

Biochemical composition of the Atlantic pearl oyster *Pinctada imbricata* (Bivalvia: Pteriidae) in suspended culture: Influence of environmental factors

Composición bioquímica de la ostra perla del Atlántico *Pinctada imbricata* (Bivalvia: Pteriidae) en cultivo en suspensión: Influencia de factores ambientales

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Resumen.- La ostra perlera del Atlántico *Pinctada imbricata* es un bivalvo con gran potencial de cultivo en ambientes tropicales y subtropicales. En este estudio el metabolismo energético de la ostra perla *Pinctada imbricata* fue analizado durante un ciclo de cultivo en el Golfo de Cariaco, Venezuela. El cultivo se realizó usando individuos juveniles con una longitud total de la concha de $27,40 \pm 2,70$ mm. Los ejemplares se cultivaron a 3 m de profundidad en un *long-line*, entre mayo 2012 y enero 2013. Mensualmente se obtuvieron los datos biométricos, el índice metabólico de Fulton (K), el contenido de proteína, carbohidrato y lípido del músculo aductor, la glándula digestiva y el manto los cuales se relacionaron con las variables ambientales de temperatura, clorofila a, material total en suspensión (TPM), materia inorgánica particulada (PIM), materia orgánica particulada (POM), y el índice de surgencia (UI). La longitud de la concha incrementó progresivamente hasta alcanzar $46,23 \pm 3,79$ mm en el octavo mes del cultivo, mientras que el incremento del peso promedio de los tejidos blandos estuvo asociado a la disponibilidad de alimento. Los promedios de las variables biométricas y bioquímicas del músculo, glándula digestiva y manto presentaron diferencias significativas entre meses. El análisis de componentes principales (PCA) indicó que hubo relaciones significativas del contenido de carbohidratos con la temperatura; las proteínas del manto y la glándula digestiva con TPM, PIM, POM y UI; del contenido de lípido y la proteína del músculo con K. Los compartimientos de energía se asociaron a factores ambientales, principalmente a la alta disponibilidad de alimento debido a los procesos de surgencia estacional de la localidad.

Palabras clave: Bivalvos, crecimiento, lípido, glándula digestiva, metabolismo, surgencia

Abstract.- Atlantic pearl oyster *Pinctada imbricata* is a bivalve with high potential for cultivation in tropical and subtropical environments. In this study, *P. imbricata* energetic metabolism was analyzed throughout a culture cycle at Gulf of Cariaco, Venezuela. Juvenile individuals (shell length 27.40 ± 2.70 mm) were cultured in a long line at 3 m depth between May-2012 and January-2013. Monthly data of biometric measurements, Fulton index (K) and protein, carbohydrate, and lipid content of the adductor muscle, digestive gland, and mantle were estimated, and were related with environmental variables: temperature, chlorophyll a (Ch a), total particulate matter (TPM), particulate inorganic matter (PIM), particulate organic matter (POM) and upwelling index (UI). Shell length increased steadily reaching 46.23 ± 3.79 mm on the 8th month of culture, while average soft tissue weight increased associated to food availability. Biometric data and biochemical substrates from the muscle, digestive gland and mantle presented significant differences among months. Principal Component Analysis (PCA) showed positive relationships between carbohydrate content and temperature; mantle and digestive gland protein content with TPM, PIM, POM, UI; lipid and protein in muscle with K. Energy allocation was associated with environmental factors, mainly through high food availability given the seasonal upwelling pulses in the area.

Key words: Bivalve, growth, metabolism, lipid, digestive gland, upwelling



INTRODUCTION

Some species of Pteridae family are appreciated for nacre luster and pearl production (O'Connor & Lawler 2004, Southgate & Lucas 2008). Particularly, Atlantic pearl oyster *Pinctada imbricata* (Röding, 1798) which is a tropical and subtropical species that lives in clusters on coarse sand and rocks fixed by the byssus (Verginelli & Prieto 1991, MacKenzie *et al.* 2003). In the early 16th century, this species raised a pearl rush at Margarita and Cubagua islands (Venezuela) and almost 450 years of exploitation of the natural banks decimated its populations, bankrupting Caribbean pearl industry (MacKenzie *et al.* 2003, Romero 2003, Southgate & Lucas 2008). Nowadays, this species is harvested in artisanal fisheries, mainly as bycatch in *Arca zebra* shellfish fishery (Licet *et al.* 2009).

Atlantic pearl oyster populations are easily harvested in clusters (MacKenzie *et al.* 2003), nevertheless pearl extraction and meat consumption threatened to reduce natural populations. For this reason, regulatory measures established a minimum harvestable shell length (50 mm) in order to avoid the exacerbated extraction of organisms during maturation and spawning months (Lodeiros & Lovatelli 2019). Populations of *P. imbricata* from the northeastern coast of Venezuela have a gametogenic cycle favored by primary productivity and food availability natural cycles (Rueda-Roa & Müller-Karger 2013, Romero-Ferreira *et al.* 2017).

Improvement of bivalve aquaculture techniques is a time-consuming experience; low scale culture methods using mainly *P. imbricata* have been assayed during the past 20 years, testing conventional methods compared to customized procedures (Semidey *et al.* 2010, Lodeiros *et al.* 2011, 2016; Márquez *et al.* 2011, Pérez *et al.* 2016). Knowledge of the Atlantic pearl oyster biological cycle is necessary, as a potentially valuable species for large scale hatchery aquaculture, spat production, and population restoration (Urban 2000b, Kimani *et al.* 2006, Lovatelli & Sarkis 2011, Lodeiros & Lovatelli 2019). Because Atlantic pearl oyster has a rapid growth rate and a high secondary production biomass ratio or turnover rate (Verginelli & Prieto 1991, Urban 2000a, Vásquez *et al.* 2015), and a short culture cycle (comprising between 9-10 months), it is a valuable resource for food, nacre, and pearl production.

Biochemical composition provides information about the general physiological condition and nutritional status of species. This understanding is valuable for bivalve aquaculture, hatchery rearing, gametogenic conditioning and even marketing (Southgate & Lucas 2008). Metabolic activities of edible bivalve species reflect interactions between food availability, environmental conditions, and metabolic challenges (Barber & Blake 1991, Darriba *et al.* 2005). Balance among lipid, carbohydrate, and protein content in several organs is vital in assessing the soft-tissue quality and as an indicator of seasonal cycles of reproduction and feeding. The energetic content of the tissues varies among species, bivalve energetic substrates as lipid and glycogen are stored at the digestive gland, adductor muscle, adipose granular cells at the gonad and mantle, mainly for growth and gametogenesis (Saucedo *et al.* 2002, Gómez-Robles *et al.* 2005, Vite-García & Saucedo 2008, Mazón-Suátegui *et al.* 2013, Freitas *et al.* 2014). For example, the digestive gland in Pteridae family is involved in food digestion and storage of energy reserves (Gómez-Robles *et al.* 2005, Freitas *et al.* 2014, Romero-Ferreira *et al.* 2016); the muscle and gonad are also responsible for energy reserve (Darriba *et al.* 2005). Knowledge of energetic metabolism of *P. imbricata* is scarce, although previous studies on the gametogenic cycle suggest that there should be variations in the accumulation and transfer of high energy macromolecules (Romero-Ferreira *et al.* 2017).

Studies of the fatty acid allocation in the digestive gland and gonad of the Pacific *Pteria sterna* and *Pinctada mazatlanica*, improved hatchery cultured bivalve management, mabe and pearl harvest (Saucedo *et al.* 2002, Gómez-Robles *et al.* 2005, Southgate & Lucas 2008). Due to the paucity of information about Atlantic pearl oyster energetic metabolism, and its role on the growth, in this study the relationship between biometry and concentration of the main energetic macromolecules from the digestive gland, muscle and mantle was analyzed. Association with environmental variables during a culture cycle was also examined, to improve culture handling and physiological condition of this oyster.

MATERIALS AND METHODS

SPAT COLLECTION

Juveniles of *P. imbricata* were obtained from a natural recruitment on a tuna fishing net (1 cm² aperture net) that was kindly donated by Marine Culture Station Fernando Cervigon, Escuela de Ciencias del Mar, Universidad de Oriente, Charagato Bay, Cubagua, Venezuela (10°49'40"N-64°11'36"W). The fishing net with the spat was transported submerged in aerated seawater, on January 2012 to Turpialito Hydrobiological Station, Gulf of Cariaco (10°26'56"N-64°02'00"W). Juveniles were acclimatized during two weeks in the sea, observing high survival and growth of new periostracum scales. Individuals did not suffer stress between locations since the environmental conditions at both localities were similar throughout the year.

Fifty poly-propylene cylinders (50 cm length, 10 cm diameter, 1 cm mesh size) were used to fix 40 individuals (27.40 ± 2.70 mm in shell length) using biodegradable cotton net. Each cylinder was held on a long line, at 1.5 m depth and 3 m among cylinders, from May 2012 to January 2013. Biweekly, predatory snails *Cymatium* sp. were removed. Twenty individuals were examined monthly from four cylinders retired at random; which were removed cutting the byssus with scissors and transported in aerated seawater to the laboratory; then the cylinders were returned to the sea.

Seawater temperature was recorded monthly during the sampling period. Temperature was recorded using a thermograph (Sealog, Vemco Ltd. Halifax, Canada; ± 0.01 °C). Seawater was collected each week at 3 m depth with a Niskin bottle (5 L); seawater analyses were done triplicate on a weekly basis and data were averaged each month, using 500-1000 mL aliquots for colorimetric estimation of chlorophyll *a* concentration (µg m⁻³), gravimetric estimation of the particulate total matter (PTM), particulate inorganic matter (PIM) and particulate organic matter (POM) (mg L⁻¹) (Strickland & Parsons 1972). Upwelling index (UI; m³ s⁻¹ km⁻¹) was formerly described at the study area in order to identify low productivity (LP) and high (HP) productivity seasons. UI is a direct measure of the daily conditions that could raise or reduce food availability for filter-feeding bivalves.

BIOLOGICAL DATA

At the laboratory, the fouling on shells was eliminated and bivalves were submerged in filtered aerated seawater to clean the digestive tract (6 h), until there were no feces at the bottom of the aquarium. Biometric data corresponded to: shell length (L), shell height (H), shell width (W), shell weight (SW), soft tissue fresh weight (SF) and soft tissue dry weight (SD) after dehydration at 65 °C for 72 h (± 0.05 mm and ± 0.001 g). Biochemical analysis of the soft tissues was done in twenty individuals; shell was opened separating the adductor muscle from the shell, then mantle, muscle and digestive gland were dissected over an ice bath and stored at -20 °C. There was no segmentation between male and female individuals.

Monthly data of shell length (L) and soft tissue dry weight (SD) were analyzed using the allometric relationship $L = a SD^b$ (Wilburg & Owen 1964). Fulton condition index (K) was calculated as the ratio between total weight (WT = SW + SF) and shell length (L), according to the formula $K = 100 (WT L^{-3})$ proposed by Ricker (1975). K is an efficient proxy for growth rate and provides information on animal responses to habitat quality and environmental conditions.

BIOCHEMICAL ANALYSIS

Analyses were done by triplicate on 100 mg fresh tissue samples for each individual to estimate lipid, carbohydrate and protein contents. Extraction of lipids followed the standard method described by Bligh & Dyer (1959). Lipid assays, based on a potassium dichromate color change reaction, were done according to Pande *et al.* (1963) using a calibration curve with tripalmitin as a standard (#T5888, Sigma-Aldrich®) dissolved in chloroform. Carbohydrate content was analyzed using anthrone colorimetric method (Dubois *et al.* 1956) with glucose anhydrous (D-glucose; SIGMA, G-5767; 1 mg mL⁻¹) as standard. Protein concentration was quantified by Lowry method, using bovine serum albumin (BSA 10 mg mL⁻¹ Sigma) as protein standard (Lowry *et al.* 1951). The absorbance was recorded by means of a spectrophotometer (UV-1201 UV-VIS, Shimadzu, Tokyo, Japan).

STATISTICAL ANALYSIS

Monthly environmental variables and biometric data were analyzed using a one-way analysis of variance (ANOVA) after verification of normality and homocedasticity. Monthly biochemical data was analyzed using non-parametric statistical test of Kruskal-Wallis (Zar 2010). Principal component analysis (PCA) was performed searching for significant relationships among environmental variables (supplementary component), biometric and biochemical data (active component). Statistical analyses were carried out using STATISTICA software (Statsoft, Tulsa, OK). Significant differences were set at 95% ($P < 0.05$).

RESULTS

Monthly average environmental data, from May 2012 to January 2013 at Gulf of Cariaco (Venezuela) are shown in Table 1. Average temperature (T) oscillated between 24.36 ± 0.07 °C (January) and 29.06 ± 0.03 °C (November). Chlorophyll *a* (Ch *a*) maximum was observed in May (0.70 ± 0.13 µg L⁻³) and minimum in November (0.14 ± 0.06 µg L⁻³). Total particulate matter (TPM) was higher during May-August, as well as particulate inorganic matter (PIM), and

particulate organic matter (POM). Environmental variables (T, Ch *a*, UI, TPM, PIM, POM) presented significant differences among months ($P < 0.05$), therefore the eight months of the study were divided into high-productivity season (HP) or low-productivity season (LP), in agreement with maximum primary productivity, temperature and upwelling index (UI) monthly oscillations (Table 1). Therefore, high productivity seasons (HP) were established from May-2012 to July-2012 and December-2012 to January-2013; and low productivity season (LP) from August-2012 to November-2012.

Shell length (L) showed a steady increase through month, reaching 46.23 ± 3.79 mm L on the 8th-month of culture. Soft fresh tissue weight (SF) showed a progressive increase from May-2012 to January-2013, displaying maximum average values during July-2012 and January-2013. The allometric relationship between L and SF was significant ($P < 0.05$), with a regression coefficient not different from 3 ($P > 0.05$), suggesting an isometric growth, mostly during LP months. Fulton condition index (K) showed significant differences among months, presenting a steady increase between May-June, decreasing from July to September, and increasing from October until January-2013 (Table 2).

Table 1. Monthly average environmental data, from May 2012 to January 2013 at Gulf of Cariaco, Venezuela / Promedio de los datos ambientales mensuales, desde mayo 2012 a enero 2013 en el Golfo de Cariaco, Venezuela

Year & Months	Season	T (°C)	Ch <i>a</i> (µg L ⁻³)	TPM (mg L ⁻¹)	PIM (mg L ⁻¹)	POM (mg L ⁻¹)	UI (m ³ s ⁻¹ km ⁻¹)
2012							
May	HP	25.98 ± 0.12 ^b	0.70 ± 0.13 ^a	23.14 ± 0.65 ^c	5.27 ± 0.43 ^c	16.54 ± 0.55 ^b	1.58 ± 1.24 ^b
Jun	HP	24.96 ± 0.06 ^b	0.67 ± 0.09 ^a	21.20 ± 0.59 ^c	6.77 ± 0.14 ^c	14.82 ± 0.58 ^b	1.79 ± 0.41 ^b
Jul	HP	24.78 ± 0.10 ^b	0.61 ± 0.04 ^a	22.37 ± 1.35 ^c	5.58 ± 0.13 ^c	17.24 ± 0.15 ^b	1.32 ± 0.77 ^b
Aug	LP	27.89 ± 0.11 ^a	0.57 ± 0.08 ^b	24.61 ± 0.43 ^c	6.71 ± 0.19 ^c	16.56 ± 0.57 ^b	0.29 ± 0.38 ^a
Sep	LP	28.21 ± 0.11 ^a	0.40 ± 0.08 ^b	10.55 ± 0.20 ^b	4.45 ± 0.07 ^b	6.74 ± 0.09 ^a	0.36 ± 0.25 ^a
Oct	LP	28.44 ± 0.04 ^a	0.32 ± 0.07 ^b	6.84 ± 0.09 ^a	3.76 ± 0.82 ^b	6.73 ± 0.06 ^a	0.37 ± 0.33 ^a
Nov	LP	29.06 ± 0.03 ^a	0.14 ± 0.06 ^c	7.92 ± 0.09 ^a	3.60 ± 0.74 ^b	7.79 ± 0.17 ^a	1.03 ± 0.44 ^b
Dec	HP	25.87 ± 0.10 ^b	0.52 ± 0.03 ^b	6.83 ± 0.02 ^a	0.37 ± 0.12 ^a	5.97 ± 0.59 ^a	1.63 ± 0.46 ^b
2013							
Jan	HP	24.36 ± 0.07 ^b	0.69 ± 0.04 ^a	7.87 ± 0.04 ^a	0.38 ± 0.04 ^a	6.70 ± 0.07 ^a	2.25 ± 0.25 ^b

Temperature (T); Chlorophyll *a* (Ch *a*); Total particulate matter (TPM); Particulate inorganic matter (PIM); Particulate organic matter (POM); Upwelling index (UI); High productivity season (HP); Low productivity season (LP); Super scripts mean significant differences among months ($P < 0.05$)

Table 2. Biometric data of *Pinctada imbricata* analyzed throughout a culture cycle at Gulf of Cariaco, Venezuela / Datos biométricos de *Pinctada imbricata* analizados en un ciclo de cultivo en el Golfo de Cariaco, Venezuela

Year & Month	Season	Biometric data			
		L (mm)	SF (g)	b	K
2012					
May	HP	27.09 ± 2.02 ^c	0.88 ± 0.19 ^c	3.25	0.61 ± 0.04 ^b
Jun	HP	35.24 ± 2.85 ^c	1.37 ± 0.21 ^c	2.60	0.75 ± 0.09 ^a
Jul	HP	39.04 ± 3.69 ^c	3.00 ± 0.94 ^b	2.33 ^c	0.57 ± 0.08 ^b
Aug	LP	41.47 ± 2.33 ^{b,c}	2.87 ± 0.50 ^b	3.40	0.51 ± 0.04 ^b
Sep	LP	40.92 ± 3.97 ^b	2.01 ± 0.63 ^b	2.73	0.37 ± 0.04 ^c
Oct	LP	41.44 ± 2.66 ^b	1.85 ± 0.60 ^b	2.58 ^a	0.43 ± 0.06 ^b
Nov	LP	42.80 ± 3.04 ^a	2.02 ± 0.50 ^b	3.01	0.46 ± 0.06 ^b
Dec	HP	44.52 ± 3.64	2.68 ± 0.68 ^b	3.43	0.51 ± 0.04 ^b
2013					
Jan	HP	46.23 ± 3.79 ^a	3.57 ± 0.77 ^a	3.08	0.60 ± 0.06 ^b

Shell length (L), Soft tissue weight (SF), Regression coefficient (b), Fulton Condition Index (K), High productivity season (HP), Low productivity season (LP). Super scripts mean significant differences among months ($P < 0.05$)

Protein content in adductor muscle, mantle, and digestive gland showed significant differences among months with a maximum in May ($P < 0.05$). Total protein from adductor muscle values were maintained between 212 mg g⁻¹ (June) to 281 mg g⁻¹ (November); mantle and digestive gland showed intermittent variations. Carbohydrate content presented significant differences among months ($P < 0.05$), with maximum values in June and oscillations at mantle, adductor muscle and digestive gland. Adductor muscle and mantle were the tissues with higher protein concentration. Lipid content of the mantle presented significant differences and was maximum in June ($P < 0.05$), while adductor muscle and digestive gland lipid content showed no significant differences among months ($P > 0.05$) (Table 3).

PCA showed positive linear relationship among environmental variables and energetic macromolecules of the tissues. Protein content of adductor muscle, mantle, and digestive gland were positively associated with UI, TPM, POM, and PIM. Carbohydrate content of mantle, adductor muscle, and digestive gland were associated with temperature. Lipid content of the muscle showed a close relationship with K. There was no significant relationship of digestive gland and mantle with environmental parameters (Fig. 1).

Table 3. Biochemical substrates from mantle (MA), adductor muscle (MU), and digestive gland (DG) of *Pinctada imbricata* analyzed throughout a culture cycle at Gulf of Cariaco, Venezuela / Sustratos bioquímicos del manto (MA), músculo aductor (MU) y la glándula digestiva (DG) de *Pinctada imbricata* analizados durante un ciclo de cultivo en el Golfo de Cariaco, Venezuela

Year & Month	Season	Carbohydrate			Lipid			Protein		
		MA (mg g ⁻¹)	MU (mg g ⁻¹)	DG (mg g ⁻¹)	MA (mg g ⁻¹)	MU (mg g ⁻¹)	DG (mg g ⁻¹)	MA (mg g ⁻¹)	MU (mg g ⁻¹)	DG (mg g ⁻¹)
2012										
May	HP	171.66 ± 29.20 ^c	315.94 ± 15.46 ^a	344.57 ± 14.66 ^a	375.01 ± 11.73 ^a	330.56 ± 36.16 ^a	101.94 ± 8.17 ^{a,b}	85.61 ± 8.57 ^a	89.08 ± 16.99 ^a	171.66 ± 29.20 ^c
Jun	HP	479.76 ± 50.13 ^a	362.80 ± 16.53 ^a	238.85 ± 6.70 ^b	212.07 ± 50.58 ^b	262.96 ± 7.74 ^b	117.90 ± 5.37 ^{a,b}	87.10 ± 16.30 ^a	114.16 ± 2.83 ^a	479.76 ± 50.13 ^a
Jul	HP	399.28 ± 42.88 ^a	392.74 ± 35.44 ^a	192.93 ± 41.49 ^c	221.05 ± 64.28 ^b	335.19 ± 57.31 ^a	100.98 ± 29.17 ^b	80.05 ± 21.19 ^a	99.08 ± 6.96 ^a	399.28 ± 42.88 ^a
Aug	LP	157.99 ± 7.26 ^c	284.62 ± 65.11 ^{a,b}	197.57 ± 30.60 ^c	261.60 ± 25.60 ^b	249.78 ± 44.26 ^b	88.15 ± 6.87 ^{a,b}	85.45 ± 8.47 ^a	100.81 ± 1.19 ^a	157.99 ± 7.26 ^c
Sep	LP	141.25 ± 12.47 ^c	214.36 ± 20.95 ^b	202.95 ± 76.96 ^b	267.44 ± 18.58 ^b	279.54 ± 21.56 ^b	81.94 ± 3.01 ^b	90.67 ± 28.73 ^a	111.54 ± 21.16 ^a	141.25 ± 12.47 ^c
Oct	LP	188.53 ± 20.48 ^c	291.51 ± 18.44 ^{a,b}	316.35 ± 41.75 ^{a,b}	270.67 ± 8.17 ^b	323.07 ± 31.06 ^a	94.50 ± 11.18 ^{a,b}	99.69 ± 18.43 ^a	126.34 ± 32.27 ^a	188.53 ± 20.48 ^c
Nov	LP	136.13 ± 11.05 ^c	267.64 ± 44.15 ^{a,b}	278.38 ± 25.18 ^b	281.74 ± 26.31 ^b	242.21 ± 66.18 ^b	79.28 ± 6.87 ^{a,b}	84.75 ± 10.78 ^a	87.35 ± 0.91 ^a	136.13 ± 11.05 ^c
Dec	HP	362.26 ± 32.19 ^{a,b}	374.57 ± 19.76 ^a	238.49 ± 47.17 ^b	276.91 ± 21.18 ^b	211.69 ± 12.65 ^b	79.91 ± 5.83 ^{a,b}	101.02 ± 18.90 ^a	107.31 ± 2.69 ^a	362.26 ± 32.19 ^{a,b}
2013										
Jan	HP	495.85 ± 29.82 ^a	346.53 ± 34.30 ^a	282.12 ± 58.73 ^b	224.22 ± 58.01 ^b	223.78 ± 26.55 ^b	101.33 ± 13.26 ^a	80.40 ± 12.49 ^a	105.03 ± 7.68 ^a	495.85 ± 29.82 ^a

Mantle (MA); Muscle (MU); Digestive gland (DG); High productivity season (HP); Low productivity season (LP); Super scripts; significant differences between months ($P < 0.05$)

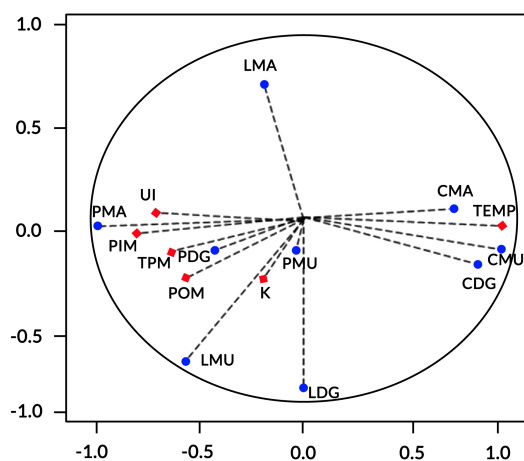


Figure 1. Principal component analysis among energetic substrates in the tissues of Atlantic pearl oyster with environmental variables. Active variables (blue circle); Supplementary variables (red square). Protein digestive gland (PDG); Protein mantle (PMA); Protein muscle (PMU); Carbohydrate digestive gland (CDG); Carbohydrate mantle (CMA); Carbohydrate muscle (CMU); Lipid digestive gland (LDG); Lipid muscle (LMU); Lipid mantle (LMA); Metabolic rate (K); Upwelling Index (UI). Temperature (TEMP). Total particulate matter (TPM). Particulate organic matter (POM). Particulate inorganic matter (PIM) / Análisis de componentes principales entre los sustratos energéticos en los tejidos de la ostra perla con las variables ambientales. Variables activas (cuadrado rojo); Variables suplementarias (círculo azul). Proteína en glándula digestiva (PDG); Proteínas en manto (PMA); Proteínas en músculo (PMU); Carbohidratos en glándula digestiva (CDG); Carbohidratos en manto (CMA); Carbohidratos en músculo (CMU); Lípidos en glándula digestiva (LDG); Lípidos en manto (LMA); Lípidos en músculo (LMU); Tasa metabólica (K); Índice de surgencia (UI); Temperatura (TEMP); Material particulado total (TPM); Materia orgánica particulada (POM); Materia inorgánica particulada (PIM)

DISCUSSION

In this study, annual oscillations of the energetic reserves of Atlantic pearl oyster long line culture, and their relationship with the environmental parameters were analyzed. The shell length growth rate agreed with previous culture trials in the Gulf of Cariaco, which reached similar size for the same time lapse: 50 mm shell length (L) at six months (Semidey *et al.* 2010), 46-56 mm L in eight months (Lodeiros *et al.* 2011), 32-34 mm L in eight months (Márquez *et al.* 2011), although faster growth rate was associated to food availability and upwelling season (Lodeiros *et al.* 2011, Pérez *et al.* 2016, Freites *et al.* 2017). As seen in previous studies, data suggest that probably a limited food availability, mainly phytoplankton biomass (chlorophyll *a* <1 µg L⁻¹), was associated to the low growth of cultivated oysters, when compared with the information reported by Romero-Ferreira *et al.* (2017) and Freites *et al.* (2017), both authors reported concentrations > 1 µg L⁻¹ for the same season. Even though there was a low primary productivity recorded in this study, the food availability could sustain this population productivity and fishery (Lodeiros & Himmelman 2000, Freites *et al.* 2012). The low mortality suggests *P. imbricata* is resilient to environmental conditions registered in the study area (Ward & MacDonald 1996, Yukihiro *et al.* 2000, Moussa-Moussa 2018).

Allometric growth model was isometric ($b = 3$), observing a maximum average of the biometric variables (L, SF, b , and k) during HP season. These variables are good indicators of bivalve species health and growth rate (Wilburg & Owen 1964, Froese 2006), showing a proportional growth between shell length and dry tissue weight. Despite the slow growth of oysters, soft tissues increased steadily. The minimum values recorded during August-October could be associated to low food availability months (León & Millán 1996).

Monthly averages of Fulton's index were maintained throughout the culture cycle, being higher during June, indicating a good condition in juvenile oysters. During the same month pearl oyster gonad development was insignificant, when compared to Romero-Ferreira *et al.* (2017). This morphometric index of condition has been scarcely used in bivalves; but it may suggest that heavier individuals for a given length are in better condition. Panta-Vélez *et al.* (2020) reported K-index values inversely associated with shell length of *Anadara tuberculosa*, but these values increased after organisms had high food availability. It is considered that upwelling pulses in the Gulf of Cariaco associated with occasional bursts of high primary productivity and other changes in environmental variables, may promote better development of *P. imbricata* under culture (Rueda-Roa &

Müller-Karger 2013).

Bivalve growth is a great concern in aquaculture; being a fast growth rate desirable. Bivalve growth can differ among seasons, according to food availability, temperature, gamete development, and handling (Urban 2000a, Semidey *et al.* 2010). A pearl oyster of 50 mm shell length (L) is the utmost goal in the shortest time-lapse, and this size is appropriate for marketing and population restoration purposes, as well (Lodeiros *et al.* 2002, Lodeiros & Lovatelli 2019). In this study, individuals reached an average of 46.23 ± 3.79 mm L in eight months. Despite the fact that individuals grew by almost 80 percent during the culture period, this growth rate was considered low if compared with individuals of the same species that were cultured in the area (Lodeiros *et al.* 2002, Semidey *et al.* 2010, Pérez *et al.* 2016) and it is possibly associated with low food availability throughout the year. Furthermore, timing for placing the seedlings in the ropes could affect the growth rate, particularly if the culture is set at the end of the upwelling season (O'Connor *et al.* 2003). Condition index K that relies upon the weight of soft tissues, was maximum during most of the sampling period. Soft tissue fresh weight content must be considered for the physiological status of the population.

Influence of seasonal changes on the proximal biochemical composition of *P. imbricata* soft-tissues was significant ($P < 0.05$), except for lipid content. The energy requirements of Pacific members of the family Pteridae can be met at low food availability and high temperature (Pouvreay *et al.* 1999, Yukihiro *et al.* 2000, Saucedo *et al.* 2002, Southgate & Lucas 2008, Vite-García & Saucedo 2008). *Pinctada margaritifera* clearance rate and absorption efficiency are high under low primary productivity, being able to grow in an oligotrophic environment (Pouvreay *et al.* 1999). Temperature may affect physiological response, feeding, respiratory rate, and growth rate of several Pteridea species, having significant differences with seasonal oscillations in water temperature (*e.g.*, *P. margaritifera* and *P. maxima*, have optimum growth rates at temperatures between 23-28 °C and 23-32 °C, respectively) (Yukihiro *et al.* 2000). *Pinctada imbricata* filter feeding occurs without selective partitioning among particles nutritive value, showing a better performance in comparison to *Arca zebra* (Ward & MacDonald 1996), an Arcidae species associated with its fishery providing substrate for spat settlement (Licet *et al.* 2009). *Pinctada imbricata* as well as some other pearl oyster species, has an excellent filter-feeding efficiency, even at low microalgae concentration, accompanied by good absorption efficiency in a wide range of temperatures (Ward & MacDonald 1996). Resilience of this species to environmental conditions makes it a good candidate for aquaculture.

Pinctada imbricata could use available resources for gonad development, arresting shell length growth, and resuming catabolic growth processes with the onset of the upwelling season. This species energy expenditure could be partitioned into early gametogenesis at the LP season (Romero-Ferreira *et al.* 2017). *Pinctada imbricata* is known to reproduce all year round, with a higher frequency of spawning during upwelling season, when there is greater availability of food, thus presenting an opportunistic reproductive strategy (Romero-Ferreira *et al.* 2017). This species initiates reproductive activity between 24-25 mm shell length (Vázquez *et al.* 2015) and gonads in both sexes showed mature gametes and spent acini between December and March (Romero-Ferreira *et al.* 2017). Bivalve growth rates generally slow down after sexual development (Southgate & Lucas 2008).

Biochemical composition of *P. imbricata* showed significant changes during the culture period, in response to primary productivity of each season. Particularly, protein and carbohydrate contents showed significant differences among months, and, to a lesser extent, the lipid content. Energetic metabolism is supported by carbohydrate energy supply at the LP season. Carbohydrate content showed the highest values in the three analyzed tissues, which could provide energy for basal metabolism, gametogenesis and storage. Carbohydrates are easily transformed for energy fulfillment, in contrast to lipid metabolism that needs additional transformation before being turned into energy (Lee *et al.* 2018). Energy allocation in *Pinctada radiata* fueled late gonad development and spawning, meeting energy requirements by glycogen to a greater extent (Moussa-Moussa 2018).

Bivalve energetic physiology relies on the muscle and digestive gland as storage organs for energy transfer and gamete development (Salah *et al.* 2012, Romero-Ferreira *et al.* 2017, Lee *et al.* 2018). The main energy storage organs in bivalves, particularly digestive gland, adductor muscle and mantle, provide energy for gamete quality and reproduction (Gómez-Robles *et al.* 2013, Freitas *et al.* 2014, Pan *et al.* 2021). Energy transfer for gonad development in bivalves comes from protein and lipid reserves of adductor muscle and digestive gland (Vite-García & Saucedo 2008). Environmental data registered during upwelling season suggest that phytoplankton concentrations and dissolved nutrients may provide the energetic requirements for growth and reproduction, increasing the protein content for metabolic functions for growth (Pan *et al.* 2021). The oscillations of lipid content during culture cycle were negligible, however the concentration of total lipids maintained in the tissues showed their role in energy supply. The family Pteridae does not have a single lipid storage organ, and lipid reserves are distributed between the muscle and digestive gland as lipid droplets with biological importance, including fatty acid associated with key metabolic processes (Freitas *et al.* 2002).

PCA suggests that *P. imbricata* adjusts its metabolic requirements to food availability, focused on the protein and carbohydrate pathways to meet growth and energetic processes. During low productivity season, phytoplankton is turned into carbohydrate reserves, acting as the main energy source for metabolic functioning, while at HP season, carbohydrate is fueled onto energy increasing protein reserves for catabolic processes.

Low growth of Atlantic pearl oyster *P. imbricata* during the upwelling season was mainly associated with low primary production. However, the increase in weight of soft tissues showed an isometric growth and high metabolic rate during the months of more intense primary productivity. Carbohydrates seem to be the main energy reserve molecules, stored by several tissues. Positive relationships were observed between carbohydrate and temperature; mantle and digestive gland protein with TPM, PIM, POM, UI; lipid and protein muscle with K.

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LITERATURE CITED

- Barber P & K Blake. 1991.** Cellular biochemistry of the mantle tissue of the mussel *Mytilus edulis*. *Aquaculture* 94: 165-176.
- Bligh G & J Dyer. 1959.** A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37: 911-917.
- Darriba S, F San Juan & A Guerra. 2005.** Energy storage and utilization in relation to the reproductive cycle in the razor clam *Ensis arcuatus* (Jeffreys, 1865). *ICES Journal of Marine Science* 62: 886-896.
- Dubois M, K Guilles, J Hamilton, P Rebers & F Smith. 1956.** Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28: 350-356.
- Freitas L, U Ubarta & M Fernández-Reiriz. 2002.** Evolution of fatty acid profiles of subtidal and rocky shore mussel seed (*Mytilus galloprovincialis*, Lmk.). Influence of environmental parameters. *Journal of Experimental Marine Biology and Ecology* 268(2): 185-204.
- Freitas L, C Lodeiros, M Guevara, J Alió & C Graziani. 2012.** Experiencias en el cultivo de organismos marinos en el Golfo de Cariaco, Venezuela. *Saber* 24(1): 5-24.
- Freitas L, C Lodeiros, D Arrieche & A Dale. 2014.** Reproductive tactics of marine shellfish species from northeastern Venezuela. In: Baqueiro-Cárdenas E (ed). *Spawning, biology sexual strategies and ecological effects*, pp. 73-105. Nova Science Publishers, New York.

- Freites L, M Rojas, A Dale, A Márquez, J Revilla, L Troccoli & C Lodeiros. 2017.** Growth and survival of the winged oyster *Pteria columbus* in suspended culture: influence of environmental factors associated to upwelling periods. *Aquaculture International* 25: 1653-1666.
- Froese R. 2006.** Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. *Journal of Applied Ichthyology* 22: 241-253.
- Gómez-Robles E, C Rodríguez-Jaramillo & P Saucedo. 2005.** Digital image analysis of lipid and protein histochemical markers for measuring oocyte development and quality in pearl Oyster *Pinctada mazatlanica*. *Journal of Shellfish Research* 24(4): 1197-1202.
- Gómez-Robles E, J Mazón-Suástegui, H Acosta, M Hawkins & P Saucedo. 2013.** Internal nutrient management associated with gonad quality and successful reproduction in the winged pearl oyster *Pteria sterna*. *Aquaculture* 412/413: 45-51.
- Kimani E, K Mavuti & T Mukiyama. 2006.** The reproductive activity of the pearl oyster *Pinctada imbricata* Röding 1798 (Pteriidae) in Gazi Bay, Kenya. *Tropical Zoology* 19: 159-174.
- León L & J Millán. 1996.** Variación del índice de condición y de la composición bromatológica de la ostra perliera *Pinctada imbricata* Röding, 1798 (Mollusca: Bivalvia) de Las Cabeceras, Isla de Cubagua, Venezuela. *Memorias de la Sociedad de Ciencias Naturales La Salle* 56(146): 23-41.
- Lee YJ, E Han, M Wilberg, W Lee, K Choi & C Kang. 2018.** Physiological processes and gross energy budget of the submerged longline-cultured Pacific oyster *Crassostrea gigas* in a temperate bay of Korea. *PLoS ONE* 13(7): e0199752. <<https://doi.org/10.1371/journal.pone.0199752>>
- Licet B, V Acosta, A Prieto & N García. 2009.** Contribución al conocimiento de los macromoluscos bentónicos asociados a la pepitona, *Arca zebra* (Swainson, 1833), del banco natural de Chacopata, Península de Araya, Venezuela. *Zootecnia Tropical* 27(2): 195-203.
- Lodeiros C & J Himmelman. 2000.** Identification of environmental factors affecting growth and survival of the tropical scallop *Euvola (Pecten) ziczac* in suspended culture in the Golfo de Cariaco, Venezuela. *Aquaculture* 182: 91-114.
- Lodeiros C & A Lovatelli. 2019.** Producción de semillas de la ostra perla *Pinctada imbricata*. Un manual práctico. FAO Documento Técnico de Pesca y Acuicultura 636: 1-96.
- Lodeiros C, D Pico, A Prieto, N Narváez & A Guerra. 2002.** Growth and survival of the pearl oyster *Pinctada imbricata* (Röding 1768) in suspended and bottom culture in the Golfo de Cariaco, Venezuela. *Aquaculture International* 10: 327-338.
- Lodeiros C, L Freites, C Graziani & J Alió. 2011.** Cultivation of bivalve mollusks in Venezuela: diversity, potential and infrastructure for seed production. In: Lovatelli A & S Sarkis (eds). A regional shellfish hatchery for the Wider Caribbean: Assessing its feasibility and sustainability. FAO Fisheries and Aquaculture Proceedings 19: 161-170.
- Lodeiros C, L Freites, A Márquez, M Glem, M Guevara & P Saucedo. 2016.** Comparative growth and survival of spat of the Caribbean Pearl oyster, *Pinctada imbricata*, cultivated indoor with microalgae diets and outdoor with natural diet. *Aquaculture Nutrition* 23(3): 511-522.
- Lowry O, N Rosebrough & L Fair. 1951.** Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* 193: 265-275.
- Lovatelli A & S Sarkis. 2011.** A regional shellfish hatchery for the Caribbean: Assessing its feasibility and sustainability. FAO Fisheries and Aquaculture Proceedings 19: 1-246.
- MacKenzie C, L Troccoli & L León. 2003.** History of the Atlantic pearl-oyster, *Pinctada imbricata*, industry in Venezuela and Colombia, with biological and ecological observations. *Marine Fisheries Review* 65: 1-20.
- Márquez A, C Lodeiros, D Semidey, M Carpio & C Graziani. 2011.** Crecimiento y supervivencia de la ostra perliera *Pinctada imbricata* (Röding 1798), bajo diferentes sistemas de confinamiento en cultivo suspendido. *Zootecnia Tropical* 29(3): 337-351.
- Moussa-Moussa R. 2018.** The potential impacts of low and high salinities on salinity tolerance and condition index of the adult pearl oyster *Pinctada imbricata radiata* (Leach, 1814). *Journal of Basic and Applied Zoology* 79: 12.
- O'Connor W & N Lawler. 2004.** Reproductive condition of the pearl oyster, *Pinctada imbricata*, Röding, in Port Stephens, New South Wales, Australia. *Aquaculture Research* 35: 385-396.
- O'Connor W, N Lawler & M Heasman. 2003.** Trial farming the akoya pearl oyster, *Pinctada imbricata*, in Port Stephens, NSW. NSW Fisheries Final Report Series 42: 174 pp. <http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0005/134519/Output-42.pdf>
- Pande S, R Khan & T Venkitesubra. 1963.** Microdetermination of lipids and serum total fatty acids. *Analytical Biochemistry* 6: 415-423.
- Panta-Vélez R, A Bermúdez-Medranda, P Mero, D Arrieche, V Acosta & R Retamales. 2020.** Reproductive cycle of *Anadara tuberculosa* (Sowerby, 1833) (Bivalvia: Arcidae) in a Mangrove System of the Chone River Estuary, Ecuador. *Advances in Environmental Biology* 14(2): 1-11.
- Pérez E, C Lodeiros, D Semidey, E Uribe & L Freites. 2016.** Crecimiento, supervivencia e influencia de factores ambientales en tres cohortes de la ostra perla *Pinctada imbricata*, en cultivo suspendido en el Golfo de Cariaco, Venezuela. *Latin American Journal of Aquatic Research* 44: 99-112.
- Pouvreau S, G Jonquières & D Buestel. 1999.** Filtration by the pearl oyster, *Pinctada margaritifera*, under conditions of low seston load and small particle size in a tropical lagoon habitat. *Aquaculture* 176: 295-314.
- Romero A. 2003.** Death and taxes: the case of the depletion of pearl oyster beds in the sixteenth-century, Venezuela. *Conservation Biology* 17: 1013-1023.
- Romero-Ferreira P, D Arrieche, V Acosta, L Pérez & C Lodeiros. 2016.** Histoquímica de la glándula digestiva en la ostra perla *Pinctada imbricata* (Pterioida: Pteriidae) durante su ciclo gametogénico, Venezuela. *Revista de Biología Tropical* 64(2): 849-858.
- Romero-Ferreira P, D Arrieche, V Acosta, L Pérez & C Lodeiros. 2017.** Ciclo gametogénico de la ostra *Pinctada imbricata* en cultivo suspendido en el Golfo de Cariaco, Venezuela. *Latin American Journal of Aquatic Research* 45(1): 139-148.

- Ricker W. 1975.** Computation and interpretation of biological statistics of fish population. *Journal of the Fisheries Research Board of Canada* 191: 1-382.
- Rueda-Roa D & F Müller-Karger. 2013.** The southern Caribbean upwelling system: Sea surface temperature, wind forcing and chlorophyll concentration patterns. *Deep Sea Research* 178: 102-114.
- Salah IB, A Bouain & L Neifar. 2012.** Gonadal cycle of the dwarf oyster *Ostreola stentina* from the south of the Gulf of Hammamet on the eastern coast of Tunisia. *African Journal of Marine Science* 34: 537-545. <doi:10.2989/1814232X.2012.689621>
- Saucedo P, I Racotta, H Villarreal & M Monteforte. 2002.** Seasonal changes in the histological and biochemical profile of the gonad, digestive gland, and muscle of the Calafia mother-of-pearl oyster, *Pinctada mazatlanica* (Hanley, 1856) associated with gametogenesis. *Journal of Shellfish Research* 21: 127-135.
- Semidey D, A Márquez & C Lodeiros. 2010.** Crecimiento y supervivencia de la madre perla *Pinctada imbricata* (Röding, 1798), bajo condiciones de cultivo suspendido, en cuerdas y cestas perleras. *Zootecnia Tropical* 28(4): 521-533.
- Southgate P & J Lucas. 2008.** The pearl oyster, 589 pp. Elsevier, Amsterdam.
- Strickland J & T Parsons. 1972.** A practical handbook of seawater analysis. *Bulletin, Fisheries Research Board of Canada* 167: 1-310.
- Urban H. 2000a.** Culture potential of the pearl oyster *Pinctada imbricata* from the Caribbean. I. Gametogenic activity, growth, mortality and production of a natural population. *Aquaculture* 189: 361-373.
- Urban H. 2000b.** Aquaculture potential of the Caribbean Pearl Oyster *Pinctada imbricata* II. Spat collection, juvenile growth and mortality in culture systems related to ambient conditions. *Aquaculture* 189: 375-388.
- Vásquez G, R Crescini, W Villalba, J Mogollón & L Troccoli. 2015.** Aspectos biológicos básicos de *Pinctada imbricata* (Bivalvia: Pteriidae) en la laguna La Restinga, Isla de Margarita, Venezuela. *Revista Ciencias Marinas y Costeras* 7: 117-132.
- Verginelli R & A Prieto. 1991.** Producción secundaria de *Pinctada imbricata* (Röding, 1798) en una población del Golfo de Cariaco. *Acta Científica Venezolana* 42: 138-144.
- Vite-García M & P Saucedo. 2008.** Energy storage and allocation during reproduction of pearl oyster *Pteria sterna* (Gould, 1851) at Bahía de La Paz, Baja California Sur, México. *Journal of Shellfish Research* 27: 375-383.
- Ward J & B MacDonald. 1996.** Pre-ingestive feeding behaviors of two subtropical bivalves (*Pinctada imbricata* and *Arca zabra*): Responses to an acute increase in suspended sediment concentration. *Bulletin of Marine Science* 59: 417-432.
- Wilbur K & G Owen. 1964.** Growth. In: Wilbur KM & CM Yonge (eds). *Physiology of Mollusca*, pp. 211-242. Academic Press, New York.
- Yukihira H, J Lucas & D Klump. 2000.** Comparative effects of temperature on suspension feeding and energy budgets of the pearl oysters *Pinctada margaritifera* and *P. maxima*. *Marine Ecology Progress Series* 95: 179-188.
- Zar J. 2010.** *Biostatistical analysis*, 944 pp. Prentice Hall, Upper Saddle River.

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