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# Evaluation of Stocking Density and Light Level on the Growth and Survival of the Pacific White Shrimp, *Litopenaeus vannamei*, Reared in Zero-Exchange Systems

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## Abstract

**The objective of this study was to evaluate the effects and interactions of stocking density and light level on the growth and survival of *Litopenaeus vannamei* in zero-exchange mixed biofloc systems. The necessity of light and its effects on stocking density could provide essential information for efficient system design in temperate regions. Twelve, 3800-L conical-bottom tanks housed in a greenhouse were filled with dechlorinated city water, adjusted to 25 ppt salinity, inoculated with mixed biofloc communities, and randomly assigned to one of four different treatment combinations. The study was a 2 × 2 factorial arrangement with main effects being stocking density (182 shrimp/m$^2$ [low density; LD] vs. 364 shrimp/m$^2$ [high density; HD]) and light (natural light [NL] vs. low level artificial [LL]) with three replicate tanks per treatment. Tanks in the NL treatment received ambient greenhouse light. For the LL treatment black plastic was used to block NL and a single 60-W incandescent bulb was hung over each low-light tank and operated on a 12:12 h schedule. Juvenile *L. vannamei* (0.40 ± 0.28 g) were stocked in rotation into each tank and fed a 35% protein diet twice daily at an initial 10% of body weight, gradually decreasing to 3% of body weight prior to harvest. After 12 wk, there was a statistically significant ($P \leq 0.05$) interaction between density and light level; therefore, data were analyzed in terms of treatment combinations. Average harvest weight of shrimp was significantly higher ($P \leq 0.05$) in the LD/NL treatment (14.5 g) than in the HD/LL treatment (12.4 g), but neither was significantly different from HD/NL (13.6 g) and LD/LL (13.4 g). Survival of shrimp was significantly lower ($P \leq 0.05$) in the HD/LL treatment (61.8%) than in the LD/NL (89.8%) and LD/LL (89.0%) treatments. Survival in the HD/NL treatment (82.7%) was intermediate and not significantly different ($P > 0.05$) from the other treatments. Harvest yield was significantly greater ($P \leq 0.05$) in the HD/NL treatment (4.1 kg/m$^2$) than in the LD/NL, LD/LL, and HD/LL treatments (2.4, 2.2, and 2.8 kg/m$^2$, respectively), which were not significantly different ($P > 0.05$) from each other. These data indicate that a combination of high stocking rate with high light levels or NL may be needed to achieve maximum production; however, relatively low levels of artificial light may be suitable at low stocking densities. Further research should investigate the type and amount of light needed to achieve optimal results.**

The Pacific white shrimp, *Litopenaeus vannamei*, is currently the leading farm-raised shrimp species in the western hemisphere (Davis et al. 2004). In recent years indoor recirculating aquaculture systems (RAS) have been evaluated for the production of penaeid shrimp and offer several potential advantages compared with earthen ponds. RAS significantly reduce water requirements per unit of production, which decreases the potential introduction of shrimp pathogens through the addition of water from an outside source (Browdy et al. 2001). These systems can also reduce biological pollution to the surrounding environment (Moss et al. 2001). In addition, indoor production of shrimp provides the opportunity for producers

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to move inland and take advantage of a wider array of markets as well as reduce the competition for land use in coastal areas. Historically, penaeid shrimp have been reared in coastal regions where extensive earthen pond production requires high levels of water exchange to mitigate the accumulation of toxic metabolites and maintain optimal water quality (Hopkins et al. 1993).

Traditionally, RAS utilized "fixed film" technology whereby autotrophic bacteria, housed inside a bioreactor, convert nitrogenous waste products into less toxic forms. A new production technology, based on mixed biofloc communities, has recently been implemented in penaeid shrimp production. Such units are known as intensive zero-exchange systems and they utilize direct carbon inputs (sugar or molasses) to promote the growth of biofloc communities (Avimelech 1999; McIntosh 2001). Mixed biofloc communities remove ammonia-nitrogen from the water and assimilate it directly into bacterial biomass. Containing as much as 34% crude protein (Tacon et al. 2002), this bacterial biomass has been shown to contribute substantially to shrimp nutrition, thereby potentially reducing feed costs, lowering production costs, and improving the overall economics of the system (McIntosh 2001; Boyd 2002; Moss 2002; Burford et al. 2004). This also reduces equipment and energy costs for the production system by eliminating the need for external components such as biofiltration, disinfection through ultraviolet light or ozonation, and possibly solid filtration. Additionally, biofloc communities are more stable than algae-based photoautotrophic communities (largely relied upon for nitrogen removal in pond-based aquaculture systems) as they are not prone to diurnal shifts, nor are they limited by sunlight for energetics (Browdy et al. 2001).

Although the use of mixed biofloc communities has its advantages, the biology of these systems remains poorly understood and may actually contain a complex mixture of photoautotrophic, autotrophic, and heterotrophic bacteria. The majority of research on mixed biofloc community systems has been performed in greenhouses in tropical or subtropical areas such as Hawaii or South Carolina. Greenhouses provide an abundance of natural light (NL) and can reduce heating expenditures in subtropical regions. Water temperatures for the culture of *L. vannamei* must be maintained between 24 and 30 C to provide for optimal health and growth (Lu-Qing et al. 2007).

Recent interest in the commercial development of indoor super-intensive heterotrophic production facilities in inland regions of the USA for rearing Pacific white shrimp prompted this line of research. In temperate regions, greenhouses with their relatively poor insulation characteristics will likely be too expensive to heat during the winter months. Agricultural-type buildings, such as those used for growing poultry or swine, are highly insulated and could be less costly to heat. However, a potential drawback of these types of buildings is their inability to provide natural sunlight. With the majority of mixed biofloc intensive shrimp research having been carried out in greenhouses, phototrophic organisms may actually be significant contributors to the functionality of these systems. Because of high energy and operating costs, maximum yields will need to be generated by indoor systems while still achieving target sizes and rapid crop turnover. Identification of optimal stocking densities will be an important component of system development.

Stocking densities of Pacific white shrimp in mixed biofloc systems have been extensively evaluated with a wide range of results. In initial trials with *L. vannamei* stocked at densities of 100–600/$m^2$, researchers reported survivals as high as 91% and harvest yields ranging from 2 to 10 kg/$m^2$ (Van Wyk 1999a; McAbee et al. 2003; Otashi et al. 2007; James Ebeling, Freshwater Institute, personal communication). In recent trials, where stocking densities were increased to 700 shrimp/$m^2$ and >800 shrimp/$m^2$, harvest yields have remained high (8.9–10.3 kg/$m^2$) although survival has been substantially lower (68–70%) (Otashi et al. 2007). As these data illustrate, stocking density can have a large impact on production. Although these trials have demonstrated some success, in many cases subsequent verification trials have not produced consistent results.

Browdy and Moss (2005) suggested that shrimp stocked at 300/$m^2$ exhibit better growth rate, survival, and average weight than those stocked at other densities; however, optimal stocking rates are not known and will likely depend on specific system and production factors.

Light level and type could directly affect the animals being cultured. Crustacean molting frequency, food consumption, cannibalism, and growth performance are directly affected by photoperiod (Aiken et al. 1983; Minagawa and Murano 1993; Minagawa 1994, Constanon-Cervantes et al. 1995; Gardener and Macguire 1998; Tidwell et al. 2001). However, results from experiments evaluating the effect of light intensity on the culture of shrimp species have varied widely. Hoang et al. (2003) found that the growth rate of *Penaeus mergiuensis* was significantly higher at 750 lx light intensity than at 75 lx, while Wang et al. (2004) reported that the growth rate of *Penaeus chinensis* under high light intensity (5500 lx) was reduced compared with shrimp cultured at lower light intensities (0, 50, 300, and 1300 lx). Prior research has indicated that *L. vannamei* raised in mixed biofloc systems without NL did not progress beyond 8 g average weight and sometimes resulted in mass mortality (Craig Browdy, Waddell Mariculture Center, personal communication). This could indicate an important, although yet undefined, role for photoautotrophic bacteria or algae in these systems, or even a direct effect of light on the shrimp themselves.

The objective of this study was to evaluate the effects of stocking density, light level, and their interactions on the growth and survival of *L. vannamei* raised in zero-exchange mixed biofloc community systems by using a $2 \times 2$ factorial arrangement with two light levels (low artificial and natural sunlight) and two stocking densities (low and high).

## Materials and Methods

### *Preparation and Stocking*

To inoculate the culture system, a 40-L alloquate of mixed biofloc culture was air shipped from a commercial shrimp farm (Ocean Boy Farms, Clewiston, FL, USA) and split into two 190-L conical-bottom tanks containing 25 ppt artificial seawater (Crystal Sea® Marine mix, Marine Enterprises International, Baltimore, MD, USA) in a greenhouse at the Aquaculture Research Center, Kentucky State University, Frankfort, KY, USA. Biofloc communities were fertilized daily with sodium bicarbonate, sugar, ammonium chloride, sodium metasilicate, and Kellogg's original All-Bran cereal at a rate of 5, 15, 1, 2, and 2 g/100 L, respectively. Multiplication and inoculation of biofloc water were utilized to the point of filling twelve 3800-L conical-bottom tanks with homogenous cultures. Water temperatures were maintained at or near 30 C using a combination of high ambient greenhouse temperatures and in-tank immersion heaters (Process Technology, Mentor, OH, USA).

Approximately 100,000 *L. vannamei* postlarvae in their eighth day of development after metamorphosis (PL-8) were shipped by air from a commercial supplier (Ocean Boy Farms) and nursed for 91 d prior to stocking the study. During the nursery phase, shrimp were fed a 45% protein shrimp diet (Zeigler Brothers, Inc., Gardners, PA, USA) at 0800, 1000, 1300, and 1600 h according to a feed chart based on shrimp biomass from samples and estimated survival.

The trial was conducted in a $10 \times 30$ m double-walled plastic greenhouse. Prior to stocking, black plastic was applied to the walls and ceiling of one-half of the greenhouse in order to block outside light. A single 60-W incandescent light bulb (GE Lighting Manufacturing Company, LLC, Cleveland, OH, USA) was hung above each of the six low-light tanks to serve as an artificial light source. A light intensity of 50 lx at the water's surface was maintained on a 12:12 h light cycle in those six tanks for the duration of the study. Six additional tanks (in the other half of the greenhouse) received NL at an intensity of 718 lx midday on a natural day/night cycle.

Three tanks in the low-light section of the greenhouse were randomly assigned a high stocking density and three tanks a low stocking density. The same procedure was used in the

natural ambient light section of the greenhouse. This yielded four different treatment combinations in a 2 × 2 factorial arrangement: low density, natural (ambient) light (LD/NL) intensity; high density, natural light (HD/NL) intensity; low density, low light (LD/LL) intensity; high density, low light (HD/LL) intensity.

At stocking juveniles from the two nursery tanks were pooled into a single 1500-L tank, graded to a similar size. A sample of 100 shrimp was blotted free of surface water and individually weighed to determine average weight ($\overline{x} \pm$ SE; $0.40 \pm 0.28$ g). Juveniles were then hand-counted in rotations of 100 animals into twelve 3.57 $m^2$ (4.38 $m^3$) conical-bottomed, polyethylene tanks (Polytank, Inc., Litchfield, MN, USA) until target stocking densities were obtained. LD tanks were stocked at 182 shrimp/$m^2$ (148 shrimp/$m^3$), and HD tanks were stocked at 364 shrimp/$m^2$ (297 shrimp/$m^3$). Individual tanks were operated as completely independent systems. Water was circulated within each tank by heavy aeration to keep solids suspended and pure oxygen was provided at a rate of 0.25 L/min through an ultrafine pore ceramic plate diffuser from a liquid oxygen tank.

### *Samples*

Shrimp were sampled weekly from each experimental tank using a small-mesh dipnet. The shrimp were group-weighed (drained weight) to the nearest 0.1 g, individually counted, and returned to their respective tank.

### *Feeds and Feeding*

During the production trial, shrimp were fed twice daily at 0800 and 1600 h using extruded sinking pellets (2.4 mm; Shrimp Growers Hyper Intensive 35) (Zeigler Brothers, Inc.) containing 35% protein and 9% fat. Feeding rates were based on a feed table (Van Wyk 1999b) beginning at 10% of estimated biomass of shrimp and gradually decreased to 3% of estimated biomass. Inputs of feed were adjusted on a weekly basis according to average sample weights and estimated survival (1% mortality per week).

### *Water Quality Management*

Pure cane sugar was applied daily to each tank at the time of feeding as a source of carbon to promote the growth of mixed biofloc communities. Sugar inputs were based on a percentage of the daily feed allotments (by weight) with application rates decreasing throughout the production cycle based on increasing total suspended solids (TSS) levels: 50% Days 1–26; 25% Days 27–31; 20% Days 32–54 and 15% Days 55–88. As mixed biofloc communities increased, the amount of sugar applied to each tank was decreased because of the regenerating ability of the floc.

Sodium bicarbonate was applied daily to each tank at the time of feeding at a rate of 15% (by weight) of the daily feed allotment. Dissolved oxygen ($O_2$), temperature (C), pH, and salinity were monitored twice daily (0830 and 1630 h) using a YSI 550 dissolved oxygen meter (YSI Company, Inc., Yellow Springs, OH, USA). Total ammonia-N (TAN) (method 385) and nitrite-N ($NO_2$-N) (method 371) were monitored using a spectrophotometer (Hach Odyssey DR 2400, Hach Company, Loveland, CO, USA) at 1000 h three times a week. Because of elevated concentrations, TAN and $NO_2$-N were monitored once daily during the first 4 wk of the study. As these levels decreased, monitoring was decreased to three times per week for the duration of the trial. Alkalinity was determined twice a week by titration (Hach digital titrator, Hach Company, Loveland, CO, USA).

TSS were measured once daily using an InsiteIG® 3150 suspended solids analyzer (Insite Instrumentation Group, Inc., Slidell, LA, USA). Levels of TSS were maintained within target ranges during the study using a bag filter system.

Target levels were ≤150 mg/L during Weeks 1–5, 200–250 mg/L during Weeks 6–9, and 250–300 mg/L during Weeks 10–12. The bag filter was operated by an airlift and used 300–1000 μm mesh bags based on floc characteristics. Bags were hung to dry overnight, and then drained weights of harvested floc were recorded.

### *Shrimp Performance*

After 12 wk, all tanks were drained to 0.5 m depth, and shrimp were removed by dipnet, bulk weighed, and counted. To evaluate possible nutritional differences in grazed biofloc from dark and light treatments, the hepatopancreas from each of these shrimp was removed and immediately frozen with liquid nitrogen in glass vials and submitted to a commercial analytical laboratory for fatty acid analysis (Tidwell et al. 1998) (Eurofins Scientific Inc., Des Moines, IA, USA).

In order to identify specific organisms contributing to floc composition, two homogenized samples containing water from each replicate tank were taken from both the high-light and low-light treatments and stored in 250-mL Nalgene bottles. One sample from each treatment was preserved in a 0.25% glutaraldehyde solution for algae analysis. The second sample from each treatment was preserved in a 30% ethanol solution for zooplankton analysis. Upon collection and preservation, all samples were immediately sent to a consulting firm (PycoTech, Inc., St. Joseph, MO, USA) for identification.

Data on shrimp growth, survival, feed conversion ratio (FCR), harvested biomass, and water quality were analyzed as a $2 \times 2$ factorial (main effects of density and light level) and tested for significant interactions ($P \leq 0.05$) between main effects using Statistix Version 7 (Statistix Analytical Software 2000, Tallahassee, FL, USA). Significant statistical interactions ($P > 0.05$) between main effects of stocking density and light were identified for a number of variables indicating that light and stocking density could not be analyzed independently. Therefore, data were analyzed in terms of the four treatment combinations and evaluated for significant differences ($P \leq 0.05$) using analysis of variance (Steel and Torrie 1980). FCR was calculated as weight of feed fed (g)/live weight gain (g). If significant differences were indicated, treatment combination means were separated using Fisher's least significant difference test. All percentage and ratio data were transformed to arc sin values prior to analysis. Data are represented in the untransformed form to facilitate interpretation.

## Results

### *Water Quality*

There were no significant differences ($P > 0.05$) in measured water quality parameters in terms of water temperature, morning and afternoon dissolved oxygen, TSS, and total alkalinity, among treatments. Overall means ($\pm$SE) were water temperature, $29.8 \pm 0.0$ C; morning dissolved oxygen, $6.4 \pm 0.1$ mg/L; afternoon dissolved oxygen, $6.5 \pm 0.1$ mg/L; total alkalinity, $167.6 \pm 1.7$ mg/L; and TSS, $181.6 \pm 18.7$ mg/L (Table 1).

Water quality parameters including TAN, $NO_2$-N, and pH were significantly impacted by the different treatment combinations ($P \leq 0.05$). Overall concentrations of TAN were significantly higher ($P \leq 0.05$) in the HD/NL ($0.10 \pm 0.0$ mg/L) and HD/LL ($0.11 \pm 0.0$ mg/L) than in the LD/NL ($0.07 \pm 0.0$ mg/L) and LD/LL ($0.05 \pm 0.0$ mg/L) treatments. Concentrations of $NO_2$-N were significantly higher ($P \leq 0.05$) in the LD/NL ($5.8 \pm 0.3$ mg/L) and HD/NL ($5.8 \pm 0.3$ mg/L) treatments than in the LD/LL ($4.9 \pm 0.0$ mg/L) and HD/LL ($5.1 \pm 0.0$ mg/L) treatments. Significantly lower ($P > 0.05$) pH levels were observed in the HD/NL ($7.7 \pm 0.1$) and HD/LL ($7.7 \pm 0.0$) treatments than in the LD/NL and LD/LL treatments ($7.8 \pm 0.1$ and $7.9 \pm 0.1$, respectively). Total harvested biofloc was significantly higher ($P \leq 0.05$) in HD/NL and HD/LL treatments ($34.1 \pm 4.2$ and $28.6 \pm 7.5$ kg per tank, respectively) than in the LD/NL and LD/LL treatments ($9.4 \pm 1.3$ and $10.1 \pm 2.3$ kg per tank, respectively) (Table 1).

There was a significant difference ($P \leq 0.05$) in total harvested biofloc in relation to density. A total of $7.8 \pm 1.0$ and $6.6 \pm 1.7$ kg of biofloc/$m^3$ was harvested from the HD/NL and HD/LL treatments, respectively. Only $2.3 \pm 0.9$ and $2.1 \pm 0.5$ kg of floc/$m^3$ was harvested from the LD/LL and LD/NL, respectively.

### *Shrimp Performance*

After 12 wk, significant differences ($P \leq 0.05$) were observed in average weight, growth rate, survival, harvest yield, and FCR of shrimp among the four treatment combinations (Table 2). Average weight of shrimp was

TABLE 1. *Treatment combination means*[1] *of water quality variables measured after 12 wk of growth of* Litopenaeus vannamei *stocked at either LD (182/m*$^2$*) or HD (364/m*$^2$*) and reared under either NL or LL (50 lx) intensity.*

| | Treatment | | | |
|---|---|---|---|---|
| Variable | LD/NL | HD/NL | LD/LL | HD/LL |
| Temperature (C) | 29.8 ± 0.2$^a$ | 29.8 ± 0.1$^a$ | 29.7 ± 0.2$^a$ | 29.8 ± 0.0$^a$ |
| $O_2$ AM (mg/L) | 6.3 ± 0.2$^a$ | 6.3 ± 0.1$^a$ | 6.6 ± 0.1$^a$ | 6.3 ± 0.2$^a$ |
| $O_2$ PM (mg/L) | 6.4 ± 0.2$^a$ | 6.3 ± 0.2$^a$ | 6.7 ± 0.2$^a$ | 6.5 ± 0.2$^a$ |
| Total suspended solids (mg/L) | 164.2 ± 6.3$^a$ | 194.1 ± 11.4$^a$ | 167.1 ± 2.6$^a$ | 201.0 ± 38.8$^a$ |
| Harvested biofloc (kg) | 9.4 ± 1.3$^b$ | 34.1 ± 4.2$^a$ | 10.1 ± 2.3$^b$ | 28.6 ± 7.5$^a$ |
| Alkalinity (mg/L) | 169.3 ± 8.9$^a$ | 166.1 ± 3.9$^a$ | 169.2 ± 4.9$^a$ | 165.8 ± 2.9$^a$ |
| Total ammonia-N (mg/L) | 0.07 ± 0.0$^b$ | 0.10 ± 0.0$^a$ | 0.05 ± 0.0$^b$ | 0.11 ± 0.0$^a$ |
| Nitrite (mg/L) | 5.8 ± 0.3$^a$ | 5.8 ± 0.3$^a$ | 4.9 ± 0.0$^b$ | 5.1 ± 0.0$^b$ |
| pH | 7.8 ± 0.1$^a$ | 7.7 ± 0.1$^b$ | 7.9 ± 0.1$^a$ | 7.7 ± 0.0$^b$ |

LD = low density; HD = high density; NL = natural light; LL = low light.

[1]Means (±SE) of three replicate tanks; means within a row followed by different superscript letters were significantly different ($P \leq 0.05$).

significantly higher ($P \leq 0.05$) in the LD/NL (14.5 ± 0.5 g) compared with the HD/LL (12.2 ± 0.4 g) treatment. However, neither the LD/NL nor HD/LL was significantly different ($P > 0.05$) from the average weight of shrimp in the HD/NL (13.6 ± 0.4 g) and LD/LL (13.4 ± 0.5 g) treatments.

Average growth rate of shrimp (Table 2) was significantly lower ($P \leq 0.05$) in the HD/LL (1.0 ± 0.4 g/wk) than in the LD/NL (1.2 ± 0.1 g/wk) but not significantly different ($P > 0.05$) than growth rates in the HD/NL and LD/LL treatments (1.1 ± 0.3 and 1.1 ± 0.2 g/wk, respectively). There were no significant differences ($P > 0.05$) in weekly sample weights during the first 2 wk of the study (Fig. 1). By Week 3, the LD/NL treatment had a significantly higher ($P \leq 0.05$) average sample weight than the HD/LL treatment and was mathematically higher than other treatments from Week 3 through harvest (Week 12).

Survival of shrimp was significantly lower ($P \leq 0.05$) in the HD/LL treatment (61.8 ± 12.3%) than in the LD/NL (89.8 ± 1.3%) and LD/LL (89.0 ± 4.8%) treatments. Survival in the HD/NL treatment (82.7 ± 1.7%) was

TABLE 2. *Treatment combination means*[1] *of average harvest weight, growth rate, survival, harvest yield, and FCR measured after 12 wk of growth of* Litopenaeus vannamei *stocked at either LD (182/m*$^2$*) or HD (364/m*$^2$*) and reared under either NL or LL (50 lx) intensity.*

| | Treatment | | | |
|---|---|---|---|---|
| Variable | LD/NL | HD/NL | LD/LL | HD/LL |
| Average weight (g) | 14.5 ± 0.5$^a$ | 13.6 ± 0.4$^{ab}$ | 13.4 ± 0.5$^{ab}$ | 12.4 ± 0.2$^b$ |
| | (14.5 ± 0.5$^a$) | (13.6 ± 0.4$^{ab}$) | (13.4 ± 0.5$^{ab}$) | (12.2 ± 0.3$^b$) |
| Growth rate (g/wk) | 1.2 ± 0.1$^a$ | 1.1 ± 0.3$^{ab}$ | 1.1 ± 0.2$^{ab}$ | 1.0 ± 0.4$^b$ |
| | (1.2 ± 0.1$^a$) | (1.1 ± 0.3$^{ab}$) | (1.1 ± 0.2$^{ab}$) | (0.9 ± 0.4$^a$) |
| Survival | 89.8 ± 1.3$^a$ | 82.7 ± 1.7$^{ab}$ | 89.0 ± 4.8$^a$ | 61.8 ± 12.3$^b$ |
| | (89.8 ± 1.3$^a$) | (82.7 ± 1.7$^{ab}$) | (89.0 ± 4.8$^a$) | (74.1 ± 2.8$^b$) |
| Harvest yield (kg/m$^2$) | 2.4 ± 0.2$^b$ | 4.1 ± 0.2$^a$ | 2.2 ± 0.1$^b$ | 2.8 ± 1.9$^b$ |
| | (2.4 ± 0.2$^c$) | (4.1 ± 0.2$^a$) | (2.2 ± 0.1$^d$) | (3.3 ± 0.2$^b$) |
| FCR | 1.7 ± 0.0$^b$ | 1.9 ± 0.0$^{ab}$ | 1.7 ± 0.0$^b$ | 3.0 ± 0.0$^a$ |
| | (1.7 ± 0.0$^c$) | (1.9 ± 0.0$^b$) | (1.7 ± 0.0$^c$) | (2.3 ± 0.0$^a$) |

FCR = feed conversion ratio; LD = low density; HD = high density; NL = natural light; LL = low light.

Data within parenthesis represent statistical analysis based on the removal of one replicate in the HD/LL treatment because of a mortality event.

[1]Means (±SE) of three replicate tanks; means within a row followed by different superscript letters were significantly different ($P \leq 0.05$).

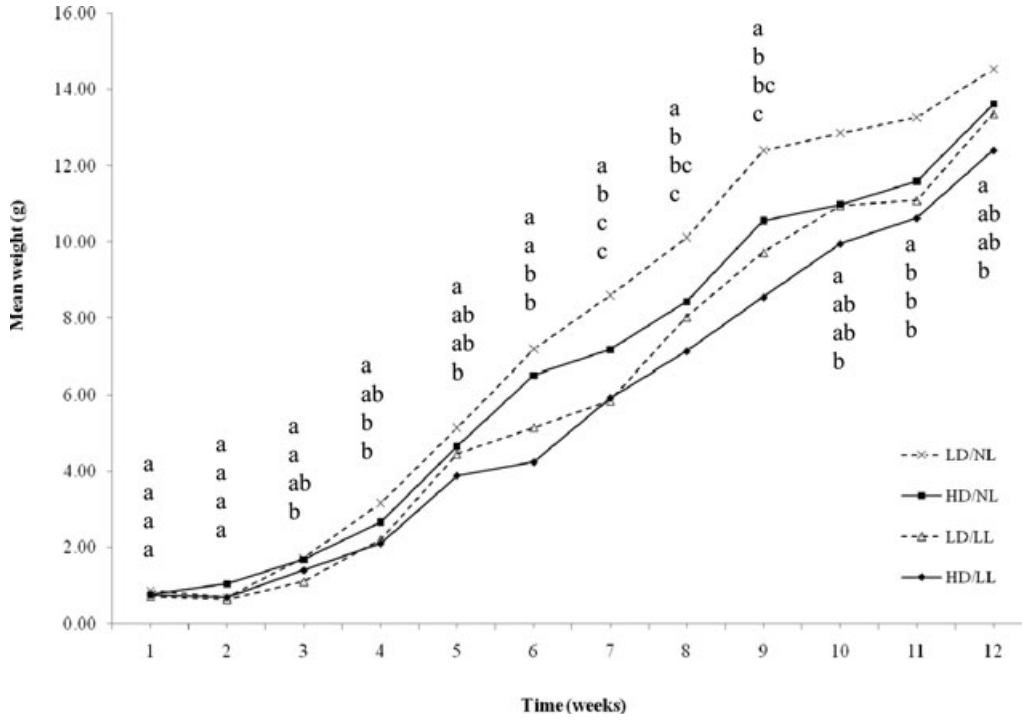


FIGURE 1. *Treatment means of weekly sample weights during the 12-wk grow-out of* Litopenaeus vannamei *stocked at either low density (LD; 182/m$^2$) or high density (HD; 364/m$^2$) and reared under either natural light (NL) or low light (LL; 50 lx) intensity. Different letters indicate significant difference (*$P \leq 0.05$*).*

intermediate and not significantly different ($P > 0.05$) from the other treatments.

Harvest yield was significantly greater ($P \leq 0.05$) in the HD/NL ($4.1 \pm 0.2$ kg/m$^2$) treatment than in the LD/NL, LD/LL, and HD/LL treatments ($2.4 \pm 0.2$, $2.2 \pm 0.1$, and $2.8 \pm 1.9$ kg/m$^2$, respectively). Harvest yield was not significantly different ($P > 0.05$) among the LD/NL, LD/LL, and HD/LL treatments.

FCR was significantly greater ($P \leq 0.05$) in the HD/LL ($3.0 \pm 0.0$) than in the LD/NL and LD/LL treatments ($1.7 \pm 0.0$ and $1.7 \pm 0.0$, respectively) but was not significantly different ($P > 0.05$) from the HD/NL treatment ($1.9 \pm 0.0$), which was intermediate and not significantly different ($P \leq 0.05$) than that of shrimp in the other treatments.

Proximate analysis of the biofloc determined the moisture level to be 87% and percent protein, crude fiber, ash, and crude fat were 3.4, 2.8, 4.3, and 0.2%, respectively (25.7, 21.5, 33.3, and 1.76% dry weight, respectively). Microorganism analysis of biofloc water samples obtained from the NL portion of the greenhouse contained a greater abundance of microalgae, zooplankton, and rotifers with average densities of 10,496/mL, 723,458/mL, and 260,582/mL, respectively (Fig. 2). Samples from the low light portion of the greenhouse contained much lower concentrations of microalgae, zooplankton, and rotifers with average densities of 4,362/mL, 292,584/mL, and 27,429/mL, respectively.

### *Fatty Acid Analysis*

The concentrations (% of total lipid) of selected fatty acids in biofloc samples and the hepatopancreases of shrimp from the same treatments are presented in Table 3. Analysis of fatty acid composition of the floc identified no significant differences ($P > 0.05$) between treatments in terms of arachidonic acid (20:4 n-6), eicosapentaenoic acid (20:5 n-3), and

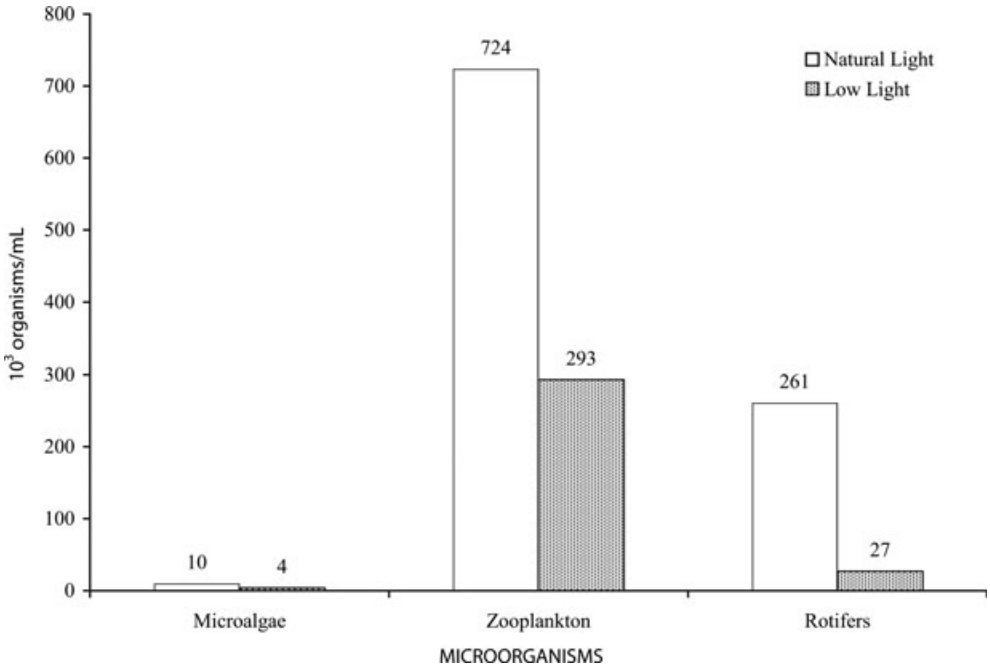


FIGURE 2. *Biofloc composition of microorganisms during the 12-wk grow-out of* Litopenaeus vannamei *stocked at either low density (182/m$^2$) or high density (364/m$^2$) and reared under either natural light or low light (50 lx) intensity.*

TABLE 3. *Treatment combination means*[1] *of concentrations for fatty acids (% of total lipid) of heterotrophic biofloc*[2] *and* Litopenaeus vannamei *hepatopancreas measured during the 12-wk grow-out of* L. vannamei *stocked at either LD (182/m$^2$) or HD (364/m$^2$) and reared under either NL or LL (50 lx) intensity.*

| | | Treatment | | | |
|---|---|---|---|---|---|
| Variable | Fatty acid | LD/NL | HD/NL | LD/LL | HD/LL |
| Biofloc | ARA (20:4 n-6) | 0.8 ± 0.7[a] | 0.6 ± 0[a] | 0.7 ± 0.2[a] | 1.3 ± 0.9[a] |
| Biofloc | EPA (20:5 n-3) | 2.2 ± 0.4[a] | 3.8 ± 0.6[a] | 2.4 ± 0.4[a] | 2.9 ± 1.8[a] |
| Biofloc | DHA (22:6 n-6) | 1.9 ± 1.8[a] | 3.2 ± 1.6[a] | 3.1 ± 0.8[a] | 3.0 ± 2.1[a] |
| Hepatopancreas | ARA (20:4 n-6) | 2.3 ± 0.1[a] | 1.8 ± 0.0[a] | 1.7 ± 0.0[a] | 2.3 ± 0.7[a] |
| Hepatopancreas | EPA (20:5 n-3) | 7.0 ± 1.0[a] | 5.3 ± 0.2[a] | 4.8 ± 0.1[a] | 6.7 ± 3.2[a] |
| Hepatopancreas | DHA (22:6 n-6) | 8.2 ± 0.2[a] | 7.8 ± 0.2[a] | 7.5 ± 0.5[a] | 8.2 ± 1.5[a] |

LD = low density; HD = high density; NL = natural light; LL = low light.

[1]Means (±SE) of three replicate tanks; means within a row followed by different superscript letters are significantly different ($P \leq 0.05$).

[2]Proximate analysis determined biofloc to contain 0.23% crude fat.

docosahexaenoic acid (22:6 n-3) concentrations with overall averages of 0.9 ± 0.3, 2.8 ± 0.7, and 2.8 ± 0.6%, respectively. There were also no significant differences ($P > 0.05$) between treatments in terms of heptopancreas concentrations of arachidonic acid (20:4 n-6), eicosapentaenoic acid (20:5 n-3), and docosahexaenoic acid (22:6 n-3) with overall averages of 2.0 ± 0.3, 5.9 ± 1.0, and 7.9 ± 0.3%, respectively.

## Discussion

### *Water Quality*

Concentrations of TAN, pH, dissolved oxygen, total alkalinity, and temperature were

within recommended ranges for the growth of *L. vannamei* throughout the course of the experiment (Van Wyk and Scarpa 1999; Whetstone et al. 2002). Concentrations of TAN were significantly higher, and pH was significantly lower in the two HD treatments compared with the two LD treatments. High $NO_2$-N concentrations (18–24 mg/L) were encountered in the early phase of the study (Days 1–19) yet these concentrations did not exceed levels considered toxic to penaeid shrimp (Chien 1992; Lin and Chen 2003). The presence of $NO_2$-N in the system indicates that the system was not solely driven by mixed biofloc communities because nitrite is not an intermediate product of their processes. Apparently nitrogen-oxidizing bacteria were present within each static system. After Week 2, $NO_2$-N concentrations exhibited a sharp decline and remained within the optimal range for shrimp production for the remainder of the experiment.

TSS were maintained within set parameters for all treatments until Week 11 of the study, at which time levels of one replicate in the HD/LL treatment rose sharply to 545 mg/L, followed by a mass-mortality event. A single sample of biofloc water from this replicate tank was immediately sent to Dr. Donald Lightner (Department of Veterinary & Microbiology and Wildlife & Fishery Science, University of Azrizona) for analysis. Dr. Lightner reported the presence of a colorless bluegreen algae known as *Leucothrix mucor* in wet mounts of the biofloc. This type of algae is known to attach to gill lamellae of shrimp, inhibit respiratory function, and may result in suffocation (McKee and Lightner 1982). *L. mucor* commonly inhabits marine waters and can be a severe problem in shrimp culture facilities; however, the source of this occurrence at the Aquaculture Research Center was not determined.

The higher levels of harvested biofloc in HD treatments are likely related to increased feed allocation and biomass densities in these treatments. This could be biologically and economically important as these values represent approximately 200% of the total harvest weight of the crop (shrimp) and represent a potential liability in terms of waste removal and disposal. However, it could also potentially represent a resource as a feed ingredient.

### *Shrimp Performance*

The combination of HD and NL significantly increased production over other treatments. The HD/NL treatment increased production by >48% compared with the same density with low light with no significant reduction in survival, average weight, or feed conversion. While it cannot be determined from this study whether light had a direct effect on the shrimp themselves, it is obvious that light had a direct positive impact on the availability of rotifers and zooplankton species (Fig. 2), which are known to be well-utilized natural food sources for shrimp (Thompson et al. 1999). Results do not show large differences in the availability of microalgae between the natural-light and low-light treatments. This could be because of the rapid grazing of microalgae by the greater number of zooplankton in the natural-light systems.

At harvest, average weight was higher in the LD/NL (14.5 g) than the HD/LL treatment (12.4 g), but was not significantly different than average weights observed in the HD/NL and LD/LL treatments (13.6 and 13.4 g, respectively). These data suggest that at relatively low stocking densities light levels did not appear to be a predominant factor; however, at higher stocking densities NL appears to be advantageous. At similar stocking densities, mean average weights of shrimp attained in the present study were within range of, or greater than, average weights reported in previous trials (Van Wyk 1999a; Samocha et al. 2004; Browdy and Moss 2005) yet are slightly less than those reported by Otoshi et al. (2007).

Mean growth rate of Pacific white shrimp observed in the present study was within range of initial super-intensive studies reported by Browdy (1999), Moss (1999), and Van Wyk (1999a). However, recent studies by these and other researchers have observed higher weekly growth of shrimp at stocking densities greater than those used in the present study (Browdy and Moss 2005; Otoshi et al. 2007). High nitrite

levels during the initial weeks of the trial may have negatively impacted growth.

Shrimp FCR was within optimal range in the LD/NL (1.7), HD/NL (1.9), and LD/LL (1.7) treatments. Poor FCR was observed in the HD/LL treatment (3.0) and was largely because of the sudden mortality event after 11 wk of feeding.

Survival of Pacific white shrimp in the LD/NL, HD/NL, and LD/LL treatments was within range, or better than, reported in other super-intensive shrimp studies (Van Wyk 1999a; McAbee et al. 2003; Samocha et al. 2004; Browdy and Moss 2005; Otashi et al. 2007). Low survival in the HD/LL (61.8%) is suspected to be the result of the proliferation of *L. mucor* within a single replicate tank in that treatment (survival; 34%). *L. mucor* led to the formation of larger bioflocculants which may have inhibited gill function of shrimp. *L. mucor* was observed in a majority of the tanks in the low-light section of the greenhouse but not in the NL section. Analyzed data (Table 2) include this low survival replicate but also provides results in parenthesis with that replicate removed. Previous occurrences of *L. mucor* during the production of Pacific white shrimp in zero-exchange systems were not found in the literature.

## Conclusion

Because of the relatively high operating costs associated with indoor recirculating systems, maximum production rates are an important component to economic feasibility. Optimal stocking densities in commercial operations will likely be based on intrinsic market factors and production costs associated with different technologies. Clearly, NL provides some growth advantage in mixed biofloc community systems, particularly at HD, as indicated in the present study. However, relatively low levels of artificial light may be suitable for indoor production of Pacific white shrimp when stocked at relatively low densities. Further research should investigate different light spectrums and intensities needed to achieve maximum production efficiency.

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