AN EVALUATION OF MANAGEMENT CRITERIA FOR BIOFLOC SYSTEMS USED IN HIGH-DENSITY EARLY LIFE STAGE REARING OF MARINE PENAEID SHRIMP AT LOW AND HIGH SALINITY

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Gregory Cottingham

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Department of Life Sciences

Corpus Christi, Texas

APPROVED: _____ DATE: _____

Dr. Addison L. Lawrence, Co-Chair

_____

Dr. Joe M. Fox, Co-Chair

_____

Dr. Anthony J. Siccardi, Member

_____

Dr. John Scarpa, Member

_____

Dr. David Moury, Department Chair

Format: *Aquaculture*

ABSTRACT

Biofloc technology is a proven, environmentally-sustainable method that reduces potentially-harmful discharge, capital costs for facility construction, and expensive feed inputs. Therefore, the use of biofloc technology, normally applied to grow-out production, was applied and evaluated for shrimp *Litopenaeus vannamei* nursery systems. The objective of the study was to evaluate nitrogen management in a shrimp nursery biofloc system at 1) low and high salinity (8 and 28 ppt), and 2) low and high biomass density (~20 g/m^2 and 300 g/m^2). Three 14-15 day nursery trials were conducted with postlarval or juvenile shrimp. Mean TAN levels were 0.63±1.17 and 0.85±1.17 mg L^{-1} in Trial 2 and 0.18±0.29 and 0.17±0.29 mg L^{-1} in Trial 3 for the 8 and 28 ppt treatments, respectively. There was no difference in TAN concentration between treatments in either trial ($p = 0.46$ and 0.77 Trial 2 and 3, respectively). Mean NO_2 levels were 3.00±1.63 and 4.68±2.54 mg L^{-1} in Trial 2 and 1.64±0.17 and 2.72±0.23 mg $L^{-}1$ in Trial 3 for the 8 and 28 ppt treatments, respectively. Nitrite was significantly higher (both $p < 0.01$) in the 28 ppt treatment of both Trials 2 and 3. Results suggest that nitrogen can be controlled at either salinity and at either low or high biomass density. In addition, the system described in this study showed good potential for stocking substantially higher densities (up to 0.49g/L vs 0.03 g/L currently stocked) of postlarvae. Biofloc-managed systems will be key in increasing the carrying capacity of nursery systems, but certain management issues (e.g., stocking procedure, establishing and maintaining ideal biofloc level and cost:benefit ratio) still remain to be investigated.

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1. Introduction

According to the FAO (2014), shrimp was the single largest fisheries commodity in value, accounting for approximately 15% of the total value of internationally traded fishery products. Farming of shrimp represented 55% of global shrimp production in 2011 (FAO 2012). Improved methods for growing shrimp, such as enhanced biosecurity via elimination or significant reductions of water exchange or stocking of high-health and genetically improved shrimp, have facilitated the intensification of its production (Browdy and Moss 2005, Mishra et al. 2008, McIntosh and Avnimelech 2006, Chamberlain 2010). To meet increasing global demand, specific techniques to mitigate high operational costs, such as labor, energy and land costs, as well as technological and infrastructure needs, while ensuring high production per unit area must be further developed.

At the Texas A&M Shrimp Mariculture Project (TXSMP), methods have been established for the successful nursery phase production of juvenile *Litopenaeus vannamei* in minimal water exchange systems at depths of 30 cm or less (U.S. Patent No. 8,335,498, 2013, CHN Patent No. 201310461821.0, 2013, EU Patent No. 242982, 2014, U.S. Patent No. 8,985,055 B2, 2013) and at salinities of 28-30 ppt (Crockett et al. 2014a, Crockett et al. 2014b, Crockett et al. 2014c, Crockett et al. 2014d, Lawrence and Crockett 2014, Lawrence et al. 2014, Moeckel et al. 2012a, Moeckel et al. 2012b). Use of an intermediate nursery phase in commercial shrimp aquaculture has the potential to increase crop availability, biosecurity and survival during grow-out by 10% (Kumlu et al. 2001, Yta et al. 2004, Fóes et al. 2011). Limited exchange reduces water usage, increases environmental sustainability, and if appropriately managed, provides supplemental

nutrition in the form of bioflocs, aggregates of organic material colonized by micro-organisms (Avnimelech 1999, Hari 2006, Avnimelech 2007, Azim and Little 2008). The use of shallow water raceways further improves upon nursery culture by providing the opportunity to stack culture units. While building design and, accordingly, associated costs still requires consideration, stacking would make it possible to increase production while avoiding significant increase in horizontal footprint. It has been suggested that using shallow water systems increases productivity through stacking (i.e., biomass produced per footprint area) and eases control of water quality parameters (Øiestad 1999, Labatut and Olivares 2004). As with all shrimp culture systems, both indoor and outdoor, environmental factors affect production performance of targeted species. A major factor influencing growth of marine shrimp is salinity, which can affect both the cultured species and the community of microorganisms present (Bray 1994, Decamp et al 2003, Fontenot et al 2007). This factor is of current importance due to growing interest in establishing super-intensive culture facilities for shrimp near market centers (Treece 2014, NSTC Interagency Working Group on Aquaculture 2014), which are often far inland.

1.1 Nursery Culture

A technique that is commonly used in the intensive production of shrimp involves inclusion of nursery systems between hatchery and grow-out phases (Samocha et al. 1993a, Peterson and Griffith, 1999, Samocha 2010). Typically, only stronger shrimp survive the duration of the nursery phase; as a result early mortality during the grow-out phase is reduce, allowing for more effective use of grow-out area. Additionally, the nursery phase gives producers the ability to assess animal condition before stocking into

grow-out systems, giving them insight into the status of the standing crop and providing the opportunity to manage feeding rates more efficiently.

The nursery phase of shrimp production utilizes smaller ponds, raceways or tanks, which are typically operated at higher stocking densities (Sturmer et al., 1992). Early-life stage shrimp can be held in an indoor or greenhouse-enclosed nursery when grow-out conditions are subpar, permitting the extension the grow-out season, which is especially useful in temperate and subtropical areas where the production season is limited. Nursery systems typically produce juvenile shrimp in the range of 0.1 – 2.0 g in weight and are used in integrated high-density production facilities, as opposed to extensive or semi-intensive farms, which stock postlarvae (PLs) typically weighing less than 0.01g directly into ponds (Sturmer et al., 1992, Yta et al., 2004, Zelaya et al., 2007). Larger initial stocking size means less time in grow-out ponds, which allows for more crops per year leading to more efficient use of grow-out production systems. The nursery phase increases overall shrimp survival, improves facility utilization and increases production per unit area, per time period with better control over shrimp growth and feed utilization (Mishra et al. 2008, Samocha 2010).

Nursery production strategies vary. A typical goal is to produce reasonable numbers of large (1-3g), healthy juvenile shrimp; however, some managers choose to simply focus on production of greater numbers of smaller (0.5-1g) animals, so that more animals are available for stocking when conditions are ideal for grow-out. Regardless of strategy, production systems should be managed using flexible approaches to changes in physical, environmental and biological criteria in order to maximize growth and survival.

1.2 Minimal Exchange Biofloc Systems and Inorganic Nitrogen Control

Minimal water exchange biofloc nurseries have been confirmed as a culture strategy for rapid growth of postlarval and juvenile penaeid shrimp (Arnold et al. 2009, Fóes et al. 2011, Emerenciano et al. 2012). Bioflocs are assemblages of particulate organic matter (e.g., feces, detritus) and uneaten feed colonized by microorganisms (i.e., algae, bacteria, protozoans) that form inside the culture unit as a result of extended water residence time and nutrient loading; in particular, brown-water biofloc systems are characterized by a microbial community dominated by bacteria (Hargreaves, 2013). Limited exchange of culture water in intensive biofloc shrimp production systems allows for accumulation of inorganic nitrogen, which is controlled via nitrification by chemoautotrophic bacteria or assimilation by heterotrophic bacteria (Burford et al., 2003, Cohen et al., 2005, Hargreaves 2013). Both processes occur simultaneously, but levels of intensity depend on the extent of respective bacterial populations. Nitrification will increase in the presence of high densities of chemoautotrophic bacteria (autotrophic phase), whereas uptake of carbon-based substrates and immobilization of nitrogen increase as levels of heterotrophic bacteria rise (heterotrophic phase, Avnimelech 2015).

Assimilation of organic material and subsequent cellular reproduction results in increased availability of microbial protein, which can be consumed by juvenile shrimp, thus recycling feed nutrients (Avnimelech 2015). It can also reduce the daily requirement for feed-sourced dietary protein by shrimp. A study conducted by Xu et al. (2012) in a limited-exchange production system showed that reducing a typical feed protein level by 10% (i.e., from 35 to 25%) did not significantly affect growth and survival of shrimp in biofloc systems. Assimilation of nitrogen by the heterotrophic community is encouraged

by adjusting the feed or feed inputs to a high C:N ratio (>10, Avnimelech 2015). When bacteria utilize high C:N ratio substrates (e.g., carbohydrates) they assimilate dissolved inorganic nitrogen from the water column for protein synthesis (i.e., cellular growth) resulting in increased biomass (Avnimelech 1999, Avnimelech 2015, Schneider 2006).

Biofloc technology for production of shrimp was developed from a need to intensify culture operations while reducing initial investment and ongoing operational costs (Hopkins et al., 1993, Avnimelech 1999, Avnimelech 2007, Krummenauer et al., 2014 Avnimelech 2015). Zero or minimal water usage in systems allows producers to reduce water costs while improving biosecurity (Avnimelech 2015). Because low water usage systems have reduced potential for discharge of pollution (i.e., nutrients, toxins or pathogens), they are considered environmentally sustainable (Wasielesky et al., 2006, Samocha et al., 2006, Mishra 2008, Avnimelech 2009). Moreover, low water usage increases the viability of culturing marine shrimp far from coastal regions, which improves proximity to consumer markets (Tallamy and Moss 2006). Intensive inland shrimp farming typically presents higher economic viability than farms located in coastal areas largely due to high cost of land (e.g., competition with recreational activities, housing developments) and the rigorous environmental protection legislation of these regions that often include wetlands, wildlife refuges, and preserves (Maica et al., 2012, Flaherty et al., 2000). Economic viability can be further improved through the use of shallow water depths (i.e., stacking of tanks to reduce overall footprint) and decreased salinity (decreased capital costs associated with acquiring water from the ocean); however to the author's knowledge there are no developed cost analyses for these types of systems at time of writing. Reduction of salinity in shrimp production systems,

especially those located inland, would decrease operational expenditures associated with use of expensive synthetic sea salts and allow for easier management of wastewater, both leading to an overall reduction in cost (Schuler and Boardman. 2010, Maica 2012). This is an important point as one of the major objectives of the aquaculture industry is to move away from expensive coastal land, while maintaining viability and environmental sustainability.

The Pacific white shrimp *Litopenaeus vannamei* has become the most widely cultured shrimp in the world because it is fast growing and tolerant to a wide range of environmental and stocking conditions (Briggs et al., 2004, FAO 2014). With respect to salinity, *L. vannamei,* a euryhaline species, has been cultured experimentally and commercially at salinities 0.5-45 ppt (Van Wyk et al., 1999, Leal et al., 2010). This wide tolerance and osmoregulatory capacity has been shown to be largely influenced by age and gill development in the later postlarval stages (Nunes and Lopez 2001; McGraw et al., 2002): shrimp $\geq PL_{10}$ (postlarvae 10 days past metamorphosis) are equally able to acclimate to salinities as low as 1 ppt in as little as 5 hours, but survival increases significantly at PL_{15} (McGraw et al., 2002). Although the commercial culture of *L. vannamei* has been documented at various salinities and inland in Arizona in raceways at salinities less that 2 ppt (Samocha et al., 2004, Roy et al., 2010), few studies have addressed biofloc management under such conditions or at variable stocking densities.

1.3. Rationale

The present research project was undertaken because there is a need to add value to U.S. produced shrimp by growing them closer to local markets. Closer proximity to market reduces transport and marketing cost (Tlusty 2002, Jagger and Pender 2001) and

research has shown that consumers are willing to pay a premium for fresh and locally sourced products (Dasgupta et al., 2010, Davidson et al., 2012, Loureiro and Hine 2014) To accomplish this, production will need to be increased while reducing feed costs and water usage. In order to reach this level of sustainable production it must be demonstrated that shrimp PLs can be reared successfully at high densities at low salinity. While it has been documented that *L. vannamei* can tolerate lower salinities (Samocha et al., 2004, Roy et al., 2010, Van Wyk et al., 1999, Leal et al., 2010 McGraw et al., 2002), this study aims to address carrying capacity of a nursery system for the production of juvenile shrimp at lower salinities. Nursery systems will also have to become more environmentally sustainable. If postlarval shrimp are to be reared at lower salinity it must be further demonstrated that management of biofloc systems at low salinity is feasible and specific procedures have to be developed.

1.4 Objective

The objectives of this research project were to evaluate nitrogen management of a low water-usage biofloc system for shrimp postlarvae at 1) low and high salinity, and 2) low and high biomass density.

2. Methods and Materials

2.1 Research Site

Trials were conducted at the Texas A&M Shrimp Mariculture Project (TXSMP) facility in Port Aransas, Texas. Seawater was pumped from the Corpus Christi Ship Channel adjacent to the facility and filtered to 50 microns (Diamond Water Systems Holyoke, MA, USA). Freshwater to lower salinity was obtained from municipal sources and treated by reverse osmosis (RO).

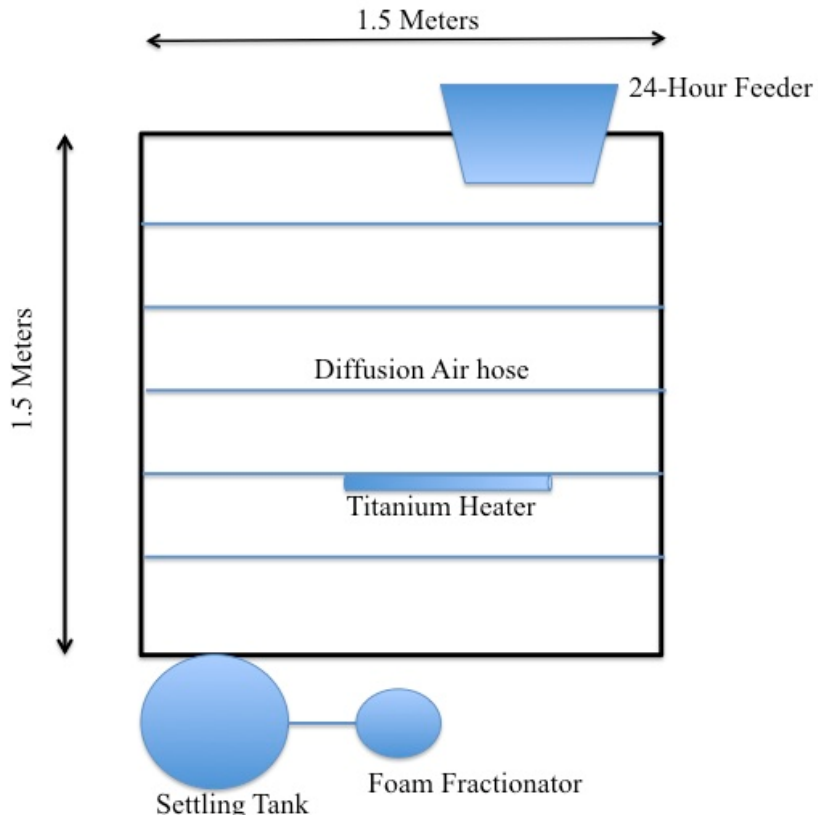
2.2 Source and Acclimation of Shrimp

Two cohorts of specific-pathogen-free *L. vannamei* were obtained as postlarvae (PLs) from Shrimp Improvement System (SIS), Inc. (Islamorada, Florida, USA) in two different shipments. The PL shipments (Cohorts 1 and 2) arrived on June 18 and October 29, 2014, respectively. It was assumed that numbers of animals per shipment quoted by SIS were correct. Upon arrival to the TXSMP facility, shipping bags containing PLs were immediately checked for levels of oxygen saturation, inorganic nitrogen, temperature, salinity, and pH. Shipping bags were then floated in either an acclimation tank or a maturation tank (both systems referred to as "lab tank"). Water from lab tanks was added to shipping bags every 2-5 minutes until temperature in shipping bags and lab tanks differed by less than 1 degree C and pH differed by 0.5 or less at which point PLs were poured from shipping bags into lab tanks and reared until experimental use or stocked from a lab tank into experimental systems immediately after acclimation. Postlarvae in lab tanks were hand-fed 10% of their daily ration twice daily with the remainder of the feed distributed through the use of a belt feeder over a 24-hour period.

2.3 Experimental System

Experimental trials took place in four 1.5 ×1.5 ×0.5 m tanks (total available volume=1125 L/tank), equipped with five equally spaced ~1.4 m length diffusion aeration hoses attached to PVC and suspended ~2cm above tank bottom. Diffusion air hoses were used to maintain minimum dissolved oxygen (DO) levels of 5 mg L^{-1} and keep particulate matter suspended in the water column. Tanks also had a submersible titanium heater, a settling tank (solids removal), foam fractionator system, and a 24-hour automatic feeder (Fig 1).

Fig. 1: Schematic representation from above of experimental tank (depth 0.5 m) showing settling tank, foam fractionator, air hoses and belt feeder.



2.4 Bacterial Dominance, Biofloc and Nitrogen Control

Conditions (i.e., maintaining C:N ratio of ~7.4:1 of the microbial community, based on feed protein level) to promote the abundance of chemoautotrophic bacteria were maintained for all experiments until a majority of tanks reached a minimum biofloc level of 2.5 ml L^{-1} (measured using Imhoff cones). This was achieved using a 40% protein commercial shrimp feed as well as the addition of a nitrifying bacteria inoculum (NB) (Bacta-Pur XLSW, IET-Aquaresearch Ltd, QC, Canada) to the culture water. Inorganic nitrogen levels (TAN, NO_2, NO_3) and alkalinity were measured twice daily with the aid of Tetra 6-1 EasyStrips and Tetra Ammonia Easy Strips (Tetra, Blacksburg, VA, USA). If TAN was greater than 2.0 mg L^{-1}, 90 mL (0.13-0.2ml L^{-1}) of NB was applied. An additional 90 mL was applied if the level of NO_2 reached a level of 3.0 mg L^{-1} or greater. This was done to establish the population of nitrifying bacteria at a level to maintain total TAN and NO_2 at or below 2.0 mg L^{-1} and 3.0 mg L^{-1}, respectively. Nitrifying bacteria application rate and nitrogen levels were recommended by inoculum supplier and modified based on anecdotal data gathered at the TXSMP from 2011-2014.

Heterotrophic bacteria dominance was promoted when a majority of tanks contained a sufficient amount of biofloc (2.5 ml L^{-1}) by shifting the carbon to nitrogen ratio to ~14:1 (based on feed protein level). This was done by reducing feed protein content from 40% (commercial feed, Rangen, Angleton, TX) to ~20% (18-23%, semi-purified feed prepared in lab) and supplementing the system with organic carbon (short-chain fructooligosaccharide or scFOS, Sigma-Aldrich, St. Louis, MO) in place of adding NB. Organic carbon was applied at a rate sufficient to induce bacteria to remove nitrogen

and maintain levels of TAN at ≤ 2 mg L^{-1}, NO_2 at ≤ 3 mg $L^{-}1$, and a residual NO_3 level of 35 mg L^{-1}. Nitrogen levels were set to mirror those for the chemoautotrophic phase.

Chemoautotrophs use carbon (CO_2 or HCO_3) for energy and produce hydrogen ions (H^+) during nitrification. In addition, aerobic respiration by both chemoautotrophic and heterotrophic bacteria produces CO_2. Chemical processes such as these reduce alkalinity and eventually pH. To maintain alkalinity, sodium bicarbonate ($NaHCO_3$) was added if alkalinity levels fell below 180 mg L^{-1} as $CaCO_3$. Application levels were determined using the following formula:

$$\frac{\left(\frac{Deficiency\ in\ alkalinity\ (mg\ L^{-1})}{Concentration\ of\ HCO_3\ in\ NaHCO_3(\text{i.e.},0.72646)} \times Tank\ volume\ in\ L\right)}{1000\ mg\ g^{-1}}$$

$$= grams\ of\ NaHCO_3\ needed\ per\ tank$$

Imhoff cones (1L) were used to determine settleable solids and used to estimate biofloc levels twice daily. One liter of water from each tank was collected and placed into one of four Imhoff cones and allowed to settle for 20 minutes before a reading was taken (Hargreaves 2013, Avnimelech 2015). Upon observing biofloc levels of 25 ml L^{-1} or greater, biofloc was reduced to 20 ml L^{-1} using settling tanks and foam fractionators. It has been suggested that at solids concentrations of approximately 20 ml L^{-1} or greater oxidation of ammonia to nitrate is limited (Ray et al., 2011). Thus, this level was chosen for the present study as it was presumed that a higher concentration of nutrients would be available for the production of single cell microbial protein. Settled biofloc and fractionated biofloc water (referred to as "BExchange" and "FExchange", respectively) was quantified, disposed of and replaced with filtered seawater or RO water to reach the

appropriate volume and salinity. The amount of biofloc to be removed was calculated according to the following:

$$\frac{(Desired\ reduction\ in\ biofloc\ (ml\ L^{-1}) \times Liters\ in\ the\ system)}{1000\ ml\ L^{-1}}$$

2.5 Feeding

For each experiment a feed curve, based on expected growth of the number of shrimp stocked per square meter, was used to quantify daily feed offered per tank (Ray et al., 2011, Lawrence and Crocket 2014). The number of shrimp stocked into each tank was adjusted for expected daily mortality, 0.7-1.4%/day or 10-20% for the entire nursery period. The adjusted population estimate was multiplied by the expected weight gain and multiplied by the daily feed conversion ratio (DFCR, Lawrence and Crocket 2014) to calculate the daily ration.

$$Number\ of\ animals\ X\ expected\ daily\ weight\ gain\ X\ DFCR$$

$$=\ amount\ of\ feed\ per\ day$$

Daily FCR ranged from 0.8-1.7 and was set each day such that the mean DFCR equaled the expected food conversion ratio for the entire experimental period (i.e., 1.2).

$$\frac{\sum DFCR(i.e\ 1.7-0.8)_{Day\ X}}{Total\ Days\ in\ Experiment(i.e.14-15)} = Expected\ FCR(i.e.,1.2)$$

Early in the experiment, DFCR was set high (i.e., 1.7, overfeeding) in order to provide more feed than the shrimp could consume, so as to provide substrate for biofloc formation. DFCR was gradually reduced until it reached 0.8 (i.e., underfeeding) as it was assumed biofloc would make up for the difference. The concept of using DFCR as presented here is based on methods established at the TXSMP (Lawrence and Crockett 2014).

Feed was offered by a 24-hour belt feeder. Feed dropped into tanks and was dispersed by water movement created by aeration. All shrimp were fed a size 0 Rangen (Rangen Inc, Angleton, TX) 40% protein diet daily during the chemoautotrophic phase. In addition, in all three trials, all tanks were fed 25 g of fine (<355 μm) feed particles once daily on Days 1-3 in order to promote biofloc formation. Fines were obtained by shaking milled feed (45% protein semi-purified standard reference diet made on-site, formulated by Dr. Addison Lawrence) through a series of sieves (355-2,360 μm).

2.6 Feed Preparation, Diet Ingredients and Nutrients

The exact preparation and ingredients of the Rangen 40% protein commercial diet are proprietary, but proximate analysis indicated the protein content was ~40% (Table 1). Two lower-protein (18 and 23% protein) feeds were manufactured on site. These were used to fine-tune system nitrogen for controlling heterotrophic dominance. Proximate analysis and ingredient composition for the 18% protein diet are shown in Tables 2 and 3 and in Tables 4 and 5 for the 23% protein diet, respectively. The 18% protein feed was prepared as a single ~28 kg mix that was parceled into ~2 kg batches for extrusion. Parceling was done to ensure the extruder was not overloaded. All ingredients 1% or less were placed in a V-mixer for one hour and then transferred to a food mixer (Model A-200m, Hobart Corporation, Troy, OH). In a separate bowl, alginate and sodium hexametaphosphate were added to deionized (DI) water and mixed using a hand mixer for approximately 45 seconds. The alginate mix was then added to the dry ingredients and mixed at room temperature (~24°C) for an additional 60 seconds to achieve a mash consistency appropriate for extrusion.

Table 1. Proximate analysis and phosphorous content of trial feed (Rangen 40%, Rangen, Inc., Angleton, Texas, USA).

Nutrient	Percent (as-fed)*	Percent, Dry Weight
Crude protein	40.0	43.1
Crude fat	8.0	9.97
Crude fiber	4.0	2.9
Ash	1.0	9.06

* Values determined by Midwest Laboratories, Inc. (Omaha, Nebraska). Values represent a single analysis.

Table 2: Crude protein, crude fat, crude fiber and ash levels (%) for 18% protein feed.

Nutrient	Dry Weight
Crude protein	18.67
Crude fat	5.58
Crude fiber	1.55
Ash	14.92

* Values determined by Midwest Laboratories, Inc. (Omaha, Nebraska). Values represent a single analysis.

Table 3: Ingredient values for 18% protein diet (as fed).

Ingredient	%
Plant ingredients	45.8
Animal ingredients	29.7
Vitamin/Mineral premix	15.5
Lipid premix	6.0
Binder, alginate	2.0
Binder, sodium hexametaphosphate	1.0
	100.0
Calculated Protein Level	18%

Table 4: Crude Protein, crude fat, crude fiber and ash levels (%) for 23% protein feed

Nutrient	Dry Weight
Crude protein	23.90%
Crude fat	7.15%
Crude fiber	1.99%
Ash	19.10%

* Values determined by Midwest Laboratories, Inc. (Omaha, Nebraska). Values represent a single analysis.

Table 5: Ingredient values for 23% protein diet (as -fed).

Ingredient	%
Plant ingredients	45.8
Animal ingredients	29.7
Vitamin/Mineral premix	15.5
Lipid premix	6.0
Binder, alginate	2.0
Binder, sodium hexametaphosphate	1.0
	100.0
Calculated Protein Level	23%

Extrusion was accomplished at room temperature using a meat chopper attachment (Model A-800, Hobart Corporation, Troy, OH) fitted with a 3-mm die. Moist feed strands were dried on wire racks in a forced air oven at 35°C to a moisture content of 8-10%. After a 24-hour drying period, feed was milled (High Speed Grain Mill, Lehman's, Dalton, OH powered by a 1.5 hp electric motor) and sifted for appropriate size, bagged and stored at 4°C until used. The 23% protein feed was prepared as a single ~28 kg mix that was parceled into ~2 kg batches for extrusion using the same procedure used for the 18% protein feed.

2.7 Experimental Approach

2.7.1 Trial 1, 28 ppt (Initial trial)

Four 1.5-m square tanks were stocked with 3,696 PL shrimp/m^2 (3.14 mg/PL initial wt, Cohort 1) to achieve stocking and biomass densities of 18,480/m^3 and 11.6 g/m^2, respectively. Approximately 500 animals (from same lab tank) were randomly selected; hand counted and weighed gravimetrically (analytical balance, Mettler Toledo, Columbus OH) as a group (i.e., one replicate) to obtain an initial estimated mean weight (g) for the population. In conjunction with gravimetric weighing to determine initial weight of the population, a photometric counter (XperCount, Xpertsea, Ontario, Canada) was used for stocking of tanks. Tanks were stocked with shrimp all from the same lab tank on the day of their arrival. Totals given by the counter were used to determine feed rates.

The feed curves for this experiment (Appendix A) were based on expected growth of shrimp stocked at ~3,696 animals per square meter. In order to promote production of

heterotrophic bacteria, feed protein percentage was reduced to ~20% (1:1 mixture of 23/18%).

Culture tanks were filled to a water depth of 20 cm (450 L/tank) and maintained at 28±1°C and 28 ppt. Water lost via evaporation was periodically replaced with RO water (RO Added). All tanks were operated as minimal exchange biofloc tanks. Water quality parameters (DO, temperature, and salinity) were measured daily using a YSI 85 oxygen/conductivity instrument (YSI, Yellow Springs, Ohio, USA) and shown in Appendix B. A YSI pH 100 (YSI, Yellow Springs, Ohio, USA) was used to monitor pH twice daily (maintained between 7.5-86 with additions of $NaHCO_3$; Loyless and Malone 1997). The nitrifying bacteria (NB) inoculum used was BactaPur 8500 (BactaPur, QC, Canada) applied at a rate of 0.2 ml L^{-1} according to the method specified in section 2.4. Initial experimental tank management targeted nitrification via addition of chemoautotrophic bacteria (NB inoculum) until Day 4 at which point production of heterotrophic bacteria was promoted via addition of scFOS.

Trial 1 was conducted for 14 days (June 18-July 2, 2014). At trial termination (July 2, 2014) a single random sample of shrimp from each tank was counted and weighed to estimate final mean weight per animal.

2.7.2 Trial 2: Salinity Comparison (8 vs 28 ppt high biomass density)

All experimental methods for this trial mirrored that of the first with the following exceptions. Experimental tanks were stocked with larger shrimp (47 mg initial wt, Cohort 1) and at a density of 3,000 shrimp/m^2 (10,000 shrimp/m^3, biomass density of 150 g/m^2 vs 11.6 g/m^2 for first trial). These shrimp were all stocked from the same lab tank. Approximately 100 animals were hand counted and weighed gravimetrically all at once

(i.e., one replicate) to obtain an initial estimated mean weight (g) for the population. Due to the relative ease of enumerating bigger animals compared to PL_{12} in the first trial, stocking densities were determined gravimetrically.

The feeding curve (Appendix C) was based on expected growth of shrimp (47mg/shrimp) stocked at 3,000 per square meter (vs 3,696 PL/m^2 in Trial 1).

Culture tanks were filled to a water depth of 30 cm (675 L) and maintained at 27±1°C. Culture water in two tanks was maintained at a salinity of 28 ppt and in the other two it was reduced to 8 ppt by gradual addition of RO water over three days (Appendix D). Daily water quality measurements (DO, temperature, and salinity and pH) are shown in Appendix E.

Nitrifying bacteria inoculum was applied at a rate of 90 ml (0.13 ml L^{-1}) for total TAN greater than 2.0 mg L^{-1} and 90 mL for NO_2 greater than 3.0 mg L^{-1}. Conditions considered conducive to chemoautotrophic dominance were maintained only for day 1. Heterotrophic dominance was promoted on Day 2.

Trial 2 was conducted for 14 days (July 8-July 22, 2014). At trial termination (July 22, 2014) a single random sample of shrimp from each tank was counted and weighed to estimate final mean weight per animal.

2.7.3 Trial 3: Salinity Comparison (8 vs 28 ppt low biomass density)

All experimental methods for this trial were similar to that of the second trial with the following exceptions: experimental tanks were stocked with 3,960 juvenile shrimp/m^2 (2.73 mg initial wt, Cohort 2) to achieve stocking and biomass densities of 13,200 shrimp/m^3 and 10.8g/m^2, respectively; feed curves were adjusted accordingly (Appendix F). After checking shipping bags for levels of oxygen, inorganic nitrogen, temperature,

salinity, and pH, a bag was floated in each of the four tanks. Water from experimental tanks was added to shipping bags every 2-5 minutes until temperature in shipping bags and tanks differed by less than 1 C and 0.5 pH units. At this point, PLs were transferred directly into tanks. In order to determine mean initial weight of the population, approximately 500 animals were randomly selected from a shipping bag received in the same shipment (i.e., same cohort) not used to stock this trial, hand-counted and weighed all at once (i.e., one replicate). Salinity was slowly reduced by addition of RO water to achieve a level of 8 ppt in two tanks, whereas the other two were maintained at 28 ppt (Appendix G).

Trial 3 was conducted for 15 days (October 29-November 13, 2014, 15 days). The extra day was to ensure there was staff to aid with termination. At trial termination (November 13, 2014) a single random sample of shrimp from each tank was counted and weighed to estimate final mean weight per animal.

2.8 Statistical Analyses

For Trial 1, a repeated-measures ANOVA was used to detect differences ($P < 0.05$) in nitrogen levels while a one-way ANOVA was used to detect differences ($P < 0.05$) in selected water quality factors between tanks. For Trials 2 and 3, a Students T-test was used to determine differences ($P < 0.05$) in shrimp performance, water quality and nitrogen levels. All tests were performed after the confirmation of normality of data distribution (Shapiro-Wilk test) (Quinn and Keough, 2002).

3. Results

3.1 Trial 1: Initial Experiment

3.1.1 Water Quality

Water quality factors remained within acceptable parameters (Van Wyk and Scarpa 1999, Lazur 2007) throughout the experimental period and were similar for all tanks (Table 6). No water was exchanged.

3.1.2 Survival and Weight Gain

Survival ranged from 106-146% with a mean of 132% (Table 7). Estimated weight gain/shrimp among experimental tanks varied from 27 mg to 55 mg with a mean of 36±12 mg. Final mean weight ranged from 30 mg to a maximum of 59 mg with a mean of 40 mg.

3.1.3 Nitrogen Management

Mean (±s.d.) TAN level ranged from 0.76±1.29 to 1.25±2.15 mg L^{-1} (Table 8). The highest mean TAN was observed in Tank 1, but was not significantly different from other tanks (p-value=0.65, Table 8). A peak in TAN (5 mg L^{-1}) was observed on Day 7 for Tank 1 and declined afterwards (Fig. 2). Total ammonia nitrogen peaked on Day 4 in Tank 2 and Day 5 in Tanks 3 and 4 (3, 3.5 and 3 mg L^{-1} respectively) and generally declined after Day 5 (Fig. 2).

Mean (±s.d.) levels of nitrite ranged from 2.24±1.89 to 4.29±2.73 mg L^{-1}. Nitrite levels did not differ significantly between tanks (p-value=0.44). Nitrite peaked on Days 5 and 6 in Tanks 2 and 4 respectively (10 and 7 mg L^{-1}) and on Day 7 in Tanks 1 and 3 (6 and 8 mg L^{-1}). A second peak (i.e., maximum level) in nitrite occurred in Tank 2 on Day 7. Levels fluctuated but the general trend was a decline in nitrite after Days 6-7 (Fig. 3).

Table 6. Means of select daily water quality and management factors for a 14-day shrimp (*L. vannamei*) biofloc nursery trial (Trial 1)[1]

Factor	Tank 1	Tank 2	Tank 3	Tank 4
pH	7.76 ± 0.16	7.73 ± 0.03	7.69 ± 0.03	7.70 ± 0.03
Akalinity (mg L^{-1})	196 ± 30	198 ± 6	192 ± 4	188 ± 5
Salinity (ppt)	29.2 ± 2.1	29.6 ± 0.3	29.2 ± 0.4	29.3 ± 0.4
Temperature (C)	27.4 ± 1.3	27.5 ± 0.2	27.5 ± 0.2	27.6 ± 0.2
RO Added/Day (L)	2.94 ± 1.84	3.08 ± 0.21	2.37 ± 0.25	2.39 ± 0.28
Feed/Day (g)	37.8 ± 20.2	29.9 ± 2.5	31.0 ± 2.5	29.7 ± 2.4

[1]Values are means ± standard deviation (SD) of daily values. For pH and alkalinity, n = 28 per tank. N=14 per tank for all other factors. No mean values within factors were significantly different.

Table 7. Growth and performance factors for a 14-day shrimp (*L. vannamei*) biofloc nursery trial (Trial 1)[1]

	1	2	3	4	Mean[3]
Stocked[2]	7,149	6,008	6,298	5,993	6,363±543
Initial weight (mg)	3.14	3.14	3.14	3.14	3.14
Initial Biomass Total (g)	22.45	18.87	19.78	18.82	19.98±1.70
Juveniles Harvested	7,627	8,802	8,642	8,292	8,341±521
% Survival	106	146	137	138	132±5
Mean Weight Gain (mg)	55.34	30.02	27.2	35.15	36.93±12.71
Total Biomass Harvested (g)	446	292	262	317	329±81
Harvest Biomass (g/m^2)	198	129	116	141	146±36

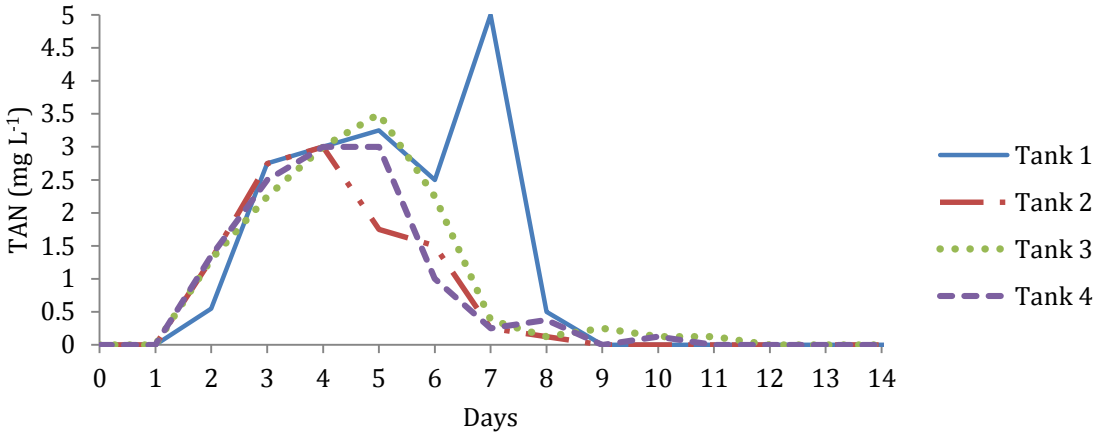
[1] Experimental goal to maintain salinity and temperature in all tanks at approximately 28ppt and 27°C, respectively. [2]Determined using photometric counter [3]Mean±standard deviation (SD

Table 8. Mean±SD concentrations (mg L^{-1}) of nitrogen species in each tank for a 14-day shrimp (*L. vannamei*) biofloc nursery trial (Trial 1)[1]

Tank	TAN	NO_2	NO_3
Tank 1	1.25±2.15	2.24±0.34	9.64±1.70
Tank 2	0.72±0.23	4.29±0.48	16.11±1.97
Tank 3	0.95±0.24	3.13±0.35	13.42±1.85
Tank 4	0.83±0.22	3.88±0.41	16.44±1.97

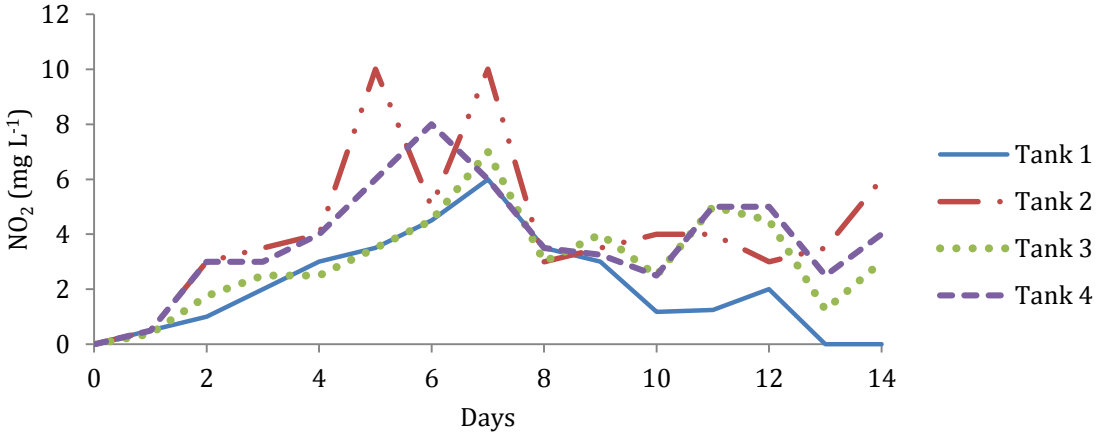
[1]No significant differences between tanks ($p<0.05$) as analyzed by repeated-measure ANOVA.

Fig. 2. Total ammonia nitrogen in an experimental 14-day shrimp (*L. vannamei*) biofloc nursery trial (Trial 1)[1]



[1]Daily values are the average of morning and afternoon measurements.

Fig. 2. Nitrite in an experimental 14-day shrimp (*L. vannamei*) biofloc nursery trial (Trial 1)



[1] Daily values are the average of morning and afternoon measurements.

Mean (±s.d.) nitrate levels ranged from 9.64±9.62 to 16.4±11.16 mg L^{-1}.

Nitrate levels in Tanks 2 and 4 were higher than that of Tank 1 although not significantly higher (p-value=0.8689). Nitrate peaked on Day 5 and 7 in both Tanks 2 and 4 and only on Day 7 in Tanks 1 and 3. Similar to nitrite, levels of nitrate fluctuated but tended to decrease after Day 5 (Fig. 4).

In order to promote nitrification, Tanks 2 and 4 required substantially higher volumes (810 mL) of NB inoculum than Tanks 1 and 3 (540 and 630 mL, respectively, Table 9). This trend was generally similar in terms of addition of scFOS, with the exception of Tank 1, which required much lower addition (139 g vs >172 g for others, Table 9). Addition of sodium bicarbonate was highest in Tank 2 (Table 9).

3.1.4 Biofloc

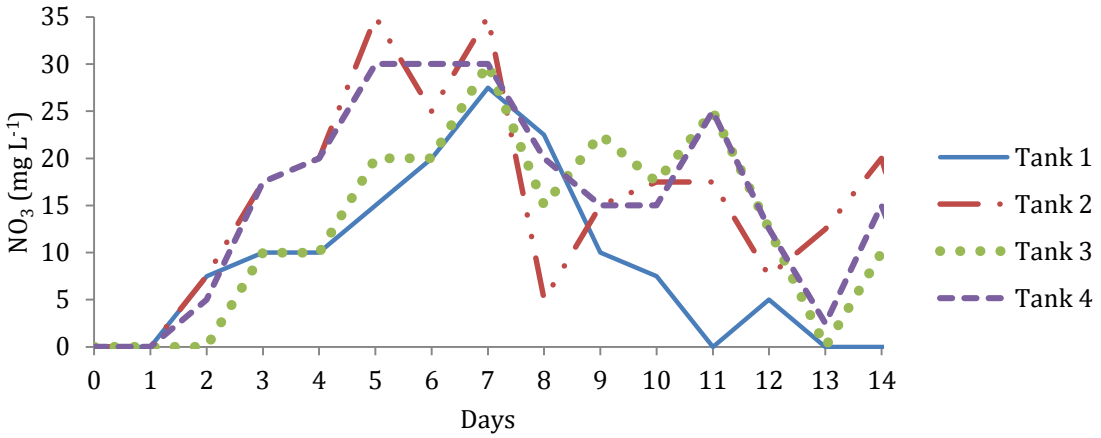
Biofloc remained below maximum acceptable levels (<25 ml L^{-1}) throughout the culture period and did not necessitate reduction (removal). Levels typically increased throughout the experimental period (Fig. 5). There were no differences (p =0.13) in biofloc levels among tanks for the experimental period.

3.2 Trial 2: Salinity Comparison (8 vs 28 ppt)

3.2.1 Water Quality and General Tank Management

Water quality parameters remained within acceptable levels throughout the experimental period, although average pH was significantly higher (8.0, p <0.01) at 8 ppt compared to the 28 ppt treatment (7.75). Means of water quality and management parameters for the entire experimental period are shown in Table 10.

Fig. 3. Nitrate in an experimental 14-day shrimp (*L. vannamei*) biofloc nursery trial (Trial 1) [1]

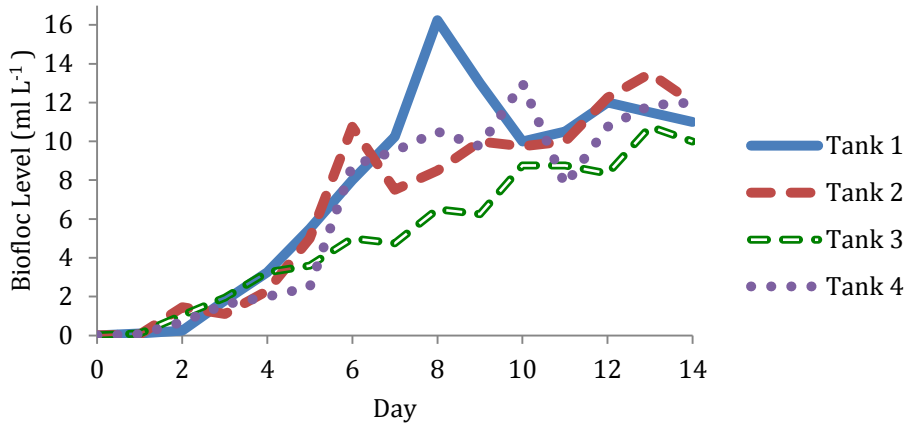


[1] Daily values are the average of morning and afternoon measurements.

Table 9. Total amounts of nitrifying bacteria (NB) inoculum, short-chain fructooligosaccharide (scFOS), and $NaHCO_3$ added to each tank during an experimental 14-day shrimp (*L. vannamei*) biofloc nursery trial (Trial 1)

Tank	Total NB Added (mL)	Total scFOS Added (g)	Total $NaHCO_3$ Added (g)
1	540	139	75.59
2	810	191	81.78
3	630	172	64.09
4	810	184	63.00
Mean	697.5	171	71.12

Fig. 5. Biofloc densities for four tanks during an experimental 14-day shrimp (*L. vannamei*) biofloc nursery trial (Trial 1) [1]



[1] Daily values are the averages of morning and afternoon measurements.

Table 10: Mean ± s.d. select daily water quality and tank management factors for two treatments (8 and 28 ppt) for a 14-day shrimp (*L. vannamei*) biofloc nursery trial (Trial 2)[1,2]

Factor	8 ppt	28 ppt
pH	8.04 ± 0.12[a]	7.75 ± 0.13[b]
Alkalinity (mg L^{-1})	189 ± 35	191 ± 27
Salinity (ppt)	8.8 ± 0.3[b]	28.4 ± 0.2[a]
Temperature (C)	27 ± 0.64	27.1 ± 0.51
RO Added/Day(L)	4.58 ± 3.30	3.79 ± 4.74
Foam Fractionator Exchange/Day (L)	0.75 ± 0.5[b]	2.23 ± 0.96[a]
Biofloc Water Exchange/Day (L)	2.67 ± 0.58	4.7 ± 1.27
Feed/Day (g)	122 ± 8	119 ± 10

[1]Values are means ± standard deviation (SD) of daily values. For pH and Alkalinity, n = 56 per tank. N=28 per tank for all other factors. [2]Means in a row without a common superscript letter differ ($P<0.05$) as analyzed by a Student's T test.

3.2.2 Growth and Survival

Survival of shrimp in Trial 2 was higher, although not significantly (p-value=0.12), for shrimp at 8 ppt (78%) versus those reared at 28 ppt (61%) (Table 11). Weight gain of shrimp at 8 and 28 ppt was 199.8±41.9 mg and 252.0±64.1 mg, respectively (Table 11). Weight gain at 28 ppt was not significantly higher (p-value= 0.44), however shrimp reared at 8 ppt resulted in higher amounts of total harvested biomass (597 vs 577 g/m^2) and achieved lower mean FCR (1.57 vs 1.71) (Table 11). Differences in biomass and FCR were not significant different (p=0.44 and p=0.63, respectively).

3.2.3 Nitrogen Management

Total ammonia nitrogen increased rapidly to a peak of ~3 mg L^{-1} in tanks maintained at both experimental salinities (Fig. 6) and then declined rapidly over the next two days to less than 0.5 mg L^{-1} where it stayed for the remainder of the experiment. Mean TAN levels were 0.63±1.17 and 0.85±0.1.17 mg L^{-1} (mean± SD) for the 8 and 28 ppt treatments, respectively. There was no significant difference in mean TAN concentration between the two treatments ($p<0.05$).

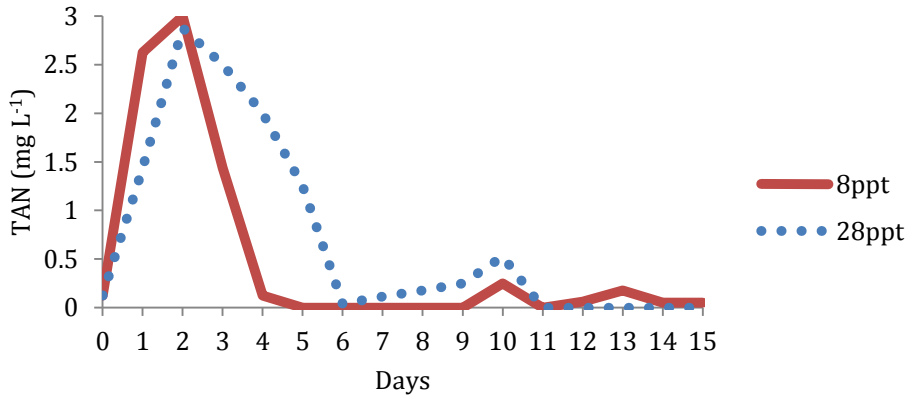
Mean nitrite levels were 3.00±1.63 and 4.68±2.54 mg L^{-1} for the 8 and 28 ppt treatments, respectively and were significantly different between treatments (p=0.003). Nitrite tended to fluctuate in experimental tanks maintained at 8 ppt and achieved its highest level (~5 mg L^{-1}) toward the end of the trial (Fig. 7). Nitrite levels in tanks maintained at 28 ppt rose rapidly to a peak of ~14 mg L^{-1}on Day 9. Afterwards, nitrite levels declined rapidly and stabilized at ~4 mg L^{-1}.

Table 11. Growth and performance factors for a 14-day shrimp (*L. vannamei*) biofloc nursery trial (Trial 2)[1]

	8 ppt	28 ppt
Total Shrimp Stocked	14,093±7	14,085±4
Initial Weight (mg)	46.77	46.77
Initial Biomass Total (g)	659.13±0.31	658.75±0.18
Total Shrimp Harvested	10,959±331	8,637±640
% Survival	78±5	61±9
Mean Weight Gain (mg)	199.8±41.9	252.0±64.1
Total Biomass Harvested (g)	1,344±330	1,298±640
Harvest Biomass (g/m2)	597±66	577±38
FCR	1.57±0.27	1.71±0.04

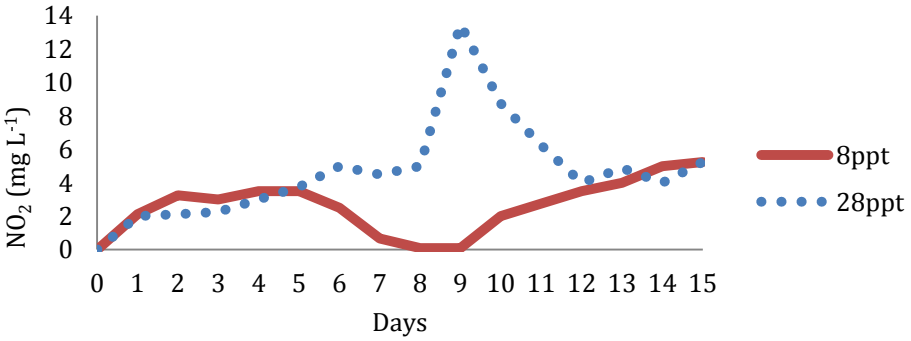
[1]Values are mean±SD, two tanks per treatment

Fig. 6. Total ammonia nitrogen in an experimental 14-day shrimp (*L. vannamei*) biofloc nursery trial (Trial 2)[1]



[1]Daily values are the average of morning and afternoon measurements from two tanks per salinity treatment.

Fig. 7. Nitrite in an experimental 14-day shrimp (*L. vannamei*) biofloc nursery trial (Trial 2)[1]



[1]Daily values are the average of morning and afternoon measurements from two tanks per salinity treatment.

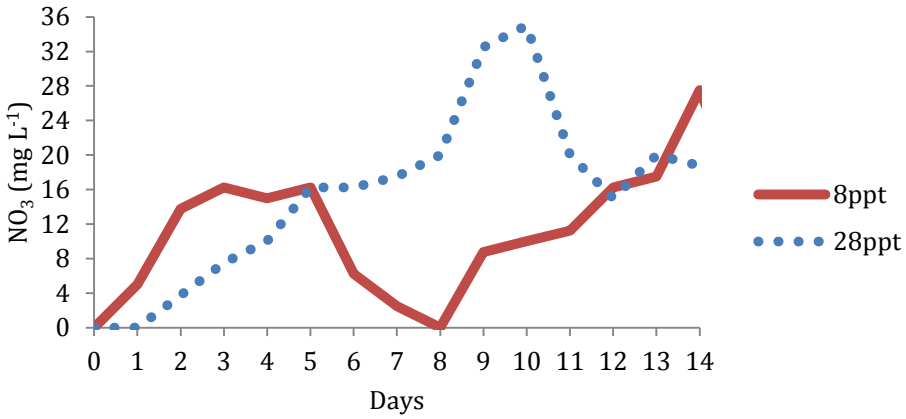
Nitrate concentrations displayed a trend similar to nitrite over the culture period, with a peak on Days 9-10 of ~36 mg L^{-1} for the 28 ppt tanks (Fig. 8). Tanks maintained at 8 ppt showed a reverse trend in which NO_3 rapidly increased to 16 mg L-1 on Days 2-5, declined to ~0 mg L^{-1} and again increased rapidly to ~28 mg L^{-1} toward the end of the trial (Fig. 8). Mean levels of nitrate were 12.16±7.03 and 10.83±6.68 mg L^{-1} for the 8 and 28 ppt treatments, respectively; means were not significantly different ($p<0.05$). Overall, inorganic nitrogen levels in experimental tanks remained within acceptable ranges for normal growth and survival of postlarval shrimp, with exception of nitrite levels in the 28 ppt tanks (peaked at ~14 mg L^{-1}).

Management of nitrogen in the 28 ppt treatment tank cultures required substantially less addition of NB inoculum than 8 ppt tanks (1,080 vs 1,280 mL); however, they required approximately twice the amount of scFOS (383 vs 686 g). Total sodium bicarbonate added to the 8 ppt treatment was about twice that of the 28 ppt treatment (Table 12). Individual daily observations of nitrogen species, water quality observations and systems management parameters are listed in Appendix E.

3.2.4 Biofloc

Biofloc levels increased rapidly over the first three days (Fig. 9). On Day 4, biofloc density appeared to plateau, but declined in the 8 ppt treatment while remaining at approximately 17-18 ml L^{-1} in the 28 ppt treatment. Reduction in the 8 ppt treatment resulted from mechanical removal. More biofloc was removed from the 28-ppt tanks; however, the total volume removed from any of the treatment tanks was quite small (22.2 and 11.8 L Total FExchange+BExchange, 8 and 28 ppt treatments, respectively). Means per treatment are shown in Table 10. Actual values are shown in Appendix E.

Fig. 8. Nitrate in an experimental 14-day shrimp (*L. vannamei*) biofloc nursery trial (Trial 2)[1]



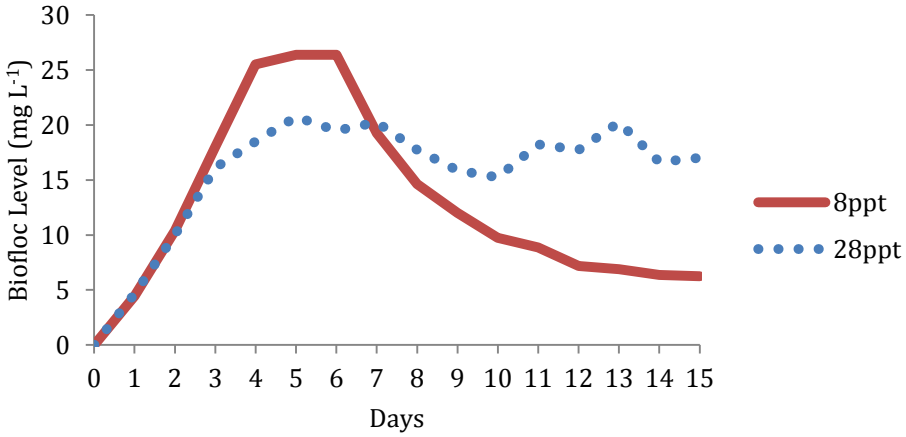
[1]Daily values are the average of morning and afternoon measurements from two tanks per salinity treatment.

Table 12. Total amounts[1] of nitrifying bacteria (NB), short-chain fructooligosaccharide (scFOS), and $NaHCO_3$ used to manage two experimental treatments (8 and 28 ppt) (Trial 2)[1]

Treatment	Total NB Added (mL)	Total scFOS Added (g)	Total $NaHCO_3$ Added (g)
8 ppt	1,260	383	369
28 ppt	1,080	686	178

[1]Values are the sum of quantities added to two experimental tanks per treatment.

Fig. 9. Biofloc densities for two treatments (8 and 28 ppt) during an experimental 14-day shrimp (*L. vannamei*) biofloc nursery trial (Trial 2)[1]



[1]Daily values are the average of morning and afternoon measurements from two tanks per salinity treatment.

3.3 Trial 3: Lower Density Salinity Comparison (8 vs 28 ppt)

3.3.1 Water Quality

Mean daily water quality observations and selected management parameters for experimental tanks maintained at 8 and 28 ppt with low initial shrimp biomass (10.8g/L) are shown in Table 13. Water quality factors remained within acceptable ranges throughout the experimental period; there were no significant differences between treatments.

3.3.3 Growth and Survival

Survival (Table 14) of postlarval shrimp in the 8ppt and 28ppt treatments (107±5% and 99±25%, respectively) was not significantly different between treatments (p-value=0.81). Shrimp reared at 28 ppt gained an estimated 26.8±5.0 mg while those reared at 8 ppt gained an estimated 31.5±0.0 mg (Table 14). This difference was not significant (p-value=0.53). There was no significant difference in harvested biomass (134 and 112 g/m2, for the 8 and 28 ppt treatments, respectively; p-value=0.72) or FCR (1.13 and 1.38 for the 8 and 28 ppt treatments, respectively; p-value=0.58) (Table 14).

3.3.3 Nitrogen Management

Total ammonia nitrogen trends were fairly similar (mean TAN levels 0.63±1.17 and 0.85±0.1.17 mg/L, 8 and 28 ppt respectively, p=0.46) for both salinity treatments (Fig. 10). Average TAN increased to a maximum of 0.7-0.8 mg L^{-1} on Day 3 in 8 ppt tanks and Day 4-5 in the 28 ppt tanks. After this, levels decreased rapidly to less than detectable levels where they remained for the remainder of the trial.

Table 13. Mean ± s.d. selected daily water quality and tank management factors for two treatments (8 and 28 ppt) for a 15-day shrimp (*L. vannamei*) biofloc nursery trial (Trial 3)[1,2]

Factor	8ppt	28ppt
pH	8.07 ± 0.98	8.02 ± 0.10
Alkalinity (mg L^{-1})	198 ± 50	203 ± 46
Salinity (ppt)	12.1 ± 1.2[b]	29.7 ± 3.2[a]
Temp (C)	26.8 ± 1.4	26.6 ± 1.3
RO Added (L)	3.31 ± 0.41	3.51 ± 0.25
Feed/Day (g)	21.1 ± 1.7	21.1 ± 1.7

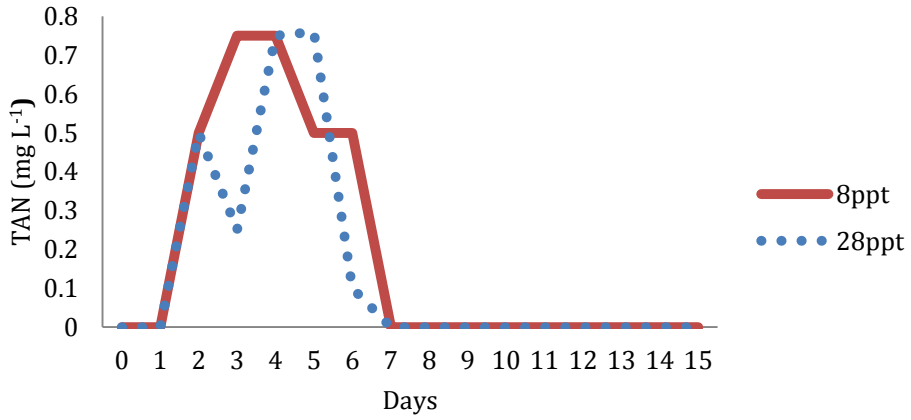
[1]Values are means ± standard deviation (SD) of daily values. For pH and Alkalinity, n = 56 per tank. N=28 per tank for all other factors. [2]Means in a row without a common superscript letter differ (P<0.05) as analyzed by a Student's T test.

Table 14. Growth and performance factors for a 15-day shrimp (*L. vannamei*) biofloc nursery trial (Trial 3) [1]

	8 ppt	28 ppt
Stocked	16336	16336
Initial Weight (mg)	2.73	2.73
Initial Total Biomass (g)	44.6	44.6
Harvested	17615±601	16289±2979
% Survival	107±7	99±37
Mean Weight Gain (mg)	31.5±0.00	26.8±0.01
Total Biomass Harvested (g)	602±17	503±148
Harvested/m^2 (g)	134±8	112±66
FCR	1.13±0.07	1.38±1.13

[1]Values are mean±SD, two tanks per treatment

Fig. 10. Total ammonia nitrogen concentration in an experimental 15-day shrimp (*L. vannamei*) biofloc nursery trial (Trial 3)[1]



[1]Daily values are the average of morning and afternoon measurements from two tanks per salinity treatment.

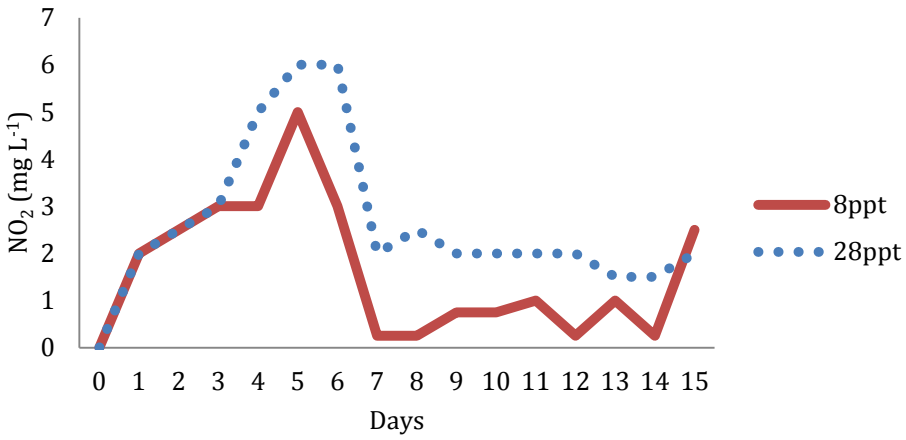
Nitrite levels increased steadily until Day 5 peaking at 5 and 6 mg L^{-1} for the 8 and 28 ppt treatments, respectively. After Day 5 nitrite levels declined sharply and in general varied by less than 66 and 25% per day in the 8 and 28 ppt treatments, respectively until the end of the experiment (Fig 11). Nitrite concentration in 28 ppt tanks averaged ~1 mg L^{-1} higher than that of 8 ppt tanks (1.64 vs 2.72). Nitrate rose steadily throughout the experimental period to level of 25 mg L^{-1} in both treatments (Fig 12.)

The 8 ppt treatment required slightly more (~100 mL) NB inoculum, but about 1/3 the scFOS (Table 15) as compared to the 28 ppt treatment. Total sodium bicarbonate added to the cultures in the 8 ppt treatment was 72% greater than that added to the 28 ppt treatment tanks. Daily water quality observations and management parameters are listed in Appendix H.

3.3.3 Biofloc

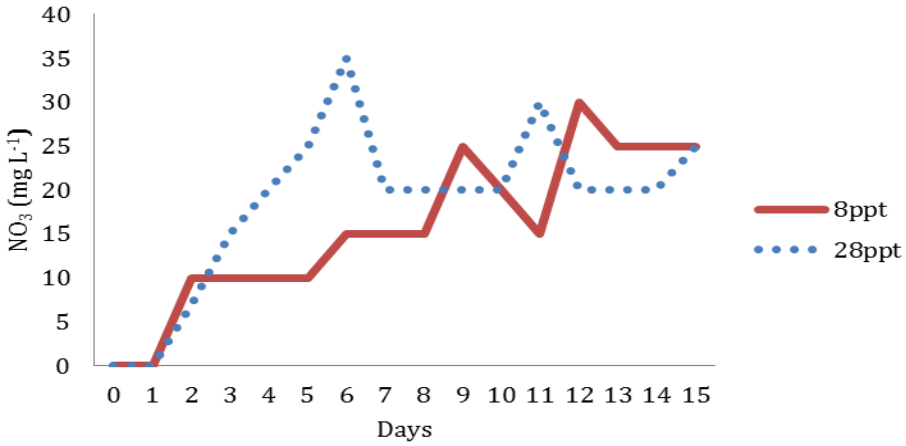
Biofloc levels rose gradually throughout the experiment for both salinity treatments, achieving a maximum of ~2 and 1 ml L^{-1} for 8 and 28 ppt, respectively (Fig. 13), which was not significantly different ($p=0.053$).

Fig. 11. Nitrite concentration in an experimental 15-day shrimp (*L. vannamei*) biofloc nursery trial (Trial 3)[1]



[1]Daily values are the average of morning and afternoon measurements from two tanks per salinity treatment.

Fig. 12. Nitrate concentration in an experimental 15-day shrimp (*L. vannamei*) biofloc nursery trial (Trial 2) [1]



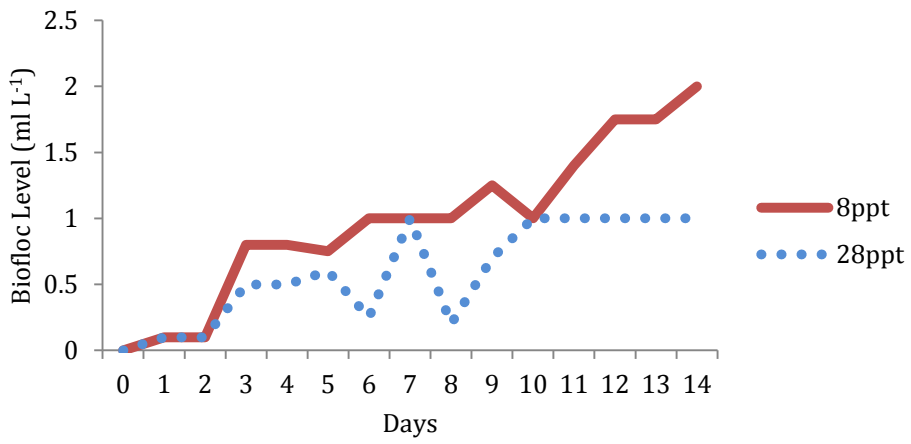
[1]Daily values are the average of morning and afternoon measurements from two tanks per salinity treatment.

Table 15. Total amounts[1] of nitrifying bacteria (NB), short-chain fructooligosaccharide (scFOS), and $NaHCO_3$ used to manage two experimental treatments (8 and 28 ppt) (Trial 3)

Treatment	Total NB Added (mL)	Total scFOS Added (g)	Total $NaHCO_3$ Added (g)
8 ppt	1,350	33.72	276.96
28 ppt	1,260	99.22	160.76

[1]Values are the sum of quantities added to two experimental tanks per treatment.

Fig. 13. Biofloc densities (ml L^{-1}) for two treatments (8 and 28 ppt) during an experimental 15-day shrimp (*L. vannamei*) biofloc nursery trial (Trial 3)[1]



[1]Daily values are the average of morning and afternoon measurements from two tanks per salinity treatment.

4. Discussion

4.1 Trial 1 (Initial Trial)

The goal of the initial trial was to evaluate high-density culture of postlarval *L. vannamei* under biofloc enhanced conditions in static tanks. A major issue was encountered when stocking the tanks. It would seem that user error, counter errors, or some combination of the two led to ambiguity regarding initial stocking numbers and densities of shrimp. For this reason mean daily feed input for Tank 1 was substantially higher than other tanks, as it was presumed that more animals were stocked into this tank. This most likely explains the higher weight gain in this tank. The errors in stocking numbers would indicate that automated technology for counting shrimp of this size (~2-3 mg) requires more development. Nonetheless, management of this culture system for nitrogen control and provision of nutrition via heterotrophic bacteria resulted in a harvest density ranging from 3,389 – 3,912 juveniles of ~50 mg final weight/m^2 in 450L of water or 16,947 – 19,560 juveniles/m^3. These results are substantially higher than that commonly achieved in commercial production of young juvenile shrimp (20-100 mg harvest weight), which typically result in harvest densities ranging from 1,000-8,000 juveniles/m^3 (Samocha 2010, Martinez-Cordova et al., 2011, Kungvankij et al., 2002).

Of significance is that the nursery system used in this study was comprised of four 2.25 m^2 tanks compared to 500-2,000 m^2 nursery ponds used in Latin America (Kungvankij et al., 1986, Flores-Nava, 2007) where shrimp nursery practices are prevalent. This represents an approximate 196– 200% difference in areal footprint (4 tanks vs 1 pond). Production of this level (19,560 animals/m^3) achieved commercially in a 50m^2 area could yield enough juvenile shrimp to stock 3-16 grow-out raceways (200-

1,000m^2 bottom area) with 300 shrimp/m^2, a typical stocking density in commercial super intensive raceways (Venero et al., 2009, Emerenciano et al., 2013, Zeigler et al., 2015).

Final weight of shrimp produced in this trial (20 – 50 mg) was less than that predicted by the feeding curve used to manage feed additions to culture tanks (100 mg, see APPENDIX A). For this reason, FCR values were higher than expected (1.20 – 1.65), due to overfeeding. Water temperature possibly influenced growth and thus FCR, as temperature has a significant effect on penaeid shrimp growth (Staples and Heales, 1991, O'Brien, 1994, Wyban et al., 1995, and Henning and Andreatta 1998). In the present study, water temperature was maintained at ~27 °C, not considered appropriate for optimum growth of postlarval *L. vannamei* (Wyban et al., 1995, Ponce-Palafox et al. 1997) in order to promote higher survival. Survival is considered the most important factor during the nursery phase (Fóes et al., 2011, Samocha et al., 2000, Yta et al., 2004) and has been shown to increase with decreased temperature (ASEAN 1978, Limsuwan and Ching, 2012, Hostins et al., 2015). Increased survival in the nursery phase improves availability of juvenile shrimp for subsequent stocking of the grow-out phase. If increased survival rates are to be realized via reduction in culture temperature, appropriate feed and growth curves should be developed for the production of shrimp under this condition

It is likely that stocking density also affected growth. The stocking density used in the present study (3,696 PL/m^2 in 450L of water or 18,480 PL/m^3) was twice that of most commercial operations and higher than those reported for several nursery studies with penaeid shrimp of similar sizes (1,500-13,200/m^3; Foes et al., 2011; Correia et al., 2013; Esparza-leal 2015, Ray et al., 2011, Samocha et al., 2007; Wasielesky 2010) although

densities of up to, 28,000 PL/m^3 have been reported (Yta et al., 2004). In addition, the duration of culture was 14-15 days in the present study whereas culture duration approximates one month (30 days) commercially (Zeigler et al., 2015). The effect of culture density of postlarval shrimp in biofloc systems has been documented by Wasielesky et al. (2013). In this study, postlarval *L. vannnamei* were stocked at densities of 1,500, 3,000, 4,500 and 6,000 PL_{10}/m^2 in a recirculating biofloc system for durations of 30 and 35 days. After 30 days, shrimp stocked at 6,000 PL/m^2 had a mean weight significantly lower than those stocked at 1,500 PL/m^2. After another 35 days, shrimp stocked at 6,000 PL/m^2 had a mean weight significantly lower than other treatments whereas those stocked at 3,000 and 4,500 PL/m^2 had a mean weight significantly lower than those stocked at 1,500 PL/m^2. Results of Wasielesky et al. (2013) suggest that, under the conditions of their study, final mean weight decreases significantly with increasing density. Harvest results of the present study indicate true initial densities were close to the range of 3,400-4,000 PL/m^2, so it is reasonable to assume that density affected final weight of shrimp. Other studies have shown stocking density to have an inverse relationship on rate of weight gain of shrimp. Maguire and Leedow (1983) showed that increased stocking density of *Metapenaeus macleavi* (Haswell) led to decreased space and natural food source availability. Other studies have shown that higher stocking densities can result in degradation of water quality (Nga et al., 2005) and accumulation of undesirable sediment (Arnold et al., 2005, 2006a), which may work in concert to reduce growth of juvenile shrimp cultured.

The benefit of maintaining postlarval/juvenile production systems with high levels of biofloc is to reduce feed cost, a major operational component (Tacon 2002,

Burford et al, 2004). For the present study, the FCR values reported were not substantially lower than that of most outdoor, semi-intensive or intensive shrimp production systems. Food conversions ratios ≤ 1.0 are standard for high-intensity nursery raceways stocked with 6,000-8,000 PL ~4m initial weight/m^3 (Zeigler et al., 2015). Food conversion ratios in the present study (1.20-1.65) compare to a study by Wasielesky et al. (2013), which reported a FCR of 1.6 when PL_{10} (3mg) were stocked at 16,666/m^3 and cultured for 35 days in biofloc nursery trial. Biofloc levels in Trial 1 did not reach levels prohibitive to shrimp growth (Schveitzer 2013, Avnimelech 2015). Mean biofloc levels reported for Trial 1 were approximately half that of maximum management levels described in the methods above (10-11 vs 20-25 ml L^{-1}). Temperature may be partially responsible for lower than expected biofloc levels, which probably decreased microbial activity. Hostins et al. (2015) in a nursery study with *Farfantepenaeus brasiliensis*, observed a significant increase in biofloc level with increased temperature (21-33 °C).

Schveitzer et al. (2013) documented the effect of biofloc level on the ability to control nitrogen in tanks with juvenile shrimp (mean wt =6.8g) stocked at 390 juveniles/m^2. In this study, biofloc levels (measured via total suspended solids, TSS) were maintained at 200, 400-600 and 800-1,000 mg L^{-1}. Under the conditions of this study, biofloc levels of ~200 mg L^{-1} led to variability in the concentrations of ammonia and nitrite which required application of a carbohydrate source (i.e., molasses) to induce nitrogen assimilation by heterotrophic bacteria. Biofloc levels in Trial 1 were similar to those of the 200 mg L^{-1} treatment, assuming biofloc level (ml L^{-1}) × 10 = TSS (mg L^{-1}) (Avnimelch, 2015). In Trial 1, ammonia and nitrite in Tank 1 exhibited a similar trend in terms of temporal flux. Schveitzer et al. (2013) suggested that biofloc levels ≥400 mg L^{-1}

led to the complete oxidation of ammonia to nitrate. While the complete oxidation of ammonia may seem ideal from the standpoint of water quality, the management goal of the present study was to purposely use a carbohydrate source in order to favor production of heterotrophic bacteria and rely on microbial protein as a feed supplement. These results suggest the possibility of improving system management in the present study by establishing slightly higher biofloc levels (e.g., 20-40ml L^{-1}) that maximize both nitrification and production of microbial protein via heterotrophic assimilation. Correia et al (2014) recently evaluated the use of a carbohydrate source (i.e., molasses) to control inorganic nitrogen in nursery systems (PL_{10}, ~1mg, stocked at 5,000/m^3) offered feeds containing two different dietary protein levels (30 vs 40%). In this study, biofloc was managed at a maximum of 15 ml L^{-1} in a 62-day nursery feeding trial. Molasses (500 ml, 24% carbon) was added every other day through Days 10-18 to promote development of heterotrophic bacteria. After this, molasses was added to provide 6 g of organic carbon for every 1 g of TAN. Molasses prevented significant accumulation of NH_3 but not NO_2 in the culture medium. In the present study, scFOS (~41% carbon) was used to give ~5 mg of carbon for every 0.8 mg of TAN and ~5 mg of carbon for every gram of nitrite. Results from Trial 1 indicated that nitrogen levels in culture tanks were managed to the extent that few instances of toxic levels of nitrite occurred (Fig. 3). Total ammonia nitrogen and nitrate were maintained at levels typically below those considered toxic by manipulating C:N ratio via low protein and carbohydrate addition. Three out of four tanks had a maximum TAN level $\leq$3.5 mg L^{-1}, which is the "safe" value suggested by Lin and Chen (2001). Initial increases in TAN are typical in biofloc systems and largely due to feed inputs required prior to establishment of adequate levels of biofloc. Also, in biofloc

systems, bacteria typically oxidize TAN in the later stages of a production cycle. Any acute increases in TAN in the present study were typically corrected within 36 hours.

4.2 High Density Nursery, Trial 2 (8 vs 28 ppt)

A major obstacle of biofloc technology is acceptance by farmers, due in part to the fact that biofloc is not fully predictable (Crab et al., 2012); especially considering that production strategies differ (e.g., varying salinities). Low salinity biofloc aquaculture has practical applications, however studies regarding such systems maintained at low salinity are limited (Maica et al., 2012), and, to the author's knowledge, do not address the nursery phase. Thus, Trial 2 was conceived to evaluate effect of low (8 ppt) and higher (28 ppt) salinity on ability to control of nitrogen and production of adequate levels of biofloc. Biomass stocking density for Trial 2 was much higher than that of Trial 1 (and Trial 3) in that 47 mg juvenile shrimp were used. This resulted in an initial or stocking biomass density of 0.49g/L versus 0.03 g/L in Trial 3. Initial biomass density could not be determined in Trial 1 due to counting error, but was likely similar to that of Trial 3.

Survival of juvenile shrimp at 8 ppt was higher, although not significantly different, than that at 28 ppt (77.8% vs 61.3%), possibly leading to a density effect resulting in greater total biomass in the 28 ppt treatment (252 g) compared to the 8 ppt treatment (~200 g). The difference in survival was likely due to an acute increase in nitrite concentration on Day 9 (from ~4 to ~14 mg L^{-1}). Rapid decline in TAN followed immediately by a spike in NO_2 suggests increased activity of *Nitrosomonas* spp. in the 28 ppt treatment. Otherwise, management of nitrogen was possible by addition of scFOS, removal of excess biofloc or both and it is likely that had procedures for nitrogen management not been followed NO_2 concentrations (and nitrogen in general) would have

been more irregular. Differences in harvest biomass and estimated FCR were likely due to this difference in survival between the two treatments. Survival results were similar to that reported in other studies with *L. vannamei* under various conditions (Samocha et al., 2007; Samocha 2000; 2013; 2015; Cohen et al., 2005, Foes et al. 2011, Mishra et al., 2008, Krummenauer et al, 2011). Maica et al. (2012) observed an inverse relationship between survival and salinity for *L. vannamei* cultured in a zero exchange setting, with significant mortality only at levels of 4ppt and below. Beyond this, there is little published information available of shrimp performance in minimal exchange systems with reduced salinity. It is likely that without having experienced high levels of nitrite in the higher salinity treatments, shrimp biomass production would likely have been similar.

A comparison of biofloc production in 8 vs 28 ppt treatment tanks indicated that removal was necessary in 8 ppt treatment tanks at or near Day 6 due to high biofloc concentration starting Day 4 (~25 ml L^{-1}). From Day 6 forward, biofloc concentration declined exponentially and never recovered to a normal, maintenance level (<20-25 ml L^{-1}). This sudden decrease in biofloc was likely due to excessive removal of biofloc on Day 8 without further addition of substrate (i.e., carbon) for "re-formation" of biofloc. Removal of biofloc on Days 6-7, as prescribed in the methods above, resulted in a decrease in biofloc level from 40 to 20 ml L^{-1} in one of the 8 ppt tanks (Tank 2, Appendix E). In an attempt to negate any possible increase in biofloc density in Tank 2 the culture water was settled preemptively on Day 8. This was very likely an overcorrection and a contributing factor to the decrease in biofloc level, as previously mentioned. It should be noted that the volume of water removed via foam fractionation was substantially higher for the 28 ppt treatment (22.3 vs 3.8 L in 8 ppt treatment). Foam may form on the surface

of culture tanks as a result of agitation (i.e., aeration) of water with high concentrations of dissolved organic matter. In Trial 2 it is likely that decaying organic matter and overfeeding (possibly exacerbated by lower survival) led to higher dissolved organic matter concentrations and thus increased need for foam fractionation. It is not known what affect biofloc removal and foam fractionation ultimately had on survival or weight gain of shrimp; however, weight gain of shrimp at 28 ppt was higher, although not significantly different, than that at 8 ppt as previously mentioned. The present study appears to suggest that production of juvenile shrimp in biofloc-managed systems at high densities and at a range of salinity from 8-28 ppt is possible. This has positive implications for siting of juvenile raceways at locations in which availability of seawater might be an issue. The opposite consideration is also true (i.e., lack of freshwater). Results also suggest that a much higher stocking density of postlarval shrimp (~3mg) could be possible.

4.3 Low Density Trial, Trial 3 (8 vs 28 ppt)

Results from Trial 3 indicated an average survival of >100% for the four treatment tanks. This means that initial biomass stocking density information was not correct for Trial 3 and that survival information is not valid. Nonetheless, mean harvest weight was 20% higher in the 8 ppt tanks vs the 28 ppt. Assuming that each tank was stocked with a similar number of shrimp, the 8 ppt treatment yielded higher total harvest biomass at a higher biomass density. In comparison to Trial 3, total biomass produced was more than two-fold higher in Trial 2 due primarily to higher initial stocking weight of shrimp (~47 mg vs ~ 3mg in Trial 3).

Compared to Trial 1, mean daily feed offered was 10-20 g lower in Trial 3

resulting in lower FCR values, despite having much lower mean biofloc levels (10.0 – 11.0 vs 0.5-1.0ml L^{-1}, respectively), suggesting feed was managed more efficiently in Trial 3. Had biofloc levels been maintained in excess of 10-11 mL L^{-1}, FCR values for Trial 3 could have been substantially lower.

Trial 3 (as with Trial 2) results tend to reinforce the results of Decamp et al. (2003, 2012), in which nitrogen dynamics were not significantly impacted by salinity in a minimal exchange shrimp production system. In a biofloc study on rearing of postlarval shrimp at four salinities (0, 2, 4 and 25 ppt), Maica et al. (2012) found that nitrification was intensified (i.e., lower levels of TAN and higher levels of NO_2 and NO_3) at higher salinities when *L. vannamei* were cultured at 300 PL_{10} /m^2, although there were no significant differences between treatments. This seems to compare favorably with the present study. Levels for all species of nitrogen (TAN, NO_2, NO_3) in Trial 3 were relatively similar at both salinities, with similar trends in flux. This was likely the result of addition of relatively similar volumes of NB to experimental tanks. Although inoculation was a reactionary measure, in both salinity trials a slightly lower volume (720 vs 630 L in 8 ppt treatment) of inoculum was required in the 28 ppt treatment suggesting a bacterial population capable of oxidizing ammonia was established slightly faster at higher salinity. It should be noted that scFOS application was 3 times higher in the 28 ppt treatment compared to the 8 ppt treatment of Trial 3 (2 times higher in Trial 2). A greater amount of scFOS was added to the 28 ppt tanks in an attempt to control levels of NO_2. Application of carbohydrate to control nitrite in biofloc systems is not a novel approach. Ray et al. (2011), while culturing PL_{12} *L. vannamei* in a biofloc nursery system, applied sucrose to maintain NO_2 levels below 2 mg L^{-1}. In general, levels of all nitrogen species

were much lower (50-113% different) in Trial 3 than those observed in Trial 2. Low levels of TAN (~0.7 mg L^{-1}) are probably the cause for the very low levels of biofloc observed in Trial 3 (1.0 – 1.5 mL L^{-1}) vs 18 mL L^{-1} in Trial 2. Trial 2 levels of TAN approximated 3 mg L^{-1}. With the lower amount of nitrogen available as a substrate for synthesis of cellular amino acids (i.e., protein), potential for production of biofloc was greatly reduced in Trial 3. According to Avnimelech (personal communication, 2015), there is no specific minimal concentration required for an active heterotrophic community; however the flux of TAN is essential. Static low levels of TAN will result in low levels of microbial biomass, which are not effective below 1 mg biofloc L^{-1}.

The effect of salinity on ability to maintain biofloc levels in the present study is not clear. Mean biofloc levels (measured via settleable solids) in Trial 2 mirrored trends documented by Maica et al. (2012), which reported significant increase in levels of TSS with increasing salinity (0, 2, 4 and 25 ppt). Mean biofloc levels maintained in Trial 3 were only slightly higher in the lower salinity treatment despite equal amounts of feed input. Interpretation of results from Trial 3 is further complicated given the three-fold higher input of scFOS in 28 ppt tanks, although this only led to a slight difference in mean combined C:N ratios (feed and carbohydrate) for the 8 and 28 ppt treatments (mean C:N ratio of 12 and 13, respectively). Addition of scFOS was used to maintain high C:N ratio of biofloc in the culture system. This had no apparent effect on nitrogen management as both treatments showed somewhat similar levels of TAN, NO_2, and NO_3. Clearly, further research is needed on characterizing biofloc in minimal water exchange biofloc systems in general, not to mention the effect of salinity on microbial composition.

The inclusion of analysis of C:N ratio of biofloc in these systems would provide a better understanding of the efficacy of use of carbon substrates such as scFOS.

A major issue in Trial 3 was the inability to maintain "adequate" levels of biofloc at either salinity. Despite having low levels of biofloc in treatment tanks, TAN levels were typically less than 1.0 mg L^{-1}. This result suggests that system microbiota was not heterotrophic in make-up, rather autotrophic. As previously mentioned this is most likely due to insufficient ammonia to support heterotrophic production of biofloc. This result cannot be confirmed, as counts of bacteria were not obtained. Trial 2 inputs of scFOS, NB and feed were much higher than those in Trial 3, necessarily due to higher initial stocking biomass (~330 g/tank vs ~ 22g/tank in Trial 3). In comparison to Trial 3, Trial 1 (also stocked with ~3 mg shrimp and at approximately the same harvest biomass/biomass density) required 2-6 times more scFOS. For this reason, it is postulated that success in management of culture systems of this type for adequate biofloc production is highly dependent upon limiting standing levels of nitrifying bacteria to a minimum. Possibly, low levels of TAN limited nitrogen availability as a substrate for growth of heterotrophic bacteria. More likely, low biofloc production was the consequence of low levels of feed input (~450 total g/per tank in Trial 1 vs 147 total g/ per tank in Trial 3). In this case organic material was completely consumed by shrimp resulting in insufficient substrate for bacterial assimilation (McIntosh 2000).

Shrimp performance and water quality factors remained unchanged at both salinities, demonstrating the feasibility of culture at lower salinity. An obvious advantage of biofloc technology regards the lower capital and operational costs resulting from not having to construct and maintain a separate biofiltration system. In addition there is a

potential savings in feed costs because biofloc technology "upcycles" nutrients into microbial protein, increasing efficiency of protein utilization by a factor of two (Avnimelech 1994).

For the system used in the present study, economic benefits could be further compounded considering the shallow depth at which these studies were undertaken. If postlarval shrimp can be reared at similar biomass densities (per unit volume) as employed by current commercial technology, but at a depth of only 20-30 cm, then these rearing tanks could be stacked, reducing the overall system areal footprint. This, in turn, would reduce capital costs in terms of land acquisition and the savings would be then distributed among capital costs associated with supportive systems such as seawater and aeration distribution systems and air-water temperature management systems.

The present study also demonstrated that production of juvenile shrimp under biofloc technology is facilitated at a lower salinity. By reducing salinity, it becomes more economically feasible to culture shrimp away from the ocean and increases the possibility of reducing costs associated with artificial sea salts (inland systems that use less sea water), permitting (through reduced water treatment requirements), land purchase (acquisition of low cost land, relative to coastal real estate) and biosecurity (reducing the need for chemicals and antibiotics through reduced water exchange; i.e., exposure to potential vectors). It is recommended that further research be undertaken to address these potential economic advantages. Other research should include better definition of minimal water depth. Research should also address the definition of optimum biofloc level and methods for maintaining levels. With characterization and estimation of the

population of the microbial community, achieving even higher stocking densities and further maximize shrimp performance factors might be possible.

5. Conclusions

- Stocking densities of ~16,000 postlarval *Litopenaeus vannamei*/m^3 with juvenile harvest biomass densities of >900 g/m^3 can be achieved using a biofloc-dominated nursery system and management procedure similar to the one in this study (assuming appropriate scaling). It is possible that much higher levels could be achieved with improved management of biofloc.
- Using the experimental approach and biofloc management strategy of the present study, concentrations of nitrogen species (TAN, NO_2, NO_3) can be controlled without significant additional daily dilution (i.e., mean addition of new make-up water to the system <2%/day) or use of external nitrification systems.
- Using the experimental system in the present study, growth and survival of postlarval *Litopenaeus vannamei* appears to be relatively salinity-independent (range of 8 – 28 ppt) with proper acclimation of postlarval shrimp. This has implications for expansion of culture systems away from natural seawater sources (potentially better siting with respect to seafood markets).
- A controlled experimental analysis of the economic benefits of postlarval biofloc production systems is required to confirm its advantages over more traditional methods.
- Biofloc management, as per this study, needs to be further refined to optimize nutritional benefits of biofloc (i.e., what are “adequate” maintenance levels of

biofloc/heterotrophic bacteria?) in order to improve performance indicators such as FCR and mean weight gain.

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Appendix A
Feed Curves for Trial 1

Tank 1 # Stocked: 7149 Survival: 90%

Date	Day	Size	Weight Gain (g)	Population	FCR	Fines 45% Protein (g)	Feed % Protein (g)	Feed (g)
18-Jun-14	0	0.0032	0.0019	7149	1.7	25	40	23.40
19-Jun-14	1	0.0051	0.0016	7095	1.6	25	40	18.45
20-Jun-14	2	0.0068	0.0016	7042	1.5	25	40	16.90
21-Jun-14	3	0.0084	0.0019	6989	1.4	25	40	18.10
22-Jun-14	4	0.0102	0.0028	6937	1.3		40	25.25
23-Jun-14	5	0.0130	0.0029	6703	1.2		23/18	23.33
24-Jun-14	6	0.0159	0.0041	6653	1.1		23/18	30.00
25-Jun-14	7	0.0200	0.0055	6603	1		23/18	36.32
26-Jun-14	8	0.0255	0.0065	6553	0.9		23/18	38.34
27-Jun-14	9	0.0320	0.0085	6504	0.8		23/18	44.23
28-Jun-14	10	0.0405	0.0105	6265	0.8		23/18	52.63
29-Jun-14	11	0.0510	0.0132	6218	0.8		23/18	65.67
30-Jun-14	12	0.0642	0.0163	6172	0.8		23/18	80.48
1-Jul-14	13	0.0805	0.0195	6125	0.8		23/18	95.56
2-Jul-14	14	0.1000		6079				
							Total feed=	668.63

Expected FCR= 1.13

Tank 2 # Stocked: 6008 Survival: 90%

Date	Day	Size	Weight Gain (g)	Population	FCR	Fines 45% Protein (g)	Feed % Protein (g)	Feed (g)
18-Jun-14	0	0.0032	0.0019	6008	1.7	25	40	19.66
19-Jun-14	1	0.0051	0.0016	5963	1.6	25	40	15.50
20-Jun-14	2	0.0068	0.0016	5918	1.5	25	40	14.20
21-Jun-14	3	0.0084	0.0019	5874	1.4	25	40	15.21
22-Jun-14	4	0.0102	0.0028	5830	1.3		40	21.22
23-Jun-14	5	0.0130	0.0029	5583	1.2		23/18	19.43
24-Jun-14	6	0.0159	0.0041	5541	1.1		23/18	24.99
25-Jun-14	7	0.0200	0.0055	5500	1		23/18	30.25
26-Jun-14	8	0.0255	0.0065	5458	0.9		23/18	31.93
27-Jun-14	9	0.0320	0.0085	5417	0.8		23/18	36.84
28-Jun-14	10	0.0405	0.0105	5150	0.8		23/18	43.26
29-Jun-14	11	0.0510	0.0132	5111	0.8		23/18	53.97
30-Jun-14	12	0.0642	0.0163	5073	0.8		23/18	66.15
1-Jul-14	13	0.0805	0.0195	5035	0.8		23/18	78.54
2-Jul-14	14	0.1000		4997				
							Total feed=	571.17

Expected FCR= 1.18

Tank 3 # Stocked: 6298 Survival: 90%

Date	Day	Size	Weight Gain (g)	Population	FCR	Fines 45% Protein (g)	Feed % Protein (g)	Feed (g)
18-Jun-14	0	0.0032	0.0019	6298	1.7	25	40	20.61
19-Jun-14	1	0.0051	0.0016	6250	1.6	25	40	16.25
20-Jun-14	2	0.0068	0.0016	6203	1.5	25	40	14.89
21-Jun-14	3	0.0084	0.0019	6157	1.4	25	40	15.95
22-Jun-14	4	0.0102	0.0028	6111	1.3		40	22.24
23-Jun-14	5	0.0130	0.0029	5856	1.2		23/18	20.38
24-Jun-14	6	0.0159	0.0041	5812	1.1		18	26.21
25-Jun-14	7	0.0200	0.0055	5768	1		18	31.73
26-Jun-14	8	0.0255	0.0065	5725	0.9		18	33.49
27-Jun-14	9	0.0320	0.0085	5682	0.8		18	38.64
28-Jun-14	10	0.0405	0.0105	5440	0.8		18	45.69
29-Jun-14	11	0.0510	0.0132	5399	0.8		18	57.01
30-Jun-14	12	0.0642	0.0163	5358	0.8		18	69.87
1-Jul-14	13	0.0805	0.0195	5318	0.8		18	82.96
2-Jul-14	14	0.1000		5278				
							Total feed=	595.92

Expected FCR= 1.16

Tank 4	# Stocked	5993			Survival:	90%			
Date	**Day**	**Size**	**Weight Gain (g)**	**Population**	**FCR**	**Fines 45% Protein (g)**	**Feed % Protein (g)**	**Feed (g)**	
18-Jun-14	0	0.0032	0.0019	5993	1.7	25	40	19.61	
19-Jun-14	1	0.0051	0.0016	5948	1.6	25	40	15.46	
20-Jun-14	2	0.0068	0.0016	5903	1.5	25	40	14.17	
21-Jun-14	3	0.0084	0.0019	5859	1.4	25	40	15.17	
22-Jun-14	4	0.0102	0.0028	5815	1.3		40	21.17	
23-Jun-14	5	0.0130	0.0029	5557	1.2		23/18	19.34	
24-Jun-14	6	0.0159	0.0041	5515	1.1		18	24.87	
25-Jun-14	7	0.0200	0.0055	5474	1		18	30.11	
26-Jun-14	8	0.0255	0.0065	5433	0.9		18	31.78	
27-Jun-14	9	0.0320	0.0085	5392	0.8		18	36.67	
28-Jun-14	10	0.0405	0.0105	5153	0.8		18	43.28	
29-Jun-14	11	0.0510	0.0132	5114	0.8		18	54.01	
30-Jun-14	12	0.0642	0.0163	5076	0.8		18	66.19	
1-Jul-14	13	0.0805	0.0195	5038	0.8		18	78.59	
2-Jul-14	14	0.1000		5000					
							Total feed=	570.42	
Expected FCR=			1.17						

Appendix B
Daily values for water quality measurements in trial 1

Tank	NH3		N02		N03		pH		ALK		DO	Sal.	Temp	Imhoff		NAHCO3		NB		RO		FOS	
6/18	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
Sq 1		0		0		0		7.4		100	6.73	34.3	24.5	0	0		50.4	90	90				
Sq 2		0		0		0		7.4		100	6.72	34.4	24.4	0	0		50.4	90	90				
Sq 3		0		0		0		7.4		100	6.63	34.3	24.6	0	0		50.4	90	90				
Sq 4		0		0		0		7.4		100	6.54	34.4	24.7	0	0		50.4	90	90				

Tanks	NH3		N02		N03		pH		ALK		DO	Sal.	Temp	Imhoff		NAHCO3		NB		RO		FOS	
6/19/14	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
Sq 1	0	0	0.5	0.5	0	0	7.72	7.8	180	200	6.73	34.5	24.3	0.2	0	18.89				3	3		
Sq 2	0	0	0.5	0.5	0	0	7.73	7.8	180	180	6.73	34.6	24.5	0.1	0	6.19				3	4		
Sq 3	0	0	0.25	0.5	0	0	7.73	7.67	200	200	6.55	34.4	24.8	0	0.2	6.19				3	3		
Sq 4	0	0	0.5	0.5	0	0	7.6	7.74	200	200	6.63	34.5	24.8	0	0.15					3	3		

Tanks	NH3		N02		N03		pH		ALK		DO	Sal.	Temp	Imhoff		NAHCO3		NB		RO		FOS	
6/20/14	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
Sq 1	0.1	1	1	1	0	15	7.8	7.73	190	250	6.07	28.1	28.4	0.2	0.3								
Sq 2	0.1	2.5	3	3	0	15	7.84	7.8	185	250	6.32	28.5	28.4	2.3	0.6			90	180	30			
Sq 3	0.1	2.5	2	1.5	0	0	7.77	7.67	200	200	6.35	28.1	28.1	1.1	1			90	90				
Sq 4	0.2	2.5	3	3	0	10	7.79	7.74	180	250	6.36	28.4	28.4	0.7	0.7			90	180	24s			

Tanks	NH3		N02		N03		pH		ALK		DO	Sal.	Temp	Imhoff		NAHCO3		NB		RO		FOS	
6/21/14	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
Sq 1	2.5	3	2	2	10	10	7.81	7.74	200	200	5.95	28.7	27.4	1.1	2.5			180	180	42			
Sq 2	2.5	3	4	3	15	20	7.82	7.72	190	250	6.18	28.6	27.6	0.7	1.5			180	180	36			
Sq 3	1.5	3	2	3	10	10	7.69	7.62	200	250	5.96	28.7	28.2	1.4	2.5			90	180	42			
Sq 4	2	3	3	3	15	20	7.81	7.74	190	200	6.11	28.6	27.9	1.2	2			180	180	36			

Tanks	NH3		N02		N03		pH		ALK		DO	Sal.	Temp	Imhoff		NAHCO3		NB		RO		FOS	
6/22/14	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
Sq 1	3	3	3	3	10	10	7.91	7.85	200	250	6.14	28.4	27.5	3	3.5			180	180	24s			
Sq 2	3	3	4	4	20	20	7.89	7.81	190	190	6.17	28.5	28.1	2.1	2.5			180	180	30s			
Sq 3	3	3	3	2	10	10	7.86	7.84	190	190	6.24	28.5	27.7	3	3.5			180	90	30s			
Sq 4	3	3	4	4	20	20	7.87	7.82	190	200	6.19	28.5	27.7	2	2			180	180	30s			

Tanks	NH3		N02		N03		pH		ALK		DO	Sal.	Temp	Imhoff		NAHCO3		NB		RO		FOS	
6/23/14	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
Sq 1	3.5	3	4	3	20	10	8.08	7.57	200	250	6.08	28.5	27.6	3.5	7.5					30		37.22	
Sq 2	3.5	0	10	10	40	30	8.04	7.5	190	180	6.21	28.6	27.7	2.6	7.4					36		54.47	
Sq 3	4	3	4	3	20	20	7.98	7.54	190	190	6.14	28.5	28.1	2.7	4.5					30		40.64	
Sq 4	4	2	6	6	30	30	8	7.47	190	190	6.08	28.5	28.4	1.5	3.5					30		46.39	

Tanks	NH3		N02		N03		pH		ALK		DO	Sal.	Temp	Imhoff		NAHCO3		NB		RO		FOS	
6/24/14	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
Sq 1	3	2	4	5	20	20	7.98	7.69	250	200	6.2	28.9	27.9	6	10					54		33.36	
Sq 2	3	0	4	6	20	30	7.86	7.65	200	185	6.26	29.1	27.9	9.5	12					1m 6s		33.36	
Sq 3	3.5	1	4	5	20	20	7.85	7.64	190	190	6.35	28.9	27.6	4	6					54s		37	
Sq 4	2	0	10	6	30	30	7.9	7.6	180	180	6.28	29.1	27.7	5.5	12					1m 6s		43.33	

Tanks	NH3		N02		N03		pH		ALK		DO	Sal.	Temp	Imhoff		NAHCO3		NB		RO		FOS	
6/25/14	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM			AM	PM	AM	PM
Sq 1	10	0	8	4	25	30	7.86	7.68	190	190	6.14	28.6	28.5	7.5	13					36		30.29	11.5
Sq 2	0.5	0	10	10	40	30	7.74	7.64	140	200	6.27	28.7	28	7.5	7.5	25.19				42		32.4	28.76
Sq 3	0.75	0	8	6	30	30	7.75	7.65	190	185	6.35	28.5	27.8	4.5	5					30		28.47	17.26
Sq 4	0.5	0	8	4	30	30	7.74	7.6	140	185	6.2	28.6	28.3	10	9					36		26.65	11.5

Tanks	NH3		N02		N03		pH		ALK		DO	Sal.	Temp	Imhoff		NAHCO3		NB		RO		FOS	
6/26/14	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM			AM	PM	AM	PM
Sq 1	0.5	0.5	3	4	25	20	7.7	7.76	185	170	6.03	29	27.9	16	16.5	6.3				120		15.15	
Sq 2	0	0.25	2	4	0	10	7.76	7.77	200	190	6.09	29.1	27.6	9	8					40		13.32	
Sq 3	0	0.25	3	3	20	10	7.7	7.65	190	190	6.22	29	28.1	7	6					68			
Sq 4	0.25	0.5	3	4	20	20	7.7	7.67	250	170	6.17	29.2	28	11	10	6.3				90		15.15	

Tanks	NH3		N02		N03		pH		ALK		DO	Sal.	Temp	Imhoff		NAHCO3		NB	RO		FOS	
6/27/14	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM		AM	PM	AM	PM
Sq 1	0	0	4	2	20	0	7.77	7.6	190	180	6.26	28.1	27.5	15	11						11.5	
Sq 2	0	0	4	3	15	15	7.78	7.7	200	200	6.26	29.1	27.9	11	9				66		11.5	
Sq 3	0.25	0.25	4	4	20	25	7.71	7.59	190	190	6.29	28.4	27.9	6	6.5				24		13.32	6.66
Sq 4	0	0	3.5	3	20	10	7.69	7.56	190	180	6.31	28.3	27.8	10	9.5				18		10.07	

Tanks	NH3		N02		N03		pH		ALK		DO	Sal.	Temp	Imhoff		NAHCO3		NB		RO		FOS	
6/28/14	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM			AM	PM	AM	PM
Sq 1	0	0	0.35	2	5	10	7.61	7.6	180	185	5.64	28.5	28.2	10	10					60			
Sq 2	0	0	4	4	15	20	7.51	7.48	180	190	5.82	28.5	28.1	9.5	10					0		5.75	5.75
Sq 3	0	0.25	2	3	15	20	7.55	7.63	180	185	5.75	28.6	28	8	9.5					59s			
Sq 4	0	0.25	2	3	10	20	7.48	7.48	180	180	5.81	28.6	28.3	15	11					49s			

Tanks	NH3		N02		N03		pH		ALK		DO	Sal.	Temp	Imhoff		NAHCO3		NB		RO		FOS	
6/29/14	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM			AM	PM	AM	PM
Sq 1	0	0	0.5	2	0	0	7.6	7.58	180	200	5.88	27.9	27.7	11	10					29			
Sq 2	0	0	4	4	15	20	7.5	7.52	180	250	5.92	29	28.4	10	10					41		5.75	
Sq 3	0	0.25	4	6	20	30	7.5	7.33	170	200	5.94	27.9	27.8	8	9.5	7.5				47		8.63	
Sq 4	0	0	4	6	20	30	7.6	7.5	180	180	5.98	28.3	27.7	8	7.5					45		8.63	

Tanks	NH3		N02		N03		pH		ALK		DO	Sal.	Temp	Imhoff		NAHCO3		NB	RO		FOS	
6/30/14	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM		AM	PM	AM	PM
Sq 1	0	0	3	1	10	0	7.96	8.06	200	185	6.05	27.9	27.8	13	11							
Sq 2	0	0	3	3	5	10	7.96	8.03	200	250	6.06	28.9	28	11.5	13				54s			
Sq 3	0	0	7	2	25	0	7.84	8.07	190	190	6.04	27.7	28	7.7	9						20.14	
Sq 4	0	0	8	2	25	0	7.89	8.11	180	185	6.03	28	28.4	10	11.5						23	

Tanks	NH3		N02		N03		pH		ALK		DO	Sal.	Temp	Imhoff		NAHCO3		NB	RO		FOS	
7/1/14	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM		AM	PM	AM	PM
Sq 1	0	0	0	0	0	0	7.81	7.97	200	180	5.62	28.5	28.4	12	11					30		
Sq 2	0	0	4	3	10	15	7.84	7.86	200	250	6.01	29.5	27.5	14	13					1m 30		
Sq 3	0	0	2	0.5	0	0	7.69	7.93	190	250	5.87	28.2	27.9	10	11.5							
Sq 4	0	0	3	2	5	0	7.76	7.8	170	250	6.03	28.6	27.7	10.2	13.5	6.3				36s		

Tanks	NH3		N02		N03		pH		ALK		DO	Sal.	Temp	Imhoff		NAHCO3		NB	RO		FOS	
7/2/14	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM		AM	PM	AM	PM
Sq 1	0		0		0		7.56		200		6.03	28.5	28	11								
Sq 2	0		6		20		7.59		250		6.16	28.9	28.3	12								
Sq 3	0		3		10		7.52		200		6.22	28.5	27.5	10								
Sq 4	0		4		15		7.53		180		6.2	28.5	28.4	12								

Appendix C

Feed curve used for all tanks in trial 2

Trial 2 # Stocked: 7500 Survival: 90%

Date	Day	Size	Weight Gain (g)	Population	FCR	Fines 45% Protein (g)	Feed % Protein (g)	Feed (g)
8-Jul-14	0	0.0406	0.0106	7500	1.7	25	40	135.15
9-Jul-14	1	0.0512	0.0134	6750	1.6	25	40	144.72
10-Jul-14	2	0.0646	0.0168	6075	1.5	25	40	153.09
11-Jul-14	3	0.0814	0.0213	5468	1.4	25	40	163.04
12-Jul-14	4	0.1027	0.0267	4921	1.3		40	170.80
13-Jul-14	5	0.1294	0.0338	4429	1.2		23/18	179.63
14-Jul-14	6	0.1632	0.0426	3986	1.1		23/18	186.77
15-Jul-14	7	0.2058	0.0537	3587	1		23/18	192.63
16-Jul-14	8	0.2595	0.0677	3229	0.9		23/18	196.71
17-Jul-14	9	0.3272	0.0853	2906	0.8		23/18	198.28
18-Jul-14	10	0.4125	0.1076	2615	0.8		23/18	225.11
19-Jul-14	11	0.5201	0.1357	2354	0.8		23/18	255.50
20-Jul-14	12	0.6558	0.1711	2118	0.8		23/18	289.94
21-Jul-14	13	0.8269	0.2157	1906	0.8		23/18	328.97
22-Jul-14	14	1.0426		1716				
							Total feed=	2920.35

Appendix D
Salinity Reduction schedule for trial 2

NRS
14-08
Salinity Calculator

down to 8ppt

final salinity 8
incoming salinity 31.8
depth incoming 7.547169811

gms salt 5366.25

liters per cm 22.5

time	desired salinity	depth	liters	calculated salinity	
9:00	31.8	7.5	168.75	31.8	
9:30	28.8	8.28	186.328125	28.8	add R.O. water
10:00	25.8	9.24	207.994186	25.8	add R.O. water
10:30	23.8	10.02	225.4726891	23.8	add R.O. water
11:00	21.8	10.94	246.1582569	21.8	add R.O. water
11:30	19.8	12.05	271.0227273	19.8	add R.O. water
12:00	17.8	13.40	301.4747191	17.8	add R.O. water
13:00	15.8	15.09	339.6360759	15.8	add R.O. water
14:00	14.8	16.11	362.5844595	14.8	add R.O. water
15:00	13.8	17.28	388.8586957	13.8	add R.O. water
16:00	12.8	18.63	419.2382813	12.8	add R.O. water
17:00	11.8	20.21	454.7669492	11.8	add R.O. water
8:00	10.8	22.08	496.875	10.8	add R.O. water
9:00	9.8	24.34	547.5765306	9.8	add R.O. water
10:00	8.8	27.10	609.8011364	8.8	add R.O. water
11:00	8	30.00	675	8.0	add R.O. water

Appendix E
Daily values for water quality measurements in trial 2

Tanks	TAN		N02		N03		pH		ALK		D.O.	Sal.	Temp	Imhoff		NAH CO3		NB		RO	FOS		FF Ex.	BIo Ex.
8-Jul	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	AM	PM		
Sq 1		0.3		0.0		0.0		7.51		120	5.88	35.0	28.0	0.0	0.0		27.88		90					
Sq 2		0.3		0.0		0.0		7.38		80	7.17	18.8	24.7	0.0	0.0		46.46		90					
Sq 3		0.0		0.0		0.0		7.46		16	6.65	35.5	27.6	0.0	0.0		9.29		90					
Sq 4		0.0		0.0		0.0		7.34		80	6.63	19.1	24.9	0.0	0.0		46.46		90					

Tanks	TAN		N02		N03		pH		ALK		D.O.	Sal.	Temp	Imhoff		NAH CO3		NB		RO	FOS		FF Ex.	BIo Ex.
9-Jul	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	AM	PM		
Sq 1	2.0	2.0	2.0	2.0	0.0	0.0	7.68	7.74	200	180	5.91	34.3	25.5	1.1	6.0	0.00	0.00	180	180					
Sq 2	2.5	3.0	2.0	2.0	0.0	10.0	7.88	7.72	180	170	6.42	17.8	26.7	1.8	5.5	0.00	0.00	180	180	2				
Sq 3	1.0	0.8	2.0	2.0	0.0	0.0	7.85	7.52	180	190	5.46	36.5	27.5	3.0	8.5	0.00	0.00	90	90					
Sq 4	2.0	3.0	2.5	2.0	0.0	10.0	7.91	7.76	180	170	6.02	18.4	27.2	2.0	8.5	0.00	0.00	180	180	3				

Tanks	TAN		N02		N03		pH		ALK		D.O.	Sal.	Temp	Imhoff		NAH CO3		NB		RO	FOS		FF Ex.	BIo Ex.
10-Jul	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	AM	PM		
Sq 1	2.5	2.5	2.0	2.0	0.0	0.0	7.64	7.69	200	180	5.79	36.2	25.7	8.0	6.5		0.00	180		4	35.94			
Sq 2	3.0	2.5	4.0	2.0	15.0	15.0	7.92	7.74	180	110	6.84	14.0	27.0	8.5	7.0		32.52	180		3	50.04			
Sq 3	4.0	2.5	2.5	2.0	5.0	10.0	7.58	7.45	200	170	5.85	36.8	27.0	14.5	11.0		4.68	180		4	54.49			
Sq 4	4.0	2.5	4.0	3.0	10.0	15.0	7.80	7.76	190	110	6.72	13.8	27.0	14.0	12.0		32.52	180		3	60.96			

Tanks 11-Jul	TAN		N02		N03		pH		ALK		D.O.	Sal.	Temp	Imhoff		NAH CO3		NB		RO	FOS		FF Ex.	BIo Ex.
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	AM	PM		
Sq 1	3.0	2.5	2.0	2.0	0.0	10.0	7.91	7.86	180	160	7.67	28.5	27.3	10.5	12.0		18.58			1	41.41			
Sq 2	2.5	0.3	3.0	3.0	10.0	20.0	8.02	7.98	170	160	8.65	9.0	27.1	10.5	18.0	9.29	18.58			1	40.26			
Sq 3	2.0	2.5	2.0	3.0	10.0	10.0	7.76	7.74	190	160	7.65	29.0	26.9	20.0	22.0		18.58			1	0.00			
Sq 4	3.0	0.0	3.0	3.0	15.0	20.0	8.04	8.02	160	170	8.69	9.2	27.0	14.5	29.0	18.58	9.29			1	45.72			

Tanks 12-Jul	TAN		N02		N03		pH		ALK		D.O.	Sal.	Temp	Imhoff		NAHCO3		NB		RO	FOS		FF Ex.	BIo Ex.
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	AM	PM		
Sq 1	1.5	2.0	3.0	3.0	10.0	10.0	7.81	7.82	200	170	6.20	28.4	27.2	15.0	13.0	9.29				1				
Sq 2	0.3	0.3	4.0	3.0	15.0	15.0	8.05	8.00	170	180	7.13	9.0	27.0	22.0	26.0	9.29				1				
Sq 3	2.0	2.5	3.0	3.0	10.0	10.0	7.71	7.66	180	170	6.04	28.8	27.6	23.0	23.0	9.29				1				
Sq 4	0.0	0.0	4.0	3.0	15.0	15.0	8.03	8.05	140	170	7.03	9.1	27.1	27.0	27.0	19.61				1				

Tanks 13-Jul	TAN		N02		N03		pH		ALK		D.O.	Sal.	Temp	Imhoff		NAH CO3		NB		RO	FOS		FF Ex.	BIo Ex.
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	AM	PM		
Sq 1	2.5	0.3	4.0	4.0	10.0	20.0	7.95	7.80	180	180	8.50	28.3	26.7	15.0	17.0					0	44.57			
Sq 2	0.0	0.0	4.0	3.0	20.0	15.0	8.17	7.99	120	170	7.38	8.9	27.2	28.0	35.0	29.42				1	17.26			
Sq 3	2.0	0.3	4.0	3.0	20.0	15.0	7.87	7.74	190	170	6.40	28.6	27.7	26.0	25.0					1	39.11			
Sq 4	0.0	0.0	4.0	3.0	15.0	15.0	8.16	7.97	190	190	7.34	9.0	27.1	23.0	19.5					1	17.26			

Tanks 14-Jul	TAN		N02		N03		pH		ALK		D.O.	Sal.	Temp	Imhoff		NAH CO3		NB		RO	FOS		FF Ex	BIo Ex.
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	AM	PM		
Sq 1	0.0	0.0	6.0	5.0	20.0	10.0	7.57	7.57	170	160	6.09	28.4	27.3	19.0	21.0	4.90				1	25.88			
Sq 2	0.0	0.0	4.0	0.5	15.0	0.0	7.87	7.91	130	170	6.99	9.0	27.6	40.0	29.0	24.50				1	17.26		1.00	3.00
Sq 3	0.2	0.0	5.0	4.0	20.0	15.0	7.49	7.56	120	180	6.17	28.5	27.5	17.0	21.0	29.40				1	23.21		2.55	
Sq 4	0.0	0.0	3.5	2.0	10.0	0.0	7.86	7.89	190	180	7.03	8.9	27.0	17.0	19.5	0.00				1	15.10			

Tanks 15-Jul	TAN		N02		N03		pH		ALK		D.O.	Sal.	Temp	Imhoff		NAH CO3		NB		RO	FOS		FF Ex.	BIo Ex.
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	AM	PM		
Sq 1	0.2	0.3	5.5	5.5	20.0	20.0	7.78	7.55	180	190	5.98	28.4	26.8	21.0	20.0					1				
Sq 2	0.0	0.0	0.2	0.0	5.0	0.0	8.14	7.77	180	200	6.96	9.0	26.7	25.0	20.0					1			0.50	2.00
Sq 3	0.0	0.0	4.0	3.0	20.0	10.0	7.80	7.54	200	180	6.03	28.4	27.2	20.0	20.0					1				
Sq 4	0.0	0.0	2.0	0.5	5.0	0.0	8.07	7.78	170	250	6.82	8.8	26.8	16.0	16.0	25.88				1				

Tanks 16-Jul	TAN		N02		N03		pH		ALK		D.O.	Sal.	Temp	Imhoff		NAHCO3		NB		RO	FOS		FF Ex.	BIo Ex.
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	AM	PM		
Sq 1	0.5	0.0	8.0	6.0	30.0	30.0	7.65	7.62	190	190	5.99	28.5	26.7	17.0	14.0					1	39.97	12.92		
Sq 2	0.0	0.0	0.1	0.1	0.0	0.0	8.00	7.91	190	250	6.87	9.0	27.5	20.0	10.0					1				3.00
Sq 3	0.0	0.2	3.0	3.0	10.0	10.0	7.69	7.63	180	160	6.04	28.4	27.2	20.0	20.0					1				
Sq 4	0.0	0.0	0.1	0.1	0.0	0.0	8.03	7.93	250	250	6.78	8.9	27.2	15.0	13.5					1				

Tanks 17-Jul	TAN		N02		N03		pH		ALK		D.O.	Sal.	Temp	Imhoff		NAH CO3		NB		RO	FOS		FF Ex.	BIo Ex.
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	AM	PM		
Sq 1	0.0	0.3	3.0	8.0	40.0	40.0	7.60	7.65	180	250	6.05	28.3	26.9	12.0	14.0							18.64		
Sq 2	0.0	0.0	0.1	0.1	20.0	0.0	8.03	8.03	190	190	7.15	8.1	26.7	10.0	8.0									
Sq 3	0.0	0.8	3.0	40.0	20.0	30.0	7.64	7.70	180	190	6.09	28.4	27.1	20.0	17.5									
Sq 4	0.0	0.0	0.1	0.1	15.0	0.0	8.00	8.05	190	190	6.92	8.1	27.4	15.0	15.0									

Tanks 18-Jul	TAN		N02		N03		pH		ALK		D.O.	Sal.	Temp	Imhoff		NAH CO3		NB		RO	FOS		FF Ex.	BIo Ex.
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	AM	PM		
Sq 1	0.1	0.0	10.0	10.0	40.0	40.0	7.61	7.71	130	250	6.57	27.9	26.8	11.0	12.0	46.46					44.23	22.12		
Sq 2	0.0	0.0	2.0	2.0	10.0	10.0	8.02	8.06	130	200	7.44	8.9	27.3	8.5	8.5	46.46				1				
Sq 3	2.0	0.0	10.0	5.0	30.0	30.0	7.59	7.60	180	190	6.45	28.5	27.6	18.0	20.0					1	64.99	19.99	1.50	
Sq 4	0.5	0.5	2.0	2.0	10.0	10.0	7.93	7.87	190	190	7.42	8.7	27.0	11.0	11.0					1				

Tanks 19-Jul	TAN		N02		N03		pH		ALK		D.O.	Sal.	Temp	Imhoff		NAH CO3		NB		RO	FOS		FF Ex.	BIo Ex.
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	AM	PM		
Sq 1	0.0	0.0	10.0	2.0	30.0	15.0	7.76	7.52	200	250	6.15	28.1	26.8	14.0	17.0						43.14		2.75	
Sq 2	0.0	0.0	3.0	2.0	10.0	10.0	8.11	7.95	250	275	7.15	8.9	26.9	8.5	8.0					1			1.50	
Sq 3	0.0	0.0	10.0	3.0	20.0	15.0	7.70	7.49	190	250	6.16	28.3	27.3	15.0	27.0						43.14		3.50	
Sq 4	0.0	0.0	3.0	3.0	10.0	15.0	8.03	7.78	190	250	6.98	8.6	27.5	10.0	9.0					1				

Tanks 20-Jul	TAN		N02		N03		pH		ALK		D.O.	Sal.	Temp	Imhoff		NAH CO3		NB		RO	FOS		FF Ex.	BIo Ex.
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	AM	PM		
Sq 1	0.0	0.0	3.0	6.0	10.0	20.0	7.72	7.75	250	250	6.19	28.3	27.0	11.0	18.0								1.25	
Sq 2	0.0	0.3	3.0	3.0	10.0	20.0	8.05	8.05	200	250	7.17	8.7	27.0	4.2	6.0					1			0.50	
Sq 3	0.0	0.0	4.0	3.0	10.0	20.0	7.65	7.71	250	160	6.27	28.4	27.0	25.0	17.0						17.26		2.25	5.60
Sq 4	0.0	0.0	4.0	4.0	20.0	15.0	7.97	7.78	190	170	7.17	8.6	26.9	10.0	8.5					1	17.26		0.25	

Tanks 21-Jul	TAN		N02		N03		pH		ALK		D.O.	Sal.	Temp	Imhoff		NAH CO3		NB		RO	FOS		FF Ex.	BIo Ex.
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	AM	PM		
Sq 1	0.0	0.0	5.0	3.5	20.0	20.0	7.87	7.84	200	250	6.23	28.5	27.4	28.0	13.5					1	21.57		4.00	3.80
Sq 2	0.5	0.0	4.0	4.0	15.0	20.0	8.21	8.00	250	250	7.28	8.7	27.4	5.5	7.0					1	22.72			
Sq 3	0.0	0.0	5.0	6.0	20.0	20.0	7.83	7.68	190	250	6.37	28.7	27.3	22.0	17.5					1	21.57	12.94	1.50	
Sq 4	0.2	0.0	4.0	4.0	15.0	20.0	8.09	7.83	190	200	7.10	8.6	27.8	8.0	7.0					1	19.40			

Tanks 22-Jul	TAN		N02		N03		pH		ALK		D.O.	Sal.	Temp	Imhoff		NAH CO3		NB		RO	FOS		FF Ex.	BIo Ex.
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	AM	PM		
Sq 1	0.0	0.0	4.0	3.0	15.0	15.0	7.97	7.81	250	190	6.24	28.4	27.0	12.0	14.5					1	17.26		1.50	
Sq 2	0.0	0.0	4.0	4.0	20.0	30.0	8.24	8.14	250	250	7.14	8.7	27.1	6.5	6.5					1	17.26			
Sq 3	0.0	0.0	5.0	4.0	15.0	30.0	7.91	7.81	190	200	6.35	28.4	26.9	20.0	20.0					1	21.57		1.50	
Sq 4	0.2	0.0	6.0	6.0	30.0	30.0	8.17	8.04	200	250	6.99	8.5	27.7	6.0	6.5					1	28.07	14.03		

Tanks 23-Jul	TAN		N02		N03		pH		ALK		D.O.	Sal.	Temp	Imhoff		NAH CO3		NB		RO	FOS		FF Ex.	BIo Ex.
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	PM	AM	PM	AM	AM	PM		
Sq 1	0.0		5.0		20.0		7.96		200		6.13	28.3	27.6	13.0										
Sq 2	0.1		4.5		10.0		8.25		250		7.18	8.6	26.8	6.5										
Sq 3	0.0		5.5		20.0		7.90		200		6.38	28.3	27.2	21.0										
Sq 4	0.0		6.0		20.0		8.15		200		7.07	8.5	27.4	6.0										

Appendix F
Feed curve used for all tanks in trial 3

Trial 3 # Stocked: 7425 Survival: 80%

Day	Size	Weight Gain	Population	FCR	Fines 45% Protein (g)	Feed % Protein (g)	Feed (G)
0	0.0035	0.0008	7425	1.7	25	40	10.098
1	0.0043	0.001	7284	1.2	25	40	8.74071
2	0.0053	0.0012	7146	1	25	40	8.57463651
3	0.0065	0.001278	7010	1	25	40	8.958480113
4	0.007778	0.001722	6877	0.9		40	10.65732338
5	0.0095	0.00235	6746	0.8		40	12.6823386
6	0.01185	0.00265	6618	0.8		40	14.0296347
7	0.0145	0.0033	6492	0.8		23	17.1389194
8	0.0178	0.00373	6369	0.8		23	19.00410126
9	0.02153	0.00447	6248	0.8		23	22.34163923
10	0.026	0.0061	6129	0.8		23	29.90930722
11	0.0321	0.0074	6013	0.8		23	35.59403686
12	0.0395	0.0085	5898	0.8		23	40.10822654
13	0.048	0.012	5786	0.8		23	55.54753445
14	0.06		5676	0.8		23	
						Total Feed=	287.96

Appendix G

Salinity reduction schedule used in trial 3

NRS 14-13

Salinity Calculator

down to 8ppt

time	desired salinity	depth	liters	calculated salinity		final salinity	8
						incoming salinity	31.8
						depth incoming	7.547169811
9:00	31.8	7.5	168.75	31.8		gms salt	
9:30	28.8	8.28	186.328125	28.8	add R.O. water	5366.25	
10:00	25.8	9.24	207.994186	25.8	add R.O. water		
10:30	23.8	10.02	225.4726891	23.8	add R.O. water	liters per cm	
11:00	21.8	10.94	246.1582569	21.8	add R.O. water	22.5	
11:30	19.8	12.05	271.0227273	19.8	add R.O. water		
12:00	17.8	13.40	301.4747191	17.8	add R.O. water		
13:00	15.8	15.09	339.6360759	15.8	add R.O. water		
14:00	14.8	16.11	362.5844595	14.8	add R.O. water		
15:00	13.8	17.28	388.8586957	13.8	add R.O. water		
16:00	12.8	18.63	419.2382813	12.8	add R.O. water		
17:00	11.8	20.21	454.7669492	11.8	add R.O. water		
8:00	10.8	22.08	496.875	10.8	add R.O. water		
9:00	9.8	24.34	547.5765306	9.8	add R.O. water		
10:00	8.8	27.10	609.8011364	8.8	add R.O. water		
11:00	8	30.00	675	8.0	add R.O. water		

down to 28 ppt

time	desired salinity	depth	liters		final salinity	28
					incoming salinity	31.8
					depth incoming	26.41509434
9:00	31.8	7.5	168.75		gms salt	
9:30	31.8	8.28	186.328125	add salt water	18900	
10:00	31.8	9.24	207.994186	add salt water		
10:30	31.8	10.02	225.4726891	add salt water	liters per cm	
11:00	31.8	10.94	246.1582569	add salt water	22.5	
11:30	31.8	12.05	271.0227273	add salt water		
12:00	31.8	13.40	301.4747191	add salt water		
13:00	31.8	15.09	339.6360759	add salt water		
14:00	31.8	16.11	362.5844595	add salt water		
15:00	31.8	17.28	388.8586957	add salt water		
16:00	31.8	18.63	419.2382813	add salt water		
17:00	31.8	20.21	454.7669492	add salt water		
8:00	31.8	22.08	496.875	add salt water		
9:00	31.8	26.40	594	add salt water		
10:00	30.8	27.10	609.8011364	add R.O. water		
11:00	28	30.00	675	add R.O. water		

Appendix H

Daily values for water quality measurements in trial 3

Tank 30-Oct	NH3 AM	NH3 PM	N02 AM	N02 PM	N03 AM	N03 PM	pH AM	pH PM	ALK AM	160 160	DO	Sal.	Temp	Imhoff AM	Imhoff PM	NAHCO3 AM	NB AM	NB PM	RO AM	FOS AM	FOS PM
Sq 1	0.0	0.1	2.0	2.0	0.0	0.0		8.00	170	160	5.98	35.40	23.90	0.10	0.10	40.00					
Sq 2	0.0	0.0	2.0	2.0	0.0	5.0		8.12	170	160	6.07	35.60	24.00	0.10	0.10	4.00					
Sq 3	0.0	0.1	2.0	2.0	0.0	5.0		8.02	170	160	6.04	35.40	24.10	0.10	0.10	40.00					
Sq 4	0.0	0.0	2.0	2.0	0.0	0.0		8.01	170	160	5.85	35.50	24.00	0.10	0.10	4.00					

Tank 31-Oct	NH3 AM	NH3 PM	N02 AM	N02 PM	N03 AM	N03 PM	pH AM	pH PM	ALK AM	160 160	DO	Sal.	Temp	Imhoff AM	Imhoff PM	NAHCO3 AM	NB AM	NB PM	RO AM	FOS AM	FOS PM
Sq 1	0.5	0.3	2.5	2.0	10.0	5.0	8.13	8.02	250	160	7.24	14.80	27.40	0.10	1.00	18.58					
Sq 2	0.5	0.0	2.5	2.0	7.0	10.0	8.02	7.93	180	160	6.48	35.20	27.10	0.10	0.70	10.00					
Sq 3	0.5	0.3	2.5	3.0	10.0	10.0	8.21	8.00	180	160	7.37	15.60	27.10	0.10	0.80	18.58	90.00				
Sq 4	0.5	0.3	2.5	2.0	5.0	10.0	8.10	7.95	180	160	6.54	35.20	27.00	0.10	0.80	10.00					

Tank 1-Nov	NH3 AM	NH3 PM	N02 AM	N02 PM	N03 AM	N03 PM	pH AM	pH PM	ALK AM	160 160	DO	Sal.	Temp	Imhoff AM	Imhoff PM	NAHCO3 AM	NB AM	NB PM	RO AM	FOS AM	FOS PM
Sq 1	0.8	1.0	3.0	3.0	10.0	15.0	8.07	8.13	160	160	8.02	7.70	27.60	0.80	0.80	18.58	90.00	90.00			
Sq 2	0.3	0.5	3.0	3.0	15.0	10.0	8.04	7.97	300	160	7.15	28.30	27.40	0.50	0.50		90.00	90.00	30		
Sq 3	0.3	0.5	3.0	3.0	20.0	20.0	1.00	8.11	160	160	8.10	8.30	27.30	0.60	0.70	18.58	90.00	90.00	30		
Sq 4	0.8	0.5	3.0	3.0	10.0	15.0	8.05	7.98	180	160	7.14	28.10	27.40	0.50	0.60		90.00	90.00	20		

Tank	NH3		N02		N03		pH		ALK	160	DO	Sal.	Temp	Imhoff		NAHCO3	NB		RO	FOS	

2-Nov	AM	PM	AM	PM	AM	PM	AM	PM	AM	160				AM	PM	AM	AM	PM	AM	AM	PM
Sq 1	0.8	1.0	3.0	3.0	10.0	10.0	8.17	8.12	160	160	7.06	7.90	26.50	0.80	0.70	18.58	90.00	90.00			
Sq 2	0.8	0.8	5.0	4.0	20.0	15.0	8.04	8.16	300	160	6.20	28.60	27.00	0.50	0.60		90.00	90.00	100		
Sq 3	0.3	0.8	3.0	3.0	10.0	25.0	8.16	8.01	300	160	7.02	8.40	26.80	0.60	0.60		90.00	90.00	100		
Sq 4	0.8	0.8	4.0	4.0	20.0	20.0	8.06	8.20	180	160	6.23	28.40	27.00	0.70	0.80		90.00	90.00	90		
										160											
Tank	NH3		N02		N03		pH		ALK	160	DO	Sal.	Temp	Imhoff		NAHCO3	NB		RO	FOS	
3-Nov	AM	PM	AM	PM	AM	PM	AM	PM	AM	160				AM	PM	AM	AM	PM	AM	AM	PM
Sq 1	0.5	0.8	5.0	5.0	10.0	20.0	8.09	8.32	120	160	7.63	8.00	27.00	0.75	1.00	35.75	90.00	90.00			
Sq 2	0.8	0.8	6.0	7.0	25.0	30.0	8.03	7.99	170	160	6.81	28.20	27.10	0.60	0.80	9.29	90.00	90.00			
Sq 3	0.5	0.8	5.0	3.0	15.0	20.0	8.22	8.29	120	160	7.70	8.30	27.90	0.70	1.00	35.75	90.00	90.00			
Sq 4	0.9	0.8	5.0	10.0	25.0	40.0	8.10	8.00	170	160	6.92	28.20	27.20	0.70	0.90	9.29	90.00	90.00			
										160											
Tank	NH3		N02		N03		pH		ALK	160	DO	Sal.	Temp	Imhoff		NAHCO3	NB		RO	FOS	
4-Nov	AM	PM	AM	PM	AM	PM	AM	PM	AM	160				AM	PM	AM	AM	PM	AM	AM	PM
Sq 1	0.5	0.3	3.0	2.0	15.0	15.0	8.39	8.13	180	160		8.10	26.80	1.00	0.70		90.00	90.00	20	12.94	
Sq 2	0.1	0.0	6.0	7.0	35.0	40.0	7.95	7.77	180	160		28.20	26.60	0.25	0.30		90.00	90.00	30	25.88	15.10
Sq 3	0.3	0.3	3.0	3.0	15.0	20.0	8.28	8.06	180	160		8.10	27.50	0.75	0.80		90.00	90.00	20	12.94	7.84
Sq 4	0.3	0.0	5.0	5.0	40.0	40.0	8.00	7.93	120	160		28.20	26.90	0.80	1.00	55.75	90.00	90.00	30	21.57	10.79
										160											
Tank	NH3		N02		N03		pH		ALK	160	DO	Sal.	Temp	Imhoff		NAHCO3	NB		RO	FOS	
5-Nov	AM	PM	AM	PM	AM	PM	AM	PM	AM	160				AM	PM	AM	AM	PM	AM	AM	PM
Sq 1	0.0	0.0	0.3	0.1	15.0	15.0	8.23	8.24	180	160	7.53	8.00	27.80	1.00	1.10	9.28			20		
Sq 2	0.0	0.0	2.0	2.0	20.0	25.0	7.87	7.86	170	160	6.60	28.10	26.90	1.00	0.50	9.28			30		
Sq 3	0.0	0.0	0.3	0.0	15.0	15.0	8.23	8.07	170	160	7.47	8.10	27.10	1.00	0.60	9.28			40		
Sq 4	0.0	0.0	0.8	1.0	10.0	25.0	8.11	8.05	180	160	6.61	28.20	27.30	1.25	1.00	9.28			40		
										160											
Tank	NH3		N02		N03		pH		ALK	160	DO	Sal.	Temp	Imhoff		NAHCO3	NB		RO	FOS	

6-Nov	AM	PM	AM	PM	AM	PM	AM	PM	AM	160				AM	PM	AM	AM	PM	AM	AM	PM
Sq 1	0.0	0.0	0.3	0.5	15.0	15.0	8.27	8.31	180	160	7.47	8.20	27.30	1.00	1.00				30		
Sq 2	0.0	0.0	2.5	3.0	20.0	25.0	7.87		170	160	6.58	28.20	26.60	0.20	0.30				50		12.94
Sq 3	0.0	0.0	1.0	1.0	18.0	15.0	8.17	8.13	175	160	7.38	8.10	27.00	0.40	0.50				30		
Sq 4	0.0	0.0	1.5	2.0	25.0	25.0	8.10	8.03	170	160	6.63	28.20	27.10	0.75	1.00				50		
										160											
Tank	NH3		N02		N03		pH		ALK	160	DO	Sal.	Temp	Imhoff		NAHCO3	NB		RO	FOS	
7-Nov	AM	PM	AM	PM	AM	PM	AM	PM	AM	160				AM	PM	AM	AM	PM	AM	AM	PM
Sq 1	0.0	0.0	0.8	0.5	25.0	15.0	8.34	8.36	180	160	7.74	8.00	27.10	1.25	1.25				30		
Sq 2	0.0	0.0	2.0	2.0	20.0	25.0	7.91	7.95	170	160	6.74	28.00	26.90	0.70	0.80	9.29			20		
Sq 3	0.0	0.0	1.0	1.0	15.0	20.0	8.22	8.16	180	160	7.46	8.00	27.60	0.75	0.80				30		
Sq 4	0.0	0.0	1.5	2.0	25.0	30.0	8.13	8.08	190	160	6.59	28.20	27.90	1.00	1.25				82		
										160											
Tank	NH3		N02		N03		pH		ALK	160	DO	Sal.	Temp	Imhoff		NAHCO3	NB		RO	FOS	
8-Nov	AM	PM	AM	PM	AM	PM	AM	PM	AM	160				AM	PM	AM	AM	PM	AM	AM	PM
Sq 1	0.0	0.0	0.8	0.8	20.0	20.0	8.23	8.21	180	160	6.63	8.10	27.70	1.00	1.00						
Sq 2	0.0	0.0	2.0	1.8	20.0	18.0	7.76	7.74	160	160	5.97	28.10	27.00	1.00	1.25						
Sq 3	0.0	0.0	1.0	1.0	18.0	20.0	8.26	8.04	180	160	6.74	8.00	27.30	1.25	1.00						
Sq 4	0.0	0.0	1.5	1.3	30.0	30.0	8.11	8.02	180	160	5.91	28.10	27.60	0.75	1.00						
										160											
Tank	NH3		N02		N03		pH		ALK	160	DO	Sal.	Temp	Imhoff		NAHCO3	NB		RO	FOS	
9-Nov	AM	PM	AM	PM	AM	PM	AM	PM	AM	160				AM	PM	AM	AM	PM	AM	AM	PM
Sq 1	0.0	0.0	1.0	1.0	15.0	15.0	8.34	8.24	300	160	7.04	8.20	27.00	1.40	1.40				194		
Sq 2	0.0	0.0	2.0	2.0	30.0	40.0	8.02	8.05	160	160	6.35	28.50	26.90	1.00	1.10	18.58			92		
Sq 3	0.0	0.0	1.0	1.0	15.0	15.0	8.34	8.21	180	160	7.09	8.10	27.30	1.25	1.25				140		
Sq 4	0.0	0.0	1.8	1.8	30.0	40.0	8.09	8.07	300	160	6.26	28.40	27.20	1.50	1.50				112		
										160											
Tank	NH3		N02		N03		pH		ALK	160	DO	Sal.	Temp	Imhoff		NAHCO3	NB		RO	FOS	

10-Nov	AM	PM	AM	PM	AM	PM	AM	PM	AM	160				AM	PM	AM	AM	PM	AM	AM	PM
Sq 1	0.0	0.0	0.3	0.0	30.0	30.0	8.25	8.31	190	160	6.59	7.90	26.80	1.75	2.00				30		
Sq 2	0.0	0.0	2.0	3.0	20.0	25.0	8.07	8.03	190	160	5.78	28.30	27.00	1.00	1.00				81		12.94
Sq 3	0.0	0.0	1.0	1.0	20.0	15.0	8.28	8.21	180	160	6.42	8.00	27.70	1.25	1.25				40		
Sq 4	0.0	0.0	1.0	1.0	35.0	35.0	8.11	8.05	180	160	5.75	28.10	27.20	1.25	1.80				56		
										160											
Tank	NH3		N02		N03		pH		ALK	160	DO	Sal.	Temp	Imhoff		NAHCO3		NB	RO	FOS	
11-Nov	AM	PM	AM	PM	AM	PM	AM	PM	AM	160				AM	PM	AM	AM	PM	AM	AM	PM
Sq 1	0.0	0.0	1.0	1.0	25.0	25.0	8.28	8.33	180	160	6.41	7.90	27.00	1.75	1.25				30		
Sq 2	0.0	0.0	1.5	2.0	20.0	25.0	8.01	8.01	180	160	5.69	28.00	27.00	1.00	1.10				30		
Sq 3	0.0	0.0	0.5	0.5	25.0	25.0	8.24	8.23	180	160	6.28	8.00	27.80	1.50	2.00				30		
Sq 4	0.0	0.0	1.5	2.0	30.0	30.0	8.10	8.08	180	160	5.71	28.00	27.00	1.00	1.20				30		
										160											
Tank	NH3		N02		N03		pH		ALK	160	DO	Sal.	Temp	Imhoff		NAHCO3		NB	RO	FOS	
12-Nov	AM	PM	AM	PM	AM	PM	AM	PM	AM	160				AM	PM	AM	AM	PM	AM	AM	PM
Sq 1	0.0	0.0	0.3	0.3	25.0	25.0	8.24	8.40	180	160	6.46	7.90	26.80	2.00	2.40	5.00			30		
Sq 2	0.0	0.0	1.5	2.0	20.0	20.0	8.03	8.07	160	160	6.06	28.20	27.60	1.00	1.25	5.00			50		
Sq 3	0.0	0.0	1.0	0.8	25.0	25.0	8.21	8.24	180	160	6.86	8.10	27.30	1.75	2.00	5.00			50		
Sq 4	0.0	0.0	1.0	2.0	30.0	40.0	8.00	8.08	190	160	6.13	28.00	26.90	1.25	2.00	5.00			30		
Tank	NH3		N02		N03		pH		ALK		DO	Sal.	Temp	Imhoff		NAHCO3		NB	RO	FOS	
13-Nov	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM				AM	PM	AM	AM	PM	AM	AM	PM
Sq 1	0.0		0.0		25.0				250		6.62	8.10	27.00								
Sq 2	0.0		2.0		25.0				300		5.98	28.40	26.80								
Sq 3	0.0		2.5		25.0				250		6.64	8.20	27.30								
Sq 4	0.0		2.0		40.0				260		5.84	28.40	27.20								