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Influence of spat origin and environmental parameters on biochemical composition and biometry of the brown mussel *Perna perna* (Linné, 1758), under culture conditions

Influencia del origen de las semillas y de los parámetros ambientales sobre la composición bioquímica y biometría del mejillón marrón *Perna perna* (Linné, 1758), bajo condiciones de cultivo

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Resumen.- La composición bioquímica, el crecimiento y la supervivencia del mejillón marrón (*Perna perna*), de orígenes intermareal y submareal fueron comparados después de que ambos grupos fueron colocados en un 'long line', donde crecieron hasta tamaño comercial, en el Golfo de Cariaco, Estado Sucre, Venezuela. Durante el período de muestreo fueron registrados para ambos grupos sus respectivas longitudes, masa seca de los tejidos blandos y de la concha, así como también su condición reproductiva. Los componentes bioquímicos analizados fueron: proteínas, carbohidratos y lípidos. Simultáneamente, se llevaron registros de las condiciones medioambientales representadas por la clorofila *a*, salinidad, temperatura y el seston. Al inicio del período experimental, que duró 213 días, los contenidos de lípidos y carbohidratos fueron significativamente más altos en los mejillones de origen submareal mientras que no hubo diferencias significativas en las proteínas entre ambos grupos de mejillones. Estas diferencias fueron mantenidas hasta el día 15 para los lípidos y el día 21 para los carbohidratos. En contraste, no se observaron diferencias significativas entre ambos grupos en cuanto al crecimiento (longitud y masas de los tejidos blandos) e índice de condición, mostrando así un potencial similar para su cultivo bajo condiciones suspendidas. Con respecto a la influencia de variables medioambientales, la temperatura y clorofila *a* mostraron una influencia marcada en la composición bioquímica de ambos grupos de mejillones.

Palabras clave: Bivalvo, carbohidratos, lípidos, proteínas, reproducción

Abstract.- Biochemical composition, growth, and survival of brown mussels (*Perna perna*) collected from subtidal and intertidal origins were compared after both groups of mussels were placed on a long line and grown to commercial size in Golfo de Cariaco, Sucre state, Venezuela. During the sampling period, data on length, dry mass of soft tissues and shell, as well as reproductive condition were collected for both groups. Proteins, carbohydrates and lipids were the biochemical components analyzed. Simultaneously, environmental conditions represented by chlorophyll *a*, salinity, temperature, and seston were registered. At the beginning of the experimental period, which lasted 213 days, lipid and carbohydrate contents were significantly higher in mussels from subtidal origin while no significant differences were observed in proteins between both mussel groups. These differences were only observed until day 15 for lipids and day 21 for carbohydrates. In contrast, no significant differences were observed between groups in growth (length and mass of soft tissues) and condition index, therefore showing similar potential for their use in suspended culture. Regarding the influence of environmental variables, temperature and chlorophyll *a* showed the strongest effects on biochemical composition of brown mussels.

Key words: Bivalve, carbohydrates, lipids, proteins, reproduction

Introduction

One of the main reasons why mussel culture has developed exponentially in some parts of the world is due to its establishment in areas where spat availability is abundant (Mason 1976, Hickman 1992). For the brown mussel *Perna perna* (Linné, 1758) in Venezuela it is expected that, as the commercial culture of this species develops, increased spat demand from rocky shores may

affect the renewal of this important fishery resource. Eventually, the use of artificial collectors for spat fixation, as used, for example, in the culture industry of *Mytilus galloprovincialis* in Galicia, Spain (Pérez-Camacho *et al.* 1995), could be a viable alternative for increasing spat availability. In the Galician mussel cultures, differential growth in spat has been attributed to heterogeneity in the different sources used. From the initial stages of development of the Galician mussel culture, better growth

performance was observed for spat from subtidal origin. These differences in growth were attributed to variations in ingestion rates (Pérez-Camacho *et al.* 1995), which would result from adaptations to marked local variability in the quantity and quality of available seston (Theisen 1977, Bayne *et al.* 1984, Navarro *et al.* 1991).

One of the main factors affecting biochemical composition of mussels and other bivalves from temperate intertidal zones is the frequent periods of air exposure, which reduces food availability and has an effect similar to starving (Hummel *et al.* 1989). For example, *Crassostrea gigas* individuals subjected to feeding stress (*i.e.* starving) showed not only a reduction of carbohydrates, but also a decrease of 44% and 23% of proteins and lipids, respectively (Whyte *et al.* 1990). Also, Freitas *et al.* (2003) observed that *Mytilus galloprovincialis* spat of subtidal origin had larger absolute content of proteins, carbohydrates and glycogen than those of intertidal origin.

Several studies have shown that differences in the biochemical composition of mussel populations located in zones with distinct environmental conditions were due to qualitative and/or quantitative differences in food availability of phytoplanktonic origin (Pérez-Camacho *et al.* 1995, Fernández-Reiriz *et al.* 1996, Okumus & Stirling 1998). With regards to natural populations of mussels of the genus *Mytilus* spp., annual fluctuations in the different components of the biochemical composition have been related to environmental parameters and the reproductive cycle (Pieters *et al.* 1979, 1980, Zurburg *et al.* 1979, De Moreno *et al.* 1980, Kluytmans *et al.* 1980, Zandee *et al.* 1980, Bressan & Marin 1985, Fernández-Reiriz *et al.* 1996, Okumus & Stirling 1998).

Accordingly, these studies agreed with the theory established by Bayne (1976), whereby after feeding, a series of metabolic processes come into play, from which the energy obtained from the food could be initially accumulated as reserve tissue and thereafter destined for gametogenesis and/or used in periods of low food availability. This would result in a biochemical cycle and, consequently, a reproductive cycle.

In the north-eastern coast of Venezuela, tide levels affect mussels fixed in the upper limit of the intertidal zone, where they may be exposed to air for up to 8 hours in periods of maximum tidal intensity. The main objective of this study was to determine the influence of these environmental conditions on initial biochemical composition and its variations, as well as survival and growth, of spat (subtidal and intertidal origin), placed under culture conditions until attainment of commercial size.

Material and methods

Spat origin, experimental design, and sampling

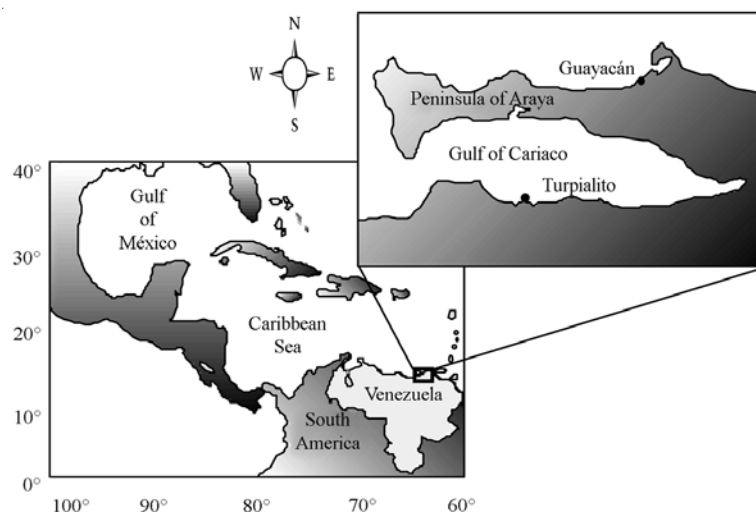
Perna perna spat were collected from a natural bed in Guayacán, (10°39'N; 63°49'W), in the north coast of Sucre state, Venezuela (Fig. 1). Spat were manually collected from the subtidal and intertidal rocky shore zones. It was estimated that the latter mussels were exposed at least 8 hours/day and consequently had not been feeding during the exposure time. Both spat groups were transported in insulated containers to the Estación Hidrobiológica de Turpialito (Instituto Oceanográfico de Venezuela - Universidad de Oriente), located at 10°27'30"N; 64°01'52"W, on the coast of Golfo de Cariaco, Sucre state (Fig. 1), where they were placed on a long-line culture system 45 m from the coast at approximately 10 m depth.

The experimental mussel population from both habitats (subtidal and intertidal) consisted of juveniles with lengths and tissue dry mass varying between 3 and 5 cm and 0.48 and 0.63 g, respectively. A total of 240 individuals from each habitat were separated randomly in 12 replicates of 20 individuals. These replicates were later attached to 1.5 m culture lines using biodegradable cotton net. Once attached, the mussels from both origins were suspended at random along the long line at a depth between 2 m and 3.5 m.

A sample was taken at the beginning of the experiment, later weekly samples were taken during the first month and fortnightly samples during the rest of the study period (from May to October 2003). Each sample collected consisted of a random selection of three experimental replicates from each habitat. These samples were placed in separate plastic bags, and transported to the laboratory for determination of tissue and shell dry mass, total shell height and mussel organic material content and biochemical composition.

Environmental variables

During mussel sampling water samples were collected with a Niskin bottle, stored in isothermic containers and transported to the laboratory for chlorophyll *a* and seston analyses. For chlorophyll *a* determination seawater was pre-filtered through a 250 µm sieve and then filtered onto Whatman GF/F (0.7 µm) filters with Millipore equipment. Chlorophyll *a* concentration was obtained using spectrophotometric techniques following methodology described by Strickland & Parsons (1972). Total seston, including its inorganic and organic fractions, was obtained by the gravimetric method after combustion in a muffle (450°C, 4 h). Salinity was measured *in situ* using

**Figure 1**

Geographic location of sampling (Guayacán) and culture area of the mussel *P. perna* (Ensenada de Turpialito)

Ubicación geográfica del área inicial de muestreo (Guayacán) y el área de cultivo del mejillón *P. perna* (Ensenada de Turpialito)

a hand refractometer ATAGO S/Mill (range 0-100%). Temperature was recorded continuously with a Sealog (Vemco Ltd, Halifax) thermograph placed at the experimental depth.

Corporal growth of mussel

Growth of *Perna perna* spat was estimated from measurements with a digital caliper (0.01 mm) of total shell height from 3 samples of 15 individuals each from the experimental replicates. To obtain dry mass of soft tissues and shell, these components were placed in previously weighed containers and placed in an oven at 60°C until reaching constant weight. Once dried, only the soft tissues were finely ground and stored in a refrigerator for biochemical analysis.

Condition index

This index was estimated for each individual from the following formula: $CI = (\text{soft tissue dry mass} \times 100) / \text{Total dry mass}$

Survival of cultured mussels

Survival was determined from counts of dead individuals at each sampling period.

Reproductive stages of cultured mussels

Qualitative assessment of reproductive stages was made by identifying morphochromatic gonad characteristics

according to the visual scale reported by Nakal (1979), that is: immature (I), developing (II), mature (III), spawned (IV), and gonadal regression (V).

Organic matter

From each experimental replicate tissue samples from 5 mussels previously oven-dried and weighed were placed in a muffle (JELRUS Two-Stage Temp-Master L) at 450°C for 4 hours and organic matter content was calculated from weight differences.

Biochemical analysis

For the remaining individuals from each experimental replicate, proteins from tissue dry mass were quantified by the method of Lowry *et al.* (1951), while total lipids were estimated using the gravimetric method following Overturf & Dryer (1967). Carbohydrates were estimated by the phenol sulphuric method (Dubois *et al.* 1956). Biochemical composition data were expressed as absolute organic matter content (mg mussel⁻¹) and relative levels (organic matter percent per mussel). In order to avoid the effects of growth on changes observed in biochemical components content, results were standardized by interpolation to a standard individual of 225 mg (dry tissue mass) which represents the average value between initial and maximum value of mass obtained from both mussels groups (see Fig 3C).

Statistical analysis

Biometric, biochemical and environmental variables results were presented in terms of the mean value \pm standard deviation.

The estimates of different parameters for both groups of mussels were compared with a one-way analysis of variance (ANOVA), after testing for homogeneity of variances with Bartlett's test, using a 95% significance level. When parameter values did not satisfy the condition of variance homogeneity data were transformed to log biomass, arcsine percentage reproductive stages (Zar 1984).

In order to analyze possible relationships between reproductive stages and biochemical composition with environmental parameters Pearson's correlation coefficient and the partial correlation coefficient were used. To account for false positives in a multiple comparison framework and considering the conservative nature and low power of Bonferroni type corrections, the False Detection Rate was assessed by calculating q values as proposed by Storey (2002). Also, a regression model was used to assess the influence of spat origin and environmental parameters on biochemical composition (carbohydrates, lipids and proteins).

Results

Environmental variables

Temperature showed minimum values (25.8°C) at the end of May and at the beginning of June (Fig. 2A), afterwards there was a sustained increase until maximum values (28.9°C) were reached in September and remained relatively high until the end of the study period. Salinity mean values in Turpialito showed relatively small variations between 34.00 ± 0.01 to 37.67 ± 0.58 , with minimum values occurring in September (Fig. 2B).

Organic and total seston showed high variability during the experimental period, with minimum values in mid-May and June, beginning of July, and during September (Fig. 2C). A strong increase of total and organic seston, reaching mean values of 11.03 ± 1.67 , 8.49 ± 0.61 , 23.49 ± 3.68 and $13.03 \pm 1.11 \text{ mg L}^{-1}$, was observed at the end of June, mid-July, mid-August, and end of October, respectively. Highest chlorophyll a concentrations were observed in the second and third weeks of May with mean values of $6.28 \pm 2.29 \mu\text{g L}^{-1}$ and $7.06 \pm 0.70 \mu\text{g L}^{-1}$, respectively (Fig. 2D). These values diminished sharply in the following sample and showed a decreasing trend until the end of the experiment when a minimum of $0.11 \pm 0.04 \mu\text{g L}^{-1}$ was registered.

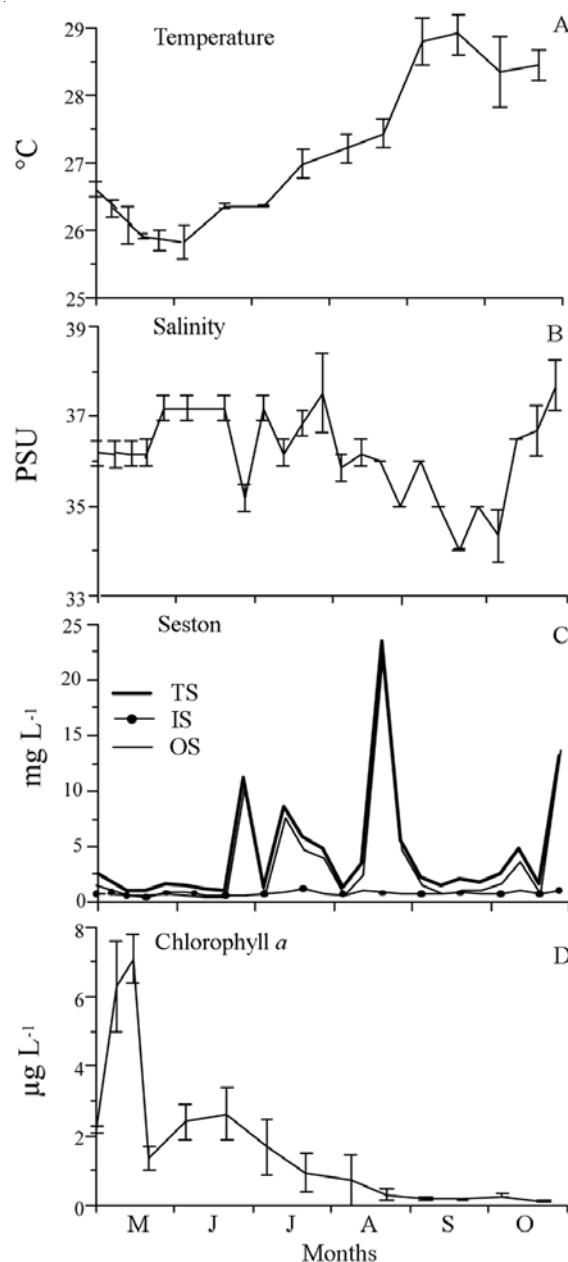


Figure 2

Variations in temperature (A), salinity (B), seston (C) and chlorophyll a (D) during the study period in Ensenada de Turpialito. Horizontal bars represent standard deviation.

TS (total seston), IS (inorganic seston) and OS (organic seston)

Variación de la temperatura (A), salinidad (B), seston (C) y clorofila a (D) durante el lapso de estudio en la Ensenada de Turpialito. Las barras horizontales representan las desviaciones estándar de los datos. TS (seston total), IS (seston inorgánico) and OS (seston orgánico)

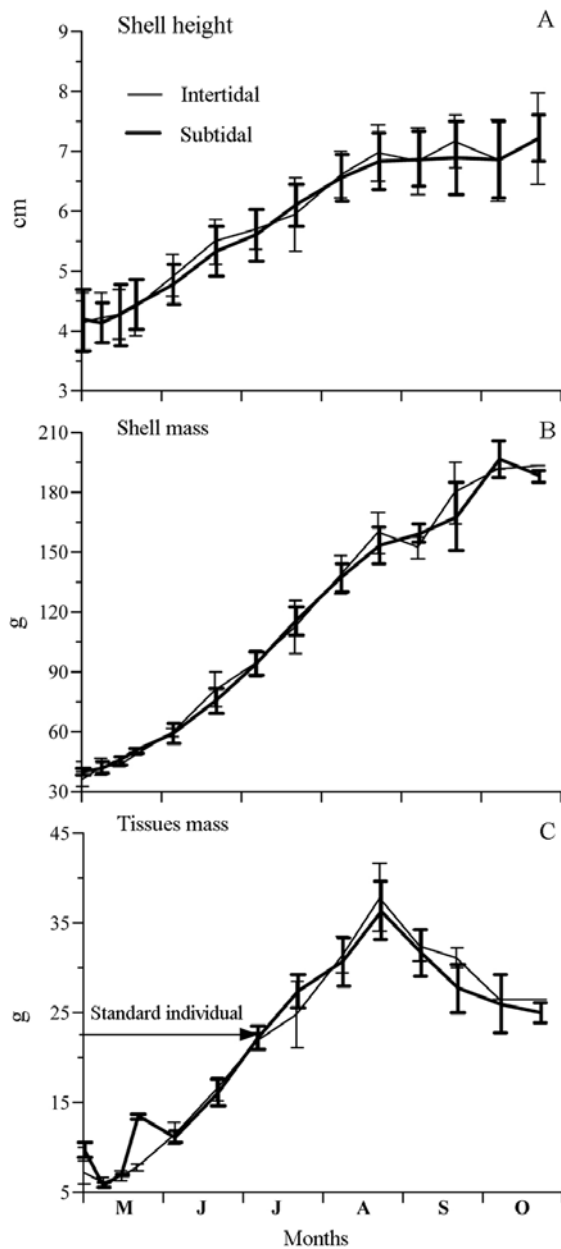


Figure 3

Variation in mean values of shell height (A) shell mass (B) and (C) tissue mass of the mussel *P. perna* from intertidal and subtidal origin during experimental period in Ensenada de Turpialito. Horizontal bars represent standard deviation

Variación del promedio de la altura de la concha (A) masa de la concha (B) y (C) masa de los tejidos del mejillón *P. perna* de origen intermareal y submareal, durante el período experimental en la Ensenada de Turpialito. Las barras horizontales representan las desviaciones estándar de los valores

Corporal growth of mussels

At the beginning of the experiment no significant differences in height and mass were observed between the two groups of spat. The initial mean heights from both origins were 4.13 ± 0.51 cm (intertidal) and 4.15 ± 0.51 cm (subtidal), while initial mean dry shell masses were 35.68 ± 3.84 g (intertidal) and 39.22 ± 1.71 g (subtidal). At the end of the study period the mussels from intertidal and subtidal habitat origins reached average heights and shell masses of 7.19 ± 0.76 cm and 7.20 ± 0.39 cm and 193.00 ± 0.07 g and 186.97 ± 3.05 g, respectively. In general, growth rate in height was fast during May and August (Fig. 3A); later growth rate showed a decrease for the rest of the study period. In contrast, growth rate of shell dry mass was relatively constant during most of the experiment (Fig. 3B). At the end of the study there were no significant differences between groups in the biometric variables analyzed.

Condition index

The individuals from subtidal origin showed a significantly higher condition index at the beginning of the study period (ANOVA, $P < 0.05$). The condition index varied between 11.64 to 19.49% for subtidal origin and between 12.06 to 19.1% for intertidal origin (Fig. 4).

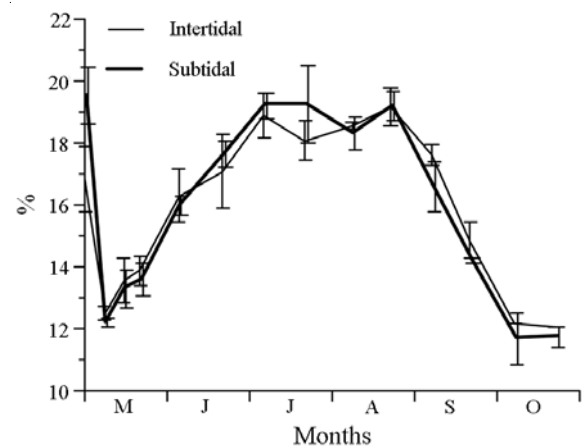


Figure 4

Variation in mean condition index (CI) of the mussel *P. perna* from intertidal and subtidal origin during experimental period in Ensenada de Turpialito. Horizontal bars represent standard deviation

Variación del promedio del índice de condición (IC) del mejillón *P. perna* de origen intermareal y submareal, durante el período experimental en la Ensenada de Turpialito. Las barras horizontales representan las desviaciones estándar de los valores

However, no significant differences were observed between subtidal and intertidal values of the index after mussels were placed on long-lines in subtidal conditions (Fig. 4). Additionally, this parameter showed for both groups a decrease at the start of the study, an increase to a maximum between July and August and finally a decrease until the end of the experiment.

Survival

The overall survival of both groups of mussels was greater than 90% (Fig. 5). Even though individuals of intertidal origin showed a higher survival from June onwards, no statistically significant differences were observed between the two groups during the experimental period. It is worth noting the lower survival rate observed for both groups in the first two weeks of the experimental period, which is likely due to loss of spat not properly attached to the culture rope.

Reproduction

Both groups of mussels showed a 1:1 sex ratio and sexual development was first apparent at approximately 41 mm shell length. Spawning occurred during the whole study period (Fig. 6). However, there were two apparent peaks in spawning activity. A first relatively weak spawning event was observed in May, while a much stronger second event was observed between August and October. Additionally, individuals from both origins showed

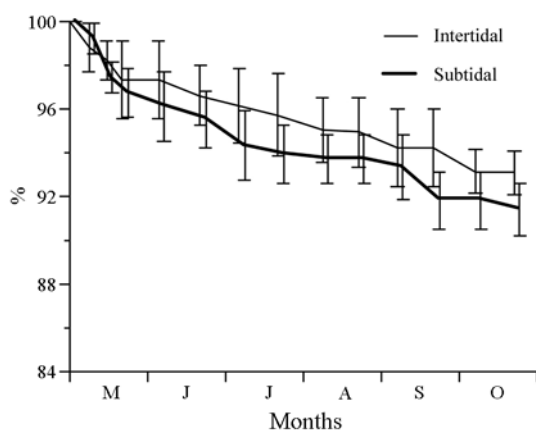


Figure 5

Survival of the mussel *P. perna* from intertidal and subtidal origin in Ensenada de Turpialito. Horizontal bars represent standard deviation

Supervivencia del mejillón *P. perna* de origen intermareal y submareal, en la Ensenada de Turpialito. Las barras horizontales representan las desviaciones estándar de los valores

asynchronous reproduction as at least three reproductive stages were present simultaneously during most of the study period.

Relations between reproductive stages and environmental variables

A number of significant Pearson correlation coefficients were observed between the different reproductive stages and environmental variables (Table 1). In particular, there was a direct and highly significant ($q < 0.001$) correlation between stage IV (spawning) and temperature for both groups of mussels. This suggests that this variable may

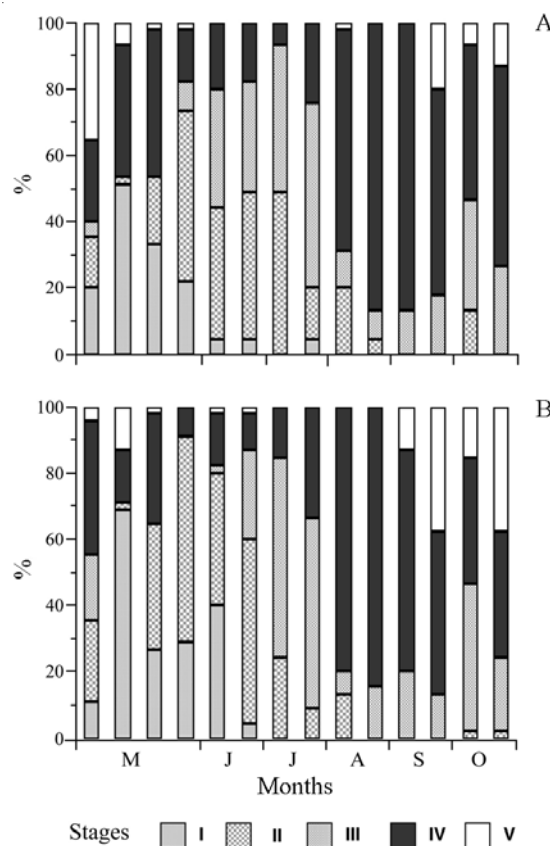


Figure 6

Histograms of reproductive stage frequencies of mussel *P. perna* from intertidal (A) and subtidal (B) origin in Ensenada de Turpialito, Sucre State. Reproductive stages: I=Immature, II=gametogenic stage, III=Mature, IV=Spawning, V=gonad regression

Histogramas de frecuencia de los estadios reproductivos del mejillón *P. perna* de origen intermareal (A) y submareal (B) cultivado en la Ensenada de Turpialito, Estado Sucre. Estadios reproductivos: I= Inicio reproductivo, II= Inmaduro, III= Maduro, IV= Desove, V= Regresión gonádica

Table 1

Pearson and partial correlation coefficients between reproductive stages and environmental parameters for mussels *P. perna* from intertidal (I) and subtidal (S) origin (* $q < 0.05$, ** $q < 0.01$, * $q < 0.001$). Values in parenthesis correspond to partial correlation coefficients**

Coefficientes de correlación de Pearson y parciales establecidos entre los estadios reproductivos del mejillón *P. perna* de procedencia intermareal (I) y submareal (S) y los parámetros ambientales (* $q < 0.05$, ** $q < 0.01$, *** $q < 0.001$). Valores en paréntesis corresponden a los coeficientes de correlación parciales

	Stage II	Stage III	Stage IV
Temperature	I -0.770*** (-0.284) S -0.752*** (-0.484)	I -0.120 (0.232) S 0.176 (-0.145)	I 0.500*** (0.237) S 0.589*** (0.464)
Salinity	I 0.508*** (-0.306) S 0.566*** (-0.157)	I 0.002 (-0.730)*** S -0.040 (-0.097)	I -0.683*** (-0.531)* S -0.547*** (0.201)
Chlorophyll	I 0.184 (0.317) S 0.360* (0.150)	I -0.225 (0.177) S -0.409 (0.165)	I -0.193 (0.337) S -0.458** (-0.160)
Organic seston	I -0.368* (0.481) S 0.360* (-0.073)	I -0.180 (-0.073) S -0.157 (0.052)	I 0.174 (0.128) S -0.251 (0.056)
Total seston	I -0.384* (-0.493) S 0.367 (0.062)	I -0.174 (0.051) S -0.156 (-0.063)	I 0.158 (-0.142) S -0.248 (-0.046)

act as a trigger mechanism for reproductive activity. However, partial correlation coefficients between temperature and reproductive stages were not statistically significant and significant negative partial correlations were only observed between salinity and reproductive stages III and IV of mussels from intertidal origin (Table 1).

Biochemical composition

Proteins were the biochemical component that showed the highest contents (Fig. 7A). Also, at the start of the sampling period, mussels from the subtidal origin showed significantly higher values of carbohydrates and lipids. This result is more evident for lipids, for which values were twice as high for subtidal individuals when compared to mussels from intertidal origin. These differences for carbohydrates and lipids were observed during the first two or three samples. Afterwards, there were some significant differences for carbohydrates

during July and October, but the differences were relatively small.

Furthermore, temporal variations in the biochemical composition of both groups of mussels followed similar patterns during the study period. Proteins showed fluctuations around 1000 mg mussel⁻¹ (Fig. 7A), with decreases in June-July and September-October which may be related to spawning considering the parallel decreases in lipids and increments in spawning activity (see Fig. 6). For carbohydrates, minimum values were observed for both groups of mussels at the onset of the study (Fig. 7B), later these values increased until August and then showed a sustained decrease until the end of the experimental period. In general, protein values remained above 1200 mg mussel⁻¹. Lipids showed similar patterns to those described for proteins, except at the beginning of the experiment when a sharp decrease was observed, especially in individuals of subtidal origin (Fig. 7C).

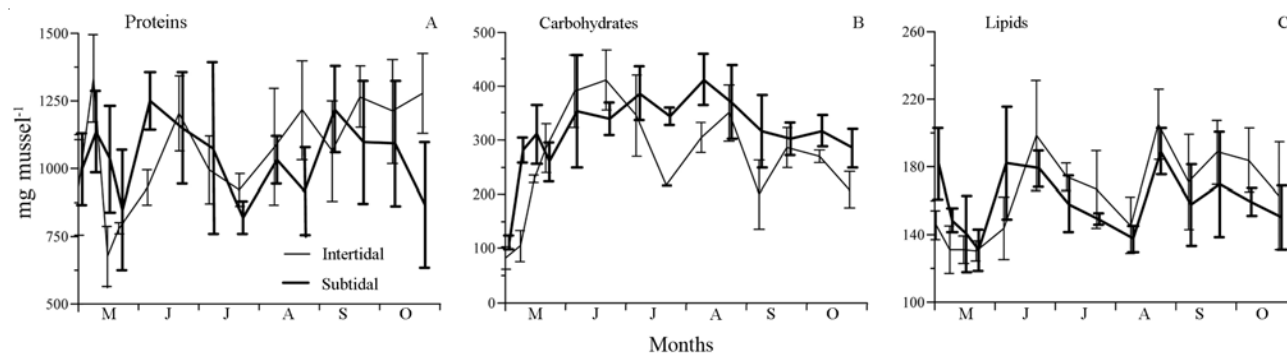


Figure 7

Absolute content (mg mussel⁻¹) of protein (A), carbohydrate (B), and lipid (C) of the mussel *P. perna* of intertidal and subtidal origin during the study period in Ensenada de Turpialito. Horizontal bars represent standard deviation

Variación de las proteínas (A), carbohidratos (B) y lípidos (C) expresados en contenidos absolutos (mg mussel⁻¹) del mejillón *P. perna* de origen intermareal y submareal, ocurrida durante el lapso de estudio en la Ensenada de Turpialito. Las barras horizontales representan las desviaciones estándar de los valores

Table 2

Pearson correlation coefficients between biochemical parameters of the mussel *P. perna* of intertidal (I) and subtidal (S) origin and environmental variables (*q<0.05, **q<0.01, *q<0.001). Values in parenthesis correspond to partial correlation coefficients**

Coefficientes de correlación de Pearson entre los parámetros bioquímicos del mejillón *P. perna* de procedencia intermareal (I) y submareal (S) y los parámetros ambientales (*q<0,05, **q<0,01, ***q<0,001). Valores en paréntesis corresponden a los coeficientes de correlacion parciales

		Proteins		Carbohydrates		Lipids
Temperature	I	0.589*** (0.404)	I	-0.789*** (-0.133)	I	0.037 (0.270)
	S	0.795*** (-0.310)	S	-0.830*** (0.103)	S	-0.402** (-0.242)
Salinity	I	0.042 (0.150)	I	0.266* (-0.051)	I	-0.057 (0.096)
	S	-0.368* (-0.141)	S	0.462** (0.244)	S	0.079 (-0.183)
Chlorophyll	I	-0.552*** (0.082)	I	0.305* (0.203)	I	0.315* (-0.087)
	S	-0.656*** (-0.240)	S	0.401** (-0.091)	S	0.498*** (0.049)
Organic Seston	I	0.342* (-0.030)	I	-0.481** (0.261)	I	-0.070 (-0.001)
	S	-0.249 (0.016)	S	0.278 (0.046)	S	0.029 (-0.008)
Total Seston	I	0.353* (0.025)	I	-0.506*** (-0.265)	I	-0.044 (-0.003)
	S	-0.247 (-0.018)	S	0.280 (-0.036)	S	0.027 (-0.003)

Table 3**Linear model analysis of carbohydrate, lipid, and protein levels as a function of environmental variables and spat origin of the brown mussel *P. perna***

Análisis del modelo lineal de los niveles de carbohidratos, lípidos y proteínas, como una función de las variables ambientales y del origen de los juveniles del mejillón marrón *P. perna*

Variable	Coefficient	Standard error	t value	P value
Carbohydrates				
Intercept	1296.09	210.86	6.15	3.14×10^{-8}
Temperature	-38.78	3.64	10.66	6.91×10^{-17}
Chlorophyll <i>a</i>	-117.02	43.78	2.67	9.15×10^{-3}
Salinity	-1.38	3.51	0.39	0.69
Total Seston	16.86	47.83	0.35	0.72
Origin	5.01	2.61	1.92	0.06
Lipids				
Intercept	-115.93	422.77	0.27	0.78
Temperature	3.81	7.29	0.52	0.60
Chlorophyll <i>a</i>	323.91	87.78	3.69	4.13×10^{-4}
Salinity	-5.13	7.03	0.73	0.47
Total Seston	77.08	95.90	0.80	0.42
Origin	5.48	5.23	1.05	0.30
Proteins				
Intercept	-1119.40	376.04	2.98	3.88×10^{-3}
Temperature	38.33	6.48	5.91	8.47×10^{-8}
Chlorophyll <i>a</i>	-249.91	78.08	3.20	1.98×10^{-3}
Salinity	15.02	6.26	2.40	1.88×10^{-2}
Total Seston	-142.75	85.30	1.67	0.10
Origin	-12.76	4.65	2.74	7.54×10^{-3}

Relations between biochemical components and environmental variables

Several significant correlations were observed between biochemical components and environmental variables (Table 2). Particularly, positive relationships were observed between proteins and temperature and between chlorophyll *a*, carbohydrates, and lipids, while strong negative relationships appeared between temperature and carbohydrates and between chlorophyll and proteins. Most of the estimated correlations were of the same sign and similar values for both spat origins. However, these relationships did not hold when partial correlation coefficients were estimated between biochemical components and environmental variables (Table 2).

Influence of environmental variables and spat origin on biochemical composition

The linear models indicated that temperature and chlorophyll *a* had the strongest effect on biochemical

composition (Table 3). Particularly, chlorophyll *a* presented relatively large negative effects on carbohydrates and protein and a relatively large positive effect on lipids, representing the only environmental variable statistically related to this biochemical group. The effects of temperature on carbohydrates and proteins were highly significant, but the effect was not as large as that of chlorophyll *a*, while spat origin had a significant negative effect on protein content only. All regression models were statistically significant, especially for carbohydrates (multiple $R^2 = 0.69$; $F_{(5,78)} = 34.23$; $P = 2.38 \times 10^{-18}$) and proteins (multiple $R^2 = 0.60$; $F_{(5,78)} = 23.60$; $P = 2.28 \times 10^{-14}$) and less so for lipids (multiple $R^2 = 0.20$; $F_{(5,78)} = 3.83$; $P = 3.70 \times 10^{-3}$).

Discussion

Results show that at the beginning of the study period, mussels from subtidal and intertidal origins showed no significant differences in initial absolute values of protein in contrast with significant differences in absolute values

of carbohydrates and lipids, differences that remained for at least the first 15 days. These results correspond with previous studies in which the biochemical composition of bivalves reflects conditions prevailing in the habitats where initial development occurs (Whyte *et al.* 1990, Napolitano *et al.* 1992, Fernández-Reiriz *et al.* 1996, Okumus & Stirling 1998).

However, mussels from both groups showed no significant differences in the biometric variables analyzed (height, dry masses shell, and condition index) throughout the study period. These results contrast with those obtained by Babarro *et al.* (2000) in which mussels from subtidal origin reached significantly higher lengths at the end of their study. In our study, both groups of mussels attained commercial sizes in only six months of culture, despite restricted growth in length and soft tissue dry mass during the last two months (September-October).

The observed decrease in growth rates, shell length and condition index during the last months of the experimental period may have been due to low food availability of phytoplanktic origin. In general, the results obtained agree with other growth studies of different species of bivalves in the same location and period where reduced growth is associated to high temperatures and low food availability (Lodeiros *et al.* 1993, Lodeiros & Himmelman 1994, Freitas & Núñez 2001). Another possible cause of the decrease in dry mass and condition index of both groups of mussels observed in September-October may be related to reproduction and the subsequent loss of biomass in the form of gametes. Ansell *et al.* (1980) and Mathieu & Lubet (1993) report that reproduction in different species of mussels is associated with considerable energy expenditure and that biomass loss during spawning may reach values between 30 and 60%.

Regarding survival, the cumulated value of this variable remained above 91% at the end of the study despite the initial mortality associated with inadequate fixing of spat to the long-line ropes. These relatively high survival rates (> 91%) represent an added element to the suspended culture potential of this species in Golfo de Cariaco.

The analysis of reproductive stage frequencies showed that spawning (stage IV) occurred during most of the study period, with a higher proportion occurring between July and October. These results coincide with the studies by Carvajal (1969), Vélez (1971) and Prieto *et al.* (1999) for the same species. Another important characteristic of this species is the temporal coexistence of individuals in different reproductive stages. In some samples all reproductive stages were present, which is indicative that *Perna perna* shows asynchronous

reproduction. Other tropical bivalve species such as *Nodipecten nodosus* (Vélez *et al.* 1987) have been shown to exhibit this type of reproduction, which contrasts with temperate species that are generally characterized by synchronic reproduction (Román *et al.* 2001).

The higher carbohydrate and lipid contents and condition index of subtidal mussels at the onset of the study indicate the more favorable conditions prevailing in this habitat, as they probably were not affected by stress factors such as direct solar radiation, high temperatures, and starvation. Additionally, while no significant differences were observed when comparing initial protein contents of both groups of mussels, the linear model analysis indicated a slight negative effect of subtidal origin on protein content. These results can be contrasted with the study by Freitas *et al.* (2003), in which individuals of *Mytilus galloprovincialis* from intertidal origin showed significantly higher protein content during the first 36 days of the experiment, while higher carbohydrate levels were observed in subtidal mussels during at least 7 more days than in the present study. These differences between both species may be due to the longer exposure period related to wider tidal fluctuations for the temperate species. However, it can not be discounted that these differences may also be related to food quantity and quality in different environments and/or with species-specific mechanisms for capturing, ingesting, and assimilating food.

Carbohydrates were the second-most important component in terms of absolute content (see Fig. 7). Carbohydrate content presented a progressive increase from the beginning of the experiment (May) until August and then a decrease for the rest of the study period. The observed decrease was probably related to low food availability of phytoplanktic origin observed during the period August-October (see Fig. 3D). This agrees with results presented by Zandee *et al.* (1980) and Okumus & Stirling (1998), who also observed in the mussel *Mytilus edulis* a decrease in carbohydrates and lipids during periods of low food availability. However, we can not discount that the observed decrease in carbohydrate levels may have been caused by the high temperatures registered during the August-October period. According to Cognetti *et al.* (2001), high temperatures increase metabolic rates and consequently carbohydrate consumption in poikilothermic invertebrates. In this context it is worth noting that the linear model of carbohydrate content as a function of environmental variables showed significant negative effects of temperature and chlorophyll. However, the negative effect of chlorophyll in this model may be explained by collinearity between this variable and temperature, as a linear model (not shown) of carbohydrates as a function of chlorophyll showed a

significant positive effect.

Chlorophyll appeared as the main environmental variable with a significant direct relationship with lipid content. In general, the range of variations and trends of lipid content in both groups of mussels were similar, except for the higher levels in subtidal mussels at the start of the experiment. Both groups of mussels presented an initial decrease, albeit of different magnitude, in lipid content which may be related to the experimental manipulation to which they were submitted. After this initial decrease, two periods of accumulation and reduction of lipids were observed which occur with parallel processes of gametogenesis/reproductive maturity and spawning, respectively. This suggests that changes in content may have been related to the formation of gametogenic tissue (structural lipids) and/or the accumulation of energetic reserves in gametes (neutral lipids), while reductions were probably related to spawning. Also, protein content decreases have been observed as a result of spawning activities in the bivalves *Mytilus edulis* (Zandee *et al.* 1980), *Mytilus galloprovincialis* (Bressan & Marin 1985), *Argopecten irradians irradians* (Epp *et al.* 1988), *Ostrea edulis* (Ruiz *et al.* 1992) and *Crassostrea iridescens* (Páez-Osuna *et al.* 1993).

Finally, despite the initial significantly higher carbohydrate and lipid content of subtidal mussels, the observed differences in biochemical composition between both groups did not extend beyond the first three weeks of the culture experiment. Also, considering that no differences were observed in growth (length and mass) and condition index between both groups, the high percent survival (> 90%), and the attainment of commercial size in only 6 months, are all indicative of the high potential of the mussel *Perna perna* for commercial culture in Golfo de Cariaco.

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