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Comparing biofloc, clear-water, and hybrid nursery systems (Part I): Shrimp (*Litopenaeus vannamei*) production, water quality, and stable isotope dynamics

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ABSTRACT

Indoor, intensive, nursery-based recirculating aquaculture systems (RAS) can provide high-quality juvenile shrimp for indoor or pond-based production systems in a biosecure manner. However, it is unclear what type of RAS is most appropriate for indoor shrimp nurseries. This study compared three types of RAS nurseries: biofloc (BF), clear-water (CW), and hybrid (HY). Each treatment included four, randomly assigned 160 L (0.35-m^2) tanks that were stocked with 3000 post-larvae shrimp m^{-3}. The post-larvae (PL10) shrimp had an initial average weight of 7 ± 0.0 mg and were grown for 48 days. The BF tanks included external settling chambers as the only filtration mechanism. The CW tanks had settling chambers, foam fractionators, and external biofilters to fully clarify the water and process nitrogenous waste. Hybrid tanks included settling chambers, and external biofilters to maintain some suspended solids along with external biofiltration. Overall, the CW treatment had significantly higher dissolved oxygen (DO) and pH levels than the BF and HY systems. The HY treatment had significantly higher DO than the BF treatment. Nitrite concentration was significantly higher in the HY treatment than the CW treatment. Turbidity in the BF treatment was significantly higher than the other treatments. On the final sample date, the BF treatment had significantly higher nitrite and nitrate concentrations than the other treatments. Differences between treatments in terms of shrimp survival, mean harvest weight, specific growth rate, and feed conversion ratio were not significant. The final weight of the shrimp at 48 days for the BF, CW, and HY were 670 mg, 640 mg, and 590 mg respectively. A stable isotope mixing model indicated that, in the BF treatment, 13% of the C and 34% of the N in harvested shrimp tissue may have originated from biofloc material, signifying some nutrient recycling. The nitrification process was more effective with the inclusion of an external biofilter. All three system types appear suitable for RAS shrimp nursery production although consideration should be given to water quality consistency and filtration costs.

1. Introduction

Recirculating aquaculture systems (RAS) are capable of producing high-value species while limiting water exchange rate, reducing waste discharge, and enhancing biosecurity (Losordo et al., 1998; Rurangwa and Verdegem, 2015). Furthermore, these systems can be used to raise marine shrimp and other tropical fish species inland using a smaller spatial footprint, which may be profitable in metropolitan markets with fresh, never-frozen local seafood (Ray, 2012).

Biofloc (BF) systems are a form of RAS that contain algae, bacteria, protozoans, uneaten feed, feces, and other organic matter in the water column (Hargreaves, 2013). Bonded together by bacterial secretions and electro-chemical forces, these components form particles (biofloc) that provide internal biological filtration largely through nitrification and algal and bacterial assimilation (Ray et al., 2009). The microbes and biofloc particles can provide supplemental nutrition (such as proteins and lipids) for culture species (Emerenciano et al., 2013). Consumption of biofloc particles may enhance growth performance of the cultured species and reduce feed costs over time (Avnimelech, 2015). In addition, start-up costs in biofloc systems may be lower than other forms of RAS, because no external biological filtration is required (Ray, 2012). Often the only filtration used in BF systems is a solids filter to

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Abbreviations: ANOVA, analysis of variance; APT, aquaculture production technologies; BF, biofloc; CW, clear-water; D, diameter; DO, dissolved oxygen; FBW, final body weight; FCR, feed conversion ratio; FF, foam fractionator; HY, hybrid; IBW, initial body weight; MBBR, moving bed biofilm reactor; NO_2-N, nitrite-nitrogen; NO_3-N, nitrate-nitrogen; NTU, nephelometric turbidity unit; PL, post-larvae; PL10, 10-days post-larvae; RAS, recirculating aquaculture systems; SGR, specific growth rate; TAN, total ammonia nitrogen

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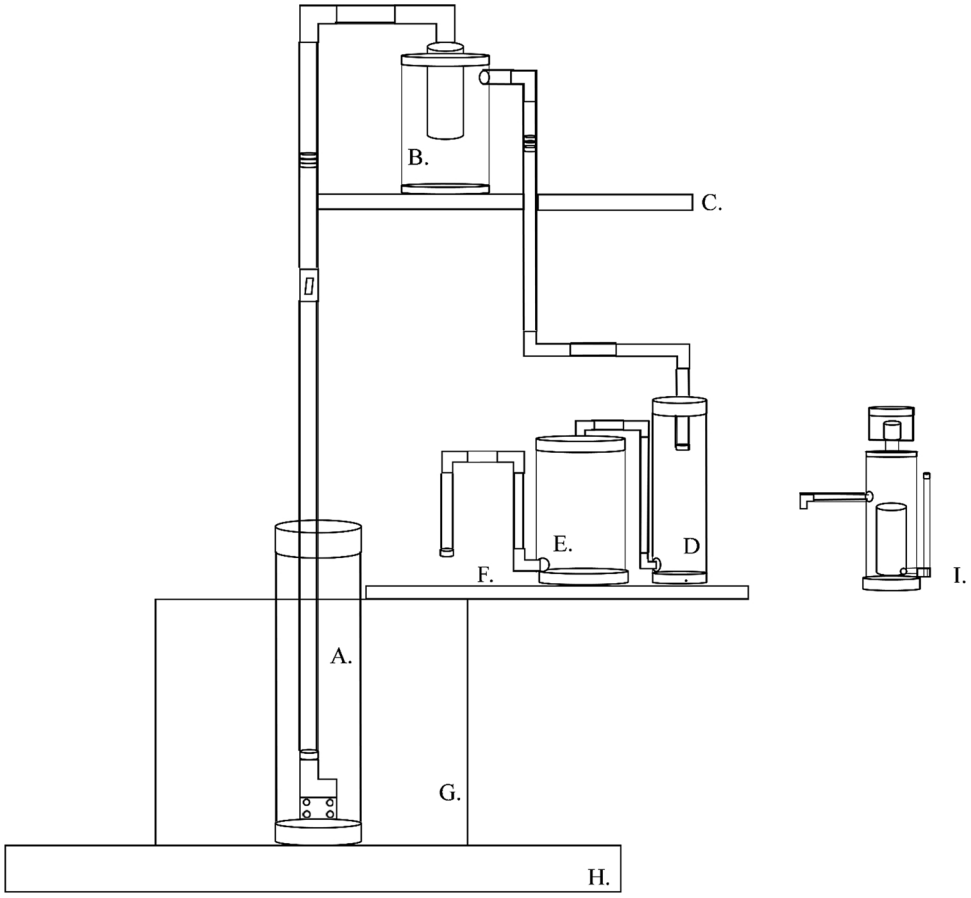

Fig. 1. RAS system design. A. pump located inside pump basket, B. settling chamber, C. level two platform, D. pseudo foam fractionator, E. biofilter or pseudo biofilter, F. level one platform, G. culture tank, H. wooden base platform, I. functional foam fractionator.

control accumulation of particles. A settling chamber is one inexpensive device for removing solids (Ray et al., 2010a). These low-tech chambers include a central baffle to reduce incoming water velocity, allowing settleable solids to fall to the bottom where they can be purged from the system later. Some limiting factors to biofloc systems include abrupt changes in water quality, potential *Vibrio sp*. outbreaks, buildup of solids, and high energy costs attributable to robust aeration (Hargreaves, 2013; Prangnell et al., 2016). Additional research may provide more information to make this a profitable production method for shrimp.

Clear-water systems (CW) are another form of RAS that utilize intensive solids and biological filtration to remove solids and ammonia waste. Foam fractionators are used in brackish-water RAS because they can remove a wide range of suspended solids and dissolved organic compounds efficiently and lower turbidity and oxygen demand (Ebeling and Timmons, 2012; Losordo et al., 1998; Malone, 2013). External biofilters contain a large amount of surface area in an aerobic environment for bacteria to accumulate, facilitating the process of nitrification (Timmons and Ebeling, 2007). External filtration such as those used in RAS allows for greater control of water quality, which is important for the production of a high-value species like Pacific white shrimp (*Litopenaeus vannamei*). However, increased filtration and energy consumption (for pumping and heating requirements) leads to higher start-up and operational costs (Timmons and Ebeling, 2007).

While RAS production has increased in popularity, there is still a need to improve production systems and reduce operational limitations (Martins et al., 2010). If the positive aspects of the BF and CW system types could be integrated to provide the reliable bio-filtration of a CW system with the nutritional contribution of a BF system, additional benefits may be realized. Such a hybrid (HY) system could be useful for indoor shrimp nurseries.

Shrimp nurseries allow the following: production of hardier juveniles, extended culture seasons, biosecurity, improved utilization of space, and higher yields for grow-out production. (Arnold et al., 2006; Samocha, 2010a). Some traditional nurseries are abandoning the coast for inshore locations due to real estate costs, periodic storm events, and rising sea levels (Gutierrez-Wing and Malone, 2006; Tidwell, 2012). A recent study indicated that CW shrimp nurseries may produce greater mean harvest weights in comparison to BF, but did not find significant differences in survival (Esparza-Leal et al., 2015). Results from a recent study indicated that CW shrimp nurseries may produce greater mean harvest weights in comparison to BF without significant differences in survival (Esparza-Leal et al., 2015). To date, a HY system has not been tested, but post-larval growth may improve using components from both BF and CW systems.

One metric to consider with biofloc systems is the nutritional contribution of floc particles. Stable isotope ratios for carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) can be measured in floc particles, shrimp feed, and shrimp tissues to determine the contribution of biofloc to shrimp nutrition (Ray and Lotz, 2017). Data from isotopic fractionation studies indicate that animals store a greater portion of heavy isotopes in their tissues while preferentially excreting lighter isotopes (Gannes et al., 1997). Thus, the isotopic similarity between shrimp tissues and potential food items is relative to the contribution of C and N obtained from that food item (Fry, 2006; Michener and Kaufman, 2007).

This manuscript is part one of a two part series; both manuscripts describe studies examining differences in water quality and animal performance in three types of RAS nurseries (BF, CW, and HY). Part one describes a shrimp (*L. vannamei*) project, and part two describes a tilapia (*Oreochromis niloticus*) project; both species are important candidates for intensive RAS production.

2. Materials and methods

2.1. Systems and experimental design

A 48-day study was conducted to compare shrimp growth and survival as well as water quality and stable isotope dynamics in three nursery system types (BF, CW, HY) for the production of Pacific white shrimp. The experiment was conducted at Kentucky State University's Aquaculture Production Technologies (APT) building in Frankfort, KY, USA. The APT is a climate controlled (~25 °C), 1,207-m^2 insulated building with fluorescent lighting. Twelve, 160-L polyethylene, tanks measuring 77-cm (L) x 46-cm (W) x 46-cm (functional height) were randomly assigned to one of three treatments (BF, CW, and HY) with four replicate tanks each. Wooden platforms held the tanks 41-cm off the ground, and two platforms above the tanks held the various filters (Fig. 1). All tanks contained one 10.2-cm (D) pipe with large holes cut into it wrapped with 1000-μm pore size mesh to prevent entry by the young shrimp. Pumps were placed inside this pipe to move water through filtration components.

All tanks included one 25-cm (D) x 36-cm (H) settling chamber with a functional volume of 12 L of water. The chambers included a central 10.2-cm diameter baffle suspended 10-cm above the bottom to reduce water velocity and allow solids to settle, similar to the design used by Ray et al. (2011). All tanks had a 15-cm ceramic diffuser receiving blown air to provide dissolved oxygen into the water and one 300-watt electric heater to maintain temperature.

2.1.1. Treatment A: biofloc (BF)

As suggested by Ray et al. (2010a), settling chambers in BF systems were utilized only if turbidity exceeded 30 NTU. In addition, the BF systems contained two pseudo filters to match the volume of water in other treatments. The pseudo foam fractionator (FF) did not include a Venturi nozzle to create foam. The pseudo moving bed biofilm reactor (MBBR) did not include any bio-media; however, it contained an air diffuser similar to the functioning biofilters in the other two treatments.

2.1.2. Treatment B: clear-water (CW)

Each CW system contained a functional settling chamber, FF, and MBBR. The FF was a Reef Octopus Classic 110 foam fractionator (Honya Co, Ltd, Guandong, China 518117) and was 15.8-cm (D) x 58.4-cm (H), with an approximate flow rate of 5-L min^{-1}. The FF had a 11.9-cm cup for foam collection that was removed for cleaning purposes as needed. The MBBR included 6-L of MB3 bio-media (Water Management Technologies, Baton Rouge, LA, USA 70809) and held 13.6-L of water at a height of 25.4-cm (water level was dictated by an external stand pipe). The top of each MBBR had a lid with 5.1-cm inlets, which allowed incoming water passing from the foam fractionators to pass through the bio-media.

2.1.3. Treatment C: hybrid (HY)

The HY systems provided an intermediate level of filtration and included a functioning settling chamber and MBBR, as well as a pseudo FF. Physical dimensions and function of the settling chamber, pseudo FF, and MBBR were the same as those in other treatments, and water was pumped through them continuously.

2.2. System management and animal husbandry

Water was pumped from each shrimp tank to the settling chamber on the top platform, which then flowed into the inlet of either the FF or pseudo FF. From there, the water flowed into the MBBR or pseudo MBBR, and back into the shrimp culture tank (Fig. 1).

2.2.1. Bacterial establishment period

To ensure that the systems had established nitrifying bacterial communities present in the biofilters or the biofloc, juvenile tilapia (*Oreochromis niloticus*) were reared in the tanks for 23 days prior to stocking the shrimp. The tanks started at 4-g L^{-1} salinity, with 5% of this water from an established HY-style shrimp nursery system in the APT building. All 12 tanks were stocked with 20 tilapia each, with a mean individual weight of 43.1 g. Over 15 days, several water exchanges were performed to increase the salinity to approximately 33-g L^{-1}, which is approximately the salinity at which post-larvae shrimp are shipped from the hatchery. All salt water was formulated using marine salt (Crystal Sea Marine Mix, Marine Enterprises International, Baltimore, Maryland, USA) and de-chlorinated municipal water that had been filtered through charcoal filters.

Fish were provided a 36% protein, and 6% lipid feed at approximately 3% of their body weight per day. The tilapia were removed from the culture tanks two days before the shrimp experiment started. To prevent high concentrations of toxic ammonia and nitrite, 15 g of sucrose was added to tanks as a carbohydrate source when total ammonia nitrogen (TAN) concentration reached 1.5-mg L^{-1} or more (Hargreaves, 2013). Over a 23 day establishment period based on TAN levels, a total of 56.5 g, 24 g, and 30 g of sucrose were added to the BF, CW, and HY systems respectively.

2.2.2. Shrimp husbandry

Shrimp were shipped in oxygenated bags from a hatchery (Shrimp Improvement Systems, Islamorada, FL, USA) as approximately 10-days post-larvae (PL10). The PL10 shrimp were slowly acclimated and placed in 55 L of water then gently mixed by hand. Five 50-mL and five 100-mL water and shrimp samples were collected and PLs counted to estimate the total number per mL. This information was then used to stock the 160 L tanks at a density of 3000 PL m^{-3} (480 PLs per tank). On the first day of the study, shrimp had an initial mean weight and standard deviation of 7 mg ± 0.0.

During the trial, post-larvae were fed initially at 12% of estimated biomass and decreased to 3% over the course of the study. Growth and uneaten feed were visually assessed daily and used to guide feed rations. All feeds used were manufactured by Ziegler Brothers, Inc (Gardners, PA, USA). A liquid *Artemia* replacement diet (EZ *Artemia* 1) with 52% protein and 17% fat initially was provided along with PL Raceway Plus Post-Larval diet (50% protein and 15% fat). After 5 days, the liquid *Artemia* diet was stopped. For the next 39 days, the Raceway Plus diet was fed as different crumble sizes based on the size of the shrimp (each size had the same nutritional profile). During the last 14 days of the trial, a 1.5-mm (40% protein and 9% fat) pelleted feed was used in combination with the Raceway Plus diet. All tanks were fed three times per day at approximately 0800, 1200, and 1600 h and received the same amount of feed.

At harvest, the total shrimp biomass for each tank was determined by measuring the bulk weight of shrimp. In addition, 50 individual shrimp and five groups of 10 shrimp were weighed and used to calculate mean individual weight. The total production output (kg m^{-3}) of each tank was calculated by dividing total biomass by the water volume (0.16 m^3). Specific growth rate (SGR) was calculated using the following formula from Bureau and Hua (2008):

$$SGR = [(\ln FBW - \ln IBW)/D] \times 100$$

where FBW is final body weight (g), IBW is initial body weight (g), and the number of days is D.

The feed conversion ratio (FCR) was calculated as the total dry weight of feed provided to each tank divided by the net wet weight of shrimp. Survival was computed by dividing the total weight from each tank by individual shrimp weight, then dividing by the initial stocking number of shrimp and multiplying by 100.

2.3. Water quality and stable isotopes

Temperature, dissolved oxygen (DO), pH, and salinity were measured twice daily, at approximately 0800 and 1500 h using a YSI

Professional Plus Multi Meter (YSI Incorporated, Yellow Springs, OH, USA). The pH was adjusted by adding 10 g of sodium bicarbonate if pH was below 7.8. Salinity was maintained at approximately 31 g L^{-1} for the duration of the experiment by adding fresh municipal water to replace evaporation and salt water to replace loss due to waste removal with the filters.

Once a week, TAN, nitrite (NO_2-N), and nitrate (NO_3-N) were measured with Hach methods 8155, 8507, and 8039, respectively, using a Hach DR6000 spectrophotometer (Hach Company, Loveland, CA, USA). Turbidity (measured as nephelometric turbidity units [NTU]) was measured with a Hach 2100Q Portable Turbidimeter weekly.

Samples of shrimp and feed were collected for C and N stable isotope analysis at the time animals were stocked and at harvest; biofloc material was collected from the BF tanks three days before harvest. Shrimp samples were dried, ground, and washed with 10% HCl acid to separate carbonate carbon from organic carbon. After the acid wash, shrimp were rinsed with distilled water, dried, and frozen until analysis. For BF systems, a water sample of 500 mL of was removed and centrifuged. The shrimp and water samples were then decanted, dried, and ground along with dried feed samples. Samples were shipped to the University of Arkansas Stable Isotope Laboratory in Fayetteville, AR, USA for analysis. Samples were combusted in an elemental analyzer and gas was delivered to a Delta Plus Mass Spectrometer, which produced δ ^{13}C and δ ^{15}N values. These values were calculated using the following equation:

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1\right) * 1000$$

where R is the ratio of heavy to light isotope (identified as $^{13}C/^{12}C$ or $^{15}N/^{14}N$) (Fry, 2006).

2.4. Data management and analysis

Statistical analyses were conducted using Statistix 10 (Statistix, Inc., Tallahassee, FL, USA) and Systat 13 (Systat Software, Inc., Chicago, IL, USA); an α-value of 0.05 was used to determine whether significant differences existed between treatments. If the statistical analyses in the results section suggest that a significant difference was found between treatments, this indicates that $P < 0.05$. The weight, total biomass output (kg m^{-3}), SGR, FCR, and survival of shrimp in all treatments were analyzed using one-way analysis of variance (ANOVA). If the normality test or equal variance test failed for production data, a Kruskal-Wallis one-way ANOVA on ranks was conducted. Since water quality data (including temperature, DO, pH, salinity, TAN, NO_2-N, NO_3-N, and NTU) were collected repeatedly, a repeated measures ANOVA was used for comparison of treatments. If the normality test or equal variance test failed for these water quality data, a Friedman repeated measures ANOVA on ranks was used to analyze data. Final TAN, NO_2-N, and NO_3-N were also analyzed on the last water quality test date using a one-way ANOVA. Differences in shrimp stable isotope δ values between the treatments were compared via one-way ANOVA.

For the CW treatment, it was assumed that the only source of C and N came from the feed. Isotope values from this treatment were used to calculate the estimated fractionation factors (Δ) using the following equation (Fry, 2006).

$$\Delta = \delta PRODUCT - \delta SOURCE$$

Where δ source corresponds to shrimp feed, and δ product is the shrimp (Ray and Lotz, 2017). Although CW shrimp may have consumed other items attached to the sides or bottom of the tanks besides the pelleted feed, it is unlikely they had access to any biofloc particles, which is the subject of interest in this case (Ray et al., 2017).

Isotope fractionation factors were subtracted from original shrimp δ ^{13}C and δ ^{15}N values to estimate the isotopic sources of C and N for the shrimp. To estimate how much C and N shrimp received from different food sources, a two-source mixing model was applied (Fry, 2006):

$$f_1 = (\delta sample - \delta source2)/(\delta source1 - \delta source2)$$

$$f_2 = 1 - f_1$$

where sample refers to shrimp tissue, source 1 is pelleted feed, and source 2 is biofloc material. Therefore, f_1 is the portion of C or N contributed to shrimp tissues by the feed and f_2 is the portion of C or N contributed by the biofloc.

3. Results

One BF tank was removed from all analyses and results because a tilapia used for the bacterial establishment period was discovered in the tank five days after the shrimp experiment began. The fish apparently had consumed some of the shrimp, thereby affecting the results from that tank.

3.1. Water quality

The CW treatment had significantly higher DO concentrations compared to the other treatments during morning and afternoon readings, while the DO was significantly higher in the HY versus BF treatment (Table 1). For pH, the CW treatment was significantly higher in both morning and afternoon readings. For turbidity, the BF treatment was significantly higher than both the CW and HY treatments, but no other significant differences were detected (Table 1, Fig. 4). During the experiment, the BF treatment received 430 g of sucrose while the CW and HY treatments received 40 g each, which was used to prevent increases in ammonia. Over the duration of the experiment, ammonia (TAN) concentrations were reasonably consistent among all three treatments and no significant differences were detected (Table 1). However, the BF ammonia increased to a mean concentration of 4.1 mg $TANL^{-1}$ 14 days into the experiment, while the CW and HY never surpassed 1 mg TAN^{-1} (Fig. 2 a). The HY treatment had significantly higher NO_2-N concentrations than the CW treatment over the total duration of the experiment, but no other significant differences regarding nitrite were found (Fig. 2b). In addition differences in nitrate (NO_3-N) concentrations were not statistically significant (Fig. 2c).

When the final concentration of TAN was analyzed, no significant differences were found between the three treatments. However, the final NO_2-N and NO_3-N levels in the BF treatment were significantly higher than both the HY and CW treatments (Table 2).

3.2. Shrimp production

No significant differences were found between treatments for any of the shrimp production metrics (Table 3). Survival ranged from 55% to 92% among the tanks; the BF treatment was numerically higher than the other treatments followed by the CW and the HY treatments (Table 3). Likewise, mean harvest weight, total biomass (kg m^{-3}), SGR, and FCR were all numerically higher in the BF treatment but not statistically significant (Table 3).

3.3. Stable isotope dynamics

No significant differences were found between treatments with respect to shrimp δ ^{13}C values. However, the δ ^{15}N shrimp tissue values were significantly higher in the BF treatment than in both the HY and CW treatments (Fig. 3) (Table 4). Using the shrimp and feed values from the CW treatment to calculate fractionation resulted in fractionation factors (Δ) of -0.2‰ δ^{13}C and 0.4‰ δ^{15}N, respectively (Table 4). Using the two-source mixing model, the shrimp in the BF treatment received an estimated 87% of their carbon from the pelleted feed source, whereas 13% came from the biofloc (Table 5). The model indicated that the BF shrimp received approximately 66% of their nitrogen from the pelleted feed and 34% from the biofloc.

Table 1
Mean ± SEM, minimum, and maximum water quality data over the entire duration of the project, analyzed using repeated measures ANOVA. Different superscript letters in a row indicate significant differences ($P < 0.05$) between treatments.

	Treatment		
Temperature °C	Biofloc (BF)	Clear-Water (CW)	Hybrid-Water (HY)
AM	27.8 ± 0.4 (25.3-30.7)	27.8 ± 0.3 (26.3-30.1)	28.0 ± 0.3 (26.5-30.6)
PM	27.9 ± 0.4 (25.4–31.5)	28.0 ± 0.3 (26.7–30.3)	28.2 ± 0.3 (26.6–30.4)
Dissolved Oxygen (mg L^{-1})			
AM	5.9 ± 0.1 (5.3–6.5)^{c}	6.0 ± 0.1 (5.4–6.5)^{a}	5.9 ± 0.1 (5.1–6.4)^{b}
PM	5.7 ± 0.1 (4.9-6.4)^{c}	6.0 ± 0.1 (4.7-7.0)^{a}	5.8 ± 0.1 (4.4–6.4) ^{b}
pH			
AM	8.0 ± 0.1 (7.8–8.3)^{b}	8.1 ± 0.0 (7.7–8.3)^{a}	8.0 ± 0.0 (7.7–8.2)^{b}
PM	8.0 ± 0.1 (7.7-8.4)^{b}	8.1 ± 0.1 (7.5–8.3)^{a}	8.0 ± 0.1 (7.8–8.2)^{b}
Salinity (g L^{-1})			
AM	31.6 ± 1.0 (25.6–36.1)	31.9 ± 1.1 (25.6–36.0)	32.4 ± 1.0 (28.5–36.0)
PM	31.6 ± 1.0 (27.0–36.3)	31.7 ± 1.1 (25.5–35.8)	32.2 ± 1.0 (27.2–35.9)
Turbidity (NTU)	15.1 ± 5.7 (2.7–45.3)^{a}	3.8 ± 1.2 (1.3–10.0)^{b}	4.1 ± 1.5 (1.0–10.8)^{b}
Ammonia (mg TAN L^{-1})	1.5 ± 0.8 (0.1–8.8)	0.3 ± 0.0 (0.1–1.1)	0.2 ± 0.0 (0.1–0.4)
Nitrite (mg NO_2-N L^{-1})	9.2 ± 4.5 (0.0–29.9)^{ab}	2.5 ± 0.3 (0.0–6.2)^{a}	5.3 ± 0.9 (0.0–12.0)^{b}
Nitrate (mg NO_3-N L^{1})	21.4 ± 8.8 (0.6–73.6)	11.3 ± 1.1 (1.0–46.0)	18.5 ± 2.3 (2.0–65.5)

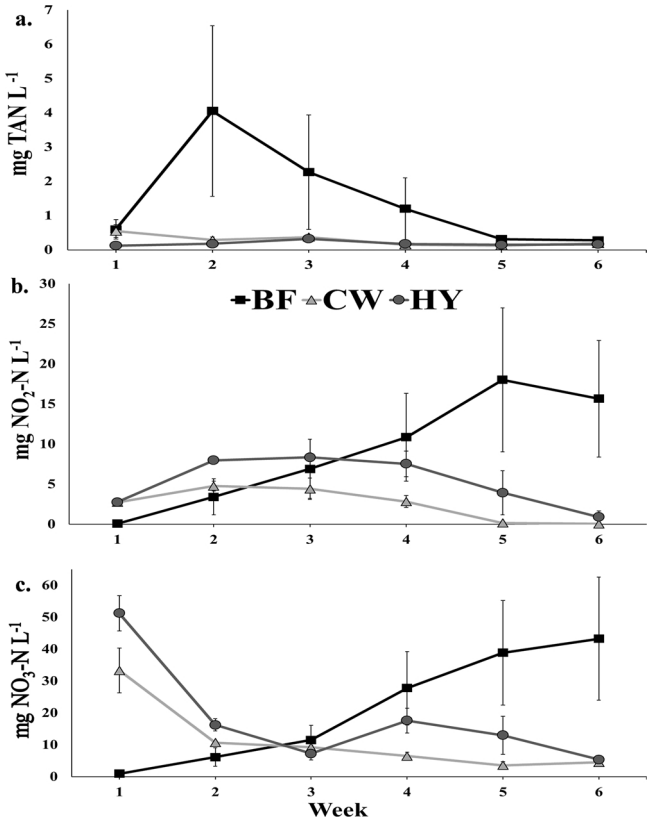

Fig. 2. Ammonia (a), nitrite (b), and nitrate (c) concentrations for biofloc (BF), clear-water (CW), and hybrid-water (HY) throughout the duration of the study. The data points represent the treatment means and error bars are one SEM.

4. Discussion

Temperature, DO, pH, and salinity all fell within suggested ranges for proper growth of *L.vannamei* (Clifford, 1985). However, differences in DO, pH, and turbidity between treatments were significant. The significantly higher turbidity levels in the BF treatment indicate a more abundant microbial community in the water column (Table 1, Fig. 4).

Table 2
Final ammonia, nitrite, and nitrite concentrations data for the three treatments represented by mean ± SEM, analyzed with one-way ANOVA. Different superscript letters in a row indicate significant differences ($P < 0.05$) between treatments.

	Treatment		
	Biofloc (BF)	Clear-Water (CW)	Hybrid (HY)
Ammonia (mg TAN L^{-1})	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
Nitrite (mg NO_2-N L^{-1})	15.7 ± 7.3^{a}	2.5 ± 0.0^{b}	5.3 ± 0.8^{b}
Nitrate (mg NO_3-N L^{-1})	43.3 ± 8.8^{a}	4.6 ± 0.7^{b}	5.4 ± 0.6^{b}

Table 3
Shrimp production data for the three treatments. The data are presented as mean ± SEM, and were compared using one-way ANOVA. No significant differences were found for any production values.

	Treatment		
	Biofloc (BF)	Clear-Water (CW)	Hybrid (HY)
Average weight (mg)	670.0 ± 0.0	590.0 ± 100.0	640.0 ± 0.0
Survival (%)	86.2 ± 1.7	80.2 ± 8.4	74.3 ± 4.0
kg m^{-3}	1.7 ± 0.1	1.4 ± 0.1	1.4 ± 0.1
FCR	1.1 ± 0.1	1.4 ± 0.2	1.4 ± 0.1
SGR (% growth/day^{-1})	1.4 ± 0.1	1.2 ± 0.1	1.3 ± 0.0

The lower pH and DO concentration observed in the BF treatment may have been due to increased respiration of the BF microorganisms, most of which consume oxygen and release CO_2, leading to reduced pH through the production of carbonic acid (Boyd and Tucker, 2014). In contrast, continual removal of solids containing bacteria in the CW and HY treatments may have reduced overall respiration in those treatments. Also, the heavily aerated environment provided by the MBBRs may have offset any microbial respiration that occurred, which also may have contributed to the significantly higher DO and pH levels in CW and HY treatments compared to the BF treatment (Ray et al., 2010b).

Based on turbidity results, the foam fractionators were more effective at clarifying the water in the CW systems than the settling chambers were in the other two treatments. The significantly higher DO and pH levels in the CW treatment may also be due, in part, to the foam

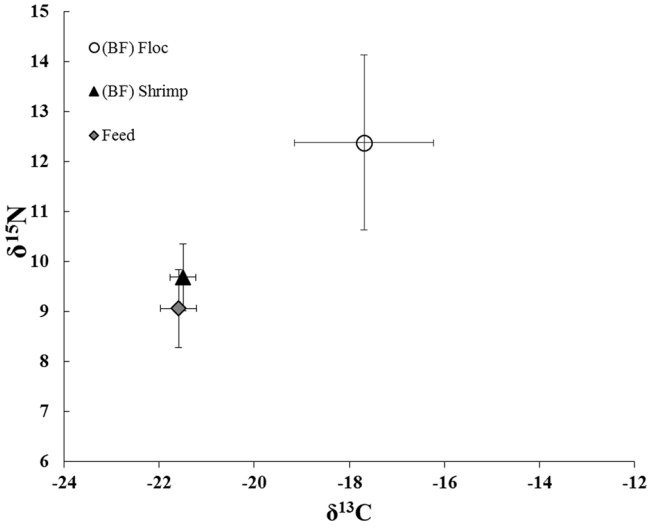

Fig. 3. Isotope values for BF shrimp, biofloc, and feed after accounting for fractionation. Data points represent the mean values and error bars are one SEM.

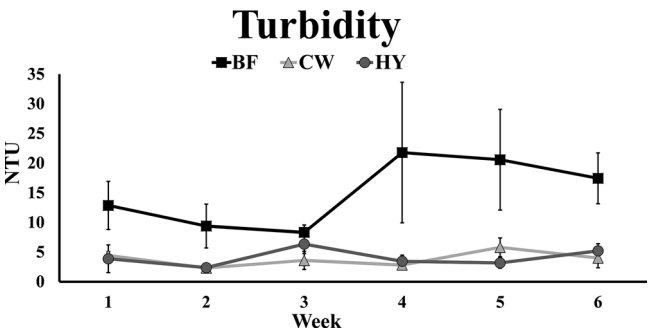

Fig. 4. Turbidity values for biofloc (BF), clear-water (CW), and hybrid-water (HY) throughout the duration of the study. The data points represent the treatment means and error bars are one SEM.

fractionator's functionality. Not only did these filters presumably remove more microbes from the water, the Venturi nozzles on the fractionators injected additional air into the water in the CW systems, which may have contributed to higher DO and greater removal of CO_2. The results of this study suggest that reducing bacterial loads through increased solids filtration combined with heavily aerated biofilters like those in the CW and HY systems may help sustain higher oxygen and pH levels.

The BF treatment may not have benefited as much from the bacterial-establishment period as the other treatments. Over the first 14 days of the experiment, the BF ammonia increased to a mean concentration of 4.1 mg TANL^{-1} while the CW and HY never surpassed 1 mg TANL^{-1} (Fig. 2a). It is possible that the water exchanges used to increase salinity during the 23-day inoculation period may have prevented adequate bacterial accumulation in the BF tanks, whereas bacteria development on the bio-media of the other treatments was not impacted. Throughout the study, the BF treatment also received 390 g more sucrose than the CW and HY treatments, which may have extended the time for chemoautotrophic bacterial proliferation, since heterotrophic bacteria are favored with carbon additions (Avnimelech, 2015; Crab et al., 2012; Hargreaves, 2006). In contrast, the CW and HY external biofilters provided a high surface area, abundant oxygen, and less environmental fluctuations; which likely enhanced the growth performance of nitrifying bacteria while limiting potential TAN spikes (Crab et al., 2007; Timmons and Ebeling, 2007).

Two weeks into the experiment, nitrite concentrations in all treatments increased above 3 mg NO_2-N L^{-1}, which is higher than the recommended level of 1.5 mg NO_2-N L^{-1} for marine post-larvae (Boyd and Tucker, 2014). The CW and HY treatments exceeded 30 mg NO_3-N L^{-1} (nitrate) at the start of the experiment, which indicates that the nitrification process had been active during the bacterial establishment period (Fig. 2c). However, the BF treatment did not surpass 30 mg NO_3-N L^{-1} until week 5 of the experiment, suggesting the nitrifying bacterial community was not as well established. Nitrate concentrations decreased in the CW and HY treatments throughout the study; this may have been due to denitrification. There may have been areas where solids settled and formed anaerobic zones, such as in the pump baskets and settling chambers. Anaerobic zones can harbor denitrifying microbes (Rijn et al., 2006; Ray et al., 2011). A total of 3 water exchanges were performed during the experiment after the external filters and pseudo filters were purged. It is possible that the water lost from these exchanges matched with replacement municipal water may have contributed to the lower NO_3-N concentrations found in the CW and HY treatments. Similar solids accumulation may have occurred in the BF pump baskets, pseudo foam fractionators, and settling chambers, however, the pumps in the BF treatment were rarely operated so the water did not pass through the baskets and filters often. This may partially explain why both the NO_2-N and NO_3-N levels increased in the BF treatment (Fig. 2, Table 2).

The significantly higher N isotope values in the BF treatment suggest that shrimp in this treatment had different dietary sources of nitrogen than the other treatments (Table 4). This finding corresponds with the large proportion of N in shrimp tissues that was attributed to biofloc (Table 5). The contribution of carbon and nitrogen estimated by the isotope mixing model may help explain why the production values in the BF treatment were slightly better than in the other treatments. Over the course of the study, a total of 430 g of sucrose was added to the BF treatment to control TAN. It is possible that the sucrose added to the BF systems may have forced the bacteria to assimilate more nitrogen, thus producing supplemental protein (Avnimelech, 2015). Future research should investigate how to increase crude protein in the biofloc

Table 5
Proportion of C and N in shrimp tissues from the biofloc (BF) treatment originating from the two potential food sources: pelleted feed and biofloc. Numerical values are rounded to the nearest tenth.

	Feed (%)	Floc (%)
Carbon	86.5	13.4
Nitrogen	66.0	33.9

Table 4
Carbon and nitrogen stable isotope dynamics for the three treatments (mean ± SEM). Significant differences ($P < 0.05$) between treatments are indicated with different superscripted letters.

	% C	δ ^{13}C	Δδ^{13}C	δ^{13}C-Δ	% N	δ^{15}N	Δδ^{15}N	δ^{15}N-Δ
BF Shrimp	48.7 ± 1.7	−21.7 ± 0.3		−21.5 ± 0.3	9.8 ± 0.4	10.1 ± 0.7^{a}		9.7 ± 0.7
CW Shrimp	49.0 ± 0.7	−22.2 ± 0.2	−0.2		10.2 ± 0.2	9.1 ± 0.3^{b}	0.4	
HY Shrimp	49.0 ± 0.2	−22.2 ± 0.1		−22.0 ± 0.1	10.2 ± 0.1	9.3 ± 0.2^{b}		8.9 ± 0.2
Feed	48.1 ± 1.3	−21.6 ± 0.4			8.0 ± 0.4	8.7		
Biofloc	7.0 ± 3.1	−17.7 ± 1.5			1.2 ± 0.3	12.4 ± 1.8		

material. If the duration of the project were extended this may enhance microbial N accumulation, possibly improving water quality, and enhancing the nutritional contribution of biofloc to shrimp (Ray and Lotz, 2017; Ray et al., 2017).

Overall shrimp production in this study was similar for all treatments. Thus, producers may want to consider using BF systems for nursery production, due to lower start-up costs than other RAS because less external filtration is required (Ray, 2012). In addition, the shrimp in the BF treatment appeared to have received some of their diet from the biofloc material, which has led to improved nursery production in the past (Emerenciano et al., 2012). With more thorough filtration, the bacterial establishment period was shorter in the CW and HY systems which resulted in better water quality than BF system. However, additional filtration components, such as the pump on the CW foam fractionators and the bio-media in the biofilters, represent additional costs (Ebeling and Timmons, 2012; Hargreaves, 2013). An important consideration for producers is that the additional oxygen demand of BF systems may require more robust aeration which could add expense. Overall, shrimp nursery managers should consider the potential for faster shrimp growth, consistency in water quality dynamics, and the costs of equipment and energy when deciding what system to use.

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