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Estimating the Nanobubble Aerated System and Stocking Density Effects on Oxygen Consumption and Survival of *Litopenaeus vannamei* (Boone, 1931) Postlarvae 8 Using Receiver Operating Characteristic (ROC) Analysis

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***Abstract*— *Litopenaeus vannamei* postlarvae (PL) 8 were usually stocked at high densities, affecting the shrimp and water quality. This condition makes stocking density becomes a challenge in shrimp cultures, and one of the solutions for this is using a nanobubble aerated system. Meanwhile, there is a lack of robust studies estimating the nanobubble and stocking density effects on shrimp survival and water quality. This study used different stocking densities of 200, 400, and 600 postlarvae/L under nanobubble treatment and control (without nanobubble) to culture *L. vannamei* PL 8 and were assessed using Receiver Operating Characteristic (ROC) analysis. The ROC showed that the values of area Under the Curve (AUC) for dissolved oxygen (DO), survival rates, and oxygen consumption were 0.826 (95%CI: 0.598-1.000), 0.722 (95%CI: 0.47-0.794), and 0.576 (95%CI: 0.28-0.873), respectively. Considering these AUC values, it can be concluded that nanobubble treatment has the possibility to affect DO and shrimp survival, although it is not the same for shrimp oxygen consumption because it has the lowest AUC values. The optimum values for DO, survival rates, and oxygen consumption of shrimp under nanobubble treatment were observed at densities of 400 postlarvae/L. The survival and oxygen consumption of *L. vannamei* PL 8 at this density were 96.83% (95%CI: 95.2-98.4) and 0.52 mg/g/h (95%CI: 0.46-0.57). Meanwhile, water DO, temperature, and EC were 4.08 mg/L (95%CI: 2.84-5.32), 27.27°C (95%CI: 27.30-27.40), and 1.43 mS/cm (95%CI: 1.40-1.46), respectively. Nanobubble has maintained DO and temperature in the suitable range for *L. vannamei* PL8 survival.**

***Keywords*— AUC; nanobubble; oxygen; postlarvae; ROC; survival**

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I. INTRODUCTION

Oxygen consumption and survival of shrimps are dependant on biological and environmental factors (such as temperature and salinity), as well as stocking densities [1]-[2]. For shrimps, oxygen consumption is a respiratory adaptation and can be used as a representative indicator of the crustacean physiological state, active movement, and routine metabolism in different nutritional or environmental situations [3]. *Litopenaeus vannamei* (Boone, 1931) postlarvae was known to have oxygen consumption that increases as the temperature rises with the oxygen consumption ranging from 0.0060 mg/g/min at 25 ppt and 20°C to 0.0183 mg/g/min at 35 ppt and 32°C [4]. Moreover, Rosas *et al.* [5] found that oxygen consumption increased in accordance with salinity reduction with high values in 5 ppt and lower values in 30 ppt.

Survival of *L. vannamei* were also influenced by numerous environmental factors. According to Bermudes-Lizárraga *et al* [6], postlarvae survivals were significantly influenced by salinity and temperature. In addition, the interaction between both factors with ultimate postlarvae survival was obtained at 30°C and 30ppt followed by 30 and 35°C at 25ppt. Postlarvae survivals were also related to suspended solids accumulation and water dissolved oxygen that may affect the growth performance of shrimp culture, where Gaona *et al* [7] reported that postlarvae survivals were at DO levels of 5.85, 5.76, 5.68 ppm and three suspended solids at low, medium, and high, were 94.79%, 84.17%, and 20.73%, respectively.

Stocking density was also an important factor influencing oxygen consumption and survival of shrimps [8], [9]. An increased stocking density will reduce the DO in water and increase oxygen consumption. Furthermore, an increase in stocking density will also reduce the survival rate of shrimps. Based on the study by Arambul-Muñoz *et al.* [10], the survival rate of shrimp postlarvae 15 weighed 0.001 g at stocking densities of 100, 300, 500, 700, and 900 shrimps/m^3 were 85%, 80%, 75%, 60%, and 50%, respectively.

Nanobubble technology has currently been used to manage the DO and oxygen consumption issues related to shrimps stocking densities. The principle of nanobubble is changing and modifying aquaculture systems to increase the concentration of DO in cultivation water by supplying DO to the water in an ultra-small liquid gas bubble [11], [12]. Nanobubble can effectively improve the DO in water and maintain DO for a longer period. Using nanobubble treatment, DO levels increased significantly from 6 to 31 ppm [13]. There was an increase of DO from 6.5 to 25.0 ppm in cultivation water using nanobubble, as observed by Mahasri *et al.* [14]. According to Rahmawati *et al.* [15], nanobubble can provide the oxygen requirements in 50 m^2 indoor raceway ponds for 81 days with shrimp postlarvae 10 stocking density weighed 0.09 g, with the area being set to 680 shrimps/m^3.

In fishery study, Receiver Operating Characteristic (ROC) has been used as a robust method to assess how an external variable can affect aquatic organisms. In the aquatic field, ROC and Area Under the Curve (AUC) have been used to evaluate the potential distribution of high-risk aquatic invasive species [16]. In Indonesia, Siregar *et al.* [17] have used ROC analysis to assess the potential fishing zones for yellowfin tuna (*Thunnus albacares*) based on yellowfin's environmental variables. Yuniarti *et al.* [18] have used ROC analysis and the AUC to evaluate various parameters for aquaculture in an intricate tropical lake system. Meanwhile, ROC assessment study on shrimp fishery and its biological aspect remains limited.

Despite the growing interest in nanobubble uses, information emphasizing the oxygen consumption and survival rate of *L. vannamei* PL 8 in various stocking densities remains limited, especially the nanobubble aerated system. This study was initially designed to assess the nanobubble and stocking density effects on water quality variables and the survival of *L. vannamei* PL 8 and determine the optimum *L. vannamei* PL 8 stocking density.

II. Materials and Method

A. Experimental Design

The experiments were conducted in August 2020 at a hatchery in Serang, Banten Province, Indonesia (Fig. 1), following the method used by Galang *et al.* [19] and Rahmawati *et al.* [15].

Two aeration treatments were used in this experiment, including nanobubble and aerator as the control, and it was conducted for 24 hours. *Litopenaeus vannamei* PL 8 with mean initial weight at 0.0024 g were transferred to the nanobubble and without nanobubble (control) tanks with three stocking densities, 200, 400, and 600 postlarvae/L. Five replications were assigned to each stocking density.

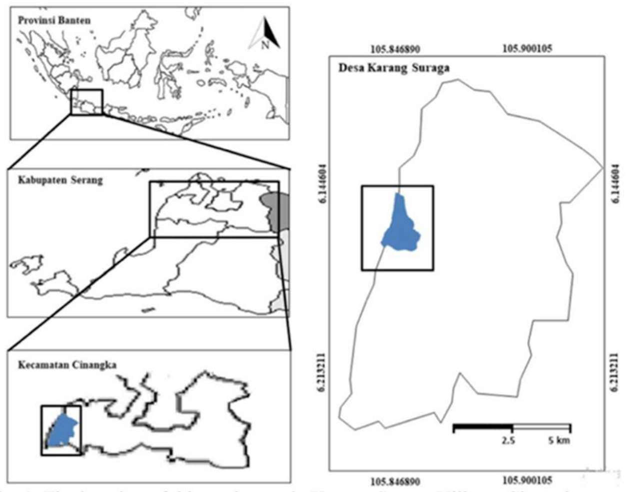

Fig. 1 The location of this study was in Karang Suraga Village, Cinangka District, Serang Regency, Banten Province, Indonesia.

B. Nanobubble Treatments

The nanobubble machinery NB S-2 with 2 horsepower developed by Nanobubble Karya Indonesia Ltd., South Tangerang, Indonesia, was used to generate oxygen bubbles with dissolved oxygen (DO) level equals 16 mg/L and bubble sizing of <200 nm. The nanobubble flow rates of water and oxygen were 60 L/min and 0.4 L/min, respectively, with 250 m3 per nanobubble unit coverage. Meanwhile, the nanobubble treatment was not applied for the control tank. Each nanobubble treatment and control tank was filled with *L. vannamei* PL 8 with a density of 200, 400, and 600 postlarvae/L.

C. Water Quality and Oxygen Consumption Measurements

The measured water quality variables include dissolved oxygen (DO), pH, temperature, and electrical conductivity (EC). The DO (mg/L) was measured using YSI 550A DO meter, pH and temperature (°C) using YSI pH100, and EC (mS/cm) using EZ 2 EC meter. The measurements of oxygen consumption (mg/g/h) followed [4] and [19].

D. Postlarvae Survival Measurements

Litopenaeus vannamei PL 8 survival rate was calculated by dividing the number of PL 8 that survived at the end of 24 hours by the initial PL 8 densities (200, 400, 600 postlarvae/L). The equation for postlarvae survival rate (SR) is as follows:

$$SR = \frac{Nt}{No} x100\% \qquad (1)$$

SR = PL 8 survival rates (%)
Nt = number of PL 8 that survived at the end of 24 hours
N0 = initial PL 8 (200, 400, 600 densities).

E. Statistical Analysis

The postlarvae survival (%) and oxygen consumption (mg/L) data at different treatments and stocking densities were analyzed using a one-way ANOVA and Tukey's test to observe a significant difference in each density within treatments [10], [20]. The differences in stocking densities were considered significant at 95%. Pearson's R [21] correlation analysis was used to test the correlation significance between stocking densities, water quality properties, *L. vannamei* PL 8 oxygen consumption, and survival rates. The Pearson's R value were ranged from 1 for very correlated to -1 for not correlated. The data were expressed as means ± standard error (SE) and 95% confidence interval.

F. Receiver Operating Characteristic (ROC)

ROC analysis was used to measure variables consisting dissolved oxygen (DO), survival rates, and oxygen consumption of *L. vannamei* PL 8. ROC analysis is an important test aiming to assess quantitative tests' accuracy or discrimination performance throughout the whole range of variables under experimental design [22]. ROC analysis may also serve to estimate the accuracy of multivariate probability scores to categorize variables as affected or unaffected by a given treatment, including control and nanobubble treatments. ROC is depicted as a curve and the results are measured based on the values of area Under the Curve (AUC). The AUC is the ranking approach to assess the treatment's performance, probability, and accuracy on the measured variables. The performance of the treatment is demonstrated by the high value of the AUC, in which the AUC's value of 0.5-0.7 is considered low, 0.7-0.9 as medium accuracy, and more than 0.9 indicates a high level of accuracy in measuring the treatment's effect [17].

III. Results and Discussion

A. Water Quality

Water quality variables including water DO, temperature, pH, and EC showed trend variations within different stocking densities between control and nanobubble treatments after 24 hours. DO in both control and nanobubble treatments were declining (Fig. 2), and the highest stocking density has the lowest DO. Despite the decline, DO in nanobubble treatments remained higher than DO in control treatments. Among nanobubble treatments, the DO level in stocking density of 200 postlarvae/L was higher compared to other treatments ($P<0.05$) (TABLE I). The highest DO in treatments was observed at 4.87 mg/L (95%CI: 4.51-5.23) for 200 postlarvae/L in nanobubble treatment, and the lowest was at 1.62 mg/L (95%CI: 1.48-1.76) for 600 postlarvae/L in the control treatment. Other water quality variables that showed declining trends were temperature (Fig. 3) and pH (Fig. 4), while EC fluctuated (Fig. 5). Temperature and EC showed differences among treatments and stocking densities ($P<0.05$), with temperature and EC values in nanobubble treatments being significantly lower than those in control treatments ($P<0.05$). The highest temperature in treatments was recorded at 28.32°C (95%CI: 28.20-28.40) for 200 postlarvae/L in control treatment, and the lowest was at 27.22°C (95%CI: 27.10-27.30) for 600 postlarvae/L in nanobubble treatment. While stocking density at 400 postlarvae/L in nanobubble treatment has the lowest EC value at 1.43 mS/cm (95%CI: 1.40-1.46), the highest EC value observed was at 1.58 mS/cm (95%CI: 1.57-1.59) for 200 postlarvae/L in the control treatment (TABLE I).

In terms of water quality variables in the control treatment (Fig. 8), it was found that there was a significantly positive Pearson's R correlation hold for DO vs. pH (R=0.56), and negative correlation hold for DO vs. density (R=-0.96) and pH vs. density (R=-0.53). In nanobubble treatment, significant positive Pearson's R correlation hold for temperature vs. pH (R=0.29), and negative correlation hold for DO vs. density (R=-0.53), pH vs. density (R=-0.43), and EC vs. temperature (R=-0.55).

B. Oxygen Consumption

The oxygen consumption trends for *L. vannamei* PL 8 among treatments and densities were reported in Fig. 6. Statistical test (Table I) indicates that there was a significant difference ($P<0.05$) between the mean of oxygen consumption of *L. vannamei* PL 8 exposed to nanobubble and control treatments. In nanobubble treatments, the oxygen consumption showed a decrease when density was increased. The oxygen consumption reduced from 0.96 mg/g/h (95%CI: 0.93-0.99) for 200 postlarvae/L to 0.37 mg/g/h (95%CI: 0.33-0.41) for 600 postlarvae/L. In contrast, in control treatment, the oxygen consumption increased from 0.49 mg/g/h (95%CI: 0.27-0.71) for 200 postlarvae/L to 0.51 mg/g/h (95%CI: 0.49-0.62) for 600 postlarvae/L.

In control treatment (Fig. 8), oxygen consumption has a slightly positive correlation with density (R=0.05) and several negative correlations were discovered with temperature (R=-0.46), EC (R=-0.25), and DO (R=-0.22). For nanobubble treatments, positive correlation of oxygen consumption was observed at DO (R=0.22), temperature (R=0.24), EC (R=0.16), and pH (R=0.53), and only a significant negative correlation with density (R=-0.94).

C. Survival Rates

Litopenaeus vannamei PL 8 survival rates showed downward trends when densities were increased (Fig. 7), even though it was not significant ($P>0.05$). In control treatments, survival rates decreased from 99.16% (95%CI: 98.6-99.8) for 400 postlarvae/L to 97.33% (95%CI: 96.5-98.1) for 600 postlarvae/L. Similarly, in nanobubble treatments, survival rates slightly decreased from 97.50% (95%CI: 96.4-98.6) for 200 postlarvae/L to 95.38% (95%CI: 92.1-98.7) for 600 postlarvae/L.

For survival rates in control treatment, negative correlation was observed for density (R=-0.11), temperature (R=-0.41), and pH (R=-0.11), while positive correlation was observed for oxygen consumption (R=0.44), DO (R=0.99), and EC (R=0.25). In nanobubble treatment, survival rate positive correlation holds for oxygen consumption (R=0.29), and DO (R=0.47), while negative correlation holds for density (R=-0.40), EC (R=-0.05), and temperature (R=-0.27).

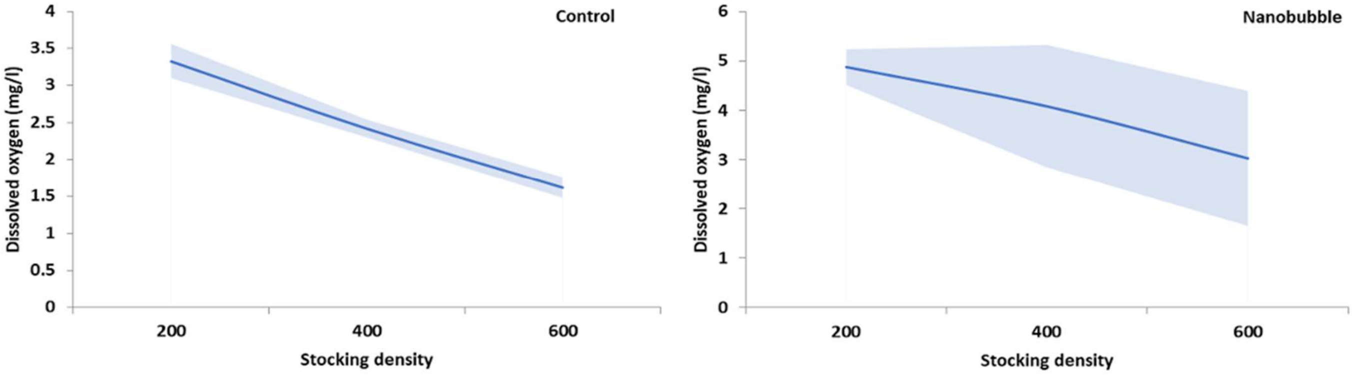

Fig. 2 Mean water dissolved oxygen (mg/L) data trends and 95% confidence interval shown by shaded areas for control and nanobubble treatments

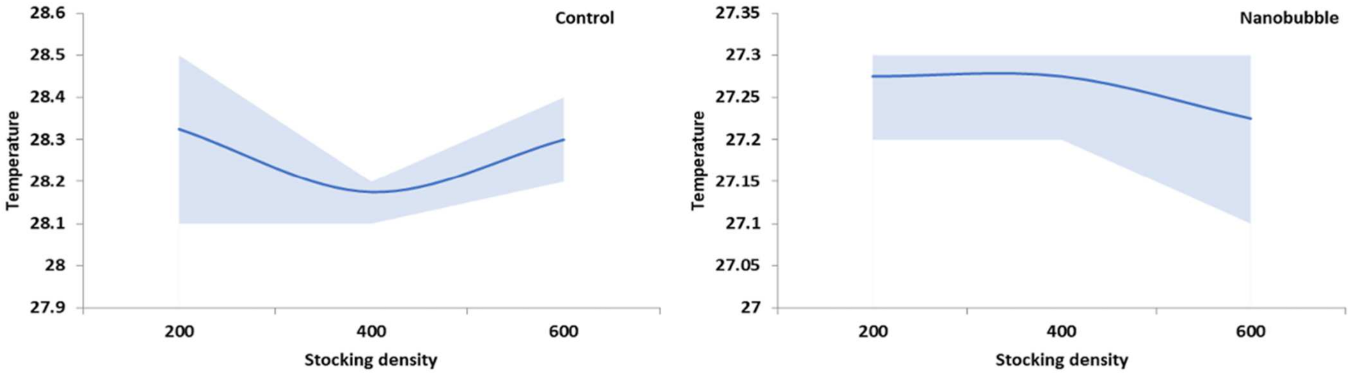

Fig. 3 Mean water temperature (°C) data trends and 95% confidence interval shown by shaded areas for control and nanobubble treatments

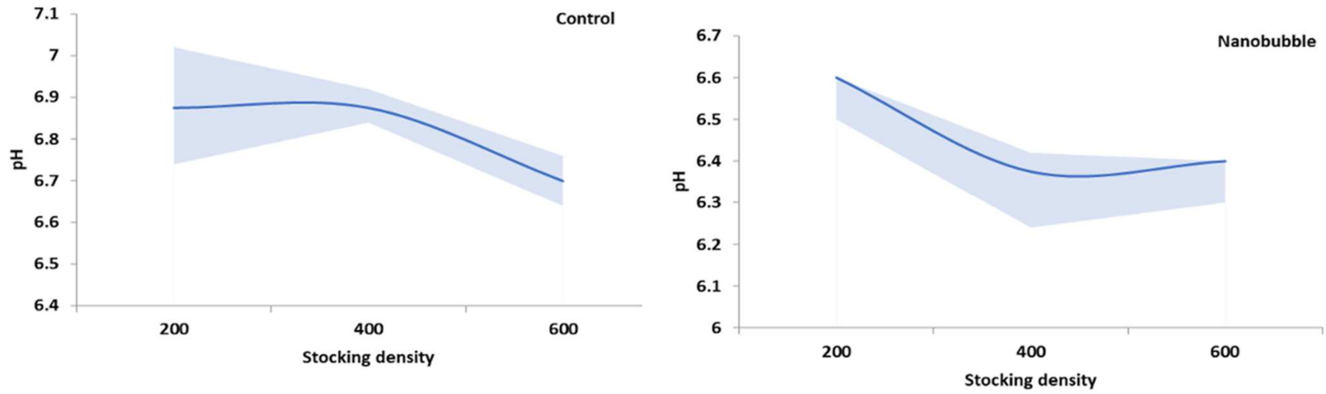

Fig. 4 Mean water pH data trends and 95% confidence interval shown by shaded areas for control and nanobubble treatments

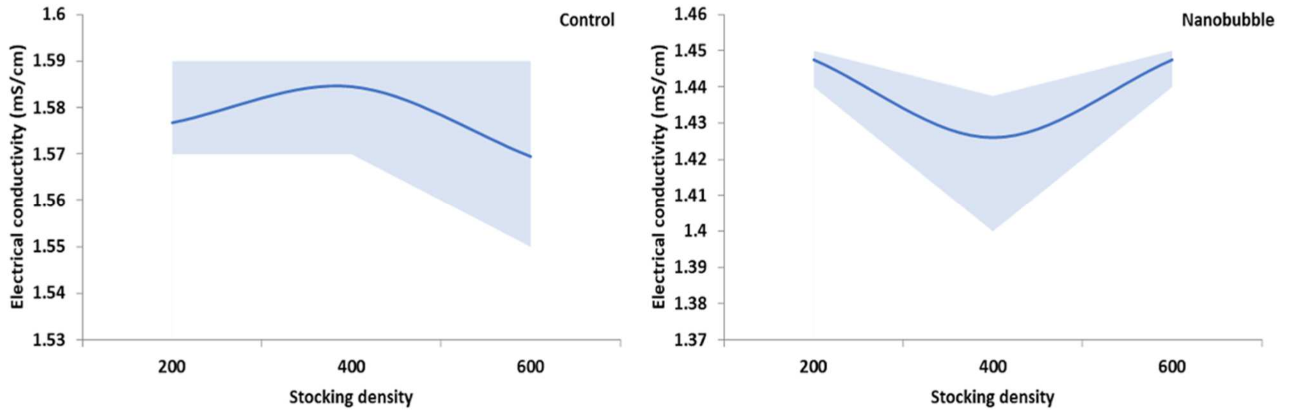

Fig. 5 Mean water electrical conductivity (mS/cm) data trends and 95% confidence interval shown by shaded areas for control and nanobubble treatments

TABLE I
MEAN ± STANDARD DEVIATION OF CONTROL AND NANOBUBBLE TREATMENTS IN EACH STOCKING DENSITY (200, 400, 600 POSTLARVAE/L)

Variables	Control			Nanobubble		
	200	400	600	200	400	600
Dissolved Oxygen (mg/L)*	3.32 ± 0.30[a]	2.42 ± 0.16[b]	1.62 ± 0.18[b]	4.87 ± 0.48[b]	4.08 ± 1.63[b]	3.02 ± 1.81[a]
Temperature (^{0}C)*	28.32 ± 0.22[a]	28.17 ± 0.05[a]	28.30 ± 0.11[a]	27.27 ± 0.05[b]	27.27 ± 0.09[b]	27.22 ± 0.09[b]
pH*	6.87 ± 0.19[a]	6.87 ± 0.05[a]	6.70 ± 0.08[a]	6.60 ± 0.00[a]	6.37 ± 0.32[b]	6.40 ± 0.00[b]
Electrical Conductivity (mS/cm) *	1.58 ± 0.01[a]	1.58 ± 0.02[a]	1.57 ± 0.02[a]	1.45 ± 0.00[b]	1.43 ± 0.05[b]	1.45 ± 0.00[b]
Oxygen Consumption (mg/g/h)*	0.49 ± 0.25[a]	0.53 ± 0.07[a]	0.51 ± 0.23[a]	0.96 ± 0.03[b]	0.52 ± 0.06[b]	0.37 ± 0.04[b]
Survival Rates (%)	97.66 ± 1.54[a]	99.16 ± 0.62[a]	97.33 ± 0.83[a]	97.50 ± 1.08[a]	96.83 ± 1.66[a]	95.38 ± 3.35[a]

Note *: significance of one-way ANOVA (P<0.05) to determine the effects of treatments and stocking densities on *L. vannamei* PL 8 and water quality variables.
[a,b]: means with different superscript letters in the same row indicate significant differences by Tukey test (P<0.05).

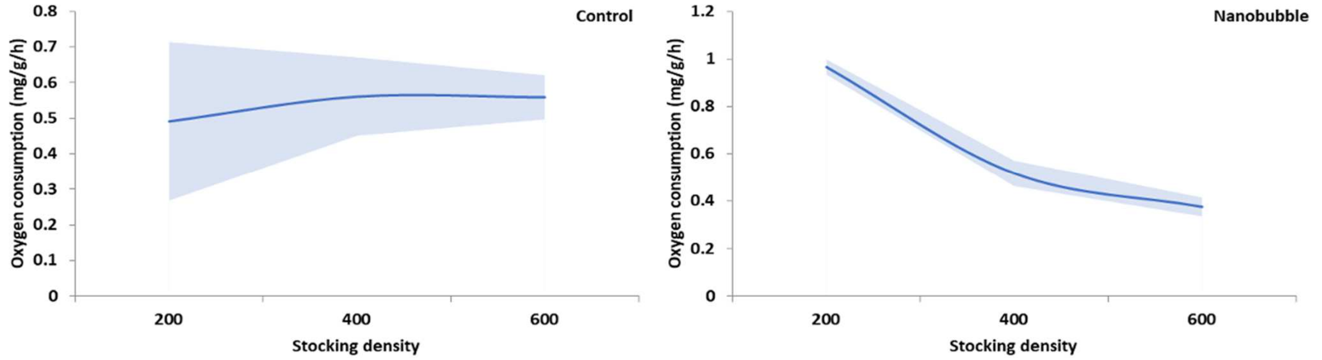

Fig. 6 Mean *L. vannamei* PL 8 oxygen consumption data trends and 95% confidence interval shown by shaded areas for control and nanobubble treatments

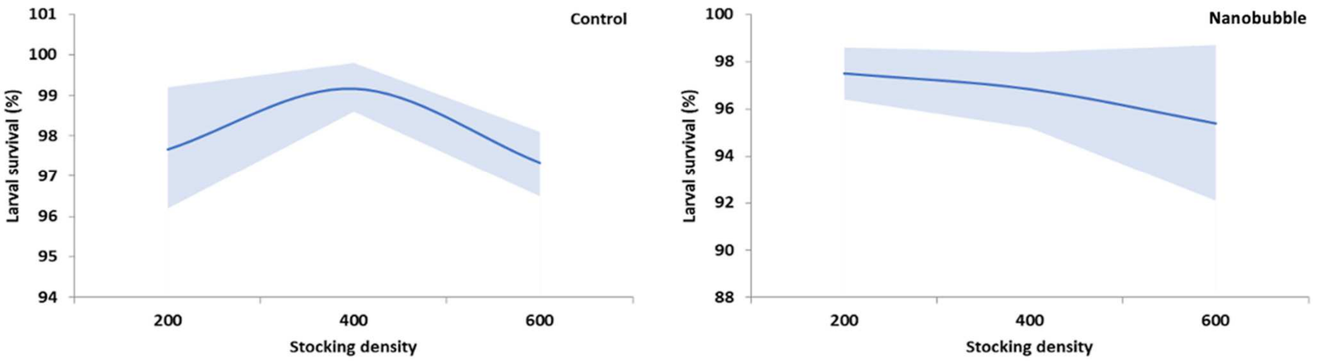

Fig. 7 Mean *L. vannamei* PL 8 survival rates (%) data trends and 95% confidence interval shown by shaded areas for control and nanobubble treatments

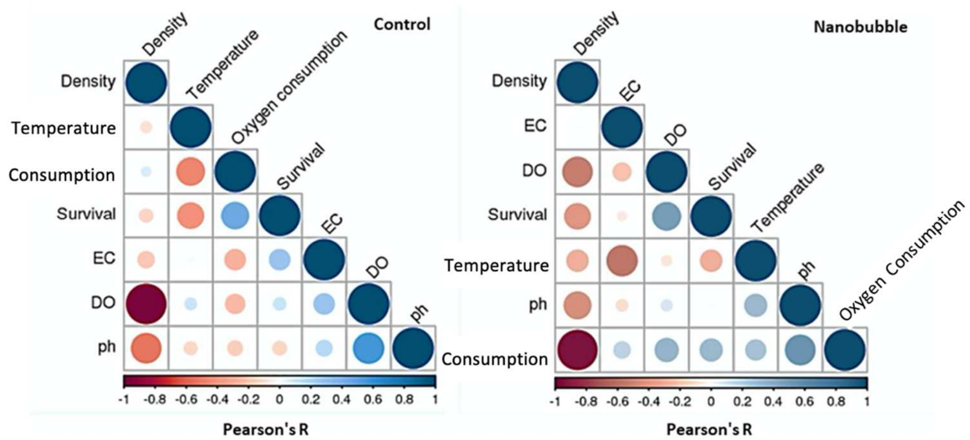

Fig. 8 Pearson's R correlation significance (-1 —1) between stocking densities, water quality properties, oxygen consumptions, and *L. vannamei* PL 8 survival rates for control and nanobubble treatments

D. Receiver Operating Characteristic

Fig. 9 shows the area Under the Curve (AUC) of Receiver Operating Characteristic (ROC) to estimate the probability of nanobubble treatment's effects on dissolved oxygen (DO), survival rates, and oxygen consumption of *L. vannamei* PL 8. The order of AUC according to measured variables was DO > survival rate > oxygen consumption. The DO variable has the highest AUC values followed by the survival rate, while the oxygen consumption variable has the lowest AUC value (TABEL II). The AUC for DO and survival rate were computed as 0.826 and 0.722, indicating adequate probability of nanobubble treatment effect on DO and survival rate of shrimps. In contrast, the AUC for oxygen consumption variable was 0.576 and this shows a low probability of nanobubble treatment effect on shrimps' oxygen consumption.

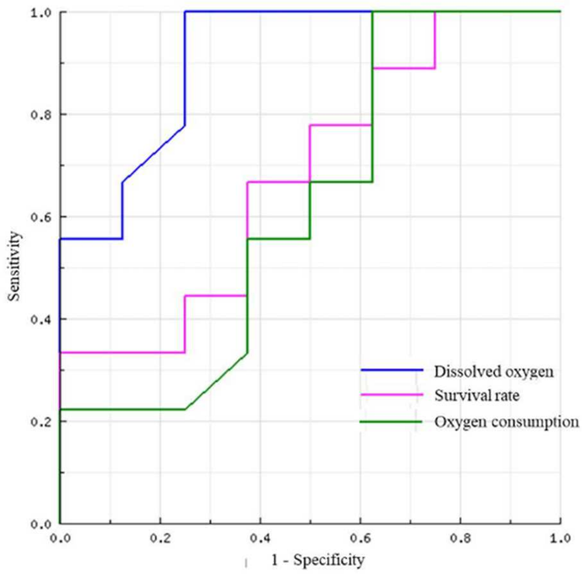

Fig. 9 Area Under the Curve (AUC) of ROC analysis to estimate the probability of nanobubble treatment effects on dissolved oxygen (DO), survival rates, and oxygen consumption of *L. vannamei* PL 8

TABLE II
VALUES OF AREA UNDER THE CURVE (AUC) OF ROC ANALYSIS (95%CI) FOR DISSOLVED OXYGEN (DO), SURVIVAL RATES, AND OXYGEN CONSUMPTION OF *L. VANNAMEI* PL 8 VARIABLES

Variables	AUC	95%CI
Dissolved oxygen	0.826	0.598-1.000
Oxygen consumption	0.576	0.28-0.873
Survival rates	0.722	0.47-0.794

The studies on shrimps' performances and water quality improvements have been reported in many literatures, and numerous treatments have been studied, including nanobubble systems. However, in the existing studies on nanobubble, the comprehensive data on the effects of stocking densities on shrimps and water quality variables are still limited [15], [19]. In addition, relationships among stocking density, shrimps, and water quality variables are not well researched. Respectively, water quality, oxygen consumption, and survival rate of particular *L. vannamei* PL 8 influenced by stocking density variations have been studied in this paper. In a recent study on shrimps, ROC analysis was used by [23] to evaluate environmental effects on the survival of *Penaeus monodon*. In this study, ROC analysis distinguished which variables can be potentially affected by nanobubble treatment.

The nanobubble and control treatments were showing a decline in DO. Despite the decline, DO in nanobubble treatment remained higher than in control. The negative correlation between density and DO was more significant in control than in nanobubble treatments. This indicates that nanobubble treatment with its stable oxygen inputs will minimize the negative effects of increasing density on DO reduction, since the DO was available and able to provide the oxygen required. A stable oxygen supply in nanobubble treatment was also reported by Rahmawati *et al* [15], where they found that nanobubble treatment has an advantage since it could maintain the stability of DO. The DO reduction due to an increase in density resulted from an increase in *L. vannamei* PL 8 individuals, biomass, and oxygen consumption [24]. However, increases in density and oxygen consumption did not reduce the DO as shown in nanobubble treatment since it had a positive correlation. There were also minimum effects of DO on density since nanobubble were able to maintain a high DO level for an extended period [25].

Water temperature recorded in this study showed that nanobubble treatment was lower than control and it was still within the range reported by other studies. The reported temperature in the other studies were between 28-33°C [26], 24-32°C [2], and 27.8-28.2°C [27]. The observed dynamics of water temperatures were related to chemical properties of water molecules, solar radiation, air temperature, and water temperature passing through the treatment units. Water consists of atoms and molecules of a mass of matter, to which energy is added, and vibrate faster, rapidly, and move slightly farther apart. As a result, the movement of atoms and molecules generates energy and heat content that raise the temperature over time.

The pH observed was influenced by density and negatively correlated, as reported by Legarda *et al* [28]. The decline in pH when density increased is most likely due to the respiration, CO_2 production, and organic matter degradation. EC in nanobubble treatment was lower than in control treatment and remained within the range recommended for shrimp cultures. The high EC values might indicate the presence of shrimp excreta and ionic substances released from biological decompositions of organic matter [29].

In this study, oxygen consumption of *L. vannamei* PL 8 (Fig. 6) agrees with results from other studies. Oxygen consumptions and temperature were observed higher in control treatments than nanobubble treatments. Ulaje *et al* [3] reported that oxygen consumption was known to have positive correlation with water temperature. Oxygen consumption rate increased significantly from 39.6 up to 90.0 mg/g/h as the temperature increased from 20 to 32°C [30]. Like juvenile and adult shrimps, postlarvae oxygen consumption also had a positive correlation with the water tempeature. According to Piña-valdez *et al* [31], the highest oxygen consumption obtained when the temperature was at 35°C.

Oxygen consumption in a particular nanobubble treatment has been studied by Galang *et al* [19]. Nanobubble treatment is known to provide higher DO and lower oxygen consumption compared to control. In this study, DO in nanobubble and control treatments for 600 postlarvae/L was 3.02 mg/L (95%CI: 1.65-4.39), and 1.62 mg/L (95%CI: 1.48-1.73) with oxygen consumption at 0.37 mg/g/h (95%CI: 0.33-0.41) for nanobubble and 0.51 mg/g/h (95%CI: 0.49-0.62) for control treatments. According to Meegoda *et al* [32], the higher DO due to stable existence and presence of long-lasting oxygen gas generated by nanobubble machine resulted in lower oxygen consumption. As nanobubble last longer in water, oxygen becomes more easily absorbed by the shrimps as observed by Galang *et al* [19].

Nanobubble treatments with DO had more significant positive correlations in survival rates compared to control. It was indicated that the oxygen provided by nanobubble had positive effect and contributed more on the *L. vannamei* PL 8 survivals. This result also correlates with the findings from Esparza-Leal *et al* [33]. The decrease in the survival rate of shrimps cultured at high densities was thought to result from increased competition for DO. In the light of the results on the relationship between stocking density and survival rate, the highest density of 600 postlarvae/L is considered unsuitable due to the higher mortality rate compared to density of 200 and 400 postlarvae/L. This finding is in agreement with [9], that culture at the highest density should be avoided due to reduction trends of shrimps' survival rate.

In this study, temperatures had negative effects on *L. vannamei* PL 8 survival rate and these were more apparent in control than in nanobubble. This was also in agreement with other studies. Crustacean survival rates were significantly decreasing at lower or higher temperatures [2]. When shrimps were exposed to water temperature of more than 33°C for longer hours, the survival rates will be impaired. Whereas, when shrimps were exposed to temperature ranging between 23.5-25.5°C, and 30-31.5°C for longer hours, the shrimp's survival rates tend to increase [1]. In this study, the nanobubble maintained the water temperature range between 27.22 and 27.27°C, and this has led to the nanobubble treatment being more suitable for *Litopenaeus vannamei* PL 8 survivals.

IV. Conclusion

In this study, treatments and stocking densities have influenced water quality variables, oxygen consumption, and *L. vannamei* PL 8 survival rates. In nanobubble treatment, *L. vannamei* PL 8 stocking densities have higher DO, and lower temperature, EC, and oxygen consumption. Among nanobubble treatments, density of 400 postlarvae/L has the most optimum DO, temperature, EC, oxygen consumption, and survival rates. The results obtained in this study can be applied in the use of nanobubble treatment in particular density of 400 postlarvae/L.

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