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Growing banana prawns, *Penaeus merguiensis* (de Man) in prawn farm settlement ponds to utilise and help remove waste nutrients

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Abstract

To assess their utility for profitable wastewater bioremediation, banana prawns, *Penaeus* (*Fenneropenaeus*) *merguiensis* (de Man), were stocked at low densities (1 – 5 m^{-2}) and grown without supplemental feeding in five commercial-prawn-farm settlement ponds (0.3 to 6.0 ha). The prawns free-ranged in the variously designed ponds for 160 to 212 days after stocking as PL15. Survival estimates ranged from 12% to 60% with production of 50 – 528 kg ha^{-1}. Over 1150 kg of marketable product was produced in the study. Exceptional growth was monitored at one farm where prawns reached an average size of 17g in 80 days. Nutrients in water flowing into (8 - 40 ML d^{-1}) and out of the settlement pond at that farm were assessed twice weekly along with routine water quality measurements. Only small differences in water qualities were detected between waters running into and out of this settlement pond. Total nitrogen levels gradually increased from 1 - 1.5 mg L^{-1} early in the season to over 3 mg L^{-1} towards the end of the season. Total phosphorus levels similarly rose from 0.1 - 0.2 mg L^{-1} to 0.3 - 0.4 mg L^{-1} in the middle of the season, but fell to 0.2 – 0.3 mg L^{-1} towards the end when approximately 12,000 prawns were harvested with a total weight of 175 kg. No significant differences ($P > 0.05$) were detected in the overall acceptability of prawns harvested from each of the 5 settlement ponds in small-scale consumer sensory analyses. The prawns from settlement ponds were rated similarly to banana prawns grown with commercial diets at two other establishments. Microbiological analyses of prawns from all farms showed bacterial levels to be well within food-grade standards and lower than prawns produced in a normal growout pond. These results demonstrate that high quality food grade banana prawns can be produced in these wastewater treatment systems.

Keywords: banana prawns, settlement ponds, bioremediation , prawn farm, wastewater, nutrients

Introduction

The environmental impacts and remediation of prawn farm waterwaters is an area of mariculture research that has received considerable recent attention (Funge-Smith and Briggs, 1998; Jones and Preston, 1999; Preston *et al.*, 2000; Jones *et al.*, 2001; Marinho-Soriano *et al.*, 2002). Whilst it is well recognised that good farm management practices can minimise the environmental impacts of prawn farms, there is a growing need to develop better water-reuse and treatment systems for more profitable and sustainable production. These considerations include reduced environmental impacts, improved biosecurity, better disease prevention abilities, lower wastes and higher efficiencies.

Strong interest is also developing in Australia regarding various aspects of the husbandry and culture potential for the banana prawn, *Penaeus* (*Fenneropenaeus*) *merguiensis* (de Man, 1888). In nature, this species mainly inhabits mud-mangrove habitats (Staples *et al.*, 1985), and has been extensively farmed in Asia for many years. In some respects, it is thought to be a better species for farming than the more commonly farmed black tiger prawn (*Penaeus monodon*), and its apparently high affinity for detritus and algae has lead to it also being proposed as a suitable species to grow in prawn farm settlement ponds in Australia (Hoang, 2001).

Prawn farmers in Australia often use sedimentation ponds to help meet discharge water quality standards set by regulators. Recent studies have shown that suspended solids and nutrients such as nitrogen and phosphorus can be reduced early in the production season with these simple water treatment systems (Preston *et al.*, 2000). However, algae senescence, settlement of particulate organic matter and bacterial decomposition later in the season can cause nutrient levels in settlement pond discharge to exceed inflow. Hence, cost-effective methods of maintaining these ponded areas in a healthy state and improving their water remediation capabilities are desired. Whilst various secondary cash crops such as seaweeds, bivalves and fish have been proposed for this purpose, prawns may offer a more convenient crop that can draw on the existing infrastructures and markets of a commercial prawn farm. Besides representing an outright sink for waste nutrients, banana prawns can help remineralise particulate organic material, and make it bioavailable for uptake by algae.

The aim of this study was to investigate the feasibility of extensively farming *P. merguiensis* in prawn farm settlement ponds without direct feeding, to improve profitability and potentially reduce waste nutrients.

Materials and methods

Seed stock production

In November 2001, approximately 285,000 banana prawn postlarvae were produced for this study at the Bribie Island Aquaculture Research Centre (BIARC). These postlarvae (23-day-old PL15) were produced from 4 families within a wider breeding program conducted for this species by the Queensland Department of Primary Industries (QDPI). Larval rearing methods used a combination of commercial production technologies (P.J.Palmer and M.J.Burke pers. com.) and methods published for this and other Penaeid species (Lim *et al.*, 1987; Luxe Enterprises Ltd., 1989). They were stocked into 5 commercial prawn farm settlement ponds (A-E) at low densities (approximately 1-5 m^{-2}) on the 13th and 14th November 2001. No artificial feeds were given directly to these settlement ponds. The stocked banana prawns were therefore required to forage for foods within the settlement ponds' ecosystems, and grow solely on the waste products entering the settlement ponds and natural feeds generated by available nutrients.

Clear pathology reports were received for each prawn family before stocking into settlement ponds (QDPI Veterinary Laboratory with NATA accreditation). Pre-stocking pathological screening involved histological examinations of no less than sixty postlarvae from each larval culture for evidence of viral infection, fouling, granuloma formation, haemocyte aggregations, muscle necrosis, appendage erosion, melanisation or bacterial infection. To predict the genetic potential for survival in farm settlement ponds, we also assessed the survival of the 4 families used in 25,000 L concrete nursery raceways after PL15. The stocking density of nursery raceways was 1 L^{-1}, which were fed commercial Penaeid starter feeds according to an estimated 10% total biomass weight updated weekly. Larviculture survival estimates were undertaken by volumetric sub samples taken from well-mixed harvest concentrations. Nursery survivals were estimated by total live biomass weight (corrected for water retention by 0.9 multiplier) divided by mean individual weight (blotted dry).

Description of each farm

The five participating prawn farms were located between Baffle Creek and the Logan River in south-east Queensland. A description of these farms' settlement ponds is provided in Table 1.

Table 1. Attributes of settlement ponds and associated production facilities in the study

Farm	Total area under cultivation (ha)	Production pond to settlement pond area ratio	Number of production ponds	Settlement pond stocking density (m^{-2})
A	10	1 : 0.30	11	2.6
B	10	1 : 0.09	12	5.5
C	2	1 : 0.15	2	4.1
D	20	1 : 0.15	15	1.1
E	17	1 : 0.35	10	1.9

All farms were primarily involved with semi-intensive *P. monodon* production using flowthrough systems for algal bloom management. Settlement ponds were screened to physically prevent the escape of the stocked banana prawns. Some of the peculiarities of the different farms and their settlement ponds for water treatment are listed below:

1. Farm A (27º 42´ S 153º 15´ E), situated on the Logan River south of Brisbane, had a single large ovoid-shaped settlement pond. This farm had installed 250 metres of vertical geotextile fabric (Geofabric® Biddum A12) to act as artificial substrate for improved benthic productivity and to baffle water flows.
2. Farm B (27º 41´ S 153º 15´ E), also situated on the Logan River, had two settlement ponds in series. Only the primary settlement pond (average depth of 2.7 metre) was used for this study. It had been fitted with approximately 60 metres of vertical nylon shadecloth (70%) to help direct water flow.
3. Farm C (27º 01´ S 153º 05´ E) was situated adjacent to Bullock Creek in the Pumicestone Passage area north of Brisbane. Its treatment system consisted of a 1-2 metre deep channel that overflowed into an ovoid-shaped settlement pond. An undetermined number of mullet of 3 different age classes were present in this settlement pond at the time of stocking the banana prawns. They had entered the prawn farm settlement system in the off-season, and had been trapped by the installation of screens on commencement of production.
4. Farm D (24º 94´ S 152º 46´ E) was situated on the Elliot River south of Bundaberg. Its water treatment system consisted of two elongated settlement ponds linked in series by a wide (approx 10 m) 2-3 metre deep channel. The stocked banana prawns therefore had open access to both of these settlement ponds.
5. Farm E (24º 53´ S 151º 98´ E) was situated on Baffle Creek north of Bundaberg. It also used shallow drains to collect production pond discharge. Two large square treatment ponds of equal size (3 ha) were linked in series by a small channel in the bank between them. Discharge from the farm flowed into the primary treatment pond at two points opposite this linking channel. Banana prawns stocked had access to both ponds.

In all 5 cases postlarvae were in excellent condition when stocked. Conditions in the settlement ponds were similar at all farms, with dense green phytoplankton blooms, some benthic algal production and variable amounts of reduced sediments noticeable around the peripheries. The settlement pond at Farm A contained juvenile prawns (up to 2 g in size) of an undetermined species (not *P. merguiensis*), and at Farm C contained sea mullet (*Mugil cephalus*) in moderate numbers when the banana prawns were stocked.

<u>Prawn growth and survival estimates</u>

Banana prawns were sampled (>30) from each settlement pond periodically using a cast net, and individually weighed. At the end of each farms production season, settlement ponds were harvested and banana prawns and other harvestable biomass were quantified.

<u>Microbiological analyses</u>

Banana prawns harvested from each settlement pond were immediately washed in clean seawater at each farm, and a random sample of these was placed inside a clean plastic bag and stored in crushed ice for approximately 5 hours prior to food-standard microbiological examinations. This involved submitting no less than 100 g of prawn flesh to NATA Accredited Laboratory Number 199, for testing total bacterial levels (standard plate count g^{-1}), *Escherichia coli* (g^{-1}) and *Salmonella* spp. ($25g^{-1}$). A random sample from a pond of dry-pellet-reared banana prawns at BIARC, was also submitted for this testing to provide a comparison with prawns taken from the settlement ponds.

Standard plate counts determined the total number of viable bacteria after plating on a non-selective growth medium and incubation at 30ºC for 72 ± 2 h (Australian Standard Method [AS] 1766.2.1 – 1991). Quantitative *E.coli* assessments used the "most probable number" method with the multiple tube technique and lauryl tryptose broth as the primary selective medium (AS 1766.2.3 – 1992). Presence/absence testing for *Salmonella* spp. (AS 1766.2.5 – 1991) involved 25g of prawn flesh incubated in a pre-enrichment medium at 37ºC for 16 to 20h. This was sub-cultured into 2 selective enrichment media - Mannitol Selenite Cystine Broth (MSCB) and Rappaport-Vassiliadis Broth (RVB) and incubated at 37ºC and 42ºC respectively for 18 to 24h. Then, aliquots of both the MSCB and RVB cultures were streak-inoculated onto the solid selective media Xylose Lysine Desoxycholate Agar (XLDA) and Bismuth Sulphite Agar (BSA). The XLDA and BSA plates were incubated at 37ºC for 24h and 48h respectively and examined for typical *Salmonella* colonies. Any typical colonies were checked for purity and subjected to biochemical and serological tests for characteristic reactions of salmonellae (personal communication Ian Miller QDPI).

Prawn consumer acceptability
Samples of banana prawns harvested from each settlement pond, washed and stored in ice as above, were assessed for sensory acceptability by a small panel of consumers. Each sample was treated with sodium metabisulphate for 30 sec (1kg in 100 l freshwater at 0-4°C) to prevent discoloration during storage. After draining for 5 min, they were individually quick-frozen (IQF) in a –20°C brine (15 min). They were coated with a freshwater glaze to prevent freezer burn, and again immersed in the IQF brine (2 min). Samples were then stored in a freezer (-28ºC) for 6-13 weeks until thawing prior to sensory testing. All treatments were thawed under ice (at 2ºC) overnight, and cooked the next day at the same time in separate mesh baskets inside a gas-fired prawn cooker (3.5% salt solution). After cooking (3 min 20 sec) they were chilled in an ice slurry (3.5% salt), and stored at 5ºC in preparation for sensory testing later the same day.

Control treatments comprised *P. merguiensis* reared on commercial feeds at BIARC (using the same post-harvest treatment described above), and *P. merguiensis* supplied in the frozen state by another commercial prawn farm. Controls were cooked as above. The mean (± se) size of prawns in the BIARC control group was 9.7 ± 0.53 g (min. 6.0 g, max. 16.7 g, n = 30), and in the commercial control was 28.7 ± 0.58 g (min. 17.0 g, max. 35.6 g, *n* = 56).

Nineteen panellists each received two whole prawns from each treatment. Panellists were asked to peel the prawns before completing the sensory assessments. Attributes were rated on both hedonic and intensity scales based on a standard rating test (AS 2542.2.2.3 – 1988) with unstructured line scales. Attributes tested on hedonic scales included odour, appearance, flavour, texture and overall acceptability. Prawn flavour and firmness were rated on intensity scales. Panellists were also able to choose standard descriptors and add comments regarding particular likes or dislikes for each sample. For each attribute, panellist scores were analysed using a two-way analysis of variance using a complete block design with panellists as blocks. Where a significant F-ratio ($P<0.05$) was found, pair wise comparisons were made using Tukeys Honest Significant Difference (HSD).

Settlement pond water analyses
Biweekly water samples were taken at the inflow and outflow points to the settlement pond at Farm A (13 Dec - 23 Mar) to investigate macronutrient levels. Sampling, undertaken by the farmer, involved splitting fresh samples into filtered (0.45µm) and unfiltered sub samples (60 ml), immediate storage on ice, then freezing. At BIARC, samples were thawed and tested for total nitrogen (TN), total phosphorus (TP), and dissolved inorganic nutrients (DIN) including total ammonia (TAN), oxides of ammonia (NOx) and phosphate (PO_4). TN and TP determinations involved the Kjeldahl digestion of known volumes of samples in concentrated sulphuric acid with a copper sulphate acid catalyst, followed by analysis with a Lachat QC8000 Flow Injection Analyser as described in the instrument manufacturers methods (QuickChem Methods, Zellweger Analytics Inc. Milwaukee WI 53218). Acid strength was increased in the digestion and carrier solution to account for the high salinity of the samples.

DIN (TAN, NOx, PO_4) were also assessed by Flow Injection according to the methods of the instrument manufacturer. Briefly, TAN was converted to blue indephenol via the Bertholot reaction, NOx was determined after reduction of NO_3 to NO_2 in a copperised cadmium column and conversion of NO_2 to a pink dye in the presence of sulphanilamide, and PO_4 was reacted with molybdate and antimonyl tatrate to form a yellow complex. NO_2 and NO_3 were analysed together as NOx. Routine water quality measurements including salinity, dissolved oxygen, temperature and pH were also measured *in situ* at the time water samples were taken. Water flow into the settlement pond was measured with a laser-calibrated flowmeter (Starflow ultrasonic Doppler instrument: model 6526B).

Prawn macro-nutrient analyses
Nitrogen and phosphorus determinations were undertaken for prawn biomass from each settlement pond at harvest (except farm C), and the culture pond at BIARC. Whole prawn samples were held on ice for up to 5 hr, frozen and stored at -18ºC for 3-5 months before analyses for total nitrogen and total phosphorous contents. Prior to chemical analysis frozen prawns were sectioned and weighed. Samples were then freeze-dried (Lindner & May Pty Ltd) for 24 hr with dry matter determined from weight change.

Nitrogen and phosphorus contents were determined for two replicates from each sample. For total nitrogen, samples (400 mg dry matter) were wrapped in nitrogen free paper and pelleted prior to analysis by the combustion method described by Sweeny (1989) using an Elementar Rapid N analyser.

The instrument was calibrated using AR grade aspartic acid, with accuracy quoted by the manufacturer as <0.5% relative standard deviation for homogeneous test samples. The ashing method was used for total phosphorus content determinations. Following ignition at 600°C for 3h and HCl digestion (A.O.A.C. 1980), phosphorus was measured by colorimetry.

Results

Survival and growth of seed stock in larval cultures and nursery systems

Survival in larvicultures ranged from medium to high levels, and survival in the nursery systems was above 50% for all families used, despite the fact that conditions in these nursery systems were not conducive for rapid growth (Table 2).

Table 2. Survival of banana prawn families in larvicultures (23 days old), and survival and growth in nursery systems at BIARC over a further 70 days.

BIARC prawn-family Code	Survival (%) in larvicultures* up to PL15	Survival (%) in concrete nursery systems* at BIARC	Mean (± se) weight (g) of juveniles** harvested from concrete nursery systems	Farm settlement ponds stocked with PL15
Pme 33	71.6	69.9	0.76 ± 0.034	A
Pme 35	60.5	53.1	0.95 ± 0.036	B & C
Pme 41	40.8	83.2	0.56 ± 0.026	D
Pme 75	94.9	66.2	0.45 ± 0.024	E

**n* = 1 ***n* = 50

Growth of prawns in settlement ponds

Banana prawns stocked into all farm settlement ponds reached a marketable size before harvest (Figure 1), and this was achieved at Farm A in less than 80 days (mean of 17.4 g; range of 13 – 23 g). Negative growth, and deterioration of condition was evident at Farm A past 80 days. Steady growth throughout the season occurred at the other four farms. Mean harvest weights ranged from 13.7 g at Farm C, to 21.7 g at Farm E (Table 3). The smallest banana prawn in harvest samples was 10.2 g from farm A, and the largest was 26.7 g from Farm E.

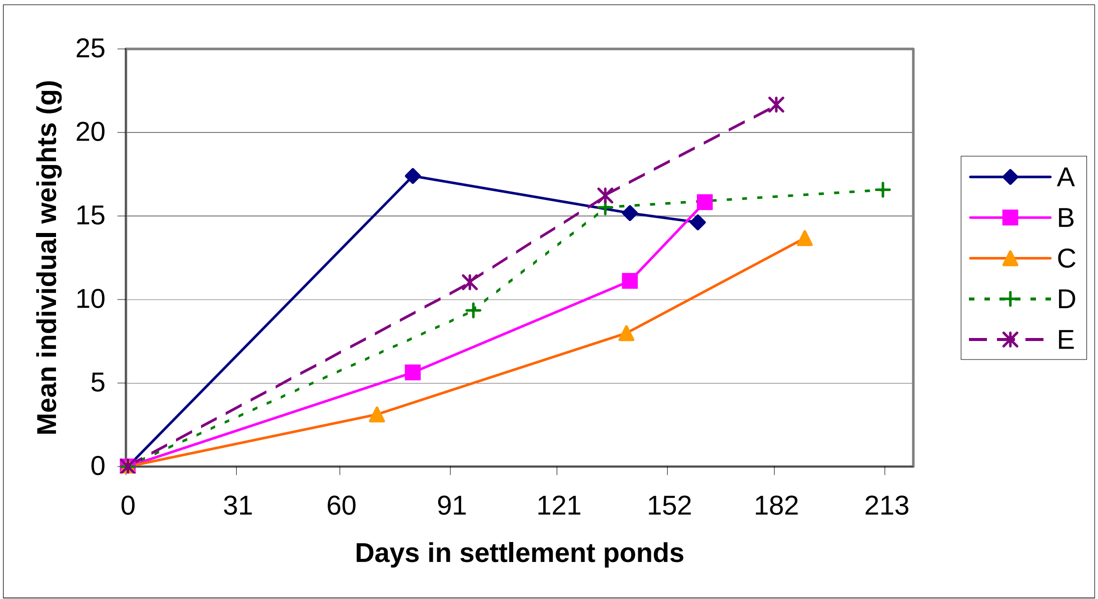

Figure 1. Growth of banana prawns in the settlement ponds of 5 commercial prawn farms (A – E).

Survival of banana prawns in settlement ponds

The study assumed that no other banana prawns had entered settlement ponds other than those that were stocked as postlarvae. Only one farm (B) displayed moderately high survival (60.5%) (Table 4). All farms reported harvest difficulties of one form or another, and all farms reported bird predation. Both of these factors could have contributed to lower recovery at harvest. Farms C and D could not complete the harvest of settlement ponds because the pond bottoms were lower than their bottom discharge pipes (regulatory control on sludge release applied by Environmental Protection Authority at some farms). Whilst Farm D attempted an estimate of the amount of unharvestable prawns, Farm C considered this too difficult to estimate.

Based on these harvest estimates, production per hectare ranged from a high of 528 kg/ha at Farm B, down to 67, 58 and 50 kg/ha in farms D, A and E respectively. Across all five farms in the study, more than 1150 kg of prawns were produced from waste nutrients and natural production in settlement ponds.

Table 3. Sizes of banana prawns at harvest from the settlement ponds of 5 commercial prawn farms.

Farm	Harvest sample date	Time in pond (d)	Mean ± se weight (g)	Minimum weight (g)	Maximum weight (g)	Number in sample
A	22/ 4/ 02	160	14.6 ± 0.36	10.2	18.3	30
B	24/ 4/ 02	162	15.8 ± 0.24	13.9	20.2	30
C	22/ 5/ 02	190	13.7 ± 0.19	10.6	19.5	56
D	14/ 6/ 02	212	16.6 ± 0.32	14.1	20.2	30
E	15/ 5/ 02	182	21.7 ± 0.36	18.4	26.7	30

Table 4. Harvest and survival estimates for banana prawns grown in the settlement ponds of 5 commercial prawn farms.

Farm	Number stocked	Mean individual harvest weight (g)	Total *P.merguiensis* harvest weight (kg)	Number of *P.merguiensis* in harvest*	Estimated survival (%)
A	78,000	14.61	175	11,978	15.4
B	49,600	15.83	475	30,006	60.5
C	12,400	13.67	Not available	-	-
D	33,800	16.58	200**	12,063	35.7
E	111,678	21.66	300	13,850	12.4

*Calculated from mean sizes and total amounts harvested.
**Estimate only due to harvest difficulties.

Species other than *P. merguiensis* harvested from the different settlement ponds are described in Table 5. *P. monodon* found in settlement ponds were mainly animals that had escaped from production pond harvest activities, except for farm D. In this case a production pond had been cleared of low-survival stocking prior to restocking, which presumably led to a population of *P. monodon* from an early stage in that settlement pond.

Over 600 kg of mullet with a range of sizes (200g – 2kg) died from lack of oxygen at farm C in mid-May 2002. This appears to have been a consequence of lowering the level of water in the settlement pond overnight. Despite this prior loss of over 99% of the mullet in this settlement pond due to low oxygen levels, banana prawns could still be captured with a cast net one week later.

Microbiological assessments of banana prawn tissues

All prawns from settlement ponds and those from the pond at BIARC were well within recommended health guidelines for frozen raw crustaceans (ICMSF, 1986) (Table 6). Interestingly, prawns fed a commercial diet had a higher level of viable bacteria than samples from the settlement ponds.

Table 5. Amounts of species other than *P. merguiensis* estimated at harvest for each of the five prawn farm settlement ponds.

Farm	*P. monodon* (kg)	Other species taken in final harvests and species noted in samples and during growout
A	5 –10	Glass shrimp *Acetes sibogae australis* (many kg) Greasy prawn *Metapenaeus bennettae* (1 kg) Various small fishes (4 –5 kg): Silver Biddy *Gerres ovatus*; Sand Whiting *Sillago ciliata*; Stripey *Lutjanus carponotatus*; Scat or Striped Butterfish *Selenotoca multifasciata* Mudcrab *Scylla serrata* (4)
B	50	Glass shrimp *Acetes sibogae australis* (many kg) Sea mullet *Mugil cephalus* (50 kg) Various small fishes (2 kg): herring presumed *Harengula abbreviata* Eels *Anguilla* spp. (6-7) Mudcrab *Scylla serrata* (3)
C	Not available	Sea mullet *Mugil cephalus* (>600 kg)* Oxeye herring *Megalops cyprinoids* (1)
D	Approx. 200	Red endeavour prawn *Metapenaeus ensis* (severa1 kg) Various small fishes: Southern Herring *Harengula abbreviata*; Silver biddy *Gerres ovatus*; Trumpeter whiting *Sillago maculate* Dusky flathead *Platycephalus fuscus* (up to 23 cm long and 85.5g) Sea hare *Stylocheilus striatus*; Bloodworms (*Marphysa* spp.)
E	5 -10	Greasyback prawn *Metapenaeus bennettae* (5 kg) Various small fishes (up to 10 kg total) including the Southern herring *Harengula abbreviata*

* Over 600 kg of sea mullet were overcome by a low oxygen event at this farm in mid-May.

Table 6. Microbiological analyses of banana prawns grown in the settlement ponds of 5 commercial prawn farms and a culture pond at BIARC.

Farm	Standard Plate Count (g^{-1})	*E. coli* (g^{-1})	*Salmonella* ($25g^{-1}$)
A	3900	<2	ND
B	2800	<2	ND
C	3800	<3	ND
D	2400	2	ND
E	4200	<3	ND
BIARC sample	7700	<3	ND

ND = not detected.

Prawn consumer acceptability

The odour of prawns from Farm C was liked significantly more (P<0.05) than that of the prawns from Farm B and the commercial control (Table 7). No other significant differences (P>0.05) in the acceptability of odour were detected.

No significant differences (P>0.05) were detected between the acceptability of appearance of prawn flesh from different settlement ponds and the commercial control. The appearance of the BIARC control treatment was liked the least, and they were liked significantly less (P<0.05) than those from farms B, D and E. Comments made by the panellists suggest the smaller size and pale colour may have contributed to this lower acceptance.

Flavour and texture did not vary (P>0.05) among treatments. The majority of panellists described the flavour of prawns from settlement ponds as fresh. All prawns from settlement ponds rated similarly for firmness, and were not rated differently (P>0.05) from both BIARC controls. The commercial control was rated as significantly firmer (P<0.05) than both the BIARC controls.

No significant differences (P>0.05) were found in the overall acceptability of any of the prawn samples. On the scale from “dislike extremely” (0) to “like extremely” (100), none of the samples scored very highly but all were above the “neither like nor dislike” (50) level (mean sensory scores ranged from 54 to 66).

Table 7. Mean sensory scores from acceptance testing of banana prawns grown in settlement ponds and control groups.

	Odour[1]	Appearance[1]	Flavour[1]	Prawn Flavour[2]	Texture[1]	Firmness[3]	Overall[1]
Farm A	58 ab	68 ab	61 a	67 a	64 a	63 ab	62 a
Farm B	55 b	74 a	64 a	63 a	69 a	64 ab	64 a
Farm C	69 a	68 ab	63 a	58 a	68 a	62 ab	61 a
Farm D	57 ab	71 a	61 a	58 a	67 a	61 ab	62 a
Farm E	58 ab	76 a	65 a	60 a	70 a	68 ab	66 a
BIARC control 1	61ab	54 c	60 a	57 a	62 a	58 b	58 a
BIARC control 2	64 ab	55 bc	67 a	65 a	66 a	59 b	65 a
Commercial control	54 b	63 abc	56 a	53 a	62 a	74 a	54 a
Tukey's HSD	13.4	13.8	16.2	18.8	14.4	14.1	16.4

[1] Scale: dislike extremely (0), neither like nor dislike (50), like extremely (100)
[2] Scale: none (0) - strong (100)
[3] Scale: very soft (0) – very firm (100)
a b c Within columns, means followed by a common letter are not significantly different (P>0.05)

Settlement pond water analyses

Figure 2 describes the dissolved oxygen concentrations and salinity, and Figure 3 describes the temperature and pH of water flowing into and out of the settlement pond at Farm A. Low rainfall throughout the 2001-2002 season in south-east Queensland caused moderately high prevailing salinities at those prawn farms that could not access significant freshwater supplies. Salinities in the settlement pond at Farm A accordingly remained in the order of 28 to 30 ppt during the study. Dissolved oxygen measurements ranged from 4.0 to 8.7 mg L^{-1}, which was above critically low levels (2-3 mg L^{-1}) that would have caused prawn mortalities in the settlement pond. Water temperatures ranged from 25 to 30ºC and pH ranged mainly from 7.2 to 8.4 (lowest inflow level of 6.5; lowest outflow level of 6.2) with no apparent trend differences between inflow and outflow water.

Water use tended to increase during the season though rates varied within weekly time frames (Figure 4). Low water usage rates (7 – 8 ML d^{-1}) were often followed by high rates, with a maximum of 40.3 ML d^{-1} recorded close to the end of the season. For nutrient export estimates (see below), the average of volumes flowing into this settlement pond between the 8 February and 23 March (17.1 ML d^{-1}) is used in conjunction with results of water analyses for this period to calculate mass quantities of nutrients discharged in settlement pond outflow between 13 December and 23 March.

Nitrogen levels gradually increased from 1 - 1.5 mg L^{-1} early in the season to over 3 mg L^{-1} towards the end of the season (Figure 5). No consistent differences were apparent between the inflow and outflow data. However, outflow levels of N were more often below inflow levels during the month of January, with this situation reversed from February where outflow was more often higher than inflow. The lowest level of nitrogen recorded was 0.9 mg L^{-1} from the outflow on the 3rd January, and the highest was 4.3 mg L^{-1} in the outflow on 25th February. The average level of nitrogen during this period in the inflow was 1.99 mg L^{-1} and in the outflow was 2.09 mg L^{-1}.

The percentage breakdown of different forms of nitrogen in water samples is provided in Figure 6. Nitrate + nitrite (NOx) represented a daily average of 3.4 % of the total nitrogen in the inflow, and 3.5 % in the outflow, during the sampled period (range of 1.6 to 6.3 % in inflow and 1.1 to 8.4 % in outflow). Total ammonia (TAN) represented a daily average of 5.0 % of the total nitrogen in the inflow and 2.8 % in the outflow (range of 0.1 to 21.6 % in inflow and 0.2 to 14.1 % in outflow). Organic nitrogen represented the majority of nitrogen in inflow and outflow, with a daily average of 91.9 % in the inflow (range of 74.8 to 99.0 %) and 93.9 % in the outflow (range of 78.6 to 98.2 %).

The average daily level of NOx in the inflow and outflow was 0.06 mg/l where the inflow ranged from 0.05 to 0.09 mg L^{-1} and the outflow ranged from 0.02 to 0.09 mg L^{-1} (Figure 7). The average daily level of TAN in the inflow was 0.1 mg L^{-1} and in the outflow was 0.06 mg L^{-1} (Figure 8). TAN in the daily inflow ranged from 0.0 to 0.24 mg L^{-1} and in the daily outflow ranged from 0.0 to 0.53 mg L^{-1}.

The average daily level of organic nitrogen measured in the inflow was 1.92 mg L^{-1} and in the outflow was 2.03 mg L^{-1} (Figure 9). Organic nitrogen in the daily inflow ranged from 0.92 to 3.38 mg L^{-1} and in the daily outflow ranged from 0.82 to 3.86 mg L^{-1}.

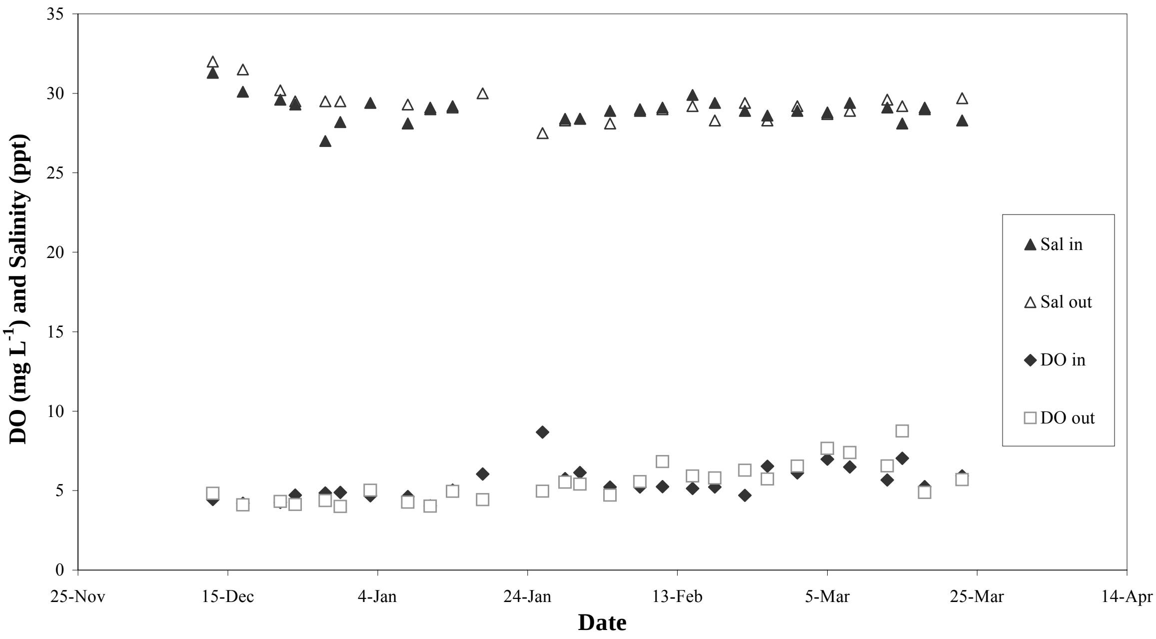

Figure 2. Dissolved oxygen concentrations and salinity of water flowing into and out of the settlement pond at Farm A.

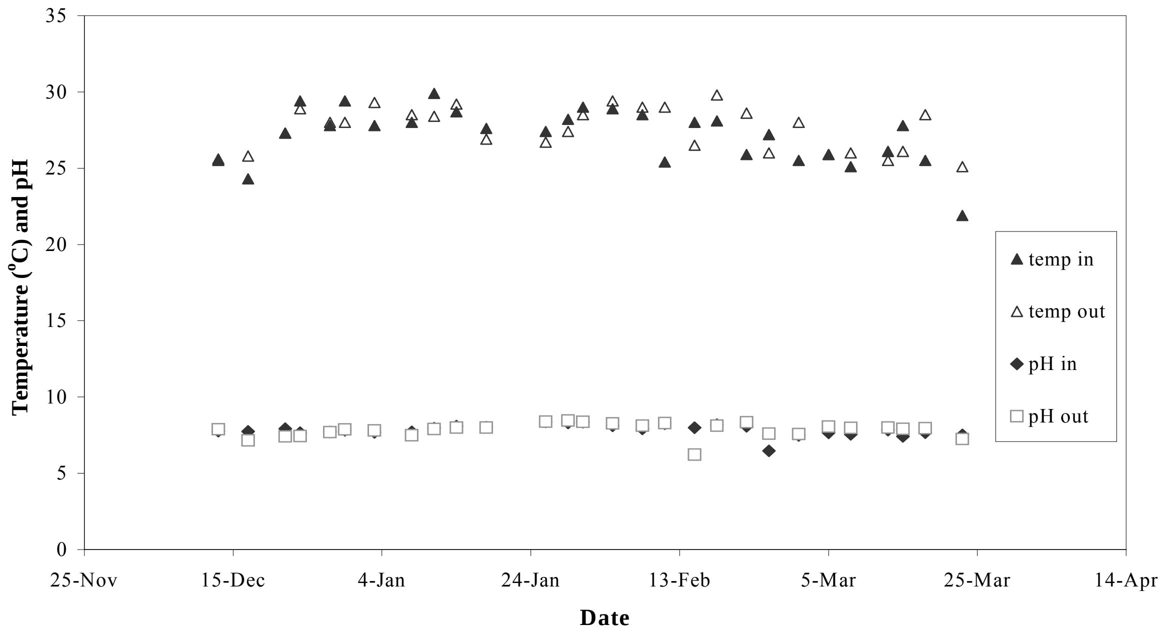

Figure 3. Temperature and pH of water flowing into and out of the settlement pond at Farm A.

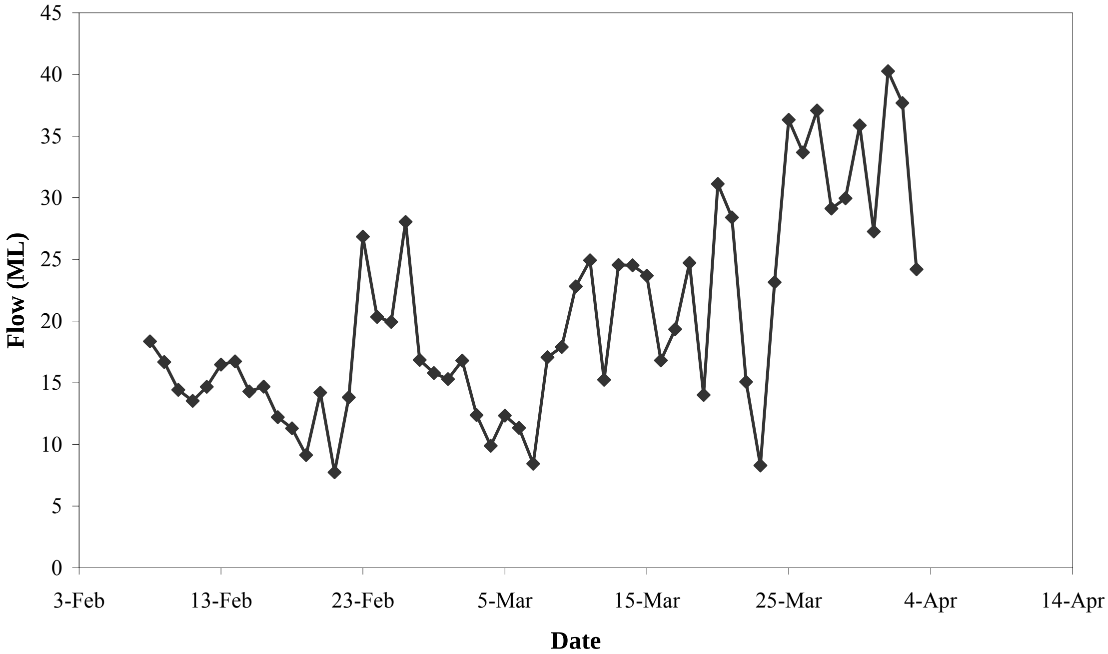

Figure 4. Daily water flow through the settlement pond at Farm A from 8/2/02 to 3/4/02.

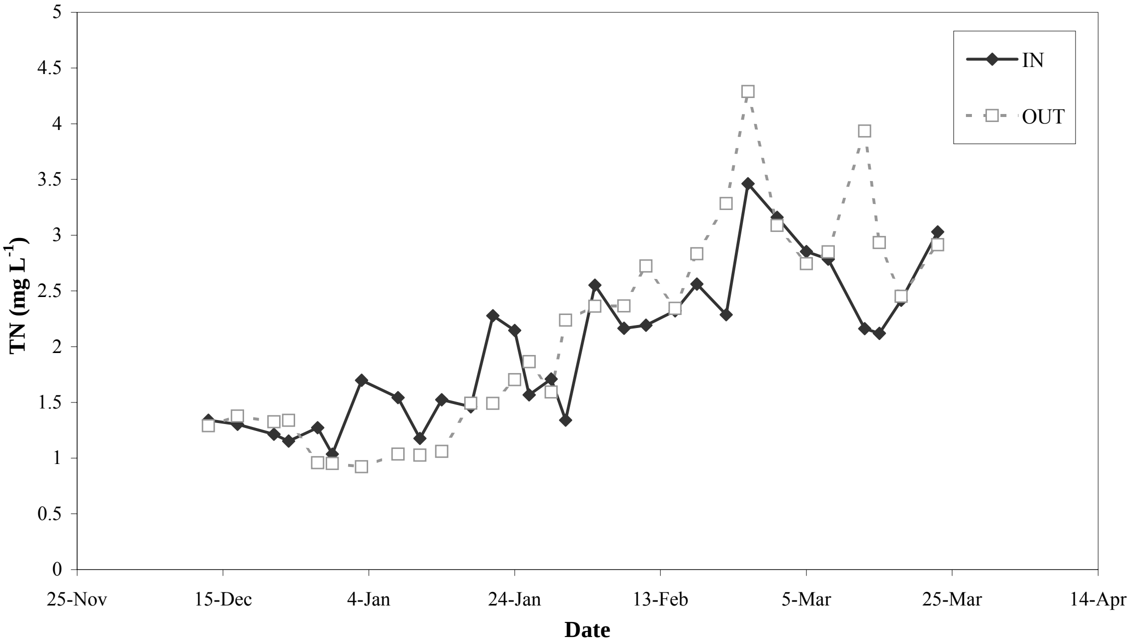

Figure 5. Total nitrogen concentrations in the inflow and outflow of the settlement pond at Farm A.

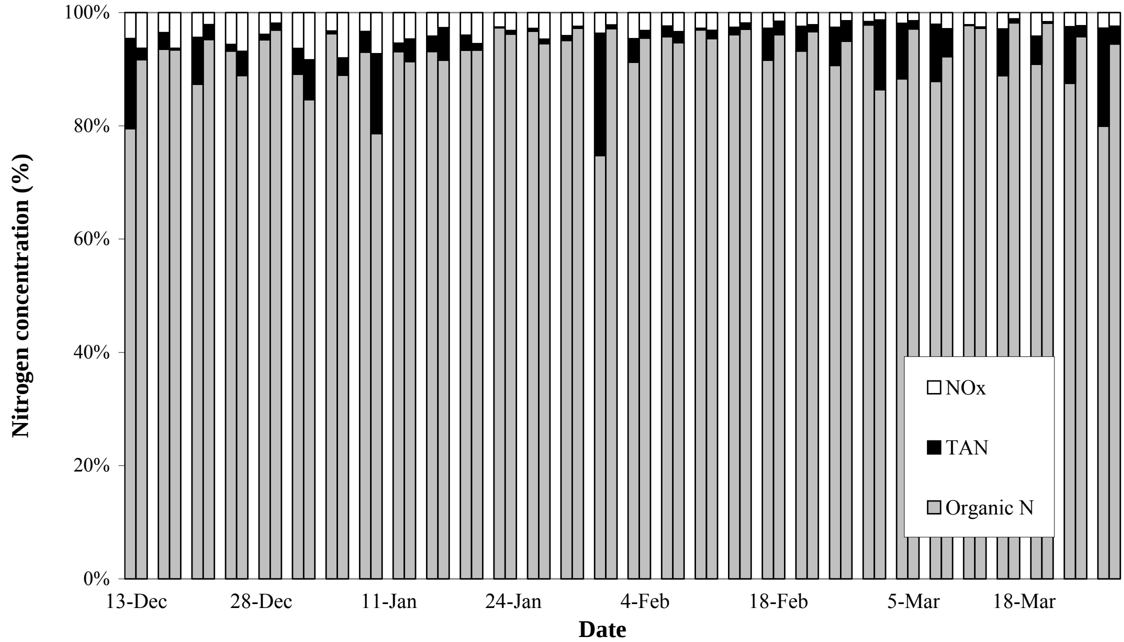

Figure 6. Nitrogen speciation as a percentage of the total in the inflow and outflow of the settlement pond at Farm A (Paired columns represent inflow on the left and outflow on the right).

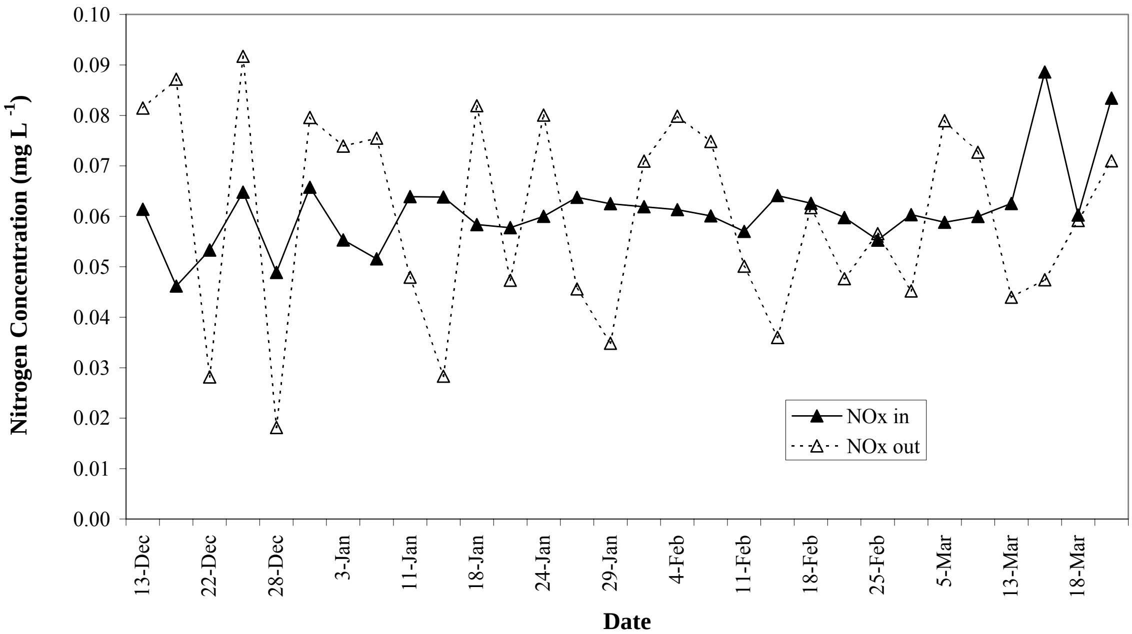

Figure 7. NOx in the inflow and outflow of the settlement pond at Farm A.

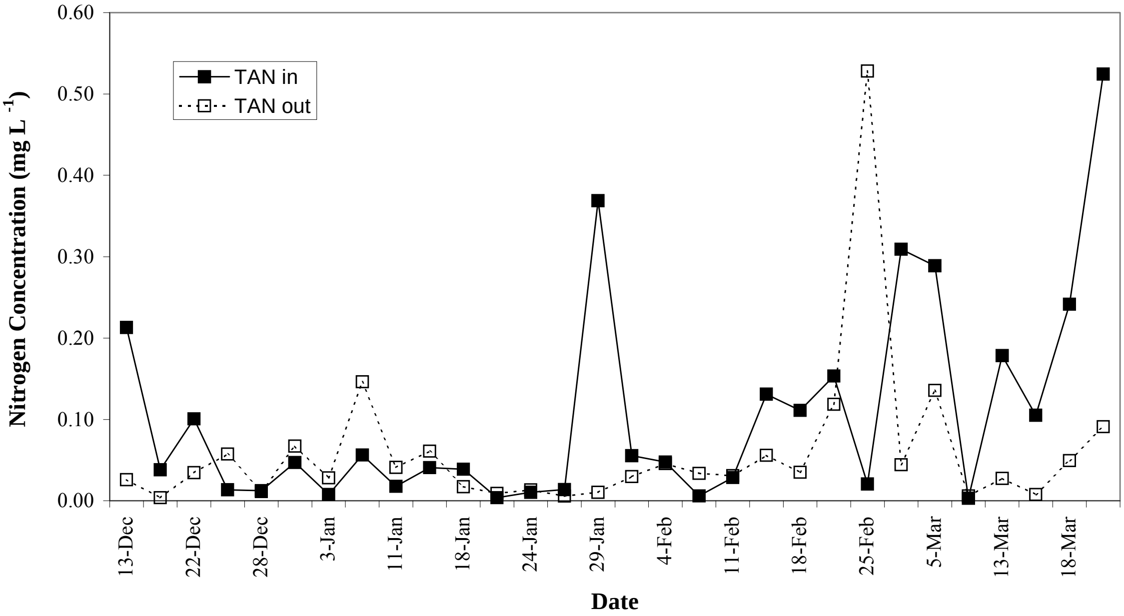

Figure 8. TAN in the inflow and outflow of the settlement pond at Farm A.

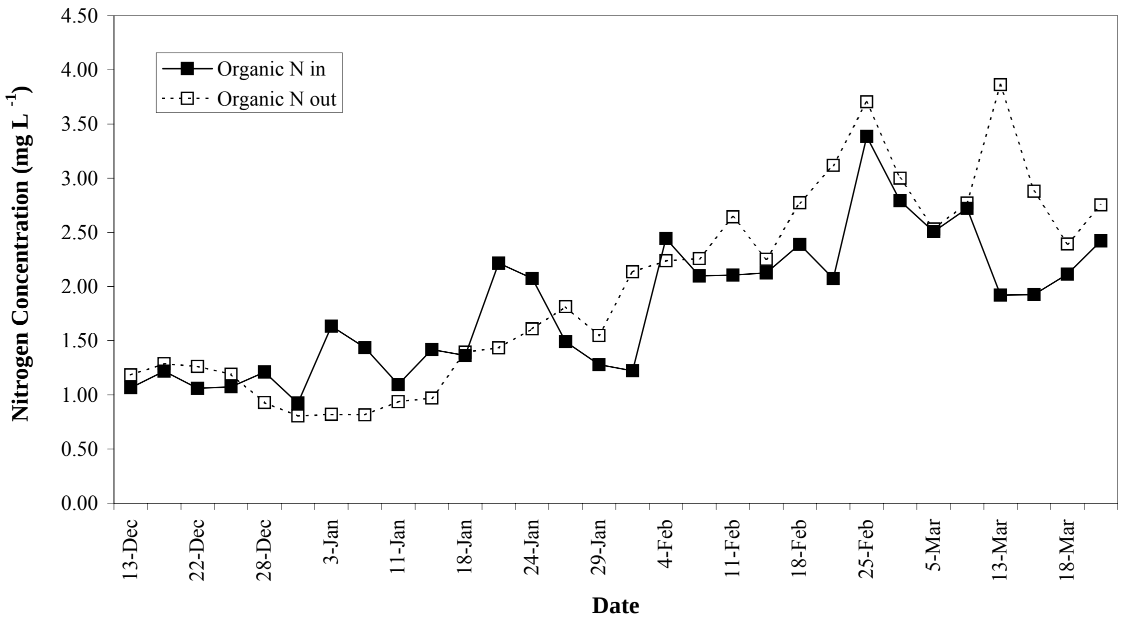

Figure 9. Organic nitrogen in the inflow and outflow of the settlement pond at Farm A.

Total phosphorus (TP) levels rose from 0.1 - 0.2 mg L^{-1} in December to peak in mid January, with a maximum inflow level (0.4 mg L^{-1}) recorded 10 days prior to the maximum outflow level (0.35 mg L^{-1} on 31st January) (Figure 10). Levels in the settlement pond appeared then to fall to 0.2 – 0.3 mg L^{-1}. Phosphorus levels in the outflow were generally lower than in the inflow until late January, but were similar thereafter. The average level of total phosphorus in inflow samples was 0.26 mg L^{-1} (range of 0.12 to 0.31 mg L^{-1}), and in outflow samples was 0.23 mg L^{-1} (range of 0.1 to 0.31 mg L^{-1}).

The percentage breakdown of different forms of phosphorus in water samples is provided in Figure 11. Inorganic phosphorus (PO4 - P), was sporadically detected on several occasions in the inflow at levels ranging from 0.0 to 0.1 mg L^{-1} (average of 0.01 mg L^{-1}), but was only detected in the outflow on one occasion at 0.02 mg L^{-1} (22 February 2002). Organic forms of phosphorus made up the majority of phosphorus in most samples.

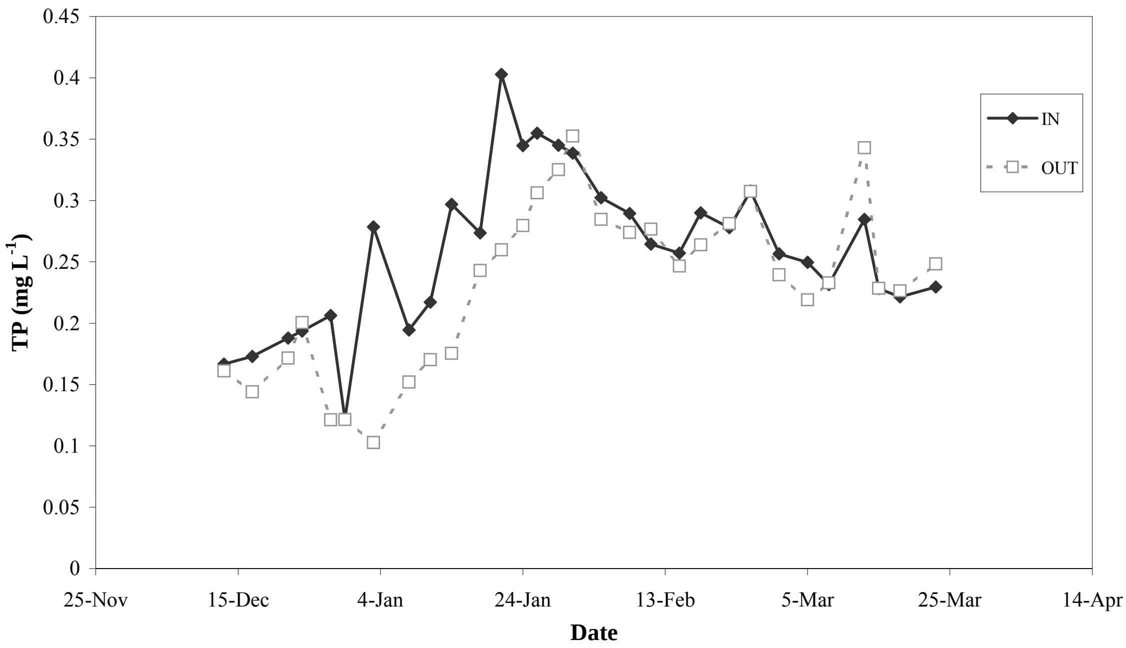

Figure 10. Total phosphorus concentrations in inflow and outflow of the settlement pond at Farm A.

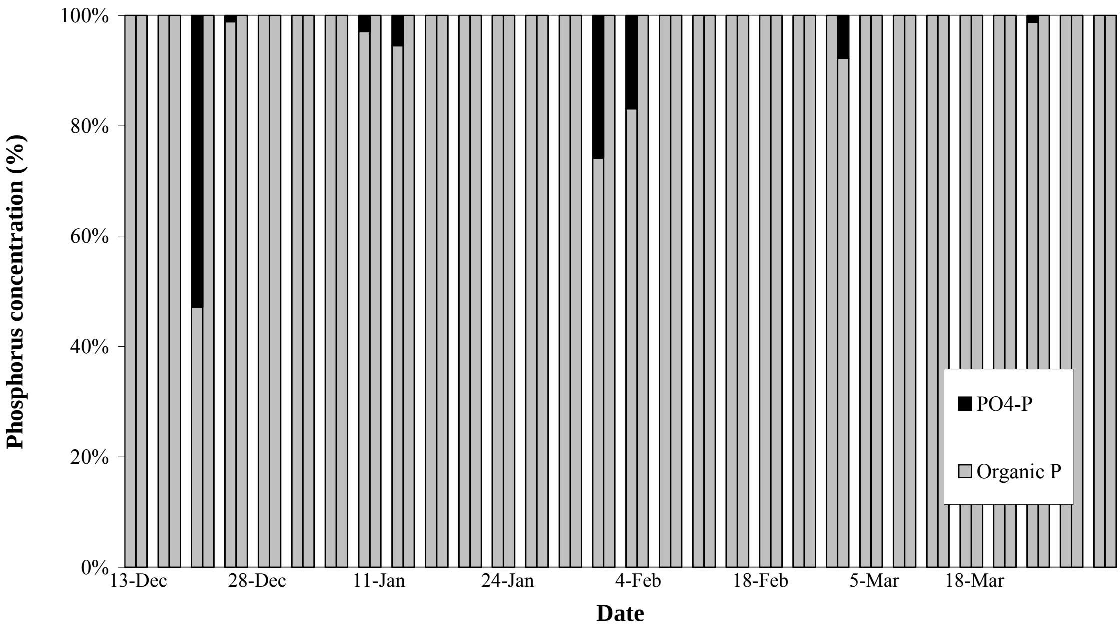

Figure 11. Phosphorus speciation as a percentage of the total in the inflow and outflow of the settlement pond at Farm A (Paired columns represent inflow on the left and outflow on the right).

Prawn macro-nutrient contents

Average dry matter contents of prawns from 4 farm settlement ponds and the culture pond at BIARC ranged from 21.8 to 26.9 % of the wet weight of samples (Table 8). Of this dry matter, an average of 11.8 to 12.5 % was nitrogen and 1.2 to 1.4 % was phosphorus.

Table 9 provides an assessment of the amounts of nitrogen and phosphorus estimated to have been incorporated into the biomass of banana prawns produced in settlement ponds during the study. The 1,150 kg of banana prawns produced represented an estimated total of 35.73 kg of nitrogen and 3.882

kg of phosphorus that was, or could have been, directly removed from these aquatic systems as prawn biomass.

Table 8. Nitrogen and phosphorus contents of banana prawns harvested from four farm settlement ponds and a culture pond at BIARC.

Source	Sample	% Dry matter	% Nitrogen*	% Phosphorus*
Farm A	Replicate 1	23.2	12.13	1.29
	Replicate 2	20.4	11.50	1.25
	Average	21.8	11.815	1.27
Farm B	Replicate 1	27.0	12.11	1.26
	Replicate 2	26.7	12.04	1.30
	Average	26.85	12.075	1.28
Farm D	Replicate 1	25.2	11.96	1.34
	Replicate 2	26.0	11.88	1.37
	Average	25.6	11.92	1.355
Farm E	Replicate 1	25.3	12.67	1.33
	Replicate 2	26.6	12.30	1.42
	Average	25.95	12.485	1.375
BIARC pond	Replicate 1	24.4	11.99	1.14
	Replicate 2	25.3	11.94	1.17
	Average	24.85	11.965	1.155

* Reported on dry matter basis

Table 9. Nitrogen and phosphorus in banana prawns produced in four farm settlement ponds.

Source	Harvest estimate (kg)*	Dry matter (kg)**	Nitrogen (kg)**	Phosphorus (kg)**
Farm A	175	38.15	4.507	0.485
Farm B	475	127.54	15.400	1.633
Farm D	200	51.2	6.103	0.694
Farm E	300	77.85	9.720	1.070

* Estimates taken from Table 4.
** Calculated using respective average values displayed in Table 8.

<u>Nutrient mass balance and export calculations</u>

Estimates of the total amount of nitrogen and phosphorus that flowed into, and then flowed out of the settlement pond at Farm A, can be calculated along with daily averages, from the average volumes and nutrient concentrations of inflow and discharge waters. Results of these calculations are presented in Table 10, which describe mass balance estimates for the period between 13 December 2001 and 23 March 2002 inclusive, spanning a total of 101 days. The average daily water volume flowing through the settlement pond calculated from Figure 4 as 17.1 megalitres per day, is used in these calculations.

Table 10. Macro-nutrient mass balance estimates for the settlement pond at Farm A over 101 days.

Parameter	Average sample concentration (mg L^{-1})	Average daily quantity (kg)	Total quantity (kg)
Inflow TN	1.99	34.029	3,436.929
Outflow TN	2.09	35.739	3,609.639
Inflow TP	0.26	4.446	449.046
Outflow TP	0.23	3.933	397.233

By comparing these inflow and outflow mass balance estimates, the results suggest that discharge waters carried 1.71 kg (5.0 %) more nitrogen out of the settlement pond than flowed in on a daily basis, which amounted to 172.71 kg over the 101-day period monitored. On the other hand, 0.513 kg (11.5 %) less phosphorus flowed out the settlement pond on a daily basis compared with inflow estimates, which amounted to 51.813 kg less phosphorus exported from the farm over the 101-day period monitored. However, these inflow and outflow differences were only relatively small when compared with the variation of levels measured during the period monitored. Additionally, the inherent errors associated with sampling and testing nutrient concentrations (without replication) coupled with high sensitivities to the effect of multipliers, suggest mass balance calculations of this nature could be misleading and should be treated with caution.

Discussion

Bioremediation of wastewater in settlement ponds

Presently in Australia, settlement in treatment ponds is a widely accepted method to reduce nutrient loads prior to discharge or reuse. Preston *et al.* (2000) have measured up to 60% removal of suspended solids, 30% removal of phosphorus, and 20% removal of nitrogen in prawn farm settlement ponds in Australia. They reported that this performance varied between farms according to a complex range of physical and biological conditions, including soil types, settlement basin size, flow rates and residence time, and the presence and activity of naturally occurring macro-algae and decomposing bacteria. That study concluded that a one-day retention time was sufficient to reduce suspended solids by the above mentioned level, but that a 2 to 3 day retention time was necessary to remove significant concentrations of nitrogen and phosphorus. As residence time is a function of settlement pond area and inflow rates, longer residence times infer the use of larger settlement ponds, in the order of 5 – 10% of farm area for each day-long retention period. The commitment therefore, of 15 – 20% or more of farm area to settlement ponds for nutrient reductions, can amount to large ponded areas that are not contributing directly to farm profitability.

Preston *et al* (2000) also made reference to a range of potential ways to reduce nutrients in prawn farm settlement ponds. Improved denitrification and volatilisation of ammonia for greater gaseous losses were proposed, along with the culture of aquatic plants and animals to incorporate available nutrients into their biomass. Whilst physical settlement of organic matter was shown to be effective at removing nutrients from discharge waters, it was also noted that bacterial mineralisation of nutrients in sediments could contribute up to 3.8 kg N ha^{-1} as ammonia each day. Therefore the effectiveness of settlement alone would rely on an ability to remove or reduce the accumulation of settled organic matter. Otherwise, periods of nutrient reductions in settlement ponds during accumulation phases, could be expected to be followed by nutrient increases from organic matter breakdown and remineralization. Drying is thought to be the best sediment management technique, and other methods (dredging or physical disturbance) for use during the cropping cycle are yet to be fully evaluated (Hargreaves, 1998).

Prawns and shrimp are known to consume particulate organic matter throughout their lives, and their activities at the soil water interface are likely to mobilise solids that would normally settle and become unavailable to most marine organisms as anaerobic sludge. Microalgal detritus and bacterial flocs can provide shrimp with substantial amounts of nitrogen and other nutrients (Moss, 2001), and biofilms with various microbial assemblages have been shown to be important food sources that improve shrimp growth (Thompson *et al.*, 2002). In particular, *P. merguiensis* is believed to have a high capability to assimilate algae and detritus, and to reduce the accumulation of organic material in culture ponds (Hoang, 2001). In tanks at high densities (1 L^{-1}), the species can reduce fouling rates on tank sides supplied with unfiltered seawater, and we have also observed them feeding directly on benthic algal mats. Additionally, the species has established markets, is easily domesticated (Hoang, 2001), and can be cultured through sensitive larval stages using well known methods like those standardised at BIARC during this study. *P. merguiensis* is therefore an ideal candidate for this research.

Despite the presence and growth of banana prawns in the settlement pond at Farm A, that pond's data reveals a typical pattern of organic accumulation followed by breakdown and nutrient release. Nitrogen and phosphorus levels were likely to be reduced through the deposition of particulate matter from early in the season (before monitoring began), until early February. Thereafter, total nitrogen increased in discharge waters and the settlement pond was less effective. Ammonia appeared in the settlement pond irregularly (see Figure 8), which can be attributed to prawn and other biomass excretions, mineralising organic matter, and diffusion from reduced sediments (Hargreaves, 1998). TAN outflow peaks appeared to be independent of inflow peaks. But ammonia could be expected to be rapidly removed by the blooms of microalgae and bacteria in the water. As nitrite and nitrate levels remained low throughout the monitored period, microalgae were probably of greater importance. This is also suggested in the present results where the settlement pond was effective at removing most of the dissolved phosphorus available for direct plant uptake (PO_4).

These results suggest that the biomass of banana prawns that grew in the pond failed to prevent the accumulation of organics so as to avoid late season nutrient remineralisation. Unfortunately, no controls could be used in this work to quantify the effect that the banana prawns had on these processes. The history of effectiveness in nutrient removal of that settlement pond was also unknown. Further, lower survival in that pond meant that the prawn biomass was low compared with other settlement ponds in the study, so as to lessen the desired effect. Despite Farm A having 23.1% of total farm area devoted to settlement ponds, the results in this trial (ie: 5 % more N, 11.5 % less P) are disappointing compared with the results described by Preston *et al*. (2000) (ie: 20 % less N and 30 % less P). However, our nutrient mass balance estimates are misleading if not viewed in the context of pond dynamics and organics that had built up in the settlement pond before biweekly monitoring had commenced. Whilst levels of nutrient reduction in excess of those levels of reduction reported in other work were detected in this study, periodic high flow rates seen in the present data (Figure 4), would have lowered retention times below the 2-3 day optima quoted, and would also have further reduced the accuracy of mass balance estimates.

Nutrients bound in prawn biomass were also low in view of the total nutrient export amounts. An estimated 1,150 kg of banana prawns produced across the 5 farms in this study, represented a total of only 35.73 kg of nitrogen and 3.88 kg of phosphorus. This was approximately equal to the nutrient that flowed from Farm A on one average day during the period monitored (see Table 10).

Mechanisms that act within natural systems to utilise available nutrients can provide useful guidance for intensifying bioremediation efforts within these existing farm facilities. Natural estuarine systems deal with eutrophication through an interplay of diverse organisms. Similarly, settlement pond ecosystems could benefit from mixing species to create functional synergies. An example could be in using other estuarine or river schooling prawns (eg: *P. bennettae* Racek and Dall, or *P. macleayi* Haswell), to better utilise niche space and capitalise on different species' habitat and resource partitioning. Some of these Penaeid species occur in natural environments adjacent to all commercial prawn farms in Australia, so local species or strains could be used at different farms to alleviate possible translocation concerns. Furthermore, if local stocks of Penaeids were being used in settlement ponds, no extra disease vectors than would have naturally occurred in farm intakes have been created, and the health of prawns in the settlement pond would act as a good monitoring tool for any potential impacts on those species in adjacent natural waters.

Other organisms that occur naturally in the vicinity of prawn farm discharge points may also be useful to incorporate into constructed treatment systems. Jones and Preston (1999) showed that populations of the Sydney rock oyster (*Saccostrea commercialis* Iredale and Roughley), which commonly occurs in estuaries in Southern Queensland, could significantly reduce levels of bacteria, phytoplankton, nitrogen and phosphorus in prawn pond effluent. However, suspended inorganic particles that often also occur in prawn farm wastewater, inhibits this oyster's filtration rates and growth. In other work, the red seaweed *Gracilaria chilensis* was shown to effectively remove dissolved nutrients from aquaculture effluents, but was shown to have little effect on particulate emissions (Chow *et al*., 2001). The particulate sources of nutrients that commonly occur at high levels in prawn farm effluent, are not available for direct photosynthetic uptake, and so this macroalgae-bioremediation strategy may only be useful if combined with processes that convert particulate matter to soluble nutrients. This is demonstrated in the laboratory scale studies reported by Jones *et al.* (2001), who combined these concepts so that silt was removed with 24 hr settlement prior to oyster filtration (24 hr), followed by absorbance (24 hr) of dissolved nutrients by the macroalga *Gracilaria edulis* (Gmelin). This provided reductions of 88 % suspended solids, 72 % nitrogen and 86 % phosphorus. Animals like banana prawns, that feed on particulate matter and convert portions of this unavailable nitrogen and phosphorus into forms more readily assimilated by plants may provide this linkage in the future.

Alternatively, other natural substrates such as the sea lettuce *Ulva* spp., have also been proposed for incorporation into these constructed ecosystems, so as to improve nutrient fixation whilst also providing surface area for micro-organisms to flourish, and possibly in turn provide natural prey items for shrimp (eg: copepods). Preliminary results with this approach at BIARC suggests that biological control of invertebrate grazers like amphipods (which are common in prawn farming systems), possibly with fish like sea mullet, may be necessary for significant accumulation and periodic removal of macrophyte biomass. Other considerations that may be necessary in the development of broad-scale macrophyte production systems in settlement ponds includes concerns that periodic die back would readily release nutrients back into the system, and result in increased biological oxygen demand associated with the decomposition of settled leaf matter. Furthermore, growing macrophytes in settlement ponds that must at times receive or release high volumes of water, could prove problematic if discharge screens became blocked or if flows were impeded by leaf matter.

Survival and growth in settlement ponds

The data displayed in Table 2 demonstrates that the prawn families used in the study could have been expected to survive at reasonable levels in the settlement ponds. These data also demonstrate the high variability in growth that can depend on environmental conditions, and that healthy populations of *P. merguiensis* can be maintained at vastly different growth rates. For example, healthy high density populations were maintained on artificial-feed rations for 70 days to produce an average size of 0.76 g, but the same family (Pme 33) grew to an average size of 17 g in 80 days when stocked as PL15 at low density in a settlement pond. This is evidence of the species' highly opportunistic growth potential and ability to be manipulated with different environments.

Comparatively high survival (60.5 %) was achieved in one of the smallest settlement ponds in the study. The lower survival in larger ponds may in part be attributable to the generally better control of bird predation possible in smaller ponds. However, predation by other animals in the ponds, soon after stocking postlarval banana prawns, may also have contributed to losses. For example, juvenile prawns of an undetermined species were present in the settlement pond at Farm A when banana prawns were stocked. They may have preyed on the vulnerable banana prawn postlarvae, and could have caused significant mortalities early in the study in that settlement pond. The rapid growth of prawns at Farm A, may also have been due to low initial survival, resulting in very low densities which would contribute to faster growth.

There is clear evidence in this study (see Table 5) that a wide variety of animals can survive and grow in prawn growout and settlement ponds. Many farmers have experienced from time to time, an accumulation of specimens that have somehow entered the production system despite preventative measures. Micron rated screens and socks with mesh sizes of 0.2 – 0.3 mm are routinely used at water supply points to protect ponds from these unwanted entries. However, occasional equipment failures (eg: holes in screens) may lead to these wild influxes, and at some developmental stages viable animals could travel through the screen apertures without fatal damage. Prawn farmers go to great lengths to minimise predators in production ponds at all times, and especially when the stock is young and vulnerable. Ponds are generally dried before refilling, to break disease cycles, to allow sediments to oxidise, and to eliminate fish and prawns that may act as predators of young stock. The same considerations apply to settlement ponds that are to be stocked with prawn postlarvae.

The aim of this study was to demonstrate significant potential profits from a secondary crop, and test and evaluate bioremediation activities that can be practically incorporated into existing treatment practices. Production of up to 528 kg of banana prawns per hectare was demonstrated at one farm (B) in the study without any infrastructure modifications other than the supply of *P. merguiensis* seed stock and some screen modifications. If higher production from settlement ponds were desired, considerations for backup aeration would be necessary to avoid losses during periodic low oxygen events. The unfortunate loss of mullet through low oxygen levels at one farm in this study, demonstrates the need to consider supplemental aeration if a large biomass is to be grown in settlement ponds. Banana prawns were shown through this chance occurrence at Farm C, to be hardier than sea mullet in a low oxygen environment (99% of the mullet died but banana prawns survived).

Boyd (1998) provides some guidance to levels of aeration necessary to support pond biomass in aquaculture operations. Oxygen levels of <2 mg L^{-1} are known to cause stress or mortalities, and only about 20% of the oxygen in a pond is generally used by the cultured species. Respiration by phytoplankton, bacteria in the water column and bottom soil organisms use the rest. Boyd suggests that 2000 kg ha^{-1} of shrimp (or prawns) can be produced in ponds without aeration, and that 1 KW of power to drive aerators is required for every additional 500 kg of production. Although this suggests that higher settlement-pond production than was demonstrated in the present study could be achieved without supplemental aeration, allowances need to be made for the greater accumulation of organics and associated oxygen debts. However, aerators generating increased water currents would reduce the effectiveness of settlement protocols for nutrient removal. In this case, aerator positioning would be important to avoid erosion of pond bottoms and embankments, and to provide sufficient areas with slow circulation to afford suspended particle deposition. Current practices in production ponds, where circular currents generated by paddlewheel aerators around the pond periphery, produce slow currents for deposition in the middle of the pond, may provide the most practical model for settlement pond design in the future. An important advantage being the ease that concentrated organics in the middle can be mechanically removed at the end of each season after ponds are drained and dried.

A number of other practical considerations are appropriate for this investigation. For example, the biomass produced in such secondary crops must be easily harvested, and be able to be completely recovered and processed using cost effective methods. This is important in countries like Australia where labour costs are high. Secondly, species selected to act as sinks for waste nutrients in discharge waters, should be easy to mass produce, survive and grow well in the target environment, have a reasonably high market value for cost recovery or profit, and fit into existing commercial operations. More importantly they need to improve the effectiveness of nutrient removal processes in these treatment ponds, or at the very least, not adversely affect their function. Although the sea mullet *Mugil cephalus* was the subject of earlier bioremediation work at BIARC, they were replaced with banana prawns in this study, because prawns are more likely to grow to market size in the time frame that settlement ponds are operated (6-8 months per year in Southern Qld), and because prawns can more easily fit into existing prawn farm infrastructures. This study demonstrates that *P. merguiensis* can survive and grow to market size in prawn farm settlement ponds, and suggests that it may be a keystone species for development of more effective nutrient-sink polycultures in the future.

Microbiological analyses

Testing the suitability of food for human consumption is generally based on results of specified microbiological assessments of sample units (ICMSF, 1986). This can either be through assessment of the presence or absence of particular organisms, or of the concentration of microorganisms. Three important microbiological attributes of prawn samples taken from settlement ponds were assessed in this study according to food standard guidelines for raw crustaceans. They were total concentrations of viable bacteria (standard plate count), the concentration of *E. coli*, and the presence or absence of *Salmonella* spp. Limits for accepting food on these bases in Australia are outlined in Food Standards for Australia and New Zealand (FSANZ, 2002), and international specifications (ICMSF, 1986) have similar levels applied. For total bacterial content of raw crustacea, Australian standards require less than 5×10^6 g^{-1}, where levels above 5×10^5 g^{-1} are considered marginal. International standards have maximum and marginal limits of 10^7 and 10^6 g^{-1} respectively for frozen raw crustaceans. All prawn samples tested in this study were well below these standard plate count limits, and prawns from all settlement ponds were more than 1000 times lower than the specified Australian maximum limit that would cause rejection.

Although levels of *E. coli* are not specified in Australian food standards for raw crustacea, molluscs other than scallops are acceptable at up to 2.3 g^{-1} but must not exceed levels of 7 g^{-1}. Internationally, levels of *E. coli* in frozen raw crustaceans above 11 g^{-1} are considered marginal and such food displaying levels above 500 g^{-1} is rejected (ICMSF, 1986). All prawn samples tested in the present study were assessed at <3 g^{-1}, which is well below levels of this coliform that would cause concerns for human consumption.

No *Salmonella* spp were detected in prawns tested in the study. Both Australian and international specifications for this specific pathogen do not allow detection at any level. These results suggest that banana prawns grown in prawn farm settlement ponds had low bacterial levels that were well within food standard guidelines, and in this regard were suitable for human consumption.

Consumer sensory comparisons

Few differences were detected between prawns grown in settlement ponds and control treatments in small-scale consumer acceptability assessments. The size of prawns appeared to influence their acceptability, as the smaller prawns in the BIARC-controls were rated less favourably, and comments made by panellists suggested that this might have been due to their smaller size. Despite the lower rating for appearance, the BIARC control prawns were not rated differently to any of the other prawns for overall acceptability. The panellists generally rated settlement pond prawns favourably, and no significant differences were found in their overall acceptability compared with controls. Accordingly, these small-scale sensory investigations show that banana prawns grown in settlement ponds can be expected to be as acceptable to consumers as banana prawns produced with commercial feeds.

Future Studies
This study could not determine whether the banana prawns had a significant effect on the qualities of water in the settlement pond at Farm A, or on the residual nutrients in that pond after draining and harvest at the end of the season. Anecdotal evidence for the development of less fouling biomass like macroalgae, tubeworms and barnacles in ponds with banana prawns, could not be proven because of different pond pre-histories. Facilities that split effluent into separate units are required to provide controls to study these factors. This could be achieved on a farm scale with parallel settlement ponds, or on a smaller scale in replicated tank systems fed with the same wastewater. Studies that investigate the effect of higher densities of captive banana prawns on nutrients in prawn farm wastewater would further evaluate the potential of the present approach.

Smaller prawns may be better than larger prawns at utilising small particulate matter that typically flows into prawn farm settlement ponds. Most Penaeid-culture operations make heavy use of the efficient and rapid growth of post-larval shrimp in eutrophic ponds with abundant plankton. In many cases the first 1-2 months growth is due to nutrition derived from the pond ecosystem rather than feed inputs. This study demonstrates rapid growth of *P. merguiensis* in settlement ponds, but did not demonstrate a high potential for nutrient removal with this species. Larger numbers of rapidly growing smaller prawns could be more effective than the low densities tested in this study. Stocking settlement ponds at high levels (eg: 40-100/m^2), with backup aeration used when necessary, and production to a smaller size for specialised food or bait markets, could be better for nutrient assimilation, than growing fewer prawns to a larger size. This could be viewed as challenging the carrying capacity of the pond so that biofilms on surfaces are regularly grazed, and so that food resources are quickly utilised and are brought into short supply. Future work could evaluate the effect on nutrients and growth rates at high densities, and attempt to demonstrate the benefits of producing larger quantities of smaller prawns that may be more efficient at stripping nutrients. If legislative frameworks could be developed in Australia to allow for the production of significant bait products at aquaculture premises, a further high value market for small banana prawns would be created, and significant pressures on wild bait prawns and juveniles that support commercial wild harvests could be alleviated.

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