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Effects of supplemental LED lighting on water quality and Pacific white shrimp (*Litopenaeus vannamei*) performance in intensive recirculating systems

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ABSTRACT

Indoor shrimp production systems allow production of fresh shrimp year-round near specific markets. However, there is typically little to no natural light available, and it is unclear whether artificial lighting may benefit systems. This study examined the effects of supplemental light-emitting diode (LED) lighting, designed for plant growth, on shrimp (*Litopenaeus vannamei*) production, water quality, and nutritional dynamics in intensive indoor shrimp systems. Four 1-m^3 round tanks with settling chambers and external biofilters were assigned to one of three treatments. One treatment received 24 h of lighting per day (Full Light, FL), another had 12 h of lighting (Partial Light, PL), and the third treatment had no supplemental lighting provided (No Extra Light, NL). All tanks were stocked with 250 shrimp with an average initial weight of 1.2 g and were harvested after 84 days. Shrimp FCR was significantly lower and total biomass was significantly higher in the FL treatment compared with PL and NL treatments. Growth rate and final individual weight were significantly greater in FL and PL treatments, and survival was significantly higher in the FL treatment than the PL treatment. Turbidity, suspended solids, and nitrate concentration were significantly lower in the FL treatment versus PL and NL treatments. Shrimp in the FL and PL treatments contained significantly higher concentrations of n-6 fatty acids and lower docosahexaenoic acid than NL shrimp. The results of this study indicate that supplemental lighting in intensive indoor shrimp systems can improve shrimp production and have effects on biofloc and shrimp nutritional characteristics.

1. Introduction

Intensive, recirculating aquaculture systems (RAS) can be used to grow shrimp indoors year-round, which may offer substantial marketing opportunities for fresh, local, consistently produced shrimp (Browdy and Moss, 2005). Biofloc-based systems are RAS that rely on the accumulation of microbes to act as a biofilter in the water column. (Burford et al., 2004; Avnimelech, 2009). Biofloc particles are formed naturally through microbial excretions and contain most of the microbes. These particles are suspended in the water column by aeration and mixing. Clear-water RAS are another option for indoor shrimp production; they rely on more filtration including intensive solids removal and external biofiltration. Because nutrients are recycled through the biofloc particles, biofloc systems can improve shrimp production compared with clear-water RAS (Emerenciano et al., 2011; Xu and Pan, 2012); however, water quality can be inconsistent, especially compared to clear-water RAS (Prangnell et al., 2016; Ray et al., 2017). In addition, clear-water RAS can have higher startup and operating costs (Luo et al., 2014). Hybrid RAS, combining positive aspects of both biofloc and clear-water systems, capitalize on the benefits of both system types (Fleckenstein et al., 2018; Tierney and Ray, 2018). Such systems accumulate a controlled amount of biofloc particles to provide supplemental nutrition for shrimp and include external biofiltration to stabilize water quality.

Shrimp aquaculture systems exposed to sunlight may perform better than indoor systems with low levels of lighting due to photosynthetic microorganism growth (Izquierdo et al., 2006; Neal et al., 2010; Coyle et al., 2011). Algae in outdoor or greenhouse-based systems can also convert waste products from shrimp into protein, fatty acids, and other bioactive compounds that can then be consumed by shrimp, increasing growth and lowering FCRs (Kent et al., 2011; Patil et al., 2007; Wasielesky et al., 2006). Some algae may even have antibiotic effects

Abbreviations: LED, Light Emitting Diode; RAS, Recirculating Aquaculture System; DO, Dissolved Oxygen; TSS, Total Suspended Solids; VSS, Volatile Suspended Solids; PAR, Photosynthetically Active Radiation; SGR, Specific Growth Rate; FCR, Feed Conversion Ratio

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and reduce susceptibility of shrimp to some diseases (Ge et al., 2017).

Indoor shrimp systems or those that do not have much natural sunlight during certain periods of the year may benefit from using supplemental lighting to drive algae growth. Spectrum, time interval, and intensity of lighting can impact system function and shrimp production. Both Coyle et al. (2011) and Baloi et al. (2013) found that metal halide lighting (MHL) increased production of L. *vannamei* by increasing algal production and altering the biofloc composition. However, the use of MHL can consume large amounts of energy relative to other lighting types (Cook, 2000). LEDs are more efficient than most other lighting and can be targeted to spectra favorable to algae production and that may have positive effects on shrimp production (Das et al., 2011).

Certain spectra of light (primarily blue light, 400–495 nm) in clear-water systems have been shown to inhibit shrimp growth by increasing activity or molting rates; however, the effect on shrimp in more turbid systems is not clear (Wang et al., 2004; Guo et al., 2011). Photoperiod length may also affect shrimp production and behavior. Wang et al. (2004) found that photoperiods of 24 h of light, 24 h of dark, 14 h light/10 h of dark, and 10 h of light/14 h of dark each influenced molting frequency, but did not have any significant effects on growth. However, other studies demonstrated that shrimp raised in absence of light have decreased growth rates whereas 24 h of supplemental lighting had positive effects (Zhang et al., 2006; Baloi et al., 2013; Sanudin et al., 2014).

Carbon and nitrogen isotope levels can be used to determine nutritional dynamics in aquaculture (Ray et al., 2017). In biofloc containing systems, shrimp are limited to only biofloc and artificial feed as their nutrient sources. Comparing carbon and nitrogen isotopes from the provided feed and biofloc allow for estimations of the dietary contributions from each source (Fry, 2006). Fatty acids play important roles in both shrimp and human nutrition, and concentrations in both biofloc and shrimp tissues have been shown to be significantly affected through culture system management (Simopoulos, 2002; Ray et al., 2019). Therefore, analyzing the fatty acid profiles of shrimp and biofloc can help identify nutritional changes and microbial dynamics due to treatment effects.

The purpose of this study was to examine the effects of supplemental LED lighting on water quality, stable isotope and fatty acid dynamics, and shrimp production in intensive, hybrid-style production systems.

2. Materials and methods

2.1. Experimental design and operation

The experiment was conducted in the Sustainable Aquaculture Development Laboratory (SADL), a 174-m^2 insulated and heated (approximately 25 °C) building, at the Kentucky State University Aquaculture Research Center. Twelve 1-m^2, round high-density polyethylene tanks were operated at 1-m depth, making the total volume 1 m^3. Tanks were randomly assigned to one of three treatments for a total of four tanks per treatment. Two of the treatments had LED light fixtures suspended 50 cm above the water surface to provide supplemental lighting. One of these treatments received 24 h of constant lighting (Full Light, FL) and the other treatment received 12 h of lighting and 12 h without light each day (Partial Light, PL). The third treatment had no supplemental lighting provided (No Extra Light, NL). The SADL had a row of standard fluorescent lighting fixtures in the center of the building; these lights were turned on for eight hours each day to ensure a safe working environment. The LED lighting units above the shrimp tanks were 300-watt grow lights designed for plant growth (Fig. 1). Photosynthetically active radiation (PAR) was measured just above the surface of the water every 16 cm from the center of each tank to the edge of the water to determine how much light was available for algae growth (Fig. 2). All PAR readings were taken using a LP-80 PAR/LAI Ceptometer (Decagon Devices, Inc. Pullman, WA, USA). A black plastic divider was hung between all tanks to ensure that light from any one tank did not reach any other tanks.

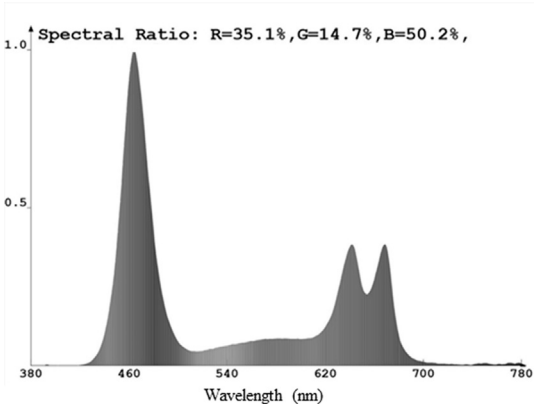

Fig. 1. A graph of the light spectrum produced by the light-emitting diode (LED) systems used in this study (Adapted from the product information sheet provided by the manufacturer: Shenzhen Bailuo Technology Co., Ltd., Guangdong, China).

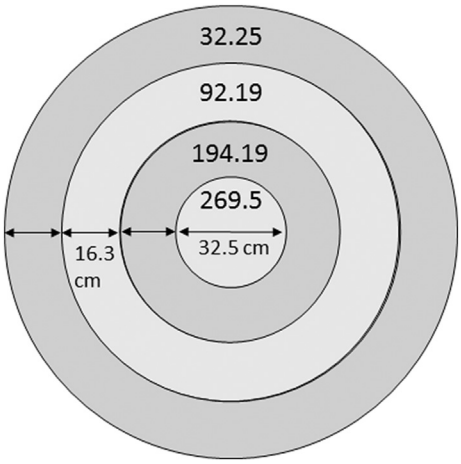

Fig. 2. The mean photosynthetically active radiation distribution across the surface of tanks with supplemental lighting.

All systems had an 18-L settling chamber similar to those described by Ray et al. (2010) to remove excess solids. A 20-L/min pump was used in every system to lift water to the settling chamber where it flowed down a central baffle to slow flow rate and encourage settling. Settling chambers were operated constantly throughout the project, but kept at low flow levels (2.5 L/min) early in the experiment to allow biofloc particles to accumulate in the water. Water from each settling chamber then flowed into an 18-L moving bed bioreactor filled with 4.5 L of plastic biomedia (Curler Advance X-1, Aquaculture Systems Technologies, LLC. New Orleans, LA, USA), which provided 4 m^2 of area for nitrifying bacteria. Of the biomedia in each bioreactor, 1.5 L was from an established biofilter connected to a commercial-scale shrimp tank to reduce the time needed to establish nitrification. A 559-watt regenerative blower provided aeration to all systems; each shrimp tank had four 15-cm long ceramic air diffusers and each biofilter had one 5-cm long ceramic diffuser. Water for the experiment was 75% dechlorinated municipal water and 25% from an existing marine biofloc shrimp system. The source of the biofloc was a 19-m^3 chemoautotrophic biofloc system that had been stocked twice with shrimp at 300 shrimp/m^3 over seven months.

2.2. Water quality

Dissolved oxygen (DO), pH, temperature, and salinity were measured twice daily at approximately 0800 and 1600 h using a YSI Professional Plus Multimeter (Yellow Springs, OH, USA). If pH levels dropped below 7.9, sodium bicarbonate was used to raise pH (Zhang et al., 2017). The initial temperature was 26 °C. The temperature was raised over eight days and maintained between 28 °C and 29 °C during the study using one 1000-watt submersible heater per tank. Salinity was maintained between 15 and 16 g/L by topping off evaporation with city water and replacing filtration water losses with mixed salt water (Crystal Sea, Marine Enterprises International, Baltimore, MD, USA). Total ammonia nitrogen (TAN), nitrite, nitrate, turbidity, total suspended solids (TSS) and volatile suspended solids (VSS) were measured weekly. The concentrations of TAN, nitrite, and nitrate were measured using Hach methods 8155, 8507, and 8039, respectively, and read on a Hach DR6000 Spectrophotometer (Hach Company, Loveland, CO, USA). The concentration of TSS and VSS were measured using Environmental Sciences Section Method 340.2 (ESS, 1993), and turbidity was measured using a Hach 2100Q Turbidimeter. Turbidity was measured weekly and used as an indicator of biofloc concentration; these data guided the use of settling chambers, as suggested by Ray et al. (2010). Settling chambers were normally operated at 2.5 L/min, but flow rate was increased to 7.5 L/min when turbidity reached 75 NTU.

2.3. Animal husbandry

Post-larvae shrimp were obtained from Shrimp Improvement Systems (Islamorada, FL, USA) and stocked at 2500 shrimp per m^3 in a 3.4-m^3 indoor "hybrid-style" nursery tank for 35 days. During the nursery phase, shrimp were fed Zeigler Brothers, Raceway Plus Diet (Zeigler Brothers, Inc., Gardners, PA, USA) with varying crumble sizes according to the size of shrimp. This diet contained 50% protein, 15% fat, 1% fiber, 10% moisture, and 7.5% ash. Beginning on day 23, shrimp were transitioned to Zeigler PL Raceway 40-9 1.5-mm diet (40% protein, 9% fat, 3% fiber, 10% moisture, and 13% ash). The shrimp were transitioned to Zeigler Hyper-intensive Shrimp 35 2.5-mm diet (35% protein, 7% fat, 2% fiber, 12% moisture, and 15% ash) at day 31, which was provided for the remainder of the project.

After a 38-day nursery phase, shrimp were stocked into the experimental tanks at a mean individual weight of 1.2 g and stocking density of 250 shrimp/m^3. All treatments were fed the same amount and each tank was equipped with a 24-h belt feeder to continually dispense feed. Feed was replenished on the belt feeder every day at 0800. Feed amounts were calculated using an estimated FCR and growth rate; feeding rates were further refined based on periodic checks for uneaten feed on the bottom of the tanks. The study was conducted for 84 days at the end of which all shrimp were harvested, weighed, and counted and final individual shrimp weight, survival, FCR, specific growth rate (SGR), and total harvest weight were calculated.

2.4. Isotope and nutritional analyses

Shrimp, feed, and biofloc material were randomly sampled for isotopic analysis during harvest. Three shrimp samples from each tank were dried at 60 °C, ground finely, washed with 10% HCl, rinsed in triplicate, and dried again. Biofloc samples were collected by harvesting freshly settled material from the settling chambers. Biofloc and feed were dried at 60 °C and ground finely. These samples were sent to the University of Arkansas Stable Isotope Laboratory (Fayetteville, AR, USA) for isotopic analysis. Samples were combusted in an elemental analyzer and gas was delivered to a Delta Plus Mass Spectrometer (ThermoFisher Scientific, Waltham, MA, USA). C and N isotope concentrations were then used to calculate $\delta^{13}C$ and $\delta^{15}N$ values as:

$$\delta = \left(\frac{Rsample}{Rstandard} - 1\right) \times 1000$$

where R is the ratio of heavy/light isotopes ($^{13}C/^{12}C$ or $^{15}N/^{14}N$).

To account for the effects of isotopic fractionation, a fractionation value was applied to both C and N isotope values (Fry, 2006). The fractionation value for C was adapted from Parker et al. (1991) due to the similarities between the systems and species used in the study. The value for N based on an earlier L. *vannamei* study at Kentucky State University (Tierney and Ray, 2018). To estimate the contribution of C and N from biofloc and feed to the shrimp tissues, a two source mixing model from Fry (2006) was used:

$$f1 = (\delta^{13}C_{shrimp} - \delta^{13}C_{biofloc})/(\delta^{13}C_{feed} - \delta^{13}C_{biofloc})$$

$$f2 = 1 - f1$$

where $f1$ is the estimated contribution of the biofloc to shrimp tissues and $f2$ is the estimated contribution of the feed.

Nutritional data was collected using in five de-headed, peeled and frozen shrimp tails collected during harvest, the feed used, and biofloc samples from the last week of the study. Samples were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA) for crude protein, moisture, crude fat, crude fiber, and ash (AOAC methods 934.01, 942.05, 978.10, 984.13, 2006; Folch et al., 1957). Fatty acid levels were analyzed and reported as a percent of total fat (AOAC method 996.06, 2001).

2.5. Statistical analyses

SigmaPlot 13.0 was used for all statistical analyses (Systat Software, Inc., San Jose, CA, USA). Differences in water quality data between treatments over time were analyzed using a repeated-measures analysis of variance (ANOVA). Final individual shrimp weight, survival, FCR, SGR, total harvest biomass, isotope, nutritional data, and the amount of sodium bicarbonate added were analyzed using a one-way ANOVA. Results were considered significant at an α-value of < 0.05. If a significant difference was found, a Tukey's HSD applied.

3. Results

Temperature, DO, and salinity were not significantly different between systems. Peak TAN values were measured during the second week of the study and remained below 3.0 mg/L in all systems (Fig. 3; Table 1). Peak nitrite levels also occurred in the second week of the study and were below 3.0 mg/L in all systems. There were no significant differences between treatments with regard to TAN or nitrite concentration. Nitrate concentration was significantly lower in FL (63.6 mg/L) systems than PL (80.9 mg/L) or NL (85.0 mg/L) systems. Mean pH levels were significantly lower in NL systems than in FL and PL systems (Fig. 4). The NL systems required a significantly greater amount of sodium bicarbonate over the course of the study to maintain targeted pH levels compared to FL and PL systems (FL: 232.5 g; PL: 260 g; NL: 365 g). The concentrations of TSS and VSS were significantly higher in NL systems than FL and PL systems. Turbidity measurements followed a trend similar to that of TSS and VSS and FL systems had significantly lower turbidity that NL or PL systems. The amount of water removed from the system when the settling chambers were emptied was minimal (< 4% total water volume throughout the duration of the study).

Average PAR readings ranged from 269.5 mol m^2/s at the center of the tank surface to 32.3 mol m^2/s at the edge of the tank (Fig. 2). Average PAR over the entire water surface was 95.8 mol m^2/s. The NL systems had an average PAR reading of 0.0 mol m^2/s at the surface of the water even when the fluorescent building lights were operating, indicating these lights had no influence on PAR. The cost per lighting system was $68 US Dollars (USD). A total of 274.2 kWh was used on FL

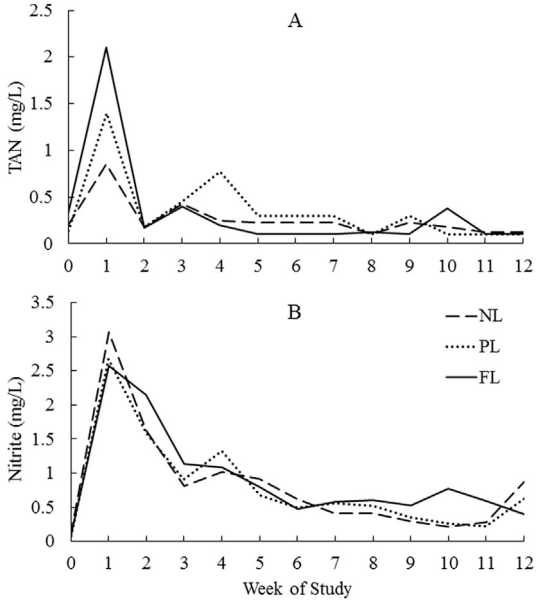

Fig. 3. Mean (A) TAN and (B) nitrite concentrations from each treatment during the study. The treatments were no extra light (NL), partial light (PL), and full light (FL).

systems and 137.1 kWh used on PL systems. Using a rate of $0.117 USD/kWh and adding the light fixture cost, the total additional cost for lights was $100.17 USD for FL systems and $84.09 USD for PL systems.

Significant differences in shrimp production were found between the three treatments (Table 2). The FL systems produced significantly greater shrimp biomass than PL or NL systems (48% increase over NL) and FL and PL systems produced significantly larger individual shrimp than NL systems. FCR was significantly lower in FL systems compared to PL and NL systems and survival was significantly higher in FL tanks versus PL tanks. Shrimp growth rate (g/week) was significantly greater in FL and PL treatments compared to NL (Table 2).

Crude protein and crude fiber levels in FL treatment biofloc were significantly higher than PL and NL biofloc samples (Table 3). Stearic acid levels in the biofloc decreased as light level increased and were significantly lower in FL systems compared to NL. Shrimp muscle tissue had no significant differences in crude fat, crude protein, crude moisture, or crude fiber between treatments (Table 3). Shrimp tissue from FL systems had significantly higher levels of linoleic acid (LA) than NL systems and significantly higher levels of alpha-linolenic acid (ALA) than PL and NL systems (Table 4). Shrimp from tanks with supplemental lighting (FL and PL) had a significantly higher amount of n-6 fatty acids than the NL treatment and likewise had a significantly higher ratio of n-6 to n-3 fatty acids than the NL treatment (Table 4). Shrimp tissue from NL systems contained significantly higher levels of docosahexaenoic acid (DHA) than FL and PL systems.

Biofloc material had significantly lower $\delta^{13}C$ values in the FL treatment than in the PL and NL treatment and the biofloc $\delta^{15}N$ values were significantly higher in FL systems than the PL treatment (Table 5). However, there were no significant differences between treatments with regard to the isotope values of shrimp tissues. Isotope analysis of sodium bicarbonate added to the systems showed a $\delta^{13}C$ value of −14.29, which was much higher than the levels found in the shrimp or biofloc (Table 5). Estimated C and N contributions from the two source mixing model showed increasing C contribution from biofloc to shrimp tissues as light increased (FL: 44%, PL: 35%, NL: 30%) and lower N contribution to shrimp tissues in FL (38%) systems compared to PL (46%) and NL (45%) systems (Fig. 5).

4. Discussion

Supplemental lighting had a significant impact on water quality, shrimp production, and the nutritional value of shrimp and biofloc material. Some of the differences in water quality may have been due to increased algae growth in the systems with supplemental lighting. Higher pH in the treatments with supplemental light and lower nitrate concentration in the FL treatment both indicate a higher abundance of algae. Algal photosynthesis reduces carbon dioxide in water, thereby reducing the amount of carbonic acid present and increasing the pH. Because bicarbonate additions were based on pH measurements, the use of bicarbonate was reduced with higher pH in the supplemental light treatments (Wurts and Durborow, 1992). Algae are also effective at utilizing nitrate as a source of nitrogen to build cellular proteins (Harlin, 1978; Mallick, 2002) and may be part of the reason nitrate was lower in the FL treatment. Nitrate accumulation is the most important factor limiting long-term water use in RAS (van Rijn et al., 2006). By reducing nitrate levels, water may be used for longer periods therefore conserving not only water, but also salt, which can be a major expense for inland RAS production. The concentrations of TAN, nitrite, and nitrate were within ranges considered acceptable for Pacific white shrimp (Schuler et al., 2010; Kuhn et al., 2010).

In addition to water quality data, some of the nutritional and isotope data collected may indicate greater algal abundance in the treatments with supplemental light. Levels of the fatty acids LA and ALA were significantly higher and DHA was lower in shrimp from the supplemental light treatments, indicating greater algal influence with regard to the nutritional contributions of biofloc (Browdy et al., 2006; Crab et al., 2010; Anand et al., 2014). Likewise, significantly higher fiber in the FL treatment biofloc material may indicate relatively higher

Table 1
Water quality parameters from each treatment over the entire study. Nitrate was measured once at the end of the study. Data are presented as treatment mean ± standard error (range). Different superscripted letters in a row denote significant differences ($P < 0.05$) between treatments. The treatments were no extra light (NL), partial light (PL), and full light (FL).

	Treatment		
	FL	PL	NL
Temperature (°C)	28.3 ± 0.2 (28.9–26.0)	28.3 ± 0.2 (28.9–26.3)	28.3 ± 0.2 (29.0–25.9)
Dissolved oxygen (DO; mg/L)	6.6 ± 0.1 (7.1–6.2)	6.4 ± 0.1 (7.1–5.2)	6.4 ± 0.1 (7.0–5.9)
Salinity (ppt)	15.7 ± 0.1 (17.7–14.8)	15.9 ± 0.1 (18.0–15.0)	15.7 ± 0.0 (17.6–14.8)
pH	8.2 ± 0.0 (8.6–7.8)[a]	8.1 ± 0.03 (8.5–7.7)[a]	8.1 ± 0.0 (8.5–7.7)[b]
Total ammonia nitrogen (TAN; mg/L)	0.4 ± 0.1 (2.1–0.1)	0.3 ± 0.08 (1.1–0.1)	0.3 ± 0.1 (1.2–0.1)
Nitrite (mg/L)	0.9 ± 0.2 (2.6–0.1)	0.9 ± 0.14 (3.1–0.1)	0.8 ± 0.1 (2.7–0.2)
Nitrate (mg/L)	63.7 ± 2.0 (72.8–14.3)[a]	80.9 ± 2.43 (91.8–11.0)[b]	87.0 ± 1.5 (97.1–13.5)[b]
Turbidity (NTU)	25.3 ± 6.6 (7.3–54.2)[a]	36.5 ± 6.78 (7.4–101.8)[b]	40.4 ± 5.5 (7.2–110.6)[b]
Total suspended solids (TSS; mg/L)	110.3 ± 22.4 (110.3–65.0)[a]	150.0 ± 43.12 (205.0–55.0)[a]	269.7 ± 23.5 (430.0–96.7)[b]
Volatile suspended solids(VSS; mg/L)	85.0 ± 21.4 (85.0–50.0)[a]	71.3 ± 19.5 (120.0–22.5)[a]	195.8 ± 53.4 (315.0–76.7)[b]
Sodium bicarbonate added (g)	222.5 ± 11.1[a]	260.0 ± 19.2[a]	365.0 ± 18.5[b]

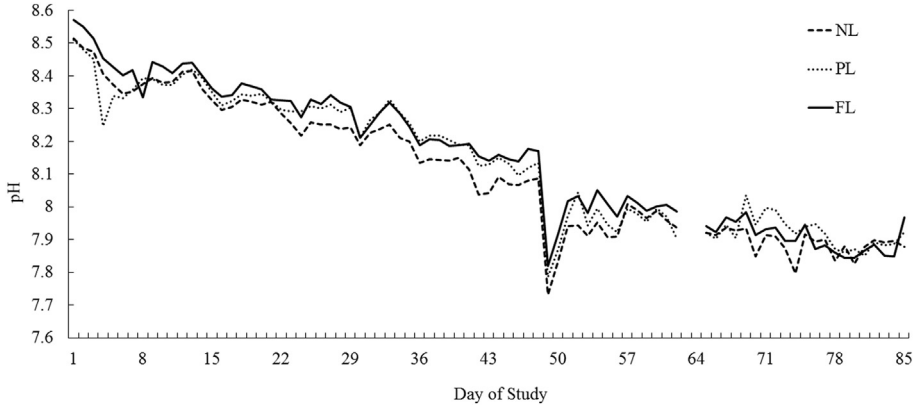

Fig. 4. Mean pH levels in each treatment during the study. The treatments were no extra light (NL), partial light (PL), and full light (FL).

Table 2
Final shrimp production metrics from each treatment. Data are presented as treatment mean ± standard error. Different superscripted letters denote significant ($P < 0.05$) differences between treatments. The treatments were no extra light (NL), partial light (PL), and full light (FL).

	Treatment		
	FL	PL	NL
Total weight (kg/m^3)	4.57 ± 0.4^a	3.4 ± 0.2^b	3.1 ± 0.3^b
Individual weight (g)	25.0 ± 1.5^a	24.2 ± 1.1^a	19.9 ± 0.5^b
Growth (g/week)	2.0 ± 0.1^a	1.9 ± 0.1^a	1.6 ± 0.0^b
Feed conversion ratio (FCR)	1.4 ± 0.0^a	1.8 ± 0.1^b	2.1 ± 0.2^b
Specific growth rate (SGR)	3.5 ± 0.1^a	3.4 ± 0.1^a	3.2 ± 0.0^b
Survival (%)	74.1 ± 5.8^a	57.2 ± 3.8^b	62.0 ± 6.2^{ab}

Table 3
Proximate nutritional percent composition from each treatment. Data are presented as treatment mean ± standard error. Different superscripted letters in a row denote significant ($P < 0.05$) differences between treatments. The treatments were no extra light (NL), partial light (PL), and full light (FL).

	Treatment			
	FL	PL	NL	Feed
Biofloc				
Crude protein	5.8 ± 0.1^a	4.6 ± 0.3^b	4.0 ± 0.6^b	35.71
Moisture	52.6 ± 4.3	53.0 ± 2.0	53.9 ± 2.2	8.73
Crude fat	0.21 ± 0.2	0.13 ± 0.1	0.36 ± 0.2	6.31
Crude fiber	1.9 ± 0.2^a	1.2 ± 0.2^b	0.9 ± 0.1^b	2.05
Ash	32.6 ± 3.6	34.6 ± 1.8	34.9 ± 2.6	9.48
Shrimp				
Crude protein	23.0 ± 0.2	23.3 ± 0.3	22.7 ± 0.2	35.71
Moisture	75.5 ± 0.1	75.0 ± 0.3	75.5 ± 0.2	8.73
Crude fat	0.6 ± 0.0	0.7 ± 0.1	0.7 ± 0.1	6.31
Crude fiber	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.05
Ash	1.5 ± 0.0	1.5 ± 0.0	1.5 ± 0.0	9.48

Table 4
Mean fatty acid levels (percent of total fat) in shrimp tissues from each treatment. Data are presented as treatment mean ± standard error, and N.D. signifies none-detected. Different superscripted letters in a row denote significant ($P < 0.05$) differences between treatments. The treatments were no extra light (NL), partial light (PL), and full light (FL).

	Treatment			
	FL	PL	NL	Feed
14:0	0.24	0.25	0.24	3.23
9c-14:1	0.00	0.00	0.00	0.13
C15:0	0.28	0.29	0.28	0.328
C15:1n5	0.00	0.00	0.00	0
16:0	18.81	18.94	19.16	26.06
9c-16:1	0.61	0.61	0.55	2.81
17:0	0.82	0.86	0.89	0.33
10c-17:1	0.30	0.28	0.29	0
18:0	9.89^a	9.98^b	10.01^b	3.63
9 t-18:1	0.19	0.22	0.19	0
9c-18:1	9.21	9.55	9.16	13.06
11c-18:1	2.04	2.19	2.11	1.89
18:2n6 (LA)	22.73^a	22.74^a	21.71^b	37.08
18:3n3 (ALA)	1.51^a	1.41^a	1.32^b	4.01
18:4n3	0.0	0.0	0.0	0
20:0	0.26	0.26	0.28	0.14
20:1n9	0.78	0.78	0.78	0.58
C20:2	1.93	1.93	1.94	0.6
20:3n3	0.27	0.26	0.28	0
20:4n6 (AA)	2.16	2.27	2.26	0.24
20:4n3	0.0	0.0	0.0	0
20:5n3 (EPA)	9.77	9.51	9.84	1.368
22:0	0.19	0.20	0.21	0
22:1n9	0.0	0.0	0.0	0
22:5n3	0.41	0.41	0.42	0
22:6n3 (DHA)	8.70^a	8.90^a	9.44^b	0.872
24:0	0.12	0.13	0.13	0
24:1n9	0.09	0.10	0.10	0
n-6/n-3 ratio	1.25:1^a	1.26:1^a	1.16:1^b	

LA = linoleic acid; ALA = alpha-linolenic acid; AA = arachidonic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

algae abundance in that treatment, since algae tend to be high in fiber (Mišurcová et al., 2010). Lastly, the significant differences in isotope levels between biofloc samples indicates some differences in the biofloc composition, although it is unclear from these data alone exactly what those differences may be.

The differences in turbidity and TSS/VSS levels could be due to increased consumption of the biofloc particles by the shrimp that is reflected in the increased growth in systems with lighting. The ability of shrimp to attain supplemental nutrition from biofloc systems is well documented and biofloc from high-light environments may improve nutritional qualities due to algae growth (Avnimelech, 2009; Ju et al., 2009; Xu and Pan, 2012; Baloi et al., 2013; Ekasari et al., 2014). Diatoms and chlorophytes can be prevalent in high light shrimp production systems, and can improve growth (Ju et al., 2009; Godoy et al., 2012). In addition, increased algae consumption by shrimp can increase digestive enzyme activity, which may have contributed to the increased growth rates in the systems with supplemental lighting (Moss et al., 2001). It is possible that the alteration of biofloc content by increased lighting could affect the rates at which the biofloc was removed from the system by the settling chambers. Although it was not quantified, the authors noted reduced amounts of solids discharged from the FL system settling chambers compared to the NL systems.

The fatty acid profiles of shrimp tissue from the different treatments may also indicate differences in biofloc consumption by the shrimp and alterations to the biofloc composition by the supplemental lighting.

Table 5
Isotope levels in shrimp tissue and biofloc samples from each treatment. Data are presented as treatment mean ± standard error. Different superscripted letters in a row denote significant ($P < 0.05$) differences between treatments. The treatments were no extra light (NL), partial light (PL), and full light (FL).

	Treatment		
	FL	PL	NL
Biofloc $\delta^{13}C$	-23.61^{a}	-22.03^{b}	-22.88^{ab}
Biofloc $\delta^{15}N$	12.80^{a}	11.74^{b}	12.08^{b}
Shrimp $\delta^{13}C$	−21.97	−21.72	−21.78
Shrimp $\delta^{15}N$	9.37	9.47	9.50

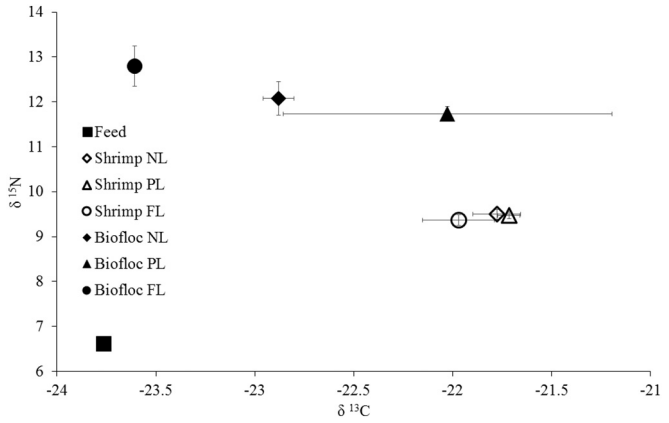

Fig. 5. Graph showing the mean $\delta^{13}C$ and $\delta^{15}N$ isotope levels from shrimp tissues, biofloc, and the feed provided. Error bars represent standard error.

Increased percentages of DHA in NL shrimp tissues could indicate higher bacterial abundance, as opposed to algae, in the biofloc. Bacterial-based bioflocs can contain high amounts of DHA whereas algae often contain relatively higher n-6 and shorter chain fatty acids (Turchini et al., 2009; Crab et al., 2010; Anand et al., 2014). The levels of LA and ALA were significantly higher in the shrimp tissues from the FL treatment, which correlated with higher growth. However, in other studies, LA and ALA have not been demonstrated to be beneficial to L. *vannamei* growth, and an elevated ratio of ALA to LA may even negatively impact shrimp performance (González-Félix et al., 2003a; González-Félix et al., 2003b). In contrast, highly unsaturated fatty acids (HUFA) are essential to shrimp nutrition (Lim et al., 1997; Zhou et al., 2007; Samocha et al., 2011). The higher DHA percentages found in NL shrimp tissues indicate higher amounts of DHA in the diet; however, this did not correspond to increased shrimp growth. Omega-6 fatty acids formed a higher percentage of the total fat in FL and PL shrimp than NL, which resulted in increased n-6:n-3 ratios. The benefits of maintaining a lower n-6/n-3 ratio in human diets is well-documented; however, all shrimp were well below the recommended ratio value of 2:1 (Simopoulos, 2002).

The lighting used in this study was designed for plant growth and consisted of red and blue LEDs. Wang et al. (2003) found that blue light increases feed intake by shrimp but also increases energy spent, decreasing the overall growth rate. Further research found that constant blue light inhibited growth of L. *vannamei*, again by increasing energy expenditure, while fluctuating spectrums of light (blue-green) can increase feed efficiency and fluctuating light frequencies caused increased molting frequencies compared to shrimp under stable light sources (Wang et al., 2004; Guo et al., 2011). In contrast, another study found that shrimp respond positively to supplemental lighting with fluctuating intensity, similar to natural daylight. Lighting that started at 900 lx and increased to 4500 lx, and then decreased back to 900 over a 14-h period resulted in increased feed intake and decreased FCR (Guo et al., 2011). Yet another study that evaluated the effects of different light intensities found that high intensity light, comparable in lux to the lighting used by Guo et al. (2011), over clear-water systems depressed growth in Chinese pink shrimp (Wang et al., 2003). An important consideration is that high amounts of biofloc in the water in this experiment may have blocked some or all light from reaching the bottom of the tank, decreasing the effects on shrimp behavior.

Although the data indicate improved shrimp growth rate corresponds with apparently higher algal abundance in the supplemental light treatments, survival was significantly lower in the PL treatment compared to the FL treatment. During the study, the authors noted substantial jumping behavior from the shrimp when the lighting turned on and off. Although the tanks were covered with mesh, some shrimp were found on the ground each morning. This behavior was noted in all systems when the LED units and overhead fluorescent lights were turned on and off, but was most substantial in PL systems, likely due to the intensity of the LEDs compared to the building's fluorescent lights. Crustaceans exhibit flight responses due to rapid fluctuations in lighting and shadows passing over (Forward Jr, 1976; Forward Jr, 1986; Liden and Herberholz, 2008). Supplemental light sources that are dimmed and intensified gradually are likely to decrease stressful effects on shrimp.

Stable isotope analysis showed differences in biofloc material between treatments. The $\delta^{13}C$ ratios were lower in FL systems than PL or NL, possibly due to effects of algae production or higher amounts of sodium bicarbonate added to the other systems. Although C contribution from biofloc increased as the duration of supplemental lighting increased, N contribution from biofloc in FL systems was lower than PL and NL. The estimated C contributions of biofloc and feed to the shrimp tissues are similar to the values found in other studies (Gamboa-Delgado et al., 2011; Ray et al., 2017). Estimated N contributions were similar to those found in pond cultures, but higher than those reported in indoor biofloc studies (Parker et al., 1989; Parker et al., 1991).

Supplemental lighting was beneficial in this study but a more complete economic model of this particular production system is warranted to determine whether the added cost of $68 USD/$m^2$ and electrical consumption (FL systems: 274 kWh, cost = $32.90; PL systems 137 kWh, cost = $16.45) is justified.

5. Conclusion

Overall Full Light systems outperformed No Light systems in shrimp production by 48% and Partial Light systems by 33%; this, in combination with the improvements in water quality with increased light, may translate to higher profits for farmers. Further research should investigate the optimum spectrum and intensity of supplemental lighting on biofloc and hybrid systems. For example, if less expensive light fixtures could produce similar effects, the cost may be reduced substantially.

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