Physiological Energetics of Blue Shrimp *Penaeus stylirostris* (Stimpson) Juveniles Acclimated To Different Salinities

Ana Denisse Re*[,1], Fernando Díaz[2], Gustavo Valdez[2], Marisela Flores[3] and Maximiliano López[1]

[1]*Laboratorio de Nutrición Funcional, Departamento de Biotecnología Marina Centro de Investigación y Educación Superior de Ensenada Km 107 Carretera Tijuana-Ensenada, Ensenada Baja California México*

[2]*Laboratorio de Ecofisiología de Organismos Acuáticos, Departamento de Biotecnología Marina Centro de Investigación y Educación Superior de Ensenada Km 107 Carretera Tijuana-Ensenada, Ensenada B. C. México*

[3]*Nutrición y Ecofisiología de Organismos Acuáticos, Instituto de Investigaciones en Ciencias Veterinarias Universidad Autónoma de Baja California Av. Obregón y Julián Carrillo s/n Mexicali B. C. México*

Abstract: The influence of salinity on the energy balance and O:N atomic ratio of the blue shrimp *Penaeus stylirostris* was determined under laboratory conditions (20, 25 and 30psu). The lowest energy expended for routine metabolism and excretion of nitrogenous products was obtained in the animals maintained at 25psu salinity. The high quantity of energy channeled to scope for growth 2757 J g^{-1} day^{-1} d.w. was obtained in the shrimp acclimated to a salinity of 25psu. The apparent heat increment fecal production and energy lost in exuviae did not differ significantly ($P > 0.05$) among the shrimp exposed to the three salinity levels. The O:N atomic ratio calculated for the juveniles indicated a catabolism of carbohydrates as the main energy substrate for the organisms maintained in the isosmotic condition (25psu). We recommend maintaining *Penaeus stylirostris* juveniles at the salinity level which is isosmotic where they are free of environmental stress; these conditions for blue shrimp juveniles would improve production in the cultivation of this species.

Keywords: Physiological energetics, salinity, O:N atomic ratio, *Penaeus stylirostris.*

INTRODUCTION

Temperature and salinity are major ecological factors, acting either singly or in concert to modify physiological responses of euryhaline penaeids, because they produce great complex biological effects [1]. The blue shrimp *Penaeus stylirostris* (Stimpson) is distributed from Punta Abreojos Baja California down to Tumbes Peru. 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 Mazatlan to the Colorado River [2]. 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) [2].

Studies of the effect of temperature and salinity on different physiological responses in penaeid have been carried out by [1-13]. However, laboratory data from published studies are not always consistent with field observations [1]. The reason is that 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 [14]. It has been noted that the osmotic work of the organism is minimal when the external medium and the body fluids are in equilibrium; besides under isosmotic conditions it is possible to cultivate the maximum number of organisms [15]. In aquatic biotechnology of the rearing of organisms it is desirable to understand the interactions between nutritional and environmental factors, the determination of the physiology energetic allows the optimization of the rations that provide the appropriate levels of energy to the species of commercial importance when covering the basic processes of the organism and therefore the protein incorporation is facilitated for the tissue synthesis [16].

Bioenergetics studies allow us to describe, explain and predict the conditions or physiological state of the organism under culture conditions through the equation $C = P + R + F + U + AHI + M$ [17] 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 digestion and food consumption and M is the energy used in the molting process. Energy balance studies have been conducted in penaeids mainly in *Penaeus vannamei* (Boone), juveniles and postlarvae to observe the effect of different levels of protein and carbohydrates in the diet, immunological conditions, ions tissue mineralization and different amounts of animal and plant protein added to the

*Address correspondence to this author at the Laboratorio de Nutrición Funcional, Departamento de Biotecnología Marina Centro de Investigación y Educación Superior de Ensenada, Km 107 Carretera Tijuana-Ensenada, Ensenada Baja California, México; Fax: 01152-6461750569;
E-mail: denisre@cicese.mx

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diet and to examine the combined effect of temperature and salinity [17- 24].

Some aspects of the energetics of the 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 salinity levels. The aquacultural potential that the blue shrimp *P. stylirostris* has in México, makes it necessary to know the effect of three salinities on elements of energy budget in juveniles; to determine if isosmotic conditions provide for a high energetics efficiency which would promote an increase in survival and growth of blue shrimp.

MATERIAL AND METHODOLOGY

P. stylirostris Pl_{21} postlarvae (n=5000) were obtained from the AQUANOVA production laboratory in Mazatlan Sinaloa México and were transported to the laboratory in polyurethane boxes. They were placed in 4 tanks of 2000 l with continuous marine water flow constant air flow and underwater heaters of 1000 W which made it possible to maintain salinity conditions of 35psu and a temperature of 28 ºC. The postlarvae remained in these conditions until they reached the juvenile stage. During this period the organisms were fed daily with Rangen commercial feed with 40% protein in two rations equivalent to 5% of the organisms' total biomass. The juveniles with a mean wet weight of 4.11 ± 1.2 g were distributed into three 500-l circular tanks to acclimate for 15 days in the different experimental conditions. These conditions were the isosmotic salinity level for *P. stylirostris* (25-26psu) a lower salinity of 20psu where the organisms were hyper-osmotic and another of 30psu where the organisms were hypo-osmotic determined by [8-9] and [11]. A temperature of 28º C was determined by [13] as optimum for this species. In order to obtain the water with the desirable salinity sea water (35psu) was diluted with fresh water. This was done daily in order to replace water lost during evaporation and avoid increases in salinity and also 50% of the total water volume in the tanks was replaced daily to maintain its quality.

In order to examine the salinity effect on the organism's different physiological responses and energy balance was determined using the [17] modified equation:

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

This was carried out with 26 organisms from each experimental condition which were distributed individually into four-liter plastic containers inside each 500-l circular tank. Two repetitions were performed for each experimental salinity condition (20, 25 and 30psu) (n=156). Such containers had screen-covered side windows to allow water exchange with the tank but at the same time preventing as well the organisms, feed and feces from exiting. Constant aeration was given and 50% of the water was changed daily. Dissolved oxygen level was maintained at 6 mg l^{-1} in all the containers (4 l). Shrimp were fed daily with a (Rs) ration size of 0.4 g^{-1} per individual of Rangen commercial food (40% protein).

The equation components were determined as follows: the daily feed ration was given and left for 2 h in the containers with the organisms. After this period the feed that was not consumed Ef (Excess food) 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 individual daily dry weight (C=Rs-Ef-Lf). Tests were carried out to obtain the solubility of the diet in each experimental condition according to methodology described by [25]; 1 g of feed was placed in each container without organisms for two hours. The data from the consumed feed were corrected by the dilution factor Lf (Leached food) of the diet.

To know the stability of the diet, due to aeration, siphoning and water movement, it was used a modification of the methodology described by [25]. One gram of the diet was placed into 10 buckets inside of the each tank of 500 l for each condition of salinity, without organisms. The food remained in the buckets for 2 hours (static method). The food was recollect with the same way that before described, later was dried in oven at 60º and it was calculated the food stability by the formula of dry matter retention.

$$\% DMR = 100 - [(WD_{bi} - WD_{as})/WD_{bi}] \times 100$$

Where: WD_{bi} = weight diet before to immersion.

WD_{as} = dry weight of the diet after immersion

Routine oxygen consumption was measured (R) and the apparent heat increment (AHI) in the shrimp for each experimental condition, with two repetitions were done and determined in a respirometer system as described by [26]. In this system 20 organisms were placed individually in a 1.0 l Erlenmeyer flask. The shrimp were placed in the respirometer system 12 h before the beginning of the measurements in order to allow their acclimation to the system as mention by [27]. The dissolved oxygen in the Erlenmeyer flasks was measured with an YSI 52 oximeter equipped with a polarographic sensor with recently fed organisms and with the organisms kept without feed for 24 h. In the first instance the organisms were fed for two hours simulating the feed and feces collection. Afterward the feed 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 was left closed for an hour since according to [28] this is an adequate time so that the dissolved oxygen 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 [29].

At the end of the respirometer experiments the organisms were weighed 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 [30].

The feces (F) were collected 2 h after feeding; this operation was repeated 6 h later and before feeding the

organisms the next day. The collection of feces was made in the same manner as the feed collection. The dry weight was determined in the same way as with the feed. The diet and feces caloric content (individual daily dry weight) was repeated five times and was determined with a PARR semi micro calorimeter model 1425 standardized with benzoic acid according to mention by [31, 32].

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 [33] 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^+ [16].

During this experimental phase a control Erlenmeyer flask was kept without organisms in order to measure the oxygen consumption and ammonium production of the microorganisms present in the respirometer system where the necessary corrections were then made.

The energy allocated for exoskeleton formation (M) was determined from the caloric content of the molts (dry basis) collected 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 feed 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 and was expressed in J g^{-1} day^{-1} d.w.

The O:N atomic ratio was estimated using the values of the shrimp's oxygen consumption and the ammonium excretion obtained in the respirometer system at the different experimental salinities. The physiological rates determined for both components were transformed to gram atoms for calculation of the O:N ratio using the respiratory thermochemical principles [34]. This ratio was used to determine if the proteins, lipids and carbohydrates were used as 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 proteins and lipids while values above 60 were attributed to the use of carbohydrates [35].

The data were tested for normality and homoscedasticity (Sigma Stat) and then the Kruskal-Wallis [36] non-parametric test was used to determine the salinity effect on the different parameters that form the energy balance equation for blue shrimp juveniles. Where significant differences were found the Dunn method was used to isolate the groups showing differences [36].

RESULTS

The energy ingested in the feed by the organism ranged from 3410 to 3455 J g^{-1} day^{-1} d.w. For the *P. stylirostris* juveniles exposed to the different salinities there were no significative differences ($P > 0.05$) among the groups with regard to ingested energy through the feed (Table **1**).

The energy that the shrimp acclimated at 25psu salinity used for routine metabolism 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 salinities.

In the shrimp maintained in the experimental salinities the energy loss in fecal production represented 5.6 to 7.0% of the ingested energy from the feed (Fig. **1**). No significant differences were found ($P > 0.05$) among the organisms exposed to the three salinities in the amount of energy lost in the production of feces.

The energy loss from the ammonium excretion by the *P. stylirostris* juveniles was increased by exposure to a salinity of 20psu and decreased in the juveniles acclimated at 25 and 30psu (Table **1**). Significant differences were found ($P < 0.05$) for different salinities in the energy allocated by the shrimp to this physiological process.

Table 1. Physiological Rates of Juveniles of *Penaeus stylirostris* Acclimated to Different Salinities. C = Ingestion, F = Fecal Production, R = Respiration, U = Ammonium Excretion, AHI = Apparent Heat Increment, M = Molting, P = Scope for Growth (J g^{-1} day^{-1} d.w). Median ± 95% Confidence Interval

	SALINITY (psu)		
	20	**25**	**30**
C	3410.27 ± 42.0[a]	3455.63 ± 52.0[a]	3445.22 ± 59.0[a]
F	237.8 ± 36.1[a]	226.5 ± 35.7[a]	191.6 ± 47.0[a]
R	215.26 ± 22.1[b]	123.62 ± 34.1[a]	260.79 ± 29.8[b]
U	64.9 ± 2.3[b]	23.97 ± 1.1[a]	39.62 ± 1.2[c]
AHI	169.2 ± 20.0[a]	135.7 ± 13.0[a]	139.21 ± 12.9[a]
M	183.81 ± 4.3[a]	189.37 ± 3.3[a]	184.69 ± 4.6[a]
P	2539.9 ± 52.0[a]	2756.47 ± 49.0[b]	2629.31 ± 39.0[a]

Values in each row followed by a different letter differ significantly with $\alpha = 0.05$.

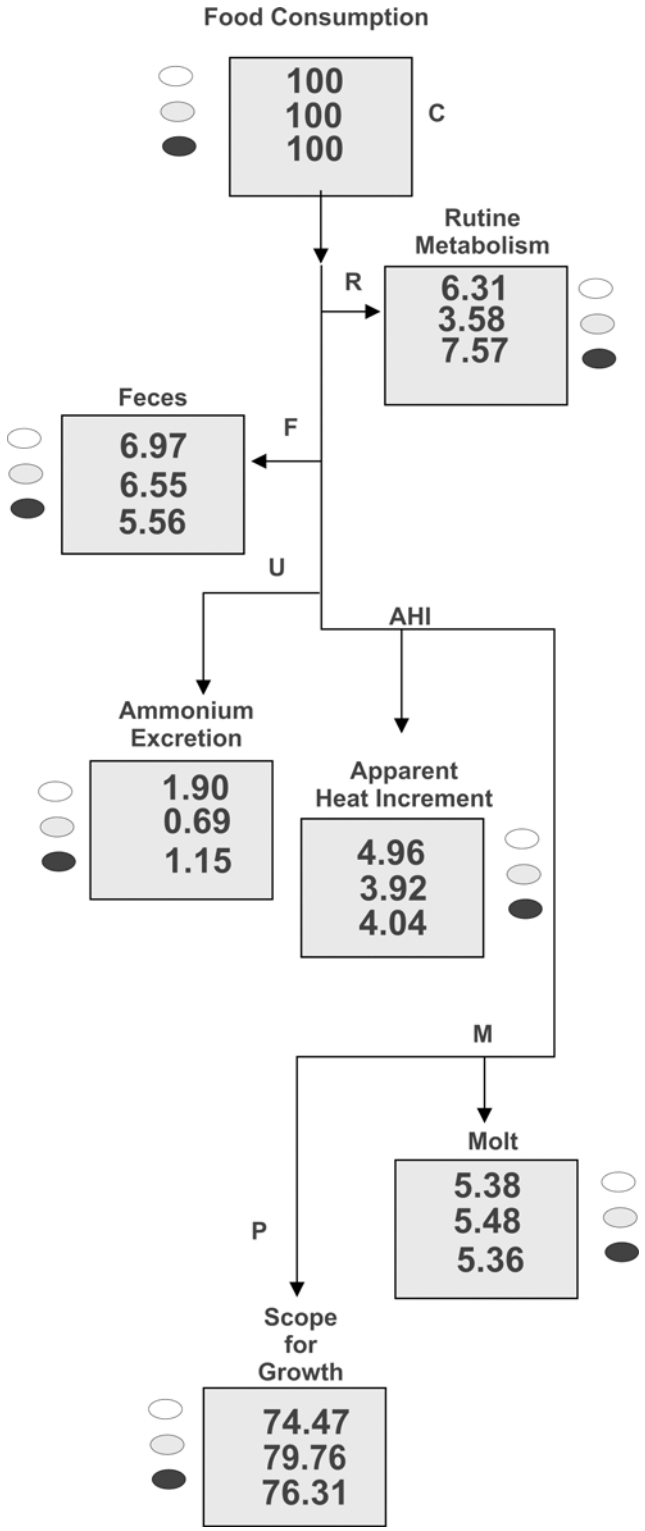

Fig. (1). Distribution percentage of energy obtained from consumed food on the different parameter of energetic balance equation of *Penaeus stylirostris* in relation to the salinity. ○ 20 25 and 30psu.

When the organisms were fed the apparent heat increment (AHI) represented an increase in the energy allocated to the metabolic process from 47.6 to 65.3% in the three salinity conditions (Fig. **1**). Such increase represented a loss of 3.92 to 4.96% of the ingested energy through feed by the organisms (Fig. **1**). No significant differences were obtained ($P > 0.05$) in the AHI among the organisms exposed to salinity of 20, 25 and 30psu.

The energy invested in molting by the shrimp acclimated at different salinities ranged 183.8 to 189.37 J g^{-1} day^{-1} d.w. (Table **1**). No significant differences were found ($P > 0.05$) in the amount of energy assigned to this process among the *P. stylirostris* juveniles exposed to the different experimental salinities.

The growth potential energy invested by the shrimp kept at a salinity of 20psu was 2539.9 J g^{-1} day^{-1} d.w. The shrimp acclimated to the isosmotic condition invested 2756.47 J g^{-1} day^{-1} d.w. The shrimp maintained at 30psu invested in this process 2629.31 J g^{-1} day^{-1} d.w. (Table **1**). The different salinity levels produced significant differences ($P < 0.05$) in the juvenile's energy allocated to growth.

The O:N atomic ratio value estimated for the *P. stylirostris* juveniles kept in the isosmotic condition was 104.6±6.0. In the organisms exposed to 30psu salinity this ratio was 90.8±2.0. The minimum value for the O:N ratio was observed in the juveniles acclimated at 20psu which was 65.6±10.0; the one-way analysis of variance showed a significative differences ($P < 0.05$). For *P. stylirostris* juveniles a change in the O:N atomic ratio was observed which indicated a change in the energy substrate used in response to variation in environmental salinity (Fig. **2**).

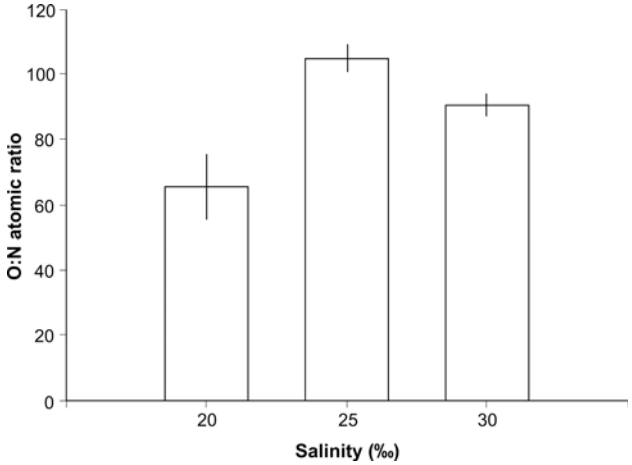

Fig. (2). O:N atomic ratio of juveniles of *Penaeus stylirostris* acclimated to different salinities.

DISCUSSION

The salinity of 25psu was that the juvenile of *P. stylirostris* used less energy to cover its metabolic processes of routine. This result was to be expected since this is the salinity that [9, 11] reported as being an isosmotic environment for blue shrimp juveniles where the organisms do not require active processes to compensate for the change in the osmotic pressure of the external environment [15]. The highest energy demand was recorded in the organisms kept at a salinity of 30psu where they were hypo-osmotic with energy demand having intermediate values at 20psu salinity where they were hyper-osmotic [5] in a similar way found in *P. vannamei* juveniles that the highest energy demands to cover routine metabolism occur when exposed to 40psu salinity.

It was found that the highest concentrations of ammonium were produced by the shrimp at salinity where they are hyper-osmotic; the organisms maintained in isosmotic condition excreted lower amounts. Intermediate values were observed in juveniles that were kept in hypo-osmotic conditions. Similar tendencies for this physiological rate in relation to salinity were reported by [6] for *P. vannamei* juveniles. These authors studied organisms maintained at salinities of 10 to 40psu and found that the lowest nitrogenous excretion rate was obtained at 26.6psu a finding which agrees with the data presented in this study and with the data reported by [7, 24] in *P. vannamei* exposed to fluctuating and constant salinities since in both works the lowest energy use due to ammonium excretion was observed in the organisms maintained at an isosmotic level of 25psu. The increased nitrogenous excretion ratio by the *P. stylirostris* acclimated to lower salinities can be explained by an increase in amino acid catabolism which is involved in the regulation of haemolymph osmotic pressure; this occurs when an active absorption of sodium is needed to compensate for loss in organisms that are found in hypo-osmotic environments. The increase in ammonium excretion favors sodium uptake possibly through the Na^+/NH_4^+ exchange pump in order to maintain the haemolymph osmotic concentration [6-11] have suggested that this physiological mechanism is the main one responsible for the maintenance of the osmotic concentration in penaeid shrimp when found in hyper-osmotic environments.

Similar results were obtained by [37] when they maintained white shrimp juveniles at 15 to 40psu salinity where no significative differences were found in the apparent heat increment since they were fed the same diet [38] mentioned that in *P. monodon* (Fabricius) juveniles acclimated at 5, 15 and 45psu salinity and fed with commercial diet there was an increase of 2 to 17% of oxygen consumption which represented 2.4 to 19.5% of the ingested energy and that no significant differences were found in the AHI among the organisms maintained at the three salinities [24] in *P. vannamei* obtained an increase of 31.6 to 37.5 in AHI in organisms exposed to three salinities which represented 7.6 to 8.9% of the ingested energy. The results obtained in the present work agree with those obtained by [24, 38] in that the *P. stylirostris* juveniles did not show differences in the magnitude of this metabolism component that could be related to salinity since the energy expended in this process represented 3.9 to 4.9% of the energy obtained through ingested feed [29] mentioned that the factors that modify the apparent heat increment magnitude resulting from feed ingestion include the nature of the diet size and ration and the chemical composition of the feed. Considering previous findings it was to be expected that no differences exist between the apparent heat increment since the organisms during this research were fed with the same diet.

The *P. stylirostris* juveniles used 5.36 to 5.48% of the energy obtained in the feed for the exoskeleton formation when they were acclimated to the different salinities. Similar values have been reported for other decapods such as *Palaemonetes pugio* (Holthuis) and *Cherax quadricarinatus* (von Martens) who invested 1 to 8% respectively of the ingested energy through feed to exoskeleton formation [16, 39]. In the blue shrimp juveniles the energy expenditure for the molting process was not affected by salinity 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 [35, 40]. The O:N ratio estimated for the organism acclimated to 25psu salinity was 1.1 and 1.7 times higher than the ratio obtained when the organisms were maintained at 30 and 20psu respectively. This indicates that the shrimp maintained at isosmotic salinity use carbohydrates as the energy substrate. When they were hypo-osmotic the main energy substrate used was a protein-lipid mixture and a salinity of 30psu where they were hyper-osmotic a mixture of lipids and carbohydrates was used as the energy substrate.

These results are similar to those reported by [37] for juveniles of *P. vannamei* kept at 15 and 40psu salinity since the higher values for the O:N ratio were obtained in the organisms kept at lower salinity being two to three times higher than that determined for organisms exposed to 40psu [41] reported that for *Farfantepenaeus paulensis* (Perez-Farfante) postlarvae kept at salinities of 25 and 34psu the O:N ratio was significantly higher than that recorded when kept at 4 and 15psu. The results of the O:N ratio values obtained in *P. stylirostris* agree with the values obtained with many other estuarine and marine species where the O:N ratio is higher for organisms found at optimum salinities and lower when found at lower salinities; this shows a change from a predominant carbohydrate metabolism at optimum salinities to a metabolism dominated by a protein-lipid mixture at lower salinities.

It has been demonstrated that in other penaeid species growth is affected in a different way by the salinity effect. The shrimp *Fenneropenaeus merguiensis* (De Man) reach a maximum biomass growth and survival when they are maintained at 25psu salinity. In *Callinectes similis* (Williams) [42] obtained the highest scope for growth when crabs were acclimated to 30psu salinity which is the optimum salinity for this species [37] found that when *P. vannamei* were exposed to salinity levels of 15 and 40psu and fed with a diet lacking carbohydrates a higher scope for growth was obtained [43] found that in *P. vannamei* juveniles acclimated to 15 and 40psu and exposed to different Na/K proportions there was a higher growth rate in the organisms at 15psu salinity [14] reported a significant interaction between salinity and temperature on growth *P. vannamei* when maintained for 40 days at different combinations of salinity and temperatures. Shrimp had a significantly greater growth at salinities higher than 20psu and temperature between 25 and 35 ºC [23] indicated that in *P. vannamei* the optimal salinity corresponding to maximum specific growth rate was about 25psu for shrimp maintained at 28 ºC. The scope for growth determined for *P. stylirostris* that were acclimated to 25psu represent the highest percentage of the ingested energy through feed.

Based on the results obtained we believe that the optimum salinity to cultivate this species is 25psu since it corresponds to the isosmotic level. According to [15] the maximum growth and survival of an organism is obtained when it is found in an isosmotic environment since in this

way the animal does not invest energy in osmotic work where it does not use this active process in maintaining the internal environmental equilibrium in relation to the external environment. Considering the above it is assumed that under these conditions the organisms will optimize their physiological process in such a way that energy expenditure will be reduced which would represent an energy saving that could be directed to growth potential.

Studies on the effect of salinity on the energy balance of crustaceans under different salinities are scarce even though they have demonstrated that this kind of analysis provides information that helps in the quantification description and explanation of the physiological condition of the organisms. Exposure of shrimp to a salinity of 25psu reduces the energy expenditure channeled to cover the routine metabolic processes and the excretion of nitrogenous products increasing the scope for growth. The O:N ratio values showed that the shrimp used primarily carbohydrates for energy a substrate which indicates that at such salinity they are free of environmental stress. We propose that *P. stylirostris* juveniles be maintained under these conditions in order to optimize culture conditions for this species.

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