COMBINED EFFECT OF BODY WEIGHT, TEMPERATURE AND SALINITY ON SHRIMP *Litopenaeus vannamei* OXYGEN CONSUMPTION RATE

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ABSTRACT

Aiming to optimize the calculations of mechanical aeration requirements in *Litopenaeus vannamei* marine shrimp cultures, oxygen consumption was quantified in combined conditions of temperature (20, 25 and 30°C) and salinity (1, 13, 25 and 37 ‰) at three body weights (2, 6 and 12 g) for juvenile *L. vannamei*. To measure oxygen consumption, shrimps were placed in a semi-open respirometry system. Results demonstrate that temperature, salinity, shrimp size and the interaction of these parameters significantly influence the specific oxygen consumption (mg O_2 g^{-1} h^{-1}). The 2-g shrimp perhaps suffered osmotic stress and consumed more oxygen at salinity 37 ‰, whereas 6 and 12-g shrimp suffered such stress at salinity 1 ‰. At 25 and 30°C oxygen consumption was more stable at salinities 13 and 25 ‰ for all groups. At 20°C and salinity below 25 ‰ oxygen consumption was higher, possibly due to the reduced hyperosmoregulatory ability in lower temperatures. The resulting regression equations allowed the calculation of *L. vannamei* shrimp oxygen consumption at the temperatures, salinities and sizes tested in this study. The equations can be used for the estimation of the environmental capacity and also the mechanical aeration requirements to secure ideal levels of oxygen in *L. vannamei* culture systems.

RESUMO

Com o objetivo de aperfeiçoar os cálculos a cerca da necessidade de aeração mecânica em sistemas de cultivo de camarão marinho *Litopenaeus vannamei*, foi quantificado o consumo de oxigênio em condições combinadas de temperaturas (20, 25 e 30°C) e salinidades (1, 13, 25 e 37 ‰) para juvenis de *L. vannamei* com três tamanhos diferentes (2, 6 e 12 g). Para medir o consumo de oxigênio por animal foi utilizado um sistema de respirometria do tipo semi-aberto. Os resultados evidenciaram que a temperatura, salinidade, peso do animal e a interação dos três fatores influenciam significativamente o consumo de oxigênio específico (mg O_2 g^{-1} h^{-1}). Animais menores (2 g) possivelmente sofreram estresse osmótico com maior consumo de oxigênio em salinidades de 37 ‰, enquanto que em animais maiores (6 e 12 g) este estresse foi observado em 1 ‰. Em temperaturas de 25 e 30°C o consumo apresentou-se mais estável em 13 e 25 ‰ em todos tamanhos analisados, sendo que em 20°C o consumo abaixo de 25 ‰ foi maior, possivelmente devido ao comprometimento da capacidade hiperosmorregulatória em baixas temperaturas. Com base nos resultados encontrados, foram obtidas equações de regressão que permitem calcular o consumo de oxigênio de *L. vannamei* para as diferentes combinações de temperaturas, salinidades e tamanhos avaliados neste trabalho. Estas equações podem ser empregadas em cálculos de capacidade suporte dos ambientes de cultivo, bem como em cálculos a cerca da quantidade de aeração mecânica necessária para manutenção de valores ideais de oxigênio dissolvido nos sistemas de produção de *L. vannamei.*

Descriptors: Oxygen consumption, Temperature, Salinity, *Litopenaeus vannamei.*
Descritores: Consumo de oxigênio, Temperatura, Salinidade, *Litopenaeus vannamei.*

INTRODUCTION

The Pacific white shrimp, *Litopenaeus vannamei*, is native of the eastern Pacific Ocean, commonly caught or farmed for human food. This specie habits the eastern Pacific, from Sonora (Mexico) to Tumbes (northern Peru), into temperature and salinity ranges of 15-28°C and 5-45 ‰ (natural environment), and 20-33°C and 0-40 ‰ (culture ponds). In America, the main sources of cultured shrimp are Ecuador, Brazil and Mexico (AQUACOP, 1984; WYBAN; SWEENEY, 1991). In Brazil, in the northeast region, all of 70,000 ton of shrimp/year produced in marine farms, is *L. vannamei* (CAVALLI et al., 2007).

Dissolved oxygen is considered the most limiting factor in semi-intensive and intensive aquaculture systems as it participates in natural

biological processes and also determines the capacity of the aquaculture environment (BOYD, 1990; VINATEA, 1997). During the shrimp grow-out, it is crucial to maintain adequate oxygen levels. Inappropriate conditions are potential stressors to animals, resulting in limited growth, increased susceptibility to diseases, and even mortalities in extreme cases (BOYD; TUCKER, 1998; JIANG et al., 2005). With the intensification of shrimp culture, oxygen from natural sources (primary photosynthetic production and atmospheric diffusion) become insufficient to provide adequate concentrations, thus mechanical aeration has become the most efficient method to increase oxygen supply allowing higher stocking densities, better effluent quality, and eventually, yield enhancement (BOYD; WATTEN, 1989; FAST; BOYD, 1992).

To determine the oxygen demand in aquaculture ponds, in addition to the animals' respiration rate, which, according to Fast and Lannan (1992), can contribute with more than 10% of the respiration losses in shrimp culture systems, it is necessary to know the water and sediment respiration rates (SANTA; VINATEA, 2007). In super-intensive cultures this demand can be more than 30% (BROWDY et al., 2001; BURFORD et al., 2003; HARGREAVES, 2006; DE SCHRYVER et al., 2008).

Water temperature and salinity are considered to be the main abiotic factors influencing oxygen consumption in aquatic animals (VERNBERG, 1983). Crustaceans' metabolism is directly influenced by temperature fluctuations of the surrounding environment, as enzymatic reactions are temperature-dependant and crustaceans' body temperature is not internally regulated (RANDALL et al., 1997). Within the tolerated temperature variation range, oxygen consumption rate increases constantly and regularly with temperature elevation. In general, an increase of 10°C results in oxygen consumption two to three times higher. Such increase is denominated thermal coefficient (Q_{10}) and represents the degree of sensibility of an organism to temperature (SCHMIDT-NIELSEN, 1999).

Salinity fluctuations also cause alterations in the metabolic rate as a result of a series of behavioral and physiological changes, such as osmoregulation. The need to maintain within strait limits the volume and the concentration of solutes in the body fluids, which invariably differ from that of the surrounding environment, compels the animals to keep adequate mechanisms to sustain the equilibrium, guarantee ionic and osmotic homeostasis, and protect internal tissues against oscillations of the surrounding environment with metabolic costs that can reflect on oxygen consumption (MANTEL; FARMER, 1983). Furthermore, there might be a complex interaction between temperature and salinity, with one variable modulating the effect of the other in the metabolic response (MANTEL; FARMER, 1983; VERNBERG, 1983).

The exact requirement of mechanical aeration in shrimp aquaculture is complex to determine since the production units present different ecological characteristics and, thus, different oxygen demands (BOYD, 1989). Therefore, the determination of the respiration rate of the white shrimp *L. vannamei* in different environmental conditions is essential for the improvement of the calculations to quantify mechanical aeration requirements (VINATEA; CARVALHO, 2007), contributing to enhance the energetic efficiency of marine shrimp culture systems. In this way, the objective of the present study was to quantify and assess the influence of temperature, salinity, and the combination of both on the oxygen consumption rate of juvenile *L. vannamei* of different body weights.

Materials and Methods

Factorial design was used combining temperatures (20, 25 and 30°C) and salinities (1, 13, 25 and 37 ‰) for juvenile *L. vannamei* of three size groups (2, 6 and 12 g). For each combination, oxygen consumption of resting animals was quantified (CECH, 1990).

A total of 306 juvenile *L. vannamei* shrimp Marine Shrimp Laboratory (LCM), Federal University of Santa Catarina (Florianopolis, Santa Catarina, Brazil) were used for the three size groups with mean body weight $\pm$ standard deviation of 11.88 $\pm$ 0.88 g, 6.17 $\pm$ 0.77 g, and 2.27 $\pm$ 0.57 g. Animals were cultured in 1000-L tanks kept inside a greenhouse and fed with 35% crude protein pelleted feed, according to their developmental stage and the LCM feeding protocols, which are based on tray consumption. Salinity and pH were weekly monitored with an optical salinometer (Aquafauna Inc.) and pH-meter (Hanna Inc.), respectively, and temperature and dissolved oxygen were daily measured with a digital oxymeter (YSI-55). About 50% of the tank water was changed at least once a week, depending on the water quality parameters.

As mentioned above, shrimps were divided into three size groups according to body weight: small (1 to 4 g), medium (5 to 8 g) and large (10 to 14 g). Each group consisted of 15 shrimps and they were exposed to the pre-determined combinations of temperature and salinity. Hundred liter tanks were partitioned in three, with plastic screen, to hold the three size groups simultaneously at a temperature of 27-28°C and salinity of 32-34 ‰, values close to those found in the original tanks. Shrimp were fed twice a day *ad libitum*.

Twenty-four hours after, shrimps were stocked and water temperature was gradually adjusted either heating with 100-W heaters or cooling with cold water coils. Natural seawater salinity was adjusted after 48 h exposure period to test temperature. Basically, initial salinity (34 ‰) was adjusted to 32 ‰ and the adjustment period protocol, adapted from McGraw and Scarpa (2004), consisted of a constant decrease of 7.9% h^{-1} for salinity 25.0 ‰ (3 h), 7.3% h^{-1} for salinity 13.0 ‰ (12 h) and 7% h^{-1} for salinity 1.0 ‰ (48 h) using a system with constant influx of dechlorinated fresh water (132 mL min^{-1}, 122 mL min^{-1}, and 117 mL min^{-1}, for 25, 13 and 1 ‰, respectively) and a drain pipe to keep tank water volume constant. Only in the treatment with salinity more than 34 ‰, sodium chloride PA was added at a rate of 2.0 ‰ h^{-1} to increase salinity to 37 ‰.

After desired salinities were reached, shrimp were kept at the combined conditions for at least 2 days before submitting to oxygen consumption measurement procedures. Only shrimp with hard exoskeleton were selected for the oxygen consumption trials. Shrimp were submitted to a 24-h fasting to reduce oxygen demand due to the digestive process (SPANOPOULOS-HERNÁNDEZ et al., 2005).

Semi-open respirometry system constant water influx during shrimp adjustment period to the system and no water influx during dissolved oxygen concentration readings were used. Polypropylene containers of three different sizes (40, 270 and 530 mL) were used as respirometers for the small, medium and large size shrimp groups. All respirometers were volume-calibrated and numbered. Each respirometer had an adapted inlet for a second connection and a water outlet, so during shrimp adaptation period constant water flow was kept proportional to the volume of the respirometer. Water flows were adjusted to 100, 270 and 530 mL min^{-1}. Respirometers were semi-submerse in the respirometry water system (30 L PVC box) in the test salinity and temperature, so as to ensure temperature was maintained constant during readings. The water used during the tests was kept in a tank right under the boxes with the respirometers and it was pumped to recirculate through each respirometer and the boxes, thus keeping the desired water quality characteristics (temperature, salinity and dissolved oxygen). The respirometry water system was covered with black plastic to avoid disturbance and to ensure the shrimp rest in standard metabolic rate.

Following adaptation period, respirometer outlet was connected to a pump system adjusted to the same flow of the respirometer inlet. Outflow water was sucked and forced through a device connected to a dissolved oxygen meter (YSI 55), calibrated according to the salinity. Then, water went through the outlet connection and returned to the respirometer through the adapted inlet. The respirometer water inlet used for acclimating shrimp and keeping high levels of dissolved oxygen was then shut and only the water volume of the respirometer and of the system connected to the pump (35 mL) circulated through the system. Readings started 1 minute after pump was on to ensure stabilization of initial values, and followed every 30 seconds until 3 minutes and every 1 minute until the final reading, i.e., when dissolved oxygen reached 70% saturation (MARTINEZ-PALACIOS et al., 1996), to avoid influence of oxygen tension on consumption, or to a maximum of 15 minutes per respirometer. For every two respirometers (two replicates) one unit was used as identical control but with no shrimp to detect the decrease during readings due to the system itself and to animal respiration. Readings in the control respirometer had the same duration as the corresponding trial unit.

After reading, shrimp were weighed in a 0.01-g precision scale and volume measured in 50 or 100 mL graduated cylinders.

To determine the time required for the stabilization of the shrimp oxygen consumption after introduction in the respirometer, consumption in time zero (immediately after introduction), and after 1, 2, 3 and 4 h inside the system was measured for the three shrimp test sizes. Four shrimp per group size with mean body weights and standard deviations of 1.68 $\pm$ 0.37g, 7.45 $\pm$ 0.59 g, and 11.64 $\pm$ 0.40 g were tested. This trial was carried out at 27°C and salinity 32 ‰.

The total oxygen consumption per shrimp was obtained using the following equation:

$$VO_2 = \frac{(\text{Shrimp } O_2 \text{ consumption} * \text{total volume}) - (\text{Control } O_2 \text{ consumption} * \text{total volume})}{1000 * \text{Time}} \quad (1)$$

where:
VO_2 = Shrimp oxygen consumption (mg h^{-1})
O_2 consumption = Final value – Initial value read by the oxygen meter (mg L^{-1}).
Total volume = (respirometer volume + reading system volume) - shrimp volume (mL).
Time = Oxygen reading time for each unit (h).

The specific oxygen consumption (mg O_2/g shrimp/h) was obtained dividing the VO_2 by the shrimp wet weight (g).

The thermal coefficient was obtained for each experimental combination by the equation (SHMIDT-NIELSEN, 1999):

$$Q_{10} = (R_2/R_1)^{10/T2-T1} \quad (2)$$

where: R_1 and R_2 are metabolic rates (specific oxygen consumption) at temperatures T_1 and T_2, respectively.

Data from the respirometer adaptation test were submitted to ANOVA ($p < 0.05$) followed by Tukey's multiple comparisons test ($p < 0.05$). For the oxygen consumption data in the combined experimental conditions, regression equations were obtained for total individual consumption and, from them regression equations derived for the specific oxygen consumption (oxygen consumption per mass unit). Individual consumption regressions were compared by ANCOVA ($p < 0.05$) followed by Tukey's multiple comparisons test ($p < 0.05$) (ZAR, 1996). The effect of temperature, salinity, shrimp size and their interactions on the specific oxygen consumption was assessed by multi-factorial analysis of variance ($p < 0.05$), as well as the effect of temperature and salinity on the specific oxygen consumption in each size group, followed by Tukey's multiple comparisons ($p < 0.05$). The thermal coefficient obtained in each shrimp size were compared by ANOVA ($p < 0.05$) followed too by Tukey's multiple comparisons test.

Analyses were performed using STATISTICA 6.0 and Excel 2003 applications.

RESULTS

Dissolved oxygen values were influenced by the flow applied in the system. As the oxygen meter probe demands constant agitation of the water over the membrane, the higher the flow, the closer would the values be to those measured in the usual manner (stirring). However, the difference between the initial and final values used for the calculation of oxygen consumption would absorb such error. Because of this result, water flow in the small respirometer was almost twice as high as the volume of the respirometer. Figure 1 shows the behavior of the oxygen values in the 50-mL respirometer at different water flows. As for the behavior of the oxygen consumption in the control unit, values were stable during the reading time with a slight reduction between the initial and final readings (Fig. 2).

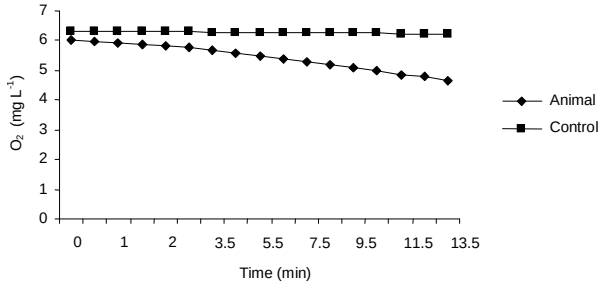

Fig. 1. Behavior of dissolved oxygen in different water flows in the 50-mL respirometer.

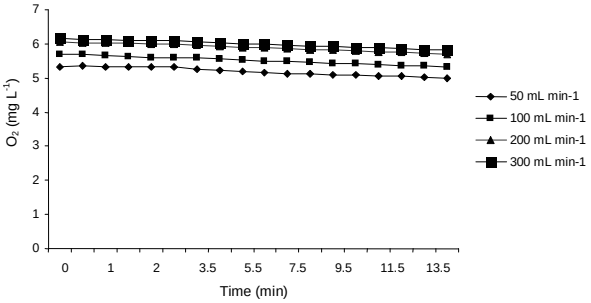

Fig. 2. Behavior of dissolved oxygen during readings in the control and trial units.

Statistical test verified that one hour after introduction to the respirometer, shrimp oxygen consumption was stabilized for the three size groups. Two hours adjustment period was adopted as the protocol before readings were commenced. Oxygen consumption rate along the hours was similar for the three size groups and followed the model presented (Table 1).

Power regressions representing oxygen consumption at the different conditions for individual consumption and consumption per specific body weight are in Table 2.

The ANCOVA test used to compare the equations for individual consumption demonstrated that inclinations are significantly different ($p < 0.05$), i.e., the variation rate of oxygen consumption per shrimp per unit of weight was influenced by the treatments, and it was followed by Tukey's multiple comparison test (Table 3).

Table 1. *Litopenaeus vannamei* mean oxygen consumption per hour inside the respirometer (n = 4).

	O_2 consumption (mg h^{-1})				
Adaptation time (h)	0	1	2	3	4
Small size group					
Mean	1.17^a	0.62^b	0.66^b	0.52^b	0.66^b
Standard Deviation	0.29	0.19	0.35	0.10	0.40
Medium size group					
Mean	5.18^a	2.85^b	2.27^b	2.49^b	2.17^b
Standard Deviation	0.22	0.59	0.48	0.53	0.48
Large size group					
Mean	7.11^a	4.07^b	3.43^b	3.36^b	3.25^b
Standard Deviation	0.60	0.67	0.71	1.04	0.59

Different letters among oxygen consumption means for each size group indicate significant difference between the hours ($p < 0.05$).

Table 2. Regression equations for *Litopenaeus vannamei* oxygen consumption and specific consumption in the different combinations of temperature and salinity.

Temperature (°C)	Salinity (‰)	N	Individual consumption rate (mg O_2 $shrimp^{-1}$ h^{-1})	R^2	Specific consumption rate (mg O_2 g^{-1} h^{-1})
30	37	29	$0.4172W^{0.887}$	0.86	$0.4172W^{-0.113}$
30	25	29	$0.3343W^{0.9835}$	0.89	$0.3343W^{-0.0165}$
30	13	27	$0.3112W^{1.0223}$	0.92	$0.3112W^{0.0223}$
30	1	24	$0.3011W^{1.1492}$	0.96	$0.3011W^{0.1492}$
25	37	29	$0.4407W^{0.6229}$	0.67	$0.4407W^{-0.3771}$
25	25	28	$0.2358W^{1.0248}$	0.77	$0.2358W^{0.0248}$
25	13	25	$0.2422W^{1.0192}$	0.80	$0.2422W^{0.0192}$
25	1	19	$0.2049W^{1.0952}$	0.80	$0.2049W^{0.0952}$
20	37	29	$0.0934W^{1.3153}$	0.87	$0.0934W^{0.3153}$
20	25	26	$0.1145W^{1.216}$	0.91	$0.1145W^{0.216}$
20	13	27	$0.1615W^{1.1378}$	0.91	$0.1615W^{0.1378}$
20	1	14	$0.1907W^{0.9388}$	0.90	$0.1907W^{-0.0612}$

W = wet weight (g).

The multi-factorial analysis of variance showed that the specific oxygen consumption is influenced by temperature, salinity, body weight and the interaction of these factors (Table 4, Fig. 3).

At 25 and 30°C oxygen consumption tended to be more stable at 13 and 25 ‰ salinity, at all sizes tested. At 1 ‰ salinity, oxygen consumption was higher in the large size group than in the small size group especially at 30°C, whereas at 25°C such effect was practically attenuated. The opposite was observed in the small size group when high oxygen consumption in those temperatures occurred at salinity 37 ‰. At 20°C specific oxygen consumption behaved differently in the different salinities for the three size groups when compared to 25 and 30°C. At 20°C oxygen consumption was higher in salinities below 25 ‰, except at 1 ‰ in the medium and large size groups.

Thermal coefficients for the temperature interval (20-30°C) analyzed in this study evidenced higher sensibility at salinity 37 ‰ for the small size group, whereas in the medium and large size groups sensibility was higher at 1 ‰ (Table 5). Based on the influence of shrimp size on the specific oxygen consumption, equations for the calculation of specific oxygen consumption per size group as a function of temperature, salinity and shrimp body weight were obtained (Table 6).

Table 3. ANCOVA and multiple comparison test between regressions for *Litopenaeus vannamei* oxygen consumption.

Inclinations (b)	Multiple Comparison (Tukey's test)			
F	30-37^{a,b}	30-25^{a,b,c}	30-13^{a,b}	30-1^{b}
3.91 ($p < 0.05$)	25-37^{c,d,e}	25-25^{a,b,d}	25-13^{a,b,d}	25-1^{a,b,d}
	20-37^{a,d}	20-25^{a,b,d}	20-13^{a,b,d}	20-1^{d,e}

Different letters indicate statistical differences between the temperature-salinity combinations ($p < 0.05$).

Table 4. Multi-factorial ANOVA for *Litopenaeus vannamei* specific oxygen consumption in the different experimental combinations.

Source	DF	SS	SM	Calculated *F*	*p*
Temperature	2	1.3487	0.6744	10603.6	***
Salinity	3	0.0161	0.0054	84.6	***
Body weight	2	0.0033	0.0017	26	***
Interaction	12	0.1205	0.0100	157.9	***
Error	270	0.0172	0.0010		

*** ($p < 0.001$).

Fig. 3. Interaction of temperature, salinity and body weight on specific oxygen consumption. Different letters indicate statistical differences between temperature and salinity combinations for each size group ($p < 0.05$). S: small, M: medium, L: large.

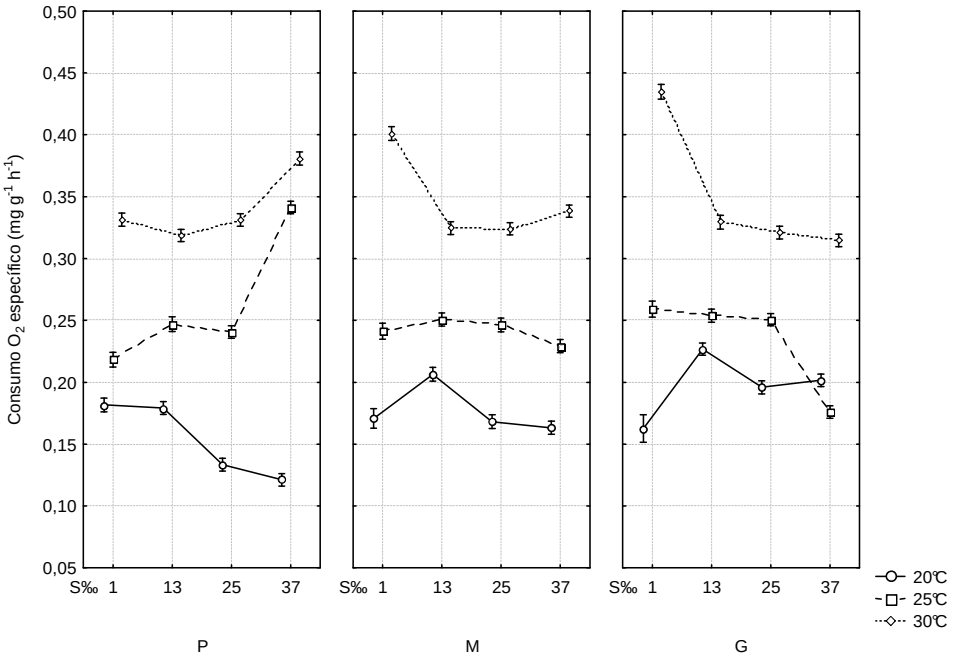

Table 5. *Litopenaeus vannamei* thermal coefficients for the three size groups per salinity tested. S = small; M = medium; L = large.

	Salinity (‰)			
Temperature (20-30°C)	37	25	13	1
S	3.14[a]	2.48[a]	1.78[a]	1.82[a]
M	2.07[b]	1.93[a]	1.57[a]	2.35[b]
L	1.56[c]	1.64[b]	1.45[a]	2.67[b]

Different letters at the same column indicate statistical differences between size groups ($p < 0.05$).

Table 6. Regression equations obtained for the calculation of the *Litopenaeus vannamei* specific oxygen consumption (mg O_2 g^{-1} h^{-1}) in the condition intervals tested. S = small; M = medium; L = large.

Shrimp size	Regression	R^2	Adjusted R^2
S	-0.2168 + 0.0188*T + 0.0008*S – 0.0080*W	0.8272	0.8222
M	-0.1717 + 0.0162*T – 0.0009*S – 0.064*W	0.9245	0.9221
L	-0.0281 + 0.0135*T – 0.0019*S – 0.0007*W	0.7749	0.7679

T= temperature (°C); S= salinity (‰); W= wet weight (g).

DISCUSSION

The result from this study demonstrated that 1 h is sufficient for the stabilization of oxygen consumption of *L. vannamei*, but 2 h was adopted as a standard protocol. Such result may reflect the high degree of adjustment of the shrimp used in the experiment to laboratory conditions, i.e., they tolerate handling and confinement very well.

According to the angular coefficient of the specific consumption regression equations, oxygen consumption per mass unit did not show any clear tendency as a function of weight in the different temperature-salinity combinations tested. The inverse relationship between metabolic rate and body mass is applicable within species and between species, as reported in studies with crustaceans such as *M. acanthurus* (GASCA-LEYVA et al., 1991), *L. vannamei* (MARTINEZ-PALACIOS et al., 1996) and *L. stylirostris* (SPANOPOULOS-HERNÁNDEZ et al., 2005). Nevertheless, according to RANDALL et al. (1997), this relation is frequently hard to demonstrate within same specie. As general, body mass variation is small compared to that between species, further to other factors that can exert superposed effects. This relation has not been observed in all treatments, which demonstrates the effect of abiotic factors, i.e., salinity and temperature on oxygen consumption, as well as their interaction, in addition to endogenous factors such as body weight, could be influenced the respiratory response at different experimental combinations. Such relation has not been observed too by Yagi et al. (1990) in *Palaemon serratus* larvae.

The effect of salinity on oxygen consumption in the present study does not follow a predicted pattern for the animal response as mentioned by Vernberg (1983), and yet the interaction between temperature and salinity seems not to affect predictably the oxygen consumption in a determined species. Little or no effect of salinity on oxygen consumption have been reported for *P. monodon* and *P. serratus* (SALVATO et al., 2001), *Penaues setiferus* (ROSAS et al., 1999), and *Farfantepenaues paulensis* (LEMOS et al., 2001), whereas others report direct influence in *F. californiensis* (VILLARREAL et al., 2003), *L. stylirostris* (SPANOPOULOS-HERNÁNDEZ et al., 2005), *L. vannamei* (VILLARREAL et al., 1994), *P. serratus* (YAGI et al., 1990), *M. acanthurus* (GASCA e LEYVA et al., 1991), *M. japonicus* (SETIARTO et al., 2004), or yet, others report the effect of the interaction of temperature and salinity on oxygen consumption in *P. serratus* (YAGI et al., 1990), *M. acanthurus* (GASCA-LEYVA et al., 1991), *L. vannamei* (VILLARREAL et al., 1994), and *L. stylirostris* (SPANOPOULOS-HERNÁNDEZ et al., 2005).

In this study, at 25 and 30°C oxygen consumption was more stable for 13 and 25 ‰ in all size groups, the latter being close to the isosmotic point for *L. vannamei* reported by Castille and Lawrence (1981): 24.7 ‰ (718 mOs kg^{-1}). The behavior of oxygen consumption in salinity 1 ‰ demonstrated that small shrimp may have more ability to osmoregulate in such condition when compared to larger shrimp. The opposite has been observed at salinity 37 ‰ with higher oxygen consumption. The behavior was inversed in the medium and large size groups, i.e., consumption at 1 ‰ was higher at 30°C, and at 25°C such effect was practically attenuated. It should be taken into account that small shrimp are better osmoregulators than larger animals (VARGAS-ALBORES; OCHOA, 1992; LEMAIRE et al., 2002). This may be due to higher gill surface-volume relation in small animals than in larger animals, which means the relation between water content and gill surface area is higher, thus facilitating ion exchange (RANDALL et al., 1997). Villarreal et al. (1994) suggested, however, that there is a possible loss in the osmoregulatory ability at 35 ‰ at 28 and 32°C, with high oxygen consumption in *L. vannamei* post-larvae in these conditions. Lemos et al. (2001) also reported that *F. paulensis* post-larvae would grow less with low

protein content at 34 ‰ and 26°C. A similar effect was observed in the small size group, suggesting that they would suffer higher osmotic stress in hyperosmotic water (37 ‰), 12 ‰ above the isosmotic point with higher oxygen consumption, whereas in larger animals the stress would be observed in extreme hyposmotic water (1 ‰), 24 ‰ below isosmotic point, as confirmed by the thermal coefficients determined in this study, when a higher sensibility of small shrimp at salinity 37 ‰ has clearly been seen, as well as with larger shrimp at 1 ‰.

Osmoregulatory ability in low salinities can vary between euryhaline species, as observed by Castille and Lawrence (1981), who reported that juvenile *Penaeus aztecus* and *P. duorarum* are better hyperosmoregulators than *P. setiferus*, *L. stylirostris* and *L. vannamei*, but no difference in hyposmoregulation was detected. Furthermore, according to Mantel and Farmer (1983), increased oxygen consumption is expected in osmoregulatory animals in hyposmotic water, i.e., due to difference in osmotic pressure the animal tends to gain water and lose salts, which demands higher metabolic expenditure due to active transportation of salt against the concentration gradient. Part of this increased oxygen consumption would be due to the increase of the ATP-ase enzymes activity involved in the cations transportation. Evidences of the increase in metabolic cost involved in osmoregulation were observed in post-larvae *L. vannamei* submitted to a diluted medium where activity of Na^+/K^+-ATPase, enzyme responsible for active transportation of Na^+ to the hemolymph, was increased (PALÁCIOS et al., 2004). Lin et al., (2000) also reported the increase in urine production by the antennal gland to regulate body volume when *Penaeus monodon* was submitted to low salinity. Another important factor observed in crustaceans submitted to low salinities and indicators of increased metabolic rate, is the increase in the ammonia excretion rate and higher utilization of proteins as substrate (ROSAS et al., 1999; LEMOS et al., 2001; SETIARTO et al., 2004).

The differentiated behavior of specific oxygen consumption at 20°C was also observed by Martinez-Palacios et al. (1996) in *L. vannamei*, where the curves for that temperature did not follow the expected model. This temperature is close to the natural limit found and these results may represent some metabolic response or osmotic disturbance in low temperatures. According to Tian et al. (2004), the ability of shrimp to adapt to low temperatures is poor. Temperature acts on the transportation of ions implicated in osmoregulation and high temperatures provide better osmoregulation than lower temperatures, also euryhaline species resist better to hyposmotic stress in high temperatures (GILLES; PEQUEUX, 1983). In the present study, at 20°C oxygen consumption in hyposmotic water was higher, suggesting a higher metabolic expense involved in the hyperosmoregulation. Lemaire et al. (2002) clearly demonstrated the effect of temperature on *L. stylirostris* osmoregulatory ability. Low temperatures compromise hypo- and hyperosmoregulatory ability, but mostly the latter. Mugnier and Soyez (2005) reported for juvenile *Litopenaeus stylirostris* that reducing temperature from 28°C to 22°C did not affect hyposmoregulatory ability at salinity 36 ‰, contributing to the idea that the osmoregulatory ability in low temperatures is mostly affected in hyposmotic water. The extreme impairment of the hyperosmoregulatory ability due to low temperature could be observed in the medium and large size groups at 1 ‰, resulting in decreased oxygen consumption. The low oxygen consumption found in these conditions could be reflecting the physiological dysfunction with metabolic depletion to the extreme conditions, since lethargic animals, reduced feed intake and high mortalities were observed during the experiment. Gilles and Pequeux (1983) reported that one stressing factor is better handled than two factors simultaneously, in this case, extreme temperature and salinity. Such fact could be minimized with a slower adaptation to salinity 1 ‰ for temperature 20°C, as temperature can interfere in the final survival results (TSUZUKI; CAVALLI, 2000). The longer adjustment period could also result in better survival rate (MCGRAW; SCARPA, 2004), mainly when considering the adaptation of juvenile shrimp to low salinity water, as it is known that 15 to 20 post-larvae stage *L. vannamei* have great osmoregulatory ability (MCGRAW et al., 2002).

The differences found in the oxygen consumption in different salinities may be reflecting physiological alterations, including not only osmoregulatory mechanisms but also feeding, reproduction and locomotion (VERNBERG, 1983). Therefore, alterations in oxygen consumption do not reflect directly the energy expenditure with osmoregulation, and energy used for this process should be duly quantified based on considerations on thermodynamics (SCHMIDT-NIELSEN, 1999).

As it could be observed, although the ability of *L. vannamei* to adapt within a wide range of temperature and salinity, these are crucial factors for the results of zoo technical performance in culture systems. Ponce-Palafox et al. (1997) reported that within the optimum temperature range, tolerance to salinity is high and growth is unaffected. Therefore, in conditions where these parameters cannot be properly controlled, the animals' physiological needs must be considered for each given condition.

Considering the temperatures, salinities and shrimp sizes tested, it can be concluded that the most adequate conditions for the comfort of juvenile *L.*

vannamei in temperatures between 25 and 30°C would be salinities from 13 to 25 ‰, where recommendation is to avoid the extreme ends, and in temperatures below 20°C salinity above 25 ‰ would be ideal due to the impairment of the hyperosmoregulatory ability observed in lower salinities.

The regression equations for each size group resulting from this study can help calculating oxygen consumption rate for temperatures between 20 and 30°C, salinities between 1 and 37 ‰, according to shrimp size divided into small (1-5 g), medium (5-10 g) and large (11-15 g). The values found can serve as basis for the calculation of the environmental capacity, as well as for the calculations on the mechanical aeration requirements in *L. vannamei* shrimp culture.

Acknowledgements

The authors are thankful to Bernauer Aquacultura Ltda. and Mr. Paulo Manso for providing the equipment required for this study; to Dr. Daniel Lemos, from Instituto Oceanografico de São Paulo, for his recommendations about shrimp respirometry techniques; to Dr. Wilson Wasielesky and Dr. Aime Rachel Magalhães, for their valuable observations; to CAPES (Coordenação de Aperfeiçoamento e Pessoal de Nível Superior, Ministry of Education) for the scholarship for the first author.

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(Manuscript received 30 September 2008; revised 11 May 2009; accepted 1 August 2009)