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Comparing biofloc, clear-water, and hybrid recirculating nursery systems (Part II): Tilapia (*Oreochromis niloticus*) production and water quality dynamics

Leo J. Fleckenstein*, Thomas W. Tierney, Andrew J. Ray

Division of Aquaculture, Kentucky State University, 103 Athletic Rd., Frankfort, KY, 40601 USA

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ABSTRACT

Indoor, recirculating aquaculture systems (RAS) can be used to nurse tilapia fry in a biosecure environment to support a variety of production systems; however, it is not understood what type of RAS may be most appropriate for this task. Clear-water and biofloc systems have advantages and disadvantages; hybrid systems combining positive features of both could optimize animal performance and minimize production costs. In this study, four replicate 160-L tanks were randomly assigned to clear-water (CW), biofloc (BF), or hybrid (HY) treatments. CW tanks had a settling chamber, foam fractionator, and external biofilter containing biomedia. BF tanks only had a settling chamber, and HY tanks used a settling chamber and external biofilter. Tilapia (Oreochromis niloticus) were stocked at 55 per tank (305 fish/m^3) at 0.17 g average weight. Total Ammonia Nitrogen (TAN) and nitrate were not significantly different between systems. Nitrite was significantly higher in BF compared to CW and HY systems during the last 4 weeks of the study. Turbidity was significantly higher in BF systems versus other treatments. Tilapia in HY systems had significantly higher average weight and specific growth rate compared to BF. Tilapia in BF systems had significantly higher feed conversion ratios and significantly lower harvest biomass versus other treatments. Diminished performance in BF systems likely resulted from inferior water quality conditions. The results indicate that CW or HY systems may be a better choice for tilapia nurseries than chemoautotrophic BF systems due to the short term periods in which nurseries operate and the volitility of nitrification in biofloc systems.

1. Introduction

Closed aquaculture systems allow a high level of control over production. Inputs such as water and feed are regulated by the producer and system parameters such as temperature, pH, and dissolved oxygen levels can be managed more easily than traditional ponds (Ray, 2012). Closed systems use less water than other aquaculture methods, thereby reducing the likelihood of environmental contamination (Verdegem et al., 2006). Such systems are versatile and can be placed indoors, allowing for increased biosecurity, production in diverse climates, and location near specific markets and consumers (Martins et al., 2010). Indoor aquaculture systems are becoming more popular, especially among tilapia (Oreochromis niloticus) producers in the United States (Watanabe et al., 2002). Tilapia producers can use indoor nursery systems for tilapia fry, increasing survival, overcoming seasonal constraints, and decreasing grow-out time when the fish are moved to a larger system such as a pond (Little et al., 2003; Bolivar et al., 2004).

Two types of closed aquaculture systems are biofloc (BF) and clear-water (CW) RAS. In BF systems biofloc particles are allowed to accumulate and are kept in suspension in the water column by aerating and mixing the water (Crab et al., 2012). These suspended particulates develop naturally and are composed of bacteria, algae, protists, zooplankton, and other organic matter (Avnimelech, 2009). The biofloc microbes assimilate dissolved nitrogen from the water or convert it to nitrate through nitrification (Browdy et al., 2012). Biofloc systems can be managed to favor the growth of heterotrophic or chemoautotrophic microbial communities. Heterotrophic bacterial growth can be encouraged by adding carbon sources to the system and raising the Carbon/Nitrogen ratio, which allows the direct assimilation of nitrogenous waste into bacterial biomass (Avnimelech, 2009). Chemoautotrophic dominant biofloc forms in systems with lower C/N ratios and may outperform heterotrophic dominated biofloc systems (Ray

Abbreviations: APT, aquaculture production technologies; BF, biofloc; CW, clear-water; DO, dissolved oxygen; HY, hybrid; MBBR, moving bed biofilm reactor; NTU, nephelometric turbidity Units; PPT, parts per thousand; RAS, recirculating aquaculture system; TAN, total ammonia nitrogen; TSS, total suspended solids levels; VSS, volatile suspended solids

* Corresponding author.
E-mail address: leo.fleckenstein@kysu.edu (L.J. Fleckenstein).

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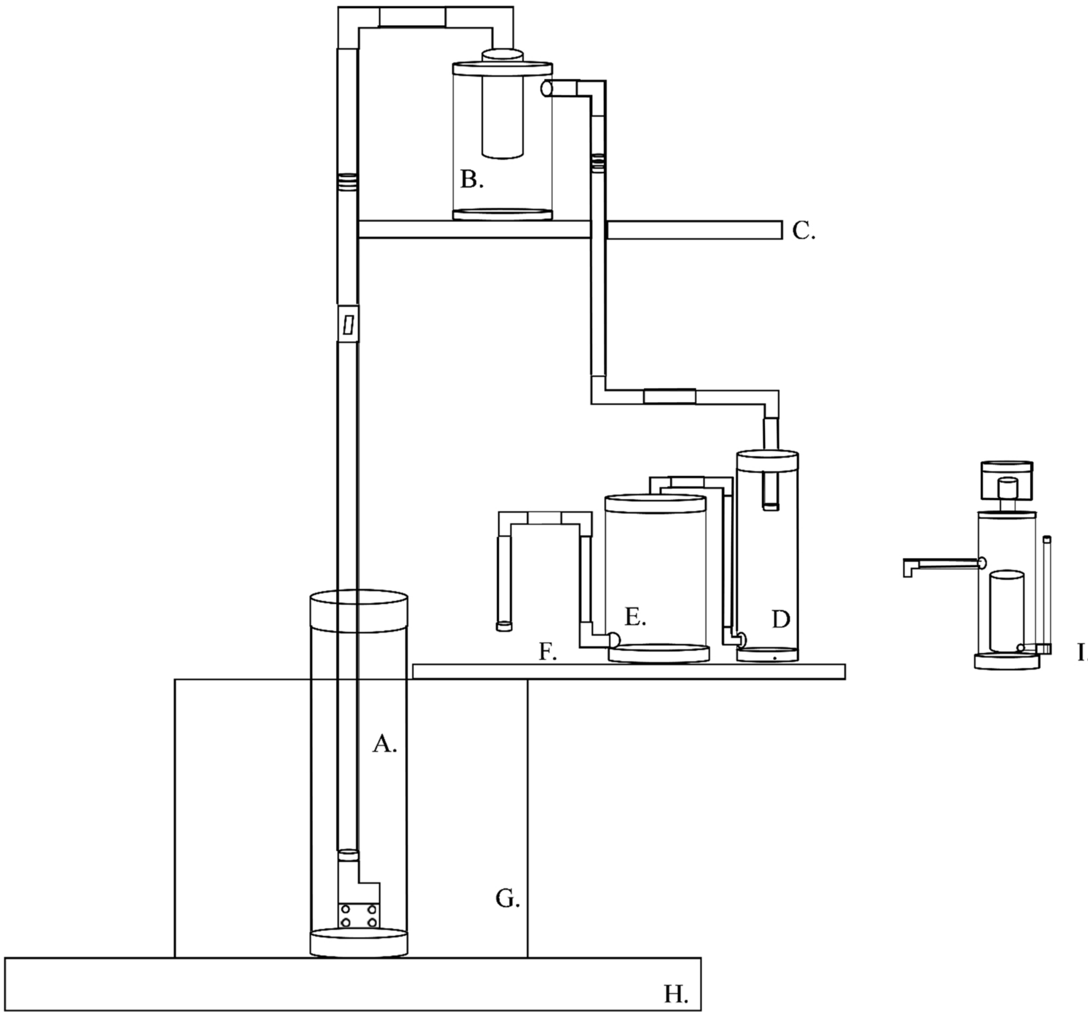

Fig. 1. Diagram showing the design for each system type.
A-Pump Basket and Water Pump
B-Settling Chamber
C-Upper Platform
D-Pseudo Foam Fractionator
E-Biofilter or Pseudo Biofilter
F-Middle Platform
G-Rearing Tank
H-Lower Platform
I-Foam Fractionator

and Lotz, 2014; Xu et al., 2016). Solids concentration in BF systems can be managed by an external settling chamber with no additional filtration (Ray et al., 2011). The biofloc particles can provide an additional in situ food source for animals such as shrimp and tilapia, thereby recycling nutrients and lowering feed conversion rates (Azim and Little, 2008; Hargreaves, 2013). Biofloc may also have a probiotic effect due to competitive exclusion of harmful microorganisms and possible enhanced digestive enzymatic activity in animals (Emerenciano et al., 2013; Kim et al., 2014). Potential drawbacks to operating BF systems include higher aeration requirements due to microbial oxygen demand and possible unstable nitrogen cycling (Hargreaves, 2013; Ray et al., 2017).

In contrast to BF systems, CW systems use more filtration components, including external biological and mechanical filters. The costs of buying and operating additional filtration components for a CW system may make this a more expensive approach. However, the nitrification cycle in CW systems may be more stable than in BF systems due to the controlled environment provided by external biofilters (Ebeling and Timmons, 2012; Ray et al., 2017). While these systems are more costly to establish, the benefits of additional stability and predictable harvests could ultimately make this technology a profitable option.

While both CW and BF systems are used to grow tilapia, adult fish produced in BF systems may have increased growth rates compared to CW systems (Azim and Little, 2008; Luo et al., 2014). Given that there are benefits and drawbacks to both the BF and CW systems, Hybrid (HY) systems could be developed that incorporate characteristics of each system type. If such a hybrid system includes external biofiltration, water quality may be more easily maintained. Similarly, if some biofloc particles are allowed to accumulate, the fish may have access to supplemental nutrition. The purpose of this project was to examine differences in fish performance and water quality dynamics between BF, CW, and HY systems as nurseries for tilapia.

2. Materials and methods

2.1. Systems and experimental design

This project was conducted in the Aquaculture Production Technologies (APT) Building at the Kentucky State University Aquaculture Research Center in Frankfort, Kentucky, USA. The APT building is a 1300 m^2, insulated facility with air temperature maintained at approximately 25 °C. There are two 1400-W regenerative blowers located outside the building that provide aeration to the entire facility. Three treatments (CW, BF, and HY) were used in this study. Each treatment was randomly assigned to four replicate tanks. Each CW tank had a setting chamber, a foam fractionator, and a moving bed biofilm reactor (MBBR). The cylindrical settling chamber was 25 cm (D) x 36 cm (H), had a functional volume of 12 L, and was intended to remove coarse solids. It included a central 10.2-cm diameter baffle suspended 10 cm above the bottom to reduce water velocity, allowing solids to settle (Ray et al., 2011). The foam fractionator (Reef Octopus Classic 110, Honya Co, Ltd, Guandong, China) removed dissolved and suspended solids (Chen et al., 1993). The fractionator was 15.8 cm (D) x 58.4 cm (H) operated with a Venturi nozzle, and had a foam collection cup, which was cleaned when full. The MBBR was the same size cylindrical container as the settling chamber, but half-filled (6 L) with plastic bio-media (Sweetwater SWX Bio-media, Pentair Aquatic Ecosystems Inc., Apopka, Florida, USA) to provide substrate (5.4 m^2) for the microbial community.

The BF tanks only had a settling chamber and the HY tanks had a settling chamber and an MBBR biofilter (these filters were the same as those described for the CW systems). To ensure that each system had the same overall water volume, each BF system had a pseudo foam fractionator and a pseudo MBBR, and each HY tank had a pseudo foam fractionator. The pseudo fractionators were built from a 10.2 cm PVC pipe and matched the volume of the fractionator from the CW system. Pseudo MBBRs were made from the same container as the functional MBBRs, but contained no biomedia. Both HY and BF systems were operated as chemoautotrophic biofloc systems. No additional carbon sources were added to the tanks other than feed. Since different feeds were added, a weighted average of the Carbon: Nitrogen ratio was determined (5.5:1).

2.2. System management

Each nursery tank (Fig. 1) had a submersible pump with a flow rate of 6.1 L/min that delivered water to the settling chamber. Water flowed via gravity from the settling chamber into the foam fractionator or pseudo fractionator, then into the MBBR or pseudo MBBR, and back into the fish tank. The settling chambers and MBBR containers had external stand pipes to maintain proper volume. Water was circulated constantly through all filtration components in CW systems. In the HY systems, if necessary, water could be bypassed around the settling chamber and diverted into the pseudo fractionator to allow biofloc particles to accumulate. Similarly, the BF system settling chamber could be operated on an as-needed basis. Settling chambers in the BF and HY systems were operated when turbidity was above 30 Nephelometric Turbidity Units (NTU). Turbidity was measured weekly and used as an indicator of biofloc concentration (Ray et al., 2010).

The twelve fish culture tanks (77 cm [L] x 46 cm [W] x 51 cm [H]) were operated at a water volume of 180 L. All 12 systems had been used previously in a marine shrimp (Litopenaeus vannamei) nursery study that evaluated the same systems and had the same experimental design as the current trial. The biomedia in the MBBRs and the biofloc in the BF tanks were thought to have established nitrifying microbial communities, as indicated by low ammonia and nitrite concentrations and elevated nitrate concentration at the end of the previous L. vannamei trial. The biomedia from that study remained in the filters and was aerated between studies to keep the microbial community alive. All systems from the previous study were aerated to keep the biofloc mixed and prevent the water from becoming stagnant. Time between the two experiments was 19 days. Nine grams of Ammonium Chloride was added to the systems 7 days after to shrimp trial ended to keep the nitrifying bacteria alive. Salinity in the systems was 32 parts per thousand (PPT) prior to this study and diluted to 10 PPT with dechlorinated municipal water. Approximately 1/3 of the water (60 L) from the previous study was retained during dilution to 10 PPT. Salinity was maintained at 10 PPT to increase the efficiency of foam fractionation and to ensure salinity was appropriate for tilapia nursery growth (El-Sayed, 2006; Wheaton, 1977). Each tank had a 300-W submersible electric heater set to 28.5 °C. All tanks had two 15-cm long ceramic air diffusers to aerate and mix the water. One diffuser was placed in the main fish tank and the other was used to aerate and prevent solids from accumulating in the MBBRs and pseudo MBBRs.

2.3. Animal husbandry

Tilapia fry produced from YY males were obtained from Louisiana Specialty Aquafarm (Robert, Louisiana, USA). The fish had a mean initial weight of 0.17 g ± 0.0 and 55 fish were stocked by hand into each tank at a density of 305 fish/m^3. During the experiment, the fish were fed Zeigler FinFish Starter Crumble #1 and Crumble #2 (Zeigler Bros., Inc. Gardners, Pennsylvania, USA), both 55% crude protein and 15% crude fat feeds, and Rangen Starter Crumble #3 (Rangen Inc. Buhl, Idaho, USA), a 55% protein and 17% fat diet. Feed rations and crumble sizes were calculated according to the recommendations of Riche and Garling (2003). Feeding rations started at 10% of the weight of fish per day and gradually decreased to 5% per day. Crumble size was determined based on the size of fish. Changes in feed were made by replacing 25% of the daily feed ration with the new feed each day until the new feed replaced the old feed entirely. Changes between feeds took place at 20 and 40 days into the study. All tanks were fed the same amount, regardless of treatment, and feed was provided at evenly spaced intervals three times per day. The experiment 9 weeks after which time all fish were counted, weighed individually, and weighed as a group. Specific growth rate and survival were determined at the end of the study. Specific growth rate (SGR) was calculated using the equation:

$$SGR = \left[\frac{\ln FinalWeight - \ln InitialWeight}{Days}\right] x100$$

Survival was calculated by dividing the number of surviving fish by the stocking number and multiplying by 100.

2.4. Water quality

Temperature, dissolved oxygen (DO), pH, and salinity were measured twice daily (morning and afternoon) in each tank using a YSI ProDSS multiparameter meter (YSI Incorporated, Yellow Springs, Ohio, USA). If pH in any tank was below 7.5, 10 g of sodium bicarbonate was added to raise pH (Loyless and Malone, 1997). Water levels were maintained with dechlorinated municipal water as needed to replace evaporation loss. Total ammonia nitrogen (TAN), nitrite, nitrate, and turbidity were each measured once weekly. TAN, nitrite, and nitrate were measured using Hach methods 8155, 8507, and 8039, respectively, and results were read on a Hach DR6000 spectrophotometer (Hach Company, Loveland, CO, USA). Turbidity was measured using a Hach 2100Q portable Turbidimeter. Total suspended solids levels (TSS) and volatile suspended solids (VSS) levels were measured using Environmental Sciences Section method 340.2 (Environmental Sciences Section, 1993).

Table 1
Water quality data from biofloc, clear-water RAS, and hybrid systems for tilapia nursery production. Not indicated in table: TSS/VSS data were significantly different between treatments in the last 4 weeks of the study. Data are presented as mean ± SEM (range). -Superscripts ([a b c]) indicate significant differences between treatments.

	Biofloc (BF)	Clear-water (CW)	Hybrid (HY)
Temperature °C			
AM	28.20 ± 0.2 (27.7-29.1)	28.35 ± 0.3 (27.5-29.8)	28.22 ± 0.2 (26.5-30.6)
PM	28.29 ± 0.2 (23.3-29.2)	28.40 ± 0.3 (22.2-29.7)	28.43 ± 0.1 (24.4-29.4)
Dissolved Oxygen (mg/L)			
AM	6.93 ± 0.05 (5.9-8.2) [a]	7.03 ± 0.04 (6.0-8.1) [b]	7.02 ± 0.03 (6.0-8.1) [c]
PM	6.76 ± 0.08 (5.6-7.8) [a]	6.90 ± 0.05 (5.7-7.4) [b]	6.87 ± 0.05 (5.6-7.8) [c]
pH			
AM	7.90 ± 0.04 (6.0-8.3) [a]	7.82 ± 0.05 (6.0-8.3) [b]	7.79 ± 0.06 (5.9-8.2) [c]
PM	7.88 ± 0.04 (7.4-8.3) [a]	7.82 ± 0.05 (7.5-8.2) [b]	7.78 ± 0.05 (7.3-8.2) [c]
Salinity (PPT)			
AM	10.53 ± 0.11 (10.0-11.1)	10.36 ± 0.16 (9.6-11.0)	10.47 ± 0.11 (9.9-11.1)
PM	10.50 ± 0.11 (9.9-11.1)	10.31 ± 0.16 (9.7-11.0)	10.42 ± 0.11 (9.9-11.1)
Turbidity (NTU)	16.2 ± 1.7 (1.6-53.3) [a]	4.4 ± 0.8 (0.9-11.1) [b]	5.0 ± 1.5 (0.8-14.3) [b]
TAN (mg TAN/L)	0.3 ± 0.1 (0.1-0.6)	0.2 ± 0.1 (0.1-0.5)	0.3 ± 0.1 (0.0-0.4)
Nitrite (mg NO_2−/L)	0.6 ± 0.1 (0.0-1.2) [a]	0.1 ± 0.1 (0.0-0.4) [b]	0.1 ± 0.0 (0.0-0.2) [b]
Nitrate (mg NO_3−/L)	25.4 ± 1.5 (7.7-51.8)	22.3 ± 2.1 (6.7-50.0)	23.4 ± 1.2 (7.0-51.6)
TSS (mg/L)	191.8 ± 45.5 (52.5-417.5)	132.1 ± 34.8 (0-342.5)	135.0 ± 58.4 (30-282.5)
VSS (mg/L)	190.0 ± 46.5 (62.5-325)	143.2 ± 40.2 (0-250)	141.1 ± 51.9 (45-202.5)
$NaHCO_3$ Added (g)	81.3 ± 7.2 (60-90) [a]	76.3 ± 3.8 (90-120) [a]	106.3 ± 6.9 (70-85) [b]

2.5. Data analyses

Statistical analyses were conducted using the program R 3.3.2 (R Core Team, 2016). An alpha value of 0.05 was used to determine whether significant differences existed between treatments. Water quality data were analyzed using a repeated measures analysis of variance (ANOVA) to account for trends in the data across the entire study. Fish production data (mean weight, biomass, specific growth rate, survival, and feed conversion ratio) were analyzed by one-way ANOVA. The total amount of sodium bicarbonate added to each tank was also analyzed via one-way ANOVA.

3. Results

There were no significant differences between the treatments in water temperature or salinity (Table 1). Average pH was significantly higher in the BF treatment versus either of the other two treatments, and pH was significantly lower in HY systems compared to CW systems. The HY systems had the lowest pH over the course of the trial and required a significantly higher amount of sodium bicarbonate to maintain pH compared to CW and BF systems (Table 1). Dissolved oxygen levels were significantly lower in BF systems compared to HY and CW systems and were significantly higher in CW systems compared to HY systems. Average turbidity was significantly higher in BF systems than in CW and HY systems (Fig. 2). Although there were no foam fractionators running on the HY systems, turbidity was never significantly higher in HY systems than CW (Fig. 2). Overall, no significant differences were found among systems for TSS and VSS levels however, TSS and VSS concentrations were significantly different between BF and HY/CW systems in the last 4 weeks of the study once the BF systems fully developed biofloc particles. Based on the repeated measures analysis, which considers the entire data set of repeated water quality measurements, TAN and nitrate concentrations were not significantly different between any of the treatments whereas nitrite concentration was significantly higher in BF systems compared to CW and HY systems (Fig. 3). Nitrate levels increased steadily over the course of the study in all treatments at a similar rate (Fig. 3C).

Average weight per tilapia, total harvest per tank, survival, SGR, and feed conversion ratio were used as metrics for assessing fish production (Table 2). Tilapia produced in the HY and CW treatments had significantly better performance metrics than those from BF systems except survival which was not significantly different among systems.

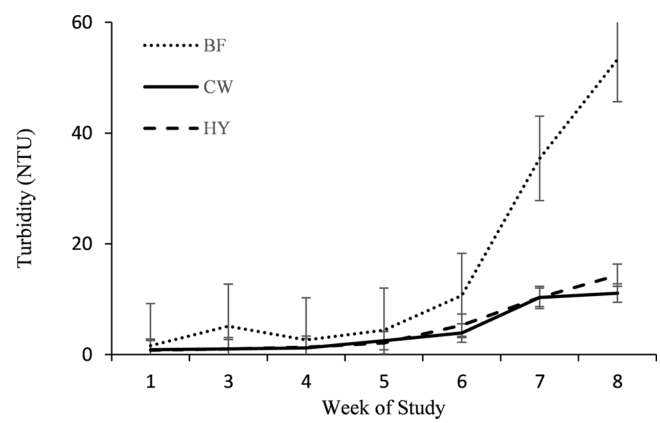

Fig. 2. Turbidity levels in all treatments over the course of the study. Each data point is the weekly average of all four replicates from each treatment.

None of the performance parameters were significantly different between CW and HY (Table 2). Tilapia produced in the HY treatment were significantly larger than tilapia from the BF treatment, and fish in both CW and HY treatments had significantly higher biomass per cubic meter than the BF systems. Feed conversion ratio was significantly lower in CW and HY treatments versus BF. The SGR was significantly higher in HY treatments compared to BF, but there was no significant difference in SGR between CW and the other treatments.

4. Discussion

Dissolved oxygen concentration, water temperature, and salinity were all within appropriate ranges for tilapia growth (DeLong et al., 2009). The lower pH in the HY systems could be due to an increased overall amount of bacterial activity compared to the other two systems. Both the external biofilter and biofloc particles in the water provided substrate for bacteria, possibly increasing the total respiration and therefore CO_2 production, which forms carbonic acid and decreases water pH. However, BF systems had the lowest DO levels over the course of the study, possibly due to increased microbial activity directly in the tanks where the DO was measured (Avnimelech, 2009). CW and HY systems had their bacterial biomass primarily concentrated in the MBBRs which were aerated to offset bacterial respiration. CW systems also had foam fractionators that are driven by venture nozzles that

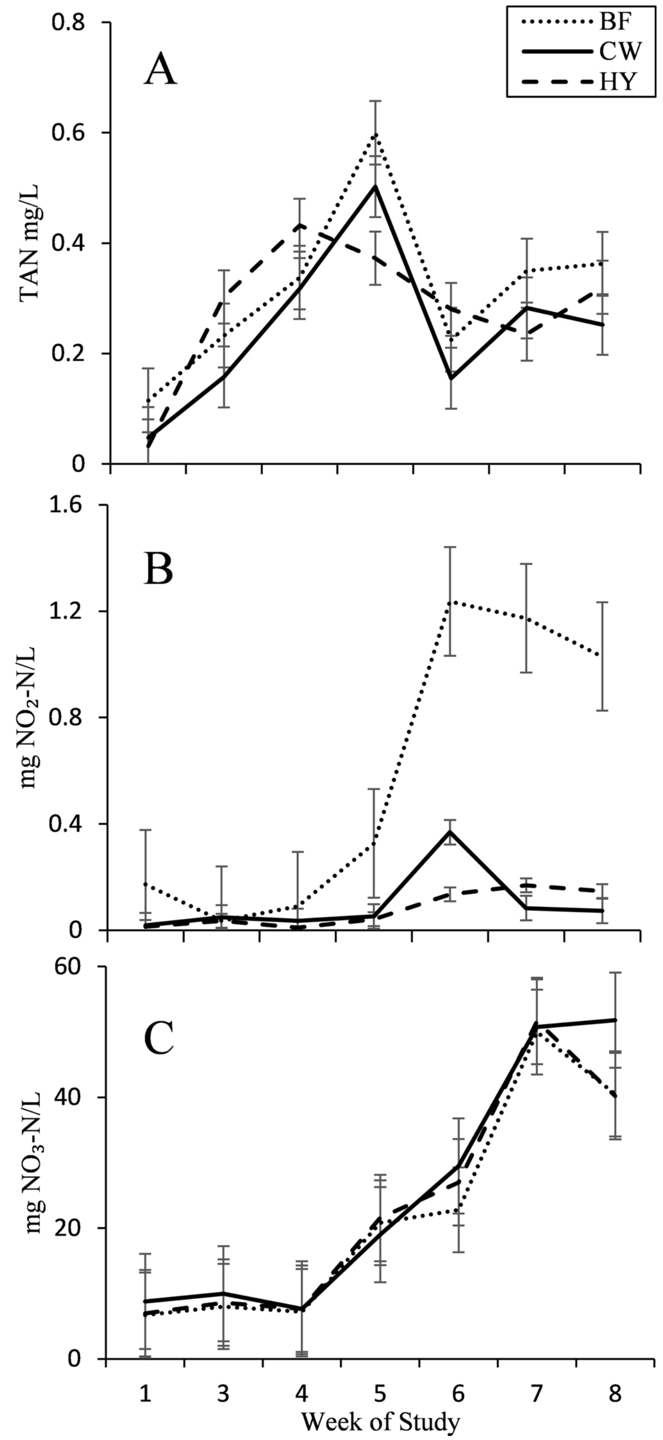

Fig. 3. Inorganic nitrogen in all treatments over the course of the study. Each data point is the weekly mean of the four replicates from each treatment. Error bars are one SEM.

heavily aerate the water, further increasing oxygenation and possibly causing CO_2 to diffuse out of the water. Turbidity was significantly higher in BF systems than CW and HY, suggesting BF systems had more microorganisms in the water column. Differences in TSS/VSS levels between treatments were likely due to biofloc formation in the BF systems. The BF systems had biofloc particles present from the previous study and despite ammonia chloride being added to feed nitrifying bacteria, biofloc concentration declined without culture animals present, as indicated by turbidity, and recovered only after tilapia were added.

Table 2
Production metrics from tilapia produced in biofloc, clear-water RAS, and hybrid systems. Data are presented as mean ± SEM. -Superscripts ($^{a\ b}$) indicate significant differences between treatments.

	Biofloc (BF)	Clear-water (CW)	Hybrid (HY)
Average weight (g)	10.7 ± 0.1^a	11.3 ± 0.3^b	11.4 ± 0.2^b
Biomass (kg/m^3)	3.3 ± 0.0^a	3.5 ± 0.0^b	3.5 ± 0.0^b
Survival	96.4 ± 0.9	95.5 ± 2.2	95.9 ± 1.7
FCR	0.9 ± 0.0^a	0.8 ± 0.0^b	0.8 ± 0.0^b
SGR	6.5 ± 0.0^a	6.6 ± 0.0 ^{ab}	6.7 ± 0.0^b

The concentration of TAN never exceeded levels considered lethal for tilapia fry in any treatment and un-ionized ammonia levels were below the recommended limit for chronic exposure (0.1 mg NH_3-N/L) (El-Shafai et al., 2004). No supplemental organic carbon was used in BF systems to select for heterotrophic bacterial assimilation of nitrogen; therefore, nitrification appeared to function well, as indicated by the accumulation of nitrate. Previous studies have indicated nitrite accumulation is a common problem in BF systems (Azim and Little, 2008; Ray et al., 2011, 2017). Tilapia fry are resistant to high nitrite levels, especially with chloride present (Atwood et al., 2001). However, tilapia performance in the BF systems may have been affected by the chronic exposure to nitrite at elevated levels. Relatively high nitrite concentrations were found in the BF systems, especially during the last half of the project, and these may have contributed to a chronic toxicity effect on the fish. Some studies have found that chronic nitrite exposure can have an effect on tilapia health which could reduce growth (Yildiz et al., 2006). In addition, all systems had elevated TAN at times; it is possible that the combination of higher TAN and concurrent high nitrite in the BF systems had a combined effect to reduce fish performance (Benli et al., 2008). Hybrid systems tended to perform better than CW in all metrics, although these differences were small and not significant. This may have been due to the biofloc particles tilapia could consume in the HY systems.

In conclusion, both CW and HY systems significantly outperformed BF systems in most production metrics. Although other studies have indicated that adult tilapia may perform well in biofloc systems, the results of this study indicate that chemoautotrophic biofloc systems may not be the most appropriate system for tilapia fry. The results of this study indicate that tilapia fry perform well in hybrid systems, which have lower startup costs than clear-water due to reduced filtration, but also include some suspended particles similar to biofloc techniques. Tilapia fry may benefit from having suspended particles in the water. However, the biofloc systems in this study had significantly higher nitrite levels than other systems and had the highest peak TAN levels, which may have contributed to lower growth rates. Other biofloc techniques, including heterotrophic systems may have lower nitrite levels than the systems used in the study. In conclusion, using external biofiltration can contribute to water quality stability in closed aquaculture systems and using hybrid systems in tilapia nursery production is a viable alternative to other established approaches. Future research should evaluate the performance of adult tilapia in these systems and should examine how suspended solids in the water column may contribute to the nutrition of tilapia.

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