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The effects of different solids and biological filters in intensive pacific white shrimp (*Litopenaeus vannamei*) production systems

Leo J. Fleckenstein*, Thomas W. Tierney, Jill C. Fisk, Andrew J. Ray

Division of Aquaculture, Kentucky State University Land Grant Program, 103 Athletic Drive, Frankfort, KY 40601, USA

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ABSTRACT

Biofloc systems rely on suspended solids in the water to house microbes that can remove or cycle nitrogenous wastes; however, nitrogen cycling can be inconsistent. In contrast, external biofilters are used in many recirculating systems to provide a more consistent environment for microbes to process nitrogen. Regardless of the biofiltration approach, solids levels must be controlled to prevent issues in shrimp such as gill fouling, low dissolved oxygen levels, and other negative impacts. The purpose of this study was to examine the effects of settling chambers versus foam fractionators for solids filtration and to compare external biofilters to the biofloc approach as biofiltration strategies. Sixteen 1-m^3 round, polyethylene tanks were randomly assigned to four treatments, each of which had four replicate tanks. Eight biofloc systems were established: four using settling chambers for solids control (BF-S) and four using foam fractionators (BF-F). The other eight tanks used external biofilters; four had settling chambers (EB-S) and the other four had foam fractionators (EB-F). All 16 systems were stocked with 250 shrimp at an average size of 4.3 g which were grown for 85 days. There were no significant differences in shrimp production between treatments; however, variability was high in biofloc systems. Nitrite levels were significantly lower in systems with fractionators compared to systems with settling chambers. The concentrations of dissolved Na, Mg, Ca, Sr and Ba in the water were significantly reduced in treatments with settling chambers. The results of this study show that filtration choices significantly impact short- and long-term water quality and reusability but may not have much effect on shrimp production in the short-term.

1. Introduction

Recirculating aquaculture systems (RAS) that utilize biofloc technology are increasing in popularity with shrimp farmers worldwide (Crab et al., 2012; Emerenciano et al., 2013; Rego et al., 2017). Biofloc systems allow for the controlled accumulation of particles that contain microorganisms such as bacteria, fungi, algae, and protists (Avnimelech, 2009). The particles provide surface area for microbes that act as a biological filter and may provide a source of supplemental nutrition for shrimp (Xu et al., 2012; Ahmad et al., 2017). The water in biofloc systems must be heavily aerated to keep particles suspended and offset the additional oxygen demand of the microbial community. Managing the concentration of biofloc particles is critical to the successful operation of systems. Biofloc levels that are too high can cause gill fouling, undesirable bacteria growth, and increased oxygen demand while low concentrations can lead to a collapse in the ammonia removal cycle and result in water quality issues (Ray et al., 2010a; Coyle et al., 2011).

In contrast to the biofloc approach, many RAS have external biofilters to control nitrogenous wastes, which may promote more stable dissolved inorganic nitrogen concentrations (Ray et al., 2017). Biological filters are widely utilized in RAS with different designs and media types (Malone and Pfeiffer, 2006). One such design is a moving bed biological reactor (MBBR), which contains inert plastic media that acts as a substrate for nitrifying bacteria and is heavily aerated to move the media, facilitate contact with water, and prevent excessive solids from settling (Tal et al., 2003; Ebeling and Timmons, 2012). Systems that include a MBBR but still allow biofloc particles to accumulate can be called "hybrid" systems. Hybrid systems help overcome the instability of nitrogen cycling in biofloc systems by combining the internal nitrogen processing of biofloc with the external nitrification of a biofilter, while potentially providing the nutritional benefits of suspended particulates to shrimp (Tierney and Ray, 2018; Fleckenstein et al., 2018, 2019).

External filters are attached to the culture systems to remove excess particles from the water (Ray et al., 2011; Hargreaves, 2013). Foam fractionators and settling chambers are two common solids filtration devices used in RAS (Losordo et al., 1998; Haslun et al., 2012). Foam

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

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fractionators work by creating a stream of small bubbles that are injected into a column of water. As the bubbles pass through the water, hydrophobic particles and molecules are adsorbed to the air/water boundary of the bubbles (Chen et al., 1993a, 1993b). These bubbles travel to the top of the water column and are channeled out of the chamber into a waste collection area (Chen et al., 2011). Settling chambers are simple filtration systems that remove suspended solids from the water through gravity. Typically, they are designed such that water enters the top of the chamber and passes down through a central baffle. The baffle is intended to slow water flow and is suspended just above the bottom of the chamber. The water outlet is located near the top of the settling chamber to ensure that water travels slowly through the entire chamber. This environment allows heavier particles to collect at the bottom of the chamber where they can be removed through a drain or by decanting the relatively clear water above.

The choice of a solids filter can affect operation costs and impact water quality (Ray et al., 2010b, 2012). Foam fractionators generally require a pump to deliver water and an additional pump or blower to provide aeration. In comparison, settling chambers can be operated using a single pump or airlift (Ray et al., 2010a; Haslun et al., 2012). Regarding water quality, settling chambers can facilitate denitrification at the interface of the anaerobic settled solids and the water (Ray et al., 2010a). This process removes nitrate and generates alkalinity, which are substantial benefits for long term water use in RAS (Haslun et al., 2012). Other water quality factors, such as the concentrations of ions, may also be affected by different filtration choices. Certain ions, such as Na, Ca, Mg, and K, are critical for physiological functions of shrimp (Saoud et al., 2003; Roy et al., 2007; Jasmani et al., 2010; Roy et al., 2010). Some dissolved ions, including K and Mg, bind to sediment (and presumably to biofloc particles) in the water through electrostatic attraction and may be removed by solids filters (Pine and Boyd, 2010).

The purpose of this study was to compare the effects of settling chambers and foam fractionators on shrimp performance and water quality in both hybrid and biofloc (with and without external biofilters, respectively) shrimp production systems.

2. Materials and methods

2.1. Experimental design and operation

This experiment was conducted in the Sustainable Aquaculture Development Laboratory (SADL) at the Kentucky State University (KSU) Aquaculture Research Center, located in Frankfort, KY. The SADL is a 174-m^2 insulated building that is heated, which allows for year-round shrimp production. Sixteen 1-m^3 round HDPE tanks were randomly assigned to four treatments: biofloc with settling chamber (BF-S), biofloc with foam fractionator (BF-F), external biofiltration with settling chamber (EB-S), and external biofiltration with foam fractionator (EB-F). All systems were under a 12 h on, 12 h off light-dark schedule using florescent lights mounted on the ceiling; no natural light reached the tanks.

The settling chambers used in this study were 18-L and designed based on those used by Ray et al. (2010a). A small pump moved water to each chamber where a 7.6-cm central baffle slowed water flow, allowing solids to settle. Flow rates were adjusted to between 2.5 L per minute (LPM) and 7.5 LPM according to turbidity levels; target turbidity was 75 Nephelometric Turbidity Units (NTU) as suggested by Ray et al. (2011, 2012) and maintained by adjusting flow rates on settling chambers or foam fractionators as needed. The foam fractionators used were Twisted Skimmer (Model 8–30; Bashsea; Ferndale, MI, USA). The fractionators were 72 cm tall and had a 20-cm diameter collection chamber. The total water volume in each fractionator was 25 L. The fractionators were each driven with an 83-LPM pump that operated a Venturi-style nozzle built into the fractionator. Both the foam fractionators and the settling chambers were cleaned weekly. The foam fractionators were cleaned by removing the collection container located at the top of the unit and emptying it. The settling chambers were cleaned by shutting off the flow to the chambers for 1 h, allowing the collected material to settle completely, and siphoning the clear water out of settling chamber back into the shrimp tank. The settled material in the chamber was then removed and the chamber connected back to the system. This minimized the amount of water lost during filter cleaning. In the hybrid systems, water from the settling chamber outlet or foam fractionator flowed into an 18-L MBBR containing 4.5 L of biomedia (Curler Advance X-1; Aquaculture Systems Technologies, LLC.; New Orleans, LA, USA) that provided 4 m^2 of additional surface area for nitrifying bacteria. Fifteen percent of the seeded media used in the study was obtained from one of the nursery systems (described in section 2.3 *Animal Husbandry*) in which the shrimp were originally stocked as post larvae (PL) from the hatchery. This was to ensure the biomedia were seeded with bacteria prior to the start of this experiment. All systems were aerated using a 560-watt regenerative blower and three 15-cm long ceramic diffusers in each shrimp tank; the biofilters were aerated using one 15-cm long diffuser. Fifteen percent of the water used in each tank was from the shrimp nursery to ensure a mature, nitrifying biofloc was present while the remainder was dechlorinated municipal water with commercial sea salt added to make 15 PPT salinity (Crystal Sea Bioassay Laboratory Formula; Marine Enterprises International, Baltimore, MD, USA).

2.2. Water quality

Temperature, dissolved oxygen (DO), pH, and salinity were measured twice daily (0800 and 1600 h) using a YSI Professional Plus Multimeter (Yellow Springs, OH, USA). Temperature was maintained at approximately 28 °C using one 1000-watt submersible heater per tank. The DO concentration was targeted at 6 mg/L and adjusted using ball valves to control air flow into the tanks. If pH levels fell below 7.8, sodium bicarbonate was added to the systems to increase pH at a rate of approximately 30 g/0.1 pH unit. Salinity levels were maintained between 14−15 g/L by topping off water lost to evaporation and filtration unit operation with dechlorinated municipal water. The amount of water added weekly to each tank was calculated by measuring the volume of water lost in each tank. Turbidity was measured once daily, at 0830 h, using a Hach 2100Q Turbidimeter (Hach Company, Loveland, CO, USA). Total ammonia nitrogen (TAN), nitrite, and nitrate were measured weekly with a Hach DR6000 Spectrophotometer using Hach methods 8155, 8507, and 8039, respectively.

2.3. Animal husbandry

The shrimp used in this study were obtained from American Penaeid Inc. (St. James City, FL, USA) and shipped to KSU at the PL12 stage. The shrimp were stocked into two 3.4-m^3 hybrid nursery systems at 3200 shrimp per m^3. The two nursery systems were 3800-L raceways, each with a 208-L MBBR and a 130-L settling chamber. The shrimp were fed Zeigler Brothers Raceway Plus Diets PL0, PL1, PL2, and PL3 (50 % protein, 15 % fat, 1% fiber, 10 % moisture, and 7.5 % ash) before being transitioned onto Zeigler Hyper-Intensive Shrimp-35 (35 % protein, 7 % fat, 2% fiber, 12 % moisture, and 15 % ash) for the remainder of the study (Zeigler Brothers, Inc., Gardners, PA, USA). The nursery phase was conducted for 46 days and the shrimp were stocked into the research systems at 250 shrimp/m^3 and an average of 4.3 g/shrimp. All treatments were fed the same amount of feed three times daily at approximately 0800, 1200, and 1600 h. The amount of feed per day was calculated using estimated growth rates (1.5 g/week) and feed conversion ratio (FCR; 1.5:1). Changes to feed amounts were also made based on water quality parameters and uneaten feed. One tank from each treatment was randomly checked each day for uneaten feed and dead shrimp which were removed immediately. If uneaten feed or dead shrimp were found, all tanks within that treatment were checked. The study lasted 85 days and all shrimp in each system were weighed and counted at harvest. The data collected from harvest were used to calculate individual shrimp weight, growth per week, total harvest per tank, FCR, and survival.

2.4. Elemental analyses

Water samples were filtered to 0.7 μm and tested for nitrate, sulfate, and several elements important for shrimp and microbial physiology or that can be toxic to shrimp. Tests for Na, Mg, P, K, Ca, Cr, Fe, Ni, Cu, As, Sr, Cd, Ba, Hg, Pb, nitrate, and sulfate were performed at the University of Georgia's Laboratory for Environmental Analysis (Athens, GA, USA). Nitrate (measured a second time to ensure accuracy) and sulfate were measured using ion chromatography and individual elements were measured using inductively coupled plasma-mass spectrometry (ICP-MS).

2.5. Statistical analyses

All final shrimp production metrics, elemental analyses, and final water quality measurements were analyzed using a two-way ANOVA with biological filter type and solids filter type as the two factors. All daily and weekly water quality measurements were analyzed using a two-way repeated measures ANOVA. All statistical tests were performed using SigmaPlot 10 (Systat Software, Inc., San Jose, CA, USA).

3. Results

There were no significant differences in shrimp production due to either biofilter or solids filter type and no interactions were found ($p > 0.05$, Table 1). Notably, mean weight, shrimp biomass per m^3, survival, and FCR were each highly variable in both biofloc treatments compared to the hybrid systems.

Temperature, DO, and pH were not significantly different between any treatments ($p > 0.05$, Table 2). Salinity was significantly lower in both treatments with settling chambers compared to treatments with fractionators ($p = 0.03$). Biofloc systems (BF-S and BF-F) required significantly more bicarbonate (90 g/week added in BF, 55 g/week added to HY) to maintain pH levels compared to both hybrid treatments ($p = 0.01$). There were no significant differences in turbidity over the course of the study. TAN concentration was not significantly different among treatments ($p > 0.05$). Nitrite levels were significantly lower in treatments with foam fractionators (BF-F and EB-F) than in treatments with settling chambers ($p = 0.05$). Nitrate levels followed a similar trend to that observed for nitrite although the differences were not significant ($p > 0.05$, Table 3). The was no significant difference in water volume change each week due to evaporation and water loss from filter cleaning (Average weekly loss of 30 L per tank). There were no interactions between solids filters or system type.

Table 1
Mean ± SD shrimp production metrics at harvest. Different superscripted letters in a row denote significant differences between treatments ($p < 0.05$) Treatment abbreviations: external biofiltration with foam fractionator (EB-F), external biofiltration with settling chamber (EB-S), biofloc with foam fractionator (BF-F), and biofloc with settling chamber (BF-S). All p-values were rounded up to the nearest hundredth.

	Treatment				p-values (filter type)	
	EB-F	EB-SC	BF-F	BF-SC	Biofilter	Solids Filter
Avg. Wt. (g)	21.3 ± 0.5	22.3 ± 1.4	22.0 ± 3.2	22.7 ± 2.5	0.63	0.79
Biomass (kg/m^3)	3.5 ± 0.5	3.4 ± 0.7	2.9 ± 1.6	2.1 ± 1.6	0.75	0.75
Survival (%)	65.2 ± 1.0	60.3 ± 6.1	52.4 ± 26.6	39.2 ± 30.4	0.79	0.85
FCR	2.5 ± 0.4	2.6 ± 0.7	5.0 ± 5.4	10.0 ± 12.4	0.52	0.31

Table 2
Mean ± SD water quality values over the course of the study. Different superscripted letters in a row denote significant differences between treatments ($p < 0.05$). Treatment abbreviations: external biofiltration with foam fractionator (EB-F), external biofiltration with settling chamber (EB-S), biofloc with foam fractionator (BF-F), and biofloc with settling chamber (BF-S). All p-values were rounded up to the nearest hundredth.

	Treatment				p-values	
	EB-F	EB-SC	BF-F	BF-SC	Biofilter	Solids Filter
TAN (mg/L)	0.2 ± 0.3	0.2 ± 0.3	0.1 ± 0.2	0.2 ± 0.2	0.15	0.36
Nitrite (mg/L)	0.5 ± 0.5^{b}	0.8 ± 0.5^{a}	0.5 ± 0.4^{b}	2.7 ± 4.1^{a}	0.14	0.05
Temperature (°C)	28.0 ± 0.5	28.3 ± 0.5	28.2 ± 0.5	28.2 ± 0.6	0.78	0.65
Dissolved Oxygen (mg/L)	6.8 ± 0.4	6.8 ± 0.4	6.8 ± 0.4	6.8 ± 0.3	0.97	0.96
pH	7.8 ± 0.0	7.9 ± 0.0	7.9 ± 0.0	7.8 ± 0.0	0.80	0.46
Salinity (g/L)	17.7 ± 4.8^{a}	16.8 ± 4.1^{b}	17.2 ± 4.7^{a}	16.8 ± 4.0^{b}	0.36	0.03
Turbidity (NTU)	76.8 ± 25.0	71.9 ± 25.1	95.9 ± 28.9	68.6 ± 17.7	0.54	0.22

Table 3
Dissolved elements, nitrate, and sulfate concentrations at harvest. All data are presented as mean concentration (mg/L) ± SD. Different superscripted letters in a row denote significant differences between treatments ($p < 0.05$). Treatment abbreviations: external biofiltration with foam fractionator (EB-F), external biofiltration with settling chamber (EB-S), Biofloc with foam fractionator (BF-F), and biofloc with settling chamber (BF-S). All p-values were rounded up to the nearest hundredth.

	Treatment				p-values	
	EB-F	EB-SC	BF-F	BF-SC	Biofilter	Solids Filter
Na	11365.0^{a} ± 515	8970.7^{b} ± 619	9778.3^{a} ± 990	9589.0^{b} ± 472	0.39	0.04
Mg	1655.0^{a} ± 150	1104.5^{b} ± 129	1330.8^{a} ± 175	1095.0^{b} ± 51	0.13	0.00
P	10.6^{a} ± 1.3	15.3^{b} ± 2.2	14.7^{b} ± 2.1	14.3^{b} ± 3.6	0.58	0.02
K	318.4 ± 3.2	265.9 ± 33.2	241.2 ± 21.7	288.7 ± 49.0	0.63	0.47
Ca	199.7^{a} ± 15.8	154.6^{b} ± 8.2	177.7^{a} ±18.0	165.5^{b} ± 6.9	0.58	0.02
Cr	0.27^{a} ± 0.0	0.26^{b} ± 0.0	0.28^{a} ± 0.0	0.26^{b} ± 0.0	0.70	0.00
Fe	24.6 ± 0.6	24.8 ± 2.8	24.1 ± 2.0	25.8 ± 1.3	0.52	0.87
Ni	0.23^{b} ± 0.0	0.24^{b} ± 0.0	0.30^{a} ± 0.0	0.31^{a} ± 0.0	0.02	0.47
Cu	0.06 ± 0.0	0.08 ± 0.0	0.06 ± 0.0	0.07 ± 0.0	0.10	0.18
Sr	8.1^{a} ± 0.6	5.8^{b} ± 0.3	7.0^{a} ± 0.8	6.3^{b} ± 0.1	0.47	0.00
Cd	0.00 ± 0.0	0.03 ± 0.0	0.02 ± 0.0	0.01 ± 0.0	0.48	0.80
Ba	0.06^{a} ± 0.0	0.02^{b} ± 0.0	0.05^{a} ± 0.0	0.03^{b} ± 0.0	0.70	0.00
Nitrate-N	81.2 ± 18.0	107.1 ± 36.2	93.3 ± 28.3	108.4 ± 17.8	0.74	0.33
Sulfate	1916.6 ± 6.8	1372.7 ± 236.4	1532.5 ± 823.3	1459.6 ± 354.7	0.71	0.44

Significant differences were found between treatments for final concentrations of measured elements (Table 3). Treatments with settling chambers had significantly lower levels of Na, Mg, Ca, Sr, and Ba compared to treatments with fractionators ($p < 0.05$). Biofloc treatments had significantly higher levels of Ni present than hybrid treatments ($p = 0.02$). There were no interactions between solids filters or system type.

4. Discussion

Shrimp production was not significantly impacted using biofilters or the use of different solids filters. However, the variability of the production metrics in biofloc treatments, specifically survival, harvest per m^3 and FCR was very high compared to hybrid treatments. The variability in the biofloc systems may be an indication of water quality inconsistency in biofloc-based shrimp production, which would create problems in business planning and farm management (Ray et al., 2017; Ferreira et al., 2020). Biofloc systems have been documented to have fluctuating or elevated nitrogenous compound concentrations, especially nitrite (Vinatea et al., 2010; Prangnell et al., 2016; Khanjani et al., 2017; Lara et al., 2017; Ray et al., 2017; Fleckenstein et al., 2018; Tierney and Ray, 2018). The increased nitrite levels in this study likely decreased feed consumption, decreasing FCRs, and any excess feed contributed to nitrogenous waste accumulation, creating a negative feedback loop. This resulted in high nitrite spikes and decreased survival, particularly in the BF-SC treatment. However, it is important to note that biofloc systems at similar levels of biofloc density have been used successfully with high shrimp survival (Krummenauer et al., 2014; Fleckenstein et al., 2020). The increased need for sodium bicarbonate to maintain pH levels in the biofloc systems is likely due to the increased FCRs and excess feed as well, caused by increased nitrification and microbial activity in the system.

The significant differences found in water quality due to the type of solids filter have important implications for shrimp producers. High nitrite levels in systems with settling chambers also contributed to the relatively poor mean survival values and increased FCRs. Shrimp are sensitive to elevated nitrite levels above 6 mg/L, which can result in poor shrimp performance (Alcaraz et al., 1999; Lin and Chen, 2003). The foam fractionators reduced nitrite levels regardless of whether they were used in hybrid or biofloc systems, due to the fractionators ability to remove proteins and other compounds from the water column (Chen et al., 1993b; Mishra et al., 2008). The removal of proteins from the water also reduced average nitrate levels found in treatments with fractionators, which also impacts long term shrimp production and water reuse, an important topic in RAS. Nitrate is a significant limiting factor in RAS because it accumulates quickly in systems with limited water exchange (Losordo et al., 1998) and can affect shrimp performance (e.g. Kuhn et al., 2010). The use of fractionators may allow for longer periods of shrimp production before the water needs to be discharged or remediated, decreasing water use, salt use, heat loss, and overall costs for producers while also reducing environmental impacts. Effluents from shrimp aquaculture can be difficult to manage, especially for inland operations, because the effluent is saline and unsuitable for most terrestrial agriculture and many water treatment facilities cannot process saline water (Whetstone et al., 2002; Boopathy et al., 2007; Browdy et al., 2012).

The impacts of the significant difference in Ni between biofiltration types is unclear; more research is needed to determine the roles of this element in shrimp performance. The reduced concentrations of salt ions in systems with settling chambers also have important implications on long-term water use and recurring costs for shrimp producers. The salt components Na, Mg, and Ca are essential for the production of marine shrimp; the lower concentration of these ions in systems with settling chambers may have negative impacts on shrimp performance over multiple crops if the ions are not replaced (Saoud et al., 2003; Roy et al., 2007; Jasmani et al., 2010; Roy et al., 2010). The elements Sr and Ba were removed as well, however the role of these elements on shrimp performance is poorly documented and should be further investigated. Fractionators seem to maintain salinity to a greater extent than settling chambers, likely due to the higher retention of salt components, most notably the alkaline earth metals. These elements appear to have been impacted by the operation of the settling chambers. In pond-based shrimp aquaculture, Na, Mg, Ca, and P can be bound in solids at the bottom of the pond (Ritvo et al., 1998; Roy et al., 2010). The settling chambers may have created a similar environment such that these ions were trapped in settled solids and removed when the settling chambers were cleaned. Ultimately, the fractionators in this experiment created a more suitable environment for long-term shrimp production with regard to the measured elemental concentrations and nitrogen concentrations compared to settling chambers.

5. Conclusion

In summary, the variability of shrimp production was lower in hybrid systems than biofloc, although there were not any significant differences in shrimp production among the treatments in this experiment. The use of foam fractionators, regardless of the type of production system, led to significantly lower nitrite and corresponded with significantly higher Na, Mg, and Ca. These findings indicate that foam fractionators may be a better choice for solids filtration with regard to nitrite/nitrate accumulation and long-term water reuse. Further research should investigate long-term effects of these filtration mechanisms, their effectiveness with increased densities of shrimp, and the potential of combining solids filtration devices.

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Ethical statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

CRediT authorship contribution statement

Leo J. Fleckenstein: Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing - original draft, Writing - review & editing. **Thomas W. Tierney:** Formal analysis, Investigation, Writing - review & editing. **Jill C. Fisk:** Formal analysis, Investigation, Writing - review & editing. **Andrew J. Ray:** Conceptualization, Funding acquisition, Methodology, Project administration, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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