The influence of nanobubble aeration system on rearing water temperature and its effects on oxygen consumption of *Penaeus vannamei* postlarvae

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Abstract. Water temperature and oxygen consumption are important indicators of physiological work for *Penaeus vannamei*. The preferred *P. vannamei* shrimp farming is indicated by low water temperature and oxygen consumption and this can be achieved by using nanobubble aeration. This study aims to assess the water temperature and oxygen consumption of *P. vannamei* postlarvae (PL) 8. In this experimental study, PL 8 at a density of 200 inds./L were treated with nanobubble aeration (16 mg/L) and compared with control (without nanobubble). The experiments were conducted with five replications. ANOVA was applied to calculate the significance level of treatments. The result showed the nanobubble influences (p<0.05) on temperature in which treatment (23.07°C; 95%CI: 22.9 to 23.3) was lower than control (27.17°C; 95%CI: 27.1 to 27.2). The apparent influences were also observed in oxygen consumption (p<0.05) in which the nanobubble can reduce the oxygen consumption up to 66%. To conclude, the nanobubble has shown a positive influence on *P. vannamei* shrimp farming as nanobubble is able to allow oxygen to last longer in water and reduce oxygen consumption as well.

Keywords: consumption; nanobubble; oxygen; shrimp; temperature

1. Introduction
Respiration is an important metabolism activity in living organisms. For animals including shrimps, respiratory adaptions in the form of oxygen consumption can be used as a representative indicator of the
crustacean physiological state since it determines the amount of energy that is available to accomplish physiological mechanisms. The consumption of oxygen is an indicator of active and routine metabolism that allows the determination of the ingested energy destinations from food in different environmental or nutritional conditions [1]. Since oxygen is the last substance or electrons in the respiratory pathway, it might be related to energy consumption as responses to water quality parameter changes. As a result, oxygen consumption of *P. vannamei* was influenced by those parameters including water salinity and temperature [2–4].

As temperature increases, *P. vannamei* postlarvae is known to consume more oxygen during normoxic conditions, with the 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 [5]. In comparison to Rosas *et al.* [6] that found there were more oxygen consumption when there was a decrease in salinity with high values in 5 ppt and lower values in 30 ppt. In spite of this, most previous studies were emphasized oxygen consumptions and water quality related parameters under conventional aeration methods [7, 8], the uses of current aeration technology like nanobubble in shrimp farming were still limited.

Recently, nanobubble is known to improve the dissolved oxygen in aquaculture water and feasible to be applied in shrimp culture [9]. A study by Rahmawati *et al.* [10] had comprehensively discussed the nanobubble technology to enhance the *P. vannamei* shrimp growth. However, their study was only emphasizing the dissolved oxygen (DO) factors. A study about *P. vannamei* shrimp oxygen consumption and DO under nanobubble treatment can be drawn from Galang *et al.* [11]. Based on their results, the aquaculture using nanobubbles system has its consequence on the oxygen content and the level of oxygen consumption in the *P. vannamei* shrimp as well. The oxygen consumption level in the nanobubble system was lower than in a conventional aerator. In reference to these previous studies, it is important to look into another vital water quality parameter that can affect oxygen consumptions, including the water temperature. This study aims to investigate the influence of the nanobubble aeration system on rearing water temperature and its effects on oxygen consumption of *Penaeus vannamei* postlarvae 8.

2. Material and methods

The experimental study was conducted from 21 to 23 August 2020 in a hatchery in Serang, Indonesia. For this study, *P. vannamei* postlarvae (PL) 8 with mean body weight 0.0024 g and length of 7–8.5 mm were used with densities of 200 ind/L. The nanobubble machinery NB S-2 used in this study was developed by Nanobubble Karya Indonesia Ltd., South Tangerang, Indonesia and able to generate oxygen bubbles with DO levels from 10 mg/L to 20 mg/L with the bubble size was <200 nm. The flow rate was 60 L per minute with coverage of 100–250 m²/nanobubble unit.

![Figure 1](image_url)

**Figure 1.** Experiment system with nanobubble, oxygen pump, and control (without nanobubble) treatments.

Treatments used in these groups consisted of DO sourced from nanobubble, oxygen pump, and control (without nanobubble) (figure 1). *P. vannamei* was placed in a closed system using plastic bags 20×40 cm [12]. In control, oxygen was sourced from atmospheric oxygen. Experimental treatments were conducted with five replications to obtain representative data for statistical analysis. Each treatment was
conducted for a 24 hour period [6]. The statistical analysis including descriptive statistics for mean calculation and a multiple regression model to measure the effect and correlation of measured water quality variables with oxygen consumption of *P. vannamei* PL 8.

Water temperature, dissolved oxygen (DO), and oxygen consumption measurement methods used in this study were in line with Villarreal *et al.* [5] and Galang *et al.* [11]. YSI 550A was used to measure both the DO level and temperature. Oxygen consumption was calculated by subtracting the DO measured after 24 hours with DO measured at the first hour. The subtraction result then divided by weight of *P. vannamei* PL 8 (0.0024 g) and observation period (hour). The obtained results were then divided by numbers of PL 8 individuals/treatment which were 200 individuals.

3. Results

The water quality parameters including DO and temperature among control and treatment groups (pump and nanobubble aerated treatments) are presented in table 1. As shown, water temperatures between the control and treatment groups are significantly different. Nanobubble treatment has the lowest water temperature (27.3°C; 95%CI: 27.0–27.3) followed by control and pumps aerated treatments. After 24 hours, water temperatures in control and nanobubble treatments were both increased (figure 2). Meanwhile, the temperature in the pump aerated group decreased. As for DO, the results showed that there were significant differences between the control and the treated groups. The average DO is observed higher in nanobubble (4.87 mg/L; 95%CI: 4.4–5.34) and pump aerated (4.94 mg/L; 95%CI: 4.4–5.48) treatments than control. After 24 hours, DO in control and all treatment groups declined (figure 3).

PL 8 oxygen consumptions were observed lower in nanobubble treatments than control and pump aerated treatments (table 1). The mean oxygen consumption values for control, pump, and nanobubble treatments were 0.813 mg/g/h (95%CI: 0.276–1.35), 7.91 mg/g/h (95%CI: 4.59–11.2), and 0.287 mg/g/h (95%CI: 0.261–0.313), respectively. In reference to the influence of water quality parameters on the particular consumption of oxygen, the calculation of this consumption per treatment as a result of DO and temperature are presented in table 2. The corresponding graph is illustrated in figure 4.

### Table 1. Mean ± standard deviations with 95% Confidence Intervals (CI) of water temperature, DO, and PL 8 oxygen consumption.

| Parameters                          | Control          | Treatments       | Nanobubble       | P      |
|-------------------------------------|------------------|------------------|------------------|--------|
| **Water temperature**               |                  |                  |                  |        |
| (°C) in 1 hour                      | 27.17±0.05       | 29.4±0.12        | 23.07±0.20       | <0.05  |
| (95%CI: 27.1–27.2)                  | (95%CI: 29.3–29.5) | (95%CI: 22.9–23.3) |                  |        |
| **Water temperature**               |                  |                  |                  |        |
| (°C) in 24 hour                     | 28.3±0.22        | 29.2±0.95        | 27.3±0.05        | <0.05  |
| (95%CI: 28.1–28.5)                  | (95%CI: 29.1–29.3) | (95%CI: 27.0–27.3) |                  |        |
| **DO (mg/L)**                       |                  |                  |                  |        |
| in 1 hour                           | 4.03±0.33        | 13.7±1.79        | 16.3±0.13        | <0.05  |
| (95%CI: 3.71–4.36)                  | (95%CI: 11.9–15.4) | (95%CI: 16.2–16.4) |                  |        |
| **DO (mg/L)**                       |                  |                  |                  |        |
| in 24 hour                          | 3.33±0.30        | 4.94±0.55        | 4.87±0.47        | <0.05  |
| (95%CI: 3.03–3.63)                  | (95%CI: 4.4–5.48) | (95%CI: 4.4–5.34) |                  |        |
| **Oxygen consumption**              |                  |                  |                  |        |
| (mg/g/h)                            | 0.813±0.54       | 7.91±3.39        | 0.287±0.01       | <0.05  |
| (95%CI: 0.276–1.35)                 | (95%CI: 4.59–11.2) | (95%CI: 0.261–0.313) |                  |        |

*P*<0.05: significance of one way ANOVA to identify the influences of treatments on the consumption of oxygen and DO, as well as water temperature.

*a,b,c*: significant differences by Tukey test (*P*<0.05) are indicated using different superscript letters.
Figure 2. Water temperature trends (°C) in each treatment for 1 and 24 hours.

Figure 3. Water dissolved oxygen trends (mg/L) in each treatment for 1 and 24 hours.

Figure 4. PL 8 oxygen consumption (mg/g/h) in each treatment.
Table 2. Multiple regression model of PL 8 oxygen consumption (y) with DO (x1) and temperature (x2) in each treatment.

| Treatments | Model | r     | Pearson’s r | Spearman rho |
|------------|-------|-------|-------------|--------------|
|            |       | x1    | x2           | x1           | x2           |
| Control    | \( y = -0.1461x_1 - 2.5961x_2 + 74.82205 \) | 0.998 | -0.258 | -0.998 | -0.100 | -1.000 |
| Pump       | \( y = 7.25293x_1 - 44.65194x_2 + 1275.02481 \) | 0.933 | 0.274 | -0.430 | 0.300 | -0.316 |
| Nanobubble | \( y = -0.05565x_1 + 0.51021x_2 - 13.48027 \) | 0.328 | -0.163 | 0.155 | -0.400 | -0.354 |

4. Discussion

Changes of water quality parameters in this study, such as water temperature and DO were also reported in other experiments where nanobubble was used. In spite of temperature increment, temperature recorded in nanobubble treatment was lower than control and was still within the ranges reported by the other studies. According to Wyban et al. [13], temperature is one of the essential aspects of shrimp growth. The reported temperature in those studies were 20–32°C [14], 28–31°C [8] and 27°C [15]. The observed dynamics of water temperatures were related to the chemical properties of water molecules, solar radiation, air temperature, and water temperature passing through the treatment units. Water consists of atoms and molecules and these mass of matter to which energy is added are vibrating faster, rapidly, and move slightly farther apart. As a result, the movements of atoms and molecules generate energy and heat content that increase the temperature over time.

DO in all treatments show declining trends after 24 hours. Despite DO reductions, DO values were different among treatments with nanobubble and pump aerated treatments, which remain to have higher DO after 24 hours. Higher DO values under nanobubble treatment observed in this study were comparable to other results obtained by Ebina et al. [16] and Rahmawati et al. [10]. According to Ebina et al. [16], nanobubble significantly increased the DO by four folds. In addition, Wu et al. [17] reported that DO increased almost by nine folds from 0.60 mg/L to over 5.00 mg/L. Similarly, Rahmawati et al. [10] reported that after 40 days, the DO level with nanobubble treatment was 5 mg/l and the control was 3 mg/L.

Oxygen consumption of *P. vannamei* PL 8 in this study was in line with results obtained from the other studies. Here, oxygen consumptions were observed high in treatments that have a higher temperature than nanobubble treatments. In control, the oxygen consumption was as high as 0.813 mg/g/h (95%CI: 0.276–1.35) with temperature equals to 28.3°C (95%CI: 28.1–28.5). When temperature was as low as 27.3°C (27.0–27.3) as observed in nanobubble treatments, the oxygen consumptions were reduced to 0.287 mg/g/h (0.261–0.313). Water temperature is known to have a positive correlation to oxygen consumption [7]. González et al. [18] reported that the consumption rates of oxygen significantly increased from 39.6 to 90.0 mg/g/h as the temperature changed from 20 to 32°C. The oxygen consumption pattern in particular postlarval shrimp can be drawn from Piña-valdez et al. [19]. Similar to juvenile and adult shrimp, oxygen consumption of postlarval also had positive correlation with the water temperature. According to them, the highest temperature at 35°C was a result of the high oxygen consumption. In addition, Boyd and Pine [20] stated that it is crucial to control the temperature as it influences the shrimp performances.

Particular oxygen consumption of shrimp in a specific nanobubble treatment has been reported by Galang et al. [11]. In their study, nanobubble treatment had higher DO and lower oxygen consumption compared to control. Similarly, DO in nanobubble and control treatments in this study were 4.87 mg/L (95%CI: 4.4–5.34) and 3.33 mg/L (95%CI: 3.03–3.63), respectively. The increment of DO was followed by a reduction of oxygen consumption as observed in nanobubble treatment and it was statistically significant as indicated by Pearson’s r and Spearman rho (table 2). Lower oxygen consumption in nanobubble treatment while having higher DO was related to the stable existence and presence of long lasting oxygen gas generated by the nanobubble machine [21]. According to Galang et al. [11], low
In this study, nanobubble treatment has the potential to reduce oxygen consumption, lower water temperature, and improve survival and growth rates of shrimp. Despite its ability to increase the DO and provide more oxygen to be consumed by shrimp, nanobubble treatment can also reduce the water temperature. In comparison to a higher temperature, the lower temperature is essential and it provides more benefits during rearing. According to Montagna [23], survival and shrimp growth were high within temperature ranges of 20–25°C and it was low at 30°C. In addition, at 30°C the shrimp biomass also reduced [24]. In this study, nanobubble treatment has the lowest temperature, thus the use of nanobubble potentially contributes to improving survival, growth, and biomass of shrimp.

The multiple regression models for every treatment group from this study can assist in predicting the consumption rate of oxygen based on DO and temperature variables [22]. The oxygen consumption resulted from the multiple regression model calculation can be used to estimate the DO and aeration requirements including the required oxygen flow rate to support P. vannamei shrimp culture.

5. Conclusions
Considering the water DO and temperature trends generated from each treatment, it can be summarized that the most appropriate environment to support P. vannamei PL 8 would be nanobubble aerated treatments. The nanobubble treatment has lower water temperature, oxygen consumption, and higher DO compare to pump aerated and control treatments. Furthermore, the nanobubble aeration system has influenced rearing water temperature due to its ability to reduce oxygen consumption of P. vannamei PL8, which is a quality indicator for shrimp optimum growth. Most recent oxygen consumption studies on nanobubble were limited to the DO variables on juvenile shrimp. However, this study has expanded the existing nanobubble research by studying both DO and an important variable, which is water temperature, to provide more valuable information for the advancement of shrimp culture.

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