Aquaculture 290 (2009) 236–242

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Growth performance of blue shrimp, *Litopenaeus stylirostris* in self-cleaning microcosm tanks

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ARTICLE INFO

Article history:
Received 17 June 2008
Received in revised form 14 February 2009
Accepted 16 February 2009

Keywords:
Microcosm tank
Shrimp
Litopenaeus stylirostris
Feed
Growth performance

ABSTRACT

Outdoor microcosm tanks were used to grow the penaeid blue shrimp, *Litopenaeus stylirostris,* in Brunei Darussalam. The tanks were cylindrical, free standing fiber glass tanks of 1827 L water holding capacity and had a self-cleaning mechanism. In three eight-week feeding trials, juvenile shrimp of 0.9–4.3 g were stocked at a density of 28 shrimp/m^2. At the end of each trial, survival rates exceeded 80%. Growth rates ranged from 1.19 to 2.46 g/week. Water quality remained stable and within suitable ranges for *L. stylirostris* growth in all trials. The tanks had algae and bacterial floc developing within a few days of starting the trials. Fourteen commercial shrimp feeds, each containing more than 40% crude protein, were tested in the trials. In spite of the presence of natural food organisms, significant feed-related differences among treatments were found in each trial. In conclusion, microcosm tanks support excellent growth and survival of *L. stylirostris* and are appropriate for conducting trials to evaluate feeds for pond growout.

1. Introduction

Aquarium tanks using flow-through or recirculated water are widely used in aquaculture experiments. The ease of replication and the ability to tightly control environmental variations are perceived advantages in the use of such systems in research. Further, the use of clear water aquaria or recirculation tank systems is necessary when evaluating feed and ingredient digestibility or conducting nutrient requirement studies that require water to be deprived of natural foods. Such systems, however, do not adequately mimic the commercial culture systems of many aquatic species, especially planktivorous, detritivorous and omnivorous species that consume natural foods generated in their culture systems. Leber and Pruder (1988) of the Oceanic Institute, Hawaii, USA, (OI) first reported that shrimp grown in tanks receiving water from a pond showed higher growth than shrimp grown in tanks receiving flow-through seawater. Freeman and Duerr (1991) reported that *Litopenaeus vannamei* in microcosm tanks designed to mimic the round experimental ponds of OI achieved an average weight gain of 1.85 g/week, which was far higher than the growth rate of penaeid shrimp grown in other tank systems. Later in the same system and species, Tacon et al. (2002) reported a weight gain of 2.16 g/week in one of the dietary treatments. The reason for this superior growth is that the microcosm tanks mimic shrimp grow-out ponds in terms of natural productivity (Tacon et al., 2002).

At the Shrimp Nutrition Research Center (SNRC), Integrated Aquaculture International, USA, and the Department of Fisheries, Brunei Darussalam, have jointly conducted feeding trials with *L. stylirostris* in microcosm tanks since early 2007. The tank design is based on dimensions of the OI microcosm tank reported by Freeman and Duerr (1991), but a number of modifications have been made to improve feeding efficiency and animal performance. The SNRC microcosm tanks do not have a sand bottom like the OI tanks. Further, the tanks have a self-cleaning mechanism that allows closer monitoring and removal of feed and fecal waste. The objectives of this paper are to describe the tank design and shrimp performance and to comment on the suitability of the tank for conducting feeding trials.

2. Materials and methods

2.1. Design and operation of self-cleaning microcosm tanks

Microcosm tanks were cylindrical, free standing fiber glass units of 1827 L water holding capacity. Various components and dimensions of the tank are presented in Fig. 1A and B. The tank inner wall and bottom were coated with black and white paint, respectively. Each microcosm tank was equipped with horizontal and vertical water circulation driven by airlift and gravity. Four airlift pipes of 5 cm diameter capped with the same size elbows were mounted in the inner periphery of the tank walls at equal intervals. These pipes created uni-directional, swirling water movement. This process swept all the solid particles (uneaten feed, feces, plankton die-offs etc.) on the bottom into the central cup of 15 cm diameter recessed within the floor of the tank. The tank bottom was sloped (10%) to make this process more efficient.

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doi:10.1016/j.aquaculture.2009.02.028

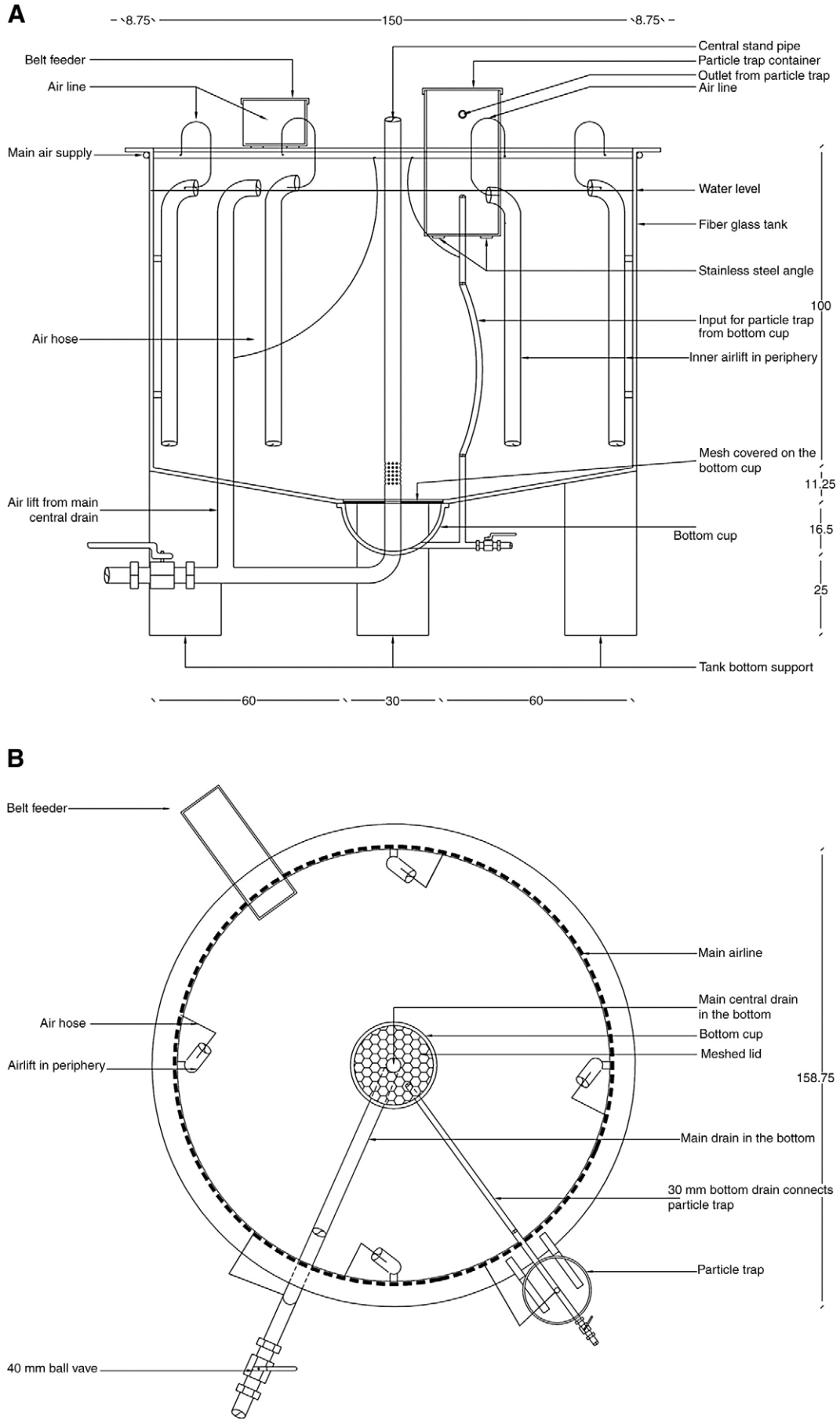

Fig. 1. A. Schematic diagram showing the components of microcosm tank – side view. B. Schematic diagram showing the components of microcosm tank – top view.

Once particles reached the bottom cup, they were air lifted through a PVC pipe (1.85 cm diameter) and a flexible hose to the particle trap at the top of the tank. The bottom cup was covered by a high density poly-ethylene (HDPE) screen to avoid escape of shrimp. Once solids reached the particle trap, most settled by gravity and only water was discharged back to the main tank. A central stand pipe of 5 cm diameter with perforations (8 mm) at the bottom was fixed on the bulk water drain. The perforations were at a height above 7.5 cm from the bottom of the tank. Outside the tank, the bulk drain was connected to an airlift which circulated water from the bottom center to the top perimeter. Flexible HDPE pipe of 2.5 cm diameter was mounted along the rim of the tank to supply air to all airlifts inside the tank.

Placement of the airlift pipes inside the tank 5 cm above tank bottom minimized any chance that settled solids are retrieved back to the surface due to the vertical water motion generated by the airlifts. The tank bottom slope of 10% was mild and not sufficiently inclined to cause the shrimp to slip along the slope towards the central drain.

A 12 h baby belt feeder, powered by a spring-wound clock mechanism, was placed on the wall of tank on its top, using a wooden support. To prevent shrimp from escaping from the tanks, the open surface of tank was covered with a HDPE netting of 1 cm mesh.

While the belt feeder on the top of the tank fed the shrimp continuously, the excess uneaten feed along with other solids at the bottom were swept to the self cleaning trap on the top of the tank. The solid matter settled in the trap was periodically removed by siphon.

2.2. Commercial feeds and analysis

Fourteen commercial feeds were obtained from a retail feed outlet in Brunei Darussalam, or directly from feed manufacturers at various times of the year. The feeds were analyzed for determination of moisture (AOAC, 2005: 930.15), crude protein (by combustion AOAC, 2005: 990.03), crude fat (by acid hydrolysis, AOAC, 2005: 954.02), and ash (AOAC, 2005: 942.05).

2.3. Feeding trial

Three trials of eight-week duration were conducted between January and October in 2007. Four commercial feeds were tested in Trial 1, and five commercial feeds were tested both in Trial 2 and 3. Each feed was randomly assigned to triplicate tanks. The tanks were stocked with juvenile *L. stylirostris*. The initial average weight ranged between 0.9 and 4.3 g. The shrimp were obtained by growing hatchery-produced high-health postlarvae in outdoor nursery tanks of 5 tonne capacity.

Two days prior to stocking, the tanks were filled with 1650 L of sea water filtered through a 500 μm screen. The water was fertilized with urea and triple super phosphate each at the rate of 1 g/tonne water. One liter each of micro algae *Thalassiosira* sp and *Chaetoceros* sp (algal concentration = 1 million cells/mL) was added to the tanks after fertilization. Fertilization at the same rate was continued until a day after stocking. A bloom of phytoplankton was reached four days after the inoculation of algae.

Each tank was randomly stocked with 50 juvenile shrimp (stocking density: 28 shrimp/m^2; 30 shrimp/m^3). Before stocking, 20 juvenile shrimp from each tank were weighed individually. The total biomass of 50 shrimp was also weighed in each tank. The day after stocking, the water had only a light phytoplankton bloom and the bottom of each tank was visible enough to count the shrimp. If some of the stocked shrimp had died in the tank, they were replaced by similar sized live shrimp. To enable easy observation of shrimp a day after stocking, only a 2-day interval between water fertilization and shrimp stocking was used.

The belt feeders were loaded with feed twice daily (08:00, 17:00 h). The self-cleaning system was not stopped during feeding.

Table 1
Feeding rate based on average body weight of shrimp.

Shrimp average body weight (ABW) (g)	Feed as % of shrimp biomass (Estimated)	
	Trial 1*	Trials 2 and 3
1–3	7	7
3–5	6	6.5
5–7	5.5	5.5
7–9	5	5
9–11	4.5	5
11–13	4	4.5
13–15	3.5	4
15–17	3	3
17–30	2.5	2.5**

* Based on the feeding guide of Oceanic Institute (Tacon et al., 2002).
** The highest ABW recorded in the trials was 23.8 g.

In Trial 1, shrimp were fed at fixed weekly feeding rations (Table 1) based on the average body weight (ABW) and water temperature, as recommended by Tacon et al. (2002). In Trial 2, daily ration was adjusted weekly, based on weight gain, survival and observations on the presence of uneaten feed in the self cleaning system. The feed ration was modified in Trial 3 (Table 1). The daily ration was divided into 35% for the morning (08:00 to 17:00 h) and 65% for evening (17:00 to 08:00 h). Feed pellets were spread evenly in the feed belt to ensure continuous distribution of feed in the tank at a rate of about 4–5% per hour. The feed pellets fell from the feeder close to the rim of the tank. Each pellet remained in water for about 30 min before being swept out of the tank to the particle trap. Ten shrimp (20% of the stock) from each tank were sampled weekly to obtain mass weight, and were returned to their respective tanks.

Water temperature, dissolved oxygen (DO), pH, and salinity were measured once every three days using YSI 556 multi probe system (YSI Environmental, Yellow springs, OH, USA). Ammonia and Nitrite were measured by HACH methods 10031 and 8153 (HACH, 1997) in Trial 3. In Trial 3, water temperature, DO and pH were measured twice daily (08:00, 17:00 h) for a period of one week continuously to determine diurnal variations. After the third week of the trial, 30% of the tank volume was exchanged and this procedure was repeated weekly thereafter on the day of sampling. Water losses due to spillage from the self cleaning system and evaporation were replaced by filtered sea water whenever needed, but never more than 10% of the total volume at a time. At the end of the trial, water was drained and shrimp were harvested, counted, and weighed to determine survival and final weight.

2.4. Performance calculations and statistical analysis

Feed and growth performances of the shrimp were calculated using the following formulas.

$$\text{Weight gain } (g) = \text{final weight } (g) - \text{initial weight } (g)$$

$$\text{Food Conversion ration (FCR)} = \text{feed offered } (g) / \text{biomass gain } (g)$$

$$\text{Daily growth coefficient (DGC)} = \left\{\left(W_f^{1/3} - W_i^{1/3}\right)/t\right\} \times 100$$

Uneaten feed removed from the particle trap was not quantified and therefore not deducted from the quantity of feed offered in calculating FCR. DGC was used to compare growth of shrimp across trials as initial shrimp weight differed considerably among the three trials.

Mean values of shrimp performance were subjected to one-way analysis of variance (ANOVA) and Duncan's multiple range test (Duncan, 1955), to determine significant differences ($P<0.05$) among dietary treatments in each trial. The statistical analyses were performed using SPSS statistical software (Ver.10 for Windows, SPSS,

Table 2
Commercial shrimp feeds used in the microcosm trials identified by their codes and proximate composition (g 100 g^{-1} dry matter).

Feed	Dry matter	Crude protein	Crude lipid	Ash
T1 F1	90.71	46.96	6.93	16.21
T1 F2	89.20	45.74	10.07	12.78
T1 F3	89.80	46.10	8.91	15.37
T1 F4	90.48	48.96	9.59	15.03
T2 F1	90.17	40.37	9.97	9.49
T2 F2	89.20	44.62	7.56	15.70
T2 F3	90.55	42.52	12.81	12.04
T2 F4	90.25	40.00	8.24	10.97
T2 F5	90.27	46.19	5.65	13.63
T3 F1	89.80	44.32	9.28	11.69
T3 F2	89.60	48.33	8.49	12.50
T3 F3	91.98	49.68	12.50	11.31
T3 F4	91.76	51.87	8.83	16.35
T3 F5	90.52	49.27	5.83	15.47

T1, T2, T3 indicate feeds used in Trial 1, 2 and 3, respectively. Within each trial, the feeds were randomly marked as F1, F2, F3, etc., and do not code a specific feed manufacturer.

Chicago, IL, USA). Limits of significance for all critical ranges were set at $P<0.05$.

3. Results

3.1. Feed composition

Proximate composition varied among feeds (Table 2). Crude protein ranged from 40.00 to 51.87% and crude fat ranged from 5.65% to 12.81%. Ash content ranged from 9.49% to 16.35%.

3.2. Water quality

The ranges of mean water quality parameters measured at dusk during the three trials are presented in Table 3. Water temperature during the three trials averaged 30.3 °C with a maximum of 32.8 °C and minimum of 28.5 °C. Throughout the trials, all physicochemical parameters were within ranges considered to be optimal for shrimp growth. Within four days after the initiation of each trial, the water typically bloomed with sufficient algae to restrict light penetration deeper than 20 cm. Blooms were stable thereafter until termination of the trials. Bacterial floc was conspicuous 10 days after initiation of each trial in all tanks, irrespective of dietary treatment.

Diurnal variations in temperature DO and pH observed over a period of seven days showed fairly uniform trends (Fig. 2). Average fluctuations for temperature, DO and pH were 2.9 °C, 0.7 mg/L and 0.6 respectively.

3.3. Growth and feed performance

Data on shrimp growth, survival and feed utilization in the trials are presented in Table 4. Only data within each feeding trial were statistically compared. There were significant differences in shrimp growth among dietary treatments in each trial. Shrimp survival rates exceeded 80% in all treatments, and generally were above 90%. Feed related differences in shrimp growth were apparent in about four weeks after the start of the experiment and remained fairly consistent after that (Fig. 3).

4. Discussion

All three growth trials were completed without interruption by water quality or disease problems. Overall survival and growth of *L. stylirostris* fed commercial diets in self cleaning microcosm tanks were excellent, indicating the potential of microcosm tanks in fully realizing the growth potential of the animal. Growth recorded in this study (range: 1.19 to 2.46 g/week; average: 1.84 g/week) is higher than that of *L. stylirostris* in commercial ponds (Hernandez-Llamas et al., 1995), tanks (Ricque-Marie et al., 1998; Tapia-Salazar et al., 2004) and cages within commercial ponds (Castex et al., 2008).

Maximum growth rates observed in *L. stylirostris* ranged from 0.42 to 2.31 g/week, averaging 1.54 g/week, in earthen ponds using pelleted feeds and organic fertilizers (Hernandez-Llamas et al., 1995). While the higher initial weight of shrimp at stocking in this study prevents direct comparison of overall growth rates between the studies, the best weekly growth rates achieved in the present study were better than those achieved in the pond study. Ricque-Marie et al. (1998) observed that *L. stylirostris* grown in 225 L rectangular tanks grew at a rate of 1.55 g/week in a 30-day trial. The animals weighed 8.41 g at the start and were fed a diet containing 53.6% protein and 40% fish meal. In our self-cleaning microcosm tanks, the best growth of shrimp at about 8 g was 2.77 g/week (Feed T3F3 in Trial 3). The best performing treatment in a recent study testing probiotics in cages within commercial ponds in New Caledonia recorded a weight gain of 1.61 g/week (Castex et al., 2008). To our knowledge, the growth rates of *L. stylirostris* reported in this study are the highest reported for the species in scientific literature.

The outstanding shrimp growth in microcosm tanks can be attributed to optimal water quality, absence of anaerobic waste on the bottom, high quality feeds, an appropriate feeding regime, and the availability of natural food organisms (plankton and bacterial floc) in the water column.

Water quality is an important determinant of shrimp growth and survival. The recommended ranges for salinity and temperature for growing *L. stylirostris* are 25–38‰ and 20–30 °C (Spanopoulos-Hernández et al., 2005), respectively. DO levels exceeding 4.0 mg/L and pH ranges between 7.0 and 8.8 are considered as optimal for penaeid shrimp (Lazur, 2007). Microcosm tanks used in this study were capable of maintaining the water quality parameters within the optimal ranges.

Temperature affects shrimp metabolism and feeding rates, directly impacts primary production in water, influences the solubility of oxygen, and affects the degree of ammonia toxicity. Exposure of microcosm tanks to direct sunlight resulted in average temperature at the upper end of the optimum (30.1 to 30.8 °C at dusk), in all three trials. Higher temperature (within the optimal range) may have resulted in high feed consumption and increased metabolic activity of the shrimps (Zhang et al., 1998). Since the volume of the water in the tank was relatively small and the tanks were above ground level, water temperature showed a uniform diurnal fluctuation of 2.9 °C. A

Table 3
Water quality parameters measured in microcosm tanks in the growth trials.

Parameters	Trial 1			Trial 2			Trial 3		
	Average	Min.	Max.	Average	Min.	Max.	Average	Min.	Max.
Temperature (°C)	30.6 ± 1.9	28.5	32.4	30.1 ± 0.9	29.1	32.8	30.8 ± 1.0	28.5	32.0
Salinity (‰)	29.7 ± 0.9	28.5	30.2	29.5 ± 0.5	28.0	30.1	20.4 ± 1.1	18.5	22.5
Dissolved oxygen (mg/l)	7.3 ± 1.0	6.8	8.3	7.1 ± 0.9	6.3	7.8	6.92 ± 0.7	5.6	7.4
pH	7.2 ± 0.5	6.9	8.3	7.2 ± 0.7	7.0	8.3	7.35 ± 0.3	6.8	8.2
Ammonia nitrogen (mg/l)	Not analyzed			Not analyzed			0.04 ± 0.02	0.01	0.2
Nitrite nitrogen (mg/l)	Not analyzed			Not analyzed			0.19 ± 0.10	0.02	0.37

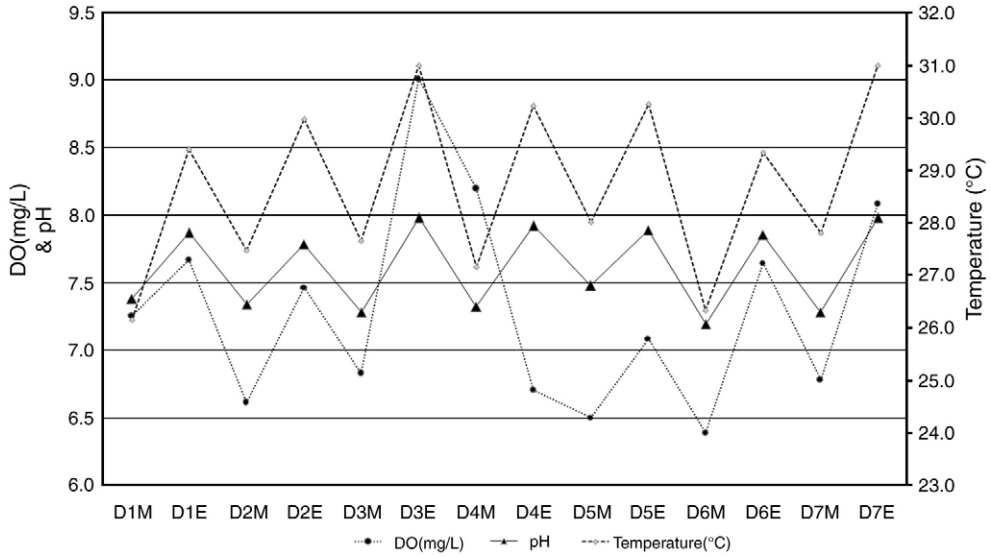

Fig. 2. Diurnal fluctuations in temperature (°C), dissolved oxygen (DO, mg/L) and pH of water in microcosm tanks for one week in Trial 3 (D = Day, M = Morning, E = Evening).

recent study (Xiangli et al., 2006) showed that, shrimp feed consumption, growth performance and feed efficiency were higher in diel fluctuating temperature than in constant temperature. Water was mixed continuously by the airlifts operating 24 h a day. DO levels were excellent (>6.9 mg/L). Shrimp are stressed when dissolved oxygen falls below 2.0 mg/L. Salinity showed minor fluctuations between the trials according to the salinity in source water which in turn was influenced by precipitation.

Ammonia concentrations studied in Trial 3 (0.04 ± 0.02 mg/L) were at the lower end of ammonia levels reported in commercial ponds. Ruiz-Fernández and Páez-Osuna (2004) reported 0.01–1.235 mg/L of ammonia nitrogen in ponds stocked with *L. vannamei*, in south of Sinaloa, Mexico. Matias et al. (2002) reported 0.18–0.317 mg/L of total ammonia nitrogen in a shrimp pond using probiotics. The observed low ammonia levels in the present study may be due to the consumption of ammonia by the phytoplankton. Hargreaves (1998) reported that ammonia is the preferred nitrogen substrate for phytoplankton. Elevated levels of nitrite coincided with minimum levels of ammonia, indicating the presence of nitrifying bacteria in the microcosm. Similar ammonia and nitrite acclimation spikes were observed by Otoshi et al. (2007) in a commercial-scale, high density shrimp production system.

Under optimum water quality, shrimps in microcosm tanks may have grown well by foraging on the natural biota in the tank as a supplement to the commercial feed offered continuously. The microcosm mimicked a natural ecosystem, where bacteria and microalgae play a major role in completing the nitrogen cycle. Water transparency was highest at the beginning of each trial and gradually declined after the first week indicating increased phytoplankton production. Bacterial floc also appeared within a short time. Though there are several studies reporting the substantial contribution of natural pond biota to nutrition of shrimp (Moss and Pruder, 1995; McIntosh and Avnimelech, 2001; Hopkins et al., 1995), only a few studies have been conducted in controlled tank conditions (Epp et al., 2002; Tacon et al., 2002; Burford et al., 2004; Moss et al., 2006). Burford et al. (2004) showed that natural pond water loaded with particulate organic matter and natural biota (micro algae and bacterial cells) yielded faster shrimp growth than clear water. Moss et al. (2006) showed that shrimp pond water had a sparing effect on

Table 4
Growth, feed utilization and survival of shrimp *Litopenaeus stylirostris* fed different commercial diets in self cleaning microcosm tanks.

	Initial weight (g)	Final weight (g)	Weight gain (g)	Feed consumed (g)	FCR	DGC	Weekly weight gain (g)	Survival (%)
Trial 1								
T1 F1	1.67 ± 0.08	16.25 ± 0.54^{b}	14.58 ± 0.46^{b}	24.48 ± 1.62^{a}	1.68 ± 0.14	2.40 ± 0.02^{b}	2.03 ± 0.07^{b}	89.33 ± 7.02
T1 F2	1.57 ± 0.04	13.52 ± 0.18^{a}	11.96 ± 0.14^{a}	20.29 ± 1.30^{a}	1.70 ± 0.09	2.18 ± 0.01^{a}	1.69 ± 0.02^{a}	95.33 ± 1.15
T1 F3	1.69 ± 0.10	18.11 ± 0.66^{c}	16.42 ± 0.76^{c}	30.60 ± 4.69^{b}	1.87 ± 0.34	2.56 ± 0.10 ^{c}	2.26 ± 0.08^{c}	83.33 ± 9.87
T1 F4	1.60 ± 0.04	16.50 ± 0.11^{b}	14.89 ± 0.15^{b}	24.29 ± 0.96^{a}	1.63 ± 0.07	2.46 ± 0.03 ^{b}	2.06 ± 0.01^{b}	93.33 ± 6.43
Trial 2								
T2 F1	1.09 ± 0.08	13.73 ± 0.77^{c}	12.64 ± 0.69^{c}	20.72 ± 1.17^{c}	1.64 ± 0.04^{abc}	2.44 ± 0.04^{bc}	1.58 ± 0.09^{c}	95.33 ± 4.16^{ab}
T2 F2	0.98 ± 0.08	12.42 ± 0.13^{b}	11.44 ± 0.17^{b}	18.30 ± 0.86^{b}	1.60 ± 0.08^{ab}	2.36 ± 0.05^{b}	1.43 ± 0.02^{b}	96.67 ± 3.06^{ab}
T2 F3	0.93 ± 0.06	10.45 ± 0.52^{a}	9.52 ± 0.57^{a}	15.73 ± 0.20^{a}	1.66 ± 0.08^{bc}	2.16 ± 0.10^{a}	1.19 ± 0.07^{a}	92.67 ± 4.16^{a}
T2 F4	1.03 ± 0.07	11.55 ± 0.48^{b}	10.52 ± 0.48^{b}	18.18 ± 0.72^{b}	1.73 ± 0.04^{c}	2.23 ± 0.07^{ab}	1.32 ± 0.06^{b}	100.00 ± 0.00^{b}
T2 F5	1.01 ± 0.07	14.45 ± 0.46^{c}	13.44 ± 0.47^{c}	20.44 ± 0.30^{c}	1.52 ± 0.07 ^{a}	2.56 ± 0.06^{c}	1.68 ± 0.06^{c}	92.67 ± 1.15^{a}
Trial 3								
T3 F1	4.38 ± 0.09	21.47 ± 1.36^{a}	17.09 ± 1.43^{a}	26.18 ± 1.06^{b}	1.54 ± 0.09	2.04 ± 0.12^{a}	2.14 ± 0.18^{a}	94.00 ± 5.29
T3 F2	4.27 ± 0.18	20.11 ± 1.41^{a}	15.85 ± 1.24^{a}	24.08 ± 0.44^{a}	1.52 ± 0.10	1.96 ± 0.08^{a}	1.98 ± 0.16^{a}	100.00 ± 0.00
T3 F3	4.23 ± 0.19	23.87 ± 1.14^{b}	19.64 ± 0.97^{b}	28.37 ± 0.21^{c}	1.45 ± 0.08	2.25 ± 0.04^{b}	2.46 ± 0.12^{b}	97.33 ± 3.06
T3 F4	4.37 ± 0.20	19.78 ± 1.36^{a}	15.41 ± 1.46^{a}	25.36 ± 0.80^{ab}	1.65 ± 0.13	1.91 ± 0.14^{a}	1.93 ± 0.18^{a}	80.67 ± 24.85
T3 F5	4.23 ± 0.13	20.93 ± 1.02^{a}	16.70 ± 1.08^{a}	25.29 ± 0.89^{ab}	1.52 ± 0.15	2.03 ± 0.10^{a}	2.09 ± 0.13^{a}	91.33 ± 8.08

Means within each feeding trial in the same column having different superscripts are significantly different ($P<0.05$).
Values are means of triplicates ± standard deviation.

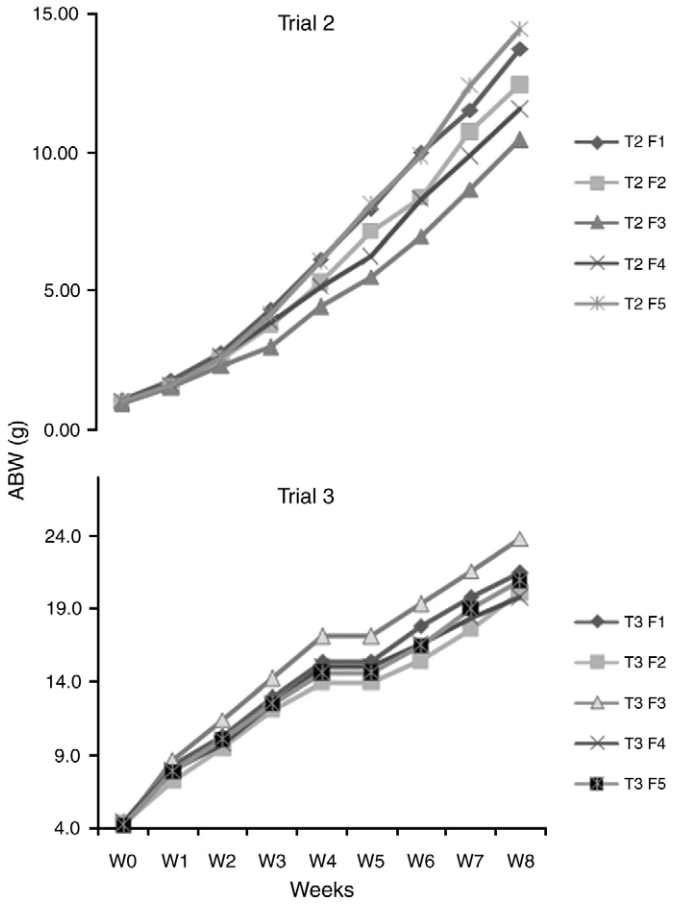

Fig. 3. Weekly growth of (average body weight; ABW) shrimp in Trial 2 (T2) and Trial 3 (T3) for eight weeks (W0–W8).

vitamins in the feeds of *L. vannamei*. Tacon et al. (2002) determined the nutrient composition of "microbial floc", produced autochthonously in zero-water-exchange tanks used for shrimp research. These particles contain a number of important macro- (calcium, phosphorus, potassium, and magnesium) and micro-minerals (copper, iron, manganese, and zinc), as well as a suite of amino and fatty acids, and appear to play an important role in shrimp nutrition. In addition, pond water is known to affect the abundance and species composition of gut microflora (Moss et al., 2000) and can stimulate digestive enzyme activity in juvenile *L. vannamei* (Moss et al., 2000; Divakaran and Moss, 2004).

There were significant diet-related differences in growth in all three feeding trials indicating the potential of microcosms to evaluate feed performance. In spite of the abundance of natural food organisms, significant diet-related differences occur in microcosms. For example, in Trial 1, shrimp receiving feeds T1F2 and T1F3 had weekly weight gains of 1.69 and 2.22 g, respectively, showing that shrimp receiving feed T1F3 had 25% excess gain when compared to shrimp receiving T1F3. Differences between dietary treatments were usually apparent in less than three weeks after the start of the trial. So, microcosms are appropriate systems to conduct feeding trials.

The differences in shrimp growth among dietary treatments even in the presence of natural biota (algae and bacterial floc) indicate that natural food satisfies only a part of the nutrient requirements. A study by Ju et al. (2008) on the composition of bacterial floc found that arginine and lysine were the two most limiting amino acids in flocs obtained at different salinity from outdoor raceways stocked with shrimp. In a rearing system which used pond water containing natural food organisms, Burford et al. (2004) observed reductions in growth rates of *P. monodon* given feeds containing low levels of crude protein. It has been suggested that natural biota are only minor contributors to the metabolizable protein and energy intake of the animals. The ^{15}N-tracer study in Burford et al. (2004) indicated that natural food contributed only 10% of the ^{15}N-nitrogen that was incorporated into shrimp.

Belt feeder and self cleaning system together acted well in continuously delivering feed to the shrimp and keeping the tank bottom clean. If the daily feed ration is given in 2 or 3 feedings per day, the shrimp would nibble on the feed slowly resulting in leaching of nutrients and disintegration of feeds in the water. Extensive leaching of nutrients has been reported as a major reason for high FCR values in shrimp (Bureau et al., 2000).

Variable feeding behavior and feeding periodicity have been observed in penaeid shrimps (Nunes et al., 1996; Focken et al., 1998; Soares et al., 2005). No studies are available on the feeding periodicity and behavior of *L. stylirostris*. The species appears to be a voracious and continuous feeder with no time preference for feeding in tank conditions.

Belt feeders delivered the feed slowly over time and increased the chances that the ration would be completely consumed by the shrimp. The feed pellets also stayed inside the tank for only approximately 30 min. If they were not consumed within 30 min, they would be collected in the self cleaning trap outside the tank. Burford and Williams (2001) reported that, dissolved organic nitrogen leached from formulated feed is one of the major sources of nitrogen in pond water. It appeared to be less effectively utilized by the microbial community and is likely to accumulate in ponds leading to poor water and sediment quality. In our microcosm tanks, uneaten feeds were rarely seen in the particle trap, indicating most of the feeds were consumed. Feeds collecting in the particle trap were siphoned out twice daily.

In Trial 1, we used the feeding rations suggested by Tacon et al. (2002) for *L. vannamei* based on average body weight of the animal and water temperature. However in Trials 2 and 3, we modified the ration size based on the observations of excess feed collected in the particle trap. The adjustments appeared to result in lower FCR in Trials 2 and 3 when compared to Trial 1.

The diet-related differences in shrimp performance could not be sufficiently explained by the proximate nutrient composition of the feeds. In general, feeds with higher crude protein performed better. There have been two studies on the effect of crude protein on *L. stylirostris* performance. A study by Baillet et al. (1997) reported that juvenile *L. stylirostris* fed the lower crude protein levels (27–31%) achieved poorer growth than those fed with feeds containing 33–43% crude protein. Based on the lack of difference among the higher protein feeds, they recommended a 33% protein feed. However Gauquelin et al. (2007) found that in adult *L. stylirostris* (21 ± 1 g) weight gain increased when dietary protein level was increased from 25 to 58%.

Some feeds with high levels of crude fat did not perform well which is consistent with the findings of Glencross et al. (2002) that crude fat levels excess of 7.5% depress feed intake and growth. The poorest growth was recorded with a feed containing 12.8% crude fat. However, the best performing feed overall in terms of weekly weight gain had a crude fat level of 12.5%. There are studies indicating that higher dietary fat levels could improve shrimp performance if the lipid is balanced with adequate levels of highly unsaturated fatty acids and phospholipids (Glencross et al., 1998; González-Félix et al., 2002; Kumaraguru vasagam et al., 2005).

5. Conclusion

L. stylirostris reared in microcosm tanks achieved growth rates as high as 2.46 g/week and survival rates exceeding 90%. Optimum water quality and the development of natural food organisms such as algae and bacteria in the tanks are likely to be the causes of excellent shrimp performance in microcosm tanks. In addition, continuous delivery of feeds through an automated feeder and removal of uneaten feeds and

feces through a self-cleaning system improved feed utilization and eliminated anaerobic sediments. In spite of the presence of natural food organisms in the tank, significant feed-related differences occurred among treatments indicating that microcosm tanks are appropriate for feeding trials.

Acknowledgement

We express our sincere thanks to Ym Dyg Hajah Hasnah Binti Ibrahim, Director, and Dyg Hajah Rosinah Binti Yussof, Head of Aquaculture Division, Department of Fisheries, Brunei for providing the space and necessary support to conduct the trials. We are grateful to Dyg Wanidawatti Binti Tamat, Awg Haji Sheikh Al-Idrus Sheikh Nikaman, and Dyg Abidah Binti Haji Mohamad Yazid, the officers of Department of Fisheries for their kindness in providing support in trial logistics and water quality analysis. We express our gratitude for Mr Chris Howell and Mr Shanmugam Muniyandi for their contribution in the design and fabrication of microcosm tanks. We thank Pg Suhanizen Pg Haji Tengah who performed day-to-day feeding and maintenance activities in all trials.

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