the chance of disease being transmitted through the intake water.

During the development of the proposal, the Principal Investigator and Matt West had extensive discussions with individual prawn farms, including SeaFarm (Alistair Dick), water quality management consultants (Baseline Australasia Pty Ltd) and government research agencies (CSIRO Marine & Atmospheric Research, Queensland Department of Primary Industries & Fisheries (DPI&F), now called Department of Employment, Economic Development and Innovation (DEEDI)). The pre-proposal was strongly supported by the Australian Prawn Farmers Association (APFA) R&D Committee and Queensland Fishing Industry Research Advisory Committee (QFIRAC) who also provided valuable input. The pre-proposal was presented to the Annual General Meeting of the APFA and received widespread support from the members and from representatives of the Queensland Government Environmental Protection Authority (EPA).



Map 1. Location of Australian Prawn Farms (APF) on the east coat of Queensland.

Photograph 1. Australian Prawn Farms, Ilbilbie, Queensland

2.1. Need

The economic viability of the Australian prawn farming industry is under threat because of the increased production of shrimp in south-east Asia and the substantial decrease in global prawn prices. The Australian industry is experiencing difficulty competing with imports – prawns can be landed from China and Thailand for about $8 per kg, compared with the current cost of production in Australia of about $10 per kg. In addition, the industry is under increasing pressure from environmental regulators to reduce the scale and impact of nutrient discharges from prawn farms. The critical need is to develop a production technology for *P. monodon* that enables Australian prawn farmers to gain the same improvements in production efficiency that have been achieved overseas with *P. vannamei* with high intensity, low discharge systems. The need for the research was to gain a better understanding of the chemical and physical processes occurring in the low water exchange ponds in order to adapt and manage this novel production system for *P. monodon*. A reduction in nitrogen input into the pond throughout the entire growing season is a widely accepted approach to managing a low water exchange pond system. This can be achieved through lower feeding rates and the use of low-protein feeds. However, this can only be achieved by managing the system so that the prawns get a substantial part of their nutrition from the microbial floc. Hence, there is a need to develop protocols to maximise the nutritional value of microbial flocs and their physical characteristics. In doing this, it will be possible to reduce the amount of feed used and its cost for the production of the prawns, thereby improving farm profitability.

In Australia, the economics of prawn farming dictate that profitability is maximised by maximising the growth rate of the prawns so that they can be harvested as 30+ g individuals before water temperatures decrease in autumn. Growth is highly influenced by temperature and by the protein content of the feed, so reducing the protein content is generally counterproductive to farm profitability. Under this scenario, it is necessary to optimise the pond management so that nutrients not retained in prawn biomass, especially nitrogen, are recycled through the pond food chain.

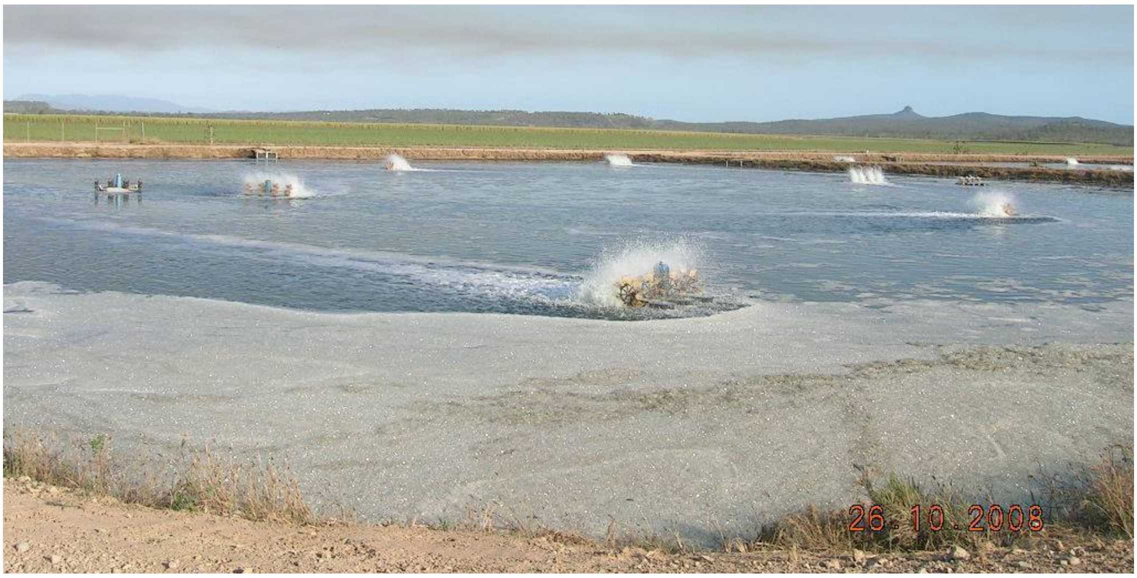
2.2. Objectives

a. Determine the effects of variations in nutrient concentrations, carbon source and water exchange on microbial floc formation, composition and persistence in commercial *P. monodon* ponds. **Significant progress has been made towards understanding the effects of variations in nutrient source. However, further work is warranted.**

b. Evaluate the effectiveness of different carbon sources for microbial floc formation in commercial ponds. **The effectiveness of different carbon sources is discussed and supported by work recently reported in the scientific literature.**

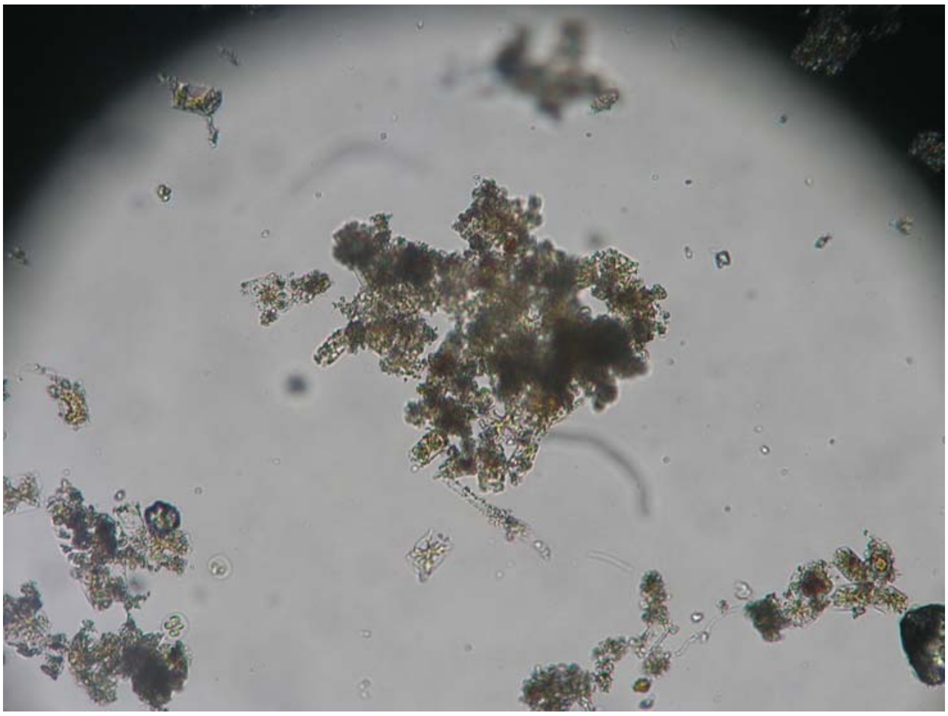
c. Determine the nutritional benefits of microbial flocs to *P. monodon* reared in ponds and quantify the associated reduction in feed costs. **This has been achieved: Improvement in feed conversion efficiency and reduction in feed costs have been quantified and are reported.**

d. Quantify the effects of low water exchange, microbial floc production system on the reduction in farm nutrient discharge and overall effects on farm profitability. **The reduction in water exchange and nitrogen discharge from the pond has been quantified and are reported**.



Photograph 2. Pond at Australian Prawn Farms showing foam generated after a dinoflaggelate bloom had crashed

Photograph 3. Biofloc (~ 0.5 mL) settling out in an Imhoff cone.



Photograph 4. Biofloc as seen through a low-power microscope.

3. METHODS

3.1. Research approach

The research approach adopted in this project was to intensively monitor the nutrients, microalgal population and biofloc density in five production ponds at APF that were being managed in the first year according to the farm's established low water-exchange protocol. This enabled the research team to acquire baseline data about the nutrient dynamics, pond water quality and biotic data that would help understand the factors that contribute to the establishment and maintenance of a stable biofloc in the ponds. From the conclusions drawn from the first year's observations, changes were made to the management protocol in the following year and the same five ponds were again intensively monitored to observe changes in the biotic and abiotic parameters. This process was again repeated in the third year of the study. Concurrently, a less intensive study was undertaken to understand the role that bacteria were playing in the formation of the biofloc and in the nutrient dynamics.

Australian Prawn Farms has allowed the research team access to farm production data and pond water quality measurements from other ponds on the farm over the three seasons. These data have been used by the team to assist them to compare and analyse the data from the five study ponds. At the end of the project, data from the whole farm was used to determine improvement in growth rate and feed conversion ratio (FCR), and to assess the reduction in pumping and water exchange, and the reduction in nutrient discharge. APF's data have also been used to assess the difference in production costs between those incurred using the low water-exchange, microbial biofloc production system and those that would have been incurred using an "open", water-exchange management system.

3.2. Pond nutrient dynamics, microalgae and biofloc

3.2.1. 2007/08 Season

In the first year the ponds were filled and fertilised using standard procedures that are widely used throughout the prawn farming industry. Once a pond was filled, fertiliser was added to it, generally every day or every second day, with between 2 and 3 kg of urea and 1 and 1.5 kg of mono-ammonium phosphate (MAP) being used at each application. The amount and frequency of application being determined by the progress of the microalgal population and prevailing weather conditions. The application of fertiliser continued for about 4 weeks and was stopped once a dense microalgal bloom was established or the Total Ammonia Nitrogen (TAN) exceeded 1 mg/L. Postlarvae (PL15) were stocked into the ponds 14 days after filling. Two ponds were stocked at the conventional stocking density of 35 PL's per m^2, two were stocked at 45 PL's per m^2, and the last pond was stocked at 60 PL's per m^2. The fertilisation program and the timing of stocking were designed to ensure that the microalgal population had developed to the point where it provided shade for the postlarvae and had supported the establishment of a zooplankton population that would be of the appropriate size to be the first food items of the postlarvae.

Intensive sampling of the five production ponds assigned to the study was carried out throughout the first and subsequent seasons, from prior to stocking to about 120 days after stocking, at which time preparations were being made to harvest ponds on the farm. This sampling gave a time series of data of pond water quality parameters. These were collected in conjunction with data on all fertilizer and feed inputs, chemical additions and assessments of the biofloc density and microalgal population status. In addition, on-farm rainfall was recorded and data were obtained from the Bureau of Meteorology on the number of hours of sunshine in Mackay for each day during the growing season. These data were used to estimate the amount of sunshine at APF. Temperature, pH and dissolved oxygen (DO) in the ponds were measured twice daily, shortly after sunrise and about mid-afternoon, using a data logger. TAN was determined on-farm using a Hach colour comparator (Hach 2428700 (NI-SA)) twice each week. Lithium hydroxide (LiOH) was added to the water to suppress interference from inorganic ions present in the seawater and hence reduce interference from other cations in the water and provide greater stability for the ammonia colour development. The biofloc volume was also assessed twice weekly using the method for determining settleable solids in water (Standard Methods for the Examination of Water and Wastewater, 2006). A 1-litre sample of water was allowed to settle in an Imhoff cone for 20 min before the amount of material that had settled out was recorded. If the settlement period was longer than 20 min, gas bubbles tended to form which increased the apparent volume of the material. The amount of solids that settled out provided an estimate of the floc volume, though at times other suspended material could contribute up to about 0.1 mL to the total. Salinity and Total Alkalinity were measured periodically as required or indicated by other parameters. The Total Alkalinity was measured on-farm using a Palintest 192 Alkavis kit.

Additional chemical analyses were carried out on pond water samples by an external analytical laboratory (ALS Laboratory Group). These analyses included Total Nitrogen, Total Kjeldahl Nitrogen (TKN), TAN, reactive silicate, and combined nitrate and nitrite (NOX), all of which were measured twice weekly in the first year and then once weekly thereafter. Total phosphorus (TP), filterable reactive phosphorus (FRP), total inorganic carbon (TIC) and total organic carbon (TOC) were measured once each week. The analysis of these constituents in the pond water was stopped shortly before harvesting, about 120 d after stocking, as the key data had been obtained by that stage.

Samples of water from each of the pond were examined at least weekly under a microscope to make a semi-quantitative assessment of the dominant microalgal species or taxa that were present. Generally no more than the five most dominant “species” were noted. Abundance was scored as: absent = 0; low density = 1, medium density = 2; and high density = 3. In this way a Diatom Score and a Blue-Green Score was obtained. In the first year, testing was also carried out twice weekly to determine the presence and relative density of *Vibrio* bacteria in the ponds using a TCBS kit (INVE Aquaculture Health).

3.2.2. 2008/09 Season

The same five ponds as were monitored in 2007/08 were studied in 2008/09. The ponds were fertilised and managed in essentially the same way as in the previous year, except that sodium silicate (waterglass, 35% sodium silicate solution) was added to the ponds in several doses during the first two weeks after filling to provide about 0.5 mg/L of reactive silica to the pond. All ponds on the farm were treated in a similar manner with sodium silicate, so there were no control ponds which could be used to compare the effectiveness of the treatment. The ponds were stocked with 50 PL’s per m^2 14 days after the ponds were filled with water.

A less intensive monitoring of the five study ponds was carried out through the season, ending shortly before harvesting, about 120 days after stocking. In this year NOX in the pond water samples was not analysed as data from the first year indicated that the levels were very low and it appeared that NOX was not a driving factor in the establishment and maintenance of the biofloc. Though twice daily measurements of DO, pH and water temperature were carried out throughout the season, the analyses of pond water samples to determine the concentration of the chemical constituents, floc volume and microalgal population structure were scaled back to just once each week.

3.2.3. 2009/10 Season

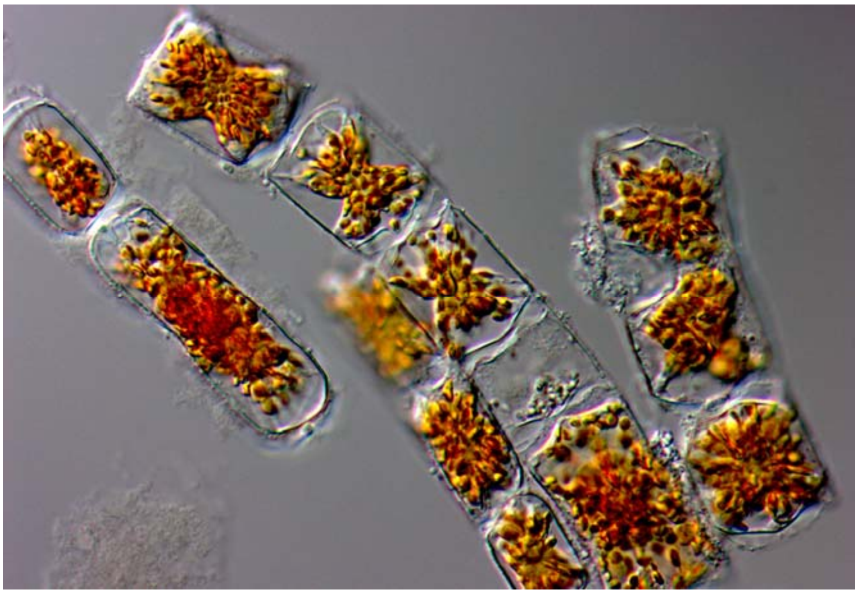
As in the previous two years, the same five ponds were assigned to the study. The ponds were fertilised and managed in a similar manner to the 2008/09 season, except that the nitrogenous fertiliser, urea, was replaced with a liquid fertiliser containing a mixture of urea and ammonium nitrate (1:1 on a nitrogen basis), (Easy N, Incitec Pivot) in most of the ponds on the farm, including the five study ponds. Six other ponds were fertilised only with urea to provide controls for the new treatment. The Easy N was applied to give the same amount of nitrogen as when urea alone was used as the fertiliser.

Due to difficulties at the hatchery at APF, there were problems with the supply of postlarvae to stock the ponds. As a result, most of the ponds on the farm were stocked at a date later than that considered optimal and significantly later than in previous years. The ponds that were to have been used as controls were stocked even later and as a result, had to be emptied and re-filled and the fertilisation regime re-started before the ponds could be stocked. Consequently, they could not be considered as valid controls. The study ponds and "control" ponds were stocked with 50 postlarvae per m^2 as in 2008/09. However, the survival of these postlarvae was lower than survival in previous years, which impacted on the amount of feed added to the ponds and hence the nutrients that entered into the pond system and, consequently, affected the development of a biofloc.

The same intensive monitoring of the five study ponds as was carried out in 2008/09 was carried out throughout the 2009/10 season, ending shortly before harvesting, about 120 days after stocking. The ponds that were to have been used as controls were not sampled, due to the cost of the analyses and due to the fact that they could not be considered as valid controls. In order to obtain some scientifically robust data to compare the effect of using Easy N instead of urea as the nitrogen-rich fertiliser, a large tank experiment was carried out at CSIRO. This experiment is reported in Section 4.3. APF had installed a Davis Weather Station – Vantage Pro2 – to measure weather conditions, including light intensity, on the farm. With this instrument, a record of the on-farm solar radiation intensity was obtained throughout the 2009/10 season. This data were compared with the daily data of hours of sunlight recorded at the Bureau of Meteorology office in Mackay to determine how well the Mackay observations correlated with the on-farm observations.

3.3. Fertiliser comparison trial

During the first two years of the project, it was found that the establishment of a bloom of diatoms within the first four to seven weeks of filling the prawn ponds contributed significantly towards the likelihood of establishing and maintaining a biofloc throughout the season. At the start of the season, the empty ponds were filled with filtered water from the inlet channel and were then fertilised with urea, MAP and sodium silicate. Bacterial action converts the urea to ammonia/ammonium ions which provides a nitrogen source for bacteria and microalgae, including the diatoms.



Photograph 5. Diatom *Bellerochia horologicalis* (Photograph courtesy of Collection of Living Microalgae, CSIRO Marine and Atmospheric Research, Hobart)

It has been reported that some species of diatoms grow faster when nitrate rather than urea/ammonia is provided as the nitrogen source (Tadros and Johansen, 1988; Thompson et al. 1989; Harrison and Berges, 2005). APF were interested in comparing the effectiveness of the two nitrogen-rich fertilisers: (a) urea and (b) Easy N (Incitec Pivot Ltd) during the 2009/10 season. Easy N is a liquid fertiliser that has approximately half its nitrogen present in urea and half in ammonium nitrate. Due to the listing of solid ammonium nitrate on the Dangerous Goods list and its limited availability, fertilisers containing mostly solid ammonium nitrate are no longer viable options for use on prawn farms. Hence Easy N provided a practical way of assessing the benefit of using a significant amount of ammonium nitrate in the fertilisation of the ponds. It had been the intention for this comparison of fertilisers to use a number of 1-hectare ponds at APF that were filled at the same time, and which were stocked with prawn postlarvae at about the same time, and that were fertilised and treated in the same way except for the type of nitrogen fertiliser. However, due to the significant delays in the availability of the postlarvae to stock the ponds assigned to the urea treatment, a scientifically rigorous comparison of the two fertilisers could not be made. As a result, it was decided to carry out this study, to compare the establishment and growth of microalgal blooms when fertilised with either urea or Easy N, using 2.5 tonne tanks in a controlled environment facility at CSIRO.

An array of 26 x 2500 L tanks in the tunnel house at CSIRO, Cleveland was used for the study (Photograph 6). The fibreglass tanks were filled with filtered seawater (20 µm) drawn from Moreton Bay and were maintained at ambient temperature of about 30°C. The tanks were aerated and mixed using two air stones that were located on opposite sides of the tanks. The air supply to these air stones was switched alternatively in 2 minute cycles to provide good mixing of microalgae within the tank. The experimental treatments comprised 13 tanks fertilised with urea (Incitec Pivot, Australia, Prill/granules; Nitrogen: 46.0%) and 13 tanks fertilised with Easy N (Incitec Pivot, Nitrogen: 42.5%). The Easy N dosage provided the same amount of total nitrogen as in the urea treatment. The tanks were set up in two rows and alternate tanks were assigned to each treatment, ensuring that any position effects were evenly distributed between the two treatments. The experiment was run for four weeks in order to observe the succession of microalgal species over a useful time interval.

The tanks were fertilised using the same materials and proportionally equivalent quantities, and at similar protocols as used at APF. On day 1, 3.54 mL of Easy N (urea + ammonium nitrate) was added to each of the 13 tanks assigned to the Easy N treatment, and 3.27 g of urea was added to the 13 tanks of the urea treatment. In addition, 1.63 g of MAP (Multi-MAP, Haifa, Israel) and 4.4167 g of sodium metasilicate pentahydrate (Metso 520, Ineos Silicas, Netherlands) were added to every tank. Thereafter, on a daily basis, the equivalent of 0.3 mL of Easy N and 0.1386 g of MAP was added to the tanks receiving Easy N, while the equivalent amounts of 0.2772 g urea and 0.1386 g MAP were added to the tanks receiving the urea treatment. After 14 days, a further addition of 4.4167 g of sodium metasilicate pentahydrate was made to every tank.

Photograph 6. The tank system used in the study to compare the effects of using the two fertilisers - urea and Easy N - to establish microalgal blooms.

To achieve fertilisation rates described above, three solutions were prepared: (a) sodium silicate, (b) combined Easy N and MAP, and (c) combined urea and MAP.

The sodium silicate solution was prepared by dissolving 176.7 g of sodium metasilicate-pentahydrate in deionised water and making it up to 2000 mL. 50 mL aliquots were dispensed into each tank (2500 L, providing 0.5 mg/L of silica (SiO_2).

The combined Easy N and MAP solution was prepared by dissolving 204 g of MAP in 1 L of water, adding 441.5 mL of Easy N (the equivalent of 582.8 g of Easy N) and making up to 2500 mL. The resulting solution contained 0.0816 g/mL MAP and 0.1766 mL Easy N/mL of solution (or 0.2331 g/mL Easy N). The Total Nitrogen in the solution was 84.4 mg/mL. At the start of the experiment, 20 mL of this solution was pipetted into each even numbered tank. Thereafter, the daily dose of 3.0 mL per tank of this solution added 0.198 mg/L nitrate and 0.068 mg/L ammonium to the water, or alternatively 0.10 mg/L Total Nitrogen.

The combined urea and MAP solution was prepared by dissolving 163.3 g MAP and 326.4 g of urea in deionised water and making it up to 2000 mL. The resulting solution contained 0.0816 g/mL MAP and 0.1632 g/mL of urea. The Total Nitrogen concentration in the solution was 84.9 mg/mL. At the start of the experiment 20 mL of this solution was pipetted into each odd numbered tank. Thereafter, the daily dose of 3.0 mL of this solution added 0.10 mg/L of Total Nitrogen to the water.

The tanks were monitored twice daily (early morning and mid afternoon) for water quality (DO, pH, salinity and temperature) using a YSI data logger. Total alkalinity was measured periodically. However, as it remained within acceptable limits (between 100 and 120 ppm $CaCO_3$) there was no need to add sodium bicarbonate to adjust the alkalinity.

Microalgal density was estimated daily using a Secchi disk and tank water samples were taken on alternate days, three times each week for examination under a microscope. The dominant microalgal species were broadly identified and their density quantified as: absent, low, medium or high density. A member of the staff from APF (Andrew Smith) came to Cleveland at the start of the experiment and demonstrated to the technicians who were carrying out the microalgal identification a practical method for scoring the microalgal populations in the tanks.

The walls of the tanks were scrubbed each week to prevent epiphytic algae becoming established in the tanks, however benthic algae and organic biomass did become established on the lower part of the walls and the bottom of the tanks as these could not be stirred or cleaned effectively.

Salinity was maintained close to 35‰ using a fresh water drip system that replaced water lost through evaporation. Total alkalinity was measured by titration to an end point at pH 4.5 using a Metrohm Autotitrator (Model 719 S Titrino) filled with standardised hydrochloric acid (0.2501 N). TAN was determined using a Saltwater Master Liquid Test Kit (Aquarium Pharmaceuticals Inc.) that had a practical detection limit of about 0.1 mg/L TAN. Nitrate concentrations were determined using a Tropic Marin Test Kit (www.tropic-marin.com) that had a practical detection limit of about 1 mg/L.

The effectiveness of the two fertilisers in encouraging a microalgal population was assessed from the daily Secchi disc readings, and the affect on encouraging a diatom population was assessed from microscope observation of the tank water samples.

4. RESULTS

4.1. Dynamics of water chemistry, microalgae and biofloc

A key objective of this project was to determine the effects of variations in nutrient concentrations and water exchange on microbial floc formation, composition and persistence in commercial *P. monodon* ponds. After the ponds were filled at the start of the season, fertiliser (urea and MAP) was added to each pond daily or every few days in small amounts (typically 2 to 3 kg of urea and 1 to 1.5 kg of MAP) to provide the nutrients to develop a microalgal bloom. These fertilisers add ammonia/ ammonium to the pond, of which only part is taken up by the microbial population, predominantly microalgae, bacteria, yeasts and protists. As a result, the TAN concentration will increase progressively. As the microalgal blooms develop, the microalgae take up both nitrogen and carbon dioxide (CO_2) from the water. The uptake of CO_2 by microalgae in turn affects the pH of the ponds. The application of fertilizer is continued until the nutrients present in the feed being distributed in the pond each day is equivalent to the minimum dosage of fertilizer. However, fertilizer applications are stopped once the TAN concentration reaches 1 mg/L. Generally this occurs 4 to 6 weeks after filling the pond. Avnemelech (2009) suggests that fertilisation of a pond to encourage microalgae is only needed if the nitrogen and phosphorus concentrations are less than 1 mg/L and 0.1 mg/l, respectively.

Throughout each of the three seasons of this study, nutrient concentrations in the ponds were monitored on a regular basis, as were the microalgal populations and biofloc density. The measurement of the concentrations of “gross” nutrients such as total nitrogen (TN), total Kejdahl nitrogen (TKN) and total phosphorus (TP) were found to be less useful than that of the reactive forms of the nutrients: TAN, reactive silica, FRP; as these are the part of the gross amount of each of these nutrient categories that is available to bacteria and microalgae. As a consequence, the focus in the discussion will be on the reactive nutrients.

4.1.1. Relationship between diatoms and biofloc

During the 2007/08 season, observations of the five ponds assigned to the project and of the other 28 ponds at APF suggested that a moderate or strong diatom bloom tended to lead to the appearance of a biofloc in the pond. This is illustrated in Figure 1 with data from Pond 8. The moving averages of over two successive samples have been used to smooth the biofloc density curves. In addition, a strong blue green algae (or cyano bacteria) bloom was also observed to be able to produce a weak floc.

The 2007/08 data from the five assigned ponds were analysed to investigate the relationship between the density of a bloom of large diatoms and the amount of biofloc in the pond, using the volume of suspended solids in 1-litre water sample as a measure of biofloc density. This showed that on 77% of the occasions when there was a medium or high density of large diatoms, a biofloc density of > 0.1 mL/L would be present. In contrast, when a medium or strong blue green algal bloom was present, on only 9% of these occasions was a biofloc density of > 0.1 mL/L detected. The 0.1 mL/L of biofloc density has been used as a cut-off point as that is the smallest measurement of settleable solids that can be made using a 1 L Imhoff cone, and that amount of settleable solids can occur through the presence of material other than biofloc.

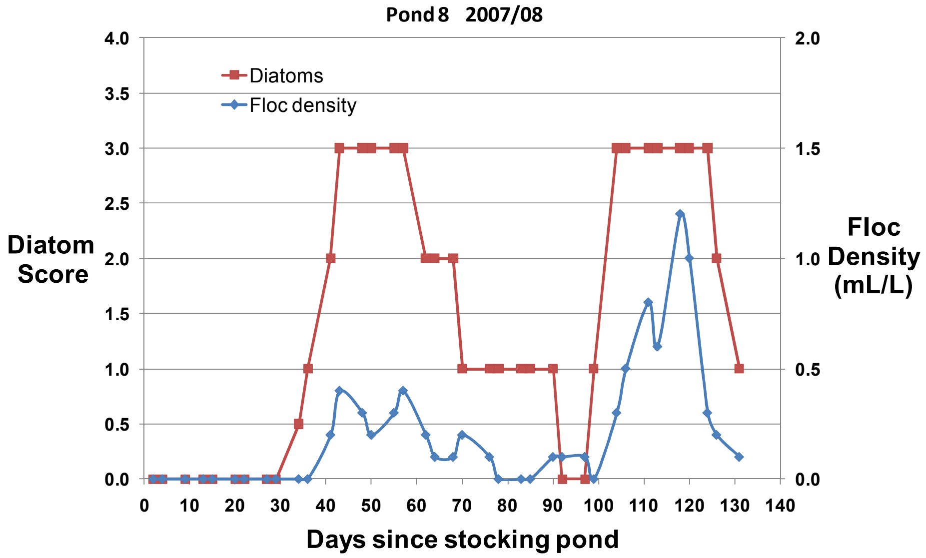


Figure 1. Relationship between Diatom Score and the moving average (n=2) of Floc volume (mL/L) for Pond 8 between 29 Sept. 2007 and 6 Feb 2008

Table 1: The frequency of occurrence and number of occurrences of the presence or absence of biofloc density of > 0.1 mL/L is shown with the concurrent occurrences of the four diatom density gradings. A significant relationship between diatom density and presence of biofloc at concentrations above 0.1mg/L was detected ($p < 0.001$).

		No Diatom	Low	Medium	High
Biofloc	No	86%	49%	32%	6%
>0.1mg/L	Yes	14%	51%	68%	94%
	Total No. of occurrences	201	55	37	63

At the end of the study, all of the data from the five assigned ponds over the three years were pooled and the relationship between the presence of a moderate or strong diatom bloom and biofloc density was re-examined using more rigorous statistical analysis. The results confirmed the relationship and were fully supported by observations from the other 28 ponds on the farm over the three seasons. The analysis shows a significant relationship between the diatom density score and the presence or absence of biofloc with a density of > 0.1mg/L ($p < 0.001$) (Table 1). This finding then enabled the research work to be focused on the factors that would be beneficial for, or encourage, the establishment and maintenance of a diatom bloom.

4.1.2. Total Organic Carbon

Total organic carbon (TOC) gives a practical indicator of microalgal biomass in the ponds. This is illustrated graphically in Figure 2. The peak in TOC that occurs at 98 days after filling the pond, which does not co-incide with a peak in diatom score, is due to a strong blue green algal bloom. Over the three seasons, there appeared to be a relationship between TOC concentration and the Diatom Score. Sharp declines after peaks of TOC indicate a microalgal population crash, as demonstrated in Figure 2. The level of TOC can suggest the need for management intervention to prevent a microalgal crash or to minimise its magnitude.

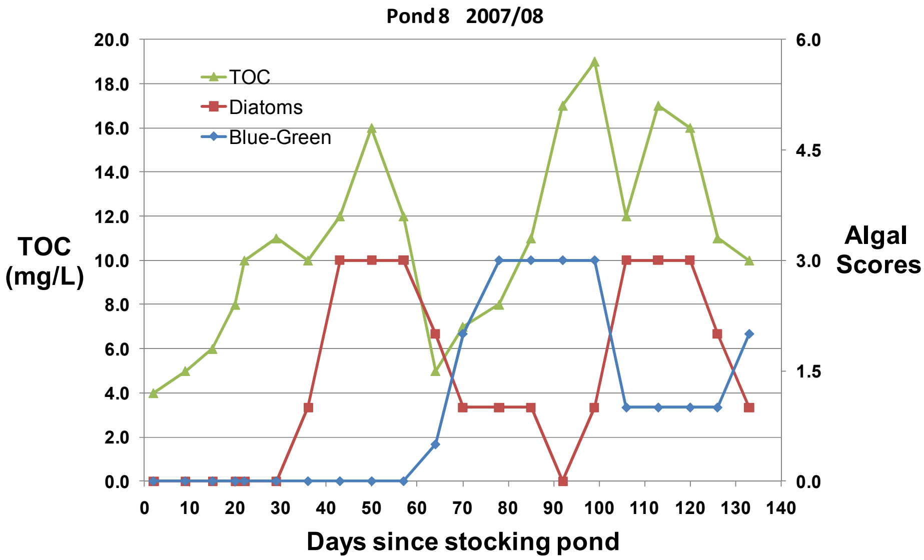


Figure 2. Relationship between Total Organic Carbon (TOC) and Diatom Score and Blue green algae Score for Pond 8 between 29 Sept. 2007 and 6 Feb 2008

The changes in TOC in the five assigned ponds over the three seasons are presented in Figures 3a, 3b, and 3c. There was a similar trend in the 2007/08 and 2008/09 seasons but a markedly different trend in 2009/10, when the ponds were filled later in the season and were fertilised with Easy N rather than urea. It appears that the nitrate in Easy N enabled a very stable bloom of pioneering Chlorella species to become established, resulting in the high levels of TOC in the first 20 days following the filling of the ponds. Though the ponds were filled later in the season in 2009/10, there was not a great difference among the water temperatures with season (Figure 4).

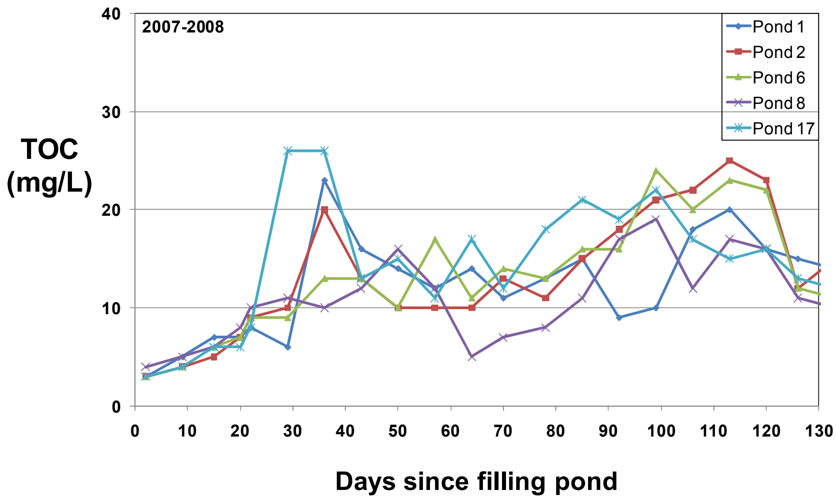


Figure 3a. Pond water Total Organic Carbon (TOC) (mg/L), 27 September 2007 to 6 February 2008

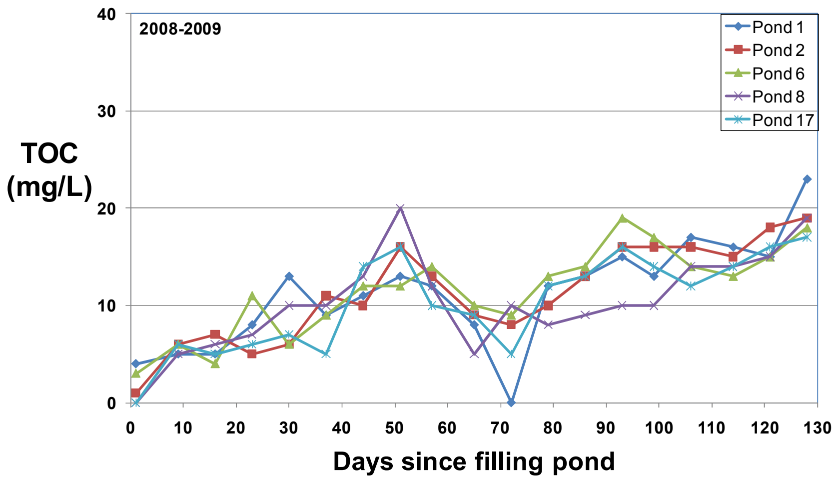


Figure 3b. Pond water Total Organic Carbon (TOC) (mg/L), 16 September 2008 to 21 January 2009

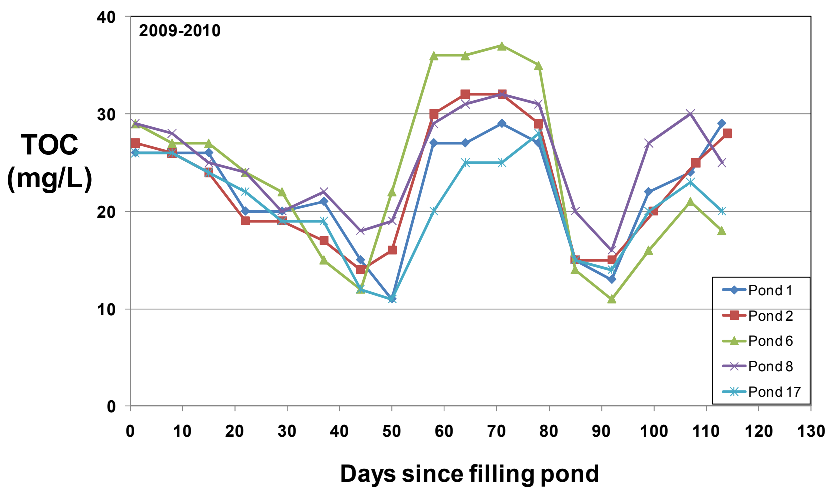


Figure 3c. Pond water Total Organic Carbon (TOC) (mg/L), 13 October 2009 to 11 February 2010

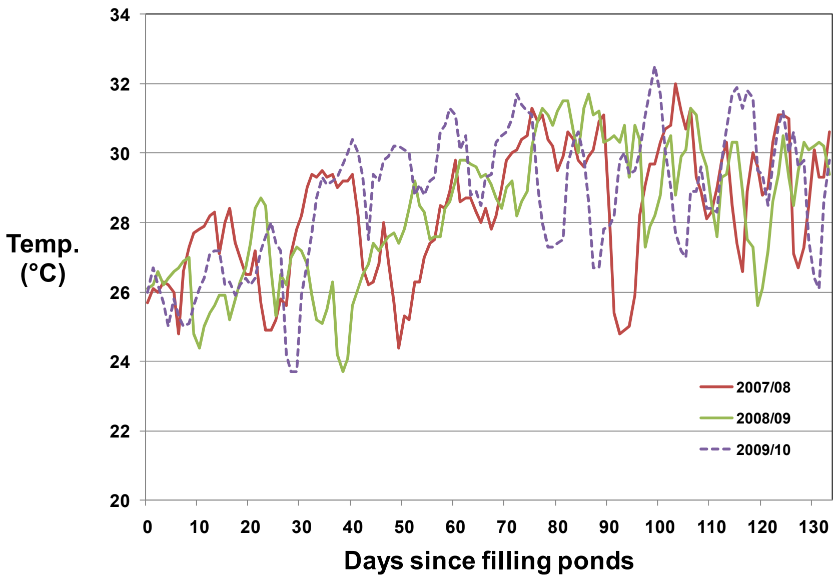


Figure 4. Pond water temperatures (°C) at Australian Prawn Farms in the 2007/08, 2008/09 and 2009/10 seasons.

4.1.3. Total Ammonium Nitrogen (TAN)

TAN concentration in water is the sum of the ammonia (NH_3) and ammonium ion (NH_4^+) concentrations. The ammonia is produced through the action of bacteria on the urea, from microbial decomposition of uneaten feed, waste matter and dead microalgal and bacteria, and from metabolic processes in organisms, particularly the prawns in the pond. TAN concentrations of about 40 mg/L result in 50% mortality of prawns, whereas a maximum "safe" level is considered to be between 3.5 and 4.3 mg/L (Allan et al. 1990, Chen, and Lei, 1990; Chen et al., 1990; Wickins, 1976).

The concentration of TAN in the five assigned ponds over the three seasons is presented in Figures 5a, 5b and 5c. In 2007/08 and 2008/09 there was a consistent increase in TAN over the first weeks of the season that reached a peak which was generally >1.0 mg/L, and which occurred between 30 to 45 days after the ponds were filled. During this period the ponds were fertilized with urea and a microalgal and bacterial population built up using some of the urea nitrogen. This increase in the microalgal and bacterial population is shown by the increase in TOC in Figures 3a and 3b. The increase in TAN was brought about through the combined effect of the fertilization and significant crashes in the microalgal populations within the pond. The addition of urea fertilizer to the pond ceased once a strong microalgal bloom was established or once the TAN had reached 1 mg/L. In 2009/10 the TAN peak did not appear in any of the ponds during the first 45 days, even though the ponds were being managed in the same way as in previous years. The difference appears to be due to the use of Easy N (50% urea, 50% ammonium nitrate) as the fertilizer rather than urea, which had been used in the previous seasons. It appears that the Easy N helped establishment of a pioneering species of algae - typically chlorella - with exceptional stability. The peaks in TAN around 60 days were due to very overcast conditions during which time there was limited photosynthesis and thus the microalgae could not take up the ammonia.

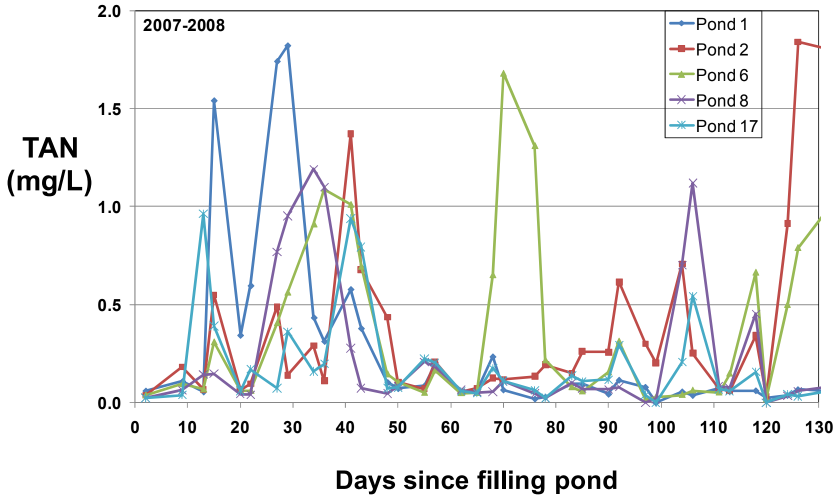


Figure 5a. Pond water Total Ammonia Nitrogen (TAN), 27 Sept 2007 to 6 Feb 2008

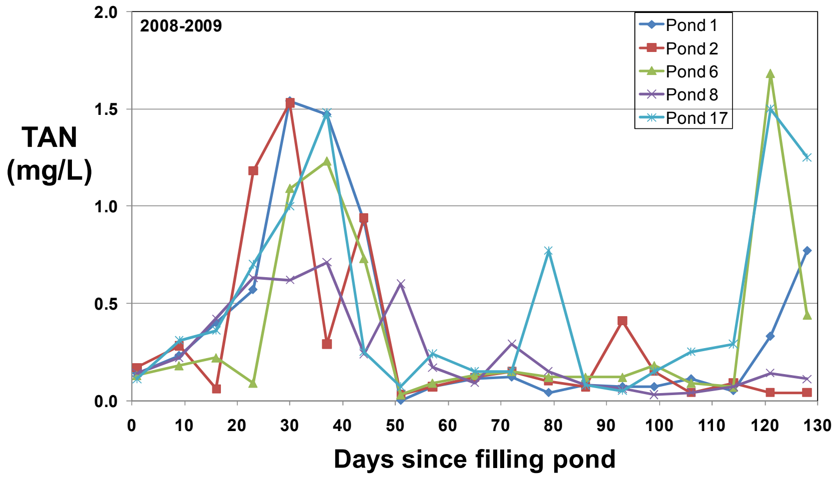


Figure 5b. Pond water Total Ammonia Nitrogen (TAN), 16 Sept 2008 to 21 Jan 2009

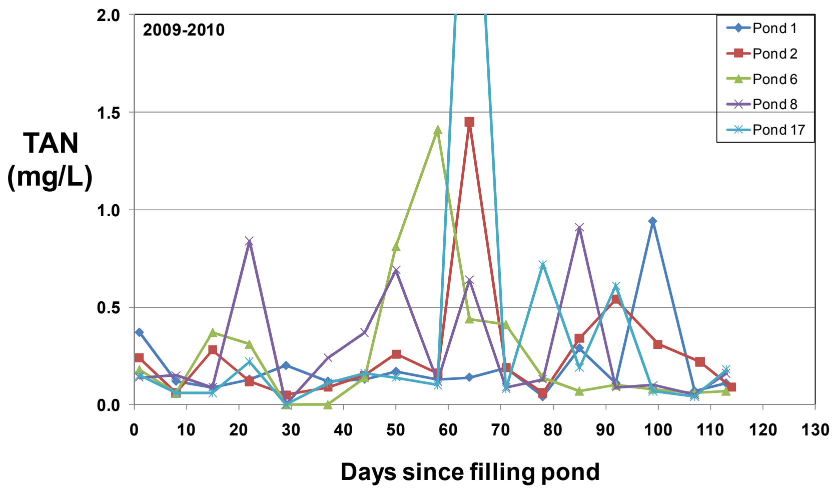


Figure 5c. Pond water Total Ammonia Nitrogen (TAN), 13 Oct 2009 to 11 Feb 2010

The peak in TAN that occurred 30 to 45 d after filling the pond appeared necessary for the development of a strong bloom of large diatoms within the following 30 to 40 days. This is illustrated in Figure 6 with data from Pond 8 in the 2007/08 season. The time that elapses between the peak in TAN and the appearance of the diatom bloom is quite variable due to many factors including the duration and intensity of sunlight, the concentrations of other nutrients required by the diatoms and the presence and density of other microalgae. Fisher's exact test was used to test the hypothesised necessity of a TAN peak above 0.9 mg/L in the first 50 days in order that a diatom density score greater than or equal to 2 be observed within 40 days of the TAN peak, or in the absence of a TAN peak, within the period 30 days to 70 days after filling the pond. The presence or absence of each event was tabulated (Table 2). Fisher's exact test showed that there was a significant relationship between a TAN peak and a diatom bloom ($p = 0.001$). Importantly, if a peak in TAN of >0.9 mg/L does not occur, it is unlikely that a medium or strong bloom of large diatoms will occur and hence it is unlikely that a biofloc will become established with an adequate density (> 0.1 mL/L). In 2009/10 season, a TAN peak of >0.9 mg/L did not occur in the first 45 days, and neither did a medium or strong bloom of large diatoms become established until late in the season, and a good biofloc failed to develop. The development of a bloom of large diatoms later in the 2009/10 season was always preceded by an increase in TAN of at least 0.9 mg/L. A biofloc can develop later on in the season when there is a large biomass of prawns in the pond and there is, consequently, a significant daily input of nutrients into the pond in the form of feed. These late-season bioflocs can also be associated with a cyanobacteria bloom.

Table 2: The number of ponds that had a TAN peak ≥ 0.9 mg/L within 50 days of filling the pond, and the number of ponds that had a diatom bloom with a density score of ≥ 2 within 40 days of the TAN peak, or in the absence of a TAN peak, within the period 30 days to 70 days after filling the pond ($p = 0.001$).

		TAN score (≥0.9 mg/L) from day 0-50		
		No	Yes	Total
Diatom density score ≥ 2	No	6	0	6
	Yes	1	8	9
	Total	7	8	15

4.1.4. pH and ammonia

Generally, the pH of prawn ponds is maintained between about 7.6 and 8.6. At high pH and at high temperatures a greater proportion of the TAN is present as un-ionised ammonia rather than the ionised ammonium ion form (Truswell, 1972; Whitfield, 1974). The pH is driven largely by the amount of dissolved carbon dioxide (CO_2) in the water, and the equilibrium between CO_2, carbonate and bicarbonate ions. Ammonia is far more toxic to prawns than ammonium ions (Smart, 1978). When the pH is at 8.2 about 10% of the TAN is present as ammonia; this increases to 50% when the pH increases to 9.3 (Figure 7). The values reported in the literature for the concentrations of un-ionised ammonia that adversely affect prawns are quite variable, due to variations in experimental conditions such as pH, temperature, salinity, oxygen concentration and age or size of the prawns. However, as a generalisation, the lethal dose where 50% of the prawns will die (LD_{50}) is 1 to 2 mg/L of un-ionised ammonia, whereas the "safe" level is generally considered to be < 0.2 mg/L (Allan et al. 1990, Chen and Lei, 1990; Chen et al., 1990; Wickins, 1976). Hence, the management of the TAN concentration and pH is critical in all prawn culture systems.

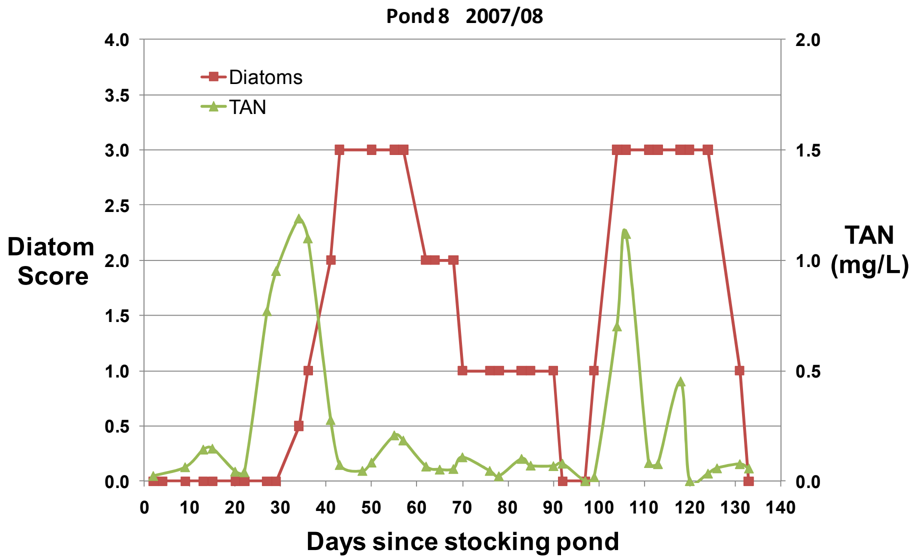


Figure 6. Relationship between Total Ammonia Nitrogen (TAN) (mg/L) and Diatom Score in Pond 8 between 29 Sept. 2007 and 6 Feb 2008

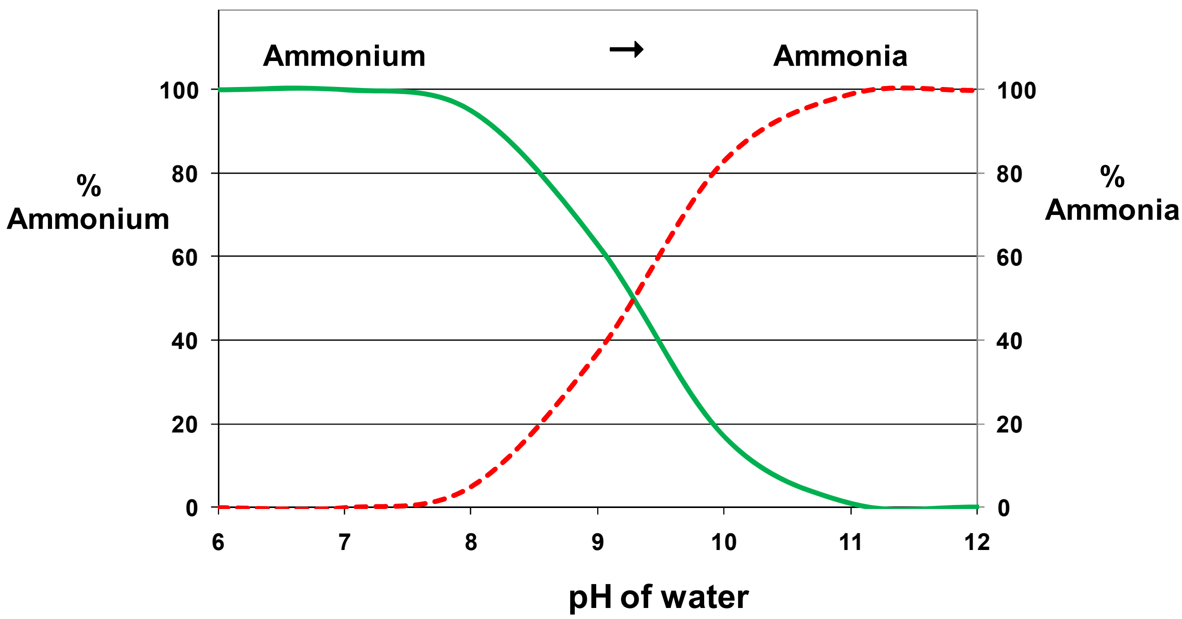


Figure 7. Relationship between pH and the percentage of ammonia and ammonium ions (expressed as a percentage of the sum of the ammonia and ammonium ion concentrations).

Over each of the three seasons, the pH of the study ponds behaved in a manner similar to that which was consistently observed in the other ponds at APF when using the low water-exchange system (Figure 8a, 8b, and 8c). The pH was observed to increase over the first 20 days from about pH 8.3 to a maximum of > 9 as the density of the microalgal bloom increased the concentration of dissolved CO_2 decreased. Thereafter, the pH tended to decline as the biomass of prawns increased and the amount of respired CO_2 increased. However, occasionally as the season progressed, farm intervention was required to increase pH through the application of sodium carbonate or hydrated lime. Sharp decreases in pH, such as illustrated at around 70 days (Figure 8a), were generally associated with either a microalgal population crash or a period of cloudy weather. During periods of cloudy weather, photosynthesis does not occur at a rate sufficient to take up the CO_2 produced by the respiration of the prawns in the pond, and so the pH decreases.

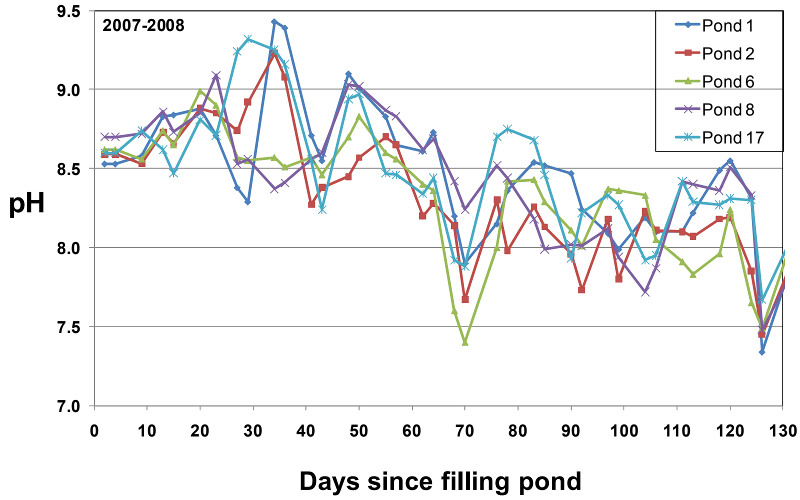


Figure 8a. Pond water pH throughout the period 27 September 2007 to 6 February 2008

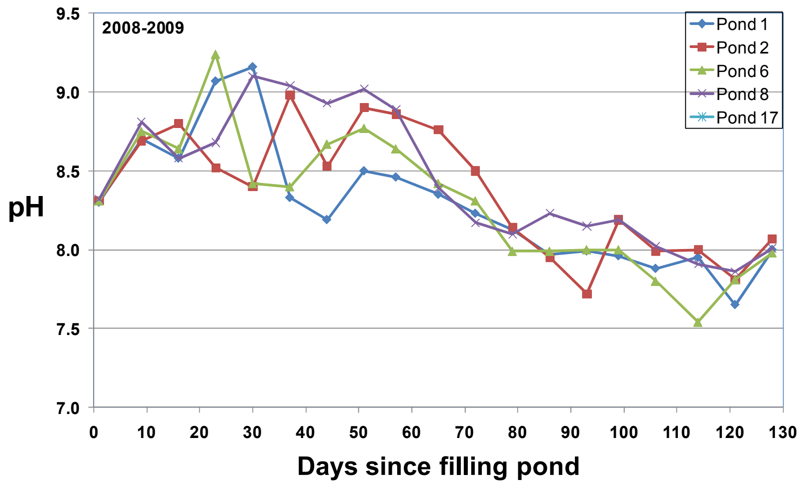


Figure 8b. Pond water pH throughout the period 16 September 2008 to 21 January 2009

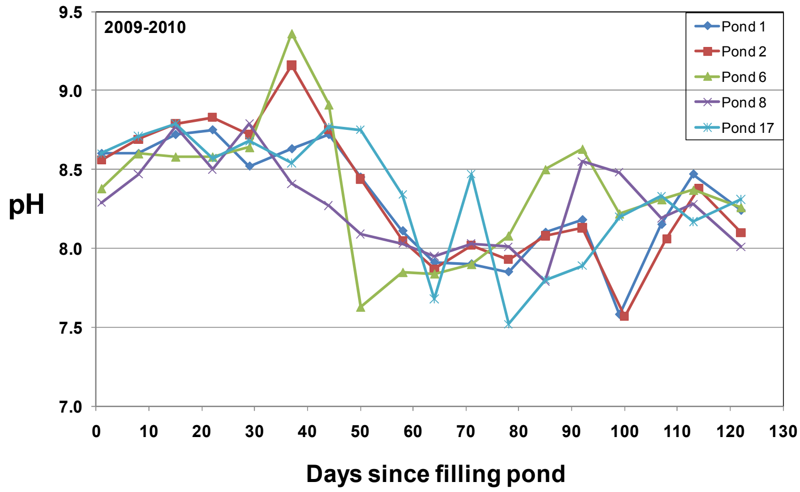


Figure 8c. Pond water pH throughout the period 13 October 2009 to 11 February 2010

At APF early in the season when the peaks in ammonia occur, the prawns were about 30 days old (30 days after they have metamorphosed to post larvae). The pond managers at APF accept TAN concentrations of up to 1.5 mg/L when the pH is between 9.0 and 9.5 for a few days. Under these conditions the un-ionised ammonia concentrations would be between 0.54 mg/ and 0.9 mg/L, depending on the pH. However, other factors that lessen the effect of the ammonia need to be considered – factors such as the dissolved oxygen level, salinity and temperature of the ponds. High dissolved oxygen, high salinity and low temperature all tend to reduce the toxic effect of the un-ionised ammonia. At APF at this time of the year the salinity is generally high (> 35 ‰) and the temperature is generally low (<28° C).

Many microalgal species do not tolerate high pH (pH > 9) very well, while some species of diatoms dominate in environments of high pH (Goldman et al. 2003a, Goldman et al., 2003b). It is possible that the peak in TAN develops as a result of the pH increasing to above the tolerance limit of species of microalgae in the ponds, resulting in some species of microalgae dying off. The dying off of the microalgae would result in less uptake of TAN and more TAN being released through the decomposition of the microalgae. It is possible that this is also occurring in other groups within the microbial community. The appearance of large diatoms following this event suggests that the large diatoms may be more tolerant to the high pH. As most conventionally managed prawn farms would not be willing to allow the pH of the ponds to reach 9.0, or let the TAN in ponds increase to the levels seen at APF, they would not see the development of blooms of large diatoms and the development of biofloc.

4.1.5. Total Inorganic Carbon (TIC)

Total inorganic carbon (TIC) is the sum of dissolved CO_2, bicarbonate (HCO_3^-) and carbonate (CO_3^-). As such, TIC is closely related to Total Alkalinity, and comprises a subset of the ions that make up Total Alkalinity. In the first 20 to 30 days after filling the ponds, the TIC declined in all ponds as the dissolved CO_2 was used by the microalgae and some of the bicarbonate was taken up by the slow-growing litho-chemoautotrophic bacteria (Figure 9a, 9b and 9c). Thereafter the pattern of TIC concentration in the ponds was quite variable due to events within the pond, but TIC and pond water alkalinity were generally maintained within defined limits (60 to 120 mg/L) by the addition of sodium bicarbonate.

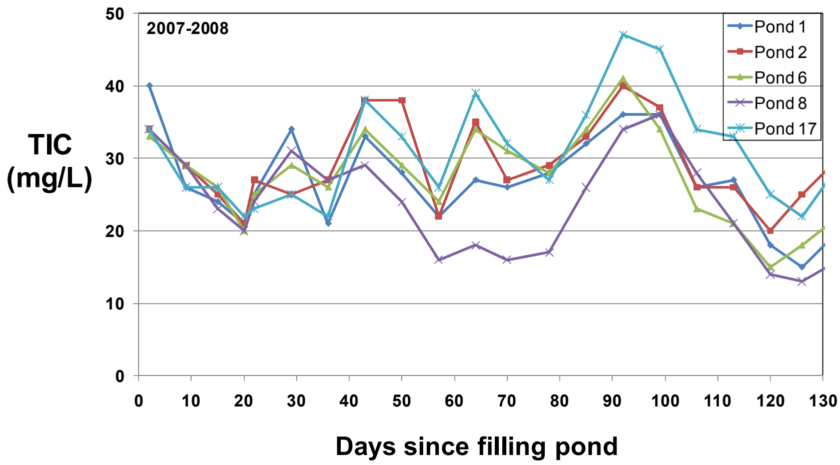


Figure 9a. Pond water Total Inorganic Carbon (TIC), 27 September 2007 to 6 February 2008

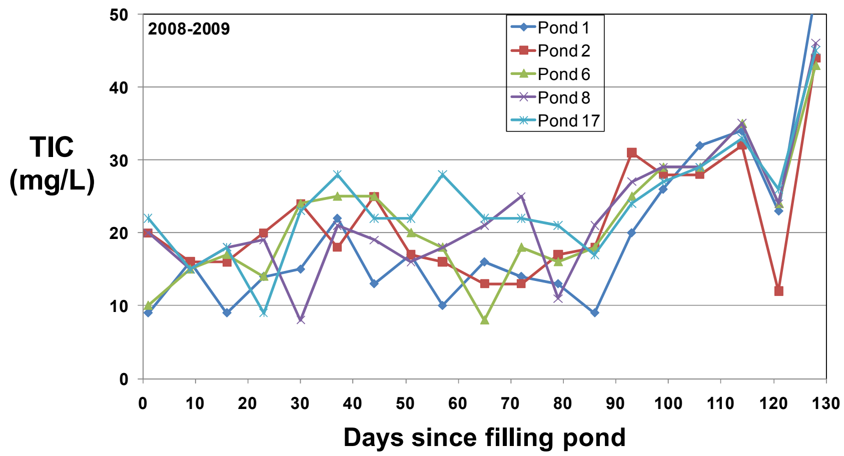


Figure 9b. Pond water Total Inorganic Carbon (TIC), 16 September 2008 to 21 January 2009

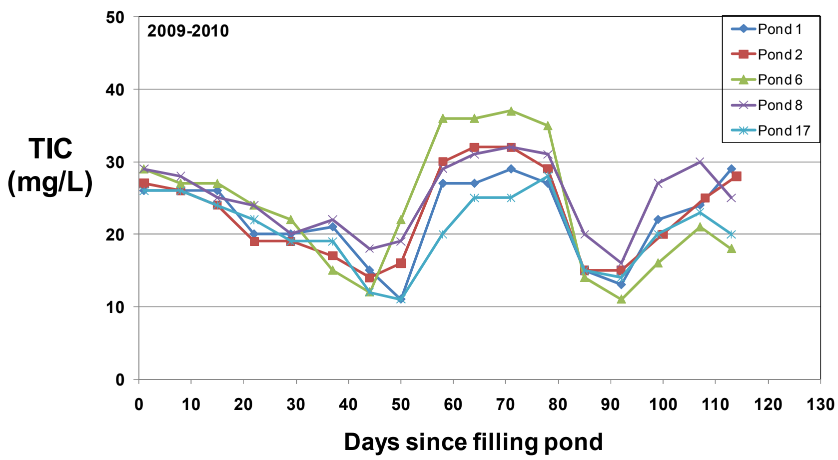


Figure 9c. Pond water Total Inorganic Carbon (TIC), 13 October 2009 to 11 February 2010

4.1.6. Silica and silicate

Silica is an essential nutrient for diatoms though it is not required by other microalgal species (Paasche, E. 1973, Becker, 1994, Gilpin et al. 2004). It is poorly soluble in water at pH < 9.0. However, it becomes increasingly soluble as the pH becomes more alkaline, with a marked increase in solubility when the pH increases above 9.0 (Iler, 1979).

The reactive silica concentration in the study ponds over the three seasons is shown in Figures 10a, 10b and 10c. There was a gradual increase in silica concentration in the early part of the season. In 2007/08 this was a result of natural processes in the pond (Figure 10a), and was probably mainly due to the increase in pH (Figure 8a) increasing the dissolution of silica from the pond sediment and from dead diatoms. In subsequent seasons the increase was brought about through both the natural processes in the pond and the addition of sodium silicate. The data were quite variable due to the dynamic nature of reactive silica in the pond system, and because of the addition of sodium silicate as part of the pond management scheme. The variability of the data illustrates the need to determine both reactive silica (silica in solution) and total silica (silica in solution and bound in microalgae and particulate matter in the water).

As observations in 2007/08 throughout APF indicated that a more stable biofloc was produced by a diatom bloom than by cyanobacteria or dinoflagellate blooms, it was decided to ensure that there was sufficient reactive silica in the ponds to support a diatom bloom. The required level of silicate in the water had not been established in 2007/08, and there are conflicting opinions in the literature on what is the optimal concentration for large diatoms. However, two hypotheses relating to silica concentration in the ponds were developed from on-farm observations and measurements made from samples of the five test ponds during the 2007/08 season (Figure 10a). The first hypothesis was that the silica concentration in the pond water needed to be greater than 0.2 mg/L for a diatom bloom to become established. The second was that the silica concentration should be about 0.5 mg/L for the diatom bloom to be established with reasonable reliability.

It was decided that during 2008/09 season the effect of adding soluble silicates (water glass, 38% sodium silicate) to the ponds would be examined to determine the effect on promoting diatom blooms. APF decided that it would add sodium silicate to all the ponds to maintain a concentration of between 0.2 and 0.5 mg/L of reactive silica in them. Consequentially, there were no control ponds in which there had been no addition of sodium silicate. Hence, it was not possible to rigorously test the effect of the silicate.

In 2009/10 APF also dosed the ponds with sodium silicate during the initial fertilisation period and intermittently during the season. The high silica concentrations in the period 40 to 80 days after filling the ponds was probably due to the fact that diatoms were not the dominant algal species, so the silica was not being taken up by the microalgae. The high TOC at this time was likely to be due to chlorella being the dominant species.

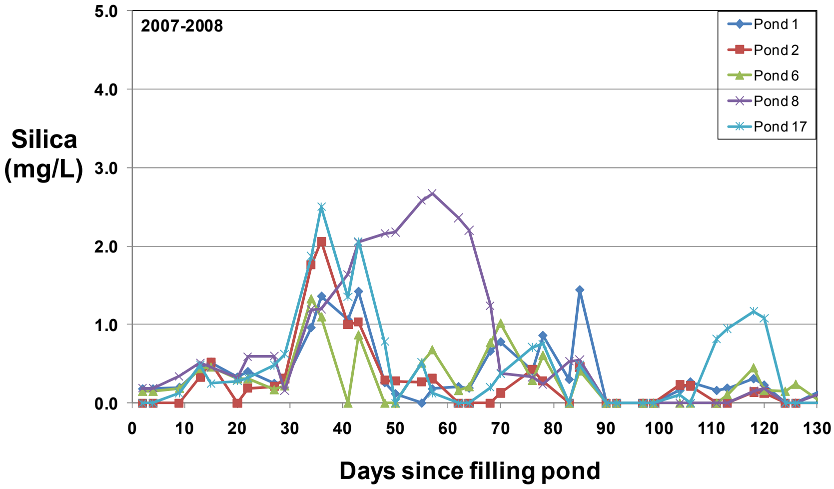


Figure 10a. Silica (SiO_2) concentrations (mg/L) in the ponds between 29 Sept. 2007 and 6 Feb 2008

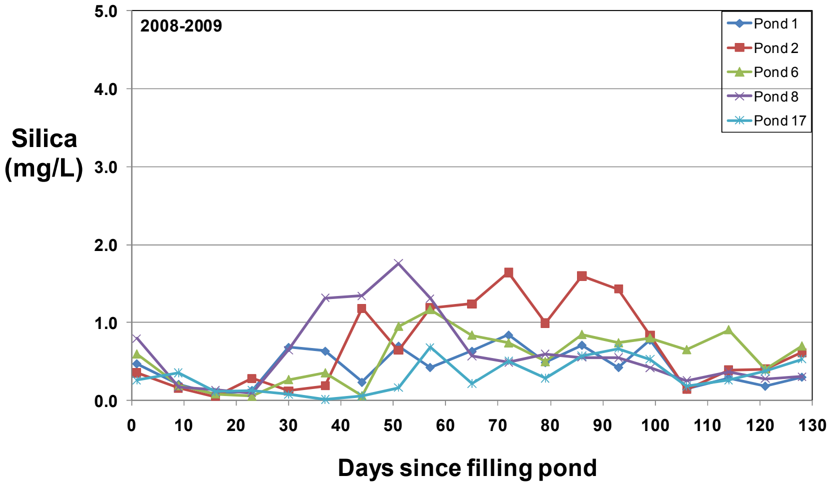


Figure 10b. Silica (SiO_2) concentrations (mg/L) in the ponds between 16 Sept. 2008 and 21 Jan. 2009

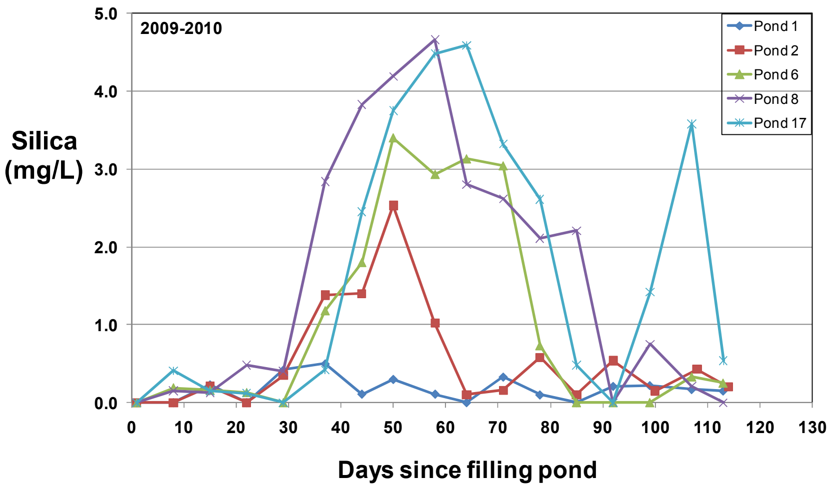


Figure 10c. Silica (SiO_2) concentrations (mg/L) in the ponds between 13 Oct 2009 and 11 Feb 2010

The reactive silica data from the five ponds that were monitored closely during 2008/09 season was quite variable (Figure 10b). Data from Ponds 6 and 8 suggest that there was little relationship between the reactive silica concentration and the dominance of diatoms, whereas data from the other ponds suggest that there was a relationship. We investigated the relationship between diatom score and the dichotomised reactive silica and FRP concentrations ($Si0_2$), of the combined data over the three seasons of the five ponds being monitored as part of this project. However, the statistical analysis showed no correlation between silica concentration and diatom density score (Table 3). The low number of ponds involved in this analysis (5 ponds x 3 seasons = 15), the periodic addition of sodium silicate, and the variability of the data would have made it difficult to detect a significant relationship between the reactive silica concentration and the subsequent appearance of a diatom bloom, if such a relationship existed. Observations by APF of their other ponds have suggested that as the silica concentrations increased the occurrence of diatoms dominating in the pond also increased. Further analysis was carried out to investigate the relationship between the presence of reactive silica of > 0.5 mg/L within 15 days of a peak in TAN (or in the absence of a TAN peak, within the period from 25 days after filling the pond to 50 days after filling the pond) and the number of ponds where there was a presence or absence of a diatom bloom with a moderate to high density (Table 4). Using Fisher's exact test, no significant relationship was detected.

Table 3. Relationship between the number of water samples with $Si0_2 \geq 1.0$mg/L and corresponding diatom score. No significant relationship was detected.

		Diatom Score				
		No Diatom	Low	Med	High	Total
$Si0_2$ ≥1.0mg/L	No	155	45	34	54	288
	Yes	45	10	5	9	69
	Total	200	55	39	63	357

Table 4: The number of ponds that had reactive silica of > 0.5 mg/L within the 15-day period following a peak in TAN, (or in the absence of a TAN peak, within the period from 25 days after filling the pond to 50 days after filling the pond) and a diatom density score of ≥ 2 in the 40 days following the TAN peak. No significant relationship was detected ($p = 0.533$).

		Reactive silica >0.5 mg/L within 15 d of TAN peak		
		No	Yes	Total
Diatom density score ≥ 2	No	0	7	7
	Yes	1	7	8
	Total	1	14	15

4.1.7. Filterable Reactive Phosphorus (FRP)

The pattern of the change in FRP concentrations in the five ponds over the three seasons is presented in Figures 12a, 12b and 12c. The pattern is very consistent in all three years, though there is a serious question about the accuracy of the zero data for all ponds on the 37th day after filling in 2008/09. In 2009/10, the FRP showed a broad peak in concentration during the first 50 days after filling, thereafter it decreased to similar levels to the ponds in previous years at the same time, except in two of the ponds where it remained at slightly higher concentrations. Statistical analysis of the relationship between diatom score and the dichotomised FRP concentrations of the combined data over the three seasons of the five ponds (Table 5) showed that there was a significant inverse relationship. Avmimelech (2009) reported that fertilisation of ponds to support microalgal blooms is only necessary if the phosphorus concentrations are less than 0.1 mg/L. Further analysis was carried out of the FRP data to investigate the relationship between the presence of FRP concentrations > 0.1 mg/L within 15 days of a peak in TAN (or in the absence of a TAN peak, within the period from 25 days to 50 days after filling the pond) and the presence or absence of a diatom bloom with a moderate to high density (Table 6). Using Fisher's exact test, no significant relationship was detected.

Table 5. Relationship between the number of water samples with FRP ≥ 0.1mg/L and corresponding diatom score. A significant inverse relationship was detected ($p<0.001$).

		Diatom Score				
		No Diatom	Low	Med	High	Total
FRP ≥0.1mg/L	No	57	33	29	45	164
	Yes	103	3	1	2	109
	Total	160	36	30	47	273

Table 6: The number of ponds that had filterable reactive phosphorus (FRP) concentration of > 0.1mg/L within the 15-day period following a peak in TAN, (or in the absence of a TAN peak, within the period from 25 days after filling the pond to 50 days after filling the pond) and diatom density score of ≥ 2 in the 40 days following the TAN peak. No significant relationship was detected ($p = 1.00$).

		FRP >0.1 mg/L within 15 d of TAN peak		
		No	Yes	Total
Diatom density score ≥ 2	No	0	8	7
	Yes	0	7	8
	Total	0	15	15

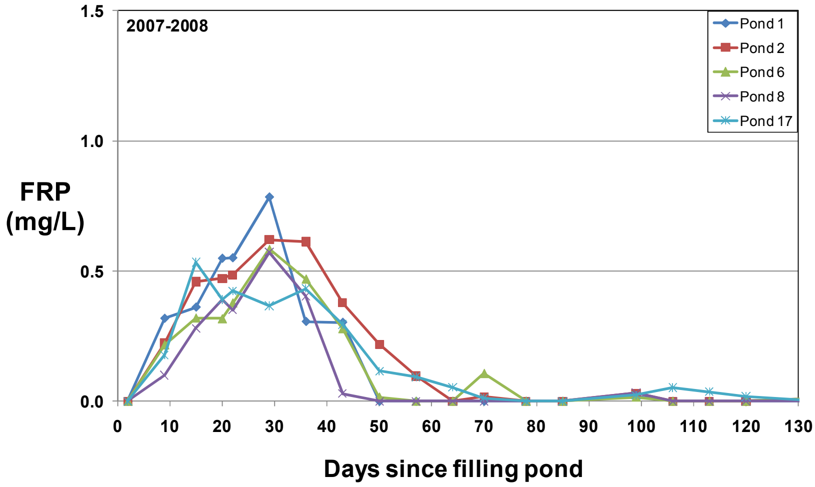


Figure 11a. Filterable Reactive Phosphorus (FRP) (mg/L) in the ponds between 29 Sept. 2007 and 6 Feb 2008

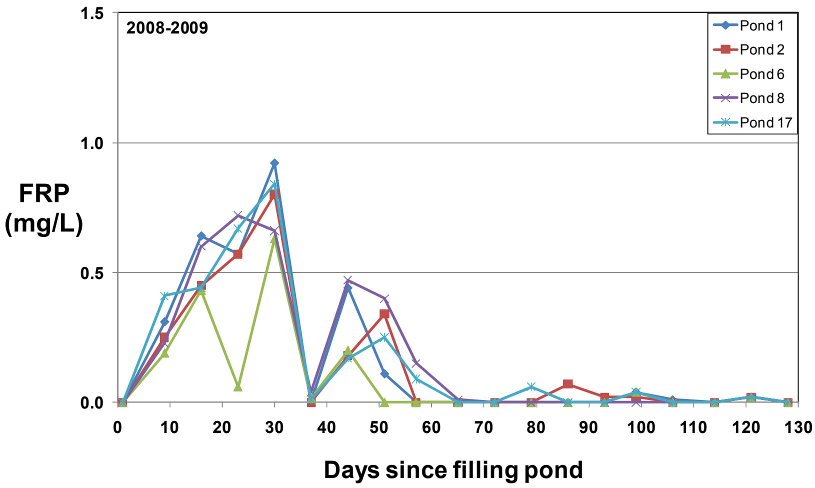


Figure 11b. Filterable Reactive Phosphorus (FRP) (mg/L) in the ponds between 16 Sept. 2008 and 21 Jan. 2009

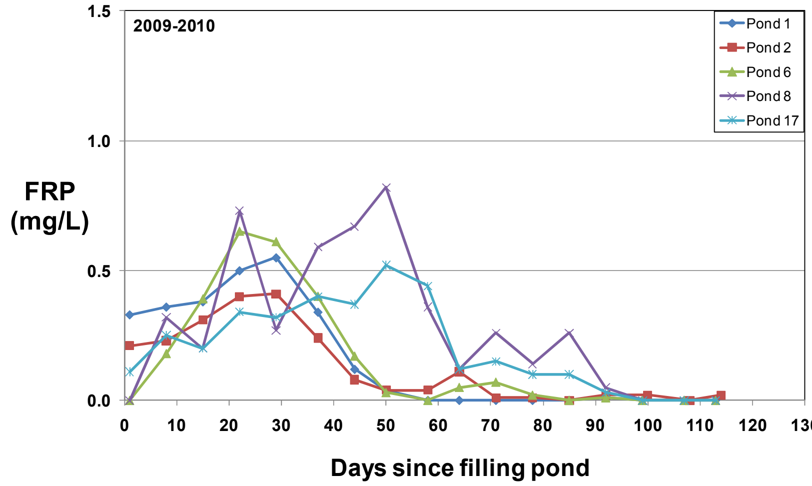


Figure 11c. Filterable Reactive Phosphorus (FRP) (mg/L) in the ponds between 13 Oct 2009 and 11 Feb 2010

4.1.8. Nitrate and nitrite in pond water

Bacteria in the pond water and sediment convert the ammonia/ammonium ions to nitrite, and then the nitrite to nitrate. Some of the ammonia/ammonium ions and the nitrate can be taken up by microalgae and used for the synthesis of proteins. The nitrate can also be converted by de-nitrifying bacteria in anaerobic sediments to nitrogen which diffuses from the water into the atmosphere. The combined concentration of nitrate-nitrogen and nitrite-nitrogen is referred to as NOX. Nitrite is the more toxic form of the oxidised nitrogen. The NOX concentrations found in all the ponds was very low (Figure 12), indicating that toxic nitrite was not a problem and suggesting de-nitrification of nitrate was occurring. This in turn indicates the presence of anaerobic sediment in the centre of the ponds which was confirmed after harvest when the ponds were drained (Photograph 7)

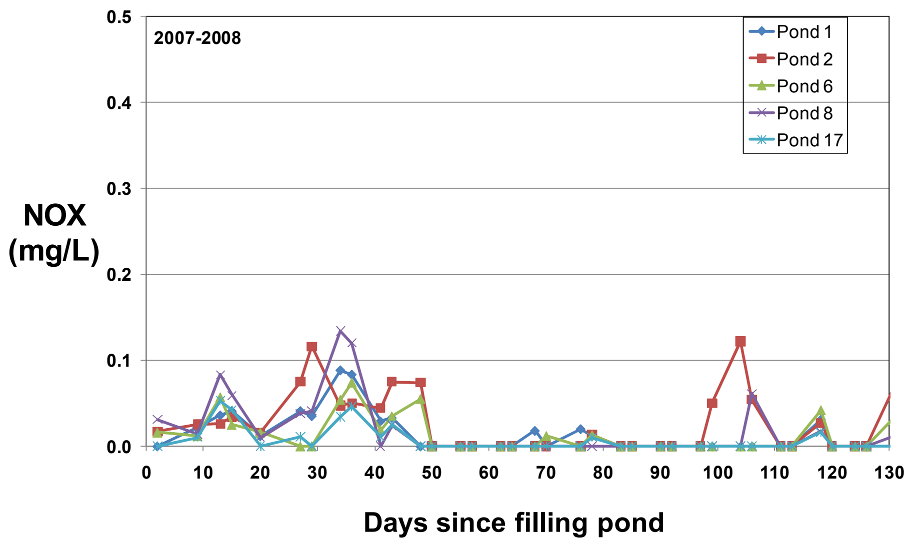


Figure 12. Combined nitrate and nitrite concentrations (NOX) (mg/L) in the ponds from 29 Sept. 2007 and 6 Feb 2008

Photograph 7. Sludge in the centre of a pond at APF after the pond was drained at the end of the season

4.1.9. Molasses, bicarbonate and biofloc

Observations throughout APF over the three years have indicated that when a good biofloc is present (>0.1 mL/L) the Total Alkalinity of the ponds will progressively decrease, requiring the addition of sodium bicarbonate to keep the alkalinity within the preferred range of 80 to 120 mg/L. If a biofloc is not present but there is a moderate to strong bloom of microalgae, the decrease in Total Alkalinity appears to be far less pronounced. Hence, this progressive decrease in alkalinity is attributed to the utilisation of bicarbonate by litho chemoautotrophic bacteria, or heterotrophic bacteria that have switched to chemoautotrophic metabolism. When there is not a good biofloc present, there is a need to add molasses to the pond to manage the microalgal bloom and the TAN by encouraging greater productivity of heterotrophic bacteria. Under these conditions the Total Alkalinity does not decrease markedly. Presented in Table 7 are the data from each of the five study ponds over the three seasons for the number of days where there was a biofloc of >0.1 mL. Also shown are the diatom and blue green algae population densities and the amount of molasses and sodium bicarbonate that were added to the pond over the season.

However, statistical analysis of these data suggested that there was no relationship between the biofloc and the amounts of molasses or bicarbonate added to the ponds. When this data were plotted (Figure 13), it does appear that there may be a weak relationship, but because of the variability of the data, with only 15 sets of data, there was not enough information available to detect a correlation if a correlation truly existed.

Table 7. Data for each pond over the three seasons for the number of weekly samples in which the biofloc was >0.1 mL/L; the number of weekly samples with a moderate or high diatom density; the number of weekly samples with a moderate or high blue green algae density, the amount of sodium bicarbonate (kg) and the amount of molasses (L) added to the pond over the season. The letter after the pond number indicates the season: a for 2007/08. b for 2008/09 and c for 2009/10.

Pond	Biofloc >0.1 mL/L	Diatom samples	Blue green samples	Bicarbonate addition (kg)	Molasses addition (L)
1 a	10	10	4	200	1240
2 a	2	4	10	100	1640
6 a	2	3	12	0	1180
8 a	8	9	7	300	1060
17 a	5	2	13	0	1490
1 b	9	11	5	875	1150
2 b	7	10	7	675	1320
6 b	2	9	9	700	1310
8 b	0	2	7	400	1010
17 b	8	11	3	700	1950
1 c	0	0	10	0	2430
2 c	0	0	7	0	2380
6 c	1	5	2	300	1360
8 c	1	4	0	550	1460
17 c	0	0	1	200	1230

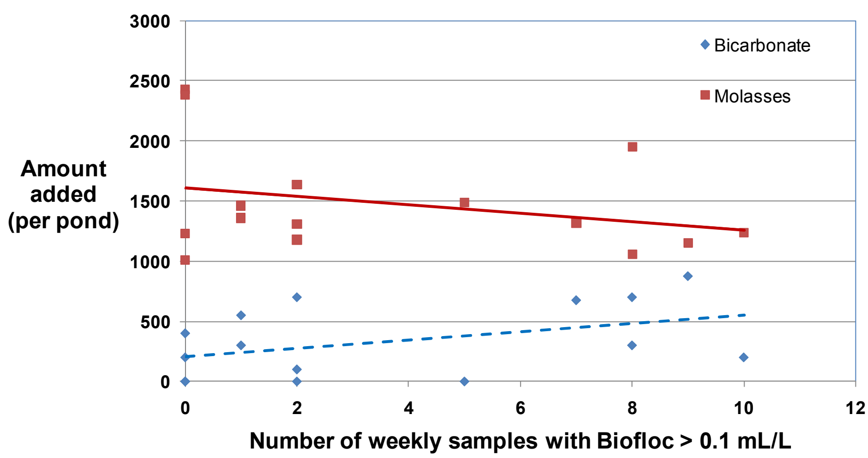


Figure 13. Relationship between number of weekly water samples in which the biofloc concentration was > 0.1 mL/L and the amount of molasses (L) and sodium bicarbonate (kg) that were added to the pond over the season. Data is from five ponds in each of the years 2007/08, 2008/09 and 2009/10.

4.1.10. Conclusions: nutrient conditions associated with establishing biofloc

This study has shown that the presence of a moderate to high density bloom of these diatoms is significantly correlated to the subsequent establishment of a biofloc of a satisfactory density (> 0.1 mL/L). The large peak in TAN (> 0.9 mg/L) that is produced within 50 days of filling the ponds is well correlated to the establishment of the bloom of large diatoms, generally about 30 days after the TAN peak. Data from the five test ponds for the reactive silica and FRP at concentrations considered necessary for diatom growth (0.5 mg/L and 0.1 mg/L, respectively) did not show that the establishment of a bloom of large diatoms was dependent on having these concentrations in the ponds. However, the data were heavily skewed in favour of adequate levels of these nutrients through the addition of fertilisers, so there was little or no data for where these nutrients were less than the concentrations shown above. However, observations from other ponds at APF and information in the literature (Becker, 1994; Thompson, 2001; Gilpin et al. 2004; Harrison and Berges, 2005; Avnimelech, 2009) suggest that it would be beneficial for the establishment of a strong bloom of large diatoms if the concentration of reactive silica was in the order of 0.5 mg/L at about the same time as the TAN peak, and that the FRP concentration be at least 0.1 mg/L at about the same time. Further work will be needed to establish the minimum concentrations of reactive silica and FRP that will support the reliable establishment of a moderate density bloom of large diatoms in the prawn ponds.

During the first 30 to 45 days after filling the ponds, the main nutrient input into the ponds is the fertilizer – the urea and MAP. As the prawns are small, there is very little feed going into the pond at this stage. Assuming that a pond has been stocked with 50 PL15 /m^2, 35 days after filling the pond (21 days after stocking) there would have been about 140 kg of urea added to the pond and 70 kg of MAP. The average feed input can be assumed to have been about 220 kg of feed with a crude protein content of 38%. Assuming that the feed contains 50% C and 6.2% N, the urea, 20% C, 46.5% N and the MAP, 0% C, 12.2% N, the C/N ratio of combined inputs of fertilizer and feed can be calculated to be about 1.5:1. This value indicates a significant shortage of organic carbon for the use of all of the nitrogen by the heterotrophic microbial community, as the typical ratio for the complete utilization of both C and N is about 20:1. As a result, the high TAN concentrations would be expected to increase and stimulate productivity of nitrifying bacteria and chemoautotrophic bacteria. In addition, it will result in depletion of CO_2 in the water with a consequential increase in pH (Figure 9a, 9b, and 9c). Observations at APF indicate that a better and more stable biofloc occurs in ponds with stocking densities > 35 prawns per m^2. Currently, ponds are routinely stocked with 50 PL/ m^2. This observation is consistent with comments by Avnimelech (2009) about the benefit of the additional feed going into the pond and the effect that this has on increasing the C:N ratio.

A key feature of the pond management protocol at APF is the chaining of the bottom of the ponds. The chaining is carried out by dragging a long loop of chain behind an outboard powered dinghy to resuspend material that has settled on the bottom (Photograph 8). This keeps the bond bottom clean and free of anaerobic sludge, except for the centre of the pond, which is not chained. Chaining of all ponds is carried out once each week, other than very early in the season when it is not chained at all. The chaining is carried out over half of the pond at a time, allowing an undisturbed area for the prawns to occupy. The chaining has a great benefit in that the organic matter is lifted off the bottom and mixed with the well-aerated water in the pond. This encourages aerobic bacteria to oxidise the organic matter. The benefit is that aerobic bacteria will oxidise the organic matter at a far greater rate than can be achieved by anaerobic bacteria - and with less oxygen demand (Avnimelech, 2009).

Photograph 8. Resuspending organic matter that has settled on the bottom of a pond at APF by dragging a chain across the bottom – i.e. "chaining"

Peaks in TAN also occur after microalgal crashes and during overcast conditions when microalgal photosynthetic activity is reduced, with a consequential reduction in their uptake of TAN. During these periods, action may need to be taken to minimise the impact of the high TAN levels. One strategy for the management of TAN is the addition of molasses to the pond, to encourage productivity of heterotrophic bacteria. The heterotrophic bacteria use the molasses as an energy source and as a source of organic carbon for biosynthesis. During that process some of the TAN from the water is used for synthesis of amino acids, protein and nucleic acids, thereby reducing the ammonia concentration in the water (Avnimelch, 2009). Another benefit of reducing the TAN concentration is that it results in less ammonium being available for microalgal productivity, which provides a management strategy to control an unsustainable increase in density of the microalgal blooms. It is this last feature that is considered by the pond manager at APF as the most important application for the addition of molasses to prawn ponds. A more detailed discussion about the use of molasses is given in Section 5.3.

4.2. Fertiliser comparison trial

4.2.1. Results

Abiotic factors - Experimental system

The salinity in the tanks was maintained as constant as practical with an average salinity of 35.2 ‰ (range 33.9 ‰ to 36.4 ‰) across all tanks and over the duration of the experiment. There was no significant difference in salinity between the two treatments. The average salinity (± Std. Dev) of tanks fertilised with urea was 35.2 ± 0.33 and that of those fertilised with Easy N was also 35.2 ± 0.32.

The average water temperatures across all tanks over the whole period of the experiment was 29.5 ± 1.61 °C with the lowest recorded temperature in an individual tank of 24.3 °C and a the highest recorded temperature of 33.9 °C. There was not a significant difference between the treatments (Table 8). The maximum difference in temperature among the 26 tanks at any one time was generally about 2 °C, but this did increase to a maximum of about 4 °C under certain wind and temperature conditions. The average diurnal variation over the whole experiment was about 2 °C with a maximum variation of 2.8 °C and a minimum of 0.7 °C.

Table 8. Average temperature, dissolved oxygen (DO) and pH of water in tanks according to treatment and time of sampling. Data are averages for the tanks over the 28 days of the experiment that was carried out to compare the effect of the two fertilisers.

Treatment	Time	Temperature (°C)	DO (ppm)	pH
Urea	am	28.6 ± 1.4	6.6 ± 0.61	8.3 ± 0.22
	pm	30.4 ± 1.3	7.5 ± 1.13	8.5 ± 0.27
Easy N	am	28.7 ± 1.4	6.6 ± 0.70	8.4 ± 0.23
	pm	30.4 ± 1.3	7.6 ± 1.09	8.5 ± 0.26
Grand Average		29.5 ± 1.6	7.1 ± 1.06	8.4 ± 0.44

The average pH (± Std. Dev) of all tanks over the duration of the experiment was 8.4 ± 0.44. There was no significant difference between treatments (Table 8, Figure 14). The diurnal variation in pH was also not significantly different between treatments with a difference of 0.15 units between the average morning pH and that of the afternoon pH. for both treatments. In only four of the 26 tanks did the pH increase to 9.0 or more. Of these four tanks, three had been fertilised with Easy N and one with urea.

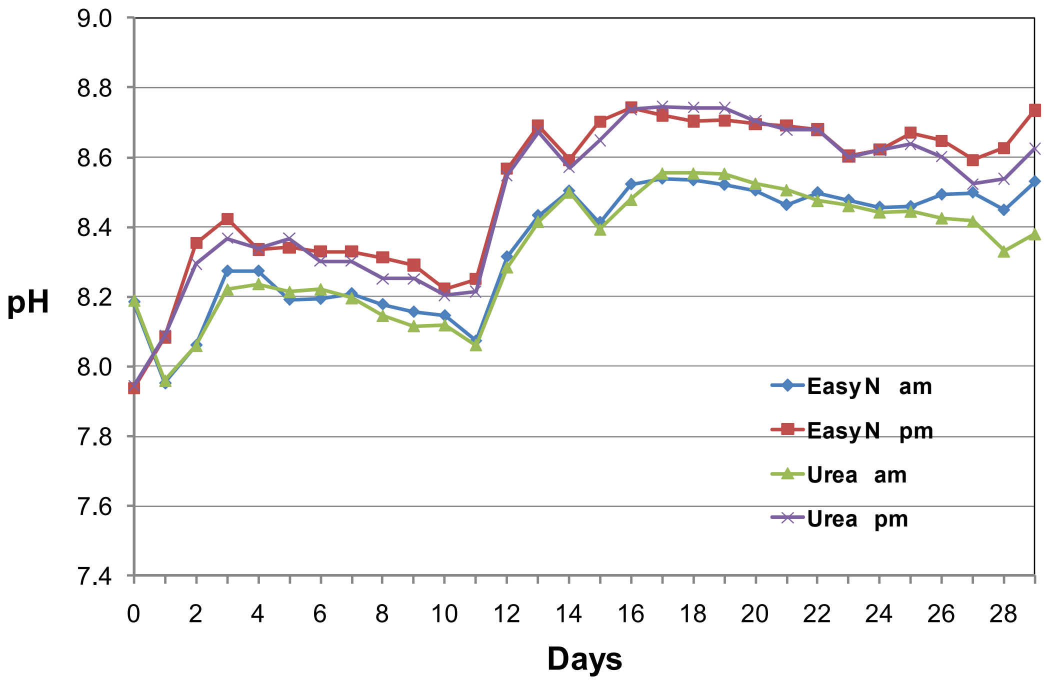


Figure 14. Change in average morning and afternoon pH of water in tanks for the two treatments over the duration of the trial

Dissolved oxygen in the tanks was consistently high throughout the experiment. Total alkalinity was measured in samples from eight randomly selected tanks at the start of the experiment. The mean alkalinity was found to be 123 ± 1.8 ppm $CaCO_3$. The alkalinity was measured a second time at Day 14 and the mean alkalinity from all 26 tanks was 120 ± 2.1 ppm CaCO3. Total ammonium nitrogen (TAN) was measured in all tanks on two occasions (Day 20 and Day 26). On both these occasions, in all of the tanks, the concentration of TAN was less than the practical detection limit of 0.1 mg/L. The nitrate concentration was measured in a random sample of 8 tanks on two occasions (Day 2 and Day 14) on both occasions the concentration of nitrate in all tanks was less than the practical detection limit of the test kit, 1 mg/L.

Microalgae

The Secchi disk measurements which were used to assess the density of microalgae in the tanks were highly variable with time. Over the period of the experiment, the average Secchi depth of tanks fertilised with urea was 78 ± 6 cm which was not significantly different from that of the tanks fertilised with Easy N (78 ± 8 cm). There did not appear to be a difference between the treatments in the rate of increase in microalgal density as measured using the Secchi disk (Figure 15)

Microalgal species composition did not appear to vary with the type of fertiliser that was used in the tank. The dominant species in the tank was variable and changed with time. This was reflected in the algal attractiveness score (Algal Score) (Figure 16). The large diatom species, *Bellerochia horologicalis*, that is strongly associated with biofloc formation at APF did not become established nor was it observed in any of the tanks during the study. Other diatoms species did appear in the tanks but did not dominate. In no instance was the diatom population in a tank scored as being "High". There were significant numbers of diatoms observed early on in the trial but after the first week they appeared less frequently and at relatively low densities. Dinoflagellates were frequently observed in the first two weeks but declined thereafter as the blue-green algae became more dominant (Figure 17).

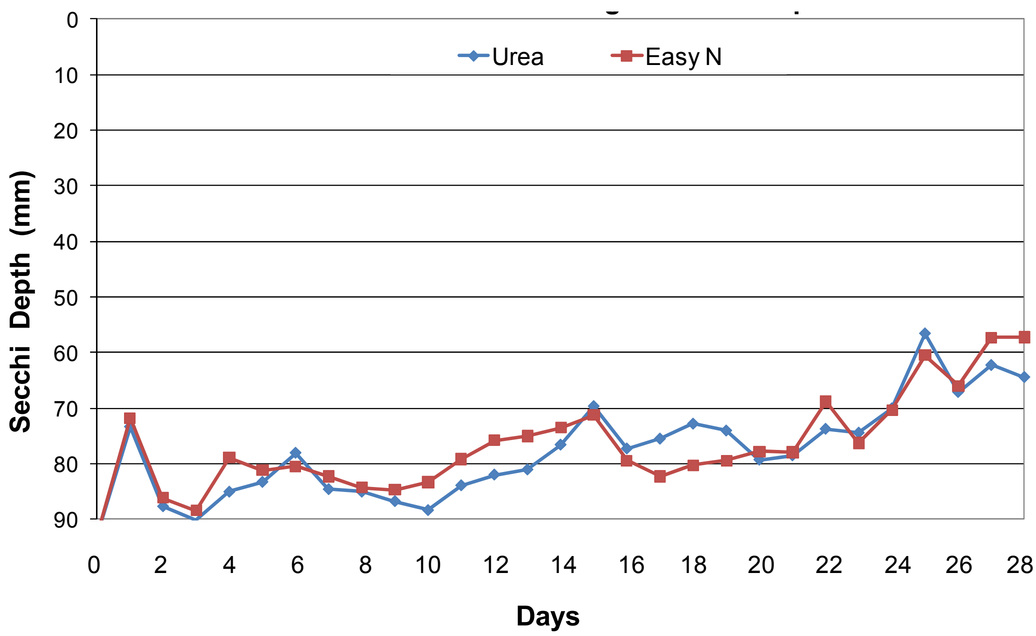


Figure 15. Average Secchi disk depths of all tanks from each treatment that were fertilised with either urea or with Easy N

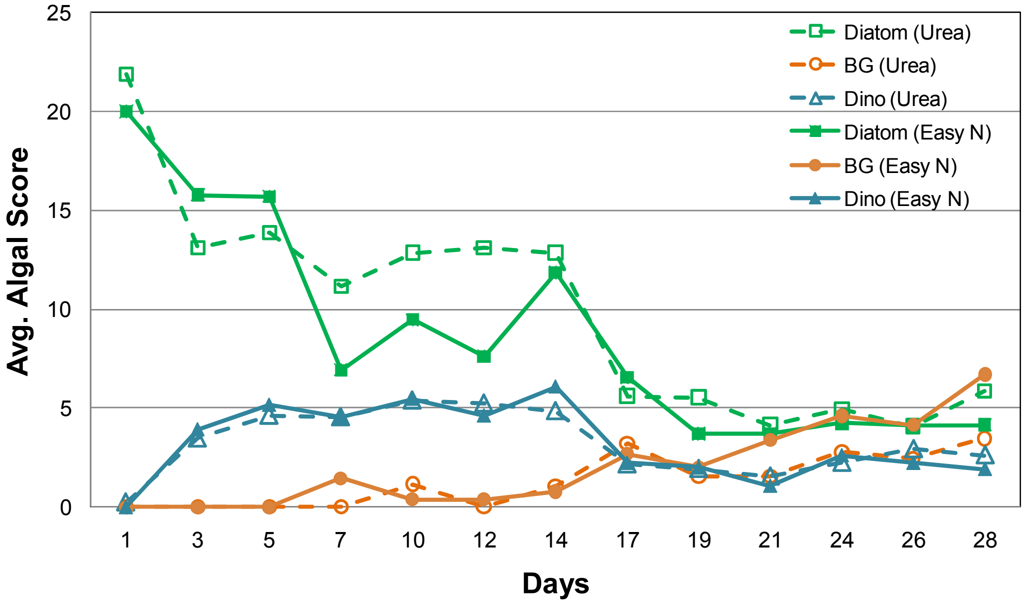


Figure 16. Average algal attractiveness scores (Algal Score), throughout the period of the experiment of all tanks fertilised with urea and with Easy N

5. DISCUSSION

The results of this study showed no difference in the effect of using either urea or Easy N as the nitrogen sources for the establishment and growth of microalgal populations in large tanks provided with full sunlight. This is in contrast to observations at APF, where the growth of microalgae in the ponds fertilised with Easy N appeared more rapid and more stable than the growth response seen in ponds fertilised with urea in previous years. In the tank trial, the temperature, salinity, pH, dissolved oxygen and alkalinity closely similar between treatments and could be considered identical. These abiotic parameters were held at, or maintained at, close to ideal levels.

The TAN was measured twice in all tanks during the study and in all cases was found to be less than 0.1 mg/L, which was the minimum practical detection limit of the ammonia analysis test kit being used. This is in contrast to measurements of pond water samples from APF where, over an equivalent initial 28 days after filling the ponds, the TAN increased from 0.1 mg/L to >1.0 mg/L. This begs the question: "What was happening to all of the nitrogen that was being added to the tanks?". The answer to this is unclear. It is possible that the nitrogen was being taken up by heterotrophic bacteria and by benthic or epiphytic algae growing on the bottom of the tanks. The calculations associated with dilution and dosing of the tanks with urea and Easy N have been checked and cross checked and the possibility that an incorrect dosage had been applied has been clearly eliminated.

The pH in the tanks increased progressively with time but with intermittent decreases that appeared to be associated with the condition of the microalgal bloom. The general pattern of change in pH was similar to what has been observed in ponds at APF. However at APF, the pH in the ponds generally starts off at about 8.6 (c.f. pH 8.2 in the tanks) but also generally increase to above 9.0 about 21 days after filling. This significant increase to pH > 9.0 occurred in only four of the 26 tanks. Of the four tanks, three had been fertilised with Easy N and one with urea.

In the first week there was a relatively high frequency of occurrence of diatoms at moderate densities in the tanks in both treatments. However, the diatoms became less prevalent after the first week and more so after the second week. The population of dinoflagellates was at its peak in the period from Day 3 to Day 14 after which it declined and the blue-green algae increased in density. Throughout the study, the algal scores for both treatments were very similar and were not significantly different.

The results from this study differ from observations made at APF. At APF ponds that had been fertilised with Easy N had exceptionally stable microalgal blooms, whereas ponds fertilised with urea appeared to have a slower response in the establishment of a bloom. In the tanks there were regular crashes in the microalgal population which resulted in the Secchi disk being clearly discernable when it was on the bottom of the tank (Secchi depth reading of > 90 cm). This did not appear to happen at the same frequency at APF. This could be a pond management issue, or be due to the buffering effect of a much larger volume of water or the presence of the earth substrate, or a combination of these factors at APF. However, regardless of the limitations of the tanks in simulating ponds, this study showed clearly that under the conditions of the experiment there was not a beneficial effect of using Easy N instead of urea as a nitrogen source for the microalgae.

5.1. Effectiveness and use of different carbon sources

One of the objectives of the project was to evaluate the effectiveness of different carbon sources for microbial floc formation in commercial ponds. The reason a carbon source is added to a pond is to provide a complementary nutrient or energy source for bacteria. This should encourage bacterial growth and hence uptake of excess ammonia in the pond. In this way, the amount of ammonia available to microalgae is limited and so their growth is controlled. At the start of this project we thought that the only type of carbon source to play a significant role in the low water exchange production system would be an organic carbon source, such as molasses or bagasse. However, during this study we came to realise that the availability of both organic carbon and inorganic carbon were very important in the management of this system. Microalgae and litho chemoautotrophic bacteria utilise bicarbonate as their carbon source, whereas heterotrophic bacteria utilise organic carbon.

Bicarbonate is a component of the group of inorganic ions that contribute to the Total Alkalinity. When a stable biofloc is established, the Total Alkalinity of the ponds decreases progressively and needs to be replenished to maintain it within the range of 80 to 120 mg/L. Generally a stable biofloc is associated with a medium to high density bloom of the large diatoms: *Bellerochia* spp, (predominantly *Bellerochia horologicalis*) and *Helicothica* spp. It appears that the presence of these large diatoms is also associated with litho-autotrophic bacteria. The litho-autotrophic bacteria appear dominant in the biofloc and, with the microalgae that are present, use the bicarbonate in the water. Sodium bicarbonate is the preferred inorganic carbon source to replenish the bicarbonate and hence increase the Total Alkalinity, if the pH does not need to be changed. If the Total Alkalinity and the pH are both too low, application of sodium carbonate will raise both of these parameters. It should be noted that the application of hydrated lime will increase both pH and Total Alkalinity: it will not add any inorganic carbon (or bicarbonate). The response of the water chemistry in the pond to the addition of these inorganic carbon sources is rapid, with the effect being observed within two to three hours.

The criteria for an effective organic carbon source are that it: (a) is degraded and utilised rapidly by bacteria; (b) is in the form of a fine powder or liquid that is readily dispersed within the pond; (c) is preferably that it is water soluble or miscible so that bacteria can absorb it rapidly; and (d) is predominantly composed of simple carbohydrates or low molecular weight lipids and with a minimum amount of nitrogen. Research partners within this project have found that the degradation rate of an alternative carbon source, bagasse, in outdoor tanks was very much slower than that of another carbon source, tapioca flour. This finding was confirmed by Avnimelech (2009) who re-calculated data from other sources, and reported that in ponds the half-life of sugars is 0.6 day; for hemicelluloses, 6.7 days; and for cellulose, 14 days. Clearly hemicelluloses- and cellulose-based products, such as bagasse, would be degraded at a rate that is far too slow to be effective in managing ammonia levels with the pond management system being used at APF. Molasses is a very practical and effective carbon source as it is more easily applied to a pond. However, it should be mixed thoroughly with water before being added to the pond for effective dispersal rather than settling on the bottom of the pond. In a review of available organic carbon sources in Australia (K.C. Williams, CSIRO, personal communication), it appeared to be the most cost-effective one available to the prawn farming industry. Given that the organic carbon source is added to a pond to help control the ammonia concentrations or algal density when they have increased to unacceptable levels, a rapid and effective response is required. If the carbon source that was being tested was not effective, it would put the crop of prawns in the pond at risk. With the information that had become available on the degradation rates of alternative carbon sources, and with APF's satisfactory experience with molasses and their evolving understanding of the nutrient dynamics in the ponds, APF were unwilling to expose their ponds to unnecessary risk by testing alternative carbon sources for themselves in the ponds but were satisfied that molasses was the best source for their application.

The approach at APF is to use molasses primarily to control fluctuations in microalgal density, rather than trying to reduce TAN concentrations. The objective is to control the algae growing on this ammonia by stimulating bacterial growth with the molasses. The addition of molasses to a pond is generally required when there is a weak or poor biofloc present. Under these situations the large diatoms, *Bellerochia* spp and *Heliothica* spp are either absent or present at low density. The molasses provides a carbon source for heterotrophic bacteria that use it as an energy source and as a source of building blocks for further biochemical synthesis. A reasonable starting population of heterotrophic bacteria is required in the pond for the application of molasses, or any carbon source, to be effective. As the heterotrophic bacterial population grows and its biomass increases, there is an increase in oxygen demand in the pond. Hence, it is important to be careful adding molasses to a pond when the morning DO's are under 3.5 mg/L. This allows for a decrease in the following morning DO's due to the increase in oxygen demand brought about by the increased bacterial oxygen demand. The addition of 100 L of molasses to a pond can reduce the ammonia by at least 0.5 mg/l, depending on the constant load on the pond, but can also decrease the following morning DO by 1 mg/L. Furthermore, the predicted cloud cover over the following days, and the amount of feed going into the pond need to be considered when making a decision on the amount of molasses to be added to a pond. Cloud cover will reduce uptake of ammonia by microalgae, and about 75% of the nitrogen in the feed put into the pond will make its way through the food chain and microbial process to end up as ammonia.

5.2. Nutritional benefits of bioflocs and reduction in feed costs

With the relatively low intensity of the low water exchange, biofloc system being used at APF, in comparison to the intensity of the system used with *L. vannamei* in the "Belize" system, the establishment of biofloc in a pond is considered to be primarily for the management of pond water quality. However, it may provide a secondary source of nutrition for the prawns. The water quality is maintained by getting a balance between microalgal productivity and bacterial productivity. For the prawns, an additional benefit of establishing a biofloc appears to be the stable environment which develops and in which the prawns are being grown. It is believed that this stable environment contributes in improved growth and better feed conversion ratios.

The nutritional benefit of bioflocs for *P. monodon* cultured in ponds is difficult to determine without the effect of the stable environment being superimposed on the outcome. Using APF production data for 2007/08, it was calculated that in ponds in which a good biofloc had been established, a harvest of 10 tonnes was obtained in 135 days with an FCR of 1.2 to 1.3. In ponds where there was only a poor biofloc, and which were given the same feed, a harvest of 10 tonnes was obtained in 145 days with an FCR of 1.5 to 1.6. Based on these data, in a system with the same management regimen, the presence of a good biofloc resulted in a saving of 3 tonnes of feed or about $6,000 per pond that is about 20% of the total feed cost. This data suggests quite clearly that the microbial floc is providing some nutritional benefit to the prawns.

Recent tank studies with *L. vannamei,* have shown that over 35 days, the weight gain of 0.4 g prawns fed diets containing biofloc was 50% greater than that of the prawns fed the control diets (Kuhn et al., 2009). At APF with the low water exchange and the microbial floc production system being used there, the feed conversion efficiency has been improved to the point where feed conversion ratios (FCR) have been reduced to as low as 1.2. This is in contrast to poorer FCRs that are typically achieved in production systems that use routine water exchanges to manage water quality, where FCRs greater than 1.8 are commonly achieved (Lobegeiger and Wingfield, 2010). To put this into perspective, a crop of 10 tonnes of prawns harvested from a conventionally managed pond with an FCR of 1.8 would have been fed 18 tonnes of feed, whereas in a pond in a low water exchange system with good floc, the 10 tonnes of prawns would have been fed about 12 or 13 tonnes of feed. This represents a saving in feed costs alone of about $10,000 per pond or about 30% of the total feed cost.

5.3. Reduction in farm nutrient discharge and effects on farm profitability

The reduction in farm nutrient discharge and the effects on farm profitability have been calculated using APF data from the 2003/04 season when an open system was being used, and from the 2008/09 season when the low water exchange system was used throughout the farm.

On a farm comprising 33 x 1 hectare ponds, operating with a conventional “open” system, an average of 10% of the water would be discharged each day over 130 days production season. This figure, together with the volume of water in the ponds that is discharged at harvest, equates to 6930 ML of water. In 2003/04, APF discharged 5282 ML. In 2006/07, when APF first started running the entire farm with a low water exchange system, they discharged 1522 ML, a reduction of over 70%. The water discharge data for 2007/08, 2008/09 and 2009/10 cannot be validly compared due to very heavy rainfall events during these seasons, but the volume of water discharged by the farm was still in the order of 2000 ML (2035 ML, 2035 ML and 1800 ML, respectively). If the volume of water required to fill the ponds is subtracted from these figures (33 ponds x 15 ML = 495 ML), the difference represents the water exchange plus rainfall and minus evaporation. On these figures, in 2006/07 there was a reduction in water exchange of 78.5%, and in three following years, the reduction was 68%, 69% and 73%.

The Total Nitrogen discharge from APF in 2003/04 was 1.36 kg/ha/d (or 9,000 kg/crop). In 2008/09 the Total Nitrogen discharge was 0.5 kg/ha/d (or 3,400 kg/crop). However productivity in the 33 ponds increased from 220 t in 2003/04 to 360 t in 2008/09. Using these production values, the Total Nitrogen discharge was reduced from 40.0 kg/t prawns to 9.4 kg/t prawns - a reduction of 77%.

The savings achieved through reduced power usage for pumping water have been calculated to be $930 per pond, The saving in feed costs are calculated to be about $6,000 per pond. The additional labour and costs associated with the application of fertilizers, molasses, silicates and other chemicals to the ponds, and the higher costs associated with the greater number of chemical analyses that are carried out are estimated to be between $1,000 and $2,000 per pond. However, the greatest benefit is the increased production from the ponds. In 2003/04 APF produced 6.7 t/pond, and estimate that if they were using an “open” system in 2008/09, they would have been producing about 8 t/pond. Using the low water exchange, biofloc system, APF’s average production in 2008/09 in ponds in which a floc had become established, was about 12 t/pond. This is an increase in production of 4 t/pond. If a farm gate price of $15 /kg is obtained, this increase in production is worth $60,000 per pond. Taking all the additional costs and savings listed above, the biofloc system can increase the production from each pond by an estimated $65,000. APF have demonstrated that they can produce more than 12 t/pond, so there is real potential for even greater productivity.

6. BENEFITS AND ADOPTION

6.1. Short term benefits

- At APF, there was increased productivity of ponds as a result of using the low water-exchange microbial floc production system, and as a consequence, increased farm profitability. A 1 ha pond in which a good biofloc is established and maintained will produce at least 12 tonnes of prawns, whereas in an "open" water exchange system under a similar management system, a production of 8 tonnes could be expected. The increased profitability from using the low water exchange system has been calculated to be about $65,000 per pond.
- APF have adopted the practice of monitoring TAN concentrations in the ponds and taking pond management action in response to the concentration of TAN, according to the history and current situation in the pond and expected weather condition. They also add sodium silicate to the ponds to encourage or support a flagging diatom population.
- APF have reduced nutrient discharge into the environment as a result of using low water-exchange microbial floc production system. In comparison with the discharge at APF prior to using the low water-exchange system, there has been a reduction of 77% in the nitrogen discharge per tonne of prawns produced.
- The identified benefits in the original proposal were (a) reduced feed costs via lower protein content and more efficient feed utilization (target between 10% and 20% reduction). Feed costs per unit weight of production have been reduced by about 30%. (b) reduced frequency of water exchanged and the amount of water exchanged (target 80%). Water exchange has been reduced by about 70%; (c) increase overall productivity – productivity increased by about 50%; (d) enhance environmental management – nitrogen discharge per unit weight of prawn produced has been reduced by 77%.
- Within the Australian prawn farming industry, at least two farms (Pacific Reef and Eimeo) are currently using this production system. Two other prawn farms are using some of the techniques and developing the required skills to run a low water-exchange biofloc system, while still using the conventional "open" system of production.

6.2. Medium term benefits

- The Australian prawn farming community will benefit directly from the results of this research in that it provides them with information to help them decide on whether to adopt a low water exchange production system, and identifies key parameters that will need to be monitored and responded to for the management of the ponds.
- Sunshine Coast TAFE ran a strategic planning workshop for farm owners and managers to equip them to make decisions about adopting this type of production system using the information generated by this project.

6.3. Long term benefits

- The Australian community will benefit in the longer term as more prawn farms adopt the technology and further reduce nutrient discharge into coastal waters.

7. FURTHER DEVELOPMENT

There is still a significant uncertainty about establishing a biofloc in the ponds. This project has identified key features that appear to be prerequisites for a biofloc to become established - a high concentration of TAN (> 0.9 mg/L) within 50 days of filling the pond, a reactive silica concentration of at least 0.2 mg/L and preferably > 0.5 mg/L occurring within 15 days of the TAN peak, and a FRP concentration of > 0.1 mg/L occurring in the pond within 15 days of the TAN peak. The requirement for the stated reactive silica and FRP concentrations has not been rigorously established, and that remains to be done. However, before that is studied, it would be very useful to find a way of creating a TAN peak at the optimal time after stocking. This could possibly be done by engineering a crash of a dense microalgal bloom. APF have considered this but are reluctant to do so in a commercial production pond.

In this study, the ratio of carbon to nitrogen input into the pond has not been examined closely. Optimising this ratio, in line with recommendations for the more intensive “Belize” system, has not been a part of the pond management strategy. In further research, it might be beneficial to examine this aspect and look towards a more heterotrophic system.

There needs to be further communication of the findings of this project to the prawn farming industry, and extension of information to them on the decision processes necessary for running a biofloc system. This training could be done in collaboration with the Sunshine Coast Institute of TAFE who provide training on a number of aquaculture topics, including the chemical analyses required for water quality monitoring and the identification of microalgae.

8. PLANNED OUTCOMES

8.1. Public Benefit Outcomes

The quantitative information produced in this project will help reduce the uncertainty about establishing and maintaining a good biofloc in the ponds. In this way more farms will be encouraged to investigate whether they want to adopt this system. As more farms are able to adopt the technology Australia will maintain its widely acknowledged global lead in the environmental management of prawn farms. Maintaining the efficiency and substantiality of Australia's prawn farming industry will contribute to providing new employment opportunities in a high value seafood industry in rural coastal regions of Australia.

8.2. Private Benefit Outcomes and Linkages with CRC Milestone Outcomes

The planned outcomes are of direct benefit to the industry participants and, as summarised below, are fully aligned with the CRC milestone outcomes.

- A reduction in feed costs through more efficient feed utilisation of between 20 and 30% per unit weight of prawn produced, depending on the farm's prior history of production. The target in the original application was 10 to 20%.
- A reduction of as much as 70% in the amount of water exchange on the farm (Original target was an 80% reduction in water exchange).
- An increase in overall productivity. Productivity at APF, when using a low water-exchange biofloc system, is estimated to be 50% greater than when they were operating using a conventional "open" water exchange system.
- Environmental management on the farm has been enhanced through a reduction of the nitrogen discharge from the farm by 77% per unit weight of prawns produced.

9. CONCLUSION

The core objectives of this study of the low water-exchange microbial floc production system at APF were; to gain an understanding of the nutrient dynamics in the prawn ponds; identify which nutrients were crucial for the development of the biofloc and; establish the required concentration of these nutrients. The results demonstrated that establishing a moderate to high density bloom of large diatoms, such as *Bellerochia horologicalis,* will consistently lead to the formation of a biofloc. A moderate or high density blue green algal bloom can also produce a weak floc. The results showed that it is necessary for the TAN in the pond to peak at a concentration > 0.9 mg/L within 50 days of filling the pond in order for a bloom of large diatoms to proliferate. The results also indicate that it is desirable to have at least 0.2 mg/L of reactive silica and 0.1 mg/Lof FRP in the pond water within a 15 day period following the peak in TAN. The results of pond trials indicate that urea is better at producing the peak in TAN than a fertiliser containing a mixture of urea and ammonium nitrate. In order to capitalise on the advances achieved in this project, further work needs to be done to increase the certainty of establishing a biofloc in a pond. The information generated during this project has provided Australian prawn farmers who have adopted the APF system with new information about how to establish and maintain biofloc in their ponds. The farmers will continue to gain the benefits of a more productive system with lower nutrient wastes.

The study has provided evidence that both organic carbon and inorganic carbon are very important in the management of a low water-exchange, biofloc system. Microalgae and litho-chemoautotrophic bacteria utilise inorganic carbon (bicarbonate) as their carbon source, whereas heterotrophic bacteria utilise organic carbon. Observations across all the ponds at APF over the three seasons have indicated that when a stable biofloc is established, the total alkalinity of the pond decreases progressively. This is probably due the uptake and utilisation of bicarbonate by litho-autotrophic bacteria. The consequence is that with a good biofloc there is a need to monitor the total alkalinity and add sodium bicarbonate to maintain the total alkalinity within certain limits (eg. 80 to 120 mg/L). However, statistical analysis of data from the five study ponds did not show a significant effect of biofloc on Total Alkalinity. However, the addition of sodium bicarbonate to the ponds in response to low alkalinity probably created too much variability in the data to detect a significant effect, if one was present.

Molasses has been demonstrated to be a very practical and effective carbon source. However, other organic carbon sources such as tapioca flour were not evaluated in ponds at APF, though other studies have given a clear indication of their comparative effectiveness. In a review of available organic carbon sources in Australia (K.C. Williams, CSIRO, personal communication), it appeared that molasses was the most cost-effective organic carbon source available to the prawn farming industry in Australia. APF have established some guidelines to the use of molasses in ponds. As the addition of molasses to a pond can reduce the following early morning DO concentration, care must be exercised in its use. When deciding on the amount of molasses to add to a pond, consideration should be given to the TAN in the pond, the species and state of the microalgal bloom, the previous day's morning and afternoon pH and DO concentrations, the amount of sunshine in previous days and the forecast amount of sunshine over the following few days, and the amount of feed going into the pond.

The study has clearly demonstrated the nutritional benefits of microbial flocs to *P. monodon* reared in ponds and quantify the associated reduction in feed costs. The feed conversion efficiency of the prawns reared in the APF system improved to be as low as 1.2. This is in contrast the results typically achieved in production systems that use routine water exchanges to manage water quality, where FCRs greater than 1.8 are commonly achieved (Lobegeiger and Wingfield, 2010). For a pond producing 10 tonnes of prawns, this represents a saving in feed costs of about $10,000 or about 30% of the total feed cost. Using APF production data for 2007/08, it was calculated that in ponds with the same management regime, the presence of a dense biofloc in a pond resulted in a saving of 3 tonnes of feed or about $6,000 per pond; that is about 20% of the total feed cost.

The reduction in farm nutrient discharge and the effects on farm profitability were calculated using data from APF from the 2003/04 season when an open system was being used, and from the 2008/09 season when the low water exchange system was used throughout the farm. These data show that in 2006/07 there was a reduction in water exchange of 78.5%, and in the three following years, the reduction was 68%, 69% and 73%. The Total Nitrogen discharge from APF in 2003/04 was 1.36 kg/ha/d (or 9,000 kg/crop). In 2008/09 the Total Nitrogen discharge was 0.5 kg/ha/d (or 3,400 kg/crop). However productivity in the 33 ponds increased from 220 t in 2003/04 to 360 t in 2008/09. Using these production values, the total nitrogen discharge was reduced from 40.0 kg/t prawns to 9.4 kg/t prawns - a reduction of 77%.

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On the basis of these results and the system management experience at APF, two additional farms are using the low water-exchange system in the 2010/11 season and at least two others are adopting techniques and learning skills in preparation for changing to this system in the future. In May 2010, Sunshine Coast TAFE ran a strategic planning workshop for farm owners and managers to equip them to make decisions about adopting this type of production system using the information generated by this project

10. ACKNOWLEDGEMENTS

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APPENDIX 1: INTELLECTUAL PROPERTY

No Intellectual Property is held for the Seafood CRC by this project.

All information generated by this project has been released into the public domain through presentations at the Australian Prawn Farmers Association conferences, at the workshop in Townsville and in personal communication to members of the public, particularly prawn farmers and barramundi farmers, who have requested information.

APPENDIX 2: STAFF

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