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Effect of dissolved carbon dioxide on oxygen consumption in the Pacific white shrimp, *Litopenaeus vannamei* (Boone 1931)

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ABSTRACT

A failure in the aeration system of *Litopenaeus vannamei* rearing with biofloc technology can decrease the oxygen concentrations rapidly and also increase the carbon dioxide (CO₂) concentrations at the same rate. We report here an evaluation of the effect of CO₂ on the oxygen consumption of *L. vannamei*. We used a continuous-flow respirometer with water recirculation equipped with a digital fiber-optic oximeter. Eight juveniles of *L. vannamei* (12.1 ± 1.4 g) were used in each treatment with one per respiratory chamber (0.6 L). The shrimp were exposed to six concentrations of CO₂ (5, 30, 60, 95, 150, and 300 mgCO₂/L) with an acute exposure time of six hours. Upon treatment with 5–30 mgCO₂/L, we observed a consumption of oxygen of 0.233 ± 0.129 and 0.33 ± 0.072 mgO₂/g/h, respectively. Upon treatment with 60 mgCO₂/L, an increase was observed in the oxygen consumption (0.521 ± 0.098 mgO₂/g/h). Upon treatment with 95, 150, and 300 mgCO₂/L however, the shrimp decreased their oxygen consumption and lost their equilibrium. The CO₂ should therefore be maintained at a maximum of 5 mgCO₂/L during shrimp rearing.

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White shrimp; *Litopenaeus vannamei*; carbon dioxide; hypercapnia; oxygen consumption

Introduction

Oxygen consumption data are important for calculations of the power required for aeration systems installed in shrimp culture tanks for a particular species and to provide data on the metabolism of organisms under different stressful conditions that can occur during cultivation (Bett & Vinatea 2009; Campos et al. 2014). An increase in the respiration rates under environmental stress conditions may indicate an increase in energy consumption required to maintain homeostasis. Upon studying *Farfantepenaeus paulensis* oxygen consumption, Tsuzuki and Cavalli (2000) stated that respiration rates can be influenced by intrinsic factors such as shrimp size, age, molting stage, sex, and acclimation. Numerous studies have been conducted using marine shrimp to determine the rate of oxygen consumption as a function of a series of environmental variables (Bett & Vinatea 2009; Ponce-Palafox et al. 2013; Campos et al. 2014).

The Pacific white shrimp, *Litopenaeus vannamei*, is the most frequently reared shrimp species in highly intensive culture systems that apply biofloc technology (BFT). In these shrimp cultures, the stocking densities range from 150 to 600 shrimp/m², and the biomass ranges from 2 to 8 kg/m² (Silva et al. 2013). During the biofloc formation, there is an increase in the suspended solids and organic matter to levels above 1000 mg/L. The removal of excess biofloc by filtration is thus required to maintain the levels below 500 mg/L (Gaona et al. 2011; Schweitzer et al. 2013). In situations where power shortages occur, the aeration system is deactivated, and the levels of dissolved oxygen (DO) then diminish rapidly to critical levels (0.65 mg/L) in approximately 30 min (Vinatea et al. 2009). Doses of hydrogen peroxide can be applied during such periods as an emergency source of oxygen for the shrimp to avoid mass mortality (Furtado et al. 2014). Furtado (2014) also confirmed that an elevation of dissolved carbon dioxide (CO₂) concentrations occurs over time under these conditions, and these levels may be even greater depending on the biomass of the shrimp and bioflocs.

When CO₂ concentrations in the water are more than 20 mg/L of CO₂, the excretion of CO₂ through the gill epithelium becomes more difficult, resulting in a decline in the pH of the hemolymph, which negatively affects the capacity of the respiratory pigment hemocyanin to transport oxygen, thus reducing the oxygenation of tissues and increasing the ventilation rate (Taylor & Whiteley 1989; Van Wyk & Scarpa 1999). Van Wyk and Scarpa (1999) found that CO₂ levels below 5 mg/L are optimal and levels up to 20 mg/L are considered acceptable for penaeid shrimp, whereas concentrations between 20 and 60 mg/L affect the CO₂ exchange in the gills.

The oxygen solubility decreases and oxygen consumption of the shrimp and micro-organisms increases when marine shrimp farms experience high temperatures. Under these conditions, shrimp can experience a state of hypoxia. In hypoxic conditions, the toxicity of CO₂ is potentiated because it interferes with the oxygen absorption capacity (Boyd & Tucker 1998). The purpose of this study was therefore to evaluate the effect of CO₂ on the oxygen consumption of juvenile *L. vannamei*.

Materials and methods

Site and facilities

The study was performed at the Multidisciplinary Unit for Teaching and Research of the School of Sciences of the National Autonomous University of Mexico (*Unidad Multidisciplinaria de Docencia e Investigación da Facultad de Ciencias da Universidad Nacional Autónoma de México* – UNAM), at the Sisal seaport (21°9'55. 22 N, 90°1'54. 93 W) Hunucmá, Yucatán, Mexico.

Water, shrimp, and experimental design

The juvenile *L. vannamei* used in this study were obtained from an intensive farming system (150 shrimp/m³) with BFT conducted at the UMDI facilities. The shrimp were reared for 110 days in a circular tank with a 5 m diameter (15 m³) and fiber glass base covered with a geomembrane, which was protected with a black shade cloth (70%) to reduce light intensity and limit bird predation. The tanks were filled with saltwater at 38 g/L salinity and filtered through a sand filter. Intensive aeration was provided with an air pump (Sweetwater® 5 HP). The drainage was modified to exclude bottom sediments concentrated at the center of the

tank, and the total daily renewal rate of the tank volume was 1%. The volume of bioflocs measured in the Imhoff cones was 12 ml/L throughout the grow-out and a higher concentrations of CO₂ measured was 5 mg/L.

The shrimp were fed four times a day with a commercially available feed (Api-Camarón[®]) containing 35% crude protein and 8% lipids, which were donated by Malta Clayton[®] Mexico, according to the feeding table of Agribrands Purina[®] and feed trays methodology (Seiffert & Andreatta 2004). The shrimp remained in the grow-out area for marine shrimp until a mean weight of 12.1 ± 1.4 g was attained.

The shrimp were transferred to 10 experimental units with a working volume of 30 L. Ten shrimp were placed in each rectangular polyethylene tank (bottom area 0.20 m²) in a stocking density of 50 shrimp/m², and the animals were then fasted for 12 h before they were transferred to respirometry chambers. In the bottom of each experimental unit, an air flow diffuser was placed to provide oxygen to the water at a saturation level.

The experiment consisted of six treatments with carbon dioxide concentrations of 5, 30, 60, 95, 150, and 300 mgCO₂/L for six hours at a salinity of 33 g/L. The shrimp remained in fasting conditions during the entire experimental period. The treatment with 5 mg CO₂/L was considered as a “Control” because no CO₂ was added to the water.

Physical and chemical parameters of water quality

The photoperiod of the experimental room was a 12-Light/12-Dark cycle with artificial light. The temperature of the experimental room was set to 26 °C, and the water temperature was 25 °C. Measurements of pH, temperature, and CO₂ were performed every 15 min over the six hours of exposure to CO₂ using a YSI 100 pH meter (Yellow Springs, OH, USA), Hach[®] carbon dioxide test kit (Hack Company, Loveland, Colorado, USA) and CO₂ Analysis Salt[®] software (Timmons & Ebeling 2010), respectively. The salinity was measured daily with an optical refractometer (Atago[®], Japan), and alkalinity was assessed daily following the methodology proposed by the APHA (1998).

Oxygen consumption

Carbon dioxide was injected into the respirometry chambers using a 30 kg pressurized gas cylinder with a manometer, regulator valve, and set of fluxometers specific for carbon dioxide (scale of 0–15 L/min). A recirculation system consisting of 10 0.6-L respirometry chambers was set-up with a 250-L rectangular reservoir containing a submerged pump (1750 L/h, Boyu[®]) (Figure 1). The pump transferred water from the reservoir to a pipe with stop valves to control the water flow at the entry of the respirometry chambers, which is where the water returned to the reservoir by gravity. The 250-L reservoir contained three air-flow diffusers to provide aeration as well as an additional diffuser to provide CO₂ originating from the pressurized-gas cylinder.

A 10-Channel Fiber Optic Digital Oxygen Transmitter, Model OXY 10 from Precision Sensing GmbH (Pre Sens[®], Germany) was used with optical oxygen sensors interfaced with a computer with OXY 10 Software. The device was programmed to record the DO levels each minute. A sensor was placed at the entryway of the water into the system to record DO measurements prior to the water entering the chambers. Individual sensors were placed at the exit of each chamber that contained the shrimp as well as the control chamber, which

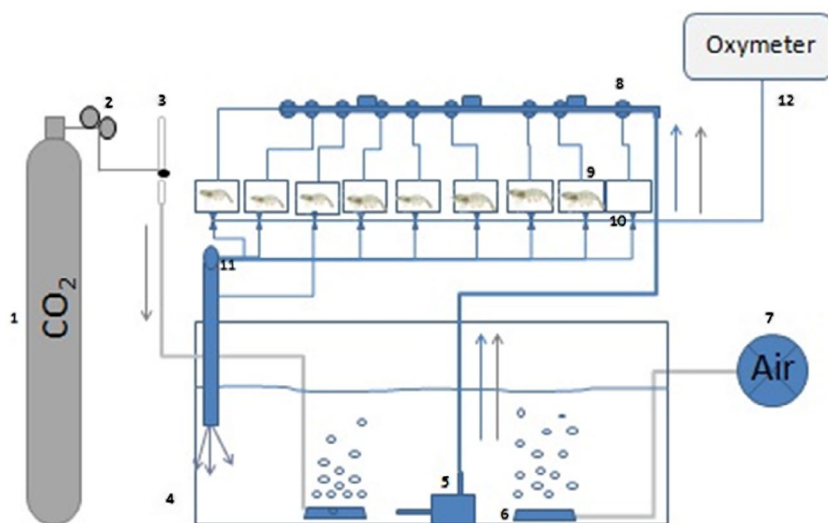


Figure 1. Diagram of the experimental system: (1): carbon dioxide tank (CO₂); (2): manometer and regulator valve; (3): fluxometer; (4): reservoir with a working volume of 250 L; (5): submersed pump; (6): air flow diffuser to oxygenate water and inject CO₂; (7): air pump; (8): stop valves to control the water flow; (9): respirometry chambers where the shrimp were stocked in a 0.6-L working volume; (10): optical sensors; (11): pipe for transfer of the water from the respirometry chambers to the reservoir; and (12): digital oxygen transmitter connected to computer.

did not contain shrimp, to measure the DO levels upon exit from the chamber, which also indicated the concentration of DO in the chamber. The flow of the exit water was set to 5.3 ± 0.7 L/h to promote a difference between the oxygen concentrations at entry and exit. The same flow was set for all of the chambers.

Only shrimp within the intermoult period were used for respiratory trials. The shrimp were individually placed in eight respirometry chambers that had been filled with water according to their respective treatments and connected to the recirculation system. The chambers were sealed to prevent air bubbles from entering the chamber. The size and volume of the chambers were the same for all of the assessed shrimp. One of the chambers did not contain any shrimp and was used as a control for each treatment.

The shrimp spent a total of 24 h inside of the respirometry chambers. Bett and Vinatea (2009) found that in 1 h, the shrimp were already acclimated to the experimental conditions of the respirometry chambers. We nevertheless standardized the procedures to 2 h of acclimation before initiating the measurements of oxygen consumption. We standardized the procedures in this study by setting acclimation to 18 h for handling stress and activity associated with photoperiod. There was 1 h of adjustment to the carbon dioxide concentrations after acclimation according to each treatment and 5 h of oxygen consumption for each CO₂ concentration. Once the measurements had been performed, the shrimp were removed from the chambers, dried with a paper towel, and weighed (live weight) on a digital balance (Scout Ohaus®, NJ, USA with a 600 ± 0.1 g capacity).

The oxygen consumption data were expressed in mgO₂/g/h following the method of Lomholt and Johansen (1979):

$$VO_2 = ([O_2e - O_2x] \times F_{shrimp}) - ([O_2e - O_2x] \times F_{control})/W$$

where VO_2 = oxygen consumed in $mgO_2/g/h$, O_{2e} = oxygen concentration in mg/L at chamber entry, O_{2x} = oxygen concentration in mg/L at chamber exit, F = flow in L/h , W = wet body weight (g).

Statistical analysis

The software program STATISTICA 7.0® (StatSoft, Tulsa, Oklahoma, USA) was used for the statistical analyses of the data. After confirming the homoscedasticity of variances (Levene) and normality of the data distribution (Kolmogorov–Smirnov), an analysis of variance (ANOVA) was performed to confirm possible significant differences in the data obtained. When significant differences were found between the treatments ($p < 0.05$), Tukey's test was performed for a comparison of the means (Zar 1999).

Results

Physical and chemical parameters of water quality

The physical and chemical parameters for the water quality monitored throughout the experiments are presented in Table 1. The values for carbon dioxide and pH were significantly different ($p < 0.05$) between the treatments, as was predicted in the experimental design. The concentration of DO was directly and inversely proportional to the concentration of CO_2 , and there was a lower concentration of DO ($p < 0.05$) in the treatments with greater concentrations of CO_2 . The temperature and salinity did not exhibit significant differences between the treatments ($p > 0.05$) at any point throughout the study. The alkalinity did not vary between the treatments and remained at $290\text{ mgCaCO}_3/L$.

Oxygen consumption

The mean values of DO at the entry and exit of the respirometry chambers upon the treatments with 5, 30, 60, 95, 150, and $300\text{ mgCO}_2/L$ are presented in Figures 2(A)–(F), respectively. In Figure 2(F), the data for DO are not shown for a period of close to 2 h of CO_2 exposure as a result of shrimp mortality. Oxygen consumption in the different treatments exhibited significant differences ($p < 0.05$) (Figure 3). The mean results for oxygen

Table 1. The mean value \pm standard deviation of the physical and chemical parameters of water quality measured over 6 h, with juvenile *L. vannamei* submitted to different concentrations of dissolved carbon dioxide (CO_2).

| Treatments | Parameters | | | | |
|------------|------------------|----------------------|----------------------|-------------------|------------------|
| | $T^\circ C$ | DO (mg/L) | CO_2 (mg/L) | pH | Salinity (g/L) |
| 5 mg/L | 25.03 ± 0.17 | 6.60 ± 0.08^a | 5.04 ± 1.98^a | 7.65 ± 0.10^a | 33.62 ± 0.37 |
| 30 mg/L | 25.00 ± 0.24 | 6.57 ± 0.09^a | 30.01 ± 3.53^b | 7.18 ± 0.05^b | 33.67 ± 0.36 |
| 60 mg/L | 25.14 ± 0.13 | 6.48 ± 0.10^{ab} | 59.29 ± 4.31^c | 6.88 ± 0.04^c | 33.72 ± 0.34 |
| 95 mg/L | 25.10 ± 0.11 | 6.34 ± 0.15^b | 95.16 ± 4.14^d | 6.36 ± 0.05^d | 33.80 ± 0.23 |
| 150 mg/L | 25.04 ± 0.12 | 5.96 ± 0.29^{bc} | 152.02 ± 8.67^e | 6.14 ± 0.05^e | 33.84 ± 0.30 |
| 300 mg/L | 25.08 ± 0.15 | 5.80 ± 0.24^c | 300.04 ± 14.10^f | 5.90 ± 0.03^f | 33.90 ± 0.28 |

Note: Data correspond to a mean value of eight replicates \pm standard deviation. Different superscripts in the same row indicate that the means were significantly different ($p < 0.05$). Temperature ($T^\circ C$), dissolved oxygen (DO), and dissolved carbon dioxide (CO_2).

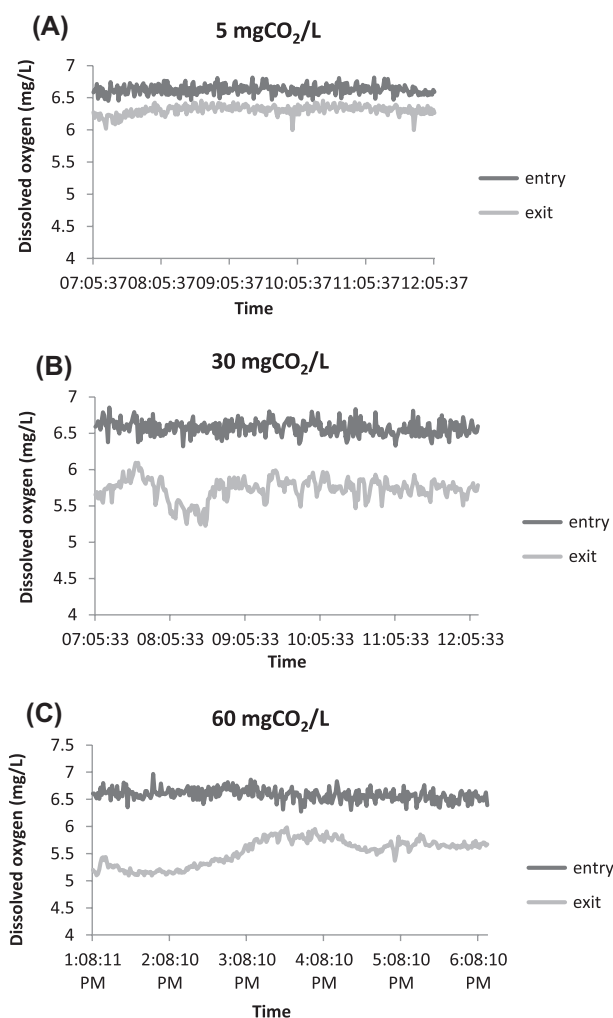


Figure 2. Mean value of the concentration of DO (mg/L) at the entrance (upper line) and exit (lower line) of the respirometry chambers ($n = 8$ per treatment) throughout the 5 h of exposure to different CO₂ concentrations.

consumption in the treatments with 5, 30, 60, 95, 150, and 300 mgCO₂/L were 0.233 ± 0.129 , 0.330 ± 0.072 , 0.521 ± 0.098 , 0.460 ± 0.12 , 0.360 ± 0.118 , 0.30 ± 0.049 mgO₂/g/h, respectively.

There was an increase in the oxygen consumption up to the 60 mgCO₂/L treatment, whereas in the 95, 150, and 300 mgCO₂/L treatments, the shrimp showed reduced oxygen consumption and exhibited a change in behavior with a loss of equilibrium, as if they had been anesthetized. The shrimp with symptoms of anesthesia during the treatments with 95–150 mgCO₂/L exhibited 100% survival after 24 h the period of exposure to CO₂. The shrimp in the 300 mgCO₂/L treatment, however, showed a 62.5% mortality rate (Figure 2(F)).

Discussion

The water temperature during the experiment remained within the appropriate range of 24–32 °C for penaeid shrimp growth (Van Wyk & Scarpa 1999). The mean salinity remained

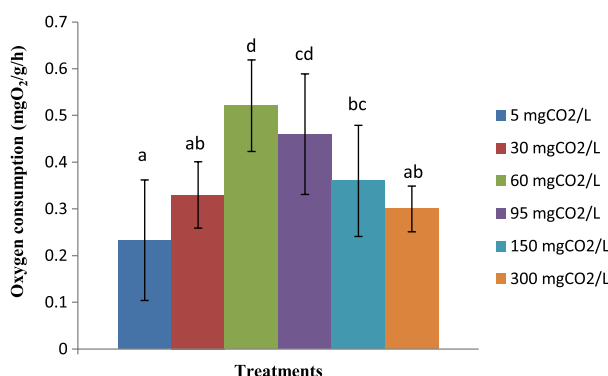


Figure 3. The mean value \pm standard deviation of oxygen consumption (mgO₂/g of shrimp/h) by juvenile *L. vannamei* ($n = 8$ per treatment) exposed for 5 h. The different letters written above the bars indicate significant differences between the treatments ($p < 0.05$).

within the range of 0.5–35 g/L indicated for the culture of this species (Van Wyk & Scarpa 1999). The concentrations of DO in the study were close to 6.0 mg/L, which is considered ideal for the cultivation of penaeid shrimp (Van Wyk & Scarpa 1999). The parameters of temperature, salinity, and DO were thus favorable for the survival of shrimp.

According to Ponce-Palafox et al. (2013), the respiratory metabolism of the Pacific white shrimp can be influenced by temperature, salinity, size, and interactions among these factors, with temperature exhibiting a stronger influence than salinity. For example, Zhang et al. (2009) assessed the effect of different salinities (5, 15, 25, 35, and 40 g/L) on the oxygen consumption of *L. vannamei* juveniles with a mean weight of 6 g at 20 °C. The authors confirmed a lower oxygen consumption for the shrimp at a salinity of 5 g/L (0.25 mgO₂/g/h) and a higher consumption at a salinity of 25 g/L (0.35 mgO₂/g/h).

During the treatments with 5–30 mgCO₂/L, the oxygen consumption values were similar to those found by Zhang et al. (2009) at a salinity of 35 g/L, despite differences in the shrimp weight and water temperature. Bett and Vinatea (2009) assessed the oxygen consumption rate for juveniles of *L. vannamei*, with a mean weight between 10 and 14 g at different salinities and temperatures and found the following equation: specific oxygen consumption (mgO₂/g/h) = $-0.0281 + 0.0135 \cdot T - 0.0019 \cdot S - 0.0007 \cdot W$. When the mean for treatments with the lowest CO₂ level (0.233 mgO₂/g/h) were compared with the equation of Bett and Vinatea (2009) calculated with our data for the temperature, mean weight, and salinity (0.238 mgO₂/g/h), we observed that they were similar, and the values of 5 mgCO₂/L did not negatively affect the respiratory rate of *L. vannamei*. These data support the safe level calculated at 5.9 mgCO₂/L by Furtado (2014).

In the treatments with a higher level of CO₂, a significant reduction was observed in the levels of DO, although not sufficient for the shrimp to experience hypoxia (Diaz & Rosenberg 1995). When assessing the consumption of oxygen under the conditions of hypoxia, Ponce-Palafox et al. (2013) found a reduction in the rate of oxygen consumption, with the shrimp (20 g) adopting an oxyconforming behavior. Oxygen consumption under conditions of hypoxia becomes extremely dependent upon the oxygen concentration, and the metabolic capacity of the shrimp can be reduced by 26–34% (Villarreal et al. 1994).

Another parameter that exhibited a significant difference was the pH, which showed an inverse relationship with increasing CO₂ levels. The pH, however, showed values of 5.87 in the 300 mgCO₂/L treatment and did not come close to the pH of 4.5 at which the juvenile *L. vannamei* exhibited mortality (Furtado 2014). Zhang et al. (2006) concluded that body weight, temperature, salinity, pH, and nutritional conditions have significant effects on the lethal DO level for *L. vannamei*. In the conditions used in their study, the authors found that the relationship between the lethal DO level (LDOL) and pH was $LDOL = 0.1487 \text{ pH}^2 - 2.2488 \text{ pH} + 8.8806$ ($r^2 = 0.99$, $p = 0.0055$). Using the most acidic pH obtained in this study, the lethal DO level was therefore calculated to be 0.804 mg/L. The pH and DO level can thus be eliminated as the cause of death observed in the 300 mgCO₂/L treatment.

Furtado (2014) assessed the toxicity of CO₂ in juvenile *L. vannamei* and found values of LC₅₀ and confidence intervals of 95% at 24, 48, 72, and 96 h of 130.05 (104.2–162.1), 77.2 (73.8–80.02), 69.65 (65.47–74.32), 59.12 (53.08–66.07) mg/L of CO₂, respectively. During this study, a loss of equilibrium was observed in the shrimp in the 95, 150, and 300 mgCO₂/L treatments. The same behavior was also observed by Kikkawa et al. (2008). In addition, the shrimp in the 300 mgCO₂/L treatment exhibited the same symptoms of anesthesia during the first 115 min of exposure to CO₂. The shrimp began exhibiting mortality at a rate that ultimately rose to over 60% thereafter.

Molecular CO₂ is diffused directly through cell membranes. Thus, when the concentration of CO₂ in the water is elevated, the excretion of CO₂ by the gill epithelium is hampered, which results in a decline of pH in the hemolymph. The main mechanism of acid–base equilibrium is based on the exchange of ions across the gills, where the entry of HCO₃[−] and exit of Cl[−] occur. The hydration of CO₂ is subsequently catalyzed by the enzyme carbonic anhydrase and frees an H⁺ that will be exchanged for an Na⁺. These exchanges are conducted by a Na⁺/K⁺-basolateral ATPase and possibly by an apical H⁺-ATPase (Henry et al. 1981). A disruption in the acid–base equilibrium leads to a reduction of the hemolymph pH and can produce reactive oxygen species that can generate oxidative damage to cell structures such as lipid membranes, proteins, and DNA (Wang et al. 2012).

According to the results obtained in this study as well as those obtained by Hall and Van Ham (1998), Pacific white shrimp are particularly sensitive to an acute/short-term increase in CO₂ concentration. Farming nurseries should therefore be managed to avoid concentrations higher than 5 mgCO₂/L (Van Wyk & Scarpa 1999; Furtado 2014). To maintain CO₂ concentrations within favorable limits for shrimp and fish farming, degasification columns (Moran 2010) and aeration systems with dimensions appropriate for the biomass being reared should be installed. A series of alkalizing compounds, such as calcium hydroxide, carbonate, and sodium bicarbonate can be applied (Furtado et al. 2011).

Conclusion

This study confirmed an increase in the oxygen consumption in juvenile *L. vannamei* exposed to concentrations of carbon dioxide above 30 mg CO₂/L and the toxic effects of dissolved carbon dioxide in *L. vannamei*. As a result we recommend that the CO₂ levels be maintained at less than 5 mgCO₂/L during *L. vannamei* culture to reduce oxygen consumption and avoid hypercapnia.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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