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Comparing salinities of 10, 20, and 30‰ in intensive, commercial-scale biofloc shrimp (*Litopenaeus vannamei*) production systems

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Abstract

Minimal-exchange, intensive biofloc aquaculture systems offer a viable means of culturing marine animals at inland locations due to very low rates of water use. Fresh, never-frozen shrimp can be provided to metropolitan markets; however, the cost of artificial salt can be substantial. The purpose of this project was to examine commercial-scale biofloc shrimp production at three different salinities. Nine raceways were randomly assigned to three salinity treatments: 10, 20, and 30‰ (LS, MS, and HS), each treatment contained three raceways operated at 50 m^3. The raceways were operated as heterotrophic biofloc systems, with daily additions of sucrose to raise the C:N ratio. Temperature, dissolved oxygen, pH, and salinity were all maintained at consistent levels. Spikes of ammonia and nitrite occurred in all tanks but nitrate remained low, with a peak value of 8.7 mg NO_3-N L^{-1}. There were no significant differences in any shrimp production metric. Mean shrimp growth rate was 1.8, 2.0, and 2.0 grams $week^{-1}$ in the LS, MS, and HS treatments respectively. Mean feed conversion rate was 1.6, 1.2, and 1.2 in the LS, MS, and HS treatments respectively, and mean final weight ranged from 17.8 to 19.3g. The only time water was removed from the systems was when settling chambers were emptied, resulting in a total mean water replacement of 5.2% or less per raceway. The mean volume of full strength seawater used to produce shrimp was 104, 159, and 235 L kg^{-1} of shrimp in the LS, MS, and HS treatments respectively. Although there were no significant differences in shrimp production metrics between treatments, these values were noticeably lower in the LS treatment due to human error. Operating at the low salinity of 10‰ reduces salt use by about 50% over the MS treatment which implies substantial cost savings for production facilities. This study helps to illustrate the range of salinity options for shrimp production in commercial-scale biofloc systems.

Keywords: Biofloc; RAS; white shrimp; *L. vannamei*; salinity

Running Title: Salinity and commercial-scale biofloc systems

Introduction

Minimal-exchange, intensive, biofloc-based systems undergo low rates of water exchange. This greatly enhances biosecurity, reduces or eliminates pollution from effluent, and may facilitate inland culture of marine animals (Hargreaves, 2013). These systems are stocked at high animal densities, reducing the area needed to culture animals and making indoor, temperature controlled production possible (Prangnell et al., 2016). As a result of low water exchange, high stocking densities, and because only crude solids filters are needed, a dense microbial community develops in the water column. This microbial community is responsible for remediating nutrients, some of which are potentially toxic to shrimp (De Schryver et al., 2008). The microbial community contained on flocculated (biofloc) particles may also provide nutrition to shrimp, thereby helping to recycle expensive nutrients from feed (Burford et al., 2004; Moss 1995; Wasielesky et al., 2006).

These systems can be sited away from the coast, using less expensive land and in close proximity to urban areas where demand for fresh, never-frozen shrimp can be substantial (Browdy and Moss, 2005). Even with minimal-exchange systems some water must be replaced due to solids management and controlling the accumulation of contaminants such as nitrate and metals (Kuhn et al., 2010, Frías-Espericueta et al., 2001). Therefore, the cost of artificial sea salts or imported seawater can be a substantial expense at inland facilities and systems should be operated at the lowest salinity possible to optimize financial returns.

The isosmotic point for *L. vannamei* has been reported to be 24.7‰ salinity (Castile and Lawrence, 1981). At this salinity, shrimp should not have to expend energy hypo- or hyper-osmoregulating to maintain haemolymph osmolality. At lower salinities *L. vannamei* must

hyperosmoregulate; a function performed partially by the renal organ and antennal gland, but to the largest extent by the gills (Roy et al., 2010).

There are conflicting reports of whether *L. vannamei* growth rates are compromised at salinities below the isosmotic point. Bray et al. (1994) found no difference in shrimp growth between animals grown in 5 and 15‰ salinity and no difference in growth between shrimp grown in 25 and 35‰ salinity. However, the shrimp cultured in the two lower salinities grew faster than those at the two higher salinities. This is in contrast to a more recent study by Yan et al. (2007) who found that as salinity was increased from 11 to 21 to 31‰, there was an increasing trend in shrimp growth rate. Both studies found no difference in shrimp survival between these salinities.

In the United States, *L. vannamei* has been produced at salinities of 1-15‰ in Alabama, Arizona, Florida, and Texas (Roy et al., 2010). However, these are relatively low intensity operations, stocking shrimp at roughly 10 to 40 m^{-3} in outdoor ponds. There is a lack of information on low or moderate salinity shrimp culture in intensive biofloc systems, and especially little research on a commercial scale. Operating low salinity biofloc systems may pose unique challenges due to fluctuations in concentration of inorganic nitrogen compounds (Browdy et al., 2012). The toxicity of inorganic nitrogen compounds, including ammonia, nitrite, and nitrate, has inverse relationships with salinity (Schuler et al., 2010; Kuhn et al., 2010).

The ionic composition of saltwater can vary based on its source (Roy et al., 2010) but only the concentration and ratios of a few major ions are most important for *L. vannamei* culture. Saoud et al. (2003) demonstrated that potassium, magnesium, and sulfate concentrations were all positively correlated with shrimp survival. Roy et al. (2007) found that by decreasing the

sodium: potassium ratio, shrimp growth and survival were increased. They also demonstrated that low concentrations of magnesium resulted in higher shrimp respiration and lower survival rates. Roy et al. (2007) recommended maintaining a Na:K ratio of 28:1, and a Mg:Ca ratio of 3.1:1 (approximately equal to that of seawater).

Using 150-L tanks, Maicá et al. (2012) explored the effects of changes in salinity on the microbial communities in biofloc shrimp systems. They found that at higher salinity flagellates and diatoms were more abundant, while chlorophytes and ciliates were more abundant in lower salinity treatments.

The purpose of this study was to compare water quality and shrimp production in a commercial-scale, biofloc-based culture facility operated at three different salinities.

Materials and Methods

Shrimp Source, Nursery, and Feeds

Eight-day-old postlarvae (PL 8) *Litopenaeus vannamei* were obtained from Shrimp Improvement Systems, LLC (Islamorada, Florida, USA) and stocked at 4000 shrimp m^{-3}. All raceways used for this project, including the nursery raceway, were 30.1 m x 3.2 m (L x W). Raceway walls were constructed of concrete surrounding a sand floor and lined with woven polyethylene pond liners. Raceways were contained in dome-shaped greenhouse structures covered in clear plastic. The nursery raceway was operated at a depth of 0.5 m and contained a central wall made of plastic sheeting; water was propelled around this wall using 6 airlift devices as described by Ray et al. (2011b) and Ray (2012a). Each airlift device had three, 15.2 cm long ceramic diffusers receiving air from a 746 W regenerative blower (Sweetwater®, Pentair Aquatic Ecosystems Inc., Apopka, Florida, USA). The airlift devices comprised (1) a PVC frame, (2) the

diffusers and (3) a rubber deflector. The PVC frame held the diffusers approximately 6 cm above the raceway floor. Above the diffusers was a sheet of EPDM rubber held by the PVC frame and oriented at an approximately 35° angle relative to the water movement. Air from the diffusers traveled vertically and contacted the EPDM, which deflected the air, and the water traveling with it, horizontally forward.

Shrimp were kept in the nursery raceway for 54 days at a salinity of 25‰. Sucrose was added periodically to raise the C:N ratio and stimulate heterotrophic bacterial assimilation of ammonia on an as-needed basis. On four occasions water exchanges were conducted due to ammonia concentrations over 5 mg TAN L^{-1}; throughout the nursery phase a total exchange of 140% of the system volume occurred.

For the first 16 days of the nursery phase, shrimp were fed freshly hatched *Artemia* sp. (INVE Aquaculture, Inc., Salt Lake City, Utah, USA) at a rate of 5,000 *Artemia* sp. L^{-1} day^{-1}. Beginning the first day of the nursery phase, 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 diet contained 50% protein, 15% fat, 1% fiber, 10% moisture, and 7.5% ash according to the manufacturer. Beginning on day 23, Zeigler PL 40-9 Vpak 1.5 mm diet was provided (40% protein, 9% fat, 3% fiber, 10% moisture, and 13% ash). On days 34 through 54, Zeigler Hyperintensive-35, 2.4 mm diet was provided (35% protein, 7% fat, 2% fiber, 12% moisture, and 15% ash). During the nursery phase, shrimp were fed based on a percent of the assumed shrimp biomass, starting at 15% of biomass and gradually decreasing to 8.8%.

Experimental Systems and Design

At the end of the nursery phase shrimp were sampled using the methods described by Ray et al. (2011a), and weighed 1.22 ± 0.02 g (mean ± SEM). At this time shrimp were enumerated by weight and 12,500 shrimp were placed into each of 9 production raceways. These raceways were identical to that used for the nursery phase, except that only 4 airlift devices were contained in each raceway, a 560-W water pump at each raceway helped to circulate and aerate the water, and the raceways were only filled to a depth of 0.26 m (25 m^3 volume) making the initial stocking density 500 shrimp m^{-3}. The water pump was connected to a 5-cm diameter pipe that encircled the inside of each raceway. At 10 locations throughout each raceway (Fig. 1) a 1.3 cm diameter pipe connected Venturi nozzles (Turbo-Venturi®, Kent Marine, Franklin, Wisconsin, USA) to the 5-cm diameter pipe. Each Venturi was located just below the 5-cm diameter pipe. Connected to the outflow of the Venturi was 1.3 cm diameter piping that extended down to approximately 3 cm above the bottom of the raceway before turning 90 degrees and extending out 14 cm parallel to the bottom of the raceway. Each Venturi had tubing attached to the gas injection point which then attached to another pipe, 2.5 cm in diameter that circumvented the raceway. This pipe had two valves to allow ambient air to be drawn in and a point where pure oxygen gas could be injected, allowing air, pure oxygen, or a combination of the two to be injected into the Venturi nozzles.

The 5-cm diameter water pipe that circumvented each raceway supplied water to 4 spray bars. Spray bars were 1.3-cm diameter pipes with 2, 2-mm diameter holes drilled in them, and the spray bars were located about 0.5-m behind the airlift mechanisms (Fig. 1). Water from the spray bars contacted the water surface on both sides of the airlifts, as thick mats of surface sludge had accumulated in these areas during previous experiments. The 5-cm diameter water pipe also supplied water to a 1.9-cm pipe that carried water to the settling chambers at each raceway.

This experiment was conducted during winter months at the University of Southern Mississippi's Thad Cochran Marine Aquaculture Center (CMAC), located in Ocean Springs, Mississippi, USA. To control temperature two 500,000 BTU boilers (RBI, Division of Mestek Canada, Inc., Ontario, Canada) heated fresh, clean water to 66° C. This water was continuously pumped through a central line that passed near each raceway. Adjacent to each raceway a digital controller received a signal from a temperature probe submerged in the raceway. The controller regulated a small pump that, when on, sent hot water from the central line through a 3.7-m long, 8-bar, titanium heat exchanger located in each raceway.

An 8-day "rest" period was initiated after moving the shrimp to the experimental raceways, allowing shrimp to recover from any stress incurred during stocking. During this time the shrimp were kept in 25 m^3 of 25‰ salinity water, temperature was maintained at 24° C initially, and gradually increased to 28° C. The 9 raceways were each randomly assigned to 1 of 3 treatments: a low salinity (10‰) treatment (LS), a medium salinity (20‰) treatment (MS), and a high salinity (30‰) treatment (HS).

After the 8-day rest period, clean water was slowly added to the raceways to bring them to the correct salinity and depth. Salinity was measured using a YSI Model 556 Handheld Instrument (YSI Incorporated, Yellow Springs, Ohio, USA). Water at a salinity of approximately 20‰ was obtained from Davis Bayou, a tributary of The Mississippi Sound adjacent to the CMAC. This water was bleached, then aerated and sodium thiosulfate was added to dechlorinate it. Salinity was increased to approximately 35‰ using Fritz Super Salt Concentrate (Fritz Pet Products, Mesquite, Texas, USA) and sodium chloride (Morton® Purex® Salt, Morton® Salt, Chicago, Illinois, USA). Dechlorinated municipal water was used as a source of fresh water. Combinations of fresh and salt water were used to reach the desired salinity in

each raceway. This process was carried out over an additional 8 days, after which 50 shrimp from each raceway were weighed in groups of 10 to estimate mean individual shrimp weight and the experiment began; this was considered time point 0.

Water Quality

At time point 0 water samples were collected, filtered with 0.7 µm pore size filters, and analyzed for Na, Mg, K, and Ca concentration. These samples were sent to the Clemson Agricultural Services Laboratory (Clemson, South Carolina, USA) for analysis using inductively coupled plasma mass spectrometry.

Twice per day, at approximately 0730 and 1600 h, temperature, dissolved oxygen (DO), pH, and salinity in the raceways were measured using the YSI Model 556 Instrument. Once per week ammonia, nitrite, alkalinity, total suspended solids (TSS), volatile suspended solids (VSS), turbidity, and settleable solids were measured in each experimental raceway. At time point 0, week 4, and week 8 nitrate concentration was measured in each raceway. At weeks 1, 2, 4, and 6 phosphate (orthophosphate) concentration was measured. Five-day biochemical oxygen demand (BOD_5) was measured at weeks 2 through 6, and chlorophyll-a concentration was measured at weeks 0, 3, 4, 7, and 8.

Ammonia (TAN) was assessed using Hach method 8155 (Hach Company, 2003) and nitrite (NO_2-N) was measured using the spectrophotometric procedure outlined by Strickland and Parsons (1972). The concentration of NO_3-N was determined using the chemiluminescence method described by Braman and Hendrix (1989). The concentration of PO_4 was measured using the PhosVer 3 method as outlined in Hach Method 8048. Alkalinity was measured following the Potentiometric Titration to Preselected pH procedure outlined in section 2320 B by the APHA (2005). BOD_5 was measured using the procedure described in section 5210 B by the

APHA (2005), which includes a 5-day incubation period at 20° C. Chlorophyll-a extraction was performed according to the methods described by DeLorenzo et al. (2004). Concentrations of chlorophyll-a were measured using the procedures outlined in section 10200 H by APHA (2005). TSS and VSS concentrations were measured following ESS Method 340.2 (ESS, 1993). Turbidity was measured in Nephelometric Turbidity Units (NTU) using a Micro 100 Turbidimeter (HF Scientific, Fort Myers, Florida, USA). Settleable solids was measured according to Section 2540 F (APHA, 2005).

Systems Management

After checking salinity in all raceways each morning, dechlorinated municipal water was added to replace evaporation as needed. The objective was to keep the water in each raceway within 0.5‰ salinity of the treatment salinity.

The morning pH readings were used to determine the amount of sodium bicarbonate to add to each raceway every day. If the pH in a raceway was above 7.9 no $NaHCO_3$ was added, 7.9 > pH > 7.7 = 300g $NaHCO_3$ added, 7.7 > pH > 7.5 = 500 g $NaHCO_3$ added, 7.5 > pH = 1,000 g $NaHCO_3$ added.

Sucrose (Extra Fine Granulated Cane Sugar, Sysco® Corporation, Houston, TX, USA) was added to each raceway three times per day between feedings, and through the night on 12-hour belt feeders to facilitate heterotrophic bacterial assimilation of inorganic nitrogen. The wet weight of feed added daily was multiplied by 50% to determine the amount of sucrose to add each day. When concentrations of ammonia and nitrite were high, additional sucrose was added. Overall, the amount of sucrose added was 57.4% of the wet weight of the feed, resulting in a C:N ratio of inputs of 10.9:1, calculated using C and N levels in feed and sucrose.

Each raceway was equipped with a 760-L settling chamber as described by Ray et al. (2011b). The settling chambers were operated at a flow rate of 15 L min^{-1} continuously, except that once per week water flow was terminated for approximately one hour to allow thorough settling of particles. A small submersible pump with a short hose attached was lowered into the top of the settling chambers to decant the water back to each raceway and leave the dark-colored settled material. As the pump was lowered the color of the water being pumped to the raceway was monitored. The settled material on the bottom of the chamber was then drained.

Shrimp Culture

For the first 9 days after shrimp were moved to the growout raceways, both the Zeigler PL 40-9 Vpak diet and the Zeigler Hyperintensive-35 diet were provided, after which only Zeigler Hyperintensive-35 diet was given. Equal portions of feed were broadcast evenly throughout each raceway by hand four times per day at 0730, 1000, 1230, 1500 hrs. At 1630 hr. feed was placed on two belt feeders for each raceway which delivered feed for 12 hours. Each day 70% of the feed ration was delivered by hand and 30% was placed on the belt feeders. During the 8-day rest period and the 8-day salinity adjustment period feed rations were 40% of what they were during the experiment. During the experiment feed rations were based on routine dip net sampling in each raceway. Feed rations were calculated such that no uneaten feed could be found in the raceways 30 minutes prior to each feeding. Each raceway received the same amount of feed at every feeding. Shrimp weights were estimated once per week by weighing five groups of ten shrimp from each raceway; shrimp were grown in the experiment for eight weeks.

Data Management and Analysis

Data are presented as mean ± SEM, and in most cases the range is given in parentheses. The statistical software used for this study was Systat Version 13 (Systat Software, Inc., Chicago, Illinois, USA). An alpha value of 0.05 was used to determine significant differences.

To standardize the comparison of Na, Mg, K, and Ca concentrations, the LS treatment data were multiplied by 3 and the MS treatment data were multiplied by 1.5; the data were then compared using a one-way ANOVA. The concentration of DO, ammonia, phosphate, alkalinity, BOD_5, VSS, settleable solids, and the turbidity data were all analyzed using a repeated measures (RM) ANOVA followed by pairwise comparisons. Ammonia, VSS, and settleable solids data were transformed by calculating the $\log_{10}$ values of the data prior to analysis to conform to the normality assumption of the ANOVA. Turbidity data were transformed by calculating the cosine. Morning and afternoon temperature and pH data, and salinity, nitrite, nitrate, chlorophyll-a, and TSS data could not be transformed to fit the ANOVA assumptions, therefore a nonparametric Wilcoxon signed rank test followed by pairwise comparisons was used to analyze these data according to the recommendations of Zimmerman and Zumbo (1993). The percent water exchanged data were arcsine transformed and analyzed using a one-way ANOVA. The amount of seawater used per kg of shrimp, seawater used per raceway, and the cost of artificial salt for each treatment were analyzed using a one-way ANOVA. Shrimp growth rate, FCR, final weight, biomass, and survival were analyzed using a one-way ANOVA.

Results

During week 4 of this study the 5-cm pipe on one of the HS raceways broke during a time that no one was present. This resulted in the water being drained, killing all of the shrimp in that

raceway. Because this was a mechanical failure in the middle of the study no data from this raceway are included in the results.

During the last week of the study, one of the vertical water pipes in a LS raceway was removed in an attempt to clear blockage at the last Venturi in the water line. When this occurred a large amount of thick, dark, sludge material poured into the raceway; due to the odor, it was suspected this material contained hydrogen sulfide. Later that day many dead shrimp were found in this raceway, and a 5% water exchange was performed using clean 10‰ salinity artificial seawater made as described above. The following day ammonia concentration was 2.0 mg TAN L^{-1} and nitrite was 3.5 mg NO_2-N L^{-1}. On the third day, this raceway was harvested, 3 days before the others in the study. A total of 1,366 dead shrimp, weighing 17.4 kg, were removed from the raceway over the course of three days; the remainder of the shrimp were alive at the time of harvest. All water quality data from this raceway are included, aside from the last week. Because this event was directly caused by human error just prior to the end of the experiment, all shrimp production data are included in the reported results. Shrimp production data from this raceway include only those shrimp that were alive at the time of final harvest because these were the only shrimp fit for sale.

The initial concentrations of major cations (standardized for salinity differences) are presented in Table 1. The concentration of these ions correlated with salinity. However, there were significant differences between LS and HS potassium and calcium values.

Temperature was maintained at 29° C (Table 2) with little variability using the centralized heating system during this study. There were significant differences in temperature between the treatments for both morning and afternoon measurements: MS > LS > HS, although differences in mean values were subtle (Table 2).

Dissolved oxygen (DO) was maintained at relatively consistent morning and afternoon concentrations. There were significant differences between treatments with respect to morning DO concentration: HS > MS > LS. With respect to afternoon DO concentration, there were significant differences between the LS and MS treatments and between the HS and MS treatments: HS, LS > MS (Table 2).

The pH was significantly higher in the LS treatment, followed by the MS treatment, and then the HS treatment. Morning pH was relatively steady throughout the study; afternoon pH was less consistent (Fig. 2).

Salinity in the raceways was maintained at a steady level throughout the study and was significantly different between treatments.

The concentration of ammonia increased substantially in all but one HS raceway during week 5 of the study (Fig. 3a). Ammonia concentration increased again in most raceways the last week of the experiment (Fig. 3a). There were no significant differences in ammonia concentrations between treatments (Table 2). Nitrite concentration increased to 1.4 and 1.5 mg NO_2-N L^{-1} in two HS raceways during week 6. Nitrite rose again in all but one LS and one MS raceway during the last week of the study (Fig. 3b). Nitrite concentration was significantly greater in the HS treatment than in the LS treatment and there were no significant differences between the MS treatment and the other treatments (Table 2). Nitrate concentration was generally low (Fig. 3c), and there were no significant differences in nitrate concentration between treatments.

The concentration of TSS was significantly higher in the HS treatment versus the LS and MS treatments: HS > MS, LS (Table 2). Over the course of the first two weeks TSS

concentration decreased substantially in the raceways (Fig. 4a). There were no significant differences between treatments with respect to the concentration of VSS (Fig. 4b).

The concentration of settleable solids was significantly greater in the HS treatment compared to the LS treatment (Table 2) and there were no significant differences between the MS treatment and any other treatment (Fig. 4c). There were no significant differences between treatments with respect to turbidity (Fig. 4d).

There were no significant differences between treatments regarding 5-day BOD (Table 2). The concentration of chlorophyll-a started at approximately 200 µg L^{-1} just prior to the beginning of the study, and decreased to approximately 50 µg L^{-1} after the experiment started. There were no significant differences in chlorophyll-a concentration between treatments.

The mean volume of full salinity seawater (35‰) used per kg of shrimp produced in the LS, MS, and HS treatments was 104, 159, and 235 L, respectively (Table 3). There were no significant differences among treatments with respect to the total volume of water used (52.4, 52.3, 52.6 m^3 respectively). The estimated cost of artificial sea salts (assuming only artificial salts are used), based on those used at the CMAC, is also depicted in Table 3.

Because of the mortality event that occurred in one of the LS raceways, the shrimp production in that treatment was lower than the others (Table 4). However, there were no significant differences in any shrimp production metric between treatments. Growth rate (Fig. 5) was high and feed conversion ratio (FCR) was low (Table 4). Harvested shrimp, including those in the problematic raceway, had long antennae, firm exoskeletons, and few lesions in the exoskeletons.

Discussion

This study helps to illustrate some of the benefits of maintaining relative consistency in water quality parameters and implications for commercial-scale shrimp production in biofloc systems. Overall, shrimp production metrics were at acceptable and commercially relevant levels regardless of salinity.

The water temperature during this study was apparently influenced little by solar input, even though the tanks were in greenhouses. The significant differences in temperature between treatments was likely caused by the location of raceways with respect to the hot water delivery system. The DO concentration during this study was relatively consistent, and was maintained at a high level to ensure this was not a source of stress for shrimp.

The intensive monitoring of pH and salinity and the regular inputs of sodium bicarbonate and fresh water resulted in consistent pH and salinity during this study. The consistency in temperature, DO, pH, and salinity during this study may have contributed to the high shrimp production values obtained, as a more constant physical environment can help to minimize stress for aquatic animals (Stickney, 2005).

The cause of lower pH with increasing salinity in this study is unclear. In seawater, pH typically increases with higher salinity because more carbonate and bicarbonate ions are present, contributing to a higher pH buffering capacity (Libes, 2009). Saraswat et al. (2011) found that in both laboratory and estuarine experiments, as salinity increased so did pH. However, Decamp et al. (2003) cultured *L. vannamei* in 1700-L minimal-exchange biofloc systems at 50 shrimp m^{-2}, with treatments of 9, 18, and 36‰ salinities, and found that pH was significantly lower as salinity increased. These authors attributed the relationship between salinity and pH to potentially increased photosynthesis in the lower salinity treatments. This may have been the case in the current study as well, although photosynthetic oxygen production was not measured.

Because no significant differences in chlorophyll-a concentrations were found, there were likely no substantial differences in algal abundance; however, the taxonomic composition of algae may have differed. Higher concentrations of CO_2 and consequently the weak acid H_2CO_3 may have been present as salinity increased, possibly due to increased shrimp respiration.

The ammonia spike during week five (Fig. 3a) of the study was followed by a sharp decline in BOD_5 concentration the following week. The BOD_5 drop may have been an indication that a portion of the microbial community died, and the decomposition of those microbes could have contributed to increased ammonia concentrations.

At week zero, chlorophyll-a concentration was much higher than after the study started. This is likely a result of a shift from dominance of algae to greater dominance by heterotrophic bacteria after the intensive additions of feed and sucrose began. Such a shift in microbial dominance was also noted by Browdy et al. (2001). The mean chlorophyll-a concentrations reported in the current study are approximately 15% of what was reported by Ray et al. (2012b) and 20% of that reported by Venero et al. (2009); both groups of authors operated biofloc systems with no supplemental carbohydrate additions. However, the chlorophyll-a concentrations reported here are more than five times higher than what was reported by Moreno-Ostos et al. (2008) in oligotrophic waters as a comparison.

Table 3 helps to illustrate the substantial savings in seawater by growing marine shrimp at lower salinities. At inland aquaculture facilities, these water savings translate directly to cost savings when considering the price of artificial sea salts. The low rate of water exchange during this study (Table 3) also helps to lower seawater use and justify inland shrimp aquaculture. Water was only exchanged as a result of solids removal. Several similar trials conducted at the

Oceanic Institute (OI) in Hawaii, USA were reported to have used 187, 172, and 402 liters of seawater per kg of shrimp (Otoshi et al., 2007).

Although there were no significant differences in shrimp production between treatments, the substantially lower production in the LS treatment would translate to less profit for a commercial shrimp operation. It is unclear whether the accidental introduction of anaerobic material would have caused the same level of mortality in the higher salinity treatments. However, it is clear that the estimated cost of artificial salts was 49.7% lower in the LS treatment compared to the MS treatment, which would translate into higher profit margins for farmers.

In summary, the production goals, and availability and cost of sea salt should be considered when deciding the salinity at which to culture shrimp. Fluctuations in ammonia and nitrite are common in intensive biofloc-based systems and should be considered as well. This study indicates that the three salinities evaluated can result in comparable shrimp production in commercial-scale biofloc systems.

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Table 1. The initial concentrations of major cations (LS values multiplied by 3, and MS values multiplied by 1.5 to standardize for salinity differences). Concentrations are reported in mg L^{-1} as mean ± SEM. Different superscript letters indicate significant differences between treatments.

	Treatment		
	Low Salinity	Medium Salinity	High Salinity
Na^{+}	8620 ± 184	8426 ± 183	8236 ± 82
Mg^{2+}	1017 ± 10	1009 ± 10	1006 ± 6
K^{+}	354 ± 2^{a}	346 ± 5^{ab}	335 ± 3^{b}
Ca^{2+}	312 ± 4^{a}	314 ± 4^{ab}	331 ± 1^{b}

Table 2. Water quality parameters during the 8 week shrimp production experiment. Data are presented as mean ± SEM (range), and different superscript letters in a row indicate significant differences.

	Treatment		
	Low Salinity	Medium Salinity	High Salinity
Temperature (ºC)			
AM	29.0 ± 0.0 (26.5 - 29.6)[a]	29.1 ± 0.0 (26.3 - 29.6)[b]	28.8 ± 0.1 (26.1 - 29.5)[c]
PM	29.1 ± 0.0 (27.6 - 30.1)[a]	29.2 ± 0.0 (27.7 - 30.0)[b]	29.0 ± 0.0 (27.5 - 30.0)[c]
Dissolved Oxygen (mg L^{-1})			
AM	8.6 ± 0.1 (6.7 - 11.1)[a]	8.7 ± 0.1 (6.6 - 12.5)[b]	8.9 ± 0.1 (6.0 - 12.9)[c]
PM	7.9 ± 0.1 (5.8 - 10.8)[a]	7.7 ± 0.1 (5.2 - 10.0)[b]	8.0 ± 0.1 (4.5 - 15.0)[a]
pH			
AM	7.9 ± 0.0 (7.7 - 8.2)[a]	7.8 ± 0.0 (7.6 - 8.0)[b]	7.7 ± 0.0 (7.4 - 8.0)[c]
PM	7.9 ± 0.0 (7.1 - 8.3)[a]	7.7 ± 0.0 (7.4 - 8.2)[b]	7.7 ± 0.0 (7.3 - 8.1)[c]
Salinity (‰)	10.3 ± 0.0 (9.2 - 11.0)[a]	20.2 ± 0.0 (18.2 - 21.2)[b]	30.2 ± 0.0 (27.1 - 31.9)[c]
Ammonia (mg TAN L^{-1})	0.8 ± 0.2 (0.0 - 4.0)	1.2 ± 0.4 (0.0 - 8.0)	0.7 ± 0.3 (0.0 - 4.4)
Nitrite (mg NO_2-N L^{-1})	0.3 ± 0.1 (0.0 - 3.4)[a]	0.4 ± 0.2 (0.0 - 3.5)[ab]	0.6 ± 0.2 (0.0 - 3.3)[b]
Nitrate (mg NO_3-N L^{-1})	1.4 ± 1.0 (0.0 - 8.7)	0.3 ± 0.2 (0.0 - 2.0)	0.6 ± 0.3 (0.0 - 1.5)
Phosphate (mg PO_4 L^{-1})	2.4 ± 0.3 (0.6 - 3.8)	2.6 ± 0.3 (1.4 - 4.4)	2.0 ± 0.2 (0.8 - 3.3)
Alkalinity (mg $CaCO_3$ L^{-1})	310 ± 15 (207 - 442)	320 ± 19 (200 - 509)	322 ± 25 (205 - 500)
BOD_5 (mg BOD_5 L^{-1})	180 ± 12 (72 - 243)	164 ± 9 (87 - 211)	171 ± 17 (65 - 238)
Chlorophyll-a (µg L^{-1})	80 ± 16 (24 - 200)	85 ± 18 (24 - 240)	84 ± 16 (40 - 240)
TSS (mg L^{-1})	263 ± 14 (185 - 500)[a]	286 ± 19 (175 - 510)[a]	330 ± 30 (210 - 645)[b]
VSS (mg L^{-1})	198 ± 14 (95 - 460)	189 ± (35 - 370)	191 ± 24 (90 - 490)
Turbidity (NTU)	74 ± 7 (49 - 211)	64 ± 4 (35 - 126)	61 ± 6 (41 - 127)
Settleable Solids (ml L^{-1})	7 ± 1 (3 - 18)[a]	9 ± 1 (4 - 21)[ab]	9 ± 1 (4 - 21)[b]

Table 3. Amount of salt water (adjusted to full strength (35‰) seawater) used per raceway, water exchanged, and the cost of artificial salt used per raceway. Data are presented as mean ± SEM (range), and different superscript letters in a row indicate significant differences.

	Treatment		
	LS	MS	HS
Total 35‰ Seawater Used per Raceway (m^3)	15.0 ± 0.1^a	29.8 ± 0.0^b	45.1 ± 0.0^c
35‰ Seawater Used per kg Shrimp (L kg^{-1})	104 ± 14^a	159 ± 5^b	235 ± 4^c
Total Water Exchange (%)	5.0 ± 0.3	4.5 ± 0.1	5.2 ± 0.0
Cost of Artifical Sea Salt per Raceway (USD)	653.8 ± 2.2^a	1300.8 ± 0.7^b	1964.0 ± 0.2^c

*Cost of artificial sea salt is based on using one 36 kg bag of Morton brand NaCl (Morton® Purex® Salt, Morton® Salt, Chicago, Illinois, USA) and one 19-L bucket of Fritz brand Super Salt Concentrate (Fritz Pet Products, Mesquite, Texas, USA) to make each 1,514-L of 35‰ water.

Table 4. Shrimp production in the three treatments. Data are presented as mean ± SEM.

	Treatment		
	LS	MS	HS
Growth Rate (g week^{-1})	1.8 ± 0.1	2.0 ± 0.0	2.0 ± 0.1
FCR	1.6 ± 0.2	1.2 ± 0.0	1.2 ± 0.0
Mean Weight (g)	17.8 ± 0.9	19.3 ± 0.2	19.0 ± 0.5
Biomass (kg)	149.5 ± 19.2	188.2 ± 5.7	191.5 ± 3.5
Biomass volume^{-1} (kg m^{-3})	3.0 ± 0.4	3.8 ± 0.1	3.8 ± 0.1
Survival (%)	68 ± 10	78 ± 2	81 ± 1

Figure 1. The configuration of each of the 9 experimental raceways used for this study.

Figure 2. Mean morning (a) and afternoon (b) pH.

Figure 3. Concentrations of ammonia (a), nitrite (b), and nitrate (c). Data points are treatment means and error bars are 1 standard error around the mean.

Figure 4. Concentrations of total suspended solids (a), volatile suspended solids (b), and settleable solids (c), as well as turbidity (d). Data points are treatment means and error bars are 1 standard error around the mean.

Figure 5. Shrimp weight throughout the 8 week production study. Data points are treatment means and error bars are 1 standard error around the mean.

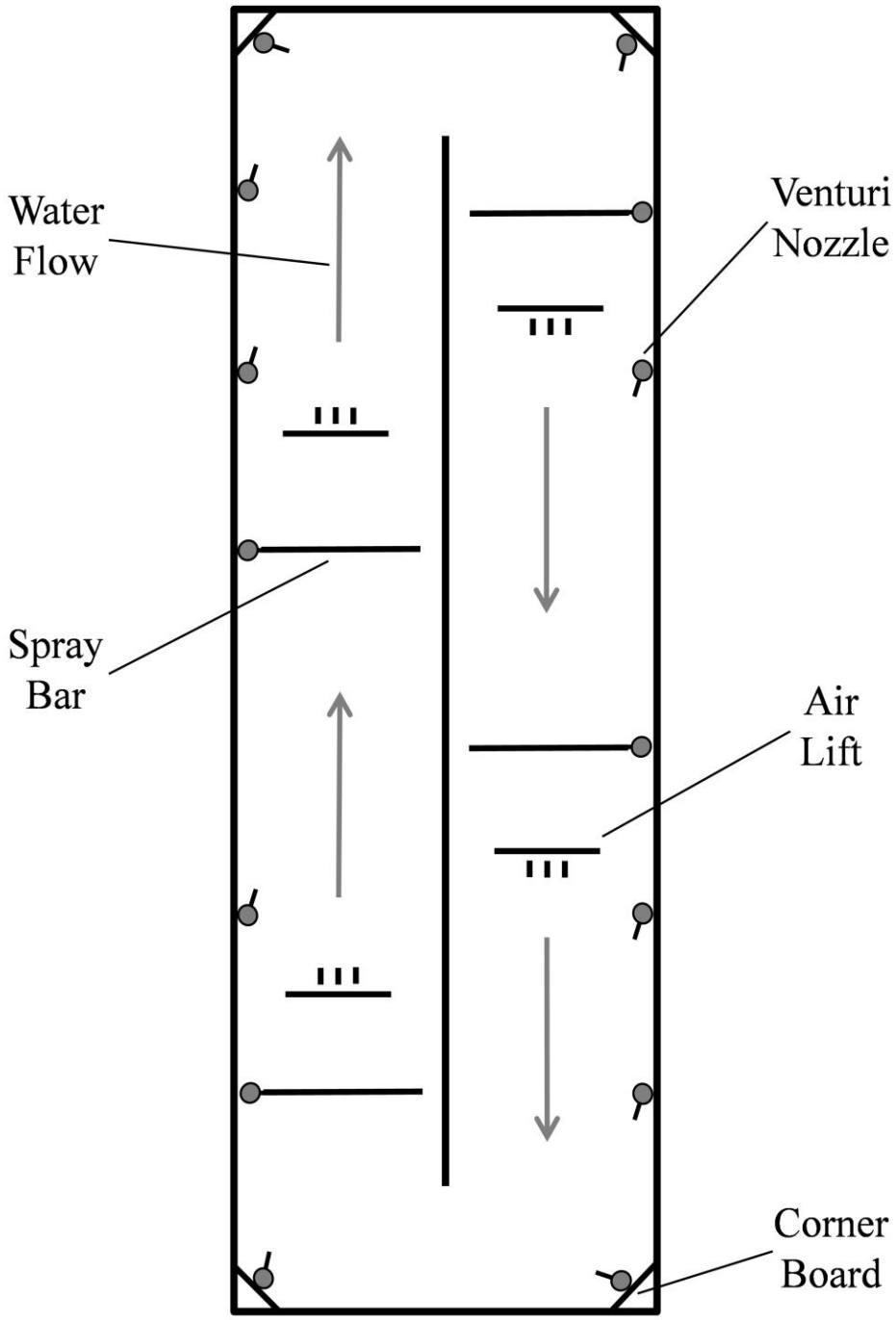

Figure 1

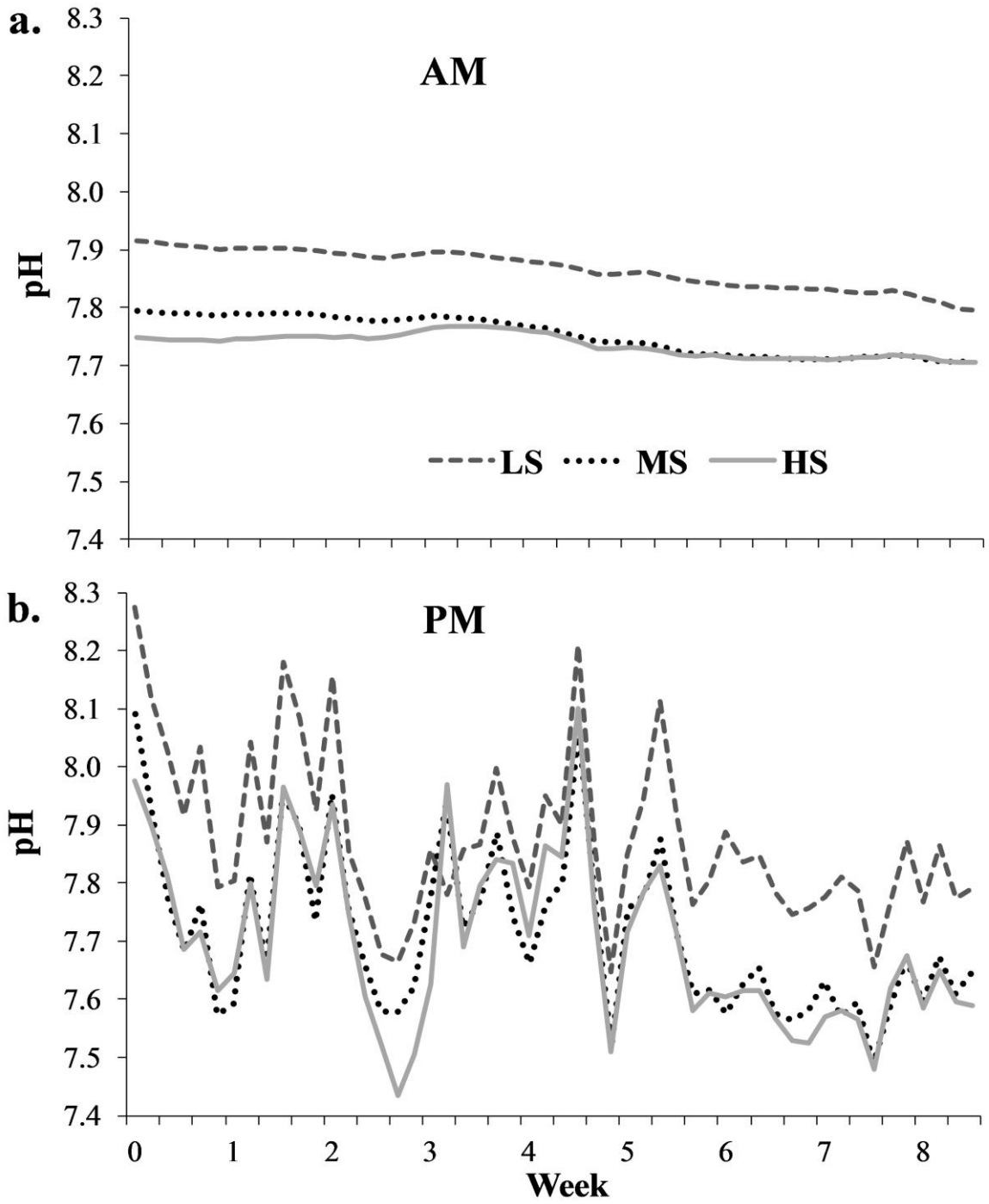
a.
AM
pH
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
LS
MS
HS
b.
PM
pH
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
0
1
2
3
4
5
6
7
8
Week

Figure 2

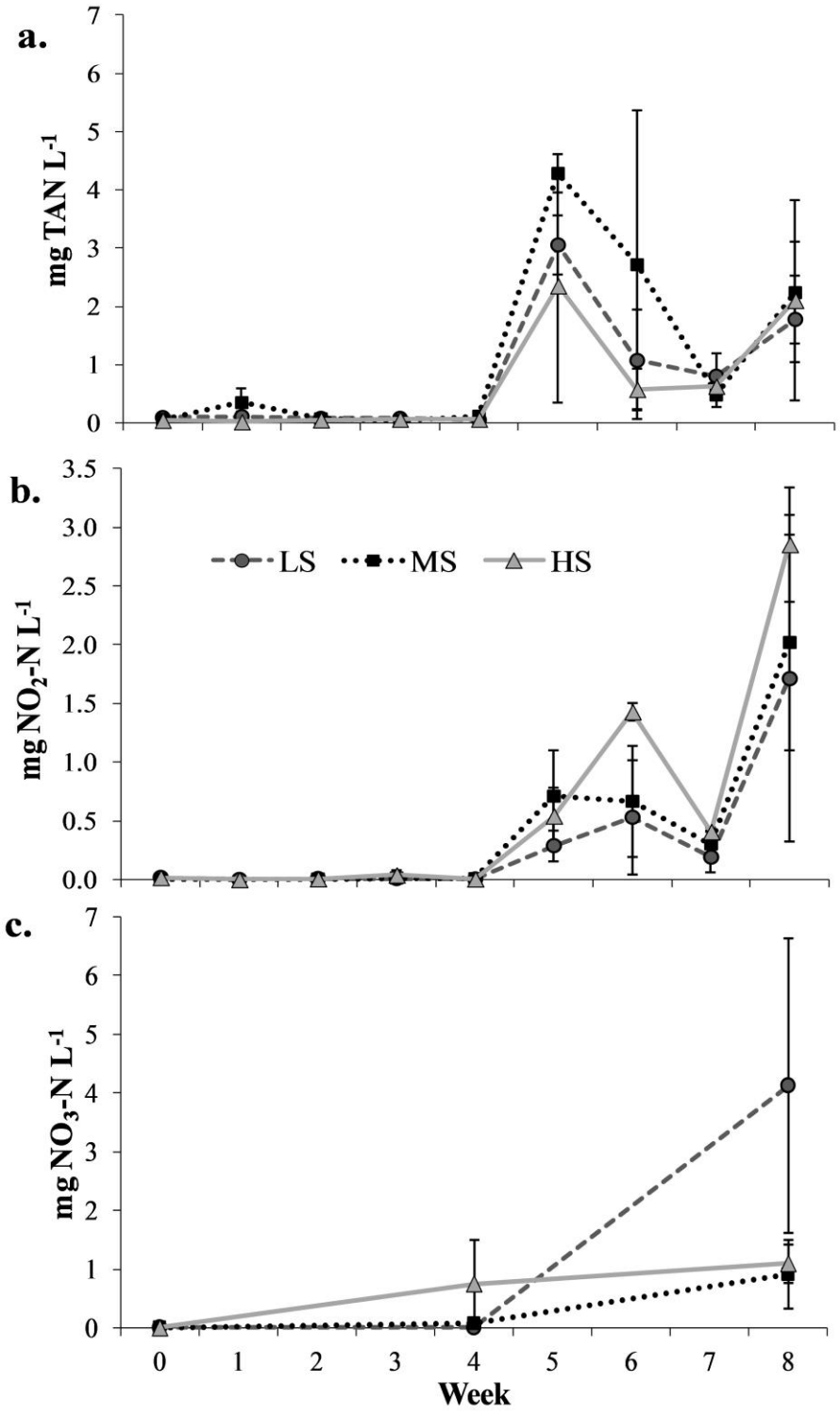

Figure 3

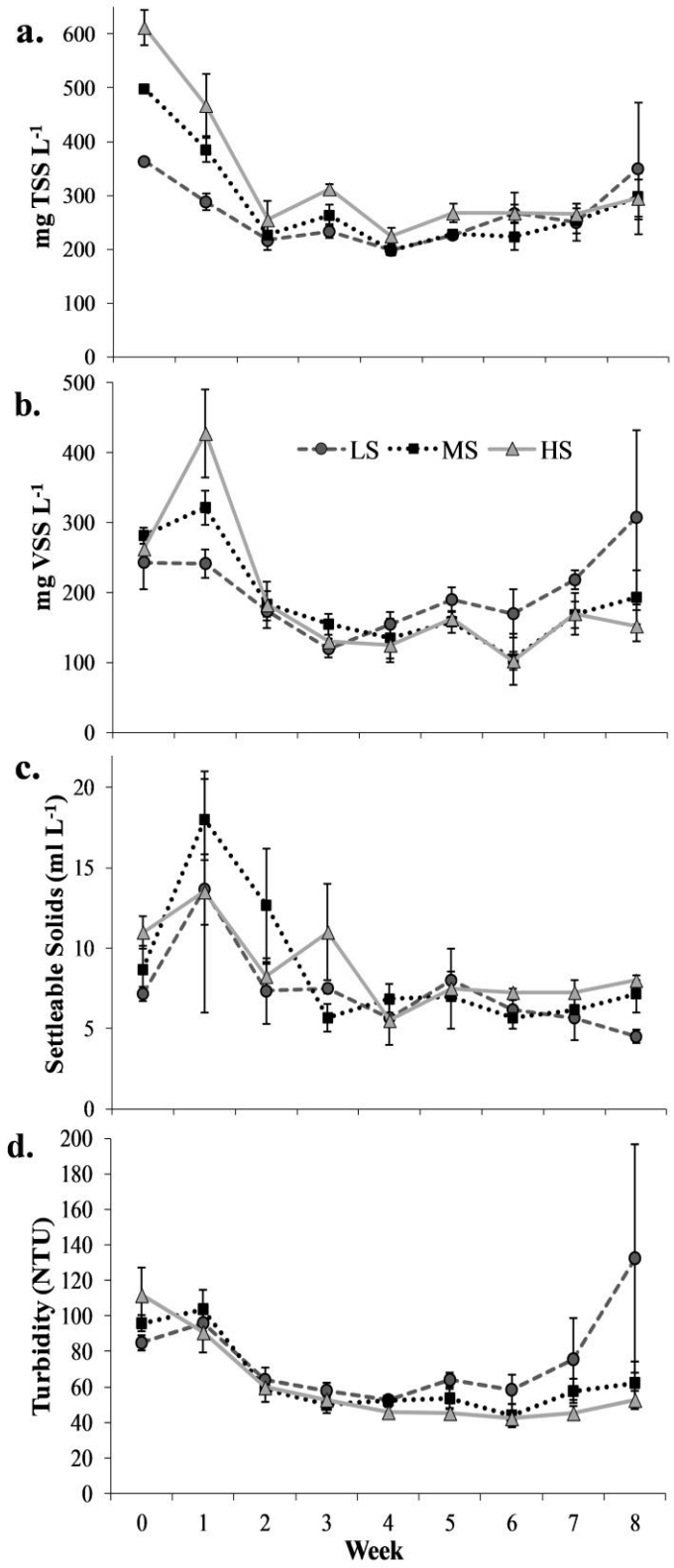
a.
mg TSS L-1
b.
mg VSS L-1
LS
MS
HS
c.
Settleable Solids (ml L-1)
d.
Turbidity (NTU)
Week

Figure 4

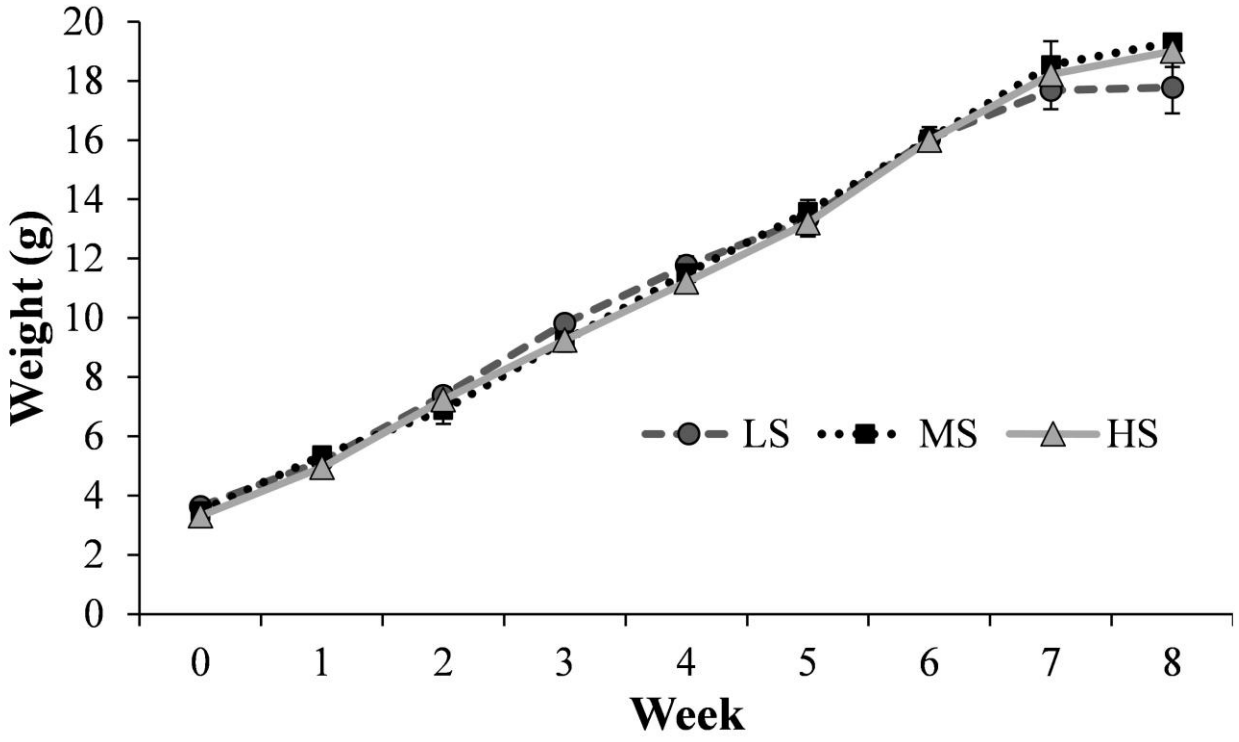

Figure 5