Evaluation of Whiteleg Shrimp (*Litopenaeus vannamei*) Growth and Survival in Three Salinities under RAS Conditions

by

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Abstract

Aquaculture provides our rising global population a multitude of social, economic, and ecological benefits. A large component of the yields is whiteleg shrimp (*Litopenaeus vannamei*), which has expanded to over three million tons annual global production. As whiteleg shrimp tolerate a wide range of salinities, a potential method to reduce impacts is freshwater inland culture, which would decrease operational and environmental costs. This study aimed to evaluate the effectiveness of such a proposal via a long-term analysis of shrimp growth and survival in freshwater, compared to brackish or saltwater (0, 12, and 35 ppt, respectively) over a 107 day period. Based on findings of previous studies, I hypothesized equal shrimp growth and survival among treatments. Growth, survival, and biomass results in freshwater were poor compared to higher salinities (0.063 g/wk compared to 0.62 and 0.091 g/wk growth, 1% versus 63 and 72% survival, and 2 g versus 307 and 1747 g final biomass, respectively). This study confirmed that many factors are vital to the successful culture of whiteleg shrimp. Multiple regression demonstrated that water quality variables (salinity, alkalinity, dissolved oxygen (DO), and pH) were significantly correlated with dependent variables (shrimp growth, survival, and biomass) to varying degrees. Growth was negatively associated with salinity and pH, and positively associated with alkalinity and DO. Survival was positively associated with alkalinity and pH, and negatively with ammonia, while biomass had a significant positive association with DO. Growth was the sole dependent variable with a significant association with salinity. Growing shrimp was most effective in brackish water under the conditions of the present experiment, but continued research on identifying drivers of this limitation are worthwhile in order to make the valuable shrimp farming industry as sustainable as possible.

Introduction

Catch trends have remained relatively stable for wild capture fisheries, at approximately 90 million tons; aquaculture production has steadily increased, and is now over eight times greater than production in the 1950s (FAO 2014; FAO 2016). Growing at a rate of eight percent per year, the yield of aquaculture has increased faster than any other animal protein production source since the 1970s (Diana et al. 2013). Aquaculture now provides over half of the world's seafood, and its value will likely continue to increase as the global population is projected to reach 9.6 billion by 2050 (FAO 2014). Aquaculture has "numerous important social, economic and environmental benefits, including increased food security and poverty alleviation impacts, increased employment opportunities within rural communities, increased seafood supply and availability, improved human nutrition and well-being, increased foreign exchange earnings, improved wastewater treatment/water reuse and crop irrigation opportunities, and improved nutrient recycling" (Tacon and Halwart 2007).

Shrimp is the most highly traded seafood product (Cao 2012) and "continues to be the largest single commodity in value terms, accounting for about 15 percent of the total value of internationally traded fishery products in 2012" (FAO 2014). Over 90% of the shrimp consumed in the US is imported (Wang 2016). Wild capture shrimp comes mainly from the Northwest and Western Central Pacific, and it peaked at 3.6 million tons in 2012 (FAO 2014). Much of the wild fishery is conducted via unsustainable trawling operations that capture substantial amounts of bycatch, and ecosystem-based management is not prioritized (Criales-Hernandez et al. 2006; Macfadyen et al. 2013; Rieser et al. 2013). Problems arise from the lack of understanding or appreciation of the value of benthic marine habitat, as well as the fact that impacts from trawling remain hidden deep in the ocean. Some have proposed modeling to designate and protect high-value benthic zones to resolve or mitigate the impacts of shrimp harvest systems (e.g., Rieser et al. 2013).

Shrimp were initially harvested mainly in the wild capture fishery, but are now also produced by aquaculture (FAO 2014). Shrimp aquaculture began incidentally centuries ago in Asia, then progressed into extensive, seasonal culture in India, Bangladesh, and Vietnam, until the twentieth century brought strides in research regarding the complex shrimp life cycle (Alday-

Sanz 2010). Whiteleg (WL) shrimp, *Litopenaeus vannamei*, primarily comes from Asia and the Americas (FAO 2016). This species dominates shrimp culture in the Western hemisphere (Laramore et al. 2001; Saoud et al. 2003; Araneda et al. 2008), but Thailand and China are the top two producers (Cao 2012). Similar to global aquaculture trends, WL shrimp culture production has undergone a great expansion, from just 8,000 tons in 1980 to over three million tons currently (FAO 2016). Shrimp prices have risen as demand has increased. Further, supply has sometimes been constrained due to presence of disease, such as in 2013 when early mortality syndrome was particularly rampant in shrimp from Asian and Latin American aquaculture (FAO 2014; FAO 2016).

Proliferation of WL shrimp aquaculture is fueled by multiple factors, including strides in research and technology development, as well as characteristics specific to WL shrimp. These suitable qualities include generalist feeding capabilities (lesser protein requirements relative to other species), hardiness in the presence of changing or poor water quality, and the availability of specific pathogen-free (SPF) postlarvae (PL), which reduce disease potential (Wyban and Sweeney 1991; Van Wyk 2013). WL shrimp are also a euryhaline species, or are able to regulate the osmotic pressure of water and salt concentrations in their bodies, enabling them to inhabit water of varying salinities (Bray et al. 1994; Saoud et al. 2003; Van Wyk 2013).

Researchers and aquaculture practitioners have noted WL shrimp distribution throughout environments of varying salinity in the wild, such as juvenile shrimp that inhabit low salinity lagoons (Bray et al. 1994; Jayasankar et al. 2009). Mair (1980) observed a potential link between salinity and shrimp migration patterns. Wyban and Sweeney (1991) noted that this ability was leading to WL shrimp culture in inland freshwater (FW) ponds in Asia and Latin America in the late 1980s, and that production was much greater than that of previously cultured indigenous species. Nonetheless, Bray et al. (1994) aptly stated that “tolerance defines geographical range but does not imply an optimal level.” Researchers have long sought an ideal salinity concentration for culture, using a variety of methods and conditions.

Research on WL shrimp culture in reduced salinity (<35 ppt) has been motivated for a variety of reasons. Coastal real estate is increasingly expensive (Laramore et al. 2001; Araneda et al. 2008) and the methods and quality of international shrimp production has been questioned and led to environmental concerns, such as mangrove destruction and SW intrusion (Dierberg and Kiattisimkul 1996; de Graaf and Xuan 1998; Sun 2009; Brown 2013; Wang 2016), as well as biodiversity loss and negative impacts on human lives such as food insecurity (Cao 2012). There is also interest in the diversification of traditional agriculture (Saoud et al. 2003). Reducing salt use in shrimp production could improve the economics of intensive indoor shrimp farming, depending on location and water replacement rates (Schuler 2008; Sun 2009), which can enhance biosecurity and environmental control (Samocha et al. 2008). Furthermore, wastewater from low-salinity culture has reclamation potential for use in other processes, such as aquaponics or irrigation.

Various researchers have set out to explore the concept of WL shrimp culture in low salinity, and the published scientific literature regarding this topic is diverse in terms of both system conditions and scope. Mair (1980) investigated what he called shrimp salinity preference, meaning the ideal salinity for rapid growth and optimal survival. His study allowed four species of Mexican *Penaeus* postlarval shrimp to 'choose' a salinity using a choice apparatus (Mair 1980). Some researchers have conducted short-term studies that focus on initial impacts of the acclimation process of WL shrimp to lower salinity levels (McGraw et al. 2002; McGraw and Scarpa 2004; Jayasankar et al. 2009). Samocha et al. (1998) conducted an acclimation study, as well as a FW stress test, to serve as a method to evaluate PL quality for hatcheries. Bray et al. (1994) tested for the effects of BW and SW concentrations, though their experimental PLs were disease-infected. Others studies have focused on the ion composition in culture water (Saoud et al. 2003; Jayasankar et al. 2009). These studies vary widely in terms of their objectives, conditions, and parameters.

Two sources that reported effective growth of shrimp in FW or very low salinity concentrations are Van Wyk (2013) and Araneda et al. (2008). Van Wyk (2013) ran three-month trials with growth rates of up to 0.7 g/wk and 88% survival in 0.7 ppt salinity. Araneda et al. (2008) reported growth of 0.38 g/wk with 77% survival in FW (0 ppt). Neither of these studies, despite reportedly decent growth and survival, had controls to compare resulting growth and survival rates at higher salinities. In contrast, Laramore et al. (2001) tested treatments of varying salinities, but found low survival of PL at salinities of < 2 ppt. Nonetheless, they acknowledge that WL shrimp have been commercially produced in FW (at 0.5 ppt).

Research on growing shrimp in FW is muddled by a multitude of factors of experimental design, including condition (type of system, indoor versus outdoor culture, source of water/shrimp/feed, acclimation protocol, feeding regime, and duration of experiment) and parameter variation (salinity concentration ranges, water quality levels, temperatures, and stocking densities). Consequently, these variables complicate a comparison of resulting survival and growth rates between studies, and reporting of results varies, as well, in that both shrimp growth and age are used as metrics (Laramore et al. 2001).

Previous studies demonstrated the considerable potential and value in growing WL shrimp in FW (Araneda et al. 2008; Van Wyk 2013); I was interested in determining quantifiable differences in growth and survival compared to other salinity levels over longer timeframes. Furthermore, various sources identified a need for a long-term investigation and further research on this topic (Briggs et al. 2004; McGraw and Scarpa 2004; FAO 2016). Therefore, I designed an experiment to evaluate WL shrimp growth and survival in three salinity treatments, including FW (0 ppt), moderate salinity (12 ppt) for a BW comparison, and SW (35 ppt), as a reference. The objective of this study was to evaluate whether shrimp in FW have growth and survival rates comparable to shrimp in BW and SW over the long term. I hypothesized that growth and survival would not be influenced by salinity, based on reported success of growing shrimp in FW (Laramore et al. 2001; Araneda et al. 2008; Van Wyk 2013).

Materials and Methods

To evaluate the effect of salinity on growth rate and survival of WL shrimp, an experiment was carried out at the School of Natural Resources and Environment, University of Michigan. The initial intent was a 30-day growout period. When that timeframe was reached, I decided low mortality rates allowed extension of the experiment to 107 days.

Three treatments were tested including FW, BW, and SW (0, 12, and 35 ppt, respectively); each condition was replicated in triplicate. Each treatment was a distinct recirculating aquaculture system, composed of three fiberglass tanks, for a total of nine experimental tanks (Figure 1). Tank dimensions were 111 cm × 50 cm × 45 cm, and were filled to 15 cm of water depth (75.7 liters). They were covered with rigid lids, and treated with artificial seawater (Instant Ocean sea salt mix), which is made up of sodium chloride (NaCl), magnesium chloride ($MgCl_2$), sodium sulfate (Na_2SO_4), calcium chloride (Ca_2Cl), and potassium chloride (KCl) to reach the specified salinity levels. Aquarium heaters were used to keep water temperature of all experimental tanks at approximately 26.5°C during the culture period. Each tank was provided with a 15 cm air stone connected to an airline for continuous aeration, and a biological sponge filter was added to provide additional solids filtration. Photoperiod was maintained on a 12:12 hour light-dark cycle using artificial luminosity.

Figure 1 Experimental setup displaying tanks and biofilters

Municipal water from Ann Arbor, Michigan was used for initial filling and water replacement, after treatment for chlorine and chloramines. Water was cycled through the three-tank system of a treatment, as well as a down-flow, nitrifying biological filter (Figure 1). Each biofilter was comprised of a column that was 20.3 cm in diameter and 91.4 cm in length, and filled with a mixture of 4 to 10-mm diameter clay spheres (Aquaclay, Keeton Industries, Wellington, CO) and 1.6 mm by 3.2 mm oval plastic beads (Aquatic Eco-system, Inc., Apopka, FL) as biofilm attachment medium (Brown 2013).

SPF WL shrimp came as ten-day-old post-larvae (PL10) from RDM Aquaculture LLC (Indiana, USA). These are free of Baculovirus, Infectious Hypodermal Hematopoetic Necrosis Virus, Taura Syndrome Virus, and White Spot Syndrome Virus (Van Wyk 2013). By 23 days of age (0.008 g/shrimp), PLs were randomly assigned to one of nine tanks. Each tank was stocked with 100 individuals, for a stocking density of 133.3 shrimp/m^2. Acclimation to the varying salinities was done following methods prescribed by Davis et al. (2004), accomplished via slow addition of filtered water. Salinity was reduced at a rate of 4 ppt/hour in the BW and FW tanks until reaching 12 and 4 ppt, respectively. The FW tanks were further reduced at a rate of 1 ppt/hr to 0 ppt.

Commercial feed was used in this experiment, containing 40% protein, as reported by the manufacturer (Rangen Inc., Buhl, ID). Feed was manually weighed once or twice daily, and distributed in feeding trays. One tray per tank was used to monitor feed consumption and estimate adjustments to rations. Daily feeding rates were started at 8% of the total stocked biomass initially, and then adjusted based on observation of shrimp feeding behavior. Feed size was also increased with shrimp size as instructed by the manufacturer.

Shrimp weight was estimated weekly by sampling at least 10% of shrimp, randomly netted from each tank. A digital scale was used to first prerecord the weight of water in a container. Excess water was removed from the shrimp by patting them dry, and they were placed into the water and batch weighed. Finally, the weight of the water and container were subtracted from total weight that was then divided by number of shrimp to obtain individual average shrimp weight. Three times throughout the experiment, every individual from each tank was counted and a random 10% sample was individually weighed. At end of the experiment all individuals were counted and weighed.

Desired water quality parameter ranges were established (Table 1). Temperature, salinity, pH and dissolved oxygen (DO) values were recorded bi-weekly with a YSI meter (Yellow Springs, OH). Based on these readings, adjustments were made in order to maintain desired ranges by manually adjusting heaters and oxygen input through air stones.

Table 1 Desired parameter ranges for culture of WL shrimp, with references in parentheses

Water Quality Parameter	**Range**
Temperature	26 – 27 C (Araneda et al. 2008)
Salinity	0, 12, 35 (± 1 ppt)
pH	7.5-9 (FAO 2014); mortality below 5.0 (ASEAN 1978)
Ammonia (NH_3)	<0.16 mg/l (Lin and Chen 2001)
Nitrite (NO_2-)	< 5 mg/l (Lin and Chen 2003; Timmons and Ebeling 2007)
Nitrate (NO_3-)	< 200 mg/l (Kuhn et al. 2010)
DO (O_2)	≥ 5 mg/l
Alkalinity	80-200 mg/l (Ching 2007)

Bi-weekly nitrogen measurements were conducted for concentrations of ammonia (NH_3) and nitrite (NO_2-) with the EPA Nessler method 8038 and the diazotization method 8507, respectively, using a digital spectrophotometer (HACH model DR 2000, HACH Company, CO, USA). Alkalinity was tested via the phenolphthalein and total alkalinity method 8203 with digital titration (HACH model 16900, HACH Company, CO, USA). Nitrate (NO_3^-) concentrations were also measured at the end of the experiment with spectrophotometry. FW exchanges were made as necessitated by these readings; a portion of the total water was cycled out and replaced with FW and salinity adjusted accordingly. Biofilters were also backwashed when accumulation of solids was visible. Maintenance also entailed regular replenishment of water levels after evaporation loss.

Three aspects of the data were of particular interest: growth rates, survival rates, and biomass. Growth rate was estimated for each density treatment as a function of weight and time with the formula:

$$GR_i = (W_{fi} - W_{oi})/t$$

where GR_i is the growth rate of treatment i; and W_{fi} and W_{oi} are average final and original weight over time t (Araneda et al. 2008). Survival was calculated as the number of shrimp per tank remaining alive divided by the original total. A biomass analysis incorporated both survival and growth. Total system biomass was calculated by multiplying tank survival by average individual shrimp weight for the four dates when I had complete population samples.

Statistical analyses were conducted with RStudio (RStudio Team 2015). Data were expressed as mean ± standard error. One-way analysis of variance (ANOVA) was used to compare independent and dependent variables between treatments. When significant, Tukey HSD a posteriori test was used to investigate which treatments differed. Dependent variables included survival, growth, and biomass; independent variables evaluated included salinity, DO, ammonia, and nitrite concentrations, as well as conductivity, alkalinity, and pH values.

Matrices were created using Pearson's correlation indicating strength of association between variables, ranging from -1 to 1 (Appendix, Table 1). I used linear mixed effect models to evaluate effects of water quality variables on growth and survival, which allows for inclusion of a random effect for tank replicates, but the tank effect was insignificant, likely due to the flow-through nature of the experimental design that resulted in homogenous water conditions for all tanks within a treatment. Therefore, stepwise multiple regression was used to determine variables significantly affecting shrimp growth, survival, and biomass. The categorical covariate of treatment (salinity level) was used as a continuous variable to achieve robust models. Residuals were evaluated for normality, and the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) tests were utilized in model selection.

Results

My null hypothesis that growth would be the same at the chosen salinities was rejected; under the conditions of my experiment, WL shrimp grew significantly slower in FW (0.063 g/wk) as compared to greater salinities (0.62 and 0.091 g/wk) (F(2,137) = 33.96, $p < 0.05$) (Figure 2). Increased growth began to occur in the BW treatment at approximately day 40; FW and SW growth more or less stagnated. Individual average shrimp weight in the BW treatment on day 107 was nearly seven times that in the SW treatment, and nine times that of the FW treatment.

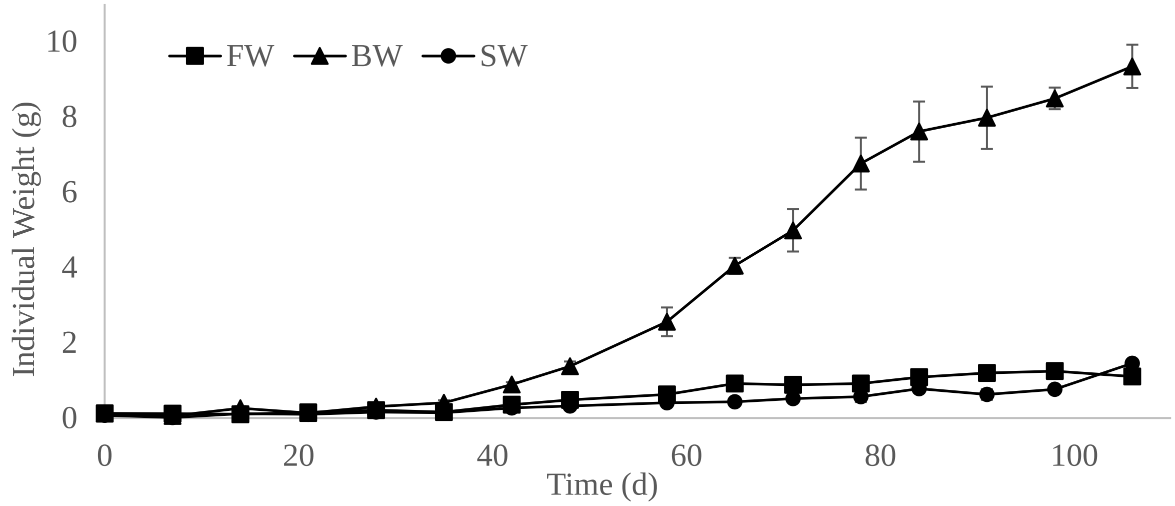

Figure 2 Mean ± SE for individual weight of WL shrimp by treatment over time

Table 2 Weekly growth rates and final survival over the 107 day growout.

Treatment	Survival	Mean Growth Rate (g/wk)
FW	1%	0.063
BW	63%	0.616
SW	72%	0.091

Exploratory data analysis showed that growth was positively correlated (r = 0.92) with ammonia concentration in the BW treatment, but not in the FW and SW treatments (r = 0.567 and -0.565, respectively). An initial multiple regression that included all potential water quality variables resulted in positive associations of ammonia and nitrite concentrations with growth. Increases in nitrogenous compounds were a result of increased growth and biomass, not a predictor of it, and therefore these parameters (ammonia and nitrite) were removed from the growth regression model. This resulted in a best fit model for mean shrimp growth and water quality, including alkalinity, pH, salinity, and DO (log transformed for normality) ($F(4,118) = 127.7$, $p < 0.05$, $r^2 = 0.812$, $r^2_{\text{adjusted}} = 0.806$). Growth was estimated as a function of the explanatory variables with the best fit model:

$$Growth = 9.851 + 0.037 \cdot Alkalinity - 1.175 \cdot pH - 0.130 \cdot Salinity + 1.917 \cdot DO$$

The second portion of my null hypothesis was also rejected: shrimp survival was affected by salinity in this experiment. Shrimp numbers steadily decreased throughout 107 days in all three salinity treatments (Figure 3), but the decline was most drastic in the FW treatment tanks, which had a final survival of 1% (Table 2). Survival differed significantly across treatments (ANOVA, $F(2,137) = 33.96$, $p < 0.05$), and a Tukey HSD post-hoc test showed that survival rates between the BW and SW treatments did not significantly differ, but both differed from survival in FW. Survival rates of shrimp were correlated with ammonia ($r = -0.914$, -0.872, and 0.674 for FW, BW, and SW, respectively) when separated out by treatment, but not when compared for all treatments combined ($r = 0.0839$).

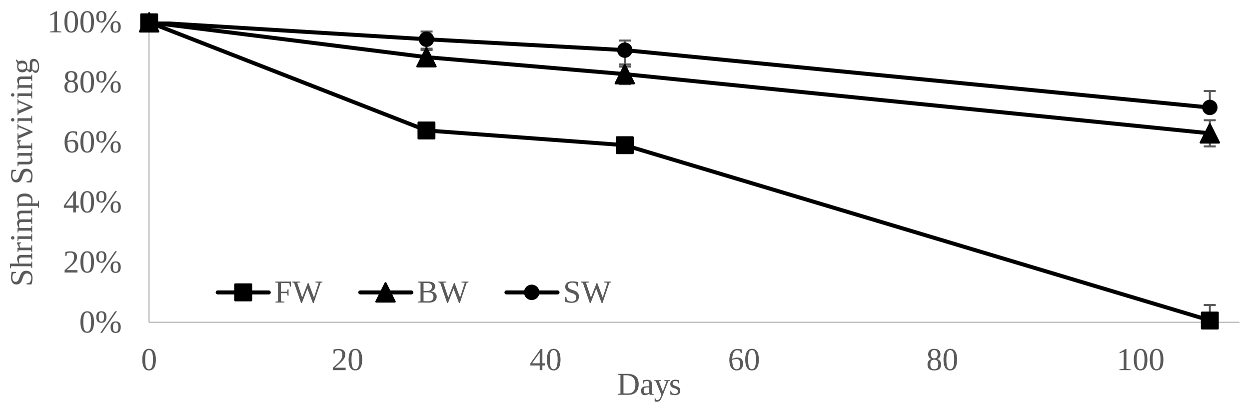

Figure 3 Mean ± SE for WL shrimp survival by treatment over time

Salinity was not one of the most significant predictors of survival. Ammonia, alkalinity, and pH were the most significant predictors of shrimp survival, and resulted in a significant regression model explaining 82% of the variance in survival (F(3,31) = 51.88, $p < 0.05$, $r^2 = 0.834$, $r^2_{adjusted} = 0.818$). The explanatory variables with best fit model was:

$$Survival = -0.970 - 0.024 \cdot Ammonia + 0.002 \cdot Alkalinity + 0.210 \cdot pH$$

Nitrogenous compounds were also positively correlated with biomass, likely due to the influence of growth and biomass on nitrogen excretion. Therefore these parameters were excluded from biomass models. Due to the combined effects of survival and growth, final biomass in the BW treatment was 5.7 times greater than in the SW treatment, and 20.4 times that in the FW treatment (Figure 4). DO was the only variable correlated with biomass, and produced a significant model that only explained 23% of the variation (F(1,30) = 10.05, $p < 0.05$, $r^2 = 0.251$, $r^2_{adjusted} = 0.226$). The best fit model was:

$$Biomass = 75.125 - 10.376 \cdot DO$$

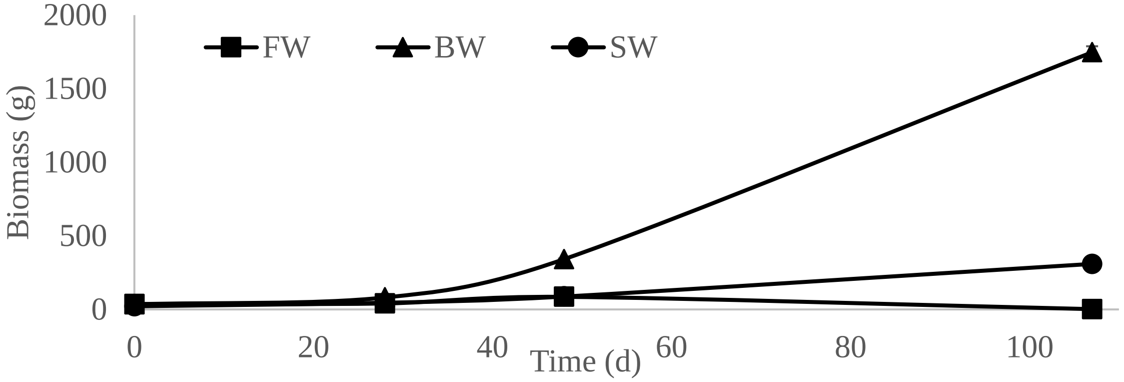

Figure 4 Mean ± SE for biomass by treatment throughout the experiment

Overall mean DO, conductivity, alkalinity, and pH differed significantly between treatments (ANOVA, p<0.05) (Appendix, Figures 1 and 2). There was variability in ammonia and nitrite concentrations across time among treatments (Figures 5 and 6). Ammonia was consistently higher than the desired range in SW tanks early in the experiment, it then tapered off at day 80, while ammonia in the BW treatment tanks followed an opposite trend. At day 50, concentrations of both ammonia and nitrite increased in the BW treatment along with growth rate.

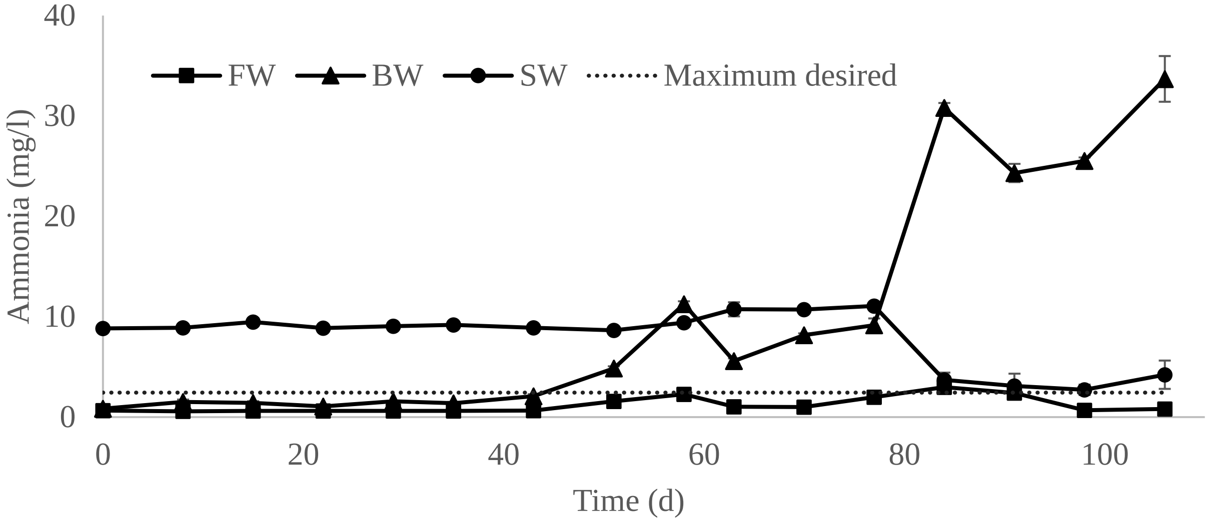

Figure 5 Mean ± SE for ammonia concentration over time by treatment, and maximum desired concentration

Nitrite levels remained low for early stages of growout, then spiked at approximately day 105 in BW and SW treatments (Figure 6). DO did not differ significantly between SW and FW treatments ($p=0.81$), and both had very slight downward trends, but mean values for both were significantly higher than the overall mean for BW ($p<0.05$) (Figure 7). As expected, ammonia and DO were highly correlated ($r = -0.805$). Nitrate concentrations stayed within the acceptable range in all treatments (4.06, 2.77, and 3.76 mg/l in FW, BW, and SW, respectively).

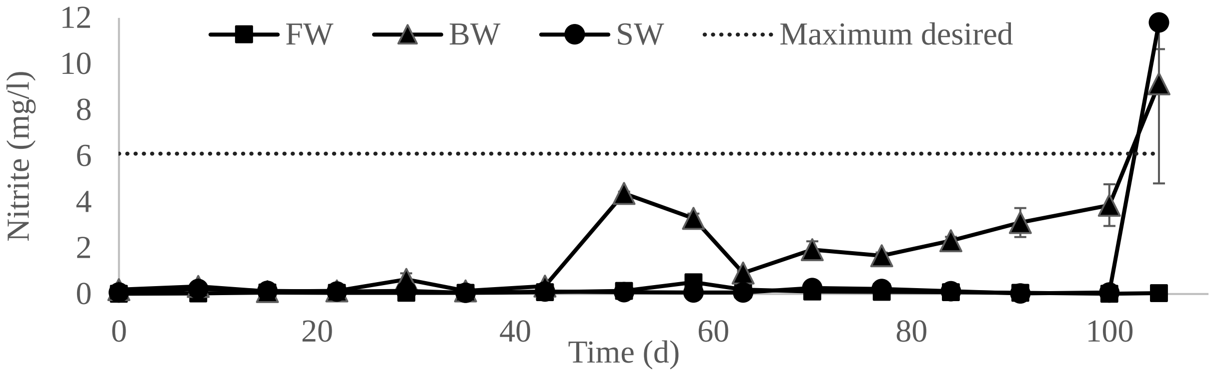

Figure 6 Mean ± SE for nitrite concentrations over time by treatment, and maximum desired concentration

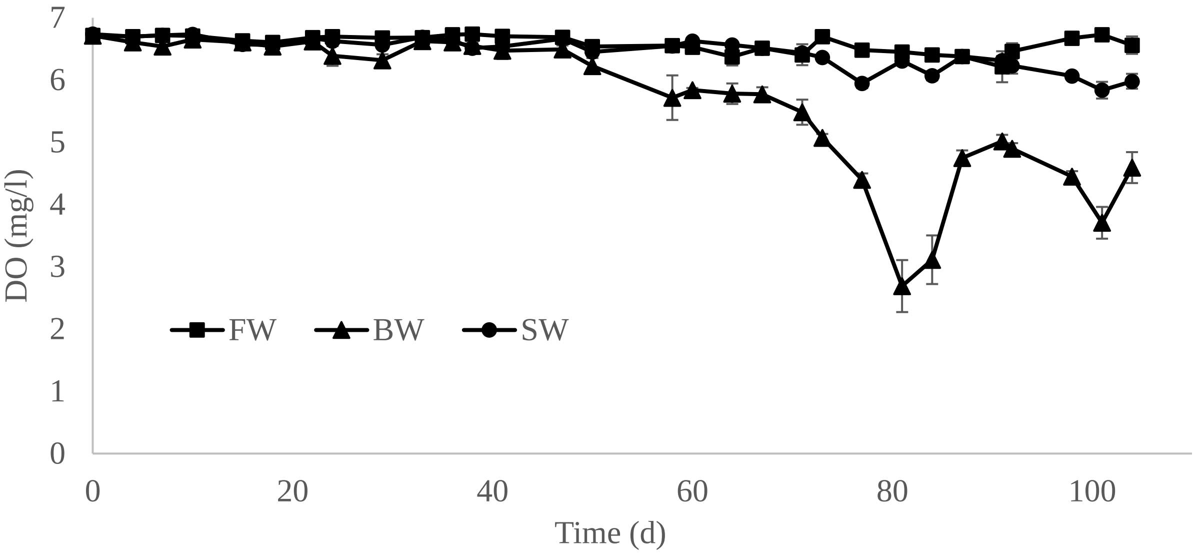

Figure 7 Mean ± SE for DO over time by treatment

Discussion

The mechanisms limiting shrimp growth and survival in FW culture are important factors influencing more biosecure, environmentally controlled, and intensive shrimp farming. Under the conditions of this experiment, WL shrimp grew poorly in FW (0.063 g/wk) as compared to greater salinities (0.62 and 0.091 g/wk). Therefore, the null hypothesis that salinity did not affect growth was rejected. WL shrimp survival was poor in FW as compared to other treatments, and I rejected the null hypothesis that survival would not vary with salinity. While salinity treatments affected survival, water quality variables also influenced growth and survival. Regression analyses indicated that water quality variables (alkalinity, pH, salinity, DO, and ammonia) were significantly correlated to growth and survival. It is likely that simultaneous suboptimal water quality parameters, as well as salinity level influences, had synergistic effects on growth and survival (Schuler 2008). Based on final regression models, growth was significantly associated with alkalinity, pH, salinity, and DO. Survival was also associated with alkalinity, pH, and ammonia, while biomass was only associated with DO. Growth was the sole dependent variable significantly associated with salinity, which indicates the importance of many water quality parameters.

Shrimp initially grew slowly in each treatment, which was expected based on known exponential growth rates, and there was little variation between treatments for the first month. After this point, growth escalated in the BW treatment, and resulted in subsequent changes in ammonia, nitrite, and DO, yet FW and SW shrimp growth and water quality stagnated in comparison. This reflects results of similar studies. Mair's (1980) choice apparatus experiment demonstrated shrimp propensity toward low salt concentrations (<10 ppt), and despite the presence of disease, Bray et al. (1994) also found improved growth at low salinities (5-15 ppt). Nonetheless, the highest growth rates, in the BW treatment, remained less than expected growth rates for WL shrimp (1-3 g/wk) (Wyban and Sweeney 1991; Briggs et al. 2004; Van Wyk 2013; Ravuru and Mude 2014; FAO 2016).

Shrimp growth in the FW treatment (0.063 g/wk) was slower than that of greater salinities (0.62 and 0.091 g/wk). Araneda et al. (2008) and Van Wyk (2013) also had comparatively poor growth rates of whiteleg shrimp in FW (0.38 and 0.4-0.7 g/wk, respectively).

This suggests that FW culture may result in poor growth of WL shrimp. Nonetheless, regression analyses indicated that other parameters were also influential on growth. Additionally, experimental design can have growth implications. For example, while my experimental setup resembled that of Araneda et al. (2008), they used a Mexican commercial feed distributed four times per day as compared to my American feed at twice per day; increased feeding frequencies can improve growth. Conversely, Araneda et al. (2008) heated tanks to just 25°C (as compared to my 26.5°C), which can slow growth. In another study, Van Wyk (2013) used a raceway system to run experiments inside passive, solar-heated greenhouses that experienced significant temperature fluctuations (22-31°C) that influenced his results. Additional differences in his study included sand biofilters, inconsistent use of varying types of commercial feeds fed four times per day, as well as a disease outbreak. The key to growing shrimp in fresh water may lie within the appropriate combination of these parameters. Modest growth in the high salinity treatment was unexpected, and may have been due to parameters specific to my experiment, such as my limited seeding of the biofilters prior to experimentation having potential implications on their effectiveness in a high salinity environment.

WL shrimp survival in FW was significantly less than in other treatments under the conditions of this experiment. Declines in number surviving were relatively constant over 107 days in all three treatments, for final survivals of 1%, 63%, and 72% in the FW, BW, and SW treatments respectively. Like growth, survival is dependent upon culture conditions; poor growth and survival results indicate suboptimal conditions occurred in the present study. Other studies reported 76-88% survival of WL in FW (Araneda et al. 2008; Van Wyk 2013), which are typical of WL shrimp aquaculture (Wyban and Sweeney 1991; Briggs et al. 2004; Jayasankar et al. 2009; FAO 2016). Successful WL survival in FW is possible, indicating that FW survival results presented here were a result of poor culture conditions beyond salinity.

In this study, shrimp growth and survival were affected by salinity and other water quality parameters. However, teasing out cause and effect becomes challenging due to compounding factors. For example, growth was most significantly associated with DO, based on the regression model, and DO is widely acknowledged as one of the most important variables in aquaculture (Lin and Chen 2003; Schuler 2008; Cobo et al. 2012). pH was secondarily predictive of growth, and most predictive of survival. This indicates that DO and pH may be the most influential parameters on growth and survival and, therefore, some of the most important to control via management. DO and pH are connected; DO consumption can increase with activity or feeding. DO consumption increases as shrimp grow and become greater in terms of biomass. Increased shrimp biomass results in greater excretion and ammonia, which leads to a faster growing nitrifying microbial community in culture tanks and biofilters that require even more DO. It may be this relationship that explains the strong statistical correlation between ammonia and DO ($r = -0.805$). We expected this process to occur in all treatment biofilters but at varying rates, such as the BW treatment which had accelerated shrimp growth. This also leads to increased CO_2 and acidity and decreased pH (Wurts and Durborow 1992). Low DO environments can cause increased shrimp respiration, as well as uptake of dissolved nitrogenous compounds, exacerbating DO effects on growth and survival. This may have been the cause of decreased growth rates in the BW treatment tanks toward the end of the experiment (days 80-107). Low pH (< 7.0) can have the same effect, of causing DO consumption to increase, which is a potential combined effect of these two variables (Pan et al. 2007).

Shrimp in the FW treatment were subject to stress at low salinity; this stress was compounded by low pH and alkalinity, which decreased over time, likely increasing adaptation stress (ASEAN 1978; Pan et al. 2007). Ann Arbor city water is softened, which reduces average alkalinity concentrations to 51 mg/l, below the desired range of 80-200 mg/l for my experiment (City of Ann Arbor, Michigan 2015). Further, literature suggests that WL shrimp are less resilient in low pH environments, indicating that tolerance to nitrogenous compounds are likely reduced (ASEAN 1978; Cobo et al. 2012). Tolerance to even slight decreases in water quality (i.e., changes of ammonia and nitrite concentrations) toward the end of the experiment was possibly at a minimum. Perhaps the combined stressors of low pH, salinity, and alkalinity over time resulted in the slow decline of shrimp condition and survival, as well as slow growth, in this treatment. pH steadily declined in the other two treatments, as well, and may have also contributed to the steady decreases in numbers of shrimp surviving.

The survival data indicate decreased WL shrimp tolerance to poor water quality at lower culture water salinity; a concept that has been noted previously (Lin and Chen 2001, Lin and Chen 2003, Brown 2013). For example, ammonia, which makes up 60-70% of shrimp excretion (Schuler 2008), increased in concentration over the course of the experiment in the BW and SW treatments, and to a lesser extent in the FW treatment. The toxicity of ammonia makes it a parameter of concern, and toxicity increases with decreasing salinity (Lin and Chen 2003; Schuler 2008; Cobo et al. 2012), as well as with temperature, pH, DO, and alkalinity (ASEAN 1978; Lin and Chen 2003; Schuler 2008; Cobo et al. 2012). Statistically, WL shrimp survival in FW and BW was strongly and negatively correlated with ammonia concentration (r = -0.914 and -0.872, respectively), and may explain low survival in the FW treatment from day 60 to 107. Comparatively, SW shrimp survival was good (72%) despite high ammonia levels, which indicates greater ammonia tolerance under seawater conditions.

There are other potential explanations for poor growth and survival of WL shrimp in FW. Increased energy expenditure required for osmoregulation may limit growth and survival (Bray et al. 1994; Van Wyk 2013), and this is potentially mitigated via acclimation at a later stage of life (Laramore et al. 2001; Jayasankar et al. 2009). My data suggest a consistent mortality rate throughout the duration of my experiment, rather than high initial mortality, which again supports the idea of experimental stress. Control of the main anions and cations (bicarbonate, sulfates, chlorides, calcium, magnesium, potassium, sodium), as well as minimum hardness levels, may also be important (Bray et al. 1994; Saoud et al. 2003; Jayasankar et al. 2009; Van Wyk 2013). Genetics may play a role, as Ecuadorian strains of WL shrimp have been seen to fare better in lower salinity than Mexican strains (Bray et al. 1994; Laramore et al. 2001). The present study supports the concept that, while salinity plays a role in shrimp growth and survival, it is not the sole limiting factor to their growth in FW.

There are various potential explanations for poor shrimp growth in high salinity. Other studies, but not all, have encountered reduced growth of WL shrimp in high salinity environments, which may be another testament to the variability in outcomes resulting from differing experimental designs (Bray et al. 1994). Bray et al. (1994) theorized that food digestibility is reduced in hypersaline conditions. In this study, the SW treatment was subject to higher concentrations of ammonia throughout the majority of the experiment (approximately day 0 to day 80), which may have been due to slower microbial colonization in the biofilter under SW conditions (Stickney 2005). The assumption that biofilters would function equally at all salinity levels may have been erroneous. Survival of shrimp was good despite high ammonia in the SW treatment, and at approximately day 85, ammonia concentrations declined and were accompanied by accelerated growth.

Results presented here indicate that shrimp growth and survival in FW does not compare with that at higher salinities. Nonetheless, there remains considerable potential in growing shrimp in FW, and FW culture will likely be more important as wild fisheries decline and aquaculture technologies continue to evolve. It is apparent, and supported by scientific literature, that management of many factors are vital to shrimp growth and survival in FW. Shrimp are an ideal aquaculture species, requiring less proteinaceous diets, having tolerance to changing or poor conditions, and being a valuable commodity; for these reasons research efforts have transformed the industry since its inception. Continued efforts to elucidate the concept of growing shrimp in FW are worthwhile in order to make the valuable shrimp farming industry as sustainable as possible. This study demonstrated that growing shrimp over the long term is currently most effective in brackish water, compared to SW and FW. Continued research on identifying drivers of this limitation, as well as the ideal salinity concentration at a finer scale is important. Briggs et al. (2004) identified growing WL shrimp in FW as an area needing further investigation, and the “continued development of biosecure, high density and low salinity culture systems” remains a high priority research endeavor for the FAO (2016).

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Appendix

Table 1. Correlation matrix, with significant results highlighted in yellow.

	DO	*Ammonia*	*Nitrite*	*Conductivity*	*Alkalinity*	*pH*	*Salinity*
DO	1						
Ammonia	-0.80535	1					
Nitrite	-0.49882	0.431314	1				
Conductivity	0.05518	0.239092	0.010844	1			
Alkalinity	-0.41927	0.687015	0.258876	0.735286	1		
pH	0.073225	0.160804	-0.01881	0.54705	0.656268	1	
Salinity	0.036369	0.262571	0.069978	0.99371	0.738797	0.539043	1

Figure 1. Mean ± SE for pH over time by treatment

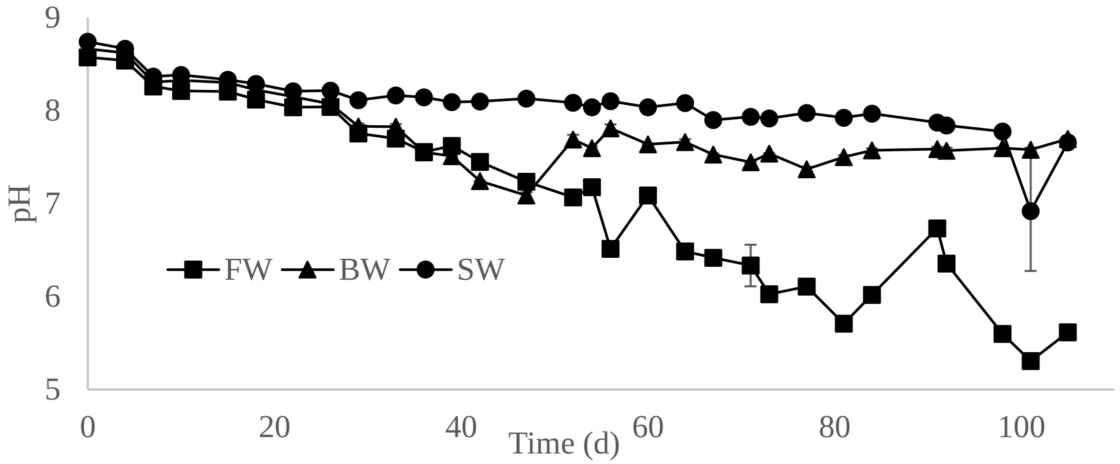

Figure 2. Mean ± SE for alkalinity over time by treatment