# Oxygen consumption of *Litopenaeus vannamei* juveniles in heterotrophic medium with zero water exchange

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Abstract – This work aimed at determining the dissolved oxygen consumption rate of *Litopenaeus vannamei* juveniles maintained in a microbial biofloc raceway system at high density with no aeration. Three 4 L bottles were filled for each treatment, sealed hermetically, and placed in an enclosed greenhouse raceway system. Four shrimp (13.2±1.42 g) were assigned to two sets of the bottles, which underwent the following treatments: light conditions with no shrimp; dark conditions with no shrimp; light conditions with shrimp; and dark conditions with shrimp. Dissolved oxygen content was measured every 10 min for 30 min. A quadratic behavior was observed in dissolved oxygen concentration over time. Significant differences for oxigen consumption were observed only at 10 and 20 min between shrimp maintained in the dark and those under light conditions. At 10 min, a higher value was observed in shrimp maintained under light, and at 20 min, in the dark. Significant differences between 10 and 20 min and between 10 and 30 min were observed when oxygen consumption was analyzed over time in the presence of light. Under dark conditions there were significant differences only between 20 and 30 min. Lethal oxygen concentration (0.65 mg $L^{-1}$) would be reached in less than one hour either under light or dark conditions with no aeration.

Index terms: *Litopenaeus vannamei*, high density, microbial biofloc, oxygen consumption, raceway system.

## Consumo de oxigênio de juvenis de *Litopenaeus vannamei* em meio heterotrófico sem renovação de água

Resumo – O objetivo deste trabalho foi determinar o consumo de oxigênio dissolvido (OD) de juvenis de *Litopenaeus vannamei* mantidos em sistema de cultivo de bioflocs bacterianos em alta densidade e ausência de aeração. Três garrafas de 4 L foram preenchidas para cada tratamento, fechadas hermeticamente e colocadas em sistema de cultivo fechado. Quatro camarões (13,2±1,42 g) foram colocados em dois dos conjuntos de garrafas. Os tratamentos aplicados foram: luminosidade, sem camarões; escuro, sem camarões; luminosidade, com camarões; escuro, com camarões. A concentração de oxigênio dissolvido foi determinada a cada 10 min durante 30 min. Foi observado um comportamento quadrático na concentração de OD ao longo do tempo. Diferenças significativas para consumo de oxigênio foram observadas apenas aos 10 e 20 min entre camarões mantidos no escuro e camarões em luminosidade. Aos 10 min, foi observada maior concentração no sistema mantido em luminosidade e, aos 20 min, no sistema mantido no escuro. Na análise do consumo de oxigênio ao longo do tempo e com luminosidade, foram constatadas diferenças significativas entre 10 e 20 min e entre 10 e 30 min. Em condições de escuridão, houve diferença significativa apenas entre 20 e 30 min. Concluiu-se que, sem aeração, a condição anóxica pode ser alcançada em menos de uma hora, tanto ao dia quanto à noite.

Termos para indexação: *Litopenaeus vannamei*, alta densidade, biofloc microbiano, consumo de oxigênio, sistema de fluxo contínuo.

## Introduction

Advances made in the super-intensive shrimp culture systems, also known as zero exchange, aerobic, heterotrophic culture systems (ZEAH), emphasize the idea that it is possible to produce aquatic organisms in a sustainable and biosecure way, at least in the environmental dimension, supported by the non-production of effluents, the use of reduced spaces, and the minimization of the dissemination of infectious diseases (McAbee et al., 2003; Burford et al., 2004; Sowers et al., 2005; Wasielesky Junior et al., 2006). Actually, using the super-intensive culture technology developed at Waddell Mariculture Center (South Carolina, USA), it is possible to produce more than 400 tones of shrimp per year in only 2.5 ha, instead of the 80 ha required to yield the same amount in the conventional semi-intensive system (McAbee et al., 2003).

Intensification of aquaculture has been demanding higher oxygen levels in the culture ponds and, consequently, more oxygen input to meet the aquatic organisms' requirements (Allan & Maguire, 1992; Boyd, 1998). It is known that the decomposition of organic matter by bacteria occurs in the pond sediment and consumes a significant portion of the dissolved oxygen available for respiratory processes (Berthelson et al., 1996; Avnimelech & Ritvo, 2003). Furthermore, phytoplankton can be responsible for the consumption of another great portion of the oxygen in the water (Boyd, 1990; Garcia & Brune, 1991). This is especially important in intensive and super-intensive cultures with zero water exchange, when survival depends on oxygen availability and suspension of solids in the water column (Hopkins et al., 1995, 1996; Peterson et al., 2001; Delgado et al., 2003).

Unexpected failure of the aeration/oxygenation systems in this type of culture could lead to fatal levels of oxygen for shrimp. Pérez-Rostro et al. (2004) demonstrated that *Litopenaeus vannamei* cannot survive at 0.2 mg $L^{-1}$ dissolved oxygen for more than one hour. Information about dissolved oxygen consumption of *L. vannamei* juveniles will allow the estimation of available time to provide emergency aeration/oxygenation supply in case of failure of the regular aeration system either during the day (with photosynthesis) or at night (without photosynthesis).

The objective of this study was to determine the dissolved oxygen consumption rate of *L. vannamei* juveniles raised in a microbial biofloc raceway system at high density in absence of aeration under light and dark conditions.

## Materials and Methods

This study took place on June 17 2008 at the Waddell Mariculture Center (South Carolina Department of Natural Resources, South Carolina, USA). The treatments applied were: bottles under light conditions, without shrimp (light control); bottles in the dark, without shrimp (dark control); bottles under light conditions, with shrimp (light shrimp); and bottles in the dark, with shrimp (dark shrimp). The bottles (4 L, n = 3) were filled with raceway water and sealed with hermetic caps without receiving aeration and were placed in an enclosed greenhouse raceway system. Four shrimp (13.2±1.42 g) were assigned to each of the non-control bottles maintained in the dark and in the light.

Shrimps were placed directly in the bottles without receiving a previous acclimation period. The reason for no acclimation, neither after shrimp manipulation nor light condition, was for simulating exactly the situation after an aeration/oxygenation system failure event in the culture system. When aeration stops, the dissolved oxygen concentration drops very fast, shrimp collide with each other, and all suspended solids go down to the bottom of the tank, resulting in an abrupt increment of luminosity.

The raceway (271 $m^2$) used to extract the water and shrimps had been stocked with *L. vannamei* post-larvae on March 13 2008 at a density of 856 shrimp $m^{-3}$ and was operated under zero-water exchange biofloc microbial raceway system. Raceway water quality parameters on the day of the experiment are shown in Table 1.

Initial dissolved oxygen concentrations were determined for each treatment. Thereafter, dissolved

**Table 1.** Raceway water quality parameters determined during the experiment and methods used for the analyses.

| Parameter (units) | Value | Method |
|---|---|---|
| Oxygen (mg $L^{-1}$) | 4.0 | Multiparameter YSI 556 MPS |
| Temperature (ºC) | 30 | Multiparameter YSI 556 MPS |
| Salinity (ppt) | 34.2 | Multiparameter YSI 556 MPS |
| pH | 7.1 | Multiparameter YSI 556 MPS |
| Alkalinity (mg $L^{-1}$) | 126 | Titration method (HCl 0.1 N) |
| Total ammonia nitrogen (mg $L^{-1}$) | 0.38 | HACH method 8155 (salicylate) |
| Nitrite (mg $L^{-1}$) | 0.23 | HACH method 8507 (diazotization) |
| Nitrate (mg $L^{-1}$) | 22.9 | HACH method 8039 (cadmium reduction) |
| Phosphate (mg $L^{-1}$) | 54.5 | HACH method 8114 (ascorbic acid) |
| Total suspended solids (mg $L^{-1}$) | 546.7 | ESS method 340.2 |
| Volatile suspended solids (mg $L^{-1}$) | 236.1 | ESS method 340.2 |
| Whole photosynthesis (mg $L^{-1}$ per hour) | 1.46 | Clear and dark BDO bottles (2 hours) |
| Net photosynthesis (mg $L^{-1}$ per hour) | 0.21 | Clear and dark BDO bottles (2 hours) |
| Water respiration (mg $L^{-1}$ per hour) | 1.07 | Clear and dark BDO bottles (2 hours) |
| Light into the water (µE) | 180 | Lightmeter LICOR (LI-1400) |
| Light out of the water (µE) | 1,080 | Lightmeter LICOR (L-1400)I |

organic content was determined every 10 min for a period of 30 min with a polarographic digital oxygen meter (YSI 556 MPS). After 30 min, when the dissolved oxygen had been almost totally consumed, the shrimp were weighted and returned to their raceway. Photosynthesis and oxygen consumption for each treatment were determined using the following equations – light control: photosynthesis $O_2$ production (mg $L^{-1}$) = ($O_2$ final - $O_2$ initial); dark control: water $O_2$ consumption (mg $L^{-1}$) = ($O_2$ initial - $O_2$ final); light shrimp: shrimp $O_2$ consumption during the day (mg $L^{-1}$) = [($O_2$ initial - $O_2$ final) - photosynthesis $O_2$ production]; dark shrimp: shrimp $O_2$ consumption at night (mg $L^{-1}$) = [($O_2$ initial - $O_2$ final) - water $O_2$ consumption].

The equations for light and dark shrimp treatments were expressed as absolute values to avoid negative numbers. The experiment had a random factorial design. Oxygen consumption values for each treatment were analyzed by one way and factorial ANOVA at 5% probability using the statistical analysis software Minitab 15 (Minitab Inc. 2007). The means were compared by a Tukey test. Data of dissolved oxygen concentration over time were submitted to polynomial regression analysis with the help of the Microsoft Excel 2007 software.

## Results and Discussion

A quadratic behavior in dissolved oxygen concentration was observed over time (Table 2). The equations for dissolved oxygen concentrations in absence of aeration allowed to determine that the lethal oxygen concentration (0.65 mg $L^{-1}$) previously reported for 13 g of *L. vannamei* (Zhang et al., 2006) would be reached at 34.7 and 31.8 min under light and dark conditions, respectively. These equations could also be useful to determine the time the emergency systems should operate to maintain dissolved oxygen levels above 2.8 mg $L^{-1}$, which is considered the limit of hypoxic conditions (Diaz & Rosenberg, 1995), and avoid nutritional, immunological, and low growth consequences resulting from sublethal oxygen (Boyd, 1990; McGraw et al., 2001; Wu et al., 2002; Jiang et al., 2005).

The photosynthesis in light control was 0.22±0.17, 0.20±0.12 and 0.83±0.23 mg $L^{-1}$ after 10, 20 and 30 min, respectively. For dark control, the oxygen consumption was 0.90±0.11, 0.17±0.16 and 0.06±0.06 mg $L^{-1}$ after 10, 20 and 30 min, respectively.

For shrimp oxygen consumption, there was significant effect of light and time factors and significant interaction between them ($p<0.05$). The oxygen consumption of shrimp maintained under light conditions was 0.55±0.10, 0.18±0.01 and 0.09±0.07 mg $O_2$ $L^{-1}$ per shrimp for 10, 20 and 30 min, respectively. The oxygen consumption for shrimp maintained in the dark was 0.23±0.11, 0.25±0.01 and 0.13±0.06 mg $O_2$ $L^{-1}$ per shrimp for 10, 20 and 30 min, respectively. There were significant differences for oxygen consumption only at 10 and 20 min between shrimp maintained in the dark and those maintained under light conditions. At 10 min, a higher value was observed in shrimp maintained under light; at 20 min, a higher value was observed in the dark. When oxygen consumption was analyzed over time in the presence of light, there were significant ($p<0.05$) differences in oxygen consumption between 10 and 20 min and between 10 and 30 min. Under dark conditions there were significant differences in oxygen consumption only between 20 and 30 min (Table 3).

Even though there was high variability in the data, it was possible to verify that after 10 min of absence of aeration the oxygen consumption rate for shrimp maintained in light was significantly higher than for those maintained in the dark. This situation was reversed after 20 min, when the shrimp maintained in the dark showed a significantly higher oxygen consumption rate. The first situation may be explained in terms of the abundance of initial oxygen in the illuminated bottles due to photosynthetic activity. The

**Table 2.** Dissolved oxygen concentrations (mg $L^{-1}$) in the treatments in absence of aeration over time, and regression equations.

| Treatment | Time (min) | | | | Regression equation | $R^2$ |
|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 30 | | |
| Light control | 4.06±0.07 | 3.84±0.11 | 3.85±0.22 | 4.68±0.46 | $y = 0.002x^2 - 0.059x + 4.089$ | 0.961 |
| Dark control | 4.20±0.04 | 3.30±0.08 | 3.13±0.23 | 3.08±0.26 | $y = 0.002x^2 - 0.098x + 4.169$ | 0.977 |
| Light shrimp | 4.01±0.14 | 2.30±0.39 | 1.38±0.24 | 1.11±0.25 | $y = 0.003x^2 - 0.179x + 3.779$ | 0.999 |
| Dark shrimp | 4.12±0.15 | 2.29±0.51 | 1.13±0.32 | 0.55±0.07 | $y = 0.003x^2 - 0.212x + 4.116$ | 0.999 |

**Table 3.** Effect of time for oxygen consumption of *Litopenaeus vannamei* juveniles (mg $L^{-1}$ per shrimp) maintained either in light or dark conditions.

| Time factor | Contrast | P |
|---|---|---|
| | Light shrimp | |
| 10 vs. 20 min | 0.55±0.10 - 0.18±0.01 | 0.004** |
| 10 vs. 30 min | 0.55±0.10 - 0.09±0.07 | 0.003** |
| 20 vs. 30 min | 0.18±0.01 - 0.09±0.07 | 0.087 |
| | Dark shrimp | |
| 10 vs. 20 min | 0.23±0.11 - 0.25±0.01 | 0.812 |
| 10 vs. 30 min | 0.23±0.11 - 0.13±0.06 | 0.247 |
| 20 vs. 30 min | 0.25±0.01 - 0.13±0.06 | 0.035* |

* and **Significant at 5 and 1% probability, respectively.

higher oxygen consumption rate after 20 min in the dark might be due to the characteristic higher activity of the shrimp in the dark with a subsequent increase in metabolism and oxygen consumption (Dall et al., 1990; Wassenberg & Hill, 1994). The existence of significant differences among the different times the shrimp were maintained under hypoxic conditions, both in the dark and in the presence of light, might also be due to the lower water oxygen concentration over time, which possibly limited the shrimp oxygen consumption.

## Conclusion

With 4.1 mg $O_2$ $L^{-1}$ in the water and in absence of aeration, critical oxygen concentrations (0.65 mg $L^{-1}$) for *Litopenaeus vannamei* in superintensive culture condition would be reached at 34.7 and 31.8 min in the presence and in the absence of light, respectively.

## Acknowledgements

To Conselho Nacional de Desenvolvimento Científico e Tecnológico and to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, for the postdoctorate grants; to Dr. Al Stokes, manager of Waddell Mariculture Center (South Carolina Department of Natural Resources), for all the aid received.

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Received on October 17, 2008 and accepted on April 30, 2009