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Nutrient levels in experimental tanks supplied with prawn pond effluent: the effect of artificial substrate and different densities of the banana prawn *Penaeus merguiensis* (de Man)

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Abstract

To experimentally investigate the effect of vertical artificial substrate and different densities of the banana prawn *Penaeus* (*Fenneropenaeus*) *merguiensis* on nutrient levels in prawn pond effluent, a time series experiment was conducted in a replicated tank system supplied periodically with discharge from a prawn production pond. Few differences ($P>0.05$) were detected between tanks without prawns, and tanks with low densities (5 prawns in 1700 litres) of prawns (10-12 g), in terms of nitrogen and phosphorus in the water column over the 28-day experimental period. Higher densities of prawns (starting at 25 or 50 per tank) caused an elevation of these macronutrients in the water column. This was partly due to prawn biomass losses from mortalities and weight reductions in the tank system. The survival and condition of prawns was significantly ($P<0.05$) reduced in tanks at these higher densities. The presence of artificial substrate (2 m^2 $tank^{-1}$) did not affect ($P>0.05$) the levels of nutrients in tank water columns, but significantly ($P<0.05$) increased the amount of nitrogen in tank residues left at the end of the trial when no prawns were present. The prawns had obviously been grazing on surfaces inside the tanks, and their swimming actions appeared to keep light particulate matter in suspension. Higher prawn densities increased microalgal blooms, which presumably kept ammonia levels low, and it is suggested that this association may provide the means for improved remediation of prawn farm effluent in the future.

Keywords: banana prawns, artificial substrate, prawn farm wastewater, nutrients

Introduction

The bioremediation of wastewater from prawn farms was the subject of farm studies conducted by the Queensland Department of Primary Industries (QDPI) at the Bribie Island Aquaculture Research Centre (BIARC) during the 2001-2002 season in southeast Queensland. These studies (Palmer *et al.* 2002) investigated the growth of low densities of the banana prawn *Penaeus* (*Fenneropenaeus*) *merguiensis* in prawn farm effluent settlement ponds to improve profitability and potentially reduce waste nutrients. Whilst it was shown that good quality food grade banana prawns could be produced in prawn farm settlement ponds, the study could not determine whether the growth of prawns had detectable effects on the levels of waste nutrients in treatment ponds. However, this work suggested that nutrient discharge from the settlement pond stocked with banana prawns might have been higher than reported in other unstocked settlement ponds. Although farm differences hinder such comparisons, this could be expected if the prawns were mobilising nutrients that would otherwise settle and accumulate. It was also suggested that higher densities of banana prawns could have had a more pronounced effect on the accumulation of organics, which invariably leads to nutrient releases late in the production season through bacterial decomposition and remineralisation (Hargreaves, 1998; Burford and Williams, 2001).

The aim of the present study was to investigate the effect of different banana prawn densities on nutrients in prawn pond wastewater. Prawn densities were evaluated in combination with the artificial substrate used in previous farm trials. The present study also quantifies the effect of these treatments on nutrients in residues left in tanks, to investigate their effect on the nutrient retention capacities of settlement ponds.

Materials and methods

Experimental system

The experiment was conducted between the 4th September and the 2nd October 2002 at BIARC. Wastewater from a prawn culture pond was pumped simultaneously and at similar rates (8.5 - 9.8 L min^{-1}) into an outdoors series of clean round plastic tanks (dia. of 1550 mm, total volume of 2000 l). Each tank was fitted with a 50 mm standpipe for screened water to overflow to waste, giving a constant water depth of 900 mm and maximum water volume of 1700 litres. Each tank was also fitted with a 4 mm airline, which delivered constant moderate aeration (0.5-0.8 L min^{-1}) to the centre of each tank in mid-water column. Following the addition of prawns and /or artificial substrate at prescribed densities, tanks were covered with fine nylon mesh (8-10 mm) to prevent bird predation and prawn escape.

Banana prawns for the study were randomly cast netted from the culture/effluent-supply pond, and held in an oxygenated holding tank for 1-2 h before allocation to treatments. Counting and distribution involved gently netting at random from the holding tank, inspecting for health and vigour, weighing the group on bulk in a tared bucket of pond water (20 sec drip time for net), and placing in respective tanks. To reduce stress, handling was minimised during transfers and weighing (30-50 sec), and prawns were handled in the same pond water from collection to distribution.

Sheets of fibrous synthetic geotextile (Biddum A64 by Geofabric Pty Ltd) were used in tanks for artificial substrate. This was suspended vertically in the water column with flotation across the top and a ceramic weight on the bottom. Pieces of this substratum (2 x 1 m^2 per tank) were positioned on either side of the aeration soon after tanks were initially filled. They had been pre-conditioned in the culture pond for 3 weeks prior to use.

The culture pond supplying effluent for the study was a square high-density-polyethylene lined pond (1600 m^2, 1.5 m deep) with 2 x 1 hp aerators operating continuously. It had a single overflow monk-drain on one side, which draws water from the pond bottom. An estimated 48,000 juvenile *P. merguiensis* were stocked into this pond several months earlier to set up a typically commercial stocking density (30 m^{-2}). Specimens removed and some uncontrolled losses during the growout phase resulted in an anticipated survival of about 50 % at the time of this experiment. Prawns in the pond at this time were fed twice daily with a commercial prawn diet (Aquafeed grower for *P. monodon* from Ridley Pty Ltd. Narangba, Queensland, Australia) at 1-2 % body weight per day. The pond was supplied with a constant inflow (50-80 L min^{-1}) of filtered (250 µm) seawater (35-36 ppt), which produced a stable green microalgal bloom that prevailed throughout the experiment. General water quality measurements and management data for the prawn culture pond, during the period in which its effluent was used for this trial, is provided in Appendix 1.

Experimental design

24 tanks were monitored following 4 equal weekly pond-water exchanges. There were 8 experimental treatments with 3 replicates of each in a completely randomised design. The treatments were:

1. No artificial substrate + 0 prawns
2. No artificial substrate + 5 prawns
3. No artificial substrate + 25 prawns
4. No artificial substrate + 50 prawns
5. With artificial substrate + 0 prawns
6. With artificial substrate + 5 prawns
7. With artificial substrate + 25 prawns
8. With artificial substrate + 50 prawns

Every 7th day after the initial fill, water from the prawn culture pond that would otherwise have flowed over the monk drain, was pumped into each tank for 4 hrs (8 am – 12 pm), thereby adding >100% of the volume of tanks on each occasion. The flow was measured and adjusted each week to ensure similar exchange volumes for all tanks. In doing this, we attempted to simulate the periodic inflow of effluent into a settlement pond which remains full, where discharged overflows are subject to volumetric displacement by inflow waters. This assumes partial mixtures of settlement pond water and production pond effluent are discharged to waste during inflow events, as would often occur in an open pond.

Prawns in each tank were counted and weighed at the beginning, and on completion of the experiment, to allow survival and weight change comparisons. In conjunction with weighing and counting prawns on completion, the condition of each prawn was assessed by recording average length of antennae, and the degree of tail bites (eg: none, low, medium, or high).

Water temperatures (max/min) were recorded daily from random tanks in the set up. Other critical water quality parameters such as salinity, dissolved oxygen and pH were monitored in each tank with a Horiba water quality probe on a daily basis, with readings taken at approximately the same time each day (12 - 1 pm).

At the end of the 4-week trial period (2nd Oct 2002), the artificial substrate in tanks was gently removed, and each tank was drained to an approximate volume of 200 l. This was undertaken in a fashion that minimised the loss of loose organic matter, which had accumulated on the tank bottoms. At this point the prawns in tanks were easily removed with a net for weighing and inspections. Then, the sides and bottoms of each tank were scrubbed to dislodge all organic matter and suspend it in the water remaining. Samples of these residues were taken after vigorous mixing for homogeneity.

To provide pictorial evidence of the effect of different treatments on organic residues and fouling organisms, photographs were taken of the residues left on each tank bottom after draining (before scrubbing), and of a representative view of artificial substrate taken from each tank.

Water sampling and nutrient analyses

Sampling for nutrient determinations involved filling a 60 ml syringe in the mid water column of tanks without unduly disturbing artificial substrate (undertaken between 12 and 1 pm). Samples were equally split into filtered (0.45 µm) and unfiltered aliquots, which were immediately placed on ice and frozen (-20°C) soon after. Two samples of pond wastewater were also collected from tank inflow points during the initial fill and later exchanges. Thereafter, water samples were taken from each tank following 2, 4, and 6 day retention times. On the day that the experiment was terminated (7th day of retention in the 4th week), water samples (120 ml unfiltered) were taken from each tank before, and after, water levels were reduced to a volume of 200 l and tanks sides and bottoms were scrubbed with a course brush. Scrubbing involved dislodging all biofilms and grime on the inside surfaces of tanks so that it was mostly washed into water retained in the tank. Tank water was then vigorously mixed to provide evenly mixed residues for sampling.

Nutrients in water samples were assessed using similar methods to those used by Palmer *et al.* (2002) in related farm studies. All samples were analysed to derive total nitrogen (TN) and total phosphorus (TP). Samples taken from tanks at the 2-, 4-, and 6-day retention times, and from the inflow during exchanges, were also analysed for the breakdown of nitrogen into nitrite + nitrate (NOx), total ammonia (TAN) and organic nitrogen (ON), and for the breakdown of phosphorus into dissolved phosphorus (PO_4) and organic phosphorus (OP). Residue samples were homogenised prior to analyses for total Kjeldahl nitrogen and total Kjeldahl phosphorus.

Statistical analyses

Analyses of variance were performed using GenStat® for Windows, Fifth Edition. Repeated measures analyses of variance were performed where data comprised time series measurements. Comparisons of means were performed with least significance difference (LSD) testing using a 5% level of significance. Binary survival data were analysed using a generalised linear model (McCullagh and Nelder, 1989) with the binomial distribution and logit link (GenStat, 2000), followed by protected t-tests to determine significant differences between the means.

Results

Water quality

Salinity ranged from 35 to 37 ppt in tanks during the experiment. Water temperatures ranged from 17.0 to 27.5ºC (Figure 1). For pH and dissolved oxygen levels in different treatments, the interaction of artificial substrate and prawn density was not significant ($P>0.05$). However, artificial substrate had significant effects on pH ($P<0.01$) and dissolved oxygen ($P<0.05$). Prawn density also had significant effects on pH ($P<0.05$) and dissolved oxygen ($P<0.01$).

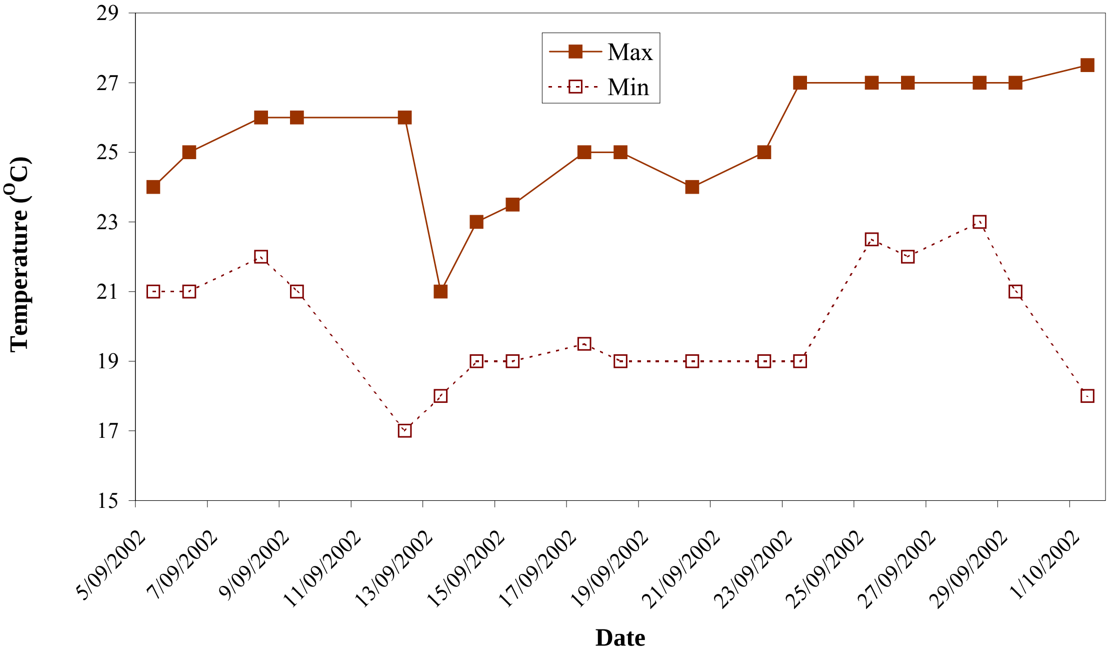

Figure 1. Maximum and minimum temperatures for water in tanks during the experimental period.

Tables 1 and 2 provide assessments of the influence of artificial substrate and prawn density respectively on pH and dissolved oxygen. Although the mean differences in pH were small, the consistency of these small differences between treatments and replicates resulted in significant ($P<0.05$) effects, where the presence of artificial substrate and increasing prawn densities increased pH levels. Similar trends are apparent for mean dissolved oxygen concentrations where levels were higher ($P<0.05$) in tanks with artificial substrate compared with those without, and the highest prawn densities produced the highest ($P<0.05$) oxygen levels.

Table 1. The mean effect of artificial substrate on pH and dissolved oxygen levels in water in tanks across the entire experimental period.

Parameter*	With artificial substrate	Without artificial substrate
pH	8.78a	8.73b
Dissolved oxygen	9.93a	9.65b

*Within rows, means followed by different letters are significantly different ($P<0.05$).

Table 2. The mean effect of prawn density on pH and dissolved oxygen levels in water in tanks across the entire experimental period.

Parameter*	No prawns	Low density	Medium density	High density
pH	8.75ab	8.72a	8.77bc	8.78c
Dissolved oxygen	9.53a	9.39a	9.95b	10.29c

*Within rows, means followed by different letters are significantly different ($P<0.05$).

Survival and condition of prawns

No mortalities occurred in the treatments with low densities (5) of prawns (Table 3). The effect of artificial substrate on survival was not significant ($P>0.05$), but the effect of prawn density on survival was highly significant ($P<0.01$).

Table 4 provides the mean starting and finishing weights derived from bulk weight estimates, and the mean weight change for individuals in each tank. Table 5 provides mean tail bite scores and antennae lengths for each tank on completion of the experiment. The weight losses and the condition of prawns following these treatments are summarised in Table 6. Prawns similarly lost weight in all tanks where no differences ($P>0.05$) were detected between treatments in terms of weight loss. The effect of artificial substrate on the tail bite score and antennae length was also not significant ($P>0.05$). Table 7 provides the pooled results for low, medium and high densities of prawns in terms of survival, tail bite score and antennae length.

Table 3. Survival of banana prawns in the tank system over the 4-week experimental period.

Treatment *	Number surviving (replicate 1, 2, 3)			Percentage surviving (replicate 1, 2, 3)			Mean (± se) percent survival
No AS + 5 prawns	5	5	5	100	100	100	100.0 ± 0.00
No AS + 25 prawns	22	19	22	88	76	88	84.0 ± 4.00
No AS + 50 prawns	27	26	30	54	52	60	55.0 ± 2.40
With AS + 5 prawns	5	5	5	100	100	100	100.0 ± 0.00
With AS + 25 prawns	23	20	19	92	80	76	82.7 ± 4.81
With AS + 50 prawns	30	32	40	60	64	80	68.0 ± 6.11

*AS refers to artificial substrate

Table 4. Weight changes in prawns in the tank system over the 4-week experimental period.

Treatment*	Mean starting weight (g)**			Mean finishing weight (g)**			Mean weight change (%)**		
No AS + 5 prawns	11.54	9.56	11.62	10.54	9.15	10.57	8.6	4.3	9.0
No AS + 25 prawns	13.10	11.86	13.12	11.25	10.97	12.25	14.1	7.4	6.6
No AS + 50 prawns	11.55	11.72	11.56	11.17	10.98	10.31	3.3	6.3	10.8
With AS + 5 prawns	11.98	12.13	12.14	10.92	11.30	11.54	8.8	6.8	4.9
With AS + 25 prawns	11.80	11.63	11.26	10.50	11.00	10.71	11.1	5.4	4.9
With AS + 50 prawns	12.38	10.84	11.81	11.65	10.05	11.11	5.9	7.3	5.9

*AS refers to artificial substrate
** Replicates 1, 2 and 3

Table 5. Mean tail bite scores and antennae lengths for each tank on completion of the experiment.

Treatment*	Mean tail bite score (0-5) (3 replicates)			Mean antennae length (cm) (3 replicates)		
No AS + 5 prawns	0.00	0.00	0.00	19.2	18.0	18.5
No AS + 25 prawns	0.41	0.21	0.09	14.4	17.6	16.0
No AS + 50 prawns	0.30	0.62	0.30	16.3	12.3	11.0
With AS + 5 prawns	0.00	0.20	0.00	19.5	19.5	19.5
With AS + 25 prawns	0.35	0.10	0.16	14.3	13.3	14.2
With AS + 50 prawns	0.23	0.13	0.45	13.8	14.5	14.4

Table 6. Weight change and condition summary for prawns in different treatments over the 4-week experimental period.

Treatment*	Mean (± se) weight loss (%)	Mean (± se) tail bite score**	Mean (± se) antennae length (cm)
No AS + 5 prawns	7.3 ± 1.51	0.0 ± 0.00	19 ± 0.4
No AS + 25 prawns	9.4 ± 2.38	0.2 ± 0.09	16 ± 0.9
No AS + 50 prawns	6.8 ± 2.18	0.4 ± 0.11	13 ± 1.6
With AS + 5 prawns	6.9 ± 1.12	0.1 ± 0.07	20 ± 0.0
With AS + 25 prawns	7.1 ± 1.97	0.2 ± 0.07	14 ± 0.3
With AS + 50 prawns	6.4 ± 0.48	0.3 ± 0.10	14 ± 0.2

*AS refers to artificial substrate
**0=none,1=low, 2=medium, 3=high

Table 7. Pooled results* for survival, tail bite score and antennae length at different prawn densities.

Prawn density	Mean survival (%)	Mean tail bite score	Mean antennae length (cm)
Low	100.0 a	0.03 a	19.1 a
Medium	83.0 b	0.22 b	15.0 b
High	61.7 c	0.34 c	13.7 b
Level of significance	P<0.01	P<0.05	P<0.001

*Within columns, means followed by different letters are significantly different.

Tanks with low densities of prawns had higher survival, lower amounts of tail bites and longer antennae than prawns in the medium- and high-density treatments at the end of the 4-week experimental period. Tanks with high densities of prawns had the lowest survival and the most tail bites. Differences in lengths of antennae between the medium and high prawn densities were not significant (P>0.05).

Nutrient analyses

The total nitrogen concentrations in water samples taken from different treatments in the experiment are displayed in continuous fashion over the entire experimental period in Figure 3. The effect of the artificial substrate on total nitrogen levels was not significant (P>0.05), but the effect of prawn density was highly significant (P<0.01). Figure 4 provides these total nitrogen results as a discontinuous time series when like prawn densities are pooled, and also displays the levels of total nitrogen in periodic exchange water (initial fill was 1.08 mg L^{-1} N).

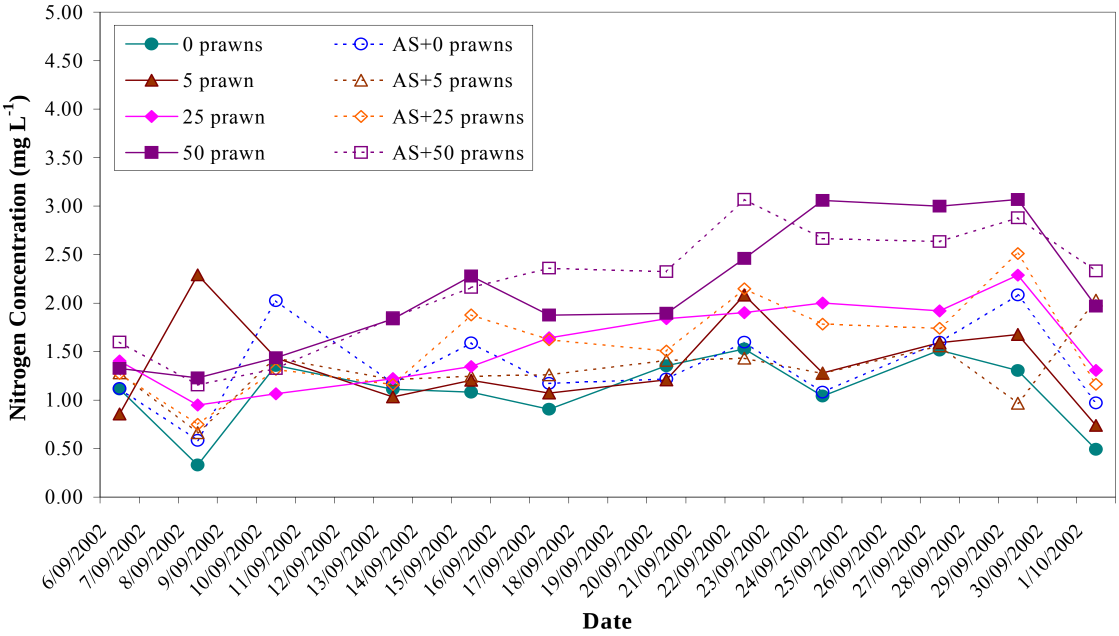

Figure 3. Total nitrogen concentrations in water samples taken from different treatments.

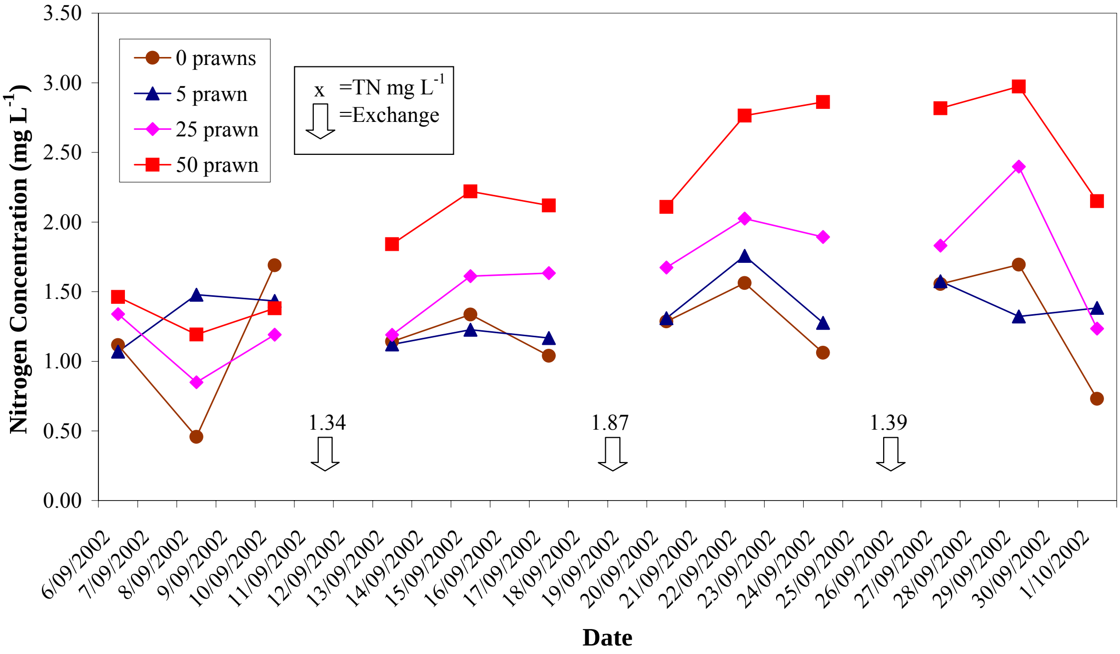

Figure 4. Total nitrogen concentrations with like prawn densities pooled.

Time series analyses for total nitrogen in the water column revealed a significant ($P<0.01$) interaction between the cycle (1-4) and prawn density. No differences between densities occurred during the first cycle, but as time progressed, the higher densities generally produced higher levels of total nitrogen in the water column (Table 8). Significant differences between the no- and low-prawn (5) treatments only occurred during the 4th cycle.

Table 8. Mean total nitrogen concentrations (mg L^{-1}) in tanks with different densities of prawns during each cycle in the time series analysis.

Prawn Density	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Mean across Cycles 1-4
0	1.088 ab	1.172 ab	1.303 b	0.884 a	1.112
Low	1.326 b	1.171 ab	1.448 bc	1.427 bc	1.343
Medium	1.126 ab	1.478 bcd	1.863 de	1.774 cde	1.560
High	1.345 b	2.060 c	2.579 f	2.647 f	2.158

*Means followed by different letters are significantly different ($P<0.05$).

The phosphorus concentrations in water samples taken from different treatments in the experiment are displayed in a continuous fashion over the entire experimental period in Figure 5. The effect of artificial substrate on total phosphorus levels was not significant ($P>0.05$), but the effect of prawn density was highly significant ($P<0.01$). Figure 6 provides these total phosphorus results as a discontinuous time series when like prawn densities are pooled, and displays the levels of total phosphorus in periodic exchange water (initial fill was 0.27 mg L^{-1} P).

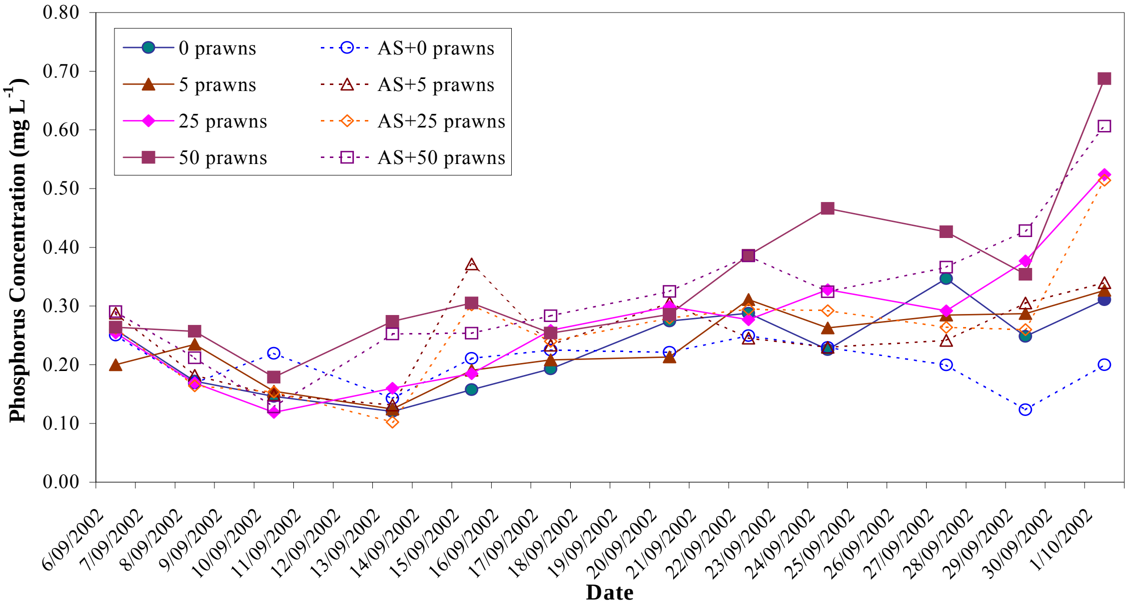

Figure 5. Total phosphorus concentrations in water samples taken from different treatments.

Time series analyses for total phosphorus in the water column also revealed a significant ($P<0.01$) interaction between the cycle (1-4) and prawn density. Again, no differences between densities occurred during the first cycle, but as time progressed, the higher densities generally produced higher levels of total phosphorus in the water column (Table 9). No differences occurred between the no- and low-prawn (5) treatments.

Table 9. Mean total phosphorus concentrations (mg L^{-1}) in tanks with different densities of prawns during each cycle in the time series analysis.

Prawn Density	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Mean across Cycles 1-4
0	0.2027 abc	0.1749 a	0.2479 bcde	0.2381 bcde	0.2159
Low	0.2013 abc	0.2099 abcd	0.2612 cde	0.2975 e	0.2425
Medium	0.1869 ab	0.2078 abc	0.2948 e	0.3716 f	0.2653
High	0.2215 abcd	0.2703 de	0.3620 f	0.4779 g	0.3329

*Means followed by different letters are significantly different ($P<0.05$).

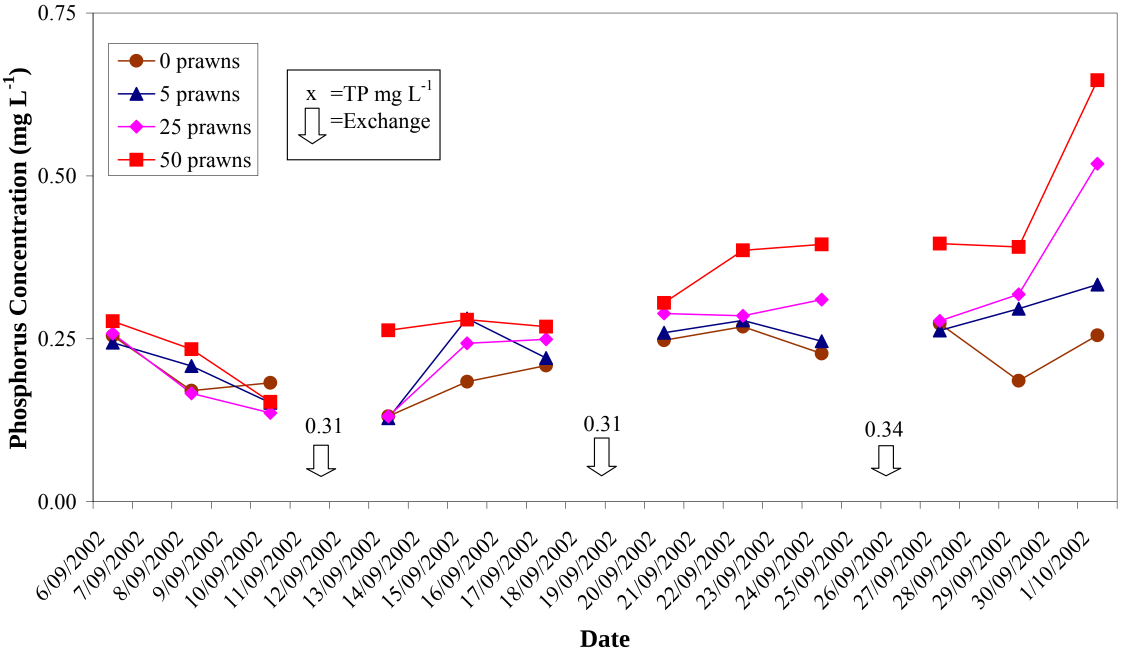

Figure 6. Total phosphorus concentrations with like prawn densities pooled.

Total ammonia nitrogen (TAN) represented a very small percentage (1.9 %) of the total nitrogen breakdown. Ammonia spikes occurred towards the end of the experiment in the high prawn density treatments, being associated with the 4th and 6th day of water retention (Figure 7) during the last cycle. Time series analyses did not reveal any significant effects of treatments on TAN.

NOx levels on average also represented a very small percentage (1.2 %) of the total nitrogen that occurred in tanks during the experiment. Although the time series analyses found significant main effects from density ($P<0.05$) and cycle ($P<0.01$), these data were somewhat compromised by the level of accuracy possible with the testing method (detection limit of 0.05 ± 0.01 mg L^{-1} NOx).

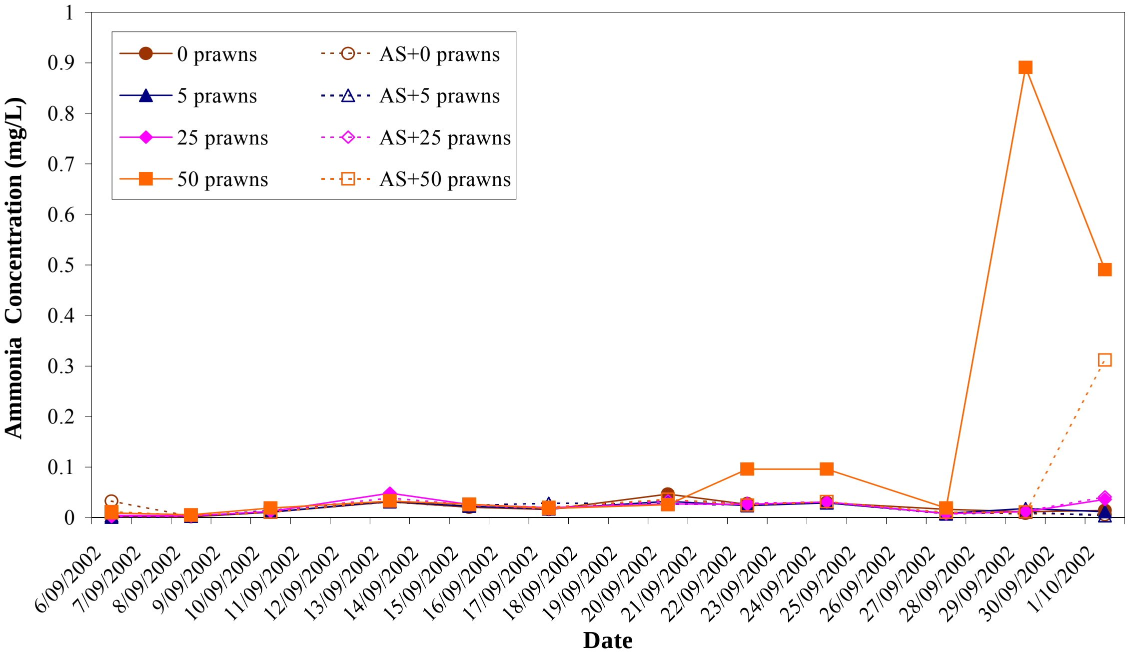

Figure 7. Total ammonia concentrations in water samples taken from different treatments.

The total Kjeldahl nitrogen (TKN) and total Kjeldahl phosphorus (TKP) in residues left in tanks at the end of the experiment are described in Figure 8. Data presented are concentrations obtained from homogenised residue samples minus the concentrations that existed in water in tanks prior to draining and scrubbing, and therefore collectively only represent nutrients in material dislodged from tank sides and in residues from tank bottoms.

There were no significant ($P>0.05$) treatment effects in terms of TKP in residues left at the end of the experiment. However, in terms of TKN, the interaction of artificial substrate and prawn density was highly significant ($P<0.001$). Compared with other treatments, nitrogen in residues was significantly higher ($P<0.05$) in the treatment with artificial substrate and no prawns (Figure 8). The next highest mean nitrogen residue level occurred in the treatment with high prawn density and no artificial substrate.

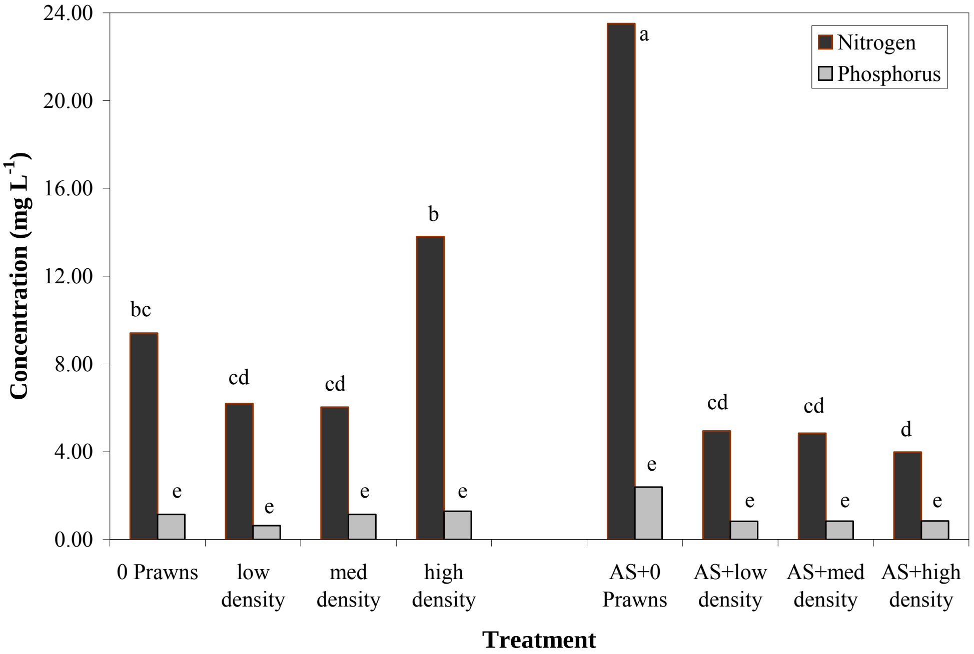

Figure 8. Mean total Kjeldahl nitrogen and total Kjeldahl phosphorus in residues left in tanks at the end of the experimental period. Within separate analyses for nitrogen and phosphorus, means with different letters are significantly different ($P<0.05$).

Nitrogen and phosphorus was lost from live prawn biomass during the experimental period, due to mortalities and losses of body weight in the tanks (Table 10). This increased with prawn density due mainly to reduced survival at the higher densities. Calculated across the 28-day experimental period, the low, medium and high prawn density treatments lost 4.4 mg nitrogen and 0.4 mg phosphorus, 75.8 mg nitrogen and 7.4 mg phosphorus, and 261.8 mg nitrogen and 25.6 mg phosphorus per day respectively from live prawn biomass.

Table 10. Mean macronutrients calculated* in biomass lost due to mortalities and prawn weight loss

Prawn density	**Mean wet weight loss from live biomass (g)**	**Nitrogen associated with mean loss of live biomass (mg)**	**Phosphorus associated with mean loss of live biomass (mg)**	**Estimated daily contribution to tanks of nitrogen (N) and phosphorus (P) lost from live biomass ****
Low	4.11	122	12	0.19 % N : 0.10 % P
Medium	71.48	2122	207	2.86 % N : 1.64 % P
High	246.80	7330	716	7.14 % N : 4.52 % P

* Wet weight of banana prawns assumed to consist of 2.97 % nitrogen and 0.29 % phosphorus after Palmer *et al*. 2002.

** Using averaged data from Tables 8 and 9 above, assuming 1700 litres per tank, and biomass loss equally over the 28-day experimental period.

Discussion

Water qualities, survival and prawn condition

The aim of this study was to experimentally simulate conditions in a settling pond, so that nutrient assessments for outflow and residues could be easily made. Each tank was completely clean at the start, and received similar exchanges, so nutrient differences at the end should only have been due to the treatments applied and natural variation. Constant low aeration was necessary to ensure oxygen levels were maintained above critical levels for the prawns, and the design created slow turbulence in the upper water column, not unlike wind generated currents in a settlement pond, with a deeper area that was less affected. Densities of prawns were designed to test low, medium and high stocking rates. The low density was similar to the densities used in the previous farm trial (1-5 m^{-2}). The medium and high densities were similar to medium and high densities used commercially in semi-intensive production ponds managed with regular water additions for algal bloom control.

Qualities of pond water can be greatly affected by algal blooms. Phytoplankton uptake and nitrification are the principal sinks for ammonia in aquaculture ponds (Hargreaves, 1998), and high algal activity can lower ammonia and orthophosphate levels and produce substantial amounts of dissolved oxygen (Krom *et. al.*, 1995; Bratvold and Browdy, 2001). Dense blooms of algae are also well known to cause high water pH in aquaculture ponds that are not well buffered (Pote *et. al.*, 1990). Whilst the biological relevance of the small differences found in treatment effects for dissolved oxygen (0.9 mg L^{-1} in the range 9.4 - 10.3 mg L^{-1}) and pH (0.1 in the range 8.7 - 8.8) is questionable, the effect of prawn density is likely to have been due to prawn excretions supplying ammonia to algal blooms. This is supported by observations that microalgal blooms in tanks appeared to be taking their own course towards the end of the trial. During the last few days, bloom colour variations in different tanks ranged from clear through various shades of green to brown, representing a variety of mixes of plankton and microalgae. Even though the effluent supply pond remained green and stable, the tank ecosystems after 1 month had diverged, so that the light green cultures related more to the low density tanks, and the thicker brown coloured blooms (40 cm Secchi depth) were predominately in tanks with the higher prawn densities.

Higher pH and dissolved oxygen levels also occurred in tanks with artificial substrate, although again, the mean differences were small and were not likely to have greatly influenced the condition of prawns or the other results of this experiment. Benthic algae were growing to varying degrees on all artificial substrate surfaces towards the end of the experiment, and through respiration and photosynthesis, it could have been responsible for these small pH and oxygen elevations. Inspections of the artificial substrate at the end of the experiment showed that substrate in the tanks without prawns had filamentous growth and yellowish-green benthic algae, whilst substrate from tanks with prawns had less filamentous algae and a greener appearance to benthic algae on surfaces. The prawns had apparently been grazing over the artificial substrate to keep filamentous growth in check, and were probably supplying the benthic algae with ammonia to produce new growth and a greener appearance. Appendix 2 provides some evidence of this effect in pictures of artificial substrate taken from tanks with different prawn densities at the end of the experiment.

Although the pH, dissolved oxygen and temperatures that prevailed in tanks during the experiment were well within suitable ranges for *P. merguiensis*, the prawns lost weight (6-9%) in all tanks during the experiment. These weight losses were not significantly affected by the presence of artificial substrate or prawn density. As density appears not to have been a driving factor in weight loss, one conclusion could be that food was plentiful but of low nutritional value to prawns of that size. The effluent sourced from the banana prawn culture pond for exchanges had a consistent green bloom with Secchi depth of 50 to 70 cm (see Appendix 1). It therefore contained algae and particulate organic matter, but the prawns do not appear to have been able to find enough suitable food in the experimental system to maintain their biomass. Faecal strands were plainly evident on the tank bottoms when the experiment was terminated, which demonstrates that the prawns were consuming available organic material, but energy expended collecting it may have outweighed the nutritional benefits it provided.

However crustaceans are well known for cannibalism, and mortalities that occurred would have provided a high protein supplement to the nutriments in the effluent so as to potentially mask the effect of density on growth. This was particularly so where survival was significantly effected by prawn density, so that prawns in the higher densities consumed more prawn biomass. Whilst banana prawns tend not to attack healthy individuals, moribund prawns and carcases are quickly consumed in semi-intensive conditions. Mortalities during the experimental period would also have added nitrogen and phosphorus to the water to complicate tank comparisons. Assessments of tail bites and antennae lengths were designed to provide an indication as to whether the prawns in different treatments were hungry enough to have reverted to cannibalism. The presence of artificial substrate did not affect this tendency, but increasing prawn density increased the level of tail and antennae damage. These results suggest that suitable feed was limited in tanks, particularly in treatments with the higher prawn densities.

Stress is another factor that may have affected the present results, particularly because the 1700 litre tanks were likely to be restricting the prawn's natural activities. Stressed prawns do not grow rapidly due to reduced feeding and delayed moulting (Funge-Smith and Briggs, 1998). Although prawns for the experiment were sourced from the same pond that was supplying the effluent, and so were well pre-conditioned to the water qualities in the experimental system when stocked, other factors may have prevented optimal vigour. For example, banana prawns in a pond can often be seen swimming with the paddlewheel-generated water currents in schools. Physically, the conditions in tanks are very different from open ponds that have space to aggregate and swim without impedances. The schooling behaviour may further provide a comfort level necessary for vigorous growth. The prawns in this study were accustomed to pond conditions when they were placed into tanks.

Nutrient levels

As discussed earlier, prawn mortalities in the higher density tanks complicated the nutrient assessments made to evaluate different treatments. Prawn weight losses would also have added nutrients to the water in tanks, and although this occurred equally for prawns in all tanks, greater biomass lost from live prawns would have occurred in treatments with more prawns. To evaluate the nutrient contributions to tanks during the experiment from these prawn biomass losses, macronutrient contents of prawns determined for the "BIARC pond" in the related farm-based study (same banana prawn stock as used in this study), were applied to estimate the amounts of nitrogen and phosphorus liberated from lost biomass. Calculations from Palmer *et al.* (2002) showed that nitrogen and phosphorus respectively made up averages of 2.97 % and 0.29 % of the wet weight of banana prawns. In the low-density treatments, no mortalities occurred, so nutrients released from lost prawn biomass was on average low. However, in the medium and high prawn densities, average survival dropped to 83.0 % and 61.7 % respectively, causing larger amounts of nutrients to be released from prawn biomass. These nutrient releases could potentially have affected both the nutrient concentrations in water samples taken periodically, and the nutrients in residual organics estimated at the end of the trial.

Nevertheless, these complications demonstrate the degree and potential for water qualities to be affected through stock mortalities. Although the timing of mortalities could not be determined in this or previous related studies, weight losses were monitored in both. In the tank trial, ammonia spikes in the final week may have been an indication of prior mortalities, whilst in the farm trial the effects of mortalities would have been largely masked by the incoming wastewaters. The apparent loss of prawn condition during this tank trial, and in one settlement pond in the farm trial provides some evidence of the development of nutritional deficits. This could occur in settlement ponds if the prawns outgrew the food in terms of their food particle-size preference or handling abilities. This would account for the pattern of growth of banana prawns in the farm trial at Farm A, which grew quickly over 80 days up to an average size of 17g, but then appeared to lose weight, dropping to an average weight of 15 g after 160 days.

To put the prevailing nutrient levels in tanks, and nutrients associated with biomass losses into perspective, average nutrient levels obtained from the different prawn densities (given in Tables 8 and 9) can be compared to the potential contribution of nutrients from prawn biomass. If biomass releases were assumed to have been released at equal rates each day during the 28-day experiment (see Table 10), comparisons suggest that only 0.19 % of the nitrogen and 0.1 % of the phosphorus (in the full tanks) could have been attributed to lost prawn biomass in the low-density treatments. However, 2.86 % of the nitrogen and 1.64 % of the phosphorus may have been added in the medium-density treatments, and 7.14 % nitrogen and 4.52 % phosphorus could have been contributed on average to the high-density treatments. These contributions do not account for the overall variation in nutrient contents of tanks with different prawn densities, but appear to proportionately add to the effect of higher prawn densities generating higher nutrient levels in the water column. These calculations also do not take into account other processes with potential to remove nitrogen like denitrification.

Density effects are well known in shrimp aquaculture where increasing stocking densities results in proportionately increased waste. For example, Martin *et. al.*, (1998) investigated the nitrogen budgets for ponds stocked with *P. stylirostris* at densities from 1 m^{-2} to 30 m^{-2}. Survival, final body weight and feeding efficiency decreased while nitrogen in suspended solids and the phytoplankton blooms increased with increasing prawn density. In that study, up to 38 % of the nitrogen that entered the pond accumulated in the sediment, but at the lowest density, nitrogen was reduced in the sediment. Natural food webs in the pond stripped nutrients and provided significant levels of feed for prawns at low densities.

Few studies have evaluated the degree to which shrimp can extract and assimilate nutrients from natural environments. Most recent work in this area has focussed on the assimilation of nutrients from artificial feeds in aquaculture systems (eg: Hargreaves, 1998; Burford and Williams, 2001). As mentioned previously, much of the prawn biomass that died in these tank trials is likely to have been consumed by other prawns, but only a portion of these nutrients would have been incorporated into the cannibal's biomass. Furthermore, not all of the nitrogen and phosphorus that is consumed as algae and detritus is assimilated. The amount of nutrients assimilated into shrimp biomass is a small fraction of total nutrients ingested, with only 18-27% of nitrogen available being assimilated (Funge-Smith and Briggs 1998). However, the level of assimilation may be much higher when the prawns are young, as suggested by the work of Velasco *et al.* (1998). They showed that postlarval *Litopenaeus vannamei* assimilated an average of 63 % of available nitrogen and 25 % of the available phosphorus in microcosm tanks designed to evaluate feed formulations. In bioremediation pursuits, the prawns should therefore be viewed not only as a sink for available nutrients while they are actively growing, but also as a means to cycle or mobilise nutrients through their physical activities and metabolic by-products. These considerations suggest that larger numbers of smaller prawns may provide a more effective nutrient sink in settlement ponds, as long as they are harvested at a small size, before they reach a point where energy expended collecting food may outweigh the nutritional benefits gained.

The artificial substrate used in this experiment did not appear to greatly influence the nutrient results. Whilst small differences in the pH and oxygen levels can be attributed to the presence of the artificial substrate, it had no detectable effects on total nitrogen, phosphorus, ammonia, and nitrite + nitrate (NOx) levels in the water columns of tanks. It's effect on phosphorus in tank residues at the end of the experiment, was also non-significant. However, total nitrogen in tank residues was higher in the treatment with artificial substrate and no prawns, than in all other treatments. A possible explanation of this is that artificial substrate baffled currents generated by the constant aeration in tanks, to reduce turbulence and allow lighter particles to settle. Without the prawn's activities, settled materials were not disturbed and hence were better retained in tanks with artificial substrate during exchanges.

Other studies have also documented a pronounced effect on pond sediments from the activity of shrimp. Martin *et al.* (1998) for example, suggested that the swimming actions of prawns at high densities caused significant erosion of pond bottoms and a greater build up of sediment deposited in pond centres. Attempts were made in the present study to simulate a settlement pond that overflowed during normal operations, and one that was to be drained for harvest of prawns. In this case, discharged volumes were the difference between inflow and evaporation over 7 days retention time. It was observed that the prawns in tanks caused a loss of organic matter with the termination drain, through their swimming activities, and this could also be expected to occur if prawns were to be harvested from a settlement pond.

In summary, few differences in nitrogen and phosphorus levels could be detected in tank systems with low densities or no prawns, but higher prawn densities caused an elevation of these nutrients in the water column. In reference to the farm trials which the present study supports, the data suggests that the low densities stocked into the farm settlement ponds (1.1 – 5.5 m^{-2}) were likely to have had insignificant effects on nutrient levels in settlement pond discharges, but are likely to have left the settlement ponds in a cleaner state at the end of the season. This final point supports the anecdotal evidence and observations of some of the farmers who participated in the farm based program.

Microalgae and bacteria play central roles in production pond and settlement pond ecosystems used for Penaeid aquaculture. These ecosystem components can be viewed as both the problem and the possible solution. Most excess nutrients eventually become either microalgal or bacterial biomass, which maintain water qualities and help remove the toxic metabolic by-products of prawns (eg: ammonia, nitrite). Whilst many microalgae and bacteria are also beneficial in prawn nutrition, their high levels in discharge waters can lead to undesirable nutrient outfall. Prawns cultured in settlement ponds appear to mobilise and drive nutrients into microalgal biomass. Many of the microalgae that quickly assimilate bioavailable forms of nitrogen can also be viewed as valuable commodities in their own right (eg: fatty acid extraction, vitamins and pharmaceutical uses: Borowitzka, 1995). Methods that remove and utilise microalgae through biological (eg: filter feeders like oysters or mussels) or mechanical means (eg: foam fractionation), will be useful in the future in conjunction with sediment recyclers to further improve profitability and environmental sustainability.

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Appendix 1. Water quality and management details for the prawn culture pond used as an effluent source.

Date	Time	PH	Temp (ºC)	Dissolved oxygen (mg/l)	Secchi depth (cm)	Feed added (kg)	Comments (eg: weather; operational)
4/9	0830	8.72	19.0	9.89	65	0	Overcast & windy
	1600	8.83	20.6	11.81	65	2	Filled experimental tanks
5/9	0915	8.78	20.9	10.54	65	2	Fine & sunny
	1530	8.86	22.3	11.57	65	2	
6/9	0830	8.77	21.2	9.66	60	2	Fine & sunny
	1515	8.80	22.8	11.44	70	2	
7/9	1100	8.78	22.2	10.28	70	2	Fine & sunny
8/9	1530	8.83	23.3	11.29	65	2	Rain overnight, sunny
9/9	0830	8.75	21.9	8.70	65	2	Fine; lots of particulate matter in water column
	1445	8.72	24.0	10.21	65	2	
10/9	0900	8.75	22.1	7.54	55	2	Fine & sunny
	1500	8.82	23.1	8.63	60	2	
11/9	1600	8.81	22.4	7.65	65	2 + 2	Fine & windy Water pumped to experimental tanks
12/9	0830	8.78	20.1	7.20	60	2	Overcast & windy
	1615	8.85	21.4	8.75	65	2	
13/9	0845	8.71	19.7	7.06	65	2	Cloudy and windy
	1500	8.81	20.7	8.13	60	2	
14/9	1400	8.83	21.3	8.45	65	2	Fine & windy
15/9	1130	8.81	21.0	8.21	50	2	Fine & sunny
16/9	0900	8.69	20.8	6.59	60	2	Fine & sunny
	1600	8.81	21.8	7.68	60	2	
17/9	0830	8.66	20.8	6.84	65	2	Fine & sunny
	1600	8.84	22.4	8.52	60	2	
18/9	0815	8.66	19.9	6.25	60	2	Rain o’night, fine & windy
	1615	8.78	21.3	8.13	55	2	Water pumped to experimental tanks
19/9	0830	8.70	19.5	7.61	60	2	Sunny & windy
	1530	8.35	25.5	6.28	60	2	Changed water quality meter
20/9	0830	7.32	19.4	7.33	60	2	Fine & sunny
	1530	7.95	21.0	10.85	60	2	
21/9	1000	8.36	21.3	8.67	65	2 + 2	Sunny
22/9	0900	8.75	22.1	7.54	65	2	Hot & sunny
	1300	8.83	23.4	10.02	65	2	
23/9	0830	8.76	22.6	7.63	60	2	Sunny
	1530	8.69	23.6	7.85	60	2	
24/9	0830	8.23	23.3	8.14	65	2	Sunny
	1600	8.41	24.6	10.19	55	2	
25/9	0830	8.23	23.5	9.12	60	2	Sunny
	1530	8.47	25.1	10.61	65	2	Water pumped to experimental tanks

Appendix 2. Pictures of one piece of artificial substrate taken from each replicate of the “No prawn” and “high-density” prawn treatments at the end of the experiment.