Effect of Different Oxygen Concentrations on Physiological Energetics of Blue Shrimp, *Litopenaeus stylirostris* (Stimpson)

Ana Denisse Re*,[1] and Fernando Díaz[2]

[1]*Laboratorio de Nutrición Funcional Departamento de Biotecnología Marina Centro de Investigación Científica y Educación Superior de Ensenada, Carretera Ensenada-Tijuana # 3918. Ensenada Baja California México*

[2]*Laboratorio de Ecofisiología de Organismos Acuáticos Departamento de Biotecnología Marina Centro de Investigación Científica y Educación Superior de Ensenada, Carretera Ensenada-Tijuana # 3918. Ensenada Baja California, México*

Abstract: The influence of different oxygen concentrations on the bioenergetics parameters, osmoregulatory capacity and O:N atomic ratio of the blue shrimp, *Litopenaeus stylirostris* (Stimpson), was determined under laboratory conditions; shrimp were acclimated at 28 ºC and 25psu and exposed to three different oxygen concentrations 2, 4 and 6 mg L^{-1}. The critical oxygen level (COL) was determined at 5.5 mg L^{-1}. Growth, oxygen consumption (R), ammonium excretion (U), scope for growth (P) and apparent heat increment (AHI) were affected significantly ($P < 0.05$) when the organisms were exposed at different oxygen concentrations. The highest energy invested for routine metabolism and ammonium excretion was obtained in the animals maintained at 2 mg L^{-1}. The exposure of organisms to oxygen concentration (2 mg L^{-1}) increased three times the feces production (23.59%). The high quantity of energy channeled to scope for growth (2756 J g^{-1} day^{-1} d.w.) was obtained in the shrimp acclimated to an oxygen concentration of 6 mg L^{-1}. The O:N atomic ratio calculated for the juveniles indicated a catabolism of proteins for the organisms maintained in the lowest oxygen concentration. In the oxygen concentrations of 2 and 4 mg L^{-1} the blue shrimp juveniles modified their osmoregulatory capacity (OC) changing the osmoregulatory pattern the isosmotic to hypo-osmotic. We recommend maintaining *Litopenaeus stylirostris* at 6 mg L^{-1} and (25psu), these conditions for blue shrimp juveniles would enhance production in the cultivation of this species.

Keywords: Physiological energetics, oxygen levels, O:N atomic ratio, *Litopenaeus stylirostris*.

INTRODUCTION

In nature many organisms are exposed to unfavorable conditions or fluctuating temperature variations in the speed of currents, increased turbidity, low concentrations of dissolved oxygen, low availability of food as well as pollutants. These changes in environmental factors acting individually or together can produce stress in organisms [1].

In the ponds, an increase in oxygen levels during the day are due to the effect of photosynthetic organisms and then decreases at night, because oxygen consumption of plants and animals [2]. Low dissolved oxygen is the major limiting water quality variable in intensive aquaculture [3]. In prolonged exposure to low concentrations of oxygen, organisms use their adaptive mechanisms to maintain normal levels of activity for foraging and reproduction. Hypoxia may operate as a significant environmental stressor particularly for brackish and seawater organisms given to the high costs of oxygen acquisition in water [4]. However, if environmental conditions are not completely adverse, organisms may adjust their internal medium even though this is a factor of stress [5]. The dissolved oxygen (DO) in water may limit the metabolic capacity of the organisms, when the production of biomass and reproduction, organisms are fed less under conditions of low oxygen concentrations [6]. Hypoxia induces a range of compensatory mechanisms aimed at maintaining sufficient O_2 uptake from the water. These include hyperventilation, lamellar recruitment, and an increased blood O_2 affinity which leads to an increased pH [7-8].

Oxygen low levels, critical or lethal, affects the relation of growth, exposition time, body weight, oxygen consumption, ammonia toxicity, molt stage and metabolic stress, some of those aspects were studied in *Penaeus monodon* (Fabricius) [9-10] in *Penaeus setiferus* (Linnaeus) [11-16] and *Penaeus vannamei* (Boone) [17-20]. Molt have been studied in *Litopenaeus stylirostris* [21, 22], beside the metabolic responses in *Palaemon elegans, adspersus* (Rathke) *and P. pugio* (Holthuis), *P. serratus* (Pennant) [23-26]. Others, researches in body composition, hardness and density shell on different oxygen dissolved concentrations were evaluated in *Homarus americanus* (Milne Edwards) [27-28], *Farfantepenaeus californiensis* (Holmes) and *Fenneropenaeus chinensis* (Perez Farfante) [29-30]. Further, studies in C*arcinus maenas* (Linnaeus) [31-32] and [11]; *Cancer pagurus* (Linnaeus) [33-34] in different oxygen levels since normal, moderated and severe hypoxia show the effects of those variable combinations.

*Address correspondence to this author at the Laboratorio de Nutrición Funcional, Departamento de Biotecnología Marina Centro de Investigación Científica y Educación Superior de Ensenada, Carretera Ensenada-Tijuana # 3918, Ensenada Baja California, México; Tel: 01152-6461750500; Fax: 01152-6461750569; E-mail: denisre@cicese.mx

1874-3366/11

However, research data from published studies are not always consistent with field observations [35]. The reason being is that those studies frequently focus on the effect of a single environmental variable, and besides, the majorities of studies use only postlarvae and do not focus on physiological responses in juveniles and adults [36].

Bioenergetics studies allow us to describe, explain and predict the conditions or physiological state of the organism under culture conditions through the equation C = P + R + F + U + AHI + M, where C is the energy ingested through food consumption, P is the fraction of energy related to the scope for growth in the juveniles or to gamete production in the adult organisms, R is the proportion of energy which is channeled through respiratory metabolism, F is the energy contained in the undigested matter, U is the energy excreted by nitrogenated products, AHI is the energy cost associated with food digestion and consumption, and M is the energy used in the molting process.

Energy balance studies have been conducted in penaeids, mainly in *Litopenaeus vannamei* in both postlarvae and juveniles, to observe the effect of different regimens of salinities on seawater Na/K ratio, survival, growth, and energy budget. In functional nutrition different experiments were conducted to measure combinations of proteins, carbohydrates, calcium, phosphorus, on the diet qualities, and were related with absorption and growth efficiency. The combined effect of temperature and salinity were evaluated on immunological conditions, energetic balance, beside were examined the effect of different amounts of animal and vegetable protein added to the diet [37-44].

The blue shrimp, *Litopenaeus stylirostris* (Stimpson), is distributed from Punta Abreojos, Baja California México, down to Tumbes, Perú America. It is the second most abundant species in the central and northern Gulf of California, and is predominant in coastal lagoons, estuaries and bays, from northern Mazatlán to the Colorado River [45]. This species is commercially cultivated in Ecuador, México and New Caledonia. It inhabits lagoons, estuaries and bays, places with a varying hydrography throughout the year, affecting organisms that inhabit these aquatic systems, due to well-defined periods of rain and drought (low water) [45, 46].

Some aspects of the energetics in blue shrimp have been studied, but the various components of an energy budget have not been well defined in the laboratory under controlled temperature and exposure to different oxygen concentrations. The aquacultural potential in which blue shrimp *L. stylirostris* has in México makes it necessary to know the effect of three oxygen levels on elements of energy budget in juveniles. In order to this would help to determine which oxygen concentrations provide a high energetics efficiency which would promote an increase growth of blue shrimp.

MATERIAL AND METHODOLOGY

The experimental postlarvae of *Litopenaeus stylirostris* (n = 5000) provided from Aqualarva S.A. de C.V. Sinaloa, and Camarón Dorado, Sonora, farms, both from Northwest México. During the maintaining phase they were placed into three oval tanks (2500 L) with constant aeration, sea water 35psu and a temperature of 28 ± 1 ºC which is the optimal temperature determined by [47]. Photoperiod was maintained 12 h light, and 12 h dark. They were fed daily with two rations 8% of their wet body weight of commercial shrimp food Rangen®. Later, on the juveniles were moved (n = 500) to the laboratory conditions and they were placed in 4 circular reservoirs of 500 L and they were acclimated for 45 days to a temperature 28 °C and to three experimental oxygen concentrations of 2, 4, and 6 mg L^{-1} and 25psu isosmotic point which was determined by [48]. After the salinity changes, 15 organisms were extracted at random from each experimental condition. From these a single haemolymph sample of 10 µl was taken to measure the osmolality and the data was expressed in mmol kg^{-1} [49]. Osmoregulatory capacity (OC) in blue shrimp juveniles was calculated as the difference between osmolality of the haemolymph and of the external medium [50].

The decrease in the oxygen concentration produced by shrimp in a closed chamber respirometric of 1.0 L was recorded with a digital oxymeter (YSI 52 equipped with a polarographic sensor (± 0.01mg L^{-1}). Before measurement, the shrimp were acclimatized for two hours in the respirometric system in order to reduce the effect of handling [14]. Oxygen consumption from individual measurements on a minimum of 20 shrimps was measured. Individual oxygen consumption was calculated taking into account the oxygen consumption of electrode in natural seawater (35psu). Data were expressed as mg O_2 h^{-1} g^{-1} dry weight (d.w.).

The COL (mg L^{-1}) was determined by the point of the inflection of the curve obtained from the relation between oxygen consumption (O_2 h^{-1} g^{-1} d.w.) and the concentration on oxygen dissolved (mg L^{-1}). A two phase linear regression was used to obtain the point of intersection of two lines which was used to indicate the COL.

To create different oxygen conditions mainly for the lowest concentrations (2 and 4 mg L^{-1} nitrogen (N_2) was injected to the water using three degassing columns PVC 3" (7.71 cm) in diameter by 40" (102 cm) long. This deoxygenating equipment was connected to the experimental systems as described by [10, 51] to maintain proper control of levels of dissolved oxygen in the containers. The nitrogen was injected into the water within the columns and was spread by diffusers at the bottom of the column, the water with levels of oxygen do get to plastic containers, organisms were exposed to every condition of dissolved oxygen during a 45 days.

To determine the different parameter of energetic balance equation, the organisms were maintained for 45 days in six of 500 L tanks. Where all were placed into 20 plastic containers. The water conditions were at 28 ºC and 25psu. The water change was 100% at rate of 35 ml min^{-1}, for every experimental oxygen saturation tank. Photoperiod was maintained at 12 h light/12 h dark. During the experimental time the organisms were fed with a shrimp Rangen® a commercial diet with 40% of protein.

The juveniles with a mean wet weight between 1.94 ± 0.26 g were distributed into three 500 L circular tanks to acclimate for 45 days in the different experimental conditions. In order to examine the oxygen effect on the organisms, different physiological responses, conforming the energy balance was determined using the [52] modified equation:

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

This was carried out with 52 organisms from each experimental condition, which were distributed individually into 26 four-liter plastic containers inside each 500 L circular tank. Two repetitions were performed for each experimental oxygen level. Such containers had screen-covered side windows to allow water exchange with the tank but at the same time preventing the organisms, and feed and feces as well, from exiting or mixing. Shrimp were fed daily with a ration of 0.4 g individual^{-1} of Rangen® commercial feed (40% protein).

The equation components were determined as follows: the daily feed ration was given (C) and remain for 2 h in the containers with the organisms. After this interval, the food that was not consumed was removed with a siphon using an 80 μm mesh placed at its distal end, and later placed in a oven at 60 ºC to obtain the dry weight. The data from the consumed food were corrected by the dilution factor of the diet [53]; 1 g of food was placed in each container without organisms for two hours.

Feces (F) were collected 2 h after feeding; this operation was repeated 6 h later and before feeding the organisms the next day. The recollection of feces was made in the same manner as the food recollection. The dry weight was determined in the same way as with the commercial diet. The caloric content of the diet and feces were determined with a PARR semi micro calorimeter model 1425 standardized with benzoic acid and was repeated five times in each evaluation.

Routine oxygen consumption was measured (R) and the apparent heat increment (AHI) in the shrimp for each experimental condition (2, 4 and 6 mg L^{-1} concentrations) was determined in a respirometric system as described by [54] with a modification by adding the Nitrogen degassing columns to maintain the experimental concentration of oxygen into the system, but only for the lowest concentrations. Shrimp were placed in the respirometric system 24 h before the beginning of the measurements in order to allow their acclimation to the system; where placed individually in a 1.0 L Erlenmeyer flask. The dissolved oxygen was measured with a digital oxymeter YSI 52 equipped with a polarographic sensor (± 0.01 mg L^{-1}), with recently fed organisms and with the organisms maintained without feed for 24 h. In the first instance, the organisms were fed for two hours simulating the feed and feces recollection. Afterward, the food that was not consumed was removed and a water sample was taken to measure the initial concentration of dissolved oxygen. The water flow in the Erlenmeyer flasks was closed and remained closed for an hour, since according to [55] this time is an adequate to avoid that the dissolved oxygen that does not decrease to below 30% and does not cause stress in the organisms. At the end, new water samples were taken from each Erlenmeyer flask to measure the final concentration of dissolved oxygen.

The routine oxygen consumption (R) was calculated from the oxygen consumed by the shrimp that were not fed. The apparent heat increment (AHI) was determined as the difference between the oxygen consumed by the organisms recently fed and the oxygen consumed by the organisms without feed [56].

The ammonium excretion (U) of the organisms for each experimental condition was assessed simultaneously with oxygen consumption determination. For this, 10 ml of water was taken from each Erlenmeyer flask. The ammonium concentration of the samples was quantified by the blue indophenol method [57] using an ELIPTICA 2000 spectrophotometer. The shrimp ammonium excretion values for the different experimental conditions were transformed to energy units using the nitrogen caloric equivalent of 5.73 cal mg^{-1} NH_4^+ [58].

At the end of the experiments of oxygen consumption, the organisms were weighed to obtain their wet weight and sacrificed. They were then labeled and placed in a 60 ºC oven for 6 days, at which time their dry weight was determined.

The difference between the initial and final gas concentration was the oxygen consumed by the organisms and was expressed in mg O_2 h^{-1} g^{-1} d.w. The routine oxygen consumption and the apparent heat increment was transformed into energy units through the use of oxycaloric equivalent of 3.53 cal mg^{-1} of O_2 consumed [59]. During the experimental phase, a control Erlenmeyer flask was maintained without organisms in order to measure the oxygen consumption and ammonium production of the microorganisms present in the respirometric system, where the necessary corrections were then made.

The energy allocated for exoskeleton formation (M) was determined from the caloric content of the molts recollected individually from each organism for each experimental condition.

The scope for growth (P) was estimated as the difference between the feed energy consumed and the sum of energy utilized in the production of feces, respiration, ammonium excretion, apparent heat increment and molts.

The data for consumed food, production of feces, routine oxygen consumption, ammonium excretion, apparent heat increment and molts were transformed into Joules using the conversion factor of 1 calorie = 4.1840 Joules [60] and was expressed in J g^{-1} day^{-1} d.w.

To obtain the growth rate the organisms were weighed in an electronic OAHUS Explorer balance and biometrics was made every week, during the experimental period. When concluding the experiments in a worksheet of electronic data EXCEL (version 6.0) the weekly averages were calculated to know the increased in weight of the shrimps for each oxygen condition and their repetitions.

The atomic ratio O:N was determined from the VO_2 and VNH_4 data using the equation given by [61]:

O:N atomic ratio = Atomic weight (NH_4^+)/ Atomic weight (O_2)/ * (VO_2)/ VNH_4^+)

This ratio was used to estimate the amounts of proteins, lipids and carbohydrates that were used as an energy source for the organisms in the different experimental conditions. Ratio values of 3 to 16 were considered as indicating oxidation of basically protein. Ratios between 50 and 60 indicated catabolism of the similar proportions of protein and lipid, while values above 60 were attributed to the use of carbohydrates [62].

The data were tested for normality and homoscedasticity (Sigma Stat). Then, the Kruskal-Wallis [63-64] non-parametric test was used to determine the oxygen concentration level effect on different parameters which form the energy balance equation for blue shrimp juveniles. The Dunn method was used to isolate the parameters which showed significant differences.

RESULTS

The critical oxygen level (COL) showed shrimp had an independent pattern behavior above 5.5 mg L^{-1} and below this concentration; the blue shrimp were oxygen conformers (Fig. **1**).

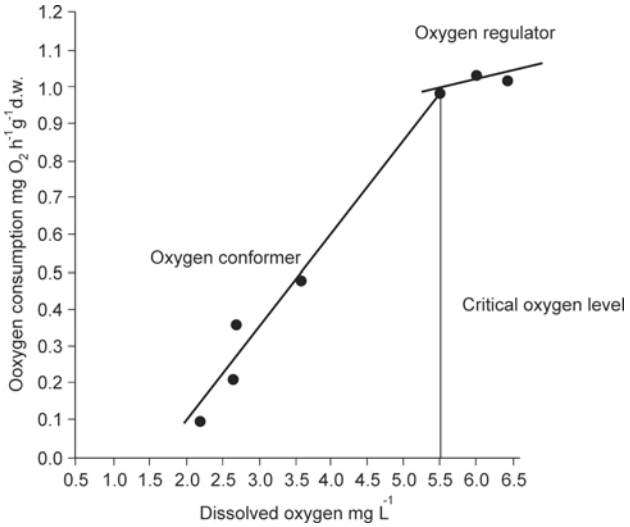

Fig. (1). Critical oxygen level, oxygen conformer and oxygen regulator. Line show the conformer behavior of juveniles of *Litopenaeus stylirostris.*

The energy ingested in the feed by the organism exposed to three oxygen concentrations which ranged from 2110 to 3445 J g^{-1} day^{-1} d.w. For the *L. stylirostris* juveniles exposed the different oxygen levels, there were significant differences ($P < 0.05$) among the groups with regard to ingested energy through the feed (Table **1**).

Table 1. Physiological Rates of Juveniles of *Litopenaeus stylirostris* Acclimated to Different Oxygen Concentrations

OXYGEN CONCENTRATION (mg L^{-1})			
	2	**4**	**6**
C	2352.6±42[a]	2110.9±47[b]	3445.22±59[c]
F	555.07±36.1[a]	393.42±35.7[b]	226.6±47.1[c]
R	189.4±26.1[a]	156.79±19.1[a]	123.62±3.8[b]
U	54.9±2.3[a]	28.11±9.1[b]	23.97±1.2[b]
AHI	129.6±12.8[a]	130.93±3.2[a]	135.7±5.4[a]
M	184.81±4.3[a]	184.37±3.3[a]	189.37±4.6[a]
P	1230.82±52[a]	1240.95±49[a]	2756.47±39[b]

C = ingestion, **F** = fecal production, **R** = routine oxygen consumption, **U** = ammonium excretion, **AHI** = apparent heat increment, **M** = molting, **P** = scope for growth (J g^{-1} day^{-1} d.w.). Median ± 95% confidence interval.
Values in each row followed by a different letter differ significantly with $\alpha = 0.05$.

The growth rate was measured by week (g^{-1} d.w) the highest rate was obtained for the organism acclimated at 6 mg L^{-1} and the 2 and 4 mg L^{-1} were drastically affected (Fig. **2**).

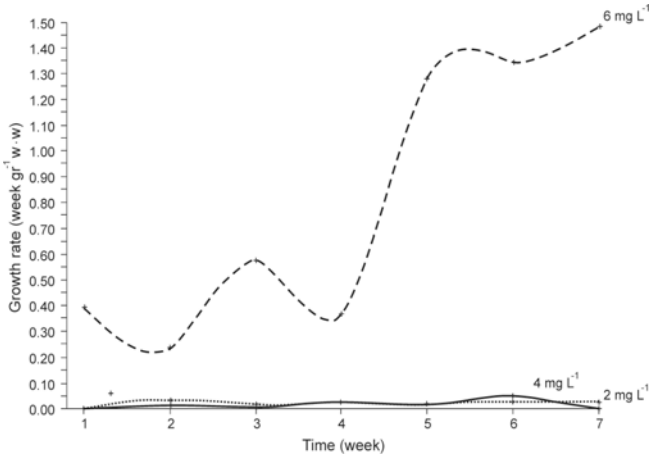

Fig. (2). Blue shrimp growth rate (weeks g^{-1} w.w.) at three oxygen levels (2, 4 and 6 mg L^{-1}).

The growth potential energy (P) by the shrimp maintained at an oxygen concentration was 2 mg L^{-1} was 1230.82 ± 52.0 J g^{-1} day^{-1} d.w. The shrimp acclimated to 4 mg L^{-1} had 1240.95 ± 49.0 J g^{-1} day^{-1} d.w. The shrimp maintained at 6 mg L^{-1} had available 2756 ± 39.0 J g^{-1} day^{-1} d.w. (Table **1**). The different oxygen levels produced significant differences ($P < 0.05$) in the juvenile's growth potential energy.

In the shrimp maintained in 2, 4 and 6 mg L^{-1}, energy loss in fecal production represented 23.6 to 6.56% of the incorporated energy from the feed (Fig. **3**). Significant differences were found ($P < 0.05$) among the organisms exposed to the different oxygen levels in the amount of energy lost in the production of feces.

The energy that the shrimp acclimated at 6 mg L^{-1} used for oxygen routine consumption was 123.62 J g^{-1} day^{-1} d.w. (Table **1**), which was significantly lower ($P < 0.05$) than that for organisms exposed to the other experimental oxygen concentrations.

The energy loss from the ammonium excretion (U) by the *L. stylirostris* juveniles were increased by the exposure to oxygen concentration of 2 mg L^{-1} and decreased in the juveniles acclimated at 4 and 6 mg L^{-1} (Table **1**). Significative differences were found ($P < 0.05$) for different oxygen levels in the energy allocated by the shrimp to this physiological process.

The apparent heat increment represented the energy allocated to the metabolic process from 135.7 to 129.6 J g^{-1} day^{-1} d.w. in the three oxygen concentrations (Table **1**). Such increase represented a loss of 3.9 to 5.9% of the ingested energy through feed by the organisms. No significant differences were obtained ($P > 0.05$) in the AHI among the organisms exposed to different oxygen concentrations of 2, 4 and 6 mg L^{-1} (Fig. **3**).

The energy invested in molting by the shrimp acclimated at different oxygen ranged 184.81 to 189.37 J g^{-1} day^{-1} d.w. (Table **1**). No significant differences were found ($P > 0.05$)

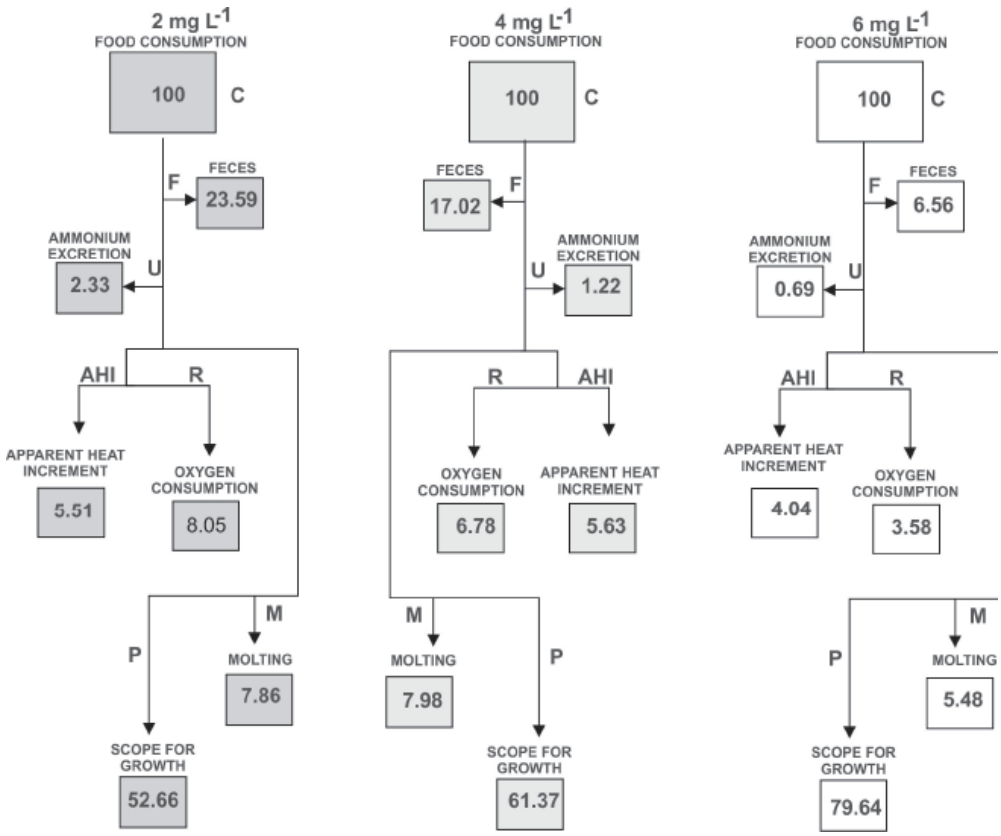

Fig. (3). Percentages of energy allocated of juveniles of *Litopenaeus stylirostris* exposed to 2, 4 and 6 mg O_2 L^{-1}.

in the amount of energy lead to this process among the *L. stylirostris* juveniles exposed to the different experimental oxygen levels.

The O:N atomic ratio value estimated for the *L. stylirostris* juveniles maintain in the 6 mg L^{-1} was 104.6. In the organisms exposed to 4 mg L^{-1}, this ratio was 19.8. The minimum value for the O:N ratio was observed in the juveniles acclimated at 2 mg L^{-1}, which was 8.5 (Fig. **4**).

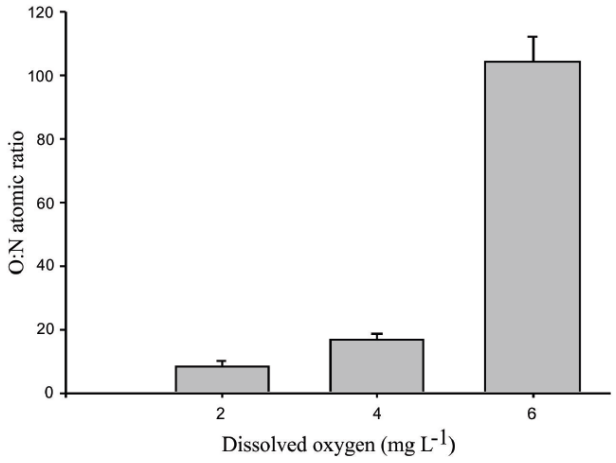

Fig. (4). O:N atomic ratio of juveniles of *Litopenaeus stylirostris* acclimated to different oxygen levels (2, 4 and 6 mg L^{-1}).

In the three oxygen levels, the organism modified their osmoregulatory capacity (OC). The exposure to low oxygen concentrations modified these osmoregulatory capabilities for organisms exposed to 2 and 4 mg L^{-1} . The values for OC was 97.5 mmol k^{-1} and 67 mmol k^{-1} respectively, changing to hypo-osmotic pattern in relation to determined in 6 mg L^{-1} were they had an isosmotic pattern (-2.68 mmol k^{-1}).

DISCUSSION

The ability of penaeids to obtain energy and distribute them effectively among the maintenance and growth requirements depends on the effect of different environmental factors. Oxygen is one of these factors that influence the metabolism of these organisms, even though [65] suggested that a threshold of DO level for maximum growth, below which growth would not improve. Many species of shrimp such as the *P. monodon*, *L. vannamei*, *Metapenaeus macleayi* (Haswell), *P. setiferus*, *Penaeus schmitti* (Burkenroad), *F. chinensis*, and *F. californiensis* [9, 12-13, 29, 51], can tolerate DO levels as low as 2.0 mg L^{-1}, but the growth is affected due the loss of energy by metabolic compensations. In this study, we can see the threshold at 4 mg L^{-1} where the growth began to be affected and maintains this adverse effect through 2 mg L^{-1}, obtained a minimum growth for those organism. Our study on the effect of dissolved oxygen on the energy balance of juvenile *L. stylirostris* had shown that O_2 affected growth due to a reduction between 21.05 and 27.53% in energy, which was available for growth. If O_2 levels are not enough to meet the costs associated with the consumption and processing of ingested food, shrimp stop eating, sacrificing the possibility of obtaining energy from food to be dedicated to growth. This obviously affects the growth strategy but ensures the

efficient use of oxygen that is needed to produce metabolic energy to maintain basic functions [14].

According to [66], dissolved oxygen (DO) is a regulating metabolic factor in aquatic organisms. DO can limit the metabolic capacity and biomass production. In general, penaeid shrimp are oxyregulators within limited DO intervals [20, 67]. Recent evidence has shown that *P. vannamei*, *Penaeus setiferus* and *P. schmitti* postlarvae (PL $_{10\text{-}18}$) are oxygenregulators at 4.5 to 5 mg L^{-1} DO corresponding with the critical oxygen level (COL), depending on salinity. Oxygen consumption below these levels becomes critical dependent on oxygen concentration, decreasing the metabolic capacity of shrimp as much as 26% and 34% [12, 20]. Below that level of oxygen consumption it becomes dependent on the concentration of oxygen, which reduces between 14 and 38% metabolizable energy. This demonstrates the controlling role of dissolved oxygen on the energy metabolism [68].

Ocampo *et al*. [29] studied the effects of dissolved oxygen and temperature on *F. californiensis* which were maintained at oxygen levels between 5.8 to 2.6 mg L^{-1}, and found that the lowest growth was obtained in the lowest oxygen level. These authors indicated that low oxygen levels significantly depressed growth. These finding agree with the data presented in this study, the COL for *L. stylirostris* 5.5 mg L^{-1}, showed the organisms are not capable of obtaining enough energy from the food and metabolizing the limited effect on the critical oxygen level**.**

The oxygen level in which the *L. stylirostris* juveniles used less energy destined to cover the oxygen routine consumption process was 6 mg L^{-1}, were oxygenregulators. The highest energy demand was recorded in the organisms which were maintained at 2 and 4 mg L^{-1}; where they were oxygenconformer. [12, 14-15] in a similar way found in *P. setiferus* and *P. schmitti* juveniles that the highest energy demands to cover routine metabolism occur when exposed to low dissolved oxygen.

In crustaceans, the largest part of excreted ammonium is the product resulting from aminoacid catabolism obtained through the diet [11]. The ammonium excretion by the *L. stylirostris* juveniles in this study was related to oxygen levels. It was found that the highest concentrations of ammonium were produced by the shrimp which were exposed oxygen concentrations of 2 and 4 mg L^{-1} where they changed their osmoregulatory pattern the isosmotic to hyposmotic. The increase in ammonium excretion favors sodium uptake possibly through the Na^+/NH_4^+ exchange pump in order to maintain the haemolymph osmotic concentration [48-49, 69-72] have suggested that this physiological mechanism is the responsible for the maintenance of the hyposmotic concentration in shrimp maintained in low oxygen concentrations.

There were no differences found in this study in on apparent heat increment among the different experimental conditions. Beamish & Trippel [56] mentioned that the factors that modify the apparent heat increment magnitude which resulted from feed ingestion include the nature of the diet, size and ration and the chemical composition of the feed. While considering previous findings, it was to be expected that no differences exist between the apparent heat increment and its duration since the organisms during this research were fed with the same diet. Similar results were obtained by [73] where non significative differences were found in the apparent heat increment they were fed the same diet. Du Preez, Chen & Hsieh [74] mentioned that where *P. monodon* juveniles were fed with a commercial diet, there was an increase of 2% to 17% of oxygen consumption, which represented 2.4 to 19.5% of the ingested energy. The results obtained in the present study in *L. stylirostris* juveniles did not show differences in the magnitude of this metabolism component, since the energy expended in this process represented 3.9 to 5.9% of the energy obtained through ingested feed. The energy expenditure for this process was not affected by oxygen levels, since values were within the range reported for different crustacean species.

The blue shrimp juveniles used 5.48 to 8.73% of the energy obtained from the food for the exoskeleton formation when they were acclimated to the different oxygen concentrations. Values have been reported for other decapods, such as *C. maenas* and *Crangon crangon* (Linnaeus) invested 10% to 17%, respectively, of the ingested energy through food to exoskeleton formation [75] [76]. Logan & Epifanio [77] mentioned that the energy loss during the molting of *H. americanus* was 10% of the ingested energy. In the blue shrimp juveniles, the energy expenditure for the molting process was not affected by oxygen concentration levels, since values were within the range reported for different crustacean species.

With the correlation between oxygen consumption rate and ammonium excretion represented in atomic equivalents, we can determine the change in the utilization of metabolic substrates when the organisms are exposed to different environmental regimes [62-78]. For *L. stylirostris* juveniles, a change in the O:N atomic ratio was observed, which indicated a change in the energy substrate used in response in the variation of oxygen concentration levels. The O:N ratio estimated for the organism acclimated to 6 mg L^{-1} which was 12.3 and 5.28 times higher than the ratio obtained when the organisms were maintained at 2 and 4 mg L^{-1} respectively. This indicates that the shrimp maintained at 6 mg L^{-1} use carbohydrates as the main energy substrate. When they were exposed to medium oxygen concentration (4 mg L^{-1}), the main energy substrate used was a protein-lipid mixture, and where they were in low oxygen concentration (2 mg L^{-1}), protein was used as the energy substrate as stress response. This values for the O:N ratio are similar to those reported by [79] for the juveniles of *Penaeus duorarum* (Burkenroad), *P. schmitti*, *Penaeus notialis* (Perez Farfante) and *P. setiferus.* The results of the O:N ratio values that obtained in *L. stylirostris* agree with the values obtained with many other estuary and marine species, where the O:N ratio is higher for organisms found at optimum oxygen concentration and lower when found at lower oxygen concentrations. Shrimps showed a change from a predominant carbohydrate metabolism at optimum oxygen concentration to a metabolism dominated by a protein-lipid mixture at suboptimal conditions [11, 29, 67].

The studies on the energy balance in crustaceans under different oxygen concentration are scarce. Even though, they have demonstrated that this kind of analysis provide information that help in the quantification, description and

explanation of the physiological condition of the organisms. Exposure of blue shrimp to optimal concentration 6 mg L^{-1} O_2 reduces the energy expenditure which is channeled to cover the routine oxygen consumption processes and the excretion of nitrogenous products, increasing the scope for growth. We propose that to optimize the energy budget in culture conditions for *L. stylirostris* juveniles be maintained under these conditions, to avoid exposure to lower oxygen concentration during sunset and sunrise where the shrimp experimented 2.76 mg L^{-1} the dissolved oxygen in cultured ponds.

REFERENCES

[1] Espina S, Vanegas C. Ecotoxicología y contaminación. In: Botello AV, Rojas Galaviz JL, Benitez JA, Sarate Lomeli D. Eds. Golfo de México contaminación e impacto ambiental. México: EPOMEX 1996; 45-68.

[2] Chen J, Kou T. Haemolymph acid-base balance, oxyhemocyanin, and protein levels of *Macrobrachium rosenbergii* at different concentrations dissolved oxygen. J Crustacean Biol 1998; 18: 437-441.

[3] Boyd CE, Watten BJ. Aeration systems in aquaculture. CRC Crit Rev Aquat Sci 1989; 1: 425-72.

[4] Reardon EE, Chapman LJ. Hypoxic stress and reproduction: Life history variation in the mouth brooding cichlid *Pseudocrenilabrus multicolor*. J Fish Biol 2009; 75 : 1795-815.

[5] Hagerman L. Haemocyanin concentration in the shrimp *Crangon crangon* (L.) after exposure to moderate hypoxia. Comp Biochem Physiol 1986; 85A: 721-4.

[6] Wourms JP, Lombardi J. Reflections on the evolution of piscine viviparity. Am Zool 1992; 32: 276-93.

[7] Jensen FB, Lecklin T, Busk M, Bury NR, Wilson RW, Wood CM, Grosell M. Physiological impact of salinity increase at organism and red blood cell levels in the European Flounder (*Platichthys xesus*). J Exp Mar Biol Ecol 2002; 274:159-74.

[8] Jensen FB. Red blood cell pH, the Bohr e Vect, and other oxygenation-linked phenomena in blood O_2 and CO_2 transport. Acta Physiol Scand 2004; 182: 215-27.

[9] Allan GL, Maguire GB, Hopkins SJ. Acute and chronic toxicity of ammonia to juvenile *Metapenaeus macleayi* and *Penaeus monodon* and the influence of low dissolved oxygen levels. Aquaculture 1990; 91: 265-80.

[10] Allan GL. Maguire G. Lethal levels of low dissolved oxygen and effects of short-term oxygen stress on subsequent growth of juvenile *Penaeus monodon*. Aquaculture 1991; 94: 27-37.

[11] Regnault M. Short term effect of hypoxia on ammonia excretion and respiration rates in the crab *Carcinus maenas*. Mar Behav Physiol 1988; 6: 257-71.

[12] Rosas C, Sanchez A, Diaz–Iglesias E, Brito R, Martinez E, Soto L. Critical dissolved oxygen level to *Penaeus setiferus* and *Penaeus schmitti* postlarvae (PL 10-18) exposed to salinity changes. Aquaculture 1997; 152: 259-72.

[13] Martínez E, Aguilar M, Trejo L, *et al*. Lethal low dissolved oxygen concentrations for postlarvae and early Juvenile *Penaeus setiferus* at different salinities and pH. J World Aquac Soc 1998; 29: 221-9.

[14] Rosas C, Martinez E, Gaxiola G, Brito R, Díaz E, Soto L. Effect of dissolved oxygen on the energy balance and survival of *Penaeus setiferus* juveniles Mar Ecol Prog Ser 1998; 174: 67-75.

[15] Rosas C, Martinez E, Gaxiola G, Brito R, Sanchez A, Soto L. The effect of dissolved oxygen and salinity on oxygen consumption, ammonia excretion and osmotic pressure of *Penaeus setiferus* J Exp Mar Biol Ecol 1999; 41-57.

[16] Alcaraz G, Espinoza V, Vanegas C. Acute effect of ammonia and nitrite on respiration of *Penaeus setiferus* postlarvae J World Aquac Soc 1999; 30: 98-106.

[17] Mauro NA, Thompson C. Hypoxia adaptation in the crayfish *Procambarus clarkii.* Comp Biochem Physiol 1984; 79: 73-5.

[18] Ross ES, Lawrence AL. Growth, feed digestibility and approximate body composition of juvenile *Penaeus vannamei* and *Penaeus monodon* grown at different dissolved oxygen levels. J World Maricul Soc 1985; 16: 333-46.

[19] Charmantier G, Soyez C, Aquacop. Effect of molt stage and hypoxia on osmoregulatory capacity in the peneid shrimp *Penaeus vannamei*. J Exp Mar Biol Ecol 1994; 178: 233-46.

[20] Villarreal H, Hinojosa P, Naranjo J. Effect of temperature and salinity on the oxygen consumption of laboratory produced *Penaeus vannamei* postlarvae. Comp Biochem Physiol 1994; 108A: 331-6.

[21] Racotta I, Palacios SE, Méndez L. Metabolic responses to short and long term exposure to hypoxia in White shrimp (*Penaeus vannamei*). Mar Fresh Behav Physiol 2002; 35: 269-75.

[22] Mugnier C, Soyez C. Response of the blue shrimp *Litopenaeus stylirostris* to temperature decrease and hypoxia in relation to molt stage. Aquaculture 2005; 244: 315-22.

[23] Taylor AC, Spicer JI. Metabolic responses of the prawns *Palaemon elegans* and *P. serratus* Crustacea: Decapoda) to acute hypoxia and anoxia. Mar Biol 1987; 95: 521-30.

[24] Taylor AC, Spicer JI. Acid-Base disturbances in the haemolymph of the prawns, *Palaemon elegans* (Rathke) and *P. serratus* (Pennant) (Crustacea : Decapoda) during exposure to hypoxia. Comp Biochem Physiol 1991; 98A: 445-052.

[25] Nielsen A, Hagerman L. Effects of short–term hypoxia on metabolism and hemocyanin oxygen transport in the prawns *Palaemon adspersus* and *Palaemonetes varians.* Mar Ecol Prog Ser 1998; 167: 177-83.

[26] Harper SL, Reiber C. Influence of hypoxia on cardiac functions in the grass shrimp (*Palaemonetes pugio* Holthuis). Comp Biochem Physiol 1999; 124A: 569-73.

[27] McMahon BR, Wilkens JL . Respiratory and circulatory responses to hypoxia in the lobster *Homarus americanus*. J Exp Biol 1975; 6: 637-55.

[28] Bayer R, Riley J, Donahue D. The effect of dissolved oxygen on the weight gain and shell hardness of new–shell American Lobster *Homarus americanus.* J World Aquac Soc 1998; 29: 491-3.

[29] Ocampo L , Villarreal H , Vargas M, Portillo G, Magallón F. Effect of dissolved oxygen and temperature on growth, survival and body composition of juvenile *Farfantepenaeus californiensis* (Holmes) Aqua Res 2000; 31: 167-71.

[30] Yuquan L, Jian L, Qingyin W. The effect of dissolved oxygen concentration and stocking density on growth and non-especific immunity factors in Chinese shrimp, *Fenneropenaeus chinensis*. Aquaculture 2006; 256: 608-16.

[31] Jouve-Duhamel A, Truchot JP. Ventilatory responses to ambient oxygen in stressed and fully settled shore crabs *Carcinus maenas* (L.) J Exp Mar Biol Ecol 1985; 90: 107-18.

[32] Lallier F, Boitel F, Truchot JP. The effect of ambient oxygen and temperature on haemolymph L-Lactate and urate concentrations in the shore crab *Carcinus maenas* Comp Biochem Physiol 1987; 86A: 255-60.

[33] Bradford SM, Taylor AC. The respiration of *Cancer pagurus* under normoxic and hypoxic conditions J Exp Biol 1982; 97: 273-88.

[34] Regnault M. Effect of a severe hypoxia on some aspects of nitrogen metabolism in the *Cancer pagurus.* Mar Behav Physiol 1993; 22: 131-40.

[35] Venkataramaiah A, Lakshmi GJ, Gunter J. Studies on the effects of salinity and temperature on the commercial shrimp, *Penaeus aztecus*, Ives, with special regard to survival limits, growth, oxygen consumption and ionic regulation. Contract report H-74-2. Gulf Coast Research Laboratory, Ocean Springs, Mississippi 1974; 134.

[36] Ponce Palafox J, Martínez Palacios CA, Ross LG. The effects of salinity and temperature on the growth and survival rates of juvenile white shrimp *Penaeus vannamei* Boone 1931. Aquaculture 1997; 157: 107-15.

[37] Pascual C, Zenteno E, Cuzon G, Sánchez A, Gaxiola G, Taboada G, Suárez T, Maldonado J, Rosas C. *Litopenaeus vannamei* juveniles energetic balance and immunological response to dietary protein. Aquaculture 2004; 236: 431-50.

[38] Zhu C, Dong S, Wang F, Huang G. Effects of Na/K ratio in seawater on growth and energy budget of juvenile *Litopenaeus vannamei*. Aquaculture 2004; 234: 485-96.

[39] Wang X, Ma S, Dong S, Cao M. Effect of salinity and dietary carbohydrate levels on growth and energy budget of juvenile *Litopenaeus vannamei.* J Shell Res 2004; 23: 231-6.

[40] Cheng K, Hu C, Liu Y, Zheng S Qi. Effect of dietary calcium, phosphorus and calcium/phosphorus ratio on the growth and tissue mineralization of *Litopenaeus vannamei* reared in low-salinity water. Aquaculture 2006; 251: 472-83.

[41] Jiménez-Yan, L, Brito A, Cuzon G, *et al*. Energy balance of *Litopenaeus vannamei* postlarvae fed on animal or vegetable protein based compound feeds. Aquaculture 2006; 260: 337-45.

[42] Wang X, Cao M, Ma S, Dong S, Yan B. Effects of salinity on survival, growth and energy budget of juvenile *Litopenaeus vannamei*. Mar Fish Res 2006; 27, 8-13.

[43] Yan B, Wang X, Cao M. Effect of salinity and temperature on survival, growth, and energy budget of juvenile *Litopenaeus vannamei*. J Shell Fish 2007; 26: 141-6.

[44] Valdez G, Diaz F, Re AD, and Sierra E. Efecto de la salinidad sobre la fisiología energética del camarón blanco *Litopenaeus vannamei* (Boone). Hidrobiológica 2008 18:105-15.

[45] Aragon NEE. Coupling the reproductive period of blue shrimp *Litopenaeus stylirostris* Stimpson 1874 (Decapoda:Penaeidae) and sea surface temperature and the Gulf of California. Rev Biol Mar Ocean 2007; 42: 167-75.

[46] Rodríguez GA, Osmoregulation and total serum proteins of two species of penaeidean shrimps from the pacific coast of México. J Crustacean Biol 1981; 1: 392-400.

[47] Re AD, Díaz F, Valdez G. Effect of salinity on the thermoregulatory behaviour of juvenile blue shrimp *Litopenaeus stylirostris* Stimpson. J Therm Biol 2006; 31: 506-13.

[48] Re AD, Díaz F, Sierra E, Gómez-Jiménez S. Oxygen consumption ammonium excretion and osmoregulatory capacity of *Litopenaeus stylirostris* (Stimpson) exposed to different combinations of temperature and salinity. Cien Mar 2004; 30: 443-53.

[49] Díaz F, Farfán C, Sierra E, Re AD. Effects of temperature and salinity fluctuation on the ammonium excretion and osmoregulation of juveniles of *Penaeus vannamei*, Boone. Mar Fresh Behav Physiol 2001; 34: 93-104.

[50] Lignot JH, Cochard JC, Soyez C, Lemaire P, Charmantier G. Osmoregulatory capacity according to nutritional status, molt stage and body weight in *Penaeus stylirostris.* Aquaculture 1999; 170: 79-92.

[51] Seidman ER, Lawrence AL. Growth, feed digestibility and proximate body composition of juvenile Penaeus vannamei and *Penaeus monodon* grown at different dissolved oxygen levels. J World Maricult Soc 1985; 16: 333-46.

[52] Klekowski RZ, Duncan A. Physiological approach to ecological energetics. In: Grodzinski W, Klekowski RZ, Duncan A. Eds. Methods for ecological energetics. Oxford: Blackwell Scientific Publications 1975; pp. 15-64.

[53] Obaldo LG, Divakaran S. Tacon AG. Methods for determining the physical stability of shrimp feeds in water. Aqua Res 2002; 3: 369-77.

[54] Díaz F, Re AD, González RA, Sánchez LN, Leyva G, Valenzuela F. Temperature preference and oxygen consumption of largemouth bass *Micropterus salmoides* (Lacépéde) acclimated at different temperatures. Aqua Res 2007; 38: 1387-94.

[55] Stern S, Borut A, Cohen D. The effect of salinity and ion composition on oxygen consumption and nitrogen excretion of *Macrobrachium rosenbergii* (De Man). Comp Biochem Physiol 1984; 79A: 271-74.

[56] Beamish FWH, Trippel EA. Heat increment: a static or dynamic dimension in bioenergetic models? Trans Am Fish Soc 1990; 119: 649-61.

[57] Rodier J. Análisis de las aguas: aguas naturales, aguas residuales y agua de mar. Barcelona: Omega 1989.

[58] Clifford III HC, Brick RW. A physiological approach to the study of growth and bioenergetics in the freshwater shrimp *Macrobrachium rosenbergii*. Proc World Maricult Soc 1979; 10: 710-9.

[59] Elliot JM, Davison W. Energy equivalents of oxygen consumption in animal energetics. Oecología 1975; 19: 195-201.

[60] Gnaiger E. Calculations of energetic and biochemical equivalents for respiratory oxygen consumption. In: Gnaiger E, Forstner H, Eds. Polarographic oxygen sensor. Berlin: Springer 1983; 337-45.

[61] Flores M, Diaz F, Medina R , Re D, Licea A. The effect of dietary astaxanthin on physiological responses of juvenile white shrimp *Litopenaeus vannamei* acclimated to low-salinity water. J Fish Intern 2008; 3: 62-9 .

[62] Mayzaud P, Conover RJ. O:N atomic ratio as a tool to describe zooplankton metabolism. Mar Ecol Prog 1988; 45: 289-02.

[63] Zar J. H. 1999. Biostatiscal Analysis. New Jersey: Prentice Hall. pp. 25-150.

[64] Sokal RR, Rohlf FJ. Biometría. Principios y Métodos Estadísticos en la Investigación Biológica. España: Blume. 1979.

[65] Brett JR, Groves TDD. Physiological energetics. In: Hoar WS, Randall DJ, Brett JR. Eds Fish Physiology VIII Bioenergetics and Growth New York: Academic Press Inc 1979; 339-52.

[66] Fry EE. Effects of the environment on animal activit. Univ Toronto Studies, Biol Ser 55. Ontario Fish Res Lab Publ 1947; 68: 15-64

[67] Villarreal H, Ocampo L. Effect of size and temperature on the oxygen consumption of the brown shrimp *Penaeus californiensis* (Holmes, 1900) Comp Biochem Physiol 1993; 106A: 97-101

[68] Cuzon G, Gaxiola G, Rosas C. Balance energético y requerimientos de energía In: Rosas C, carrillo O, Wilson R, Andreatta ER. Eds. Estado actual y perspectivas de la nutrición de los camarones peneidos cultivados en Iberoamérica. Mexico. CYTED 2006; 198-228.

[69] Jiang DH, Lawrence AL, Neill WH, Gong H. Effects of temperature and salinity on nitrogenous excretion by *Litopenaeus vannamei* juveniles. J Exp Mar Biol Ecol 2000; 253: 193-09.

[70] Lemaire P, Bernard E, Martínez-Paz JA, Chim L. Combined effect of temperature and salinity on osmoregulation of juvenile and subadult *Penaeus stylirostris*. Aquaculture 2002; 209: 307-17.

[71] Díaz F, Re AD, E. Sierra & E. Díaz-Iglesias. Effects of temperature and salinity fluctuation on the oxygen consumption, ammonium excretion and osmoregulation of the blue shrimp *Litopenaeus stylirostris* (Stimpson). J Shell Res 2004; 23: 903-10.

[72] Gong H, Jiang DH, Lightner DV, Collins C, Brock DA. Dietary modification approach to improve the osmoregulatory capacity of *Litopenaeus vannamei* cultured in the Arizona desert. Aquat Nutr 2004; 10: 227-36.

[73] Rosas C, Cuzon G, Gaxiola G, *et al*. An energetic and conceptual model of the physiological role of dietary carbohydrates and salinity on *Litopenaeus vannamei* juveniles. J Exp Mar Biol Ecol 2002; 268: 47-67.

[74] Du Preez HH, Chen HY, Hsieh CS. Apparent specific dynamic action of food in the grass shrimp, *Penaeus monodon* Fabricius. Com Biochem Physiol 1992; 103A: 173-8.

[75] Klein Breteler W CM. Food consumption, growth and energy metabolism of juvenile shore crabs, *Carcinus maenas*. J Sea Res 1975; 9: 255-72.

[76] Vernberg FJ. Crustacea. In: Pandian T.J. Vernberg F.J. Eds. Animal energetics, protozoa through insect. New York: Academic Press Inc. 1987; pp. 301-372.

[77] Logan DT, Epifanio CE. A laboratory energy balance for the larvae and juveniles of the American lobster *Homarus americanus*. Mar Biol 1978; 47: 381-9.

[78] Conover RJ, Corner EDS. Respiration and nitrogen excretion by some marine zooplankton in relation to their life cycles. J Mar Biol Assoc 1968; 48: 49-75.

[79] Rosas C, A. Sanchez, E. Diaz–Iglesias, *et al*. O_2 consumption and ammonia excretion of *Penaeus setiferus, P. schmitti, P. duorarum* and *P. notialis* postlarvae fed purifed test diets: Effect of protein level on substrate metabolisms. Aquat Living Resour 1995; 8: 161-9.

Received: September 29, 2010 Revised: November 22, 2010 Accepted: November 29, 2010