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Effect of dissolved oxygen on the energy balance and survival of *Penaeus setiferus* juveniles

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ABSTRACT: *Penaeus setiferus*, the white shrimp from the Gulf of Mexico, is an abundant species in the coastal lagoons and estuaries, where it can experience anoxic conditions. This study was designed with the purpose of measuring the effects of dissolved oxygen (DO) on the assimilation of ingested food (*AS*), by measuring the respiratory rate (*R*) and the biomass production of *P. setiferus* during its growth (*P*) process ($AS = R + P$). Postlarvae of this species (PL_{27}: 27 d after the last metamorphic molting) were exposed to 2, 3, 4 and 5.8 mg l^{-1} DO for 50 d. *P* was obtained from the transformation into energy units (J g^{-1} dw d^{-1}) of the growth rate. The respiratory rate was determined by evaluating oxygen consumption of fasting shrimp and spontaneous activity (routine metabolism: R_{rout}) and measuring apparent heat increase (R_{AHI}). Throughout the experimental periods, survival was not affected by DO levels and remained within 77 and 85%. *P* was constant between 5.4 and 4 mg l^{-1} DO but decreased at lower DO levels ($p < 0.05$). R_{rout} was affected by DO, with the highest levels observed in shrimp exposed at 4 and 5.8 mg l^{-1} DO and the lowest at 2 mg l^{-1} DO ($p < 0.05$). The opposite effect was seen in R_{AHI}, where the highest levels were registered in shrimp kept at 2 mg l^{-1} DO and the lowest at 5.8 mg l^{-1} ($p < 0.05$). The reason behind a higher R_{AHI} in shrimp kept at a lower DO level was the result of more time being invested in the mechanical and biochemical transformation of the food, which acts as a metabolical brake. *AS* was constant between 5.8 and 4 mg l^{-1} DO, but decreased with respect to a DO reduction. Accordingly, a critical level was established for *AS* at 4 mg l^{-1} DO, below which *AS* becomes dependent on DO. In contrast, the amount of assimilated energy directed to production (*P*/*AS*) increased with respect to DO reduction when the shrimp were exposed to DO levels below 4 mg l^{-1}. These results show that the side effects produced by low DO levels are generally compensated by an increase in production efficiency despite reduced respiratory efficiency.

KEY WORDS: Dissolved oxygen · Production · Oxygen consumption · Assimilation · Survival · *Penaeus setiferus* juveniles

INTRODUCTION

According to Fry (1947), dissolved oxygen (DO) is a regulating metabolic factor in aquatic organisms. DO can limit the metabolic capacity and, consequently, biomass production. In general, penaeid shrimp are oxyregulators within limited DO intervals. Recent evidence has shown that *Penaeus setiferus* and *P. schmitti* postlarvae (PL_{15-18}) are oxyregulators at 4.5 to 5 mg l^{-1} DO, depending on salinity. Oxygen consumption below these levels becomes dependent on oxygen concentration, decreasing the metabolic capacity of shrimp as much as 26% (Rosas et al. 1997).

The following is the equation that integrates the elements of energetic balance (Lucas 1993):

$$C = F + U + R + P$$

where *C* is the ingested energy from food; *F* represents the energy lost in feces; *U*, the energy lost through

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nitrogen excretion; R, the energy of total metabolism; and P, the energy accumulated in tissue production. The amount of assimilated energy may be calculated as (Lucas 1993):

$$AS = C - (F + U) = P + R$$

In this equation, R can be expressed as:

$$R = R_{std} + R_{AHI} + R_{act}$$

where R_{std} is the energy from standard metabolism, R_{AHI} represents the metabolic energy invested in apparent heat increment and R_{act} indicates the metabolic energy used in active metabolism. Because of difficulties related to its measurement R_{act} has not been measured in shrimp and R_{std} has been frequently integrated from routine oxygen consumption measurements (R_{rout}) without considering R_{AHI} (Klein 1975, Dame & Vernberg 1982, Lucas 1993, Rosas et al. 1993a, b). R_{AHI} is a measurement of metabolic activity of the post-absorptive processes following food ingestion (Beamish & Tripple 1990). R_{AHI} has been hitherto mainly studied with respect to diet type and quality, having been used as an indicator of the energetic metabolism of the post-absorptive processes that follow the ingestion of food (Du-Preez et al. 1992, Hewitt 1992, Rosas et al. 1996). In some omnivorous-carnivorous shrimp species, R_{AHI} can represent as much as 6.4% of the ingested energy, depending on the proteins in the diet (Du-Preez et al. 1992, Rosas et al. 1996).

Production (P) is an integrative response of the physiological state of organisms that represents tissue accumulation in the animals. Energetically speaking, production is a measurement of anabolism and is determined by environmental conditions. Some studies have documented that dissolved oxygen can regulate penaeid shrimp growth, depending on experimental conditions. Seidman & Lawrence (1985) have reported that constant levels below 2 mg l^{-1} DO significantly reduce growth in juvenile *Penaeus vannamei* and *P. monodon*. Allan & Maguire (1991) found that exposure to 0.5–1.1 mg l^{-1} DO during a 4 to 12 h period did not affect growth rates in *P. monodon*. Stickle et al. (1989) have reported that hypoxia tolerance in *Callinectes sapidus* and *P. aztecus* is correlated with activity level and metabolic rate, demonstrating that both species of crustaceans, which are active swimmers, were very sensitive to hypoxia. Notwithstanding reports on DO effects on survival (Stickle et al. 1989, Allan & Maguire 1991) and oxygen consumption (Liao & Chien 1994, Rosas et al. 1997) in several penaeid shrimp, there is no information concerning the effects of DO on the energetic balance of any species.

Penaeus setiferus, the white shrimp from the Gulf of Mexico, is an abundant species in the coastal lagoons and estuaries of the Gulf of Mexico and has an attractive potential for shrimp culture development in the area (Hopkins et al. 1993). Dissolved oxygen may limit the natural distribution of the species and, at lower concentrations, may be responsible for growth decrease in *P. setiferus* intensive culture (Hopkins et al. 1993). The purpose of this study was to determine the effect of DO on: (1) growth and survival, and (2) the regulatory mechanism of the assimilated energy, by evaluating some of the elements of energetic balance ($R[R_{rout} + R_{AHI}] + P$) in juvenile shrimp.

MATERIALS AND METHODS

Growth. We used 374 postlarvae (27 d old postlarvae = PL_{27}), with an initial weight of 35 ± 0.06 mg dw (mean ± SE), obtained from larvae cultured from a single spawning in the Centro Regional de Investigaciones Pesqueras de Lerma-Campeche (INP) by the Experimental Marine Biology Group of the National Autonomous University of Mexico (UNAM). The mean initial weight of the shrimp was obtained from a sample of 50 postlarvae randomly captured from the group of experimental shrimp. Once the wet weight was determined, 50 shrimp postlarvae were sacrificed and dried (60°C) until a constant weight was reached. The remaining shrimp were randomly placed in twelve 70 l tanks with a 0.28 m^2 surface and an average density of 50 shrimp m^{-2} (14 shrimp $tank^{-1}$, Martínez et al. 1998). The shrimp were exposed to 4 different DO levels of 2, 3, 4, and 5.8 mg l^{-1}. Each concentration was repeated 3 times.

Natural seawater, previously filtered with sand (20 µm) and with cartridge filters (5 µm), was used. During the experimental period, water temperature was kept at 28 ± 0.5°C, salinity at 36 ± 1‰ and pH at 8 ± 0.1. DO levels were maintained constant, according to the experimental design. Throughout the 50 d duration of the experiment, shrimp were fed *ad libitum* rations 2 times a day (08:00 and 20:00 h). Two types of balanced food were used, prepared according to protein requirements of the species: 50% protein (between PL_{27} and PL_{35}) and 40% protein (between PL_{36} and the end of the experiment) (Gaxiola 1994; Table 1). Both diets were prepared following the described procedures in Rosas et al. (1996). Casein, with 90% protein, was used as the main source of protein in the diets. L-Arginine HCl was added to the casein to improve the amino acid balance to the amino acid composition of penaeid abdominal muscle. Diets were prepared by thoroughly mixing the dry ingredients with oil and then adding water until a stiff dough was formed. The dough was then passed through a meat grinder to form 5 mm diameter pellets. The pellets

Table 1. *Penaeus setiferus*. Composition (% of dry weight) of diets used in growth and metabolic experiments

Ingredients	Gaxiolina-40[c]	Gaxiolina-50
Fish meal	45	54
Soybean meal	30	36.5
Cholesterol	0.5	0.5
Cod liver oil	1.76	–
Soybean lecithin	1.00	1.00
Vitamin C	0.50	0.50
Vitamin and mineral mix[a]	2.50	2.50
Carboxymethyl cellulose	5.0	5.0
Talc as filling	13.74	–
Protein content (%)	40.15	50
Digestible energy (J g^{-1} diet)[b]	12.78	11.64

[a]Purina de México, México City, Mexico
[b]Calculated from digestible energy provided by Nose (1979)
[c]Used in metabolic experiments

were then dried at 60°C in a electric oven. After drying, the feed was broken up, sieved to obtain a convenient pellet size, and stored at –4°C.

Feces, non-ingested food and molts were removed, twice a day, from each tank, along with a 50% water exchange. Salinity, temperature, O_2 (YSI 51B) and pH (ORION 720A) were also measured twice a day. Ammonia nitrogen was measured once a week ($N\text{-}NH_3$) with an ion-selective electrode connected to an ORION 720A multianalyzer. At the end of the experiment, the shrimp were weighed on an analytic balance (±0.00005 g), sacrificed and dried at 60°C until constant weight was achieved. The growth rate (*GR*; mg d^{-1}) was related to survival through a performance index, which was defined as:

$$PI = GR \times S$$

where *PI* indicates the growth rate in mg d^{-1} and *S* the fractionated survival value. *PI* was used as an integrative indicator of the different experimental conditions. The value of 23.96 ± 0.72 J g^{-1} dw was used to transform the growth data into production units (*P*; J g^{-1} dw d^{-1}). This value was obtained from analyzing the energy content applied to the muscle of 10 shrimp by means of a calorimeter (Parr), previously calibrated with benzoic acid.

Experimental device. For the growth experiment, a modified device, based on Seidman & Lawrence (1985), was adapted to a semiclosed circulation system. Four 6" (15.24 cm) diameter and 2 m tall PVC columns were used as mixed gas systems. Seawater was supplied through gravity by means of an elevated tank. Gaseous nitrogen was provided through diffusers placed in the bottom part of the columns, just above the water outlet leading to the experimental tanks. Each nitrogen tank was connected to the nitrogen regulator and a vertical air PVC flowmeter, which permitted the control of nitrogen flow to the mixed gas columns. The tanks were covered with a plastic sheet to avoid partial water re-oxygenation. Seawater discharged from each experimental tank was collected in a lower tank, from which the water was again pumped to the elevated deposit. This deposit was vigorously aerated throughout the experimental period. The discharge reservoir was also used for the daily seawater exchange of the system.

Oxygen consumption. Once the growth experiment was concluded, we proceeded to determine the effect of DO on oxygen consumption of *Penaeus setiferus*. Oxygen consumption was measured in 20 treatment shrimp from the growth experiment from each DO level. The weight interval of these shrimp was between 0.6 and 1.5 g dw.

Oxygen consumption in shrimp was determined individually by a continuous flow respirometer in closed circuit (Martínez-Otero & Díaz-Iglesia 1975, Rosas et al. 1995). The shrimp were placed in a 250 ml chamber with a flow of seawater filtered with sand (20 µm), cartridge filter (5 µm), and diatoms (1 µm), and sterilized with UV. In each respirometric chamber the seawater flow was 33 ml min^{-1}. DO control of the chambers was achieved with a 6" (15.24 cm) diameter and 2 m tall PVC column to allow gas interchange (nitrogen for oxygen) in the seawater, prior to its entrance into the respirometric chambers. A 24 h period was considered sufficient to decrease the stress caused by handling. Shrimp were not fed during this period. Isolation of the shrimp from laboratory staff required covering the respirometers with a black translucent plastic lamina. Once the acclimatization period was over, routine oxygen consumption measurements were carried out during fasting and spontaneous activity (R_{rout}). Oxygen consumption was calculated as:

$$VO_2 = O_{2e} - O_{2ex} \times Fr$$

where VO_2 is oxygen consumption (mg O_2 h^{-1} $shrimp^{-1}$), O_{2e} indicates oxygen concentration at the entrance to the chamber (mg l^{-1}), O_{2ex} is oxygen concentration at the exit (mg l^{-1}) and *Fr* is the flow rate (ml h^{-1}). Oxygen concentration was measured by means of a digital oxymeter (YSI 50B digital) with a polarographic sensor (±0.01 mg l^{-1}), previously calibrated with oxygen-saturated seawater at 28°C. The shrimp were afterwards fed food pellet fragments of 0.051 g each in the respirometric chambers. The same amount of food was placed in a control chamber without organisms to estimate the oxygen lost by food decomposition. Oxygen consumption in the control chamber was insignificant (between 0.01 and 0.03 mg O_2 h^{-1} chamber). Oxygen consumption of fed shrimp was mea-

Table 2. *Penaeus setiferus*. Environmental seawater characteristics during growth experiments (50 d). Mean ± SE

Dissolved oxygen (mg l^{-1}) Nominal	Real	Temperature (°C)	Salinity (‰)	pH
2.0	2.04 ± 0.01	28 ± 0.5	33 ± 1	8 ± 0.01
3.0	3.01 ± 0.01	27 ± 0.5	34 ± 1	8 ± 0.01
4.0	4.02 ± 0.01	27 ± 0.5	32 ± 1	8 ± 0.01
5.0	5.83 ± 0.02	27 ± 0.5	32 ± 1	8 ± 0.01

sured every hour for a 9 h period, between 08:00 and 17:00 h. The described procedure for non-fed shrimp was followed to determine oxygen consumption of fed shrimp. Respirometric chambers were also covered during this experimental period. Once the experiment was concluded, the shrimp were weighed, sacrificed and dried at 60°C until constant weight was attained.

Oxygen consumption values (VO_2) during fasting and feeding were related to dry weight (DW_t) of the shrimp, by means of the following equation:

$$VO_2 = a\,DW_t^b$$

The specific rate of oxygen consumption VO_2 was obtained from $VO_2 = a$, when $DW_t = 1$ (Sánchez et al. 1991). Specific rate R_{rout} (J g^{-1} h^{-1}) was estimated from the VO_2 of the unfed shrimp. Specific rate apparent heat increase (R_{AHI}: J g^{-1} h^{-1}) was estimated from the difference between VO_2 of the unfed shrimp and the maximum value attained after feeding. A 14.3 J mg^{-1} conversion factor of oxygen consumption was used to transform the unfed and fed VO_2 to J g^{-1} dw (Lucas 1993). R_{AHI} (J g^{-1} dw d^{-1}) was estimated considering the time needed for peak oxygen consumption after feeding and the number of rations fed to the shrimp per day (n = 2). R_{rout} (J g^{-1} dw d^{-1}) was estimated considering the time during the day in which the shrimp were not fed.

Energy balance. Assimilated energy (AS) was estimated using the following balanced equation (J g^{-1} d^{-1}):

$$AS = P + R$$

where P is biomass production and R indicates respiration ($R = R_{rout} + R_{AHI}$).

Statistical analysis. The effect of dissolved oxygen on growth and survival was analyzed separately by an ANOVA. An arcsine square root transformation was used before analyzing the percentage values. A multiple variance analysis applied to the regression coefficient of the relation between oxygen consumption and weight determined the statistical difference in the respiratory rate after feeding at each DO level. The effect of DO on the routine metabolic rate R_{rout} and on the R_{AHI} was analyzed separately using ANOVA as well.

RESULTS

The system used to maintain dissolved oxygen revealed high stability. Variation in the 4 DO levels was 0.3%, equivalent to 0.1–0.2 mg l^{-1} DO (Table 2). Throughout the study, temperature was between 27 and 28°C, salinity between 32 and 34‰ and pH around 8 (Table 2).

Growth, survival and performance index (*PI*)

Growth decreased (mg dw d^{-1}) in relation to DO reduction. The growth rate of shrimp maintained at 2 mg l^{-1} DO was 32% lower than observed in shrimp exposed to 3 and 4 mg l^{-1} DO ($p < 0.05$; Table 3). Growth rate of shrimp in the control group was 1.74 times higher than the growth of shrimp exposed to 2 mg l^{-1} DO ($p < 0.05$). Survival of shrimp was between 77 and 85%; there were not significant differences between treatments ($p > 0.05$). A progressive *PI* decrease was observed with respect to DO reduction. *PI* obtained in 2 mg l^{-1} was 41% less than obtained at 5 mg l^{-1}, 32% less than that obtained at 4 mg l^{-1} DO and 21% less than that at 3 mg l^{-1} DO (Table 3).

Oxygen consumption

A significant relationship existed between the oxygen consumption rate and body weight in shrimp exposed to different DO levels, during fasting and feeding and food assimilation (Table 4). Oxygen consumption rate varied directly with DO concentration. Oxygen consumption rate of fasting shrimp exposed to 2 mg l^{-1} DO was 73% less than shrimp exposed to

Table 3. *Penaeus setiferus*. Effect of dissolved oxygen and density on growth (mg dw d^{-1}), survival (%) and performance index (*PI*) obtained during 50 experimental days. DO: dissolved oxygen (mg l^{-1}). Mean ± SE. Means with the same letter are not significantly different ($p > 0.05$)

DO (mg l^{-1})	Initial weight (mg dw)	Final weight (mg dw)	Growth rate (mg dw d^{-1})	Survival (%)	*PI* (mg dw d^{-1})
2	11.8 ± 0.1	180^a ± 7	3.36^a ± 0.20	85^a ± 5	2.85^a ± 0.15
3	11.8 ± 0.1	245^b ± 19	4.66^b ± 0.40	78^a ± 5	3.63^b ± 0.23
4	11.8 ± 0.1	285^c ± 24	5.46^c ± 0.30	77^a ± 7	4.20^c ± 0.25
5.8	11.8 ± 0.1	313^c ± 20	6.02^c ± 0.60	81^a ± 5	4.87^d ± 0.24

Table 4. *Penaeus setiferus*. Oxygen consumption (mg O_2 shrimp^{-1} h^{-1}) and dry weight (g) relationship obtained at different DO levels. Data of fasting (24 h) and feeding shrimp. $y = ax^b$, $p < 0.05$

		a	*b*	r^2	p
DO (mg l^{-1})	2.0				
Fasting	0	1.72	1.11	75.1	0.000
Feeding	1	2.18	1.20	79.6	0.001
	2	3.76	1.51	99.8	0.02
	3	5.59	1.61	96.4	0.03
	4	4.68	1.43	99.8	0.02
	5	3.98	1.44	99.7	0.03
	6	2.69	1.58	82.3	0.005
	7	1.85	1.12	76.4	0.032
DO (mg l^{-1})	3.0				
Fasting	0	3.22	1.65	80.2	0.02
Feeding	1	3.11	0.96	75.3	0.03
	2	5.03	1.00	76.4	0.01
	3	4.88	1.01	76.5	0.000
	4	3.58	0.99	74.4	0.009
	5	3.55	1.11	79.7	0.01
	6	3.48	1.36	78.8	0.02
	7	3.33	1.64	80.4	0.05
DO (mg l^{-1})	4.0				
Fasting	0	5.92	2.14	97.7	0.02
Feeding	1	6.24	1.61	83.3	0.015
	2	8.38	1.63	75.6	0.03
	3	7.29	1.60	80.0	0.02
	4	6.41	1.61	85.9	0.02
	5	6.00	1.69	88.4	0.05
	6	5.88	1.81	95.7	0.04
	7	5.23	2.16	98.8	0.06
DO (mg l^{-1})	5.8				
Fasting	0	6.29	1.54	99.9	0.02
Feeding	1	7.69	1.54	93.2	0.05
	2	8.08	1.50	86.8	0.03
	3	7.69	1.49	88.5	0.04
	4	7.31	1.49	92.7	0.001
	5	7.17	1.51	96.2	0.000
	6	6.62	1.49	97.2	0.02
	7	6.55	1.55	98.2	0.003

5.8 mg l^{-1} DO for 50 d ($p < 0.05$). Oxygen consumption rate increased after feeding in each of the treatments (Fig. 1). Oxygen consumption behavior of shrimp during feeding led to 2 groups: one composed of the shrimp maintained at 5.8 and 4 mg l^{-1} DO and the other composed by those maintained at 2 and 3 mg l^{-1} DO. Oxygen consumption was significantly longer than that of shrimp maintained at 2 and 3 mg l^{-1} DO. In each case, oxygen consumption increased rapidly after feeding, and decreased afterwards until reaching similar levels to those seen at the beginning of the experiment. The time required to achieve peak oxygen consumption after feeding was higher in shrimp maintained at 2 mg l^{-1} DO (3 h) than at the other DO levels (2 h, Table 5). Time used for daily feedings proved to be 6 h in 2 mg l^{-1} DO and 4 h in the remaining treatments (Table 5). R_{AHI} of shrimp maintained at 2 mg l^{-1} (332.0 J g^{-1} dw d^{-1}) was 3.24 times higher than that obtained in shrimp maintained at 5.8 mg l^{-1} DO (102.4 J g^{-1} dw d^{-1}) ($p < 0.05$). Intermediate R_{AHI} values were achieved in shrimp maintained at 3 and 4 mg l^{-1} DO (Table 5). The R_{AHI} coefficient was significantly higher in shrimp maintained at 2 mg l^{-1} DO (4.06%) than the R_{AHI} in shrimp from the other treatments (1.40% average value) (Table 5) ($p < 0.05$).

A reduction in R_{rout} was also directly related to DO concentration (Table 6). Shrimp maintain a constant R_{rout} between 5.8 and 4 mg l^{-1} DO, with a decrease at 3 and 2 mg l^{-1} DO. The R_{rout} of shrimp kept at 2 mg l^{-1} DO (442.7 J g^{-1} dw d^{-1}) was 25% of that of shrimp kept at 4 to 5.8 mg l^{-1} DO (average of 1746 J g^{-1} dw d^{-1}). Respiratory rate (R) of shrimp (R_{rout} + R_{AHI}) was also directly related to DO concentration (Table 6). Shrimp maintain a constant R between 5.8 and 4 mg l^{-1} DO (an average value of 1866.4 J g^{-1} dw d^{-1}), followed by a reduction at 2 to 3 mg l^{-1} DO. R in shrimp maintained at 2 mg l^{-1} DO (774.7 J g^{-1} dw d^{-1}) was 41% of that in shrimp maintained at 4 to 5.8 mg l^{-1} DO ($p < 0.05$).

The amount of energy directed to production (P) varied directly with DO concentration, with the highest values being observed in shrimp kept at 5.8 and 4 mg l^{-1} DO (144.5 and 130.9 J g^{-1} dw d^{-1}, respectively) and the lowest in shrimp at 2 mg l^{-1} DO (80.7 J g^{-1} dw d^{-1}) ($p < 0.05$; Table 6). P obtained in 2 mg l^{-1} DO was 56% of that observed at 5.8 mg l^{-1} DO.

Assimilation (AS) was the result of adding R to P. AS also varied directly with DO concentration (Table 6). AS values obtained at 2 mg l^{-1} DO (855.4 J g^{-1} dw d^{-1}) were 42% of values in shrimp maintained at 5.8 mg l^{-1} DO (2044.8 J g^{-1} dw d^{-1}). Again, no significant differences were found in the AS of shrimp maintained at 4 to 5.8 mg l^{-1} DO ($p > 0.05$; Table 6).

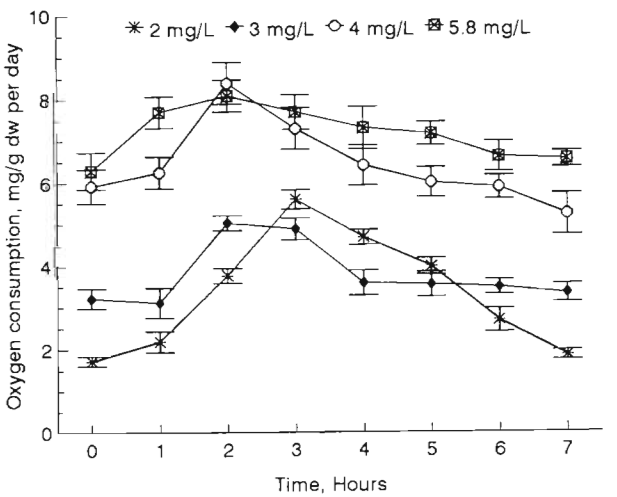

Fig. 1. *Penaeus setiferus*. Mean oxygen consumption (± SEM) related to feeding time and dissolved oxygen

Table 5. *Penaeus setiferus*. Apparent heat increment and AHI coefficient (AHI/energy content of food ingested $d^{-1} \times 100$) obtained at different DO levels. AHI values calculated from oxygen consumption (fasting and feeding) and time invested in feeding by day, considering the time to reach the peak per 2 rations per day. Means ± SE. Means with the same letter are not significantly different

DO	Oxygen consumption (mg g^{-1} dw h^{-1})		Time to reach the peak (hours feeding)	Time invested in feeding (h d^{-1})	AHI (J g^{-1} dw d^{-1})	AHI (% d^{-1})
	Fasting	Feeding				
2	$1.72^a \pm 0.12$	$5.59^a \pm 0.23$	3	6	$332.0^a \pm 23.1$	4.06 ± 0.17
3	$3.22^b \pm 0.24$	$5.03^b \pm 0.18$	2	4	$103.5^b \pm 7.7$	1.25 ± 0.04
4	$5.92^c \pm 0.41$	$8.38^c \pm 0.50$	2	4	$140.7^c \pm 9.7$	1.70 ± 0.10
5.8	$6.29^c \pm 0.44$	$8.08^c \pm 0.38$	2	4	$102.4^b \pm 7.2$	1.24 ± 0.06

Between 90 and 93% of *AS* was invested in *R* (Fig. 2). Respiratory efficiency (*R/AS*) was kept constant between 4 and 5.8 mg l^{-1} of DO (a 93% average value); *R/AS* decreased in shrimp maintained between 3 and 2 mg l^{-1} DO (average value of 90.4%). The opposite behavior was seen in net growth efficiency (*P/AS*) where the lowest values were registered in shrimp kept at 4 to 5.8 mg l^{-1} DO (average value of 6.9%) and the highest ones were found in shrimp at 2 to 3 mg l^{-1} DO (9.6% average value) (Fig. 2).

DISCUSSION

From the results in this study, 4 mg l^{-1} DO may be proposed as the critical oxygen level for the assimilation (*AS*) of ingested energy in juvenile *Penaeus setiferus*. DO primarily affected energy assimilated (*R* + *P*) as in the case for most marine invertebrates exposed to gradients of environmental factors (Guerin & Stickle 1992). Survival, biomass production (*P*), respiratory rate (*R*) and, therefore, food assimilation were kept constant at 4 to 5.8 mg l^{-1} DO. A significant reduction of the components in the energetic balance equation was seen in shrimp maintained below 4 mg l^{-1} DO. As reported (Rosas et al. 1997), juvenile *P. setiferus* are limited oxyregulators, capable of tolerating DO changes between 5.8 and 4 mg l^{-1}. Similar results were obtained by Stickle et al. (1989) in *P. aztecus* juveniles which were very sensitive to hypoxia with 28 d LC_{50} values between 60 and 80% air saturation (4.5 to 5.72 mg l^{-1}), depending on salinity. This oxyregulating ability has also been seen in postlarvae from this and other species. In a recent study, Rosas et al. (1997) documented that *P. setiferus* and *P. schmitti* postlarvae are limited oxyregulators between 6 and 4.5–5.0 mg l^{-1} of DO (93 to 70% air saturation), depending on salinity. We noted that the oxyregulatory ability of *P. setiferus* may be related to the energetic mechanisms involved in the use of the different available metabolic substrates under normoxic and hypoxic conditions. Based on O:N ratio measurements from our laboratory, it was shown that juveniles from this species are able under normoxic conditions to change the protein-lipid metabolic substrate (5.8 to 4 mg l^{-1} DO, 90 to 63% air saturation) primarily to proteins under hypoxic conditions (2 to 3 mg l^{-1} DO, 47 to 31% air saturation) (Rosas et al. in press). Herreid (1980) has noted several mechanisms

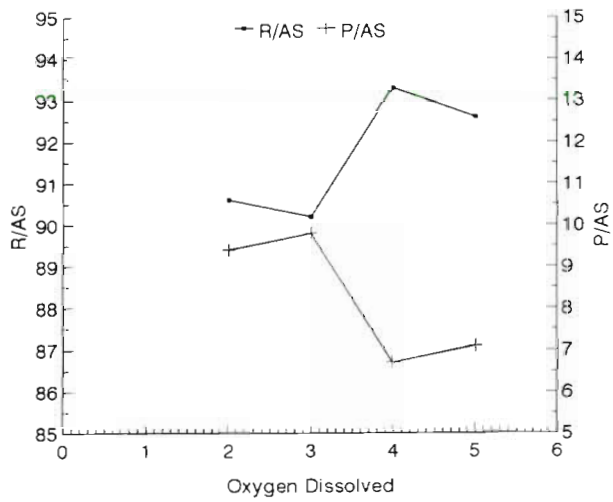

Fig. 2. *Penaeus setiferus*. Respiratory (*R*) and production (*P*) efficiencies (% of assimilation: *AS*) of shrimp exposed to different levels of dissolved oxygen

Table 6. *Penaeus setiferus*. Effect of dissolved oxygen level in the energy balance. Values in joules g^{-1} dw d^{-1}. Mean ± SE. Means with the same letter are not significantly different

DO (mg l^{-1})	*R*		*P*	*AS*
	R_{rout}	R_{AHI}		
2	$442.7^a \pm 30.8$	$332.0^a \pm 23.1$	$80.7^a \pm 4.8$	855.4
3	$920.9^b \pm 68.6$	$102.6^b \pm 7.7$	$111.7^b \pm 9.6$	1135.2
4	$1693.1^c \pm 116.8$	$139.4^c \pm 9.7$	$130.9^c \pm 7.2$	1963
5.8	$1798.9^c \pm 125.7$	$101.4^b \pm 7.2$	$144.5^c \pm 13.4$	2044.8

used by aquatic crustaceans to preserve oxygen consumption in case of a DO decrease. Changes in behavior (avoidance of hypoxic conditions) and physiological mechanisms (ventilatory activity and blood flow increase) have been mentioned as the most important ones (Stickle et al. 1989, Das & Stickle 1994). Because lipids are a readily available energy supply for respiratory metabolism (Beamish & Tripple 1990), it may be assumed that organisms would use lipids more intensely to maintain homeostasis.

Apparent heat increment (R_{AHI}), also known as specific dynamic action, has been associated with the calorigenic effect of food. This is a measurement of metabolic activity of post-absorptive processes following food ingestion (Beamish & Trippel 1990). R_{AHI} in crustaceans depends on the quality, quantity and energetic component balance of the food (Hewitt & Irving 1990, Du-Preez et al. 1992, Koshio et al. 1992, Rosas et al. 1996). Measured as oxygen consumption, R_{AHI} is a response controlled by environmental factors, such as dissolved oxygen. Results obtained in this study show that R_{AHI} increased with severity of hypoxia, with the lowest values in shrimp maintained at 5.8 mg l^{-1} DO (90% air saturation) (101.4 J g^{-1} dw d^{-1}) and the highest in those maintained at 2.0 mg l^{-1} DO (31% air saturation) (332.0 J g^{-1} dw d^{-1}). R_{AHI} estimation considered time invested by the shrimp in the feeding processes (mechanical and biochemical) and was related to the time required to reach maximum oxygen consumption after initiation of feeding. It was inferred that post-absorptive effects are highest at that time, and after that the metabolic rate also reflects food particle absorption and assimilation processes (Rosas et al. 1995). As a consequence of low DO levels, time to reach peak oxygen consumption was longer in shrimp, thereby directly affecting R_{AHI}. For R_{AHI} estimation, the 2 rations given to the shrimp per day were considered, and it was found that at low DO levels (2 mg l^{-1}, 31% air saturation), time spent on R_{AHI} was longer (6 h at low DO levels) in contrast to time per day used by shrimp kept at high DO levels (5.8 mg l^{-1} DO; 90% air saturation) (4 h). The fact that feeding produces an increased oxygen consumption rate by up to 60% in aquatic animals has been widely documented (Martínez-Palacios & Ross 1986, Du-Preez et al. 1992, Rosas et al. 1996). According to Beamish & Tripple (1990), this increase has been interpreted in terms of energy needs associated with AHI. Because DO limits the amount of available energy for general physiological functions under hypoxic conditions, it can be readily assumed that AHI will also decrease as a consequence of the reduction in the energy required for mechanical and biochemical transformations of the ingested food. The general metabolic level of juvenile *Penaeus setiferus* exposed to hypoxic conditions was not only reduced, as was AHI, but the process was also slower, increasing the time and amount of energy invested per day (R_{AHI}) with respect to those obtained under normoxic conditions. Therefore, DO under hypoxic conditions acted as a metabolic limitation in juvenile *P. setiferus*, thus implying higher energy expenditure and a reduction in available energy for growth. The energy-limited effect of low DO has been observed in *Callinectes sapidus* and *C. similis* (Das & Stickle 1994).

Juvenile *Penaeus setiferus* were able to preserve a practically constant biomass production (P) between 5.8 and 4 mg l^{-1} DO, which decreased under more severe hypoxia. Seidman & Lawrence (1985) observed that growth was constant in *P. vannamei* and *P. monodon* between 2 (31% air saturation) and 4 mg l^{-1} DO (62.5% air saturation). This information is evidence that shrimp of the genus *Penaeus* exhibit compensatory mechanisms to preserve growth, regardless of DO concentration. Results of measuring the O:N ratio in our laboratory (Rosas et al. in press) show that metabolism of mixed metabolic substrates (protein-lipids) are part of the compensatory mechanisms exhibited by the organisms to maintain P independent from DO.

At less than 4 mg l^{-1} of DO, growth was decreased. It has been observed that *Penaeus setiferus* utilizes protein as an energetic substrate under hypoxic conditions (Rosas et al. in press), partially accounting for the reduction in available amino acids for new protein formation. The activation of anaerobic mechanisms (Herreid 1980, Taylor & Spicer 1987), energy production reduction through aerobic metabolism and R_{AHI} related energetic losses help of understand the previously reported metabolic depression secondary to hypoxic conditions in aquatic organisms (Guppy et al. 1994). Even though it was not possible in this study to quantify the amount of ingested food, it was evident that shrimp maintained under hypoxic conditions ingested less food than those under normoxic conditions, thereby possibly affecting their growth. Seidman & Lawrence (1985) have also noted a reduction in general activity and amount of ingested food in juvenile *P. monodon* and *P. vannamei*.

Based on the results from this study, it is evident that respiratory efficiency (R/AS) is significantly affected by dissolved oxygen level below the critical 4 mg l^{-1} level (62.5% air saturation). In other words, the proportion of assimilated energy directed to respiration decreases as a function of the DO level. In contrast, the amount of assimilated energy directed to production (P/AS) increased with respect to DO reduction when the shrimp were exposed to DO levels below 4 mg l^{-1}. These results show that the effects produced by low DO levels are generally compensated by an increase in

production efficiency despite reduced respiratory efficiency. According to Lucas & Beninger (1985), the *P/AS* ratio is a good physiological condition index to reflect the general health status of shrimp exposed to a detrimental condition. In the present study the proportion of assimilated energy directed to production increases as a function of the DO level (Table 6). In that sense the experimental interval to which organisms were exposed (2 to 5.8 mg l^{-1} DO; 31 to 90% air saturation) can be interpreted as an interval that does not harm the general physiological mechanisms of juvenile *Penaeus setiferus* but only depresses their metabolism. Survival results indicate that no experimental condition produced massive mortality, evidence that juveniles of this species can probably recover after remaining in DO levels as low as 2 mg l^{-1}, even for long periods. In such circumstances the behavioral avoidance activities by juvenile penaeid shrimp observed by Renaud (1986) in *P. setiferus* and Stickle et al. (1989) in *P. aztecus* may temporarily allow them to escape oxygen deficient estuarine water, i.e. below 2 mg l^{-1}, without apparent physiological damage. These results agree with other reports that have proposed a limit security level of 2.3 mg l^{-1} DO for juvenile *P. setiferus* survival (Martínez et al. 1998).

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