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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.