JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 44, No. 4
August, 2013

Effects of Inorganic and Organic Fertilization on Physicochemical Parameters, Bacterial Concentrations, and Shrimp Growth in *Litopenaeus vannamei* Cultures with Zero Water Exchange

Guillermo F. Lara-Anguiano

Maestría en Recursos Naturales y Medio Ambiente Program, Instituto Politécnico Nacional-CIIDIR Unidad Sinaloa, Boulevard Juan de Dios Bátiz Paredes # 250, Guasave, Sinaloa 81101, Mexico

Héctor M. Esparza-Leal[1], Juan C. Sainz-Hernández

Departamento de Acuacultura, Instituto Politécnico Nacional-CIIDIR Unidad Sinaloa, Boulevard Juan de Dios Bátiz Paredes # 250, Guasave, Sinaloa 81101, Mexico

Jesús T. Ponce-Palafox

Laboratorio de Bioingeniería Costera, Universidad Autónoma de Nayarit, CENITT-CBAP, Escuela Nacional de Ingeniería Pesquera, Ciudad de la Cultura Amado Nervo, Tepic, Nayarit 62155, Mexico

Wenceslao Valenzuela-Quiñónez, Juan P. Apun-Molina

Departamento de Acuacultura, Instituto Politécnico Nacional-CIIDIR Unidad Sinaloa, Boulevard Juan de Dios Bátiz Paredes # 250, Guasave, Sinaloa 81101, Mexico

Mariel Gullian Klanian

Unidad Experimental Marista, Universidad Marista de Mérida, Periférico Norte Tablaje Catastral #13941, Carretera Mérida-Progreso, Mérida, Yucatán 97300, Mexico

Abstract

To identify ways to improve water quality and shrimp production in closed systems, two parallel experiments (one in tanks and one in ponds) were conducted using Pacific white shrimp, *Litopenaeus vannamei*, cultures. In both experiments, the effects of inorganic (Nutrilake®) and organic (molasses) fertilization on physicochemical parameters, bacterial concentrations, and shrimp performance under zero water exchange were evaluated. Fertilization with both molasses and Nutrilake enhanced the feed conversion rate, as well as shrimp survival and production. In tanks, the shrimp survival and production rates were highest in the molasses treatment, but this effect was not observed in ponds. In ponds, fertilization with Nutrilake increased nitrogen and phosphorus concentrations more than did the controls and molasses treatments toward the end of the experiment. In tanks, fertilization with molasses reduced ammonia concentrations toward the end of the experiment, but the same effect was not observed in ponds. In ponds, fertilization reduced the proportion of *Vibrio* spp. bacteria, which most likely reduced the incidence of disease from these potentially pathogenic organisms. In both culture systems, fertilization increased the proportion of *Bacillus* spp., which most likely enhanced food availability.

Shrimp farming is a major industry in tropical and subtropical areas around the world. According to industry sources, the global production of aquaculture shrimp was estimated at 2.3 million m.t. in 2011 (FAO-Globefish 2012). However, pollution, environmental degradation,

[1] Corresponding author.

poor pond management, and other factors have increased the occurrence of diseases, such as white spot, in most shrimp-producing countries over the last two decades (Moser et al. 2012; Rowley and Pope 2012). Researchers are interested in identifying methods to improve water quality, increase shrimp production (Rosenberry 1993; Hopkins et al. 1994), and improve ecological sustainability.

During the 1980s and 1990s, studies of shrimp culture were conducted (Aquacop 1985; Wyban and Sweeney 1990; Sandifer and Hopkins 1996) with the goals of mitigating the environmental impacts of effluent discharge and reducing the risk of contaminant and pathogen introduction into the water supply (Esparza-Leal et al. 2010; Panjaitan 2010). More recently, shrimp cultures have evolved from open systems with frequent water discharge to closed systems with limited water discharge. The main problem with closed systems is rapid eutrophication, which increases concentrations of nutrients (i.e., ammonia and nitrite) and organic matter in the system during the culture (Thakur and Lin 2003).

Some authors have reported that closed, zero water-exchange culture systems have upper limits of nutrient input and shrimp biomass, beyond which shrimp growth and survival are compromised (Tacon et al. 2002; Decamp et al. 2007; Panjaitan 2010). A balance between waste production and assimilation in the pond environment is critical to the success of closed systems (Panjaitan 2010). A promising procedure for maintaining this balance in zero water-exchange shrimp cultures involves promoting the growth of heterotrophic bacteria. Heterotrophic bacteria recycle metabolites and are sensitive to increases in the total biomass in the culture system (Panjaitan 2010). Heterotrophic bacteria in an aquaculture system most likely use nitrogen from sources other than the pond water, and the addition of monosaccharides such as glucose and carbon compounds to pond water can stimulate ammonia uptake by heterotrophic bacteria in marine waters (Wheeler and Kirchman 1986; Hoch et al. 1994) and provide single-celled sources of protein for shrimp (Avnimelech et al. 1989; Avnimelech 1999).

Molasses is an economical source of carbon because it costs approximately $0.60/kg (SoloStocks 2012). It has been widely used in aquaculture as a carbon source for denitrification and aerobic waste conversion (Burford et al. 2003; Jimenez et al. 2004; Quan et al. 2005; Samocha et al. 2007). Additionally, the low nitrogen, ash, and fiber contents of molasses make it a valuable source of carbon (Vega-Baudrit et al. 2008).

Natural food production can be increased by inorganic fertilization (Boyd 1997), and more than 50% of the diet of *Litopenaeus vannamei* comes from natural food (Anderson et al. 1987; Castille and Lawrence 1988). The application of Nutrilake® or "Chilean saltpeter" (primarily composed of sodium nitrate) to aquaculture ponds has several environmental and economical benefits. This fertilizer is a nitrogen source that, unlike ammonium fertilizer, does not produce acidity through nitrification, and it can be used by bacteria to mineralize organic matter, transforming it into CO_2 and water (Boyd 1997).

The objectives of this study were as follows: (1) to determine whether providing additional carbon via molasses applications promotes the uptake of ammonia by heterotrophic bacteria and reduce environmental ammonia concentrations; (2) to determine whether adding Nutrilake stimulates natural food production; and (3) to determine which strategy (unfertilized, molasses, or Nutrilake) is most effective at enhancing shrimp production in zero-discharge tanks and ponds.

Materials and Methods

Study Site and Experimental Design

To investigate the effects of fertilization with Nutrilake and molasses, two parallel experiments using juvenile *L. vannamei* were conducted outdoors under ambient conditions, one in tanks and one in ponds. The tank experiment was carried out at the Centro de Diagnósticos Integrales de Laboratorio, Guamúchil, Salvador Alvarado, Sinaloa,

TABLE 1. *Average values of the components of molasses made from sugar cane (reported by Vega-Baudrit et al. 2008).*

Component	Composition (%)
Water	20.0
Saccharose	35.0
Glucose	7.0
Levulose	9.0
Other reducing substances	3.0
Other carbohydrates	4.0
Ash	12.0
Nitrogen compounds	4.5
Non-nitrogenous compounds	5.0
Waxes, steroids, and phospholipids	0.4

Mexico (25°27′10″N and 108°04′56″W); the pond experiment was conducted at a commercial shrimp farm in Angostura, Sinaloa, Mexico (25°20′33″N and 108°22′41″W). The fertilization treatments at both sites were (1) 0.5 g/m^3/wk of an inorganic fertilizer, Nutrilake (the fertilizer application rates are as follows: 0.073 mg N, 0.030 mg P_2O_5, 0.018 mg SiO_2, and 0.115 mg Na/L/wk; 14.5-6-0 inorganic fertilizer with 3.5% SiO_2 and 23% Na; Nutrilake, SQM Nitratos de Mexico), (2) 1.25 g/m^3/wk of locally purchased molasses made from sugar cane (Table 1; Vega-Baudrit et al. 2008), and (3) control (unfertilized). The amount of molasses added was based on our prior experience and the reports of Avnimelech (1999) and Ebeling et al. (2006). For each treatment, three tank replicates and two pond replicates were randomly assigned. The experimental period lasted 75 d from May to July 2010.

Tank Study

Each treatment (Nutrilake, molasses, and control) was applied to three ($2 \times 1 \times 1$ m) rectangular plastic tanks with bottom areas of 2 m^2. All of the tanks contained a uniform sediment layer (7-cm deep) obtained from the original ponds where the farm experiment was conducted. Juvenile shrimp (3.3 ± 0.5 g) from the on-farm ponds were transported to the outdoor laboratory. After 1 wk, uniformly sized (4.0 ± 0.3 g) juveniles without evidence of disease or parasites were stocked at a density of 20 juveniles/m^2 in each experimental culture tank. To ensure that the shrimp were white spot syndrome virus (WSSV) negative, three pooled hemolymph samples (10 shrimp per pool, 50 μL of hemolymph per shrimp) were taken from the shrimp batch (Esparza-Leal et al. 2009), and WSSV-negative status was confirmed via nested polymerase chain reaction (PCR) prior to the start of the experiment. In addition, shrimp were examined to ensure that they were free of signs of disease such as anorexia, lethargy, and reddish discoloration of the body before the experiment was started.

For the fertilization treatments, Nutrilake and molasses were weighed and dissolved in marine water. Each solution was spread over the tank water after the first feeding and repeated once per week until the end of the study period (total = 11 times). The control treatment was conducted under the same conditions as the fertilization treatments.

Water (salinity = 32 g/L) was obtained from the ponds where the on-farm trials were conducted, and it was filtered (1.5-mm polyethylene mesh) and aerated for a week before the experiment.

Shrimp were reared under a light regime of approximately 14 h light : 10 h dark under ambient temperatures. Each tank was aerated by two airstones suspended midway in the water column. Pressurized air was supplied by a regenerative air blower (1/2 hp, Sweetwater®, Aquatic Ecosystem Inc., Apopka, FL, USA). Water was maintained at a depth of 0.85 m with zero water exchange throughout the experimental period, except for water added to maintain the water level. The shrimp were fed twice daily at 0800 and 1600 h with commercial shrimp pellets containing 35% crude protein (Purina®, Obregón, Mexico). The feed ratio was gradually adjusted (16–3%) based on demand and was monitored with feed trays and the body weight of the shrimp (Cuadros and Beltrame 1998).

To record shrimp growth, 50% of the population was sampled weekly. Samples were mass weighed, counted, and returned to the tank. Shrimp were harvested after 75 d. The survival rate was calculated as the percentage of live shrimp remaining in each tank from

the original stocked number. The production for each tank was calculated by adding the weight of all organisms harvested. The specific growth rate (SGR, % body weight/d) was calculated using the equation $SGR = 100\ [(\ln W_f - \ln W_i)]/t$, where W_f is the mean weight at the end of the period, W_i is the mean weight at the beginning of the period, and t is the time in days of the period (Ricker 1979). The feed conversion rate (FCR) was calculated by dividing the feed supplied (dry weight) by the live weight gain (wet weight) of the shrimp (Hari et al. 2004).

Pond Study

Six earthen ponds of 1 ha each (two replicates per treatment) were selected for the on-farm experiment. Ponds for the fertilization (Nutrilake and molasses) and control treatments were subject to the usual prestocking procedures of a semi-intensive shrimp farm (Martínez-Córdova 1999). Marine water for the experimental ponds was pumped from the Caimán estuary (25°17′59″N and 108°22′41″W) in the Gulf of California. The water was filtered through a 1.5-mm polyethylene mesh to remove unwanted organisms. Water was maintained at a depth of 1 m with zero water exchange throughout the experimental period, except for water added to maintain the water level. Postlarvae PL-16 (16-d-old postlarvae; 0.014 ± 0.001 mg) *L. vannamei* were purchased from a commercial hatchery and stocked in each experimental pond at 10 PL/m^2. The pond experiment began after 36 d, when each shrimp weighed approximately 4.0 ± 0.4 g and the pond density was approximately 9 orgs/m^2. Prior to starting the experiment, sampling was conducted to estimate the population density of each pond (Anónimo 1998), and the shrimp were confirmed to be WSSV-negative via nested PCR using samples taken from each pond (Esparza-Leal et al. 2009).

Nutrilake and molasses were separately dissolved in pond water and applied uniformly over the pond surfaces before the first feeding and then once per week until the end of the study (total = 11 wk).

The control treatments were conducted under the same pond conditions as the fertilization treatments. The daily feed ratio was gradually adjusted (16–3%) based on demand and was monitored with feed trays and the body weight of the shrimp until the end of the culture period (Cuadros and Beltrame 1998). Feed was distributed evenly over the pond surface twice daily at 0800 and 1600 h. Each week, 200 shrimp per pond were removed, weighed, and returned, and the average weight was used to estimate biomass. Shrimp were harvested on the 75th day of culture. The total shrimp production per pond was estimated, and subsamples were weighed and counted to calculate the average weight and shrimp survival rate. SGR and FCR were calculated using the same method as that used in the tank experiment.

Physicochemical Parameters and Bacterial Analyses

Water samples for analyses of physicochemical parameters and bacterial concentrations were collected prior to treatment. During both experiments, pH (monitored with a Hanna 213 pH meter, Hanna Instruments, Woonsocket, RI, USA), temperature (°C), and dissolved oxygen (DO; mg/L) (both monitored using a YSI 55 digital oxygen meter with an integrated thermometer, Yellow Springs Instruments, Yellow Springs, OH, USA) were measured in each tank/pond twice a day (tank experiment: 0800 and 1600 h and pond experiment: 0600 and 1600 h). Water salinity was monitored weekly with an Atago refractometer (Novatech International, Houston, TX, USA). Nitrite (mg NO_2/L), nitrate (mg NO_3/L), ammonia (mg NH_4/L), and phosphate (mg PO_4/L) were analyzed twice monthly using the method described by Strikland and Parsons (1972).

Water samples for bacterial analyses were collected twice monthly from each tank/pond following standard procedures (APHA 1995; Gómez-Gil 2006). Bacteria were spread-plated on nutrient agar to obtain bacterial counts: tryptone soy agar (TSA agar, Difco, Lawrence, KS, USA) was used for the total viable bacteria count, thiosulfate citrate bile salt agar (TCBS

agar, Difco) was used for the presumptive *Vibrio* spp. count, and mannitol-egg yolk-polymyxin agar (MYP Agar, Difco) was used for the enumeration of presumptive *Bacillus* spp. The plates were supplemented with 2.5% NaCl and incubated for 24 h at 30 C before counting (CFU/mL).

Statistical Analyses

The effect of the treatments on physicochemical parameters and the bacterial counts in each experiment were evaluated by two-way repeated measures analysis of variance (ANOVA) with treatment (separate tanks to ponds) as a main factor and sampling date as a repeated measures factor (Gomez and Gomez 1984). The effects of treatment on shrimp growth, survival, production, and FCR were evaluated using one-way ANOVA. Where significant main factor effects were identified, differences among treatments were tested with Tukey's multicomparison test of means. The results were evaluated at the 5% significance level. The analyses were conducted using Statistica package v6 (StatSoft, Tulsa, OK, USA).

Results

Physicochemical Parameters

In both the tank and pond experiments, nitrite concentration differed significantly among treatments (both $P < 0.05$; Fig. 1). No significant difference was observed between the molasses and control groups in either experiment (tanks, $P > 0.05$ and ponds, $P > 0.05$); however, nitrite concentrations were significantly lower in these groups than in the Nutrilake group (tanks, $P < 0.05$ and ponds, $P < 0.05$). The nitrite concentration did not exceed 2 mg/L in any group. In both the tank and pond experiments, the nitrite concentration was highest in the Nutrilake treatment (1.70 and 0.08 mg NO_2/L, respectively) and lowest in the control treatment (0.50 and 0.03 mg NO_2/L, respectively).

In the tanks, no significant differences in nitrate concentration were detected among the treatments ($P > 0.05$; Fig. 1); however, significant differences were observed in the ponds ($P < 0.05$; Fig. 1). Significantly higher nitrate concentrations (ca. 7 mg NO_3/L) were observed in the Nutrilake group ($P < 0.05$) compared with the molasses and control groups, which did not differ significantly ($P > 0.05$).

The ammonia concentration differed significantly among treatments in both the tank and pond experiments (both $P < 0.05$; Fig. 1). In the tanks, the Nutrilake and control groups did not differ significantly ($P > 0.05$), but both yielded significantly higher concentrations ($P < 0.05$) than molasses. In the ponds, the molasses and control groups showed no significant differences ($P > 0.05$) in ammonia concentration (0.3 mg NH_4/L), and both exhibited significantly lower ammonia concentrations than the Nutrilake group (ca. 2 mg NH_4/L, $P < 0.05$).

The phosphate concentration (<0.6 mg PO_4/L) did not differ significantly among treatments in either experiment (tanks, $P > 0.05$ and ponds, $P > 0.05$; Fig. 1), nor were any significant differences (all $P > 0.05$) in temperature (values ranged from 27.4 to 33.6 C), pH (7.9–8.2), DO (3.5–6.1 mg/L), or salinity (32–37 g/L) detected among treatments.

Bacterial Analyses

Unlike in the pond experiment ($P < 0.05$), the total bacteria count differed significantly among treatments ($P < 0.05$; Fig. 2) in the tank experiment. The fertilized treatments did not differ ($P > 0.05$); however, both yielded significantly higher counts than the control group ($P < 0.05$).

In both experiments, total *Vibrio* spp. count varied by treatment ($P < 0.05$; Fig. 2), but in different ways. In the ponds, the control group counts (ca. 30,500 CFU/mL) were significantly higher ($P < 0.05$) than the fertilized group counts, whereas in the tanks, the control tank concentrations were significantly lower ($P < 0.05$; <5000 CFU/mL) than the fertilized tank concentrations.

The total *Bacillus* spp. counts differed significantly among treatments in both the tank and pond experiments (both $P < 0.05$; Fig. 2). In the tanks, differences were observed between all treatments (all $P < 0.05$), with the highest counts in the molasses treatment and the

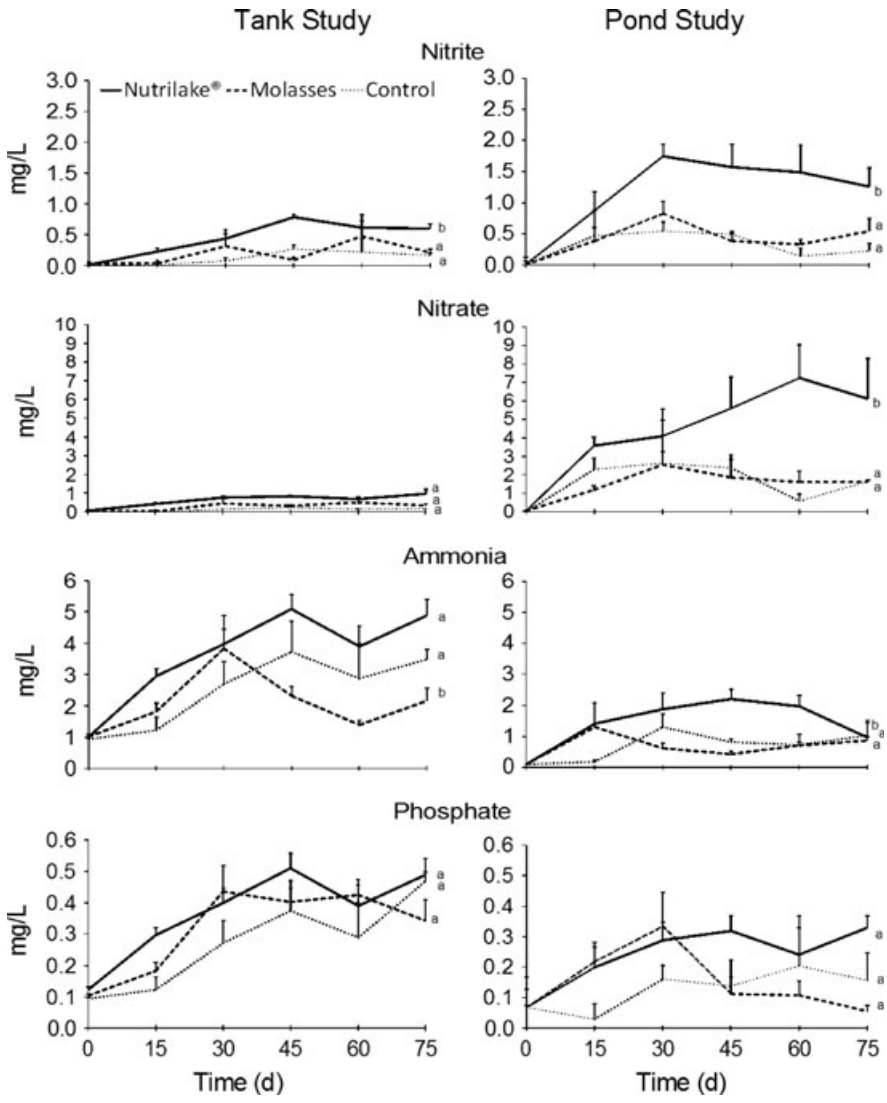

FIGURE 1. *Temporal variations in the nitrite, nitrate, ammonia, and phosphate concentrations in shrimp culture water in the tank and pond experiments across treatments: inorganic fertilization (Nutrilake), organic fertilization (molasses), and unfertilized (control) over 75 d. Each point represents the mean (±SE) of three replicates (one sample per replicate) in the tank experiment and two replicates (two samples per replicate) in the pond experiment. Letters that differ between lines indicate significant differences (*$P < 0.05$*).*

lowest counts in the control group. In the ponds, the counts did not differ significantly between the fertilized groups ($P > 0.05$), but both fertilized groups presented significantly higher ($P < 0.05$) counts than the controls. In both experiments, the lowest total *Bacillus* spp. counts (<400,000 CFU/mL, except during the last week) were recorded in the unfertilized tanks/ponds, and the highest concentrations occurred in the molasses treatment (>400,000 CFU/mL throughout the study).

Shrimp Production Parameters

In the tank experiment, the average final weight and SGR of the shrimp after 75 d in culture did not differ significantly among

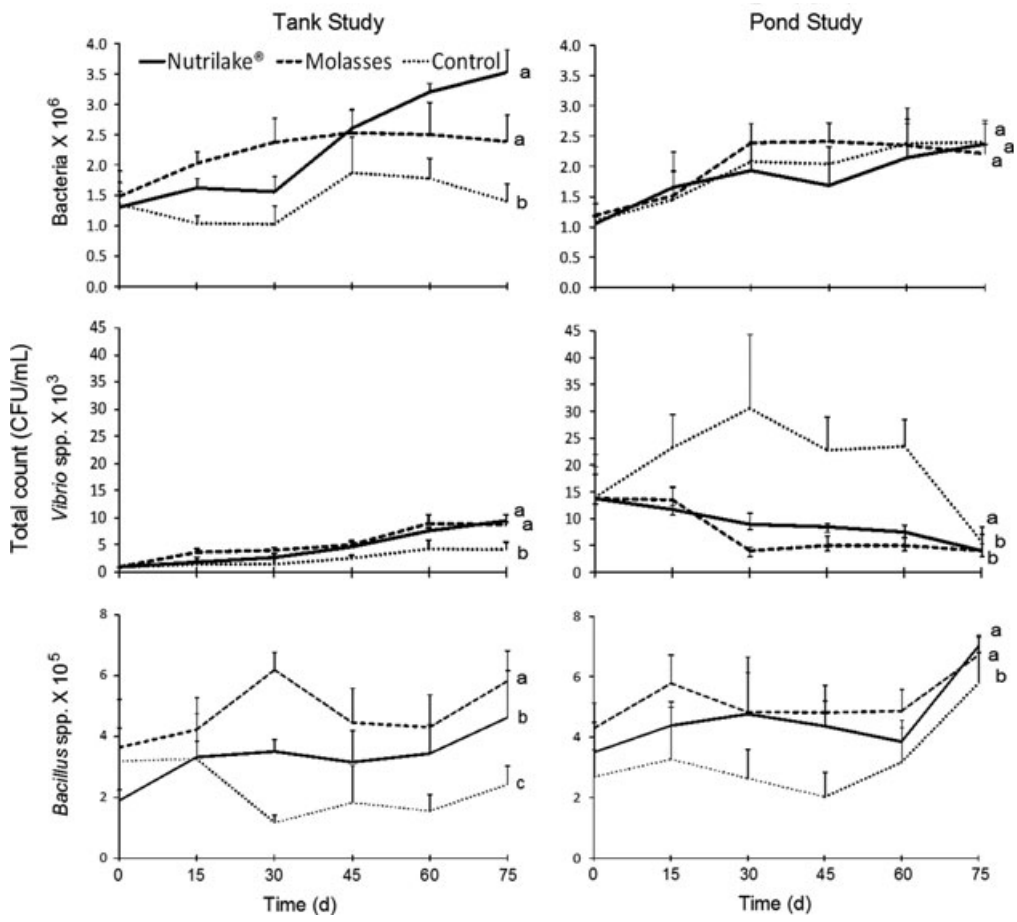

FIGURE 2. *Effect of inorganic fertilization (Nutrilake), organic fertilization (molasses), and unfertilized (control) treatments on total bacteria, presumptive* Vibrio *spp., and* Bacillus *spp. counts (CFU/mL) in shrimp culture water in the tank and pond experiments over 75 d. Each point represents the mean (±SE) of three replicates (one sample per replicate) in the tank experiment and two replicates (two samples per replicate) in the pond experiment. Letters that differ between lines indicate significant differences (*$P < 0.05$*).*

treatments (both $P > 0.05$; Table 2). In contrast, in the pond experiment, the average final weight and SGR of the shrimp differed significantly among treatments (both $P < 0.05$; Table 2). No significant differences (average final weight, $P > 0.05$ and SGR, $P > 0.05$) were observed between the Nutrilake and molasses treatments; however, both yielded significantly lower average final weights ($P < 0.05$) and SGR ($P < 0.05$) than the control group.

In both experiments, shrimp survival differed significantly among treatments (tanks, $P < 0.05$ and ponds, $P < 0.05$; Table 2). In the tanks, shrimp survival was higher ($P < 0.05$) in the molasses treatment than in the Nutrilake treatment and was higher in the Nutrilake treatment ($P < 0.05$) than in the control group. In the ponds, shrimp survival did not differ significantly between the fertilized treatments ($P > 0.05$); however, both yielded higher survival rates ($P < 0.05$) than the control group.

The total shrimp production in each experiment differed significantly (both $P < 0.05$) among treatments (Table 2). In the tanks, shrimp production was higher ($P < 0.05$) in the molasses treatment than in the Nutrilake treatment and was higher in the Nutrilake treatment

TABLE 2. *Mean (±SE) values of production parameters for shrimp (*Litopenaeus vannamei*) reared in tanks (20 orgs/m^2) and ponds (9 orgs/m^2) over a 75-d culture period*[1].

Trial	Fertilization	Initial weight (g)	Final weight(g)	SGR (%/d)	FCR	Survival (%)	Production (kg/ha)
Tanks	Nutrilake	4.1 ± 0.3^{a}	9.9 ± 0.5^{a}	0.98 ± 0.18^{a}	1.2 ± 0.1^{a}	70.2 ± 3.0^{a}	$^{2}1247 \pm 100^{a}$
	Molasses	4.0 ± 0.3^{a}	10.5 ± 0.8^{a}	1.07 ± 0.25^{a}	1.1 ± 0.1^{a}	80.0 ± 3.0^{b}	$^{2}1512 \pm 200^{b}$
	Control[3]	4.0 ± 0.4^{a}	9.3 ± 0.8^{a}	0.93 ± 0.33^{a}	1.4 ± 0.1^{b}	40.0 ± 5.0^{c}	$^{2}669 \pm 190^{c}$
Ponds	Nutrilake	4.0 ± 0.4^{a}	13.5 ± 0.9^{a}	1.35 ± 0.28^{a}	1.3 ± 0.1^{a}	85.0 ± 4.0^{a}	1147 ± 145^{a}
	Molasses	4.0 ± 0.6^{a}	14.2 ± 0.8^{a}	1.41 ± 0.39^{a}	1.1 ± 0.1^{a}	88.0 ± 3.6^{a}	1249 ± 176^{a}
	Control[3]	4.1 ± 0.3^{a}	16.5 ± 1.2^{b}	1.55 ± 0.15^{b}	1.6 ± 0.2^{b}	38.0 ± 7.6^{b}	627 ± 149^{b}

FCR = feed conversion rate; SGR = specific growth rate.

[1]Means (±SE) of three replicates (one sample per replicate) in the tank experiment and two replicates (two samples per replicate) in the pond experiment are shown. Values in the same column with different superscripts differ significantly ($P < 0.05$) within experiments.

[2]Production values were extrapolated from kg/m^2 to allow comparisons with the pond results.

[3]Control = Unfertilized.

($P < 0.05$) than in the control group. In the ponds, shrimp production did not differ significantly between the molasses and Nutrilake groups ($P > 0.05$), but shrimp production for both treatment groups was significantly higher than for the control group ($P < 0.05$).

In both experiments, FCR differed significantly (both $P < 0.05$) among treatments (Table 2). FCR did not differ between the fertilized groups (tanks, $P > 005$ and ponds, $P > 0.05$); however, both fertilized groups presented significantly lower FCRs than the control group (tanks, $P < 0.05$ and ponds, $P < 0.05$).

Discussion

Some studies have found that the addition of molasses to culture water significantly increases the total heterotrophic bacterial count (Burford et al. 2003; Schneider et al. 2006; Samocha et al. 2007; Panjaitan 2010); however, we found no significant differences in bacterial counts between the molasses and Nutrilake treatments.

Because the tanks were stocked at 20 orgs/m^2 and the ponds were stocked at 9 orgs/m^2, the feeding rates were most likely significantly different, making it difficult to directly compare the performance of the tanks and ponds. However, fertilization with molasses or Nutrilake enhanced shrimp survival, production, and FCR in both zero-water exchange systems. In the tanks, we observed higher final shrimp survival and production in the molasses treatment, which is consistent with other studies (Avnimelech 1999; Burford et al. 2003, 2004; Erler et al. 2005; Stuart et al. 2009; Panjaitan 2010), but this effect was not observed in the ponds. These findings suggest that fertilization programs may be beneficial for shrimp culture but that they likely need to be tailored to both shrimp density and the type of culture unit.

In both experiments, the lowest total bacteria counts, survival, production, and FCR were recorded in the control tanks and ponds. This finding was expected because the lack of inorganic or organic fertilization can limit microbiota proliferation (Burford et al. 2003). However, lower shrimp survival rates (<40%) in the unfertilized controls and the reduced density yielded slightly larger shrimp in the ponds, where feeding could not be adjusted for unknown mortality. According to Wang et al. (1998), the mean final size of shrimp tends to decrease with increasing density, but at a low density, the final size continues to increase until the carrying capacity of the system is reached.

In both the tank and pond experiments, the total *Vibrio* spp. counts were similar across the fertilized treatments. In the ponds, fertilization decreased the proportion of *Vibrio* spp., which most likely reduced the incidence of disease from these potentially pathogenic organisms. In the control treatments, the two experiments yielded opposite outcomes: relative to the fertilized treatments, lower concentrations of *Vibrio*

spp. were observed in the tanks, whereas higher concentrations were observed in the ponds. Although no mass mortality of shrimp occurred, a low shrimp survival rate was more evident in the control groups. This lower survival rate is most likely associated with *Vibrio* spp. concentrations because a high concentration was observed in the ponds but not in the tanks; however, no conclusions can be drawn from this study because we did not evaluate the total *Vibrio* spp. count in shrimp. However, *Vibrio* spp. have been closely associated with mortality in cultured shrimp (Song et al. 1993; Liu et al. 1996). For vibriosis to occur, an increase in pathogenic *Vibrio* spp. numbers is most likely expected but does not entail an increase in the entire *Vibrio* population (Lavilla-Pitogo et al. 1998; Sung et al. 1999). Differences among shrimp populations in their response to factors eliciting vibriosis most likely reflect differences in their capacity to resist stress, as influenced by their nutrition and physiology (Sung et al. 1999). Sung et al. (1999) reported that vibriosis in culture pond water was associated with increases in the proportion of potentially pathogenic species in the *Vibrio* population, and Roque et al. (1998) demonstrated a close relationship between the incidence of disease in shrimp and the pathogen population of *Vibrio* spp. in the surrounding water.

In the tank experiment, the total *Bacillus* spp. count differed among all three treatments, with the lowest concentrations occurring in the unfertilized tanks and ponds. Alone, these results suggest that molasses and Nutrilake provide water conditions that are more favorable to *Bacillus* species; however, in contrast to expectations (Samocha et al. 2007), molasses did not have a greater effect than Nutrilake on the *Bacillus* spp. concentrations in the ponds. Although the fertilized treatments yielded higher *Bacillus* counts than the unfertilized treatment in the latter experiment, our data do not indicate that molasses enhances *Bacillus* proliferation more than Nutrilake.

In both culture systems, fertilization enhanced the proportion of *Bacillus* spp., which most likely enhanced food availability and yielded the lowest *Vibrio* spp. concentrations. The addition of *Bacillus* spp. as a probiotic in penaeid shrimp ponds has been shown to increase shrimp survival rates and decrease luminous *Vibrio* spp. densities (Moriarty 1998). Other studies have reported that the addition of *Bacillus* spp. in the shrimp diet can reduce *Vibrio* spp. concentrations; *Bacillus* spp. most likely outcompete *Vibrio* spp. for nutrients and space and exclude other harmful bacteria (Boonthai et al. 2007; Nimrat et al. 2008).

Neither phosphate concentration, temperature, pH, and DO nor salinity varied between treatments in either of the studies, as all of the parameters remained within the tolerable level for shrimp growth (Chien 1992). However, in the ponds, fertilization with Nutrilake increased the phosphorus concentrations toward the end of the experiment above the levels of the control and molasses treatments.

Nitrogen concentrations differed among treatments in both studies. In the ponds, fertilization with Nutrilake increased nitrogen concentrations toward the end of the experiment above the level of the control and molasses treatments. As expected, the addition of Nutrilake, which is primarily composed of sodium nitrate (Boyd 1997), resulted in higher concentrations of nitrite, nitrate, and ammonia. In the tanks, molasses reduced the ammonia concentrations toward the end of the experiment, which was expected because several studies have demonstrated that molasses serves as a substrate for bacterial growth, which in turn can decrease ammonia concentrations (Avnimelech et al. 1989; Avnimelech 1999; Burford et al. 2004; Samocha et al. 2007). However, the same effect was not observed in ponds. In both zero-water exchange systems, the nitrite, nitrate, and ammonia concentrations did not exceed 2.0, 9.0, and 6.0 mg/L, respectively; the concentrations that juvenile shrimp can reportedly tolerate without affecting their growth are 8.0 (Sowers et al. 2004), 100 (Muir et al. 1991; Rijn et al. 2006), and 6.5 mg/L (Frías-Espericueta et al. 1999), respectively.

In summary, fertilization with molasses or Nutrilake enhanced shrimp FCR, survival,

and production. In the tanks, treatment with molasses caused the highest rates of survival and shrimp production and reduced ammonia concentrations in the culture system, but this effect was not observed in ponds. In the ponds, fertilization with Nutrilake increased nitrogen and phosphorus concentrations toward the end of the experiment above the level of the control and molasses treatments, and decreased the proportion of *Vibrio* spp. In both culture systems, fertilization enhanced the proportion of *Bacillus* spp. These findings suggest that fertilization programs are most likely beneficial for shrimp culture, but they need to be tailored to both shrimp density and the type of culture unit. Further research with molasses and Nutrilake fertilization should consider starting the experimental cycle with postlarval shrimp because the early juvenile stage has a faster growth rate and is more sensitive to some aspects of water quality.

Acknowledgments

Thanks to the personnel from the "Avance Acuícola" shrimp farm for their help and permission to conduct research in their facility. This study is a funded project of the Instituto Politécnico Nacional (SIP-20100145 and SIP-20110581).

Literature Cited

Anderson, **R. K.**, **P. L. Parker**, **and A. L. Lawrence**. 1987. A $^{13}C/^{14}C$ tracer study of the utilization of present feed by a commercial important shrimp *Penaeus vannamei* in a pond growout system. Journal of the World Aquaculture Society 18:149–155.

Anónimo. 1998. Muestreo poblacional en el cultivo de camarón, I parte: Uso de atarraya. Boletín Nicovita 3(3):1–2. Accessed March 15, 2010, at http://www.nicovita.com.pe/cdn/Content/CMS/Archivos/Documentos/DOC_106_1.pdf.

APHA (American Public Health Association). 1995. Standard methods for the examination of water and waste water, 19th edition. American Public Health Association, Washington, DC, USA.

Aquacop. 1985. A new approach in intensive nursery rearing of penaeid. Pages 169 *in* Y. Taki, J. H. Primavera, and J. A. Llobrera, editors. Proceeding of the First International Conference on the Culture of Penaeid Preawns/Shrimp, Iloilo City, Philippines, 4-7 December 1984. SEAFDEC, Iloilo, Philippines.

Avnimelech, **Y.** 1999. Carbon/nitrogen ratio as a control element in aquaculture systems. Aquaculture 176:227–235.

Avnimelech, **Y.**, **S. Mokady**, **and G. L. Scroeder**. 1989. Circulated ponds as efficient bioreactors for single cell protein production. Israeli Journal of Aquaculture-Bamidgeh 41:58–66.

Boonthai, **T.**, **V. Vuthiphandchai**, **and S. Nimrat**. 2007. Effect of probiotic on change of *Vibrio* and probiotic bacteria during black tiger shrimp culture. Proceeding of 45th Kasetsart University Annual Conference, 30 January–2 February, Bangkok, Thailand.

Boyd, **C. E.** 1997. Practical aspects of chemistry in pond aquaculture. The Progressive Fish-Culturist 59:85–93.

Burford, **M. A.**, **P. J. Thompson**, **P. McIntosh**, **R. H. Bauman**, **and D. C. Pearson**. 2003. Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. Aquaculture 219:393–411.

Burford, **M. A.**, **P. T. Thompson**, **R. P. McIntosh**, **R. H. Bauman**, **and D. C. Pearson**. 2004. The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero-exchange system. Aquaculture 232:525–537.

Castille, **F. L. and A. L. Lawrence**. 1988. The effect of deleting dietary constituents from pelleted feed on the growth of shrimp on the presence of natural food in ponds (Abstract). Journal of the World Aquaculture Society 20:22A.

Chien, **Y. W.** 1992. Water quality requirements and management for marine shrimp culture. Pages 144–156 *in* J. Wyban, editor. Proceedings of the special session on shrimp farming. Word Aquaculture Society, Baton Rouge, Louisiana, USA.

Cuadros, **W. and E. Beltrame**. 1998. Nuevas técnicas de alimentación con bandejas en el Brasil. Panorama Acuícola 3:8–9.

Decamp, **O.**, **L. Conquest**, **J. Cody**, **and I. Forster**. 2007. Effect of shrimp stocking density on size-fractionated phytoplankton and ecological groups of ciliated protozoa within zero-water exchange shrimp culture systems. Journal of the World Aquaculture Society 38:395–406.

Ebeling, **J. M.**, **M. B. Timmons**, **and J. J. Bisogni**. 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. Aquaculture 257:346–358.

Erler, **D.**, **P. Songsangjinda**, **T. Keawtawee**, **and K. Chaiyakum**. 2005. Preliminary investigation into the effect of carbon addition on growth, water quality and nutrient dynamics in zero-exchange shrimp (*Penaeus monodon*) culture systems. Asian Fisheries Science 18:195–204.

Esparza-Leal, **H. M.**, **C. M. Escobedo-Bonilla**, **R. Casillas-Hernández**, **P. Álvarez-Ruíz**, **G. Portillo-Clark**, **R. C. Valerio-García**, **J. Hernández-López**, **J. Méndez-Lozano**, **N. Vibanco-Pérez**, **and F. J. Magallón-Barajas**. 2009. Detection of white spot syndrome virus in filtered shrimp-farm water fractions and

experimental evaluation of its infectivity in *Penaeus* (*Litopenaeus*) *vannamei*. Aquaculture 292:16–22.

Esparza-Leal, **H. M.**, **F. J. Magallón-Barajas**, **G. Portillo-Clark**, **R. Perez-Enriquez**, **P. Álvarez-Ruíz**, **C. M. Escobedo-Bonilla**, **J. Méndez-Lozano**, **N. Mañón-Ríos**, **R. C. Valerio-García**, **J. Hernández-López**, **N. Vibanco-Pérez**, **and R. Casillas-Hernández**. 2010. Infection of WSSV-negative shrimp, *Litopenaeus vannamei*, cultivated under fluctuating temperature conditions. Journal of the World Aquaculture Society 4:912–922.

FAO-Globefish. 2012. Shrimp, Asia-March 2012. Accessed March 21, 2012, at http://www.globefish.org/shrimp-asia-march-2012.html.

Frías-Espericueta, **M. G.**, **M. Harfush-Meléndez**, **J. I. Osuna-López**, **and F. Páez-Osuna**. 1999. Acute toxicity of ammonia to juvenile shrimp *Penaeus vannamei* Boone. Bulletin of Environmental Contamination and Toxicology 62:646–652.

Gomez, **K. A. and A. A. Gomez**. 1984. Statistical procedures for agricultural research, 2nd edition. John Wiley and Sons, New York, New York, USA.

Gómez-Gil, **B.** 2006. Manual de bacteriología. CIAD-Mazatlán, Sinaloa, México.

Hari, **B.**, **B. M. Kurup**, **J. T. Varghese**, **J. W. Schrama**, **and M. C. J. Verdegem**. 2004. Effects of carbohydrate addition on production in extensive shrimp culture systems. Aquaculture 241:179–194.

Hoch, **M. P.**, **M. L. Fogel**, **and D. L. Kirchman**. 1994. Isotope fractionation during ammonium uptake by marine microbial assemblages. Geomicrobiology Journal 12:113–127.

Hopkins, **J. S.**, **P. A. Sandifer**, **and C. L. Browdy**. 1994. Sludge management in intensive pond culture of shrimp: effect of management regime on water quality, sludge characteristics, nitrogen extinction, and shrimp production. Aquacultural Engineering 13:11–30.

Jimenez, **A. M.**, **R. Borja**, **and A. Martin**. 2004. A comparative kinetic evaluation of the anaerobic digestion of untreated molasses and molasses previously fermented with *Penicillium decumbens* in batch reactors. Biochemical Engineering Journal 18:121–132.

Lavilla-Pitogo, **C. R.**, **E. M. Leaño**, **and M. G. Paner**. 1998. Mortalities of pond-cultured juvenile shrimp, *Penaeus monodon*, associated with dominance of luminescent *Vibrios* in the rearing environment. Aquaculture 164:337–349.

Liu, **P. C.**, **K. K. Lee**, **K. C. Yii**, **G. H. Kou**, **and S. N. Chen**. 1996. Isolation of *Vibrio harveyi* from diseased kuruma prawns *Penaeus japonicus*. Current Microbiology 33:129–132.

Martínez-Córdova, **L.** 1999. Cultivo de camarones peneidos. AGT Editor, D.F., México.

Moriarty, **D. J. W.** 1998. Control of luminous Vibrio species in penaeid aquaculture ponds. Aquaculture 164:351–358.

Moser, **J. R.**, **D. A. Galván-Álvarez**, **F. Mendoza-Cano**, **T. Encinas-Garcia**, **D. E. Coronado-Molina**, **G. Portillo-Clark**, **M. Risoleta-Marques**, **F. J. Magallón-Barajas**, **and J. Hernández- López**. 2012. Water temperature influences viral load and detection of white spot syndrome virus (WSSV) in *Litopenaeus vannamei* and wild crustaceans. Aquaculture 326–329:9–14.

Muir, **P. R.**, **D. C. Sutton**, **and L. Owens**. 1991. Nitrate toxicity to *Penaeus monodon* protozoea. Marine Biology 108:67–71.

Nimrat, **S.**, **S. Suksawat**, **P. Maleeweach**, **and V. Vuthiphandchai**. 2008. Effect of different shrimp pond bottom soil treatment on the change of physical characteristics and pathogenic bacteria in pond bottom soil. Aquaculture 285:123–129.

Panjaitan, **P.** 2010. Shrimp culture of *Penaeus monodon* with zero water exchange model (ZWEM) using molasses. Journal of Coastal Development 14:35–44.

Quan, **Z. X.**, **Y. S. Jin**, **C. R. Yin**, **J. J. Lee**, **and S. T. Lee**. 2005. Hidrolized molasses as an external carbon source in biological nitrogen removal. Bioresource Technology 96:1690–1695.

Ricker, **W. E.** 1979. Growth rates and models. Pages 677–743 *in* W. S. Hoar, D. J. Randall, and J. R. Brett, editors. Fish physiology, Volume VIII, Bioenergetics and growth. Academic Press, New York, New York, USA.

Rijn, **J. V.**, **Y. Tal**, **and H. J. Schreirer**. 2006. Denitrification in recirculating systems: theory and applications. Aquacultural Engineering 34:364–376.

Roque, **A.**, **J. F. Turnbull**, **G. Escalante**, **B. Gomez-Gil**, **and M. V. Alday-Sanz**. 1998. Development of a bath challenge for the marine shrimp *Penaeus vannamei* Boone, 1931. Aquaculture 169:283–290.

Rosenberry, **R.** 1993. Production drops 16 % in 1993. In World Shrimp Farming 1993. Aquaculture Digest, December 1993.

Rowley, **A. F. and E. C. Pope**. 2012. Vaccines and crustacean aquaculture – a mechanistic exploration. Aquaculture 334-337:1–11.

Samocha, **T. M.**, **S. Patnaik**, **M. Speed**, **A. M. Ali**, **J. M. Burger**, **R. V. Almeida**, **Z. Ayub**, **M. Harisanto**, **A. Horowitz**, **and D. L. Brock**. 2007. Use of molasses as carbon source in limited discharge nursery and grow-out systems for *Litopenaeus vannamei*. Aquacultural Engineering 36:184–191.

Sandifer, **P. A. and J. S. Hopkins**. 1996. Conceptual design of a sustainable pond-based shrimp culture system. Aquacultural Engineering 15:41–52.

Schneider, **O.**, **V. Sereti**, **M. A. M. Machiels**, **E. H. Eding**, **and J. A. J. Verreth**. 2006. The potential of producing heterotrophic bacteria biomass on aquaculture waste. Water Research 40:2684–2694.

SoloStocks. 2012. Melaza líquida. Accessed March 22, 2012, at http://www.solostocks.com.mx/venta-productos/agricultura-ganaderia/crianza-animales/melaza-liquida-128198.

Song, **Y. L.**, **W. Cheng**, **and C. H. Wang**. 1993. Isolation and characterization of *Vibrio damsela* infections for

cultured shrimp in Taiwan. Journal of Invertebrate Pathology 61:24–31.

Sowers, **A.**, **S. P. Young**, **P. Shawn**, **J. Isely**, **J. Jeffery**, **C. L. Browdy**, **and J. R. Tomasso**. 2004. Nitrite toxicity to *L. vannamei* in water containing low concentrations of sea salt or mixed salts. Journal of the World Aquaculture Society 35:445–451.

Strikland, **J. D. H. and T. H. Parsons**. 1972. A practical handbook of seawater analysis. Fish Research Board of Canada Bulletin, Ottawa, Canada.

Stuart, **J.**, **E. Frank**, **C. Coman**, **J. Jackson**, **and A. G. Sarah**. 2009. High-intensity, zero water-exchange production of juvenile tiger shrimp, *Penaeus monodon*; an evaluation of artificial substrates and stocking density. Aquaculture 293:42–48.

Sung, **H. H.**, **H. C. Li**, **F. M. Tsai**, **Y. Y. Ting**, **and W. L. Chao**. 1999. Changes in the composition of *Vibrio* communities in pond water during tiger shrimp (*Penaeus monodon*) cultivation and in the hepatopancreas of healthy and diseased shrimp. Journal of Experimental Marine Biology and Ecology 236:261–271.

Tacon, **A. G. J.**, **J. J. Cody**, **L. D. Conquest**, **S. Divakaran**, **I. P. Forster**, **and O. E. Decamp**. 2002. Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (boone) fed different diets. Aquaculture Nutrition 8:121–137.

Thakur, **D. P. and C. K. Lin**. 2003. Water quality and nutrient budget in closed shrimp (*Penaeus monodon*) culture systems. Aquacultural Engineering 27:159–176.

Vega-Baudrit, **J.**, **K. Delgado-Montero**, **M. Sibaja-Ballestero**, **P. Alvarado-Aguilar**, **and S. Barrientos-Ramírez**. 2008. Empleo de melaza de caña de azúcar para la obtención y caracterización de poliuretanos potencialmente biodegradables. Revista Iberoamericana de Polímeros 9:408–421.

Wang, **J. Q.**, **D. Dong**, **S. Wang**, **and X. Tian**. 1998. Experimental studies on polyculture in closed shrimp ponds. I. Intensive polyculture of Chinese shrimp (*Penaeus chinensis*) with tilapia hybrids. Aquaculture 163:11–27.

Wheeler, **P. A. and D. L. Kirchman**. 1986. Utilization of inorganic and organic nitrogen by bacteria in marine systems. Limnology and Oceanography 31:998–1009.

Wyban, **J. A. and J. N. Sweeney**. 1990. A system approach to developing intensive shrimp growout technology. Pages 91–94 in R. Hirano and I. Hanyu, editors. Proceeding of the Second Asian Fisheries Forum, Tokyo, Japan, 17-22 April 1989. Asian Fisheries Society, Manila, Philippines.