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Comparing clear-water RAS and biofloc systems: Shrimp (*Litopenaeus vannamei*) production, water quality, and biofloc nutritional contributions estimated using stable isotopes

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ABSTRACT

Indoor shrimp aquaculture systems can be used to produce fresh, never-frozen, quality shrimp near metropolitan seafood markets regardless of season and climate. However, questions still remain regarding what type of production system is best suited to maximize indoor production. In this project, two types of systems were compared: clear-water (CW) RAS and biofloc (BF) systems. Three, 1.36 m^3 tanks were assigned to each of the two treatments; CW tanks had external settling chambers, two foam fractionators, and external biofilters, all operated continuously. BF tanks had settling chambers and one foam fractionator which were operated as needed to control solids accumulation. Shrimp weighing 0.42 g were stocked in all tanks at 250 m^{-3} and grown for 55 days. Ammonia and pH levels were significantly ($P < 0.05$) higher in the CW treatment, while nitrite, nitrate, and turbidity were all significantly higher in the BF treatment, although all parameters remained within acceptable ranges for shrimp growth. Shrimp mean harvest weight was significantly higher, biomass (kg m^{-3}) was significantly greater, and FCR was significantly lower in the CW treatment; there were no significant differences in survival between treatments. Isotope levels indicated that shrimp in the BF treatment obtained a portion of the C (18-60%) and N (1-18%) in their tissues from biofloc material; however, this effect did not positively influence production in that treatment. By nearly eliminating solids from the water and using an external biofilter, substantially better water quality was maintained in the CW systems, which may have been a major contributor to the improved shrimp production in that treatment.

1. Introduction

Closed aquaculture systems have very low rates of water exchange, tightly controlled inputs, and are typically contained in smaller spaces than more traditional open ponds. Such systems are increasingly being examined as a means of enhancing biosecurity, reducing water use, and producing marine animals away from coastal zones (Browdy and Moss, 2005). Intensive closed systems can be housed indoors, which opens opportunities for consistent year-round production situated near consumers, and for production in locations with seasonal temperature fluctuations (Martins et al., 2010). This technology is gaining popularity in some parts of the world, including the United States where an indoor shrimp farming industry seems to be developing (Ray, 2015).

Two types of closed aquaculture systems are clear-water recirculating aquaculture systems (CW) and biofloc (BF) technology systems. CW systems typically have an external biofilter to provide surface area and an aerobic environment for nitrifying bacteria, they have one or more solids filters to remove most or nearly all solids from the water, and some systems have water sterilization filters such as UV lamps (Timmons and Ebeling, 2007). BF technology systems contain a substantial amount of particulates which are created by and contain a dense microbial community (Ray et al., 2009). Typically the only external filtration for BF systems is a solids filter to control the particulate abundance (Azim and Little, 2008).

CW systems usually have more filtration components, leading to relatively higher start-up costs, and potentially greater operational costs (Luo et al., 2014) compared to BF. However, by externalizing biofiltration in a container with consistent conditions, CW systems may allow greater control and stability in the system, especially with regard to nitrogen cycling (Hargreaves, 2013). BF systems, in contrast, may have lower start-up costs because less equipment is needed. Also, biofloc particles may provide supplemental nutri-

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tion for shrimp by recycling expensive nutrients from feed and lowering feed conversion rates (Avnimelech, 2012; De Schryver et al., 2008; Wasielesky et al., 2006). Biofloc is composed of a wide variety of microorganisms, the abundance and composition of which is affected by system management and environmental influences, and it may provide a diverse set of nutritional benefits (Ray et al., 2010b). Although there are fewer external filters in BF systems, robust aeration equipment is essential because the microbial community can, at times, consume more oxygen than the cultured animals (Samocha et al., 2012; Browdy et al., 2012). Additionally, biofloc systems are biologically complex and swings in toxic ammonia and nitrite concentrations, as well as bacterial abundance have been reported (Ray et al., 2011). Biofloc systems are generally more difficult to control and may have a long establishment period before adequate bacteria are present to process animal metabolites (Prangnell et al., 2016).

Examining the levels of C and N isotopes in shrimp, feed, and biofloc can provide estimates of where shrimp are obtaining these elements. Isotopes are generally measured as a ratio of heavy/light, in this case C^{13}/C^{12} and N^{15}/N^{14}, and these data are reported as a δ-value in per mil units (‰); the exact formula is presented below (Fry 2006). The closer the δ-value is to one potential food source versus the other indicates that shrimp obtained a greater portion of C or N from that source. A two-source mixing model can be used to quantify the percent C or N contribution from the two sources (biofloc and feed in this case) (Fry, 2006).

The purpose of this project was to compare CW shrimp production systems to BF systems with regard to shrimp production, water quality dynamics, and the estimated nutritional contribution of suspended biofloc particles in BF systems.

2. Materials and methods

2.1. Systems and experimental design

This project was conducted at Kentucky State University's Aquaculture Research Center (Frankfort, Kentucky, USA) in a building with sheet metal walls and a translucent, polycarbonate roof. Six identical fiberglass tanks were arranged in two rows of three; each tank had an internal diameter of 153 cm and an operating depth of 74 cm, resulting in 1.36 m^3 volume. Two, 15 cm long ceramic air diffusers were placed in each tank and blown air was supplied by a 1 HP regenerative blower. Three, 300 W submersible, electric, resistance heaters were placed into each tank and set at 28.5° C. A natural gas-powered heater was used to warm the air in the building.

Three tanks were randomly assigned to a CW treatment and three to a BF treatment. Each CW tank had an external settling chamber and two small foam fractionators (Sea Clone 100, Instant Ocean, Blacksburg, Virginia, USA) to manage solids concentration, and an external bio-filter for nitrogen control. The settling chambers and bio-filters were constructed using the same style cone bottom tanks, each with a functional volume of 100 L. Settling chambers were constructed based on the design described by Ray et al. (2011): water was pumped into a central, 10 cm diameter pipe suspended 15 cm above the bottom of the settling chamber. This pipe slowed the water velocity and solids settled near the bottom, while clarified water flowed out near the top of the chamber, and into the bio-filter. The bio-filters were a moving bed bioreactor design, filled half full (50 L) with Sweetwater SWX Bio-Media (Pentair Aquatic Eco-Systems Inc., Apopka Florida, USA). Each bio-filter had a 15 cm air diffuser in it and water passed down through the bio-media, out of a drain at the bottom, and back into the shrimp tank; an external stand pipe maintained the water level. Foam fractionators were hung on a central pipe within the shrimp culture tanks. The three filter types were operated constantly in CW tanks.

The BF systems also had a settling chamber and only one foam fractionator to help control solids accumulation as needed. These were the same style as the filters in the CW systems, and were operated based on turbidity readings. Turbidity is a fast and objective measurement of the relative clarity of water, and is well-suited to guide solids management decisions in biofloc systems (Ray et al., 2010a). The intention was to keep turbidity in the tanks under 60 Nephelometric Turbidity Units (NTU). In both treatments, foam fractionator collection cups were emptied daily and the sludge from the bottom of settling chambers was drained weekly.

Half of the water initially supplied into the experimental tanks originated from previously established shrimp production systems. The water for the three CW systems originated from a clear-water RAS shrimp nursery tank which had a settling chamber, a foam fractionator, and an external bio-filter. The biofloc water in the experimental tanks came from a biofloc shrimp tank which only had a foam fractionator to control solids. The six experimental tanks were filled with their respective water types half way, while the other half was filled with de-chlorinated municipal water mixed with salt (Crystal Sea Marine Mix, Marine Enterprises International, Baltimore, Maryland, USA) at a salinity of 26 g L^{-1}. The intention was to start the biofloc systems with an established microbial community, and to treat the clear-water systems similarly.

2.2. Animal husbandry

Litopenaeus vannamei post-larvae (PL 12) were obtained from Shrimp Improvement Systems, LLC (Islamorada, Florida, USA). The shrimp were raised in a clear-water nursery system for 30 days before being stocked into the experimental systems. A clear water nursery was used so that isotopic changes in shrimp may be more clearly observed when some were moved to biofloc systems. During the nursery phase, the shrimp were fed Zeigler Brothers, Raceway Plus Post-Larval Diet (Zeigler Brothers, Inc., Gardners, Pennsylvania, USA) with varying crumble sizes according to the size of shrimp. This is a 50% protein, 15% fat diet according to the manufacturer. At week three, feeding gradually transitioned to PL Raceway 40-9 (Zeigler Brothers, Inc.), a 1.5 mm, 40% protein, 9% fat diet. At the time shrimp were moved to the experimental tanks, they were being fed this 40% protein diet. Just after stocking the experiment, the shrimp were transitioned over three days to Zeigler Brothers Hyperintensive-35, a 35% protein, 7% fat, 2.4 mm diet; they were fed this diet for the remainder of the experiment. No supplemental carbon was added to the experimental tanks, meaning the C:N ratio was dictated by the feed which has a C:N of approximately 8.3:1.

Shrimp were stocked into the experimental tanks with a mean weight of 0.48 g; 340 shrimp were stocked by hand-counting individuals into each tank at a density of 250 shrimp m^{-3}. All tanks were fed the same amount of feed; they were fed three times per day by hand. Feeding was based on an estimated feed conversion ratio of 1.5:1 and a growth rate of 1.5 g $week^{-1}$, along with routine sampling to check for uneaten feed. If uneaten feed was observed then feed rations were reduced in all tanks. Shrimp were grown for 55 days, and at the end of the study each tank was sampled for total biomass by weighing all of the shrimp in bulk from each tank. Growth rate and individual weight were measured by sampling the final weights of 50 shrimp from each tank. Survival was enumerated by dividing the total weight from each tank by the individual shrimp weight and then dividing by the initial number of shrimp. Lastly, feed conversion ratio was calculated by dividing the total dry weight of feed given to each tank by the total wet weight of shrimp.

2.3. Water quality

A YSI Professional Plus Multi Meter (YSI Incorporated, Yellow Springs, Ohio, USA) was used to measure dissolved oxygen (DO), temperature, pH, and salinity twice daily. A Hach DR6000 spectrophotometer was used to measure total ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen concentrations; Hach methods 8155, 8507, and 8039 were respectively used to measure each of these compounds weekly (Hach Company, Loveland, CO, USA). A Hach 2100Q Portable Turbidimeter was used to measure turbidity in NTU weekly (Hach Company, Loveland, CA, USA).

2.4. Stable isotopes

Shrimp, biofloc, and feed samples were collected five times throughout the study to measure C and N stable isotope levels. These samples were collected at the start of the experiment (week 0, although no biofloc was collected at this time), weeks 1, 3, 4, and week 8 (at harvest). To collect biofloc, 500 mL water samples were centrifuged at 3,000 RPM for 5 min, and the pellets were collected. Biofloc and feed were dried and finely ground, and shrimp were dried, ground, acid washed with 10% HCl to separate organic carbon from carbonate carbon, thoroughly rinsed, dried, and homogenized. Whole shrimp were sampled because the weight of whole shrimp is measured at the end of experiments and commercial production ventures as a metric of production success. Therefore, the growth, and likewise the chemical composition, of the entire animal is important to understand. Isotope samples were then sent to the University of Arkansas Stable Isotope Laboratory in Fayetteville, AR, USA where they were placed into an elemental analyzer connected to a Delta Plus Mass Spectrometer which generated $\delta\ ^{13}C$ and $\delta\ ^{15}N$ values, calculated using the following equation:

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

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

2.5. Data management and analysis

For the purpose of stable isotope data, the CW treatment was used as a negative control. It was assumed that the shrimp in this treatment had only pelleted feed available as a potential food source, and therefore the differences in δ values between the shrimp tissue and the feed were considered the fractionation factors. Fractionation factors (Δ) were calculated using the following formula (Fry, 2006):

$$\Delta = \delta_{SOURCE} - \delta_{PRODUCT}$$

Where the source is the pelleted shrimp feed and the product is the shrimp; Δ values are expressed in per mil (‰) units. These fractionation factors were subtracted from the shrimp δ values in the BF treatment to make the BF shrimp δ values look isotopically like their food source.

It is possible that shrimp in the CW treatment had items available to eat other than pelleted feed, such as periphyton growing on surfaces in the tanks. Nonetheless, they did not likely have suspended biofloc particles available to consume from the water column, and it is the biofloc particles that are the focus of the isotopic investigation as a source of nutrition.

The following two-source mixing model equations were then used to estimate the relative amount of C and N contributed to shrimp by potential food sources (Fry 2006).

$$f_1 = \left(\delta_{SAMPLE} - \delta_{SOURCE2}\right) / \left(\delta_{SOURCE1} - \delta_{SOURCE2}\right)$$

$$f_2 = 1 - f_1$$

Where f_1 is the portion of C or N contributed by source 1, and f_2 is the portion contributed by source 2. The sample is shrimp, source 1 was feed, and source 2 was biofloc.

The statistical software used for this study was Systat Version 13 (Systat Software, Inc., Chicago, Illinois, USA), and an α-value of 0.05 was used to determine significant differences between the two treatments. Water quality data were analyzed using a repeated measures (RM) ANOVA. Shrimp isotope levels were also analyzed using the RM ANOVA. To compare isotope levels in shrimp between the two treatments, unaltered (no fractionation factors subtracted) mean isotope data were compared using a one-way ANOVA. Final shrimp production data (survival, FCR, growth rate, average weight, and biomass) were also analyzed using a one-way ANOVA.

3. Results and discussion

3.1. Water quality

Temperature, DO, and salinity all remained within acceptable ranges for the growth of *L. vannamei* (Clifford, 1985). The pH in the CW treatment remained fairly constant between 7.9 and 8.1; however, in the BF treatment, pH declined, leading to the significantly lower values in that treatment (Table 1). Although the overall mean pH in the BF treatment was 7.7, on two specific days the mean treatment value dropped to 7.3, possibly causing some stress for the animals although this was corrected by adding bicarbonate to the systems. Zhang et al. (2006) suggested that maintaining pH at approximately 7.6 was ideal for *L. vannamei*.

Turbidity was significantly lower in the CW treatment (Table 1), indicating that the intended effect of the extra filtration in that treatment was successful in keeping particle concentration low. Turbidity in the BF treatment was high at times during the study: the weekly mean treatment values ranged from 53 to 162 NTU. Ray et al. (2011) conducted a biofloc study with a high suspended solids treatment and low solids treatment. The turbidity range in the current study is much like that of the Ray et al. (2011) high solids treatment which produced a shrimp growth rate of 1.3 g wk^{-1} and survival that was not significantly different than the low solids treatment. In the current study, turbidity may have been higher than what is ideal; however, it is not unusual to have similar high turbidity levels in biofloc systems.

Ammonia was consistently higher in the CW treatment compared to the BF treatment (Fig. 1a) which resulted in a significant difference between the treatments (Table 1). All the measured ammonia concentrations were below what Lin and Chen (2001) estimated to be safe levels for rearing *L. vannamei*. By week two of this study, nitrite had decreased below 1 mg NO_2-N L^{-1} in the CW treatment, but was above 2 mg L^{-1} in the BF treatment on all but two sample dates (Fig. 1b), contributing to a significantly higher concentration in the BF treatment. Nitrate concentration did not appear to accumulate as would be expected in systems with a functioning nitrifying bacterial community (Fig. 1c), possibly indicating that the floc community was not fully established. It is unclear why that continual accumulation did not occur, although Ray et al. (2011) indicated that a substantial amount of denitrification may take place in settling chambers such as those used in this study.

3.2. Shrimp production

Shrimp grew to a significantly larger average size of 11.6 g in the CW treatment versus 11.1 g in the BF treatment (Table 2) which corresponded with significantly greater biomass production in the CW treatment. Feed conversion ratio was also significantly better in

Table 1
Water quality data and shrimp isotope values between the two treatments. Data are mean ± SEM (range), superscript letters denote significant differences (P < 0.05) between treatments.

	Treatment	
	Biofloc (BF)	Clear-Water (CW)
Temperature (°C)	29.1 ± 0.1 (24.8–33.1)	29.0 ± 0.1 (25.1–32.5)
Dissolved Oxygen (mg L^{-1})	6.4 ± 0.0 (5.6–8.2)	6.3 ± 0.0 (5.2–8.8)
pH	7.7 ± 0.0 (6.6–8.1)[a]	7.9 ± 0.0 (5.9–8.9)[b]
Salinity (g L^{-1})	28.4 ± 0.2 (21.6–36.6)	28.8 ± 0.2 (21.3–35.6)
Ammonia (mg TAN L^{-1})	0.1 ± 0.0 (0.0–0.4)[a]	0.3 ± 0.0 (0.0–0.8)[b]
Nitrite (mg NO_2-N L^{-1})	2.2 ± 0.1 (0.2–2.8)[a]	0.9 ± 0.1 (0.3–2.7)[b]
Nitrate (mg NO_3-N L^{-1})	39.3 ± 3.8 (6.8–101.2)[a]	20.5 ± 1.5 (0.5–32.7)[b]
Turbidity (NTU)	90.1 ± 8.0 (10.6–174.0)[a]	6.1 ± 0.4 (1.9–11.4)[b]
Shrimp δ C^{13} (Unaltered)	−21.2 ± 0.2 (−22.0–−20.2)[a]	−21.3 ± 0.2 (−22.2–−20.4)[b]
Shrimp δ N^{15} (Unaltered)	9.7 ± 0.2 (8.7–10.5)	9.3 ± 0.3 (8.1–10.5)

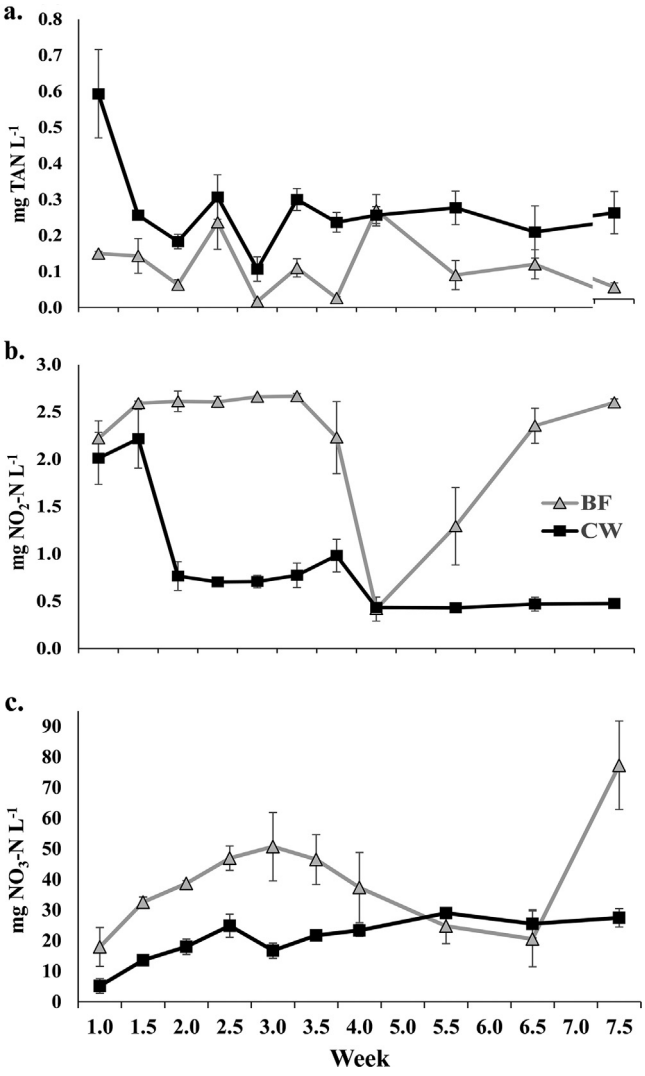

Fig. 1. Ammonia, nitrite, and nitrate concentrations over time during the project. Data points are treatment means and error bars are one SEM around the mean.

the CW treatment at 1.5:1, compared to 1.8:1 in the BF treatment. Although there were no significant differences in shrimp survival between the treatments, mean survival in the CW treatment was notably higher at 78% in contrast with 69% in the BF treatment. Based on these data, shrimp production was substantially better in the CW tanks.

One potential cause for relatively diminished shrimp production in the BF systems may have been high particulate concentrations

Table 2
Final shrimp production data between the two treatments at the end of the study. Data are mean ± SEM (range), superscript letters denote significant differences (P < 0.05) between treatments.

	Treatment	
	BF	CW
Individual Weight (g)	11.1 ± 0.2 (10.8–11.3)[a]	11.6 ± 0.3 (11.1–12.2)[b]
Biomass Produced (kg m^{-3})	1.7 ± 0.0 (1.6–1.8)[a]	2.0 ± 0.1 (1.9–2.2)[b]
Growth Rate (g wk^{-1})	1.4 ± 0.0 (1.4–1.4)	1.5 ± 0.0 (1.4–1.5)
FCR	1.8 ± 0.1 (1.7–1.8)[a]	1.5 ± 0.1 (1.3- 1.6)[b]
Survival (%)	69 ± 0.6 (68–70)	78 ± 4.3 (70–85)

indicated by the turbidity values. Excessively concentrated particles may increase oxygen demand of the microbial community, clog shrimp gills, promote nuisance microorganisms, and slow shrimp growth (Beveridge et al., 1991; Chapman et al., 1987; Ray et al., 2009). Higher nitrite and lower pH in the biofloc treatment, which are common observations in biofloc systems (Prangnell et al., 2016; Ray et al., 2011; Ray and Lotz, 2014), may have singularly, or in combination, contributed to lower shrimp production. Water quality in biofloc systems overall seems more variable than in clear-water systems, similar to the findings of Ray and Lotz (In Press).

These results appear to contradict a series of studies that indicated shrimp grown in water from ponds containing particulate matter performed significantly better than shrimp grown in clean, saline well water (Moss, 1995; Moss et al., 2001; Moss et al., 2006). These studies were conducted at much lower shrimp densities and particulate concentrations than the current study, and each study emphasized the important role that dense algal blooms and meiofauna likely played in shrimp growth. Although algae concentration was not measured in the current study, since the tanks were in a building with low light levels, the algal abundance was likely not substantial.

It has been noted that in more intensive systems the nutritional contribution of natural biota, such as biofloc, is reduced as higher animal density necessitates greater reliance on pelleted feeds (Tacon, 1993). Therefore, biofloc as a nutritional supplement may not be as important in intensive systems as it is in semi-intensive ponds whichcould be why the findings here seem to diverge from some previous studies.

3.3. Isotope dynamics

Shrimp from the CW treatment had significantly lower δ C^{13} values compared to shrimp from the BF treatment (Table 1). This indicates that shrimp from the two treatments obtained dietary

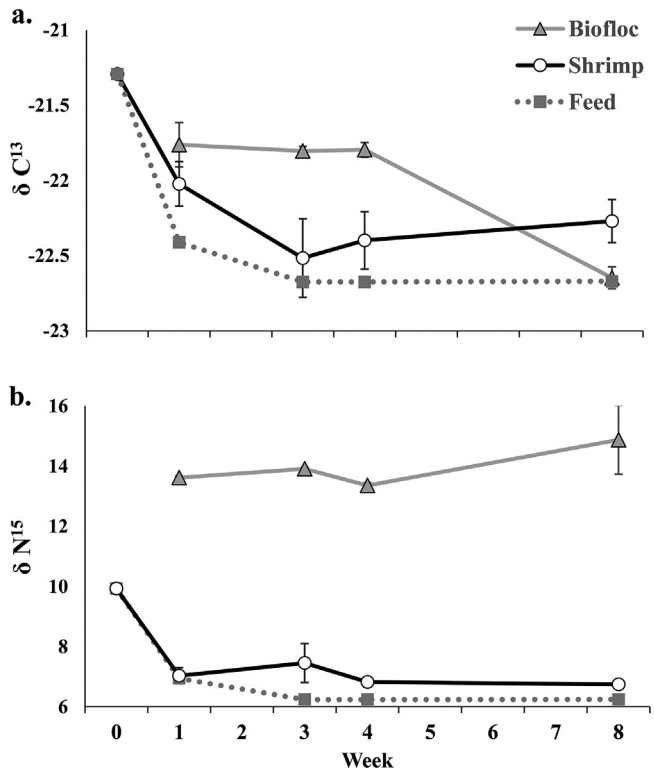

Fig. 2. Carbon (a) and nitrogen (b) isotope values over time in the biofloc treatment. Data points represent the mean values and error bars are one SEM around the mean.

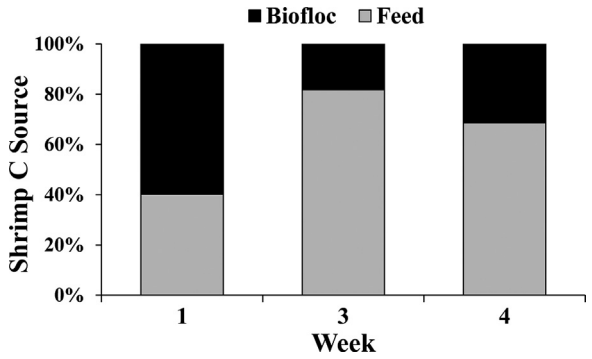

Fig. 3. The percent contribution of feed and biofloc to the carbon content of shrimp tissues according to a two-sample isotope mixing model.

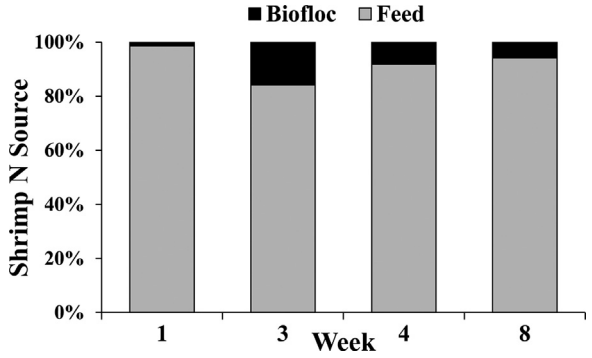

Fig. 4. The percent contribution of feed and biofloc to the nitrogen content of shrimp tissues according to a two-sample isotope mixing model.

carbon from different sources, suggesting that biofloc may have served as a source of carbon in the BF treatment. There were no significant differences between shrimp δ N^{15} values in the two treatments.

When fractionation values calculated using the CW treatment were subtracted from BF shrimp isotope values, the result was that BF shrimp values tended to fall between the biofloc and feed isotope levels (Fig. 2). On sample date zero, the shrimp looked isotopically like the feed, as they had been in a clear-water nursery with no biofloc available. Over time, the isotopic signatures of shrimp became closer to the δ-values of biofloc (Fig. 2). Mean BF shrimp δ C^{13} and δ N^{15} values fell between those of biofloc and feed on each sample date except one, indicating that shrimp may have received some C and N from the biofloc. BF shrimp δ C^{13} values (Fig. 2a) were relatively closer to the biofloc values than the δ N^{15} values (Fig. 2b), indicating that shrimp received more C from the biofloc than N. Examining the results of the two-sample mixing model indicates that shrimp received between 18 and 60% of the C (Fig. 3), and between 1 and 16% of the N (Fig. 4) in their tissues from biofloc. Shrimp may have received more C than N from biofloc due to better digestibility of C compounds or perhaps low N content of the biofloc. The temporal variability in biofloc contribution to shrimp nutrition may have been caused by shifts in the microbial community as it became established, especially during week one. Future studies should pair isotopic analyses with nutritional examinations of biofloc to better understand some of the underlying causes of these fluctuations and better take advantage of biofloc as a nutritional supplement.

Any nutritional contribution that shrimp received from biofloc did not improve shrimp production relative to shrimp in CW systems. Nitrogen, in the form of protein, is known to be the primary nutritional factor driving shrimp growth (Kuresh and Davis, 2000). Given that the estimated N contribution from biofloc to shrimp in this study was minimal, the biofloc may not have contributed much to shrimp growth. This is in contrast to the findings of Burford et al. (2004) who suggested that 18 to 29% of the N in shrimp tissues came from biofloc. Once more though, these authors obtained the biofloc in their study from a lower density pond.

4. Conclusions

In this study, CW tanks had significantly higher ammonia and pH concentrations, while BF systems had significantly higher nitrite, nitrate, and turbidity levels. Stable isotope data indicate that shrimp obtained between 18 and 60% of the C, and 1 to 16% of the N in their tissues from biofloc. However, these nutritional contributions from biofloc did not correspond to better shrimp production in the BF treatment. Individual shrimp weights, total biomass, and FCR were all significantly better in the CW treatment compared to the BF treatment. The exact reasons for differences in shrimp production are not clear; however, the dissimilarities in water quality may have played a role. The results of this study indicate that clear-water RAS may be a more productive option than biofloc systems for indoor marine shrimp production.

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