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Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize

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Abstract

Microbial and phytoplankton processes, and their effect on water quality were examined over a 3-week period in five high-intensity (120 animals m^{-2}) shrimp (*Litopenaeus vannamei*) ponds of varying crop ages at Belize Aquaculture Ltd., (BAL) in Central America. These ponds were characterized by zero water exchange throughout the crop, plastic lining and high aeration rates. Nitrogen (N) and organic carbon (C) inputs, in the form of fishmeal-based feed, grain-based feed and molasses, resulted in high concentrations of dissolved organic and inorganic N (2.29–5.56 and 0.17–10.66 mg l^{-1}, respectively) and dissolved organic C (14.20–48.10 mg l^{-1}). Phosphate levels were also high, ranging from 0.07 to 1.17 mg l^{-1}. The high nutrient concentrations promoted the growth of bacteria, phytoplankton (mostly autotrophic flagellates) and protozoa. Up to 40% of the bacteria were associated with flocculated matter. However, bacterial numbers and oxygen (O_2) consumption in the water column did not appear to increase with crop age. This may be due to a reduction in the C/N ratio below the optimum for bacterial growth. Up to 22% of the O_2 consumption was due to nitrification and there was some indication of lowering of total ammoniacal N (TAN) concentrations and an increase in nitrite and nitrate levels in older crops. Both phytoplankton and bacteria were responsible for high rates of ammonium uptake. In ponds with high nitrate concentrations, nitrate uptake rates were also high. Phytoplankton productivity remained high irrespective of crop age and ponds fluctuated between net O_2 production (autotrophy) and net O_2 consumption (heterotrophy) irrespective of crop age. This reflected the highly dynamic nature of the bacterial and phytoplankton populations with frequent blooms and crashes of individual phytoplankton species. The high mixing rates resulted in phytoplankton and other detritus remaining suspended in the water column. However, a small area of sludge (< 2% of

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pond area) did accumulate containing a high N and C content, and high pore water TAN. This study showed that despite what is generally considered as poor water quality in the ponds, i.e. high nutrient concentrations, high and unstable phytoplankton numbers, and high bacterial numbers, shrimp production was high relative to conventional ponds. There appeared to be scope for increasing bacterial production in these systems by increasing the C/N ratio, and hence C availability for bacterial growth. However, it remains to be established which microbial processes are likely to be promoted, and if the benefits of this outweigh the costs.
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Keywords: Nitrogen; *Litopenaeus vannamei*; Water quality

1. Introduction

Shrimp farming is a major industry in tropical and subtropical areas around the world with current production estimated at 1090 Mt/^{y} (FAO, 2000). However, there are concerns about the ecological sustainability of shrimp farming including the discharge of nutrient-rich waters into coastal waters that may result in the deterioration of ecosystem health (Eng et al., 1989; Naylor et al., 1998). The industry is, therefore, under increasing pressure from resource managers and non-government organizations to reduce nutrient and suspended solids discharges while still remaining viable and profitable.

Another major issue for shrimp farming in recent years has been the escalation in disease problems in many countries in Southeast Asia, and Central and South America. Many of the outbreaks have been viral in origin and are exacerbated by poor water quality and a high intensity of farms sharing farm intake and discharge waters (Kautsky et al., 2000).

One approach to improving sustainability and biosecurity has been the development of high-intensity growout systems with no water discharge over the crop cycle. This technology was developed principally at the Waddell Mariculture Center in the USA in the early 1990s (Hopkins et al., 1993; Sandifer and Hopkins, 1996). In the mid-1990s, the technology was adopted and modified at a commercial farm, BAL in Belize, Central America (Browdy et al., 2001). BAL developed an integrated approach to farming shrimp using high health, selectively bred stocks, low-protein (~ 20%) feed input, high stocking densities in ponds, no water exchange throughout the growth season and recirculation of water through treatment ponds at harvest time.

Conventional shrimp farming relies on the exchange of water from growout ponds to manage algal blooms and prevent deterioration in other water quality parameters that results from the addition of high-protein feeds. Poor water quality can impact shrimp growth and health. However, in the BAL system where there is no water exchange, the combination of high aeration and mixing, lined ponds, low-protein feeds and effective management techniques have resulted in harvest tonnages averaging 15 t ha^{-1} crop^{-1} (Browdy et al., 2001). This apparent anomaly, where shrimp production can be consistently high under conditions of poor water quality, provided the basis for a study that sought to characterize the water quality in the BAL growout ponds and compare this with conventional shrimp farming. Additionally, the role of bacteria and phytoplankton in nutrient cycling, which ultimately controls water quality, was examined.

2. Materials and methods

2.1. Facility

The BAL production facility is located in the Stann Creek District of Belize, in Central America (17°20′ N, 88°30′ W). The pond facility consists of a pilot farm with 26 growout ponds ranging in size from 0.065 to 1.6 ha, two settlement ponds (0.7 ha each) and a water reservoir (0.5 ha). The nearby production farm consists of 20 growout ponds (1.6 ha each) and a settlement pond (0.7 ha). The ponds were stocked with high health, TSV disease-resistant white shrimp (*L. vannamei*) at a typical density of 120 animals m^{-2}. All ponds were fully lined with high-density polyethylene (HDPE) liners and aerated with paddle wheels and propeller-aspirators (50 hp ha^{-1}), which created a circulating current. Ponds were maintained at 1.8 m deep without water exchanges throughout the 4-month growth season. Sludge that accumulated in the pond centre, as a result of deposition of waste products of feeding and other detritus, was partially removed periodically via central drains or siphons. Lime, iron and silicate were added throughout the season to buffer the pH, bind the sulfides released from the sludge, and promote the formation of flocculated matter. Molasses was added to promote bacterial growth. Water drained from the ponds at the pilot farm during the harvest was placed in a settlement pond for 7 days before being reused to fill another pond prior to stocking.

2.2. Feeds and feeding

Three feeds were added to the ponds: a low-protein, grain-based feed (soy meal, wheat grain, corn); and two fishmeal-based feeds (24% and 31% protein) (Jory et al., 2001). A higher proportion of the 31% protein feed was added early in the growth season which was replaced with the 24% protein feed when the shrimp are 4.5 g in weight. The grain-based feed was used throughout the season but the proportion of total feed input gradually decreased from 90% to 25%. The N and C content of two brands of 31% protein feed and the grain-based feed were analyzed using a CHN analyzer. Water stabilities of the feeds were determined by placing known weights of feed in containers of distilled water and shaking at 40 opm at 28 °C for 4 h (Smith et al., 2002). The feed was then removed, dried at 60 °C overnight and weighed. Feed that had not been placed in water was also weighed, dried at 60 °C overnight and re-weighed to determine the moisture content of the feed. Water stability was calculated based on the difference between the initial dried weight and final dried weight of the feed. Leaching of total N and total organic C from the feed was also determined by placing a known weight of feed in containers of distilled water and shaking at 40 opm at 28 °C. Samples of the water were taken at 0.5 and 4 h and frozen until analyzed for total organic C and total N using methods described in Section 2.6.

2.3. Routine water sampling

Five ponds representing crop ages from 17 to 98 days were selected for routine water sampling and measurements of ecological processes. Four of the ponds (ponds 5, 7, 9 and

18) were located at the production farm, while the fifth (pond 3) was at the nearby pilot farm. Ponds 5, 7, 9 and 18 were initially filled with oceanic water while pond 3 was initially filled with recirculated water from the treatment pond. All ponds were stocked at a density of 117–122 animals m^{-2}.

The ponds were sampled for water quality parameters twice weekly for 3 weeks from 19/6/01 to 9/7/01. Ponds were sampled weekly for oxygen consumption, primary production, nitrification and ^{15}N-ammonium uptake measurements from 19/6/01 to 9/7/01. The sampling time was 0800 h ± 30 min except on the 26/6/01 when water samples were collected at 0640 h. A previous study in intensive shrimp ponds has shown that soluble nutrients in aerated ponds are well mixed, so sampling at one location is appropriate (Burford, 1997). Flocculated matter appeared to concentrate in the pond centre, therefore a consistent sampling location (eastern side of pond) was always used to allow direct comparisons between ponds. Duplicate 1-l water samples were collected in polycarbonate bottles from just below the water surface approximately 2 m from the bank. Samples were placed in a cool, dark place and immediately transported to the on-site field laboratory for processing within 4 h. Temperature, salinity, oxygen and Secchi disc readings were monitored each day at 0600 and 1600 h by farm staff. Oxygen and salinity data were not obtained from pond 3 due to a logger breakdown. pH was measured in each pond at 0800 h ± 30 min using a multi-parameter logger (Yeokal 611). The logger was calibrated every few days.

2.4. Data logging of physical parameters

Between the 3/7/01 and 6/7/01, dissolved O_2, pH, temperature and turbidity were logged every 30 min for 24 h in ponds 3, 5 and 7 using a multi-parameter logger (Yeokal 611). The probe from the logger was positioned at mid depth approximately 2 m from the bank. Farm data for O_2, salinity, temperature (YSI dual channel loggers) and Secchi disc readings was also collected.

2.5. Processing of water samples

In the laboratory, the water samples were mixed, then a 50-ml subsample was taken from each duplicate pond sample. This was filtered through a GF/F glass fibre prefilter and a 0.45-μm cellulose acetate syringe filter for nitrate, nitrite, ammonia and dissolved N analyses. After additional mixing, a 30-ml subsample was taken from each duplicate pond sample for total N and total phosphorus analyses. For total suspended solids (TSS), chlorophyll *a* and volatile solid analyses, known volumes were filtered onto precombusted GF/F glass fibre filters. All water samples and filters were subsequently frozen.

2.6. Microbial community characterization and nutrient analyses

Water samples were also used to quantify bacteria, phytoplankton, protozoa and flocculated matter in the ponds. The samples were examined using a Sedgewick Rafter

counter under phase-contrast microscopy within 2 h of collection to observe small and fragile species. They were then fixed with 1% borate-buffered formaldehyde. Phytoplankton, flocculated matter and protozoa in fixed samples were counted using a Sedgewick Rafter counter under phase-contrast microscopy. Bacteria were counted using epifluorescence microscopy after whole water samples were stained with acridine orange (Hobbie et al., 1977). Whole water samples were also sonicated for 1 min using a sonicator probe (Branson), stained, and bacteria were counted. The number of bacteria associated with flocculated matter was calculated as the difference between the counts before and after sonication.

All nutrient samples and filters from the routine water quality sampling were transported to the CSIRO Marine Laboratories for analysis. Nutrient samples were thawed at ambient temperature on the day of sampling, then shaken. Ammonia was analyzed using the phenate method (4500-NH3 G) and filterable reactive phosphorus by the ascorbic acid method (4500-P E) (American Public Health Association, 1995). Nitrate was analyzed using a spongy cadmium method (Jones, 1983), and nitrite by removing the cadmium reduction step of the spongy cadmium method. Total N, dissolved total N and total phosphorus were measured using a modified simultaneous persulfate oxidation method (Hosomi and Sudo, 1986). The modified method involved autoclaving for two 30-min periods, with samples being removed and shaken in between autoclaving to dissolve hydroxide precipitates. After digestion, the pH of the samples was adjusted to 8.0. TOC samples were converted to carbon dioxide by catalytical combustion, then analysed using a TOC analyser with an infra-red detector (5310 A and B) (American Public Health Association, 1995).

Filters for TSS, and volatile solids determination were dried at 103–105 and 550 °C, respectively (Methods 2540 D and 2450 E, American Public Health Association, 1995). Filters for chlorophyll *a*, *b*, *c* and phaeopigment analysis were extracted by sonicating for 1 min in cold 100% acetone, adjusting the acetone concentration to 90%, then measuring the extract spectrophotometrically with and without acidification (Jeffrey and Welshmeyer, 1997).

2.7. O_2 consumption, primary production and nitrification measurements

Water samples were collected weekly from the five growout ponds, using the same procedure as for water quality sampling, for primary productivity, bacterial oxygen consumption and nitrification measurements. These parameters were each measured by changes in O_2 concentrations in BOD bottles (Bratvold and Browdy, 1998). For O_2 consumption and nitrification measurements, six replicate bottles were taken from each pond. Water samples were vigorously shaken, poured into 300-ml BOD bottles, and bubbles removed by tapping. Initial O_2 levels were measured in all bottles using an oxygen probe (TPS). Nitrification inhibitor (0.16 g), 2-chloro-6(trichloromethyl)pyridine (Hach nitrification inhibitor formula 2533), was added to each of three bottles from each pond. Bottles were incubated in the dark at 30 °C. For primary production measurements, three BOD bottles were filled with water from each pond and initial O_2 levels measured. Bottles were then placed in a rack which was partially submerged in a nearby pond, and incubated in full sunlight.

O_2 measurements were taken in the bottles periodically at intervals ranging from every 30 min to 3 h, depending on how quickly O_2 levels changed. A minimum of three O_2 measurements were obtained for all bottles without nitrification inhibitor, and the incubations were not completed until there was at least a 2 mg l^{-1} change in O_2 concentrations. The incubation period ranged from 1.5 to 9 h. Bottles with nitrification inhibitor were not measured until the end of the incubation period so as not to contaminate other samples. The rates of O_2 consumption with and without nitrification inhibitor, and O_2 production rates were calculated based on the rate of change of O_2 concentrations. Nitrification rates were calculated as the difference between O_2 consumption with and without nitrification inhibitor.

Depth-integrated O_2 productivity was calculated using Secchi disc data to calculate light attenuation (Kirk, 1983), and Steele (1962) equations to integrate oxygen production over depth. Surface productivity rates were based on incubations done in full sunlight (details described above).

2.8. ^{15}N-ammonium and nitrate uptake

Rates of ammonium and nitrate uptake by phytoplankton and bacteria were determined using ^{15}N-enriched ammonium and nitrate incubations. Water samples were collected from the five growout ponds weekly for 3 weeks using the same protocol as for the water quality parameters. Water was shaken and two replicates of 300 ml each were placed in glass bottles. ^{15}N-ammonium chloride was added to each sample at a concentration calculated to be 10% of ambient TAN concentrations. This was estimated prior to incubations using a Hach salicylate method (8155). One replicate bottle from each pond was incubated in full sunlight in a pond, the other in a dark incubator at 30 °C for 1 h. At the end of the incubation period, known volumes of water were filtered onto precombusted GF/F glass fibre filters for mass spectrometry and CHN analysis to

Table 1
N and C content, and water stability of grain-based feed and two brands of 31% feed used at BAL, and leaching rates [mean (± S.D.)] of total N (TN) and total organic C (TOC) from these feeds after 0.5 and 4 h

	Grain-based	Feed	Feed
		31% protein	31% protein
		1	2
Feed %protein	21.9	31.4	32.4
Feed %N	3.3	5.2	5.2
Feed %C	45.5	46.7	47.1
Molar C/N ratio	14.7	10.5	10.6
Feed water stability (%)	84	85	86
Leaching loss—0.5 h			
% of TN in feed	2.3 (0.5)	3.8 (0.1)	4.1 (0.1)
% of TOC in feed	2.7 (0.5)	4.5 (0.2)	3.6 (0.1)
Leaching loss—4 h			
% of TN in feed	12.0 (0.2)	9.7 (0.4)	10.6 (0.1)
% of TOC in feed	12.1 (0.2)	12.4 (0.3)	10.5 (0.3)
Molar C/N ratio of leachate	15.4	13.5	10.7

determine $^{15}N/^{14}N$ nitrogen ratios and the N content. Ammonium and nitrate uptake rates were calculated using the equations of Glibert et al. (1991).

2.9. Sludge characterization

The sludge depth was recorded at five locations in the sludge zone across both perpendicular axes of the ponds, using an observer on the pond bank as a reference point. Core samples of sludge were collected from ponds 5, 7, 9 and 18 using a PVC pipe (60 mm diameter, 1 m long) with end caps to contain the watery sludge. There was no sludge in pond 3. The water above the sludge was decanted. Three locations in the sludge zone were sampled. Three cores collected from an area of approximately 2 m^2 at each location were combined, mixed then subsampled into sample bags and frozen. Samples were freeze-dried, sieved, digested and total Kjeldahl N (TkN) and total phosphorus (TP) was analyzed using an autoanalyzer (Technicon, 1977). Subsamples were also taken for TAN pore water analysis. The sediment was filtered through glass fibre (GF/F) and membrane filters to collect the filtrate which was frozen until analyzed. TAN was analyzed using the same method as for pond water column samples.

3. Results

3.1. Feed characteristics

Feeds used at BAL (one grain-based and two brands of 31% protein) were compared for C and N content, water stability, and C and N leaching rates (Table 1). The grain-based feed had the lower % C and N (45.5% and 3.3%, respectively) than the 31% feeds (46.7–

Table 2
Mean (± S.D.) values for physical parameters and shrimp production in five *L. vannamei* growout ponds at different crop ages at BAL over a 3-week period

Parameter		Days since stocking				
		17–37	24–47	55–75	71–91	98–118
		Pond				
		3	18	9	7	5
Temperature (°C)	0600 h	29.0	28.7 (0.6)	28.8 (0.6)	29.2 (0.5)	28.7 (0.6)
	1600 h	30.0	30.4 (0.5)	30.3 (0.4)	30.5 (0.4)	30.3 (0.5)
O_2 (mg l^{-1})	0600 h		2.5 (0.5)	3.3 (0.3)	3.7 (0.6)	3.3 (0.5)
	1600 h		7.4 (1.1)	6.1 (1.2)	7.4 (2.2)	6.5 (1.7)
pH	0800 h	7.8 (0.3)	7.3 (0.2)	7.2 (0.9)	7.4 (0.2)	7.0 (0.2)
Salinity			38.1 (0.9)	41.0 (0.8)	42.1 (0.7)	42.1 (0.9)
Secchi disc (cm)		33	36 (17)	40 (12)	54 (12)	34 (9)
Shrimp weight (g)		3.0	5.5	8.2	10.1	14.7
Shrimp biomass Estimate (kg ha^{-1})		1800	8100	5900	8100	11 700

Shrimp weights and biomass estimates at the end of the sampling period (9/7/01).

47.1 and 5.2%, respectively). The molar C/N ratio was highest in the grain-based feed, i.e. 14.7 compared with 10.5 and 10.6 for 31% protein feeds 1 and 2, respectively. The total N and total organic C leaching loss after 4 h was similar for the three feeds, ranging from 9.7% to 12.0% of the N in the feed, and 10.5–12.4% of the C in the feed. The grain-based feed had the highest C/N ratio of leachate, i.e. 15.4 compared with 13.5 and 10.7 for 31% protein feeds 1 and 2, respectively. The water stability of the feeds was similar, ranging from 84% to 86%.

3.2. Physical parameter and shrimp production

Afternoon temperatures (1600 h) in BAL ponds showed little variation over the 3-week study and morning temperatures (0600 h) were only 1–2 °C lower than afternoon temperatures (Table 2). Salinity was high in all ponds, i.e. 38.1 to 42.1. O_2 concentrations were lower in the morning (2.5–3.7 mg l^{-1}) than the afternoon (6.1–7.4 mg l^{-1}). The pH, as measured at 0800 h, ranged from 7.0 to 7.8. Secchi disc readings ranged from 33 to 54 cm and there was no trend with crop age.

The data logger was used to measure changes in O_2 concentrations and temperature every 30 min for 24 h periods on different days in three ponds (ponds 3, 5 and 7). There was a distinct diel change in the O_2 concentrations in all ponds but there was little

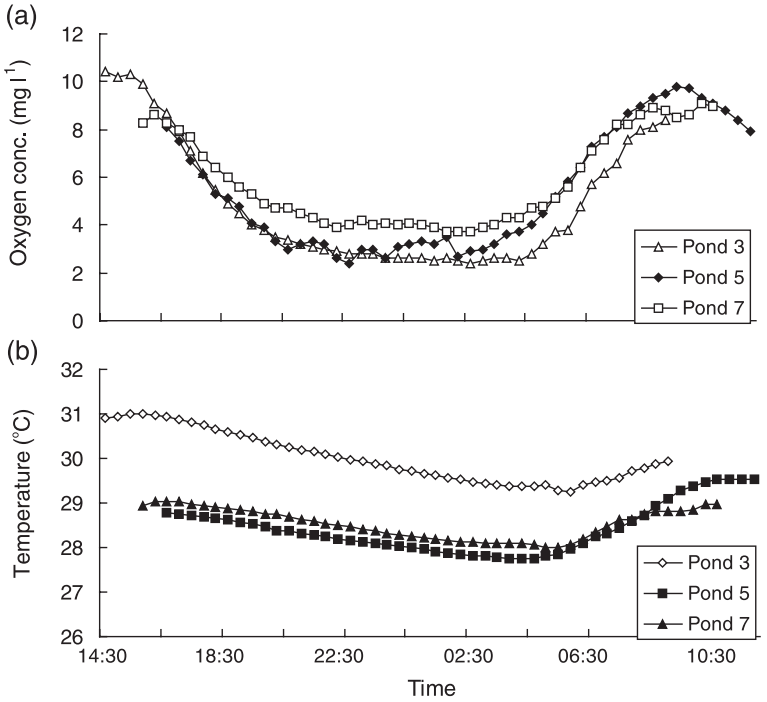

Fig. 1. Changes in (a) O_2 concentrations (mg l^{-1}) and (b) temperature (°C) over three 24-h periods in ponds at BAL.

difference in O_2 levels between ponds (Fig. 1a). Maximum and minimum temperatures only varied from 1 to 2 °C (Fig. 1b).

Individual shrimp weights ranged from 3 g in pond 3 to 14.7 g in pond 5 (Table 2). The estimated pond biomass, based on feed inputs and individual shrimp weights (a subset of a known number of shrimp was weighed), at the end of the sampling period ranged from 1800 to 11 700 kg ha^{-1}.

3.3. Bacterial processes

Mean bacterial numbers in the ponds ranged from 3.35 to 5.42 × 10^7 ml^{-1} over the 3 weeks of sampling (Table 3). More than 50% of the bacteria were free living, the remainder associated with detritus in the form of flocculated matter (1070–4310 flocculated particles ml^{-1}). Particulate N and C values ranged from 3.12 to 5.42 and 11.14 to 32.22 mg l^{-1}, respectively (Table 4). There was no correlation between chlorophyll *a* and either particulate N or C, which indicates that most of the PN and PC was not living phytoplankton. The correlation between phaeopigments, i.e. chlorophyll breakdown products, and PN ($R^2 = 0.49$, $P < 0.005$) suggests that dead phytoplankton contributed in a substantial way to PN values. DOC and DON concentrations were highly variable ranging from 14.20 to 48.10 and 2.29 to 5.56 mg l^{-1}, respectively (Table 4).

Table 3
Mean cell numbers (± S.D.) for bacteria, phytoplankton, protozoa and flocculated matter in the water column in five *L. vannamei* growout ponds at different crop ages at BAL over a 3-week period

Parameter	Days since stocking				
	17–37	27–47	55–75	71–91	98–118
	Ponds				
	3	18	9	7	5
(× 10^7 ml^{-1})					
Total bacteria	3.64 (0.66)	5.42 (1.78)	3.59 (1.06)	3.35 (0.65)	4.27 (0.70)
Free living bacteria	2.61 (0.86)	4.35 (1.39)	2.49 (0.79)	2.95 (1.05)	2.51 (0.92)
(× 10^4 ml^{-1})					
Autotrophic dinoflagellates	7.43 (4.23)	ND	0.26 (0.41)	ND	ND
Heterotrophic dinoflagellates	0.12 (0.10)	ND	0.11 (0.08)	0.17 (0.21)	0.22 (0.44)
Autotrophic nanoflagellates	ND	40.87 (44.28)	107.91 (175.62)	9.14 (10.81)	20.23 (9.29)
Cyanobacteria	0.81 (1.14)	3.63 (3.09)	5.45 (11.60)	0.07 (0.01)	ND
(ml^{-1})					
Diatoms	ND	ND	643 (1444)	ND	ND
Ciliates	132 (244)	86 (186)	433 (327)	86 (157)	129 (150)
Heterotrophic flagellates	357 (945)	729 (1039)	400 (695)	57 (151)	1157 (1024)
Rotifers	14 (38)	57 (113)	86 (107)	114 (121)	43 (113)
Heliozoa	ND	ND	43 (113)	29 (49)	43 (79)
Amoebae	ND	ND	ND	ND	29 (76)
Flocculated matter	1070 (540)	1820 (910)	2310 (1110)	1270 (670)	4310 (1840)

ND = not detected.

Table 4
Mean concentrations (± S.D.) of N, phosphorus, C, suspended solids and chlorophyll *a* in the water column and sludge in five *L. vannamei* growout ponds at different crop ages at BAL over a 3-week period

Parameter	Days since stocking				
	17–37	27–47	55–75	71–91	98–118
	Ponds				
	3	18	9	7	5
Water column (mg l^{-1})					
TAN	0.13 (0.27)	0.15 (0.22)	1.26 (0.59)	2.76 (0.33)	0.19 (0.18)
Nitrite	0.06 (0.09)	0.01 (0.01)	2.48 (1.38)	0.32 (0.28)	1.85 (3.01)
Nitrate	0.01 (0.02)	0.01 (0.01)	0.39 (0.25)	0.11 (0.07)	8.62 (2.81)
DON	5.18 (0.51)	2.29 (0.47)	3.51 (0.64)	5.56 (0.55)	5.24 (0.93)
PN	5.42 (3.25)	4.06 (1.43)	3.15 (0.90)	3.12 (1.47)	5.39 (1.54)
Total N	11.01 (2.43)	6.68 (0.90)	10.95 (0.68)	11.34 (1.06)	21.36 (1.20)
Phosphate	0.72 (0.52)	0.07 (0.07)	0.43 (0.25)	0.59 (0.06)	1.17 (0.07)
Total P	2.63 (0.42)	0.80 (0.18)	1.50 (0.13)	1.32 (0.08)	2.54 (0.29)
DOC	48.10 (20.59)	14.20 (1.40)	17.47 (2.98)	22.83 (1.75)	19.97 (1.72)
PC	32.22 (18.52)	21.57 (9.66)	14.43 (7.99)	11.14 (0.95)	25.75 (11.60)
TSS	75.33 (32.10)	84.19 (50.62)	48.46 (9.60)	38.44 (11.85)	66.71 (17.81)
Part. Org. (%)	83	76	69	75	73
Chl *a* (μg l^{-1})	435.10 (382.67)	157.41 (119.28)	134.29 (59.99)	175.41 (99.93)	224.87 (72.98)
Chl *b* (μg l^{-1})	3.51 (6.74)	5.07 (6.79)	21.30 (9.85)	19.00 (23.14)	6.77 (4.28)
Chl *c* (μg l^{-1})	242.81 (213.51)	89.19 (49.04)	97.90 (33.88)	96.54 (53.60)	123.09 (36.79)
Phaeopigment (μg l^{-1})	935.54 (745.35)	484.94 (165.83)	516.43 (165.54)	478.35 (229.24)	607.82 (166.99)
Sludge					
Total Kjeldahl N (g kg^{-1})	no sludge	16.42 (0.51)	18.65 (2.17)	18.30 (2.37)	18.25 (0.91)
Total P (g kg^{-1})	no sludge	4.55 (0.22)	5.87 (1.45)	9.73 (2.60)	7.13 (0.96)
%Organic	no sludge	44.1 (3.0)	50.4 (2.3)	49.2 (1.8)	56.3 (4.0)
Pore water NH_4 (mg l^{-1})	no sludge	28.9 (9.4)	52.0 (20.0)	149.7 (43.1)	53.9 (26.7)
%Pond area	no sludge	0.8	1.5	1.7	1.9

DON = dissolved organic N; PN = particulate N; DOC = dissolved organic C; PC = particulate C; TSS = total suspended solids; Part. Org. = particulate organics; Chl = chlorophyll.

Ammonium uptake in the dark, as measured using a ^{15}N-enrichment technique, was 11.72–40.54 μg l^{-1} h^{-1} (Table 5).

The molar DOC/DN ratio decreased from 15.5 to 3.1 with crop age (Fig. 2). The DOC/DN ratio was above that for marine bacteria (4.7, Lee, 1993) only until day 67. DOC/DN and PC/PN ratios were positively correlated with O_2 consumption rates ($R^2 = 0.93$ and 0.91, respectively) (Fig. 3). O_2 consumption ranged from 0.30 to 0.62 mg O_2 l^{-1} h^{-1} (Table 5).

Nitrification, as measured by O_2 consumption ± nitrification inhibitor, ranged from 7% to 22% of O_2 consumption and there was no trend with crop age (Table 5). Highest rates were in pond 3 (0.11 ± 0.07 mg O_2 l^{-1} h^{-1}), the only pond to contain recirculated water. Nitrification rates were compared with changes in TAN, nitrite and nitrate concentrations with crop age. In the ponds with younger crops (< 50 days after stocking), TAN, nitrite and

Table 5
Mean (± S.D.) rates of O_2 and N uptake and production in five *L. vannamei* growout ponds at different crop ages at Belize Aquaculture over a 3-week period

Parameter	Days since stocking				
	17–37	27–47	55–75	71–91	98–118
	Ponds				
	3	18	9	7	5
O_2 (mg l^{-1} h^{-1})					
Total consumption	0.62 (0.02)	0.48 (0.02)	0.42 (0.02)	0.38 (0.02)	0.30 (0.02)
Consumption due to nitrification	0.11 (0.07)	0.05 (0.03)	0.09 (0.05)	0.04 (0.03)	0.02 (0.05)
%Nitrification	17%	10%	22%	11%	7%
Net O_2 prod^n—surface	5.06 (0.18)	4.36 (0.11)	3.30 (0.13)	1.42 (0.09)	3.56 (0.09)
Depth-integrated net O_2 prod^n (g m^{-2} h^{-1})	4.59	2.80	2.85	1.36	2.42
N (µg l^{-1} h^{-1})					
Ammonium uptake					
Light	24.65 (5.45)	60.06 (26.19)	84.72 (41.65)	75.04 (44.53)	85.70 (57.91)
Dark	27.76 (11.84)	19.53 (8.68)	40.54 (45.02)	11.72 (1.60)	29.24 (8.09)
Nitrate uptake	2.18	0.33	3.32	1.27	217.09

Prod^n = production

nitrate concentrations were relatively low (<1 mg l^{-1}) (Fig. 4). TAN concentrations increased after this to a maximum of 3.10 mg l^{-1}, followed by nitrite concentrations of 7.66 mg l^{-1}. Both nitrite and TAN concentrations decreased after day 100 and nitrate concentrations increased to 10.62 mg l^{-1}. However, this trend was not entirely consistent in all ponds. In pond 7, for example, TAN concentrations remained high compared with nitrate and nitrite concentrations despite crop age (71–91 days).

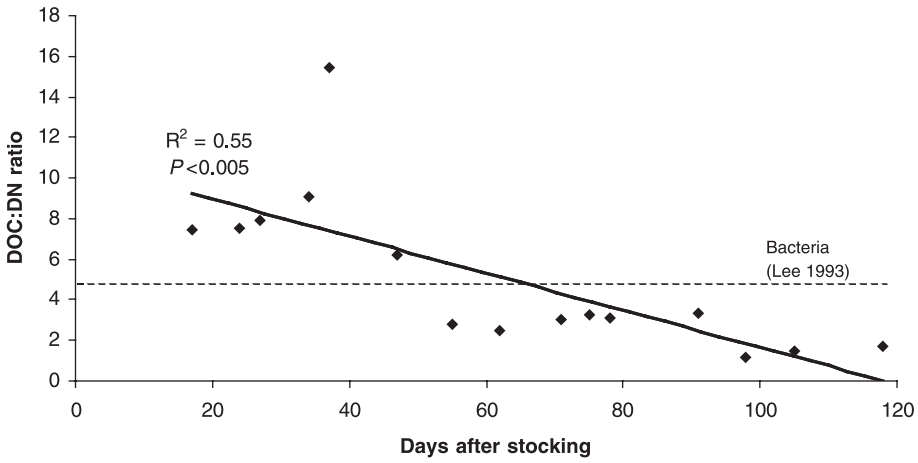

Fig. 2. Changes in molar DOC/DN ratio with days since stocking in five ponds at BAL. Dashed line shows C/N ratio for marine bacteria (Lee, 1993).

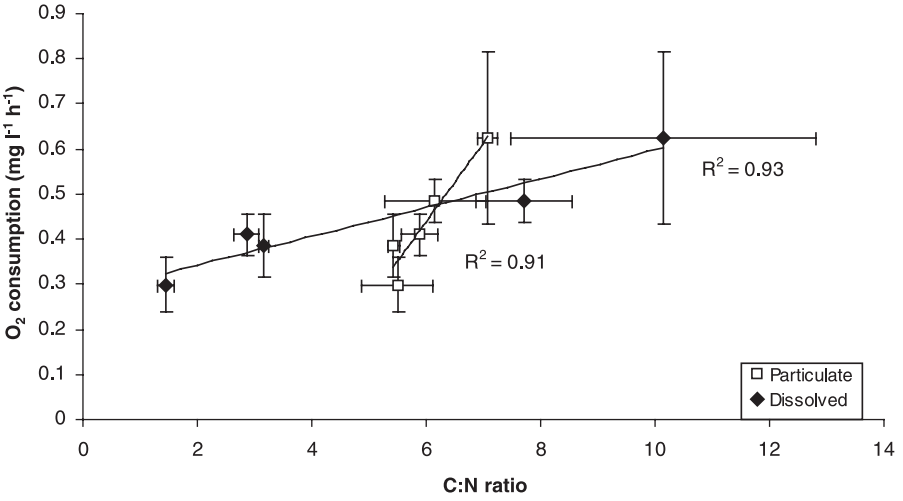

Fig. 3. Correlations between O_2 consumption (mg l^{-1} h^{-1}) with changes in the particulate (open squares, solid line) and dissolved (solid diamonds, dashed line) organic C/dissolved N molar ratios across all ponds.

There were substantial numbers of bacteriovores in all ponds (up to 2200 ml^{-1}), mainly ciliates and heterotrophic flagellates (Table 3). Due to the counting technique and fixation used, only heterotrophic flagellates >10 μm could be counted; however, substantial numbers of smaller flagellates were also observed in fresh samples.

3.4. Phytoplankton processes

Mean chlorophyll *a* concentrations ranged from 134.29 to 435.10 μg l^{-1} and phaeopigment concentrations ranged from 478.35 to 935.54 μg l^{-1} during the study

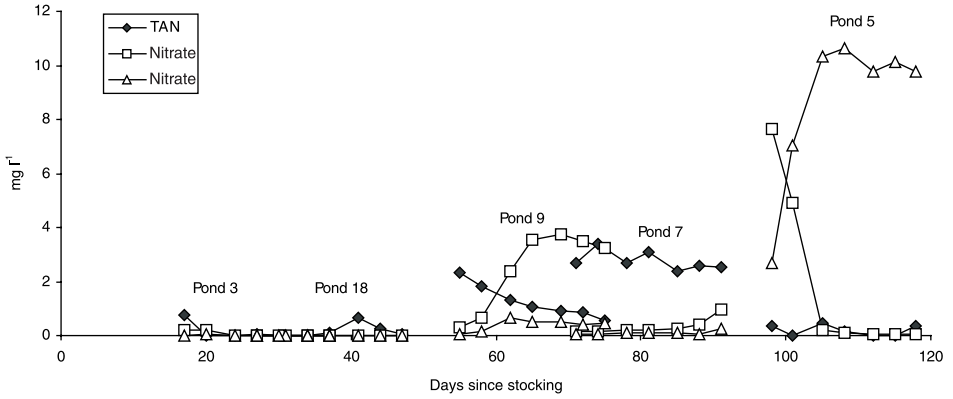

Fig. 4. Changes in TAN, nitrite and nitrate concentrations (mg N l^{-1}) with days since stocking in five ponds at BAL.

(Table 4). The dominant phytoplankton groups over the 3-week study were autotrophic and heterotrophic dinoflagellates, nanoflagellates and cyanobacteria (Table 3). Most of the nanoflagellates were <10 μm in size and the high chlorophyll *c* concentrations suggest that they were chrysophytes, cryptophytes and/or prymnesiophytes (Table 4). Diatoms were absent with the exception of low numbers in pond 9. Species dominance varied substantially over time frames of days. There was no obvious trend in phytoplankton dominance or cell numbers across the ponds of varying crop ages. Grazing of phytoplankton by rotifers and some ciliate species was observed. Rotifers and ciliates were present in all ponds (Table 3).

Net surface phytoplankton productivity, as measured by O_2 production, ranged from 1.42 to 5.06 mg O_2 l^{-1} h^{-1}, and depth-integrated productivity, calculated from O_2 surface production and Secchi disc readings, ranged from 1.36 to 4.59 g m^{-2} h^{-1} (Table 5).

Phytoplankton uptake of ammonium, as calculated from ^{15}N-ammonium uptake rates in light conditions minus dark conditions were highly variable within and between ponds, ranged from 0 to 63.32 μg l^{-1} h^{-1} (Table 5). Phytoplankton uptake of nitrate was also measured (on one occasion), and the rate (0.33 to 2.18 μg l^{-1} h^{-1}) was generally an order of magnitude lower than ammonium uptake, with the exception of pond 5 were nitrate uptake was higher (217.09 μg l^{-1} h^{-1}) than ammonium uptake. This was also the pond with highest nitrate concentrations (8.62 ± 2.81 mg l^{-1}) and relatively low TAN concentrations (0.19 ± 0.18 mg l^{-1}) (Table 4). Phosphate, the other major nutrient for phytoplankton growth, was present in concentrations ranging from 0.07 to 1.17 mg l^{-1}.

The ratio of productivity to respiration (P/R) varied substantially within ponds and between ponds (Fig. 5). Values ranged from 0.3 to 2.2 but there was no trend between ponds, irrespective of crop age. Indeed values ranged from <1 to >1 from 1 week to the next within individual ponds.

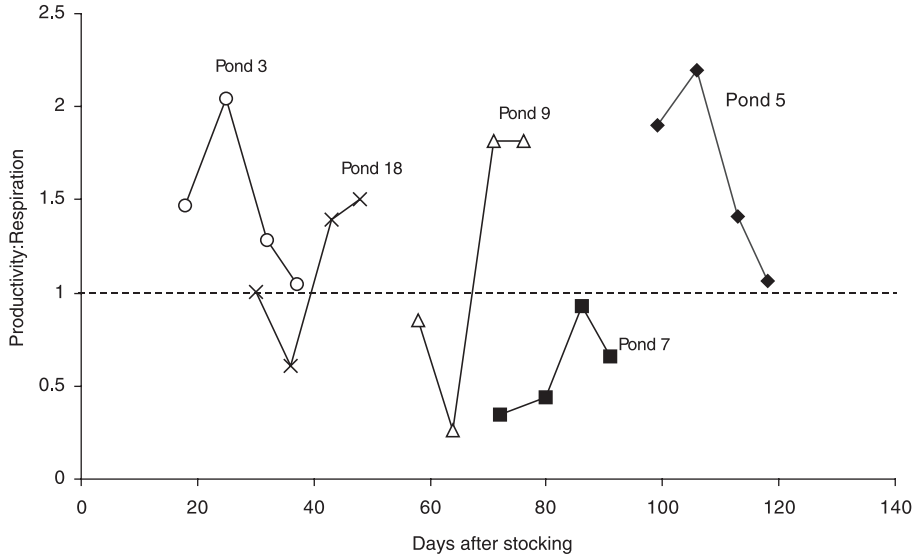

Fig. 5. Changes in productivity/respiration (P/R) ratio with increasing days since stocking in five ponds at BAL.

There was a mound of sludge in the middle of all ponds, except pond 3 (the youngest pond). The sludge area ranged from 0.8% to 1.9% of the total pond area (Table 4). It was not possible to accurately quantify the volume of sludge that accumulated over the crop as sludge had been periodically removed via central drains. However, N and phosphorus concentrations in the sludge were measured. Total Kjeldahl N (TkN) values ranged from 16.42 to 18.65 g kg^{-1}, and total phosphorus (TP) ranged from 4.55 to 9.73 g kg^{-1} with no obvious trend with crop age (Table 4). The molar TkN/TP ratios ranged from 4.3 to 8.0. Pore water TAN concentrations ranged from 28.9 to 149.7 mg l^{-1}.

4. Discussion

Concentrations of dissolved inorganic N and phosphate in the water of BAL shrimp ponds were all considerably higher than those in conventional shrimp ponds, characterized by lower stocking densities and feed inputs, earthen floors and periodic water exchange (Table 6). Ammonia and nitrite are two compounds that can be toxic to shrimp in high concentrations (Chien, 1992). In the case of TAN, the low pH in the ponds (7 to 8) would have resulted in a low proportion of the toxic, unionized form, ammonia. The low pH in these ponds is therefore beneficial in maintaining low ammonia concentrations. However, the high and consistent harvest tonnages would suggest that the high TAN and nitrite concentrations were not having major impacts on shrimp production. Whether the high TAN and nitrite concentrations affected shrimp growth rates was not established.

The high phosphate concentrations were probably the result of lining the ponds, thus removing the sediment interface. This was also reflected in the low N/P ratio in the sludge in ponds at BAL. In earthen ponds, substantial amounts of phosphorus become bound to sediment particles (Munsiri et al., 1996; Burford et al., 1998). The increased phosphorus availability is likely to have stimulated phytoplankton growth.

Phytoplankton numbers and primary production rates in BAL ponds were high relative to conventional shrimp and fish ponds (Yusoff and McNabb, 1989; Knud-Hansen et al., 1991; Burford, 1997), irrespective of crop age. There were high numbers of small (< 10 μm) flagellates and cyanobacteria, similar to conventional ponds (Burford and Glibert, 1999; Burford, 2000). The high numbers and productivity are not surprising, since the key nutrients required for growth, N and phosphorus were present in high concentrations. Additionally, the high aeration rates mixing the water column ensured that light and

Table 6
Comparison of the nitrogen and phosphorus concentrations (mg l^{-1}) in the water column of conventional shrimp ponds and this study

Study	Concentration (mg l^{-1})				
	TAN	NO_2/NO_3	Total N	Phosphate	Total P
Cowan et al. (1999)	0.56	0.10	3.48	0.03	0.33
Martin et al. (1998)	0.01	0.001	0.58	–	–
Ziemann et al. (1992)	0.06	0.03	0.69	0.04	0.23
This study	0.90	2.77	12.27	0.60	1.76

carbon dioxide did not become limiting. The most distinct difference in BAL ponds compared to conventional ponds was the low numbers of diatoms (Burford and Pearson, 1998; Tookwinas and Songsangjinda, 1999).

The phytoplankton in BAL ponds were very dynamic with short-lived blooms of individual species that were rapidly succeeded by other species. The high phaeopigment concentrations suggest high phytoplankton mortality rates. Some phytoplankton grazers were observed, most notably rotifers, and they were likely to have impacted substantially on phytoplankton numbers at times. The combination of phytoplankton and bacterial activity resulted in highly variable productivity/respiration ratios within ponds fluctuating from day to day between a net autotrophic (phytoplankton-dominated) and net heterotrophic (bacterial-dominated) state. However, there was no obvious trend of increasing heterotrophy with pond age in the five ponds studied. This is contrary to the theory that manipulating the C/N ratios by increasing the feed C input to these ponds results in a shift from an autotrophic to a heterotrophic system (Avnimelech et al., 1994; Avnimelech, 1999; Browdy et al., 2001). Only one pond was consistently heterotrophic throughout the study and this pond had higher carbon input from feeds than carbon from primary production. It may be that a heterotrophic system can only be achieved if the feed carbon input can exceed the primary production.

An additional complication is that some phytoplankton groups that were present in the BAL ponds, particularly dinoflagellates and other flagellates, are capable of mixotrophic or heterotrophic growth, allowing them to utilize organic sources of C either in addition to, or instead of, carbon dioxide and light. This adds another layer of complexity to understanding the trophic status, and interactions between phytoplankton and bacteria in the ponds.

A substantial proportion of bacteria were associated with the flocculated matter (up to 40%). The high load of flocculated matter is likely to be due to the high mixing rates suspending detrital particles in the water column. The total bacterial counts and O_2 consumption rates were comparable with those in other high-intensity, zero-exchange shrimp ponds (Bratvold et al., 1999; Bratvold and Browdy, 2001) and higher than those in conventional ponds (Moriarty, 1986; Burford, 2000). There was no trend of increasing numbers of bacteria or flocculated matter between ponds or over the 3-week study. The same was also true of O_2 consumption rates. Despite the high feed inputs, resulting in high DN and DOC concentrations and C/N ratios, bacterial growth may have become limited by the availability of C as the shrimp biomass and feed inputs increased. This is reflected in the decrease in the DOC/DN ratio with crop age below the ratio found in bacterial biomass (4.7, Lee, 1993) by day 67. C/N ratios of bacteria are an indication of the ratios of their uptake of C and N. Conversely in ponds with younger crops, the DOC/DN ratios were higher than that required by bacteria. However, another explanation for why bacterial numbers or oxygen consumption did not increase with crop age may have been control by grazers or viruses. The microbial loop in shrimp ponds is poorly understood.

The promotion of bacteria at BAL is designed to increase the breakdown of organic matter, reduce TAN levels and, in the form of flocculated matter, to provide a food source for shrimp (Avnimelech et al., 1994; Avnimelech, 1999; Browdy et al., 2001). The lack of obvious increase in DON and DOC concentrations with crop age suggests that bacterial activity was sufficient to prevent the accumulation of these compounds as the feed load increased. Other studies have found that the addition of labile carbon sources only

promoted bacteria capable of using labile carbon sources, not complex refractory compounds (Coffin et al., 1990). Therefore, it is likely that the accumulation of organic compounds is principally those refractory in nature.

In terms of ammonium uptake, bacteria and phytoplankton both appeared to play a major role. However, previous studies have shown that net uptake of inorganic nitrogen only occurs when the C/N ratio of the organic matter is higher than 10, which was not the case in this study (Lancelot and Billen, 1985). Phytoplankton uptake of ammonium is an active process requiring light (Raven, 1984). However, it is possible that mixotrophic or heterotrophic phytoplankton, in addition to bacteria, contributed to ammonium uptake in dark conditions. Ammonium uptake rates were higher than those in conventional ponds (Burford and Glibert, 1999; Burford, 2000).

TAN may also be used by bacteria for conversion to nitrite and nitrate, via the nitrification pathway. Nitrification rates were comparable with those measured in pilot scale, high-intensity, low-waste shrimp systems (Bratvold et al., 1999; Bratvold and Browdy, 2001), but nitrification was a minor contributor to oxygen consumption (22%) compared with heterotrophic bacteria. A previous study has shown that nitrification is inhibited by the addition of organic carbon (Zhu and Chen, 2001). Therefore, while the addition of grain-based feed and molasses may be effective in promoting heterotrophic bacterial growth, it may be limiting nitrification.

It should be noted that the nitrification method used in this study only measures the conversion of TAN to nitrite (Roy and Knowles, 1995). O_2 consumption by bacteria converting nitrite to nitrate was not accounted for. Compared with BAL ponds, conventional shrimp ponds appear to have little or no nitrification, since nitrite and nitrate concentrations remain low throughout the growth season (Tookwinas and Songsangjinda, 1999; Cowan et al., 1999). These differences may be due to a number of factors: water exchange rates in conventional ponds prevent slow-growing nitrifiers from becoming established; the presence of a high load of flocculated matter in BAL provides a substrate for nitrifiers to become established; and the high sludge loads in conventional ponds produce hydrogen sulfide which inhibits nitrifiers (Juliette et al., 1993). The low nitrification rates in conventional ponds, in turn, provide little substrate for denitrification (Burford and Longmore, 2001). Therefore, little of the N in the ponds is lost as nitrogen gas. Denitrification rates were not measured in BAL ponds and despite the availability of nitrate, it is not known whether redox conditions are suitable for denitrification. However, nitrate was effectively utilized by phytoplankton, especially when nitrate levels were high and TAN levels were low.

Despite the high mixing rates in the ponds, there was sludge formation due to sedimentation of detritus. In addition, products of shrimp feeding, including uneaten feed and faeces, would have contributed to the sludge layer in the pond centre. The N and phosphorus concentrations in the sludge were substantially higher (1.8% and 0.7%, respectively) than those in conventional shrimp ponds with earthen floors (0.1–0.2% and 0.01–0.05%, respectively) (Smith, 1996; Burford et al., 1998). This is consistent with less inorganic material in the particulate matter and sludge at BAL. In conventional ponds, aerators scour the outer regions of the ponds with the result that 90% of the sludge is inorganic. However, the pore water TAN concentrations were similar in the BAL ponds and conventional ponds (Burford and Longmore, 2001). In conventional ponds, high pore

water TAN concentrations resulted in high rates of TAN release from the sludge, contributing to the TAN concentrations in the water column. However, in the BAL ponds, the sludge area was < 2% compared with 15–35% in conventional ponds so the contribution to TAN production in these ponds is likely to be much smaller.

In conclusion, the high-intensity, zero-exchange shrimp ponds at BAL were characterized by high concentrations of nutrients and high numbers of bacteria, phytoplankton and protozoa. The dominant ecological processes in BAL ponds during our study were comparable with conventional ponds that have lower stocking densities, lower aeration rates, earthen floors and periodic water exchange. However, rates of nitrogen and carbon transformation are higher in the BAL ponds. Despite the additional input of carbon, in the form of grain-based feed and molasses, bacterial processes did not dominate the ponds. Rather there was a rapidly fluctuating dominance of bacterial and phytoplankton processes that reflected rapid growth and death of phytoplankton species. There may be scope to improve bacterial growth by further increases in the carbon loading later in the crop cycle; however, the benefits of this need to be weighed against increased feed costs and an increase in the oxygen demand. Additionally, it is not clear whether this will result in improved breakdown of organic matter. If the objective is to provide an additional food source for shrimp and/or improve utilization of inorganic N, increased carbon loading may be warranted. However, if the objective is to promote nitrification, carbon loading may be having a negative effect.

Despite water quality conditions that would generally be considered poor or detrimental to shrimp growth, shrimp production was high. This suggests that in the case of this species, at least, factors other than water quality are more important. A key difference between this system and conventional systems was the use of pond liners and higher aeration rates. This combination ensured that most of the pond floor was free of detritus and other material that can create poor sediment conditions. The implications of this for more traditional production systems may be that more attention should be focused on ensuring adequate sediment conditions.

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