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Interactive effects of some coastal metal contaminant and environmental variables in the performance of the tropical winged pearl oyster *Pteria colymbus* (Mollusca: Bivalvia), in hanging culture

Efectos interactivos de algunos metales contaminantes y las variables ambientales en el desempeño de la ostra alada tropical *Pteria colymbus* (Mollusca: Bivalvia), en cultivo suspendido

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Keywords
aquaculture, bioaccumulation, ecotoxicology, condition index, Cadmium, Copper, Magnesium, Zinc.

ABSTRACT | The present study aimed to evaluate the interactive effect between the concentration of several pollutants metals (Zn, Cd, Mn, and Cu) detected in the soft tissue of the winged oyster *Pteria colymbus* and the environmental variables that occurred during the culture period. Quantification of contaminants was performed by inductively coupled plasma optical emission spectrophotometry. Monthly sampling of environmental variables in the water column included total particulate matter (TPM), organic fractions (POM) and inorganic fractions (PIM), temperature, dissolved oxygen, chlorophyll *a* (Chl-*a*), and the upwelling index. The results showed a decreasing order in the levels of Zn > Cd > Mn > Cu. The principal components analysis showed a significant and direct relationship between the upwelling index and Zn, Cd, and Cu, and an inverse and significant relationship with Mn. On the other hand, this analysis also showed an inverse and significant relationship between Zn, Cu, and Cd with the temperature (cold waters), suggesting that the source of the availability of these contaminants is not local and that it depends more on the transport of water masses caused probably by the action of upwelling. Due to the high concentration levels of Zn and Cd, the consumption of *P. colymbus* cannot be considered safe according to the tolerable limits of these contaminants, which exceeded the allowed levels. However, interest in the cultivation of members of the Pteriidae family does not decay due to the great benefit of their potential to produce round and mabé pearls.

Palabras clave
acuicultura, bioacumulación, ecotoxicología, índice de condición, Cadmio, Cobre, Magnesio, Zinc.

RESUMEN | El presente estudio tuvo por objetivo evaluar el efecto interactivo entre la concentración de varios metales contaminantes (Zn, Cd, Mn y Cu) detectados en el tejido suave de la ostra alada *Pteria colymbus* y las variables ambientales ocurridas durante el periodo de cultivo. La cuantificación de los contaminantes se realizó mediante espectrofotometría de emisión óptica de plasma acoplado inductivamente. Los muestreos mensuales de las variables ambientales en la columna de agua incluyeron al material particulado total (TPM), fracciones orgánicas (POM) e inorgánicas (PIM), temperatura, oxígeno disuelto, clorofila *a* (Chl-*a*) e índice de surgencia (IS). Los resultados mostraron un orden decreciente en los niveles de Zn> Cd> Mn> Cu. El análisis de componentes principales mostró una relación significativa y directa entre el índice de surgencias y el Zn, Cd y Cu, e inversa y significativa con el Mn. Por otro lado, este análisis también mostró una relación inversa y significativa entre Zn, Cu y Cd con la temperatura (aguas frías), sugiriendo que probablemente la fuente de estos contaminantes no sería local y que depende más del transporte de masas de agua provocadas por la acción de la surgencia. Debido a los altos niveles de concentración del Zn y el cadmio, el consumo de *P. colymbus* no puede considerarse seguro de acuerdo con los límites tolerables de estos contaminantes, que excedieron los niveles permitidos. Sin embargo, el interés en el cultivo de los miembros de la familia Pteriidae no decae debido al gran beneficio que se obtiene de su potencialidad para producir perlas redondas y mabés.

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INTRODUCTION

Industrial, agricultural, and urban development is accompanied inevitably by problems of water and general environmental pollution (Azizi *et al.* 2018a,b). An increase in industrial activity and associated population has resulted in a flux of pollution into estuarine and coastal environments (Aladaileh, 2014). The marine environment is the final repository for waste from various sources, such as household, agriculture, industry, and mining (Denton *et al.* 2003; Usero *et al.* 2005). Pollution generated by contaminant heavy metals in the marine environment has become a phenomenon of global concern due to toxicity, ecotoxicity, persistence, bioaccumulation, and bio-magnification, through the food chain and the serious negative effects generated in aquatic organisms (Rajeshkumar and Munuswamy, 2011).

Contaminant heavy metals are those elements whose density is at least five times greater than that of water, i.e., chemical elements having an atomic weight between 63.55 and 200.59 (Peña-Salamanca *et al.* 2005). Pollutants such as mercury, lead, cadmium, and arsenic are the main elements that cause adverse effects on public health based on their toxicity and exposure levels (Pendergrass *et al.* 1997). These elements, when present in the environment, can undergo processes such as speciation, mobilization, and chemical and/or physical transformation and, therefore, could be incorporated into food chains depending on their bioavailability (Allen and Hansen, 1996).

In the Caribbean Sea, heavy metal pollution has been linked to the development of the steel and oil industries, generating a large negative impact on different ecosystems (Fernandez *et al.* 2007). Several studies have reported the concentrations of essential and nonessential heavy metals in various marine species in representative coastal ecosystems, such as the green mussel *Perna viridis* (Acosta and Lodeiros, 2001, Narváez *et al.* 2005, Rojas *et al.* 2009, Pinto *et al.* 2015), the clam *Tivela mactroides* (Acosta and Lodeiros, 2001), the decapod *Callinectes* sp (Pérez *et al.* 2004); white shrimp and diverse fish species (Márquez *et al.* 2008), and the ark clam *Arca zebra* (Lanza *et al.* 2011), particularly in the northeastern coast of Venezuela.

Regarding the availability of heavy metals in the study area, a geochemical study of the surface sediments of the Turpialito Cove reported that the speciation of Zn, Cd, and Mn concentrations was higher in the available fraction (Marcano, 2015). These ecosystems have great ecological and economic importance since they provide food and shelter for breeding and rearing a variety of aquatic species. Regarding the location studied (Turpialito), the gonads and adductor muscle of the brown mussel *Perna perna*, cultivated under suspended conditions, showed high levels of Cr and Ni that exceeded allowed levels (Castillo *et al.* 2005).

The accumulation of metals in marine organisms is modulated by a large number of biotic and abiotic factors that determine their rate of incorporation in the environment (Wang *et al.* 2017; Otchere, 2019). On the one hand, the reproductive status of marine bivalves, such as the mussel *Mytilus galloprovincialis*, seem to be relevant aspects in the levels of contaminant metal, because gametogenesis did not influence contaminants metals distribution between body compartments, although, gametogenesis diluted their concentrations as a direct consequence of massive reproductive tissue production (Richir and Gobert, 2014). Further, the period or season of the year affects the variability of the concentration of heavy metals in *Mytilus edulis* (Otchere, 2003; Belabed *et al.* 2013). In turn, their bioaccumulation in the different parts of individuals indicates the level of contamination in the environment. Aquatic organisms have thus been widely used to monitor the health of the environment due to anthropogenic impacts (Rajeshkumar and Munuswamy, 2011; El Moselhy *et al.* 2016).

Since more than three decades ago, different species of mollusks, especially bivalves, have been used to evaluate the spatial and temporal bioavailability of contaminant metals (Kimbrough *et al.* 2008; Rosioru *et al.* 2016). Bivalves are the most commonly used bio-indicators of pollution in the marine environment worldwide, due to their ability to tolerate and debug high concentrations of pollutants (Neuberger-Cywiak *et al.* 2003; Narváez *et al.* 2005; Rojas *et al.* 2009; Nour and El-Sorogy 2020; Yap *et al.* 2021).

Among these species of bioindicators of contamination, the Atlantic wing oyster *Pteria colymbus* (Röding, 1798), does not stand out, since there are very few studies regarding its use as a bio-indicator organism of heavy metal pollution. Nevertheless, it has certain characteristics that make it a useful and emerging bioindicator species, among others, it is abundant in the marine environment, has a sedentary lifestyle, feeds on suspended particles by filtration, and is easy to sample (Kimbrough *et al.* 2008). These type of characteristics have allowed for several species of mussels to be considered “bioindicator organisms”, to detect the temporal and spatial variation of chemical pollutants and to contribute to the knowledge of trends in marine contamination (Besada *et al.* 2014; Lacroix *et al.* 2017; Azizi *et al.* 2018a).

Pteria colymbus is an epibenthic species, belonging to the family Pteridae. It is of medium to large size (60-70 mm) normally living in the subtidal zone at depths between 3 and 10 m attached to rocks and octocorals in the Western Atlantic, from North Carolina to southern Brazil (Díaz and Puyana, 1994, Lodeiros *et al.* 1999). The importance of studying the levels of heavy metals in these species rests on the fact that they represent one of the most important economic and ecologic groups in marine aquaculture due to their low production costs and high profitability, as well as their position as primary consumers. In particular, the Pteriidae family attracts great interest due to their ability to produce pearls naturally or in aquaculture, and the edible quality meat of some species (Saucedo *et al.* 2005; Lodeiros and Freites, 2008; Lodeiros *et al.* 2011).

It has been shown that, in bivalves, the extent of accumulation of contaminant metals is a function of several biotic and abiotic variables (Everaarts *et al.* 1989; Wang and Lu, 2017; Nour and El-Sorogy, 2020), and that a direct relation between upwelling periods and the bioavailability of some contaminant metals has been observed (Segovia-Zavala *et al.* 2004; Kavun and Podgurskaya, 2009; Lino *et al.* 2016; Wang and Lu, 2017). The present study evaluated the interactive effects of coastal metal contaminants (Zn, Cd, Mn, and Cu) and the upwelling period (active and non-active) on the performance of the edible winged pearl oysters *P. colymbus*.

MATERIALS AND METHODS

Oyster culture experiment

This study was conducted from April 2012 to May 2013 at Turpialito Cove, Gulf of Cariaco, Sucre state, northeastern Venezuela (10º27′30″ N, 64º01′52″ W) (Fig. 1). Wild *P. colymbus* juveniles were harvested by hand from natural populations settled on floating cages used for fish culture in the Charagato Cove, Cubagua Island (Nueva Esparta State), Venezuela (10º49′49, 17″ N; 64º09′40.42″ O). The oysters were transferred to a research station in Turpialito site (Fig. 1), in insulated containers packed with moistened foam layers to maintain a cool environment and limit stress.

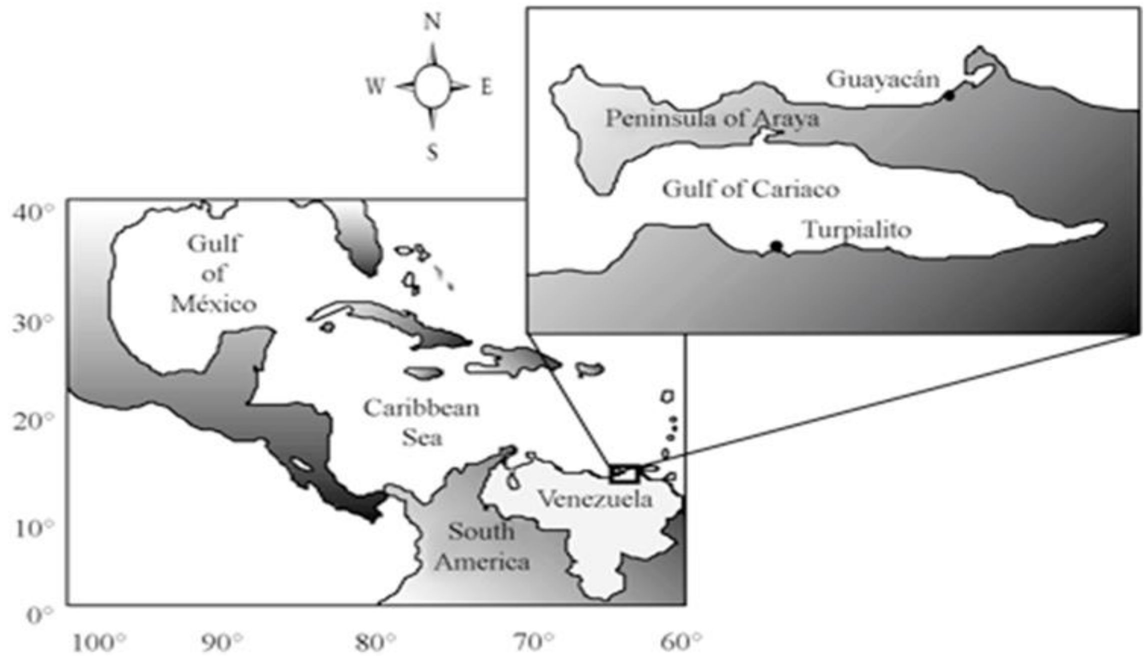

Figure 1. Geographical location of the Turpialito Cove, Gulf of Cariaco, Sucre State,
Figura 1. Ubicación geográfica de la Ensenada de Turpialito, Golfo de Cariaco, Estado Sucre,

At the beginning of the experimental period, individuals showed an initial length of the dorsal-ventral axis of 2.7 ± 0.22 cm and an antero-posterior axis length of 3.5 ± 0.24 cm. The juveniles were placed in 5 basket lantern nets (mesh Ø 0.5 cm, with base area 0.12 m^2), formed by five levels each. Initially, 56 oysters were placed in each level, with an area coverage of the bottom basket by each individual of 9.53 cm^2, and a partial coverage of the total bottom basket of 44%.

The baskets were suspended from a long line of 50 m in length and 7 m of bottom depth, located on the inner side of the cove. These baskets were deployed with a maximum depth of 2 m of the sea surface. Every 30 days, five individual oysters were sampled from each of the three experimental replicates. and transported to the Fisheries Biology laboratory, Oceanographic Institute of Venezuela. Total soft tissues were then removed from the shell of each individual using dissecting equipment and carefully separated to obtain the mass of the shell and soft tissues. All these components were dehydrated in an oven (60 ºC for 48 h) to obtain dry mass values with an error of 0.001 g. Only the soft tissues were pulverized in a ceramic mortar and sieved, placed in hermetic bags, labeled, and stored for subsequent analyses. The condition index (CI) of the winged oysters was then calculated following the recommendations described by Narváez *et al*. (2008).

Environmental variables

Weekly water samplings were made near the culture areas using a 5 L Niskin bottle to determine chlorophyll *a* using the classical methodology (Strickland and Parsons, 1972). The components of the seston: Total particle matter (TPM), particle inorganic matter (PIM), and particle organic matter (POM) were determined using a gravimetric method comparing filtered samples before and after combustion in a muffle at 400 °C for 5 h. In the culture system, a thermograph (Sealog-Vemco) was placed to record the temperature (± 0.01 °C) at 60 min intervals. One aliquot of each sample was carefully taken to avoid air bubbles for the determination of dissolved oxygen using the Winkler method within 6 h of sampling (detection limit 0.07 mg L^{-1}). For the determination of the upwelling index was considered the methodology previously described (Freites *et al.* 2017), using the formula described by Bowden (1983) and modified by Lavin *et al*. (1999).

Determination of the concentrations of heavy metals by ICP-OES

Approximately 0.4 g of dry soft tissue powder was extracted from each of the three tissue replicates taken monthly and weighed on an Ohaus analytical balance with a precision of 0.0001 g. These samples were placed in Erlenmeyer flasks containing 16 mL of concentrated nitric acid (HNO_3) for 12 h. The samples were transferred to specialized containers equipped with an internal Teflon seal and pressurized using a torque wrench, facilitating microwave-assisted acid digestion. Following the digestion process, the samples underwent filtration using Whatman #42 filters. Deionized water was incrementally added until reaching a final volume of 25 ml, after which the solution was transferred to plastic vials. The quantification of heavy metals (Zn, Cu, Mn, and Cd) in the dry tissues was conducted using inductively coupled plasma optical emission spectroscopy (ICP-OES) with Perkin Elmer equipment, model 5300DV Optima.

For the determination of metals with ICP-OES, the following conditions were used: outside flow of argon gas of 15 L min^{-1}, gas flow to the nebulizer of 0.5 L min^{-1}, flow of auxiliary gas of 0.2 L min-^{1}, radio frequency and 1300 W peristaltic pump flow of 1.5 L min^{-1}. Each metal was measured at a characteristic wavelength and detection limits were determined by a calibration curve (Meier and Zünd, 1993). For each metal, the wavelength (nm) and the detection limit (g g^{-1}) were, respectively, 206.200 and 0.101 for Zn; 327.393 and 0.050 for Cu; 228.802 and 0.041 for Cd; 257.610 for Mn.

Accuracy of the method

The accuracy of the method (5 repetitions) was verified using standard reference material of marine organisms certified by the International Atomic Energy Agency Analytical Quality Control Services (Cat # 312, lot # IAEA - . 407). The results were verified and compared with those reported by the manufacturers. Recoveries were higher than 90% for all trace metals measured.

Statistical analysis

Previous comparison of homogeneity of variance and normality of the data by the Levene and Kolmogorov-Smirnov tests, respectively, showed that these conditions were not met. Thus, the variation of metal concentration in the sampling months was determined using the non-parametric method of Kruskal-Wallis (Zar, 1999). The contaminant metal concentrations were correlated with the physical-chemical variables, condition index, and upwelling index by a principal component analysis, PCA (Chatfield and Collins 1980, Clarke and Warwick, 2001), and by a Spearman correlation analysis. Analyses were performed using the statistical software R (R Core Team, 2015).

RESULTS

Environmental variables

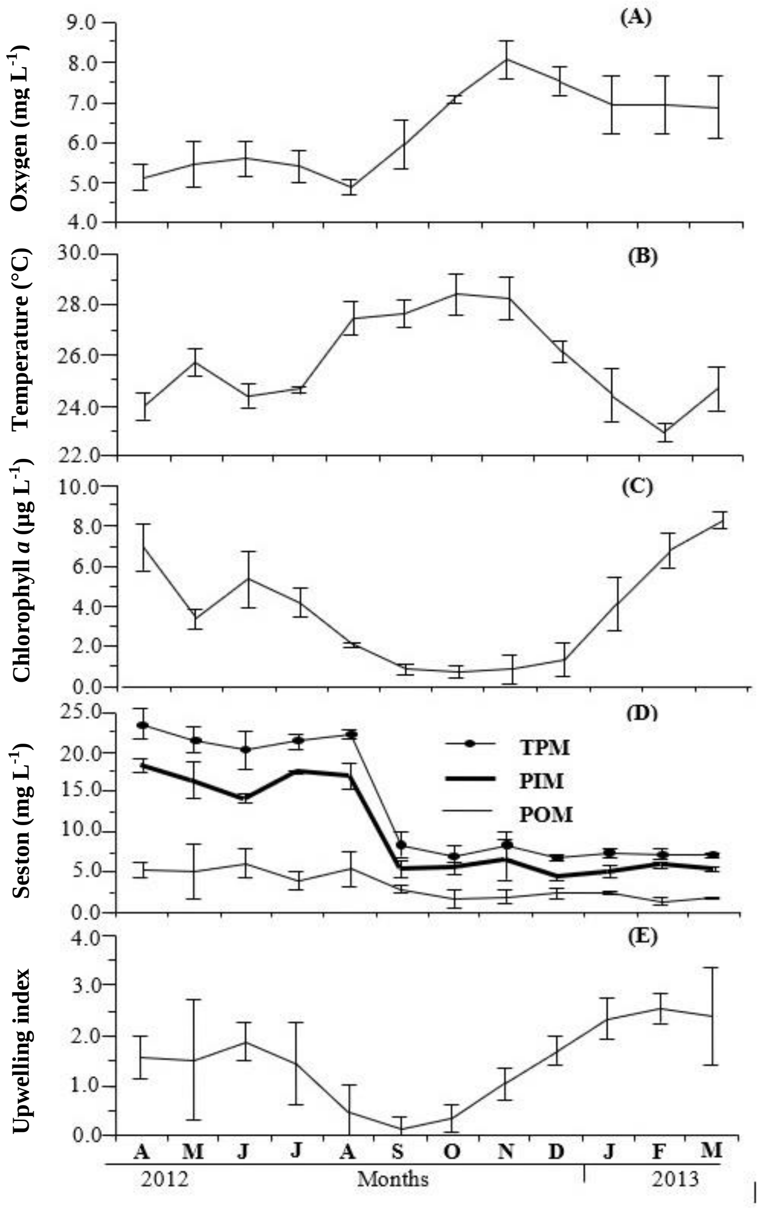

Figure 2. Variation of environmental variables during the experimental period: A) Oxygen, B) Temperature, C) Chlorophyll *a*, D) Seston, E) Upwelling index. Symbols and error bars represent mean monthly values plus the standard deviation, respectively.

Figura 2. Variación de variables ambientales durante el período experimental: A) Oxígeno, B) Temperatura, C) Clorofila *a*, D) Seston, E) Índice de afloramiento. Los símbolos y las barras de error representan los valores medios mensuales más la desviación estándar, respectivamente.

Dissolved O_2 (DO) showed large seasonal variability, from minimum values of 4.9 mg L^{-1} in August 2012 to 8.1 mg L^{-1} in November 2012 (Fig. 2A), and remained elevated until the completion of the study. Temperature also showed notable temporal variability (Fig. 2B), with the highest values of 27.4 to 28.3 °C recorded from mid-August to November 2012, and the lowest temperatures of 22.9 to 23.9 °C from April to June 2012 and between January and March 2013. Chl-*a* showed maximum values of 6.9 and 8.5 µg L^{-1} during the peak upwelling periods of April 2012 and March 2013, respectively (Fig. 2C).

Low concentrations of sestom down to 0.7 µg L^{-1} were observed in September 2012, including a decrease in the last sampling month from 9.5 µg L^{-1} (late April) to 3.12 µg L^{-1} (mid-May). TPM was dominated by the inorganic fraction (PIM) Higher values were observed during the first months of the study with a maximum of 22.3 mg L^{-1} in July 2012, of which 16.9 mg L^{-1} was organic (Fig. 2D). Organic seston (POM) remained stable from September 2012 at around 6 mg L^{-1} until the end of the study. UI presented medium values that fluctuated from 1.5 to 1.8 between April and July 2012 (upwelling season) and highest values around 2.5 (Fig. 2E), between January and March 2013. The low upwelling values (0.1 and 0.5) were observed between August and October 2012.

Heavy metals in the soft tissues of *Pteria colymbus*

During the evaluation of the heavy metal concentrations in specimens of *P. colymbus* cultivated in Turpialito Cove, significant differences were found for Zn (KW= 32.20; p <0.001), Cd (KW= 31.19; p <0.001) and Mn (KW= 25.20; p <0.009), while Cu showed no significant differences among months (KW= 18.33; p>0.05 <0.07). The highest Zn concentrations were evident during December and January (1630.850 ± 328.685 µg g^{-1} and 1659 ± 196.190 µg g^{-1} respectively) (Fig. 3A), while the lowest concentrations were observed in June, July and August (235.167 ± 50.990 µg g^{-1}, 231.924 ± 71.311 µg g^{-1} and 276.414 ± 36.216 µg g^{-1}, respectively).

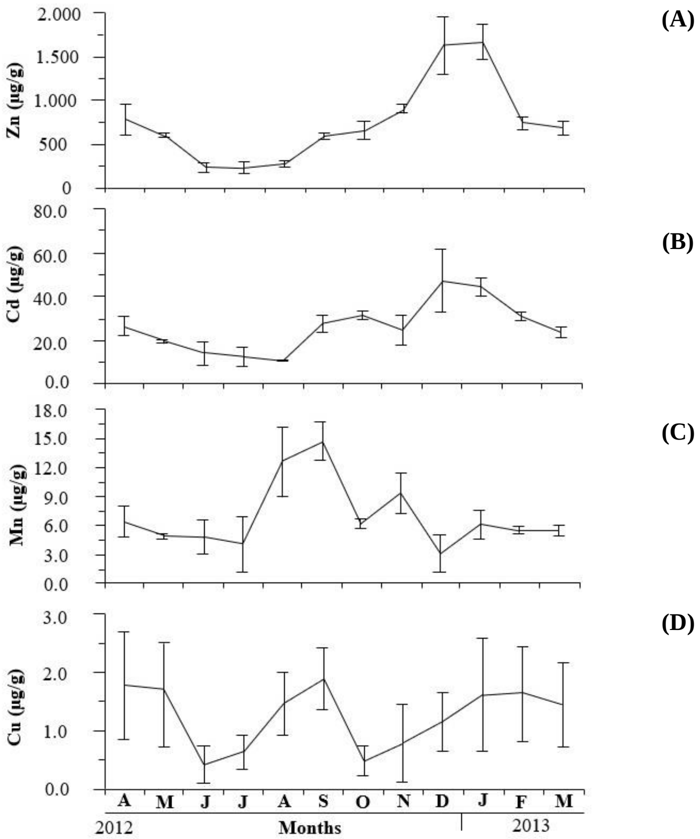

Figure 3. Monthly variation of the concentration of Zn, Cd, Mn, and Cu (µg g^{-1} dry mass) in the soft tissue of the winged oyster *Pteria colymbus* under suspended cultivation in Turpialito Cove. Symbols and error bars represent mean monthly values plus the standard deviation, respectively.

Figura 3. Variación mensual de la concentración de Zn, Cd, Mn y Cu (µg g^{-1} masa seca) en el tejido blando de la ostra alada *Pteria colymbus* cultivada en suspensión en la Ensenada de Turpialito. Los símbolos y las barras de error representan los valores medios mensuales más la desviación estándar, respectivamente.

The same pattern of seasonal accumulation was observed for Cd (Fig. 3B), with higher values in December and January (47.005 ±14.283 and 44.406 µg g^{-1} ± 4.525 µg g^{-1}, respectively), and lower contents in June, July, and August (13.972 ± 5.067 µg g^{-1}; 12.263 ± 4.325 and 10.728 µg g^{-1} ± 0.438 µg g^{-1}, respectively). On the other hand, Mn showed the highest values in the tissues of *P. colymbus* in August (12.700 ± 3.643 µg g^{-1}) and September (14.745 ± 2.051 µg g^{-1}). The lowest values were found in the remaining months analyzed with contents close to 6 µg g-1 (Fig. 3C), with December showing the lowest average content (3.083 ± 1.933 µg g^{-1}). Finally, Cu presented fluctuations over the sampling period with values ranging from 0.418 ± 0.331 µg g-1 in June to 1.891 ± 0.533 µg g^{-1} in September (Fig. 3D).

Condition index

Highest values of the Condition Index of 16-18 were observed in mid-July to August 2012 and mid-February to mid-April). The lowest CI was around 10-11 in mid-March to June 2012 and was > 8 from mid-November 2012 to mid-January 2013 (Fig. 4).

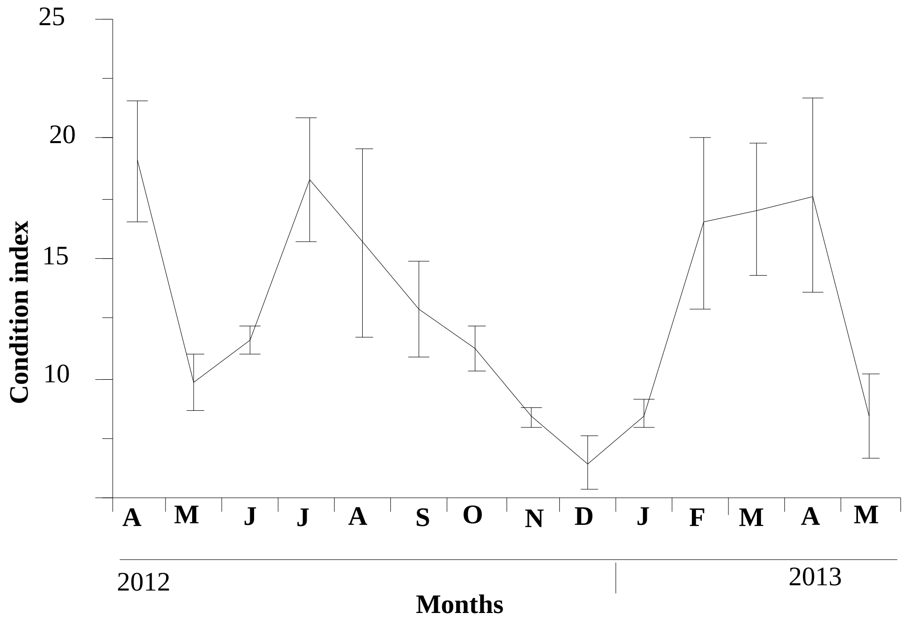

Figure 4. Monthly variation of the Condition Index in the winged oyster *Pteria colymbus* suspended under cultivation in Turpialito Cove. Symbols and error bars represent mean monthly values plus the standard deviation, respectively.

Figura 4. Variación mensual del Índice de Condición en la ostra alada *Pteria colymbus* cultivada en suspensión en la Ensenada de Turpialito. Los símbolos y las barras de error representan los valores medios mensuales más la desviación estándar, respectivamente.

Principal Component Analysis between concentrations of heavy metals, environmental variables, and biometrics

Concerning the PCA between the heavy metals in the soft tissue of *P. colymbus*, environmental variables, and Condition Index (CI), the first two components explained 63.64 % of the variation (Table 1), indicating that this is an acceptable graphical representation.

The first component showed a direct relationship between Zn, Cd, and in a minor proportion with Cu, while these three metals showed a positive and significant relationship with the upwelling index. An inverse relationship was found between these three pollutants, the Cl, and the environmental variables Chl *a*, TPM, POM, and PIM (Fig. 4). For the second component, an inverse relationship between Mn and temperature was observed about the upwelling index (UI) and Chl *a*, respectively.

Table 1. Eigenvalues and percentage of variance explained by each component of the PCA data corresponding to Zinc (Zn), Copper (Cu), Cadmium (Cd), Manganese (Mn), Particulate organic matter (POM), Particulate inorganic matter (PIM), Total particulate matter (TPM), Chlorophyll *a* (Chl *a*), Temperature (Temp), Upwelling index (UI) and Condition index (CI) of winged oyster *Pteria colymbus* suspended under cultivation in Turpialito Cove.

Tabla 1. Valores propios y porcentaje de varianza explicado por cada componente de los datos de ACP correspondientes a Zinc (Zn), Cobre (Cu), Cadmio (Cd), Manganeso (Mn), Materia orgánica particulada (POM), Materia inorgánica particulada (PIM), Material particulado total (TPM), Clorofila *a* (Chl *a*), Temperatura (Temp), Índice de afloramiento (UI) e Índice de condición (CI) de la ostra alada *Pteria colymbus* cultivada en suspensión en la Ensenada de Turpialito.

Component number	*Eigenvalues*	*% of variance explained*	*% accumulate*
1	4.15737	37.794	37.794
2	2.84249	25.841	63.635
3	1.44828	13.166	76.801
4	1.06405	9.673	86.475
5	0.450632	4.097	90.571
6	0.439525	3.996	94.567
7	0.2375	2.159	96.726
8	0.172176	1.565	98.291
9	0.132476	1.204	99.496
10	0.0554909	0.504	100.000

The PCA analysis provides equations that relate the influence of the environmental variables and heavy metals concentrations on the observed variance in the CI of the winged oyster *P. colymbus*, as follows:

0,296948*CI -0,0546866*Cu +0,0166385*Mn -0,4186*Cd -0,398662*Zn +0,170128*Chl-*a* -0,117634*Temper + 0,449085*TPM + 0,360874*PIM+0,437914*POM-0,0891068*UI.

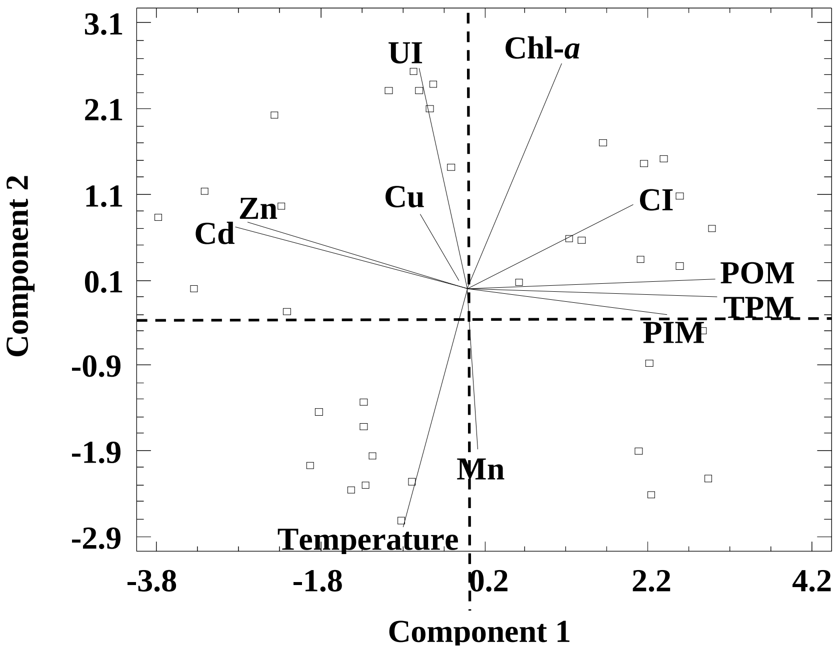

Figure 5. Two-dimensional principal component analysis plot of the variables zinc (Zn), copper (Cu), cadmium (Cd), manganese (Mn), organic seston (POM), inorganic seston (PIM), the total seston (TPM), chlorophyll *a* (Chl *a*), temperature (Temp), upwelling index (UI) and condition index (CI), of the winged oyster *Pteria colymbus*.

Figura 5. Gráfica bidimensional del análisis de componentes principales, de las variables zinc (Zn), cobre (Cu), cadmio (Cd), manganeso (Mn), seston orgánico (POM), seston inorgánico (PIM), seston total (TPM), clorofila *a* (Chl *a*), temperatura (Temp), índice de surgencia (UI) e índice de condición (CI), de la ostra alada *Pteria colymbus*.

DISCUSSION

Levels of contaminant metals Mn and Cu observed in the soft tissue of the winged oyster *P. colymbus* held under suspended culture conditions showed values under the limits established by organizations such as the Food and Agriculture Organization of the United Nations (FAO), Food and Drug Administration (FDA) of the United States of America, and Brazilian Legislation Food (BFL) (Table 2). However, Zn and Cd exceeded the allowed levels. In Venezuela there are no technical norms establishing allowed levels of any trace elements. The Comisión Venezolana de Normas Industriales (COVENIN) has only established norms for determination of Pb and Cd in food as well as in Ark shell, tuna and sardine, COVENIN (1994) and COVENIN (1998), respectively.

Table 2. Maximum allowed values for heavy metals (μg g^{-1}) by international institutions for marine edibles resources.
Tabla 2. Valores máximos permitidos de metales pesados (μg g^{-1}) por instituciones internacionales para recursos marinos comestibles.

Metal	FAO	FDA	BFL
Cu	10	---	30
Mn	---	---	---
Cd	1	4	1
Zn	50	---	50

Sources: FAO (1983); FDA: Food and Drugs Administration (1997); BFL: Brazilian Federal Legislation (1975).
Fuentes: FAO (1983); FDA: Food and Drugs Administration (1997); BFL: Brazilian Federal Legislation (1975).

The content of contaminant metals Cd, Cu, and Zn found in samples of the soft tissues of *P. colymbus* was particularly related to the periodic upwelling processes that occur in Northeastern Venezuela. However, it cannot be ruled out that part of these levels of contaminant metals come from the seston present in the study area and that they were resuspended by the action of the waves produced by the trade winds that characterize the upwelling process. Other studies observed a direct relation between upwelling periods and the availability of some contaminant metals (Lares *et al.* 2002; Segovia-Zavala *et al.* 2004; Kavun and Podgurskaya, 2009; Lino *et al.* 2016; Wang and Lu, 2017),

On the other hand, PCA showed significant negative associations between Cd, Zn, and minor proportion with Cu, with the condition index (CI) of the organism. This suggests that the physiology of *P. colymbus* probably was impacted by the high concentrations of these contaminant metals in the soft body tissues. This result agrees with those obtained by Lobel *et al*. (1991) who observed a significant and negative correlation between Cd concentrations and the condition index of the mussel *M. edulis*.

The decreasing order of accumulation of contaminant metals was Zn> Cd> Mn> Cu, contrasting with other authors that found a tendency to bioaccumulate essential metals more than non-essential metals in the clam *Tivela mactroides* (Acosta and Lodeiros, 2004) and mussel *Perna perna* (Castillo *et al.* 2005), suggesting that the degree of contamination is specific for certain metals in the study area. This could be probably related to the polluting effect of industrial drains in the areas nearby, such as shipyards, docks, and an industrial cannery, implying that the presence of metals becomes more evident due to the systems of currents and upwelling that take place in the Gulf of Cariaco. Even low concentrations imply a high degree of toxicity for different organisms present in the aquatic environment (Huanxin *et al.* 2000; Shulkin *et al.* 2003; Maanan, 2007).

Another aspect to consider regarding the high levels of contaminant metals in the soft tissues of *P. colymbus*, is the phenomenon of upwelling, a process particularly marked in northeastern Venezuela. This region is characterized by two seasonal periods: the rainy season from June/November, and the dry season from November/May (Antonius, 1980). In turn, during the dry season, strong mixing of surface waters by wind-induced upwelling occurs (Bakun and Nelson, 1991), resulting in the dispersion of heavy metals for marine organisms. This may be associated with higher concentrations of Zn and Cd during the dry season or period of upwelling, in the Northeastern of Venezuela and this study. A significant positive correlation was observed between the rate of upwelling and Zn. Likewise, the release of Cd in the environment occurs closely with the peaks of coastal upwelling in the Northeastern region of Venezuela (Acosta and Lodeiros, 2004).

Concerning the availability of heavy metals in the study area, a geochemical study of the surface sediments of the Turpialito Cove reported that the speciation of Zn, Cd, and Mn concentrations was higher in the affordable fraction (Marcano, 2015). Nevertheless, it could not be ruled out that the contents of Zn, Cd, and Mn in the soft tissues of *P. colymbus* may be the result of progressive bioaccumulation from the heavy metals absorbed from the sediment, as a result of the high degree of resuspension of the marine sediment that occurs in the cove, during the period of strong winds that occur during the upwelling period. In this way, Wright and Welbourn (1994) said that much of the cadmium added to aquatic systems accumulates in sediments where it presents a risk to benthic biota and under certain conditions may reenter the water column. This fact probably makes the contaminating metals accumulated in the sediment available to pearl oysters. Accordingly, the absorption of heavy metals by organisms from the sediment appears to be a feasible mechanism for the bioaccumulation of these contaminants in some mussel and oyster species (Shulkin *et al.* 2003; Borgmann *et al.* 2004; Maanan, 2008).

Lower concentrations of Zn and Cd were observed in June, July, and August when the annual upwelling period was ending. In contrast, the levels of Zn and Cd in the soft tissues of the winged oyster *P. colymbus* showed the highest concentrations during December and January, when the upwelling period was active. These higher concentrations of two pollutants coincide with the lowest levels of the condition index of the winged oyster *P. colymbus*. Zn levels may be related to the metabolic requirements for maintaining body growth rate, reproduction, and spawning, under suspended culture conditions, being mostly of relevance for germinal tissue (White and Rainbow, 1985; Rainbow, 2007; Lemus *et al.* 2010). Zn is an essential element for living organisms but at high concentrations can cause toxicity in marine organisms (Chan, 1988; Everaarts *et al.* 1989; Rainbow, 2007).

Possible implications regarding the highest contents of Zn and Cd in *P. colymbus* agree with previous research in areas adjacent to this study for the green mussel *Perna viridis* (Rojas *et al.* 2002; Aanand *et al.* 2010; Lemus *et al.* 2010, 2013; Zapata *et al.* 2012; Pinto *et al.* 2015), and the clam *Tivela mactroides* (Acosta and Lodeiros, 2004). The levels of heavy metals observed in the present study were lower than those cited above.

In the present study, concentrations of Cd in the soft tissues of *P. colymbus* fluctuated between averages of 10.7 and 44 µg g^{-1}, affecting the health of oysters, as reflected in the inverse and significant relationship between the CI and the contents of these pollutants in the body tissues. The values of Cd and Zn observed in the present study are comparatively high to those observed by Lemus *et al*. (2010) and Pinto et al. (2015) in the soft tissues of the *Perna viridis* mussel of 0.83 - 1.23 and 0.82 - 1.78 µg g^{-1}, respectively. In this way, static tests have shown that the average lethal concentration of Cd for a bivalve was 2.27 ug L^{-1} at 96 h (Vijayavel *et al.* 2007). Further, previous studies have shown that exposure a Cd results in oxidative damage to oyster *Crassostrea virginica* tissues exposed to 50 µg L^{-1} of Cd, at 28 °C, but not at 20 or 24 °C, as indicated by accumulation of malondialdehyde, a final product of lipid peroxidation (Lannig *et al.* 2008).

Cd and Zn showed significant positive associations. These results agree with Packer *et al*. (1980) who observed a positive correlation between the concentration in the polychaete *Arenicola marina* from the sediment for both zinc and cadmium. Therefore, the antagonistic effect of Cd on Zn (Cd/Zn) has been reported, where Cd replaces Zn biochemically in certain enzymes such as carbonic anhydrase (González *et al*. 2004).

Mn showed average values that fluctuated between 6.0 and 14.7 µg g^{-1}, observing the highest values in the soft tissues of *P. colymbus* from August to September, coinciding with the annual rainy season. This trend suggests that Mn concentrations in the tissues of *P. colymbus* were also likely to grow due to increased availability in the culture area as a result of runoff to the Cove. Mn is a minor element in seawater, but it is present in appreciable quantities in marine sediments (Sadiq, 1992). Besides, levels of Mn may thus be related to anthropogenic activities in the study area modified by climatic factors such as the rainy season. The mean concentrations of Mn recorded in this study were higher than those reported by previous studies in the study area and its surrounding using *Perna viridis*, *Arca zebra*, and *Perna perna* (Castillo *et al.* 2005, Lanza *et al.* 2011, Pinto *et al.* 2015), but were close to the Mn content in the soft tissues of the mussel *Modiolus modiolus* from the Uril Islands shelf (Sea of Okhotsk, RUSSIA), in which maximum values of 16.8 µg g^{-1} were recorded (Kavun and Podgurskaya, 2009).

Regarding the positive associations between Mn contents in the soft tissues of winged oysters and temperature, this fact probably could be related to an increase in metabolic activity of the oysters, due to the increase in temperature, which in turn could lead to a greater accumulation of metals, as was observed in the mussel *Mytilus edulis* (Mubiana and Blust, 2007) and the ophiuroid *Ophiothrix fragilis* (Hutchins *et al.* 1996) at the highest temperatures. It must be taken into account that the temperature plays a very important role in the speciation of metals which leads to greater assimilation of the dissolved phase. The toxicity of heavy metals can be influenced by their solubility which is affected by temperature (Bat *et al.* 2000). On the other hand, some studies have shown that the oyster *Crassostrea virginica* readily accumulates manganese into its ganglia and tissues (Murray *et al.* 2007). Higher concentrations of manganese disrupted the dopaminergic innervation of the gill of *C. virginica*, affecting the beating rates of the lateral cilia of the gill, and therefore, probably affecting the rate of feed filtration and oxygen acquisition (King *et al.* 2008; Nelson *et al.* 2010, 2018).

Cu presented fluctuations during the sampling months with minimum values in June and maximum values in September. This suggests that Cu levels were related to the increase of the oyster metabolism as a consequence of the temperature increment observed in September, such as was observed in the mussel *Perna viridis* (Lemus *et al.* 2010). It should be stressed that Cu as Zn is essential for the development of bivalves, acting as metabolic enzyme cofactors in the processes of electron transport, but they are also deleterious at high concentrations (Chavez *et al.* 2003). Experimental evidence showed that a considerable number of species of bivalves have sensitivity to low concentrations of these metals (Aanand *et al.* 2010). Cu levels reported in previous studies were higher than those observed in the present study (Castillo *et al.* 2005, Rojas *et al.* 2005, Lemus *et al.* 2010, Zapata *et al.* 2012, Pinto *et al.* 2015). Yet, Cu concentration in *Arca zebra* determined in Caimancito and Chacopata locations was lower than those reported in this study (Lanza *et al.* 2011).

Focusing on a broader level, comparing average levels of heavy metals found in this study with data reported in other bivalve species and areas around the world, such as *Donax trunculus* in the Gulf of Catania, Italy (Copat *et al.* 2013), *Mactra chinensis* in Shanghai, China (Lei *et al.* 2013) and *Mytilus galloprovincialis* in the Adriatic Sea (Jović and Stankovic, 2014), heavy metal levels were reported below those of the present study. In contrast, measured levels of heavy metals in *Mactra veneriformis* in Bohay Bay, China, showed Mn levels higher than those of the present study (Li *et al.* 2015). These studies linked heavy metals in the tissues of the studied organisms to the rapid increase in industrialization in the study areas.

The above discussion suggests concern regarding the increasing input of pollutants to coastal areas where aquaculture of marine organisms of great economic importance is carried out. In particular, Cd concentrations were high, being a highly toxic metal. Little is known about the content of heavy metals in *P. colymbus* in Venezuela.

The inverse and significant relationship between the concentrations of Cd and Zn contained in the soft tissues of the winged oyster and the availability of food in the water column from phytoplankton and seston origin suggests that the observed levels of contaminant metals probably did not depend on local availability. In contrast, the inverse and significant relationship between temperature and Cd, Zn, and to a lesser degree Cu, content, suggest that said availability is due to the intense exchange of water mass that occurs in the period dominated by coastal upwelling, which probably made the metals available to the organisms distributed in the coastal areas. Further research is recommended in which the oysters used as bioindicator organisms of the contamination degree by pollutant metals should include, in addition to soft tissues, also the shell, seston, and the water column, in addition to other physical-chemical factors. This would allow the characterization of the water masses and corroborate the influence of the upwelling that occurs in the northeastern zone of Venezuela on the dispersion of these contaminant metals that have been accumulated in deeper areas of the continental shelf or areas close to the locality under study, with higher levels of contamination, such as the City of Cumana, just 8 km away.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ETHICAL USE OF ANIMALS

All the procedures followed the guidelines for ethical and responsible research using in vivo animals for experiments (Kilkenny, *et al.* 2010).

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