Effects of minimum dissolved oxygen setpoints for aeration in semi-intensive pond production of Pacific White shrimp (*Litopenaeus vannamei)*

By

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A dissertation submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
May 3rd, 2024

Keywords: Aquaculture, Shrimp, Aeration, Oxygen, pond management

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Abstract

Aeration is considered one of the most critical factors in shrimp farming as it affects the metabolism of shrimp and other living organisms within a pond. Maintenance proper dissolved oxygen (DO) concentrations is critical, as low DO can cause stress, lower resistance to disease, and inhibit growth. Although the effects of single, short-tern hypoxia events have been widely studied, little to no research has examined effects of repeated exposure to hypoxia during multiple diurnal cycles that shrimp may experience in aquaculture ponds. Even though the concept is simple, proper DO management is not simple. It is dependent on numerous factors and often based on anecdotal recommendations. The present trial was conducted to help elucidate the effects of DO management strategies on shrimp performance. The trial examined the effects of three different DO set points for automatic aeration on shrimp production and water quality parameters in earthen ponds. In sixteen earthen ponds (0.1 ha), juvenile Pacific white shrimp (~0.030g) were stocked at a density of 25ind/m^2. Minimal DO setpoints that activated automatic aeration in each of the treatments were 2.5, 3.5, and 4.5 mg O_2/L, respectively. Each of the treatments was applied to 5 ponds, except for treatment 1 (2.5mg/L) which was applied 6 ponds as it was considered of higher risk. Shrimp growth performance and water quality indicators were monitored weekly. At the end of the 79–81-day trial, results showed that different aeration control treatments had no significant effect in terms of growth performance, feed inputs or productivity parameters. The final weight of the shrimp ranged between 33.3-33.6 g, with average final yields of 7,500-8,500 kg per ha. Nonetheless, mean electrical costs differed significantly between treatments, with higher DO setpoints treatments exhibiting a higher aeration cost with no discernible benefit. Water quality parameters showed no significant difference, except for morning and afternoon DO concentrations.

Acknowledgements

I extend my heartfelt gratitude to Dr. Allen Davis, for the opportunity of joining his lab and whose unwavering support and guidance have been instrumental throughout the journey of working towards my degree. Dr. Davis, your expertise, encouragement, and valuable insights have significantly shaped the direction of my research skills and professional career. Next, I would like to thank our CPMC crew. First, I'd like to thank Melanie Rhodes for being the leading figure in all our work done at Gulf Shores. Your guidance has been an asset to assure the success of many experimental trials.

I also want to thank Fernando, Khanh, and Trent, who diligently worked throughout the last summer with me, to maintain the ponds and ensure the accomplishment of this trial. Finally, I'd like to thank the rest of the Davis lab and all the people that helped during the harvest. Your unwavering support and hard work were an essential part of a fulfilling outcome.

At last, I would like to thank my family and friends for always encouraging and supporting me, during my entire academic career. Especially to my parents, who always believed in me and encouraged me to always continue growing as a professional and as a person, I would not be here if it weren't for you.

This work was supported in part by the Hatch program (ALA016-1-19102) of the National Institute of Food and Agriculture, USDA, as well United States Department of Agriculture Research Service (USDA ARS) Specific Cooperative Agreements #58-6010-0-007.

Table of Contents

List of Tables

List of Figures

1. Introduction

Aquaculture production plays a pivotal role in meeting the global demand for seafood. The Pacific white shrimp (*Litopenaeus vannamei)* stands as one of the most important crustacean species in this industry (Liao & Chien, 2011). Shrimp culture encompasses a diverse range of production systems, including traditional pond culture, intensive raceway systems and more recently, innovative systems such as biofloc technology and recirculating aquaculture systems. Each of these production systems can have its own restraints and complexities.

Semi-intensive shrimp ponds are the most common production system in top producing Asian countries, with over 40% of the total shrimp farms in countries like Thailand, Philippines, Taiwan and Indonesia (Kongkeo, 1997). Higher farm densities are directly correlated with a considerable number of adverse consequences, such as poor water quality, easier spread of pathogens, environmental degradation and increased stress on the shrimp (Kautsky et al., 2000). To ensure lucrative shrimp farming, several environmental factors need to be meticulously managed. In terms of water quality, dissolved oxygen (DO) plays an important role and at higher densities the risk of having low concentrations increases. DO concentration in the water is considered to be one of the most critical parameters influencing shrimp health and growth (Allan & Maguire, 1991; Hargreaves & Boyd, 2022; Richardson, 1999).

Oxygen constantly moves between the air-water interface by the process of diffusion. The rate or the velocity at which oxygen moves, depends on the partial pressure of its liquid and gas phases (Tucker, 2005). The greater the difference, the faster the diffusion rate. When the pressure of both phases is equal or at equilibrium, the water is said to be at saturation (Boyd, 1998). Aerators increase DO by creating fine bubbles or drops of water to increase the interfacial area. Using aerators in ponds can be problematic due to constant fluctuations in water saturation that are driven by phytoplankton photosynthesis during the day. Aeration management should be closely monitored in ponds along with other inputs, like feed, in order to maintain proper DO levels.

In pond systems, managing aeration can be particularly challenging, as it involves balancing oxygen input and oxygen consumption (stocking density, feed input, etc.). Some studies have tried to comprehend the effects of different aeration systems on production in different settings, as aeration can be supplied through different types of mechanisms (Boyd, 1998). Most pond studies suggest that the increasing use of aeration (regardless of the type of aerators used) will directly result in improved growth of the shrimp (McGraw et al., 2001; Wyban et al., 1989).

The effects of low dissolved oxygen in pond culture have also been studied for other species, such as catfish. Cumulative respiration rate in a pond is driven by multiple factors. Specific calculations of aeration requirements based on stocking and feeding rates can be unreliable, as DO concentration is also dependent on the abundance of phytoplankton. The catfish industry uses a minimum DO set point as a trigger for mechanical aeration use. Electrically powered aerators are turned on when DO concentration falls below 3 mg/L, which is considered the critical hypoxia level for these species (Yang et al., 2015; Boyd et al., 2018). Triggering aeration based on a minimum DO threshold can make management more efficient, economically speaking. Intensively ponds that base aeration management on stocking density and size of fingerlings at stocking have shown to be less feasible compared to medium-intensity ponds (Kumar & Engle, 2017; Kumar et al., 2018). The use of intensively aerated ponds for commercial catfish production has been gaining momentum in recent years in the U.S. catfish industry (Bott et al., 2015; Kumar et al. 2018; Hegde et al., 2022). Hence the importance of finding better and more efficient ways to deal with aeration management in pond systems.

Studies have also shown that sediment respiration rate can vary according to the area studied (Santa & Vinatea, 2007). Organic matter and sediment accumulation have a direct effect on the respiration rate of a pond. Multiple studies agree that sediments are the major consumer of oxygen in ponds, followed by the oxygen consumption of the water column (Madenjian, 1990; Santa & Vinatea, 2007). Organic matter content in the soil should be limited to 0.5 and 3-4% to avoid excessive oxygen consumption (Boyd, 2003).

Although many studies have examined effects of exposure to low DO on the organism and culture medium, very few have examined effects of cyclical low DO events driven by diurnal cycles in outdoor ponds under semi-intensive culture conditions. There is a scarcity of scientific information on this subject, and most producers rely on general recommendations given by empirical methods. Therefore, the objective of this study was:

- Gather data-driven understanding of the diurnal dynamics of DO in semi-intensive shrimp ponds with different aeration rates.
- Study the effect of different automatic aeration setpoints on Pacific white shrimp production parameters and water quality.

2. Materials and Methods

2.1 Post-larvae nursing

A pond trial was conducted at the Alabama Department of Conservation and Natural Resources, Claude Peteet Mariculture Center (CPMC) (Gulf Shores, AL, USA). The shrimp post-larvae (PL) were sourced from Homegrown Shrimp USA hatchery (Indiantown, FL, USA). PLs were acclimated into six, 6000 L outdoor nursery tanks. On days 0 through 2, PLs were fed 25% of their body weight (BW) Zeigler Bros. (Gardners, PA, USA) Raceways 1 (50% protein, 15% lipids). Days 3 – 5, PLs were fed 20% of their BW with a combination of Zeigler Raceway 1 and 2 (1:1 ratio). During days 6-11, PLs were fed 15% of their body weight, until day 8 using a combination of Raceway 1 and 2, and from day 8 to 11 using only Raceway 2 (50% protein, 15% lipid). Finally, on days 12 and 13, PLs were fed 10% of their BW using Raceway 2. Fourteen days after receiving the PLs, juvenile shrimp were stocked in 16 ponds of approximately 0.1 ha, at a density of 25 shrimp/m^2.

2.2 Ponds

Sixteen lined ponds (1.52mm high-density polyethylene) with a 25 cm sandy loam bottom were tilled and then filled with water from the intercoastal waterway in Gulf Shores, AL (13 ppt). Water used to fill the ponds was filtered using a 250-micron mesh sock. Before juvenile shrimp were stocked, ponds were fertilized with inorganic fertilizers (1697 mL of 30-0-0 and 303 mL of 10-34-0 for 5.7 kg/ha of N and 1.03 kg of P, respectively).

2.3 Experimental diet

During the production trial shrimp were fed using Zeigler Bros. Inc. commercial diet PL Raceway 40-9 1.5mm (40% protein, 9% lipid) and Shrimp Grower SI-35 2.4mm (35% protein, 7% lipids). The 1.5mm feed was used for only the first two weeks of production to accommodate for the juveniles' size and the 2.4 mm feed was used for the rest of the cycle.

2.4 Feed management

The first 15 days after stocking, each pond was hand fed twice a day a daily ration, with the amount increasing weekly from 1.0 kg to 1.5kg. On day 16, feed started being delivered via a timer feeder. From days 16-22, the assigned feed amount was 3.0 kg per day, and on days 23-29 the assigned amount increased to 6.0 kg. Finally on day 30 of production, sonic feeding was started using the AQ1 Systems (AQ1 Feeder, AQ1 Systems Pty. Ltd., Tasmania, Australia), after the minimum activity required by AQ1 Systems was consistently met. Acoustic activity produced by the shrimp's mandibular occlusion were perceived by a hydrophone placed in each pond. This allowed the system to determine how much feed to apply after doing a test spin. A feeding schedule from 8am to 10pm with a maximum daily limit of 16 kg (160kg/ha) was set on the software to minimize negative repercussions on the pond's water quality.

2.5 Water quality management

Water quality parameters such as DO, salinity, temperature, and pH were monitored twice per day, at 5:00-5:45 am (before sunrise) and 7:20-8:00 pm (sunset), using a YSI ProQuatro Multiparameter meter (Yellow Springs Instrument Co., Yellow Spring, OH, USA). The AQ1 system uses Analytics software to continuously monitor DO and temperature via the use of probes placed in the water of each pond, the software also causes the system to stop feeding when DO levels dropped below the respective set points for each of the treatments. The AQ1 system also initiated automatic aeration controls when DO dropped below the set points for each treatment.

Total ammonia nitrogen (TAN) was measured once per week using an ion-selective electrode for TAN, and the Orion 4-Star Plus pH/ISE (Thermo Fisher Scientific, Waltham, MA, USA), Weekly, a spectrophotometer, (WaterLink Spintouch LaMotte, Chestertown, MD, USA) was used to measure pH, ammonia, nitrite, nitrate, magnesium, calcium, phosphate, and alkalinity.

2.6 Aeration control management and treatments

Aerators were set to turn on in each treatment at a threshold of either 2.5, 3.5 and 4.5 mg/L. Two aerators were placed in each one of the ponds to provide the aeration needed. A 1-HP Aquarian Pro (Air-O-Lator, Kansas City, MO, USA) served as the primary aerator to turn on with automatic controls. This aerator was programmed to turn on when DO values were below each respective set value. A 2-HP Aire-O2(Aire-O2, Aeration Industries International, Inc., Minneapolis, MN, USA) aerator was placed in each pond and was manually turned on and off as needed. The 2.5 mg/L setpoint treatment had a total of six replicates ponds as this was considered the highest risk treatment. Treatments using a 3.5 mg/L or 4.5 mg/L setpoint had five replicates each. Back-up aerators were turned on only when DO values dropped below the set value. Early in the production cycle, ponds worked only with primary aeration, as biomass increased and DO values started getting low in the evening, secondary aeration was turned on overnight. For treatment ponds using 2.5 mg/L back up aerators started being used from day 49 forward; ponds using 3.5 and 4.5 mg/L started secondary aeration from day 53 forward, when DO was below each respective setpoint by the last evening check. This was done to minimize the risk of lethal DO level drops overnight.

2.7 Sampling and Harvest

At stocking, an initial sample was taken for proximate composition analysis, to be later used for protein retention calculations. Protein retention (PR) was calculated as follows:

$$PR = \frac{((Final\ CP\ content\ (\%) * Final\ MW(g)) - (Initial\ CP\ content\ (\%) * Initial\ MW(g)))}{Protein\ offered\ (g)}$$

Following the first two weeks of the production cycle, shrimp were sampled using cast nets and weighed every week. A minimum of 60 shrimp or a total of ten throws per pond was used as the sample. Average individual weight was calculated by dividing the sample's biomass by the total count of shrimp in the sample.

A week before harvest, two sub-samples were taken from the regular weekly sampling for hemolymph extraction and whole-body composition analysis. Hemolymph was extracted from four shrimp and mixed to create a single pooled sample. The pooled sample from each pond was then analyzed using a Vetscan® VS2 Chemistry analyzer (Zoetis, NY, USA). For whole-body composition ten shrimp were kept and frozen. Six of them were dried (at 90°C), homogenized and then sent to be analyzed by Midwest Laboratories (Omaha, NE, USA) for proximate and mineral analysis. An additional four shrimp were kept for historic samples.

All ponds were harvested on days 79-81(8/12-8/14). The ponds' water level was lowered the day before and completely drained at the time of harvest. All shrimp were removed and weighed in baskets. A random sample of 150 shrimp was taken from each pond and weighed individually.

2.8 Economic data calculations

After harvest, the production value for each of the ponds was calculated according to the size class of the shrimp and market price from Latin America in October 2022 (Urner Barry, Toms River, NJ, USA). Shrimp pricing was accounted as headless and 60% dressed weight. Partial income was then calculated by subtracting the total feed cost and electrical cost of each pond from its respective generated value.

2.9 Statistical Analysis

All data was analyzed using Statistical Analysis System for Windows (V9.4, SAS Institute, Cary, NC, USA). Data were analyzed using one-way analysis of variance (ANOVA), to determine statistical differences between treatment ($P>0.05$). Furthermore, Tukey's honest significant difference (HSD) multiple range test were performed to test for pairwise differences between treatments.

Throughout the production cycle, substantial mortality events occurred in two ponds due to low DO because of equipment failure. These ponds included two replicates from the treatment with the 3.5 mg/L set point. Hence, these two replicates were removed from statistical analysis, leaving 3 replicates of this treatment that were used to calculate mean values of production parameters. During harvest, another pond was lost from the 2.5 mg/L treatment due to human error, this was also removed from the statistical analysis.

3 Results

3.1 Effects on water quality

A summary of water quality data can be found in Table 1. The average morning (5:00-5:45am) DO values were found to be significantly different between treatments, increasing linearly as the set point increased. Evening (7:20-8:00pm) DO was also found to be significantly different, but just for the highest treatment. This data was recorded using a hand-held YSI, as opposed to continuous readings recorded by the AQ1 Analytics software. Figures 1 and 2 present this data as daily and total means where it can be seen that treatment the 2.5 mg/L was persistently lower for both morning and afternoon. Salinity was slightly greater in ponds in the 3.5 mg/L treatment. The remaining water quality variables (including total ammonia nitrogen, pH, nitrate, nitrite, phosphate, calcium, magnesium, alkalinity and secchi depth) were not significantly different between treatments and were maintained within acceptable ranges for culture of this species.

When looking into the continuously recorded DO data (every five minutes), the analysis of variance showed significant differences between treatments 1(2.5mg/L setpoint) and 3 (4.5 mg/L setpoint). Treatment 3 was found to be constantly higher during the whole production cycle. The data was also evaluated as means from the 5 replicates per treatment during the whole 11-week cycle by hour throughout the day. To explore any significant trends, a cubic regression was applied to each treatment to fit the data points (Figure 3), each data point represents the mean DO value of all replicate ponds by treatment during the whole cycle (89-91 days). As can be seen, the lowest recorded DO values were during early morning for all the treatments. It is worth noting that- the 2.5mg/L treatment was persistently lower for longer periods during the morning. Figure 4 also shows the same data in a frequency distribution plot, in which an increasing plateau can be observed as the treatment setpoint increases. It can also be noted that the concentration of points

TABLE 1. Summary of water quality during 11 weeks of Pacific white shrimp (*Litopenaus vannamei*) production in ponds with three different set points for automatic aeration activation. Juveniles (0.03 g, 25 shrimp/m^2) were stocked in 16 ponds (0.1 ha). Values are shown as the mean ± standard deviation, including minimum and maximum values below in parenthesis for dissolved oxygen (DO), salinity, total ammonia nitrogen (TAN), and temperature.

	2.5mg/L	3.5mg/L	4.5mg/L	PSE[1]	P-value[2]
Morning DO (mg/L)[3]	3.36 ± 1.28^{c} (0.86, 8.64)	3.80± 1.26^{b} (1.62, 8.86)	4.29 ±1.12^{a} (2.15, 8.42)	0.6308	<0.0001
Evening DO (mg/L)[3]	9.45 ± 2.69^{b} (1.8, 16.01)	9.79 ± 2.85^{b} (1.88, 19.80)	10.26 ± 2.83^{a} (3.66, 18.3)	0.8884	0.0001
Salinity (g/L)	12.28 ± 1.39^{b} (9.9, 15.8)	12.59 ± 1.32^{a} (10.4, 15.6)	12.37 ± 1.47^{b} (10.7, 16)	0.3957	<0.0001
TAN (mg/L)	0.59 ± 0.99 (0, 4.2)	0.29 ± 2.27 (0, 3.9)	0.45 ± 0.97 (0, 4.5)	1.8748	0.2317
Temperature (ºC)	31.47 ± 2.24 (24.8, 35.7)	31.53. ± 2.23 (24.8, 35.9)	31.53 ± 2.32 (24.5, 36.2)	0.4033	0.8337
Secchi depth (cm)	46.16 ± 26.8	47.21 ± 27.71	45 ± 25.97	3.9524	0.9023
pH	8.77 ± 0.74	8.83 ± 0.75	8.80 ± 0.71	0.2424	0.2183
Nitrate	0.74 ± 3.17	0.03 ± 0.16	0.87 ± 5.37	4.8002	0.5238
Nitrite	0.19 ± 0.57	0.25 ± 0.90	0.17 ± 0.40	1.4491	0.8724
Phosphate	15.18 ± 36.15.	17.37 ± 42.40	16.08 ± 39.03	9.7263	0.9664
Calcium	197.6 ± 132.4	182.5 ± 97.3	178.2 ± 99.8	8.2323	0.6941
Magnesium	323.2 ± 152.7	338.8 ± 139.4	332.4 ± 137.4	7.9127	0.8779
Alkalinity	100.14 ± 44.43	103.74 ± 42.43	100 ± 38.02	4.4429	0.9325

[1] PSE: Pooled Standard Error

[2] One-way ANOVA, Means not sharing any letter are significantly different by the Tukey's HSD-test at the 5% level of significance.

[3] Morning = 5:00am – 5:45am, Evening = 7:20pm-8:00pm

FIGURE 1. Daily and total average morning (5:00-5:45am) dissolved oxygen during 11 weeks of Pacific white shrimp (*Litopenaus vannamei)* production in ponds with three different set points for automatic aeration activation. Juveniles (0.03 g, 25 shrimp/m^2) were stocked in 16 ponds (0.1 ha).

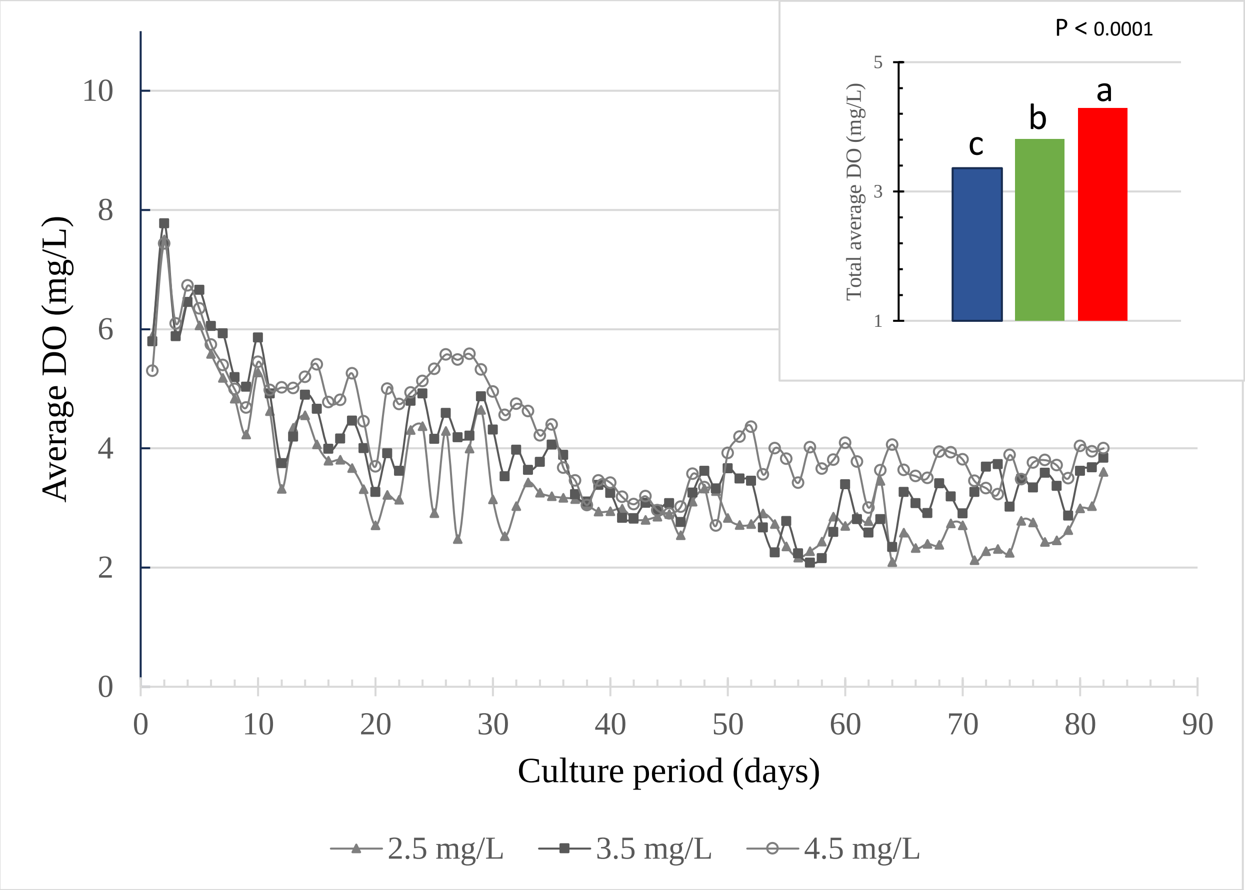


FIGURE 2. Daily and total average afternoon (7:20-8:00pm) dissolved oxygen during 11 weeks of Pacific white shrimp (*Litopenaus vannamei)* production in ponds with three different set points for automatic aeration activation. Juveniles (0.03 g, 25 shrimp/m^2) were stocked in 16 ponds (0.1 ha).

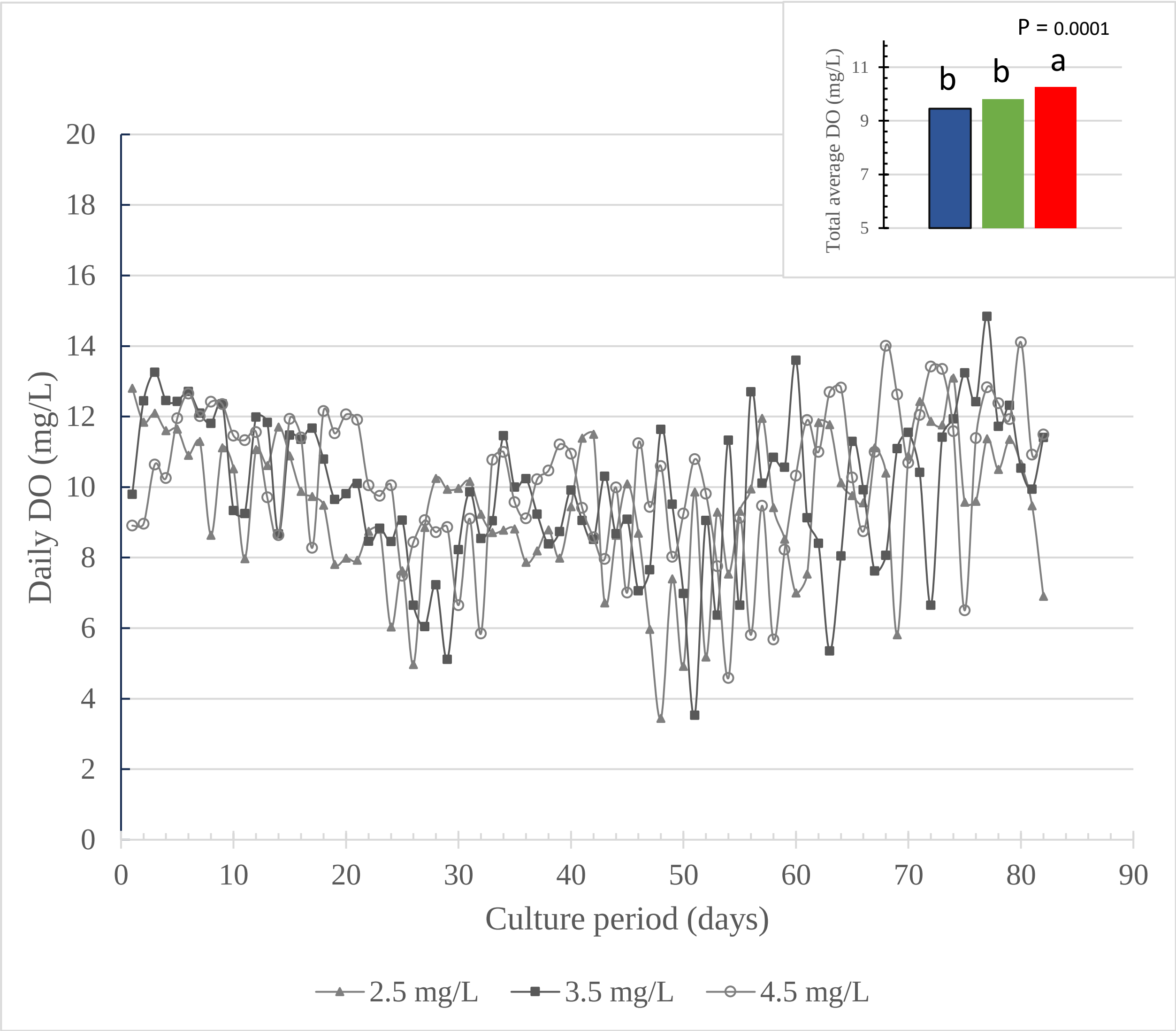


FIGURE 3. Regression analysis of the diurnal DO cycle in 5-minute intervals from all replicate ponds for three different automatic aeration setpoints during 11 weeks of Pacific white shrimp (*Litopenaus vannamei)* production. Juveniles (0.03 g, 25 shrimp/m^2) were stocked in 16 ponds (0.1 ha).

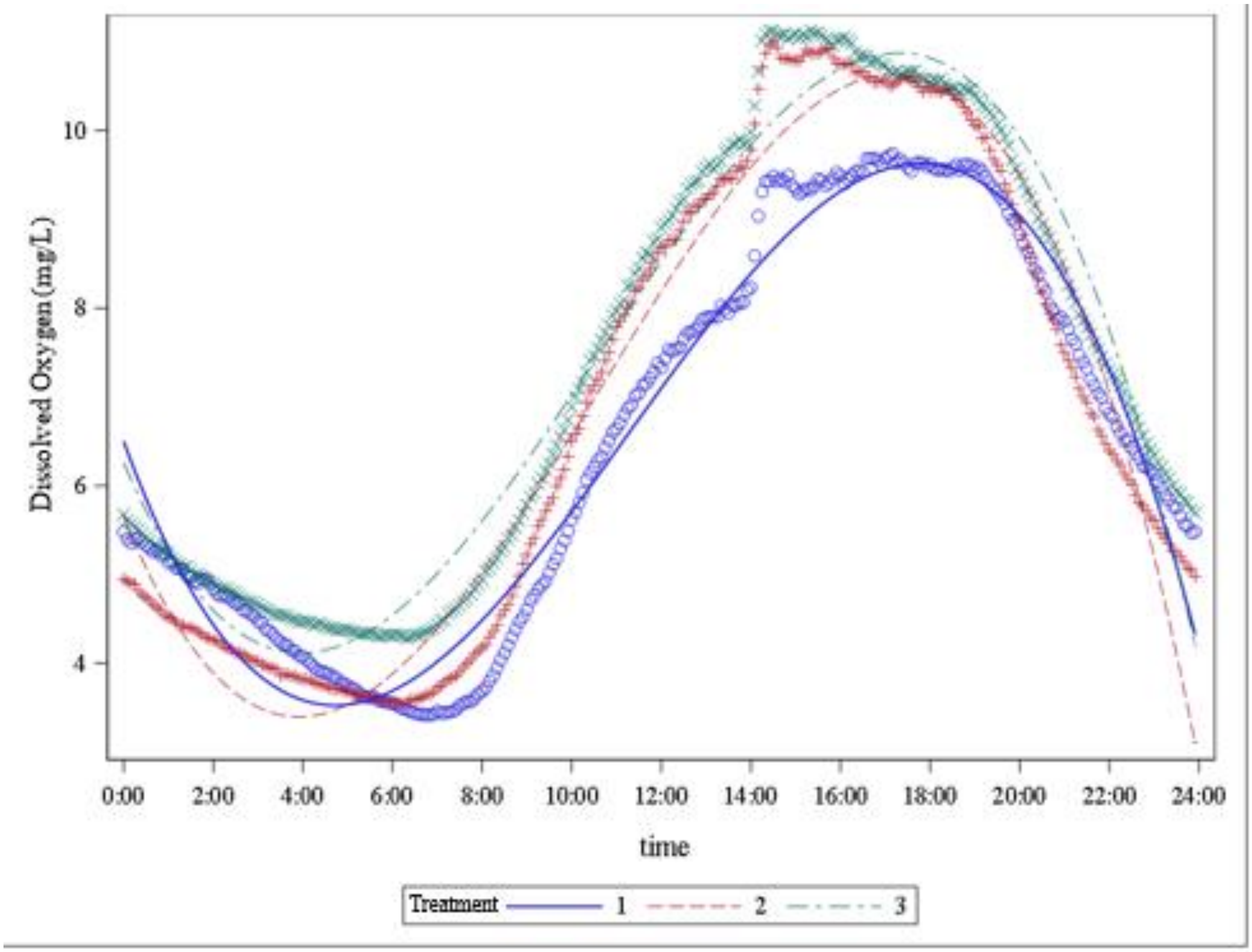
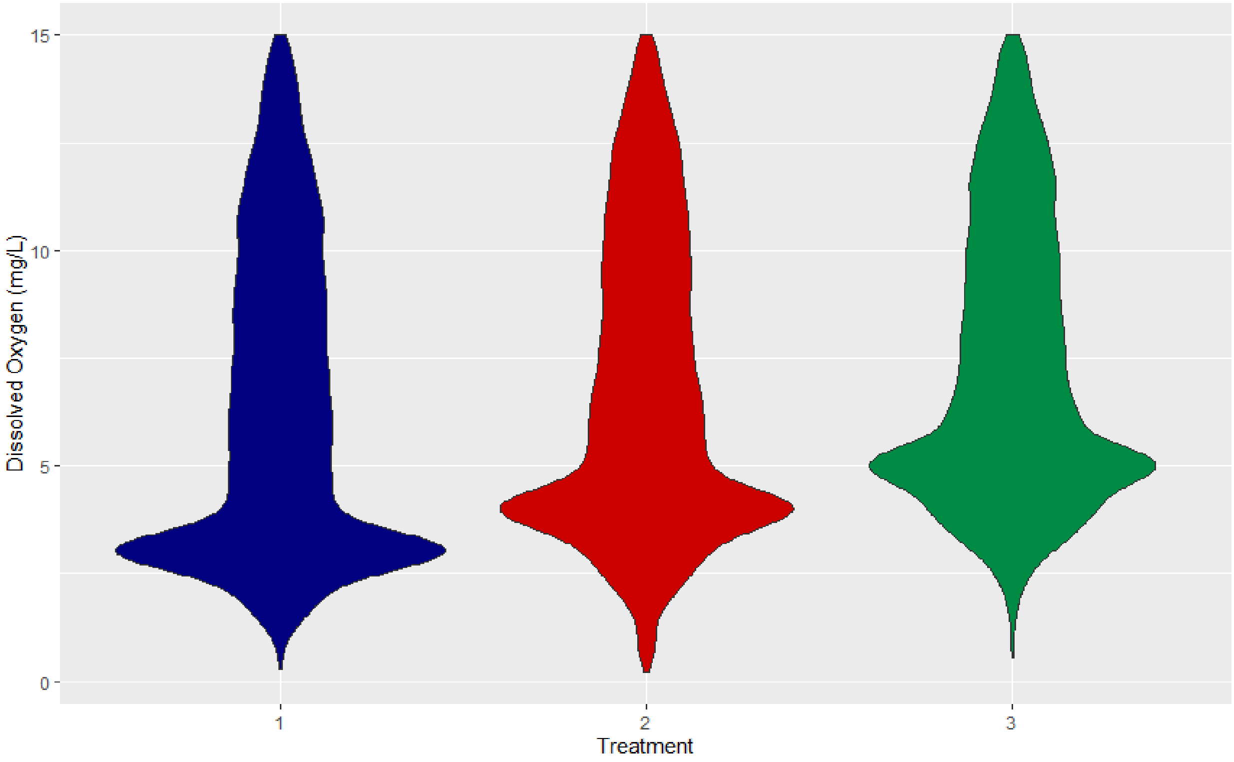


FIGURE 4. DO frequency distribution for three different automatic aeration setpoints in five replicate ponds, for 11 weeks (measurements were recorded every 5 minutes) of Pacific white shrimp (L. vannamei) production. Juveniles (0.03 g, 25 shrimp/m^2) were stocked in 16 ponds of 0.1 ha.



below that plateau also increase as the treatment increases. This can be related to the decreasing efficiency of aerators when using higher lower set points.

3.2 Effects on shrimp performance

3.2.1 Effects on production parameters

At the end of the 11-week trial, no significant difference was found for any production parameters except for electrical use and cost, and all parameters were found to be within acceptable ranges (Mohanty et al., 2017) for profitable production (Table 2). Shrimp final weight was approximately 33 g for all treatments. Growth rate ranged between 2.92-2.95 g/week for all treatments. Final density was 25.55, 24,77, and 22.54 shrimp/m^2 for the 2.5 mg/l, 3.5 mg/L, and 4.5 mg/L treatments, respectively. The average survival rate ranged from 89-100% across all experimental treatments. Feed conversion ratio was maintained between 1.0-1.1 across all treatments. Total yield per hectare was 8,505 8,360 and 7,575 kg/ha for the 2.5 mg/L, 3.5 mg/L, and 4.5 mg/L treatments, respectively. Apparent net protein retention and phosphorus retention were not significantly different and were found to be withing the usual reported values (Strebel et al., 2023; Weldon et al., 2021). Electrical use increased significantly as the set point increased. Figure 5 shows the increasing trend in electrical use and cost for the whole production cycle. Feed cost was maintained at around $12,000/ha for each treatment. Mean shrimp production value per ha decreased as the set point for automatic aeration increased, albeit this trend was not statistically significant. Mean partial income also decreased as the set point increased but did not show any significant difference.

Weekly growth rate was set side by side with average weight and weekly feed input for easier comprehension (Figures 6 and 7). It is noticeable that growth rates of 5 grams were recorded after four weeks of production and the set daily feeding limit of 160 kg/ha was reached after 8 weeks

TABLE 2. Production results of Pacific white shrimp (*Litopenaus vannamei)* reared with three automatic aeration activation set points with incremental lower limits under semi-intensive conditions in 16 outdoor ponds (0.1 ha) over an 11-week period.

Treatment	2.5 mg/L[1]	3.5 mg/L[2]	4.5 mg/L[1]	PSE[3]	p-value[4]
Individual Weight (g)	33.30	33.81	33.64	0.595	0.835
Weight Gain (g)	850.21	835.68	757.24	40.931	0.238
Growth rate (g/week)	2.92	2.97	2.95	0.079	0.813
Final density(shrimp/m^2)	25.55	24.77	22.54	1.259	0.222
Survival (%)	101.70	97.90	89.65	5.087	0.235
Yield (kg/ha)	8,505.2	8,360	7,575.5	409.305	0.252
Feed Conversion Ratio	1.05	1.03	1.12	0.073	0.656
Apparent Net Protein Retention (%)	51.41	51.41	47.23	0.912	0.525
Phosphorus Retention (%)	28.01	27.79	25.72	0.709	0.569
Electrical Use (kWh/ha)	10,960.33^{c}	14,610.6^{b}	18,610^{a}	2.687	<0.001
Feed cost/					
($)/kg of shrimp	1.49	1.48	1.59	0.168	0.689
($)/ha	12,665.4	12,354.8	12,083.2	2.006	0.455
Shrimp value ($/ha)	75,682	74,605	69,376	335.613	0.361
Partial Income ($/ha)	61,639	60,473	55,047	339.813	0.344

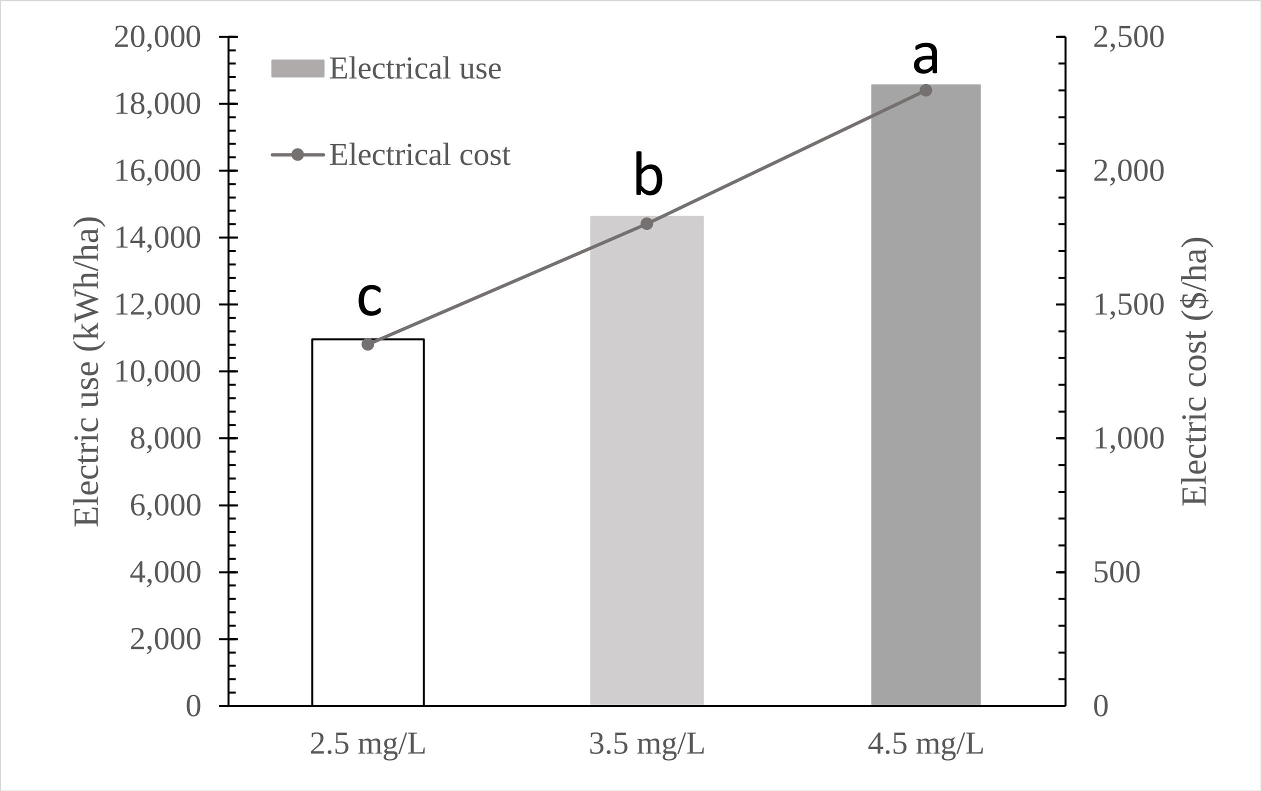
[1] N=5

[2] N=3

[3] PSE: Pooled Standard Error

[4] One-way ANOVA, Means not sharing any letter are significantly different by the Tukey's HSD-test at the 5% level of significance.

FIGURE 5. Average electric use and total cost for11 weeks of Pacific white shrimp (*Litopenaus vannamei)* production in ponds with three different set points for automatic aeration activation. Juveniles (0.03 g, 25 shrimp/m^2) were stocked in 16 ponds (0.1 ha).



of production and maintained for the rest of the cycle. These values are considered good in any commercial production setting. Lastly, the proportion of the shrimp's size class distribution was calculated. Figure 8 shows that up to 80% of the shrimp were 11-15 count per pound and around 20% were 16-20 count per pound. This figure also shows the total produce value compared to the biomass of each treatment, indicating that 3.5mg/L treatment had the highest crop value. These values were not significantly different from each other (Table 2).

3.2.2 Effects on shrimp physiology

Whole-body proximate analysis results are shown in Table 3. The moisture values ranged from 59-60% for all samples. Dry matter was between 27-29%. Whole-body analysis showed significant difference in phosphorus content, showing that phosphorus decreased as aeration increased. There were no significant differences in protein, fat, ash and sulfur, potassium, magnesium, calcium, sodium, iron, manganese, copper, or zinc between treatments. Protein values ranged from 74-77%.

Hemolymph composition showed no significant difference among treatments except for urea nitrogen, which was significantly higher in the 4.5 than 3.5 mg/L treatment (Table 4). Glucose had a decreasing pattern as the set point in each treatment increased. Potassium and total protein decreased as the aeration setpoint increased. Although these values were not significantly different from each other they could appear to be an indicator of further effects of hypoxia exposure.

TABLE 3. Whole-body composition for Pacific white shrimp (*Litopenaus vannamei)* reared in 16 ponds (0.1 ha) with three different set points for automatic aeration activation for 11 weeks. Samples were analyzed by Midwest Laboratories (Omaha, NE, USA) and all values are presented as means on a dry weight basis.

	2.5mg/L[1]	3.5mg/L[1]	4.5mg/L[1]	*PSE*[2]	P-value[3]
Moisture (%)[4]	60.81	56.88	59.01	0.279	0.2940
Protein (%)	74.97	77.94	74.94	0.977	0.0735
Fat (%)	10.58	9.43	10.35	0.508	0.2592
Ash (%)	11.65	11.94	12.10	0.528	0.8153
Sulfur (%)	0.78	0.79	0.76	0.019	0.4683
Phosphorus (%)	1.35^{a}	1.31^{ab}	1.26^{b}	0.023	0.0431
Potassium (%)	1.22	1.24	1.16	0.038	0.3916
Magnesium (%)	0.28	0.28	0.26	0.010	0.3635
Calcium (%)	2.24	2.41	2.08	0.168	0.4140
Sodium (%)	0.72	0.76	0.70	0.022	0.1479
Iron (ppm)	124.0	131.8	160.8	29.575	0.6448
Manganese (ppm)	6.23	8.40	4.96	1.672	0.3664
Copper (ppm)	87.68	81.94	88.70	2.175	0.0936
Zinc (ppm)	66.58	68.12	65.92	1.315	0.4957

[1] N=5

[2] PSE: Pooled Standard Error

[3] One-way ANOVA, Means not sharing any letter are significantly different by the Tukey's HSD-test at the 5% level of significance.

[4] Reported on as is basis.

TABLE 4. Hemolymph physiology mean values of Pacific white shrimp (*Litopenaus vannamei)* reared in 16 ponds (0.1 ha) with three different set points for automatic aeration activation for 11 weeks.

	2.5mg/L[1]	3.5mg/L[2]	4.5mg/L[1]	*PSE*[3]	P-value[4]
Alkaline phosphatase (U/L)	31.96	30.23	49.62	9.470	0.2886
Alanine aminotransferase (U/L)	218.14	177.9	182.71	24.970	0.4367
Amylase (U/L)	456.57	473.83	477.38	23.604	0.7636
Total Bilirubin (mg/dL)	0.597	0.547	0.574	0.039	0.6901
Urea Nitrogen (mg/dL)	3.58^{ab}	2.06^{b}	4.54^{a}	0.535	0.0334
Calcium (mg/dL)	30.51	26.94	26.93	1.659	0.2035
Creatinine (mg/dL)	0.99	0.68	1.04	0.142	0.2686
Glucose (mmol/dL)	51.94	39.1	37.93	6.989	0.2635
Potassium (mmol/dL)	12.42	11.94	11.73	0.585	0.6385
Total protein (g/dL)	15.09	14.56	13.90	0.663	0.3924

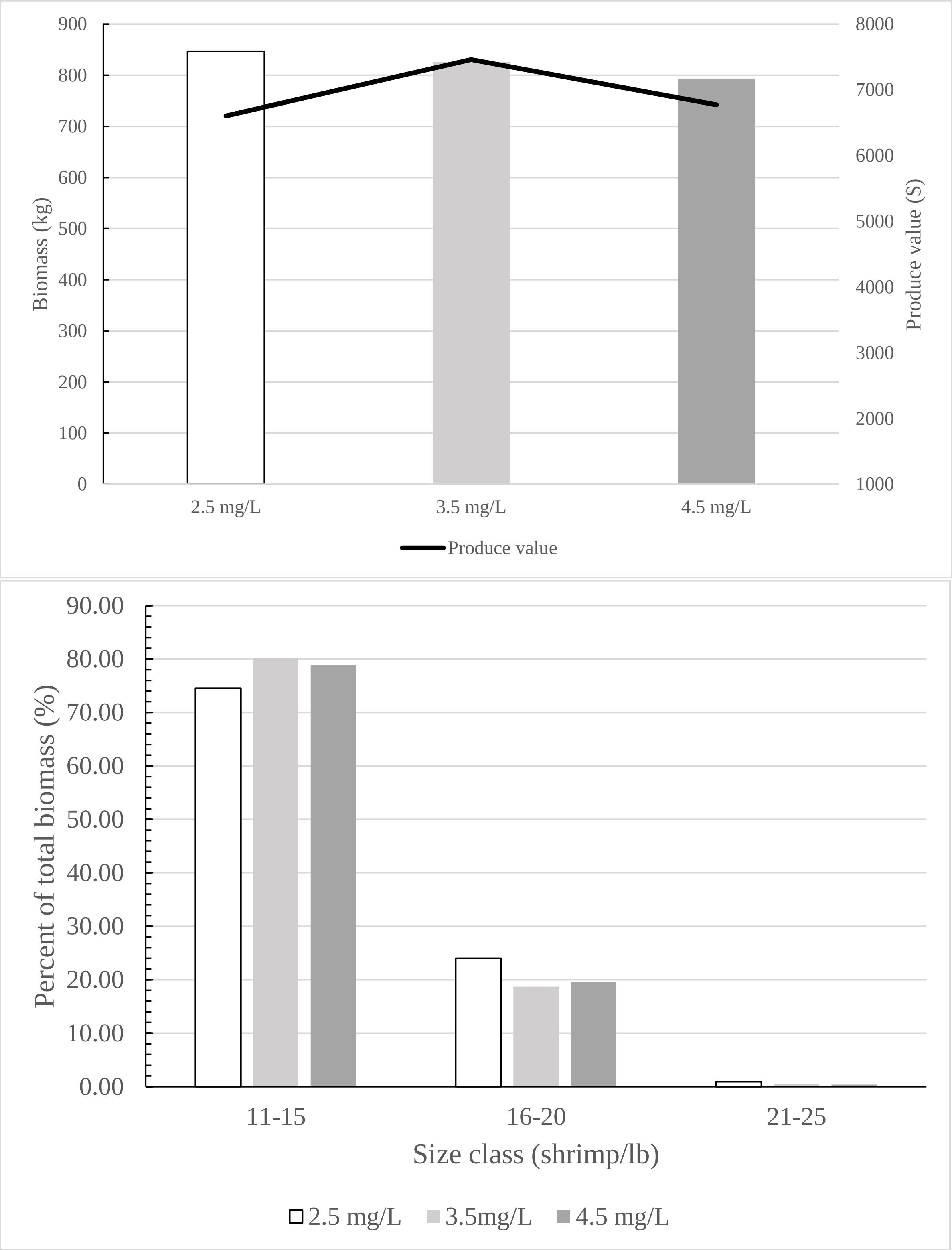
[1] N=5

[2] N=3

[3] PSE: Pooled Standard Error

[4] One-way ANOVA, Means not sharing any letter are significantly different by the Tukey's HSD-test at the 5% level of significance.

FIGURE 6. Size class distribution, biomass and produce value of Pacific white shrimp (*Litopenaus vannamei)* reared with three automatic aeration activation set points with incrementally lower limits under semi-intensive conditions in 16 outdoor ponds (0.1 ha) over an 11-week period.



4 Discussion

There are a considerable number of studies across aquaculture species looking at effects of aeration regimes. Most conclude the increased use of aeration is beneficial to production (Ruiz-Velazco et al., 2010; Boyd et al., 2018; Wyban et al., n.d.; Bott et al. 2015; Hegde et al. 2022). However, as aeration capacity is increased there is a need for increased capital and increased variable costs associated with the extra expense to run the aerators. With regards to shrimp, there are few publications in the literature that have explored this issue. Most researchers directly or indirectly cite the conclusions drawn from McGraw et al. (2001), who showed that lower thresholds for initiating aeration resulted in lower shrimp survival rates and yields. Overall survival rates were 40-60% for all aeration thresholds. The lowest treatment in this study is referred to as 15% saturation, which translates to ~1.1 mg/L (calculated based on historic temperatures and salinity of around 13ppt). This is lower than what most literature label as lethal for aquaculture species produced in semi-intensive ponds and would not be used as a lower threshold in low tolerant species. Individual growth was not affected by aeration threshold.

Martinez-Cordova et al., (1998) evaluated different aeration rates with treatments consisting of aeration for 0, 6, 12 and 24 hours per day. They found significant differences in yield and survival, with an average of 40-60% between treatments. A similar study testing the same aeration treatments (Martinez-Cordova, et al., 1998) but with Yellowleg shrimp (*Penaeus californiensis*) showed differences in morning DO, ammonia, and organic sediment, but no significant effects on growth and survival parameters were observed among treatments.

Using these previous studies as examples, we can see that most conclusions are drawn from results that are not the most desirable. To better understand these results for any given study, it is important to understand all the different physical, chemical, and biological factors and processes that may alter he availability of DO in the water. For starters, when considering DO in an aquaculture production system, the most important thing to consider would be feed input, as it will directly and indirectly becomes waste in the pond that requires oxidation to be decomposed. Hopkins et al. (1991) stated that the required aeration at a given stocking density can be pre-determined by also determining a maximum daily feed amount. Having a daily feed limit in this trial, and the fact that the ponds were only fed during high DO conditions, may be a possible reason DO crashes were prevented. Subsequently, mortality due to low DO was also reduced.

Most studies also report having higher amounts of ammonia and organic matter in lower aeration treatments and less aerated/feeding areas within the same pond (Martinez-Cordova et al., 1998; Delgado et al., 2002; Santa & Vinatea, 2007). This is because sedimentation may exceed decomposition on account of less DO availability, resulting in an accumulation of organic matter at the bottom of the pond which could contribute to higher ammonia concentrations (Steeby et al., 2004). Organic sediments were not measured in this study, and ammonia values did not show any significant differences among treatments. Since ponds bottoms were tilled before they were filled prior to stocking the experiment, it is possible that the presence of oxygen at the bottom combined with a set daily feed limit, prevented any major accumulations of organic matter in ponds with lower aeration treatments. This may also help explain the lack of differences in TAN among treatments.

After sediment, the water column is considered the second major consumer of oxygen in outdoor semi-intensive production ponds. Organisms that make up the water column are primarily zooplankton and phytoplankton. In shrimp production ponds, phytoplankton are typically predominant. During the day, phytoplankton help produce oxygen in the water during through photosynthesis. However, their presence can also be counterproductive at night (or on cloudy days) due to their respiration rate. Microalgae concentrations that are too high or too low concentrations of microalgae can be a cause of insufficient DO in ponds (<2mg/L) (Smith & Piedrahita, 1988; Drapcho & Brune, 2000). During the production cycle, secchi disk depth was measured weekly to monitor natural productivity, and to prevent eutrophication a partial water exchange was performed a week before termination. Regarding growth parameters, there's no literature that supports that aeration would directly affect growth. However, it is known that low DO stressful conditions could potentially slow down growth of the shrimp, as the organism puts energy into their immune response due to stress induction (Seidman & Lawrence, 1985; Nonwachai et al., 2011). Nonetheless, these are a result of long-term exposure to hypoxia under laboratory conditions. Zhang et al. (2006) stated that *L. vannamei* cultured at optimum conditions could withstand hypoxia for short terms of time. Using these points to interpret the results from this trial, it could be said that other than the relatively low DO conditions the shrimp were exposed to during the mornings of the diurnal cycle of the ponds that were shown to be significantly different conditions (Figure 3), the shrimp were maintained under acceptable conditions for culture during the rest of the production cycle. For that reason, it makes sense that no biological differences were found between any growth and general production parameters.

The weekly growth rate of shrimp revealed a solid performance. During the trial 5g/week of growth was the highest recorded, and a mean of 3g per week of growth was achieved across all the treatments. This is likely attributed to good quality genetics and the use of an automated feeding system. Nevertheless, other factors may have also contributed to a similarly high growth rate among treatments. For instance, the use of a daily feed input limit could have potentially limited the treatments from showing differences. Figure 6 shows weekly feed input and growth rate per treatment. At week 8, all ponds were reaching the daily feed limit while a distinguished decline in

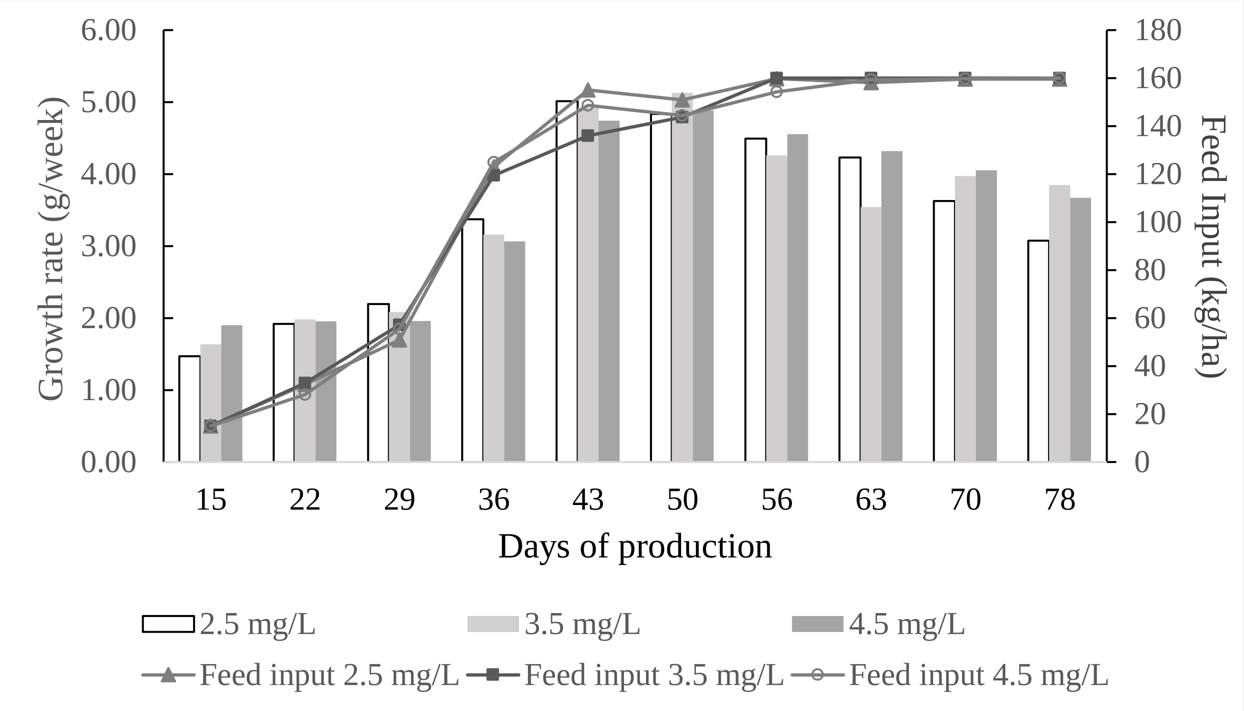


FIGURE 7. Growth rate of shrimp and feed inputs expressed in weekly intervals during 11 weeks of pond production of Pacific white shrimp (*Litopenaus vannamei)* with three different set points for automatic aeration activation. Juveniles (0.03 g, 25 shrimp/m^2) were stocked in 16 ponds (0.1 ha).

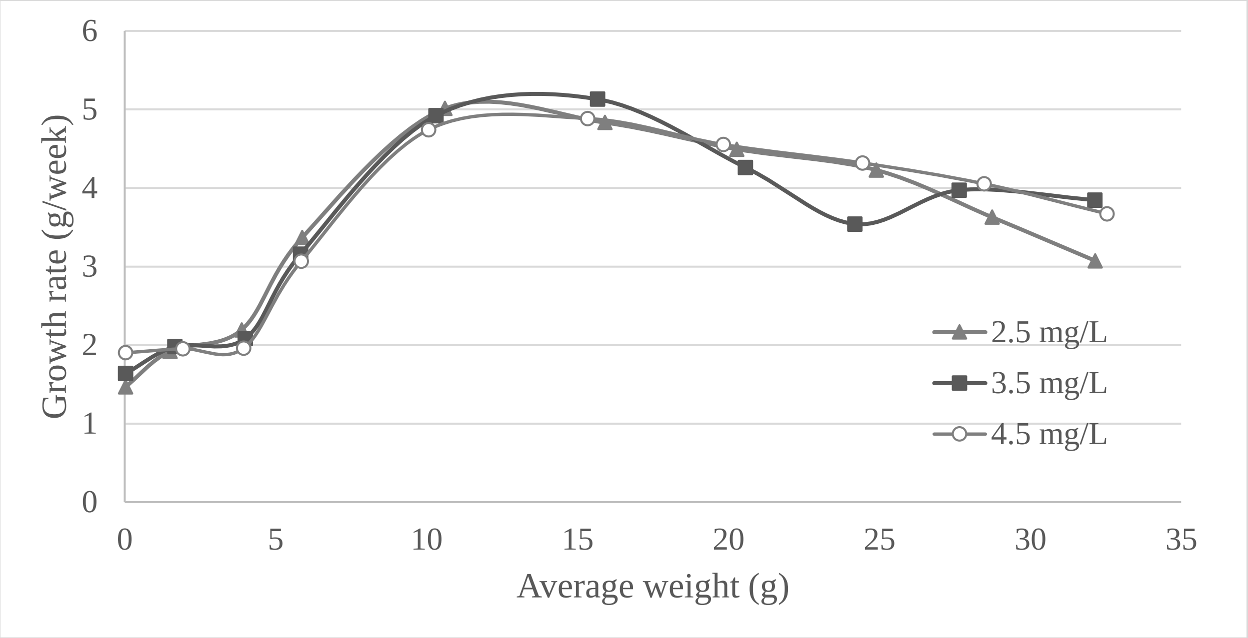
growth rate was also observed. Another factor could be the actual size of the shrimp, which has been shown to have effects on growth rate (Lee & Lawrence, 1985), in other words as the size of the shrimp increases the growth rate decreases.

Figure 7 demonstrates a comparison between the average weight and the weekly growth rate of shrimp. Growth rate jump started after shrimp attained a size of 5 g (which is also when on demand feeding was started) and slowed down after shrimp reached an average of 15 g. Chen et al. (1985) confirmed that effects on growth rate are more likely to be perceived in smaller size shrimp. On that account, if aeration time were to have any impact on growth under this trial's conditions it would have been noticeable in this period. This outcome then suggests that growth rate of shrimp is not susceptible to changes in aeration.

When talking about production parameters, general and variable costs are essential aspects to consider. Since electricity use and cost were the only production variable to have dissimilarities, it's important to discuss its implications. Boyd & McNevin (2021) stated that often the installed aerator capacity and duration of aerator operation exceed what is required in most shrimp farming operations, specially at the initial stages of grow out. Aeration costs could be considerably lowered in most situations by reducing aeration capacity and run times. Partial income for this study, although not statistically different, showed a clear increasing trend as the aeration rate decreased. This is directly correlated with the electrical use for each treatment.

Different DO concentrations can affect crude protein, lipid and ash content (Abdel-Tawwab et al., 2015). The treatment means for these specific variables in the current study were not significantly different, suggesting that there was no effect of aeration setpoint on shrimp whole-body composition. Yet phosphorus content did show difference between the lowest and highest treatment. A hypoxic acclimation mechanism has been described for other aquatic species (such as

FIGURE 8. Average weight and weekly growth rate of Pacific white shrimp (*Litopenaus vannamei)* reared with three automatic aeration activation set points with incremental lower limits under semi-intensive conditions in 16 outdoor ponds (0.1 ha) over an 11-week period.



fish and eels), where guanosine triphosphate (GTP) plays an essential role. The phosphates bind to specific sites of the organisms central cavity and stabilizes the structure in the deoxy conditions(Weber et al., 1976; Wells, 2009). This corroborates the fact that lower aeration treatments developed higher phosphorus content compared to the highest treatment and could be a possible explanation of what may have taken place.

Various components associated with hemolymph osmolality may go through changes when exposed to hypoxic conditions. Cheng et al. (2002) reported notable depressions in freshwater giant prawn (*Macrobrachium rosenbergii*) in parameters such as sodium, potassium and chlorine within 24 hour of low DO conditions. Similar to the growth variables examined, the lack of any measurable changes in hemolymph variables would likely imply the shrimp did not suffer from hypoxemia for a continuous period that was long enough to cause effects on osmoregulation.

5. Conclusion

Results of this study suggest that a lower aeration setpoint threshold of 2.5mg/L would pose the most economical strategy to achieve minimal costs and higher income compared to the other two aeration setpoints examined under the production conditions in this trial. However, this strategy carries with it a higher degree of risk that needs to be considered, particularly as it relates to low DO events, such water quality, and feed management. The results of this study do not aim to indicate that the use of 2.5 mg/L as an automatic aeration trigger would be beneficial in all pond systems. However, it was observed that under optimal culture conditions, the need for aeration can be significantly reduced, resulting in cost savings related to aerator usage.

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