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POTENTIALLY HARMFUL CYANOBACTERIA IN OYSTER BANKS OF TÉRMINOS LAGOON, SOUTHEASTERN GULF OF MEXICO

Cianobacterias potencialmente nocivas en los bancos ostrícolas de la laguna de Términos, sureste del Golfo de México

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ABSTRACT

Cyanobacteria inhabit hypersaline, marine and freshwater environments. Some toxic and non-toxic species can form harmful blooms. The aim of this study was to identify potentially harmful cyanobacterial species in the oyster banks of Términos Lagoon, the southeastern Gulf of Mexico. Six sample sites (up to 2-m depth) were monitored monthly from August 2012 to September 2013. Water temperature, salinity, pH, dissolved oxygen saturation (% DO), inorganic nutrients and abundance of cyanobacteria were determined. Temperature and salinity were characterized by marked seasonal differences (26.8 to 30.6 °C and 6.1 to 19.5, respectively). The pH values (ranging from 7.1 to 8.4) and the % DO (88.4 to 118.2 %) suggest a predominance of photosynthetic activity in the windy season (October-February). Elevated nutrient contents are associated with the period of increased river discharge, determined by water circulation and biogeochemical processes. Fourteen taxa were identified, of which *Anabaena* sp., *Merismopedia* sp., *Oscillatoria* sp. and *Cylindrospermopsis cuspis* produced blooms. Cyanobacterial abundances were on the order of magnitude of 10^6 cells L^{-1} in October 2012 at stations S1-S6, with an average value of 3.2×10^5 cells L^{-1} and a range of 2000 to 3.1×10^6 cells L^{-1} throughout the study period; however, they showed a remarkable absence during the windy season (October to January). *Anabaena* sp. and *C. cuspis* reached abundances of 1.9×10^6 and 1.3×10^6 cells L^{-1}, respectively. The latter caused the temporary closure of oyster *Crassostrea virginica* harvesting for 15 days in October 2012.

Keywords: annual cycle, cyanobacteria, eutrophication, Gulf of Mexico, harmful algal blooms.

RESUMEN

Las cianobacterias habitan en ambientes hipersalinos, marinos y de agua dulce. Algunas especies tóxicas y no tóxicas pueden formar florecimientos nocivos. El objetivo de este estudio fue identificar las especies de cianobacterias potencialmente nocivas en los bancos ostrícolas de laguna de Términos, sureste del Golfo de México. Seis sitios de muestreo (hasta 2 m de profundidad) fueron monitoreados mensualmente de agosto de 2012 a septiembre de 2013. Se midió la temperatura del agua, salinidad, pH, saturación de oxígeno, nutrientes inorgánicos y abundancia de cianobacterias. La temperatura y la salinidad se caracterizaron por marcadas diferencias estacionales (26,8 a 30,6 °C y 6,1 a 19,5, respectivamente). Los valores de pH (de 7,1 a 8,4) y la saturación de oxígeno disuelto (de 88,4 a 118,2 %) sugieren un predominio de la actividad fotosintética en la temporada de nortes (octubre-enero). Las concentraciones elevadas de los nutrientes están asociados al periodo de mayor descarga de los ríos, determinados por la circulación y los procesos biogeoquímicos. Se identificaron 14 taxa, de los cuales *Anabaena* sp., *Merismopedia* sp., *Oscillatoria* sp. y *Cylindrospermopsis cuspis* formaron florecimientos. Las abundancias de cianobacterias fueron del orden de magnitud de 10^6 células L^{-1} en octubre de 2012 en las estaciones S1-S6, con un valor promedio de 3.2×10^5 células L^{-1} y un rango de 2000 a 3.1×10^6 células L^{-1} a lo largo del periodo de estudio. Sin embargo, mostraron una ausencia notable durante la temporada de nortes (octubre a enero).

Anabaena sp. y *C. cuspis* alcanzaron abundancias de 1.9x10^6 y 1.3x10^6 células L^{-1}, respectivamente. Este último causó el cierre temporal de la colecta del ostión *Crassostrea virginica* durante 15 días en octubre de 2012.
Palabras clave: cianobacterias, ciclo anual, eutrofización, florecimientos algales nocivos, Golfo de México.

INTRODUCTION

Cyanobacteria are a diverse group of photosynthetic microorganisms with specific physiological and morphological characteristics that allow them to adapt to a wide range of habitats including pelagic and benthic environments where they are highly competitive (Liotenberg *et al*., 1996; Morvan *et al*., 1997; Oren, 2000). Some species can survive under extreme environmental conditions such as deserts, hot springs and alkaline lakes (Oren, 2000).

The growth of cyanobacteria in aquatic environments is controlled by a variety of environmental factors, and adequate nutrient conditions, temperature, hydrogen potential (pH) and illumination are required for their cultivation (Kebede and Ahlgren, 1996). Cyanobacteria can grow in both saline and non-saline environments, as well as showing a greater probability of colonizing various aquatic environments, compared to those that are strictly freshwater or halophilic (Rosales-Loaiza *et al*., 2004). In particular, planktonic cyanobacteria frequently proliferate in eutrophic freshwater environments; however, little is known about their role in tropical coastal environments with a certain degree of eutrophication.

Harmful algal blooms (HAB) caused by cyanobacteria affect water quality, fish resources, animals and human beings (Muciño-Márquez *et al*., 2015). Due to the production of excessive biomass, they can cloud the water, thereby inhibiting phytoplankton photosynthesis, and they also produce toxins, causing unpleasant odors in water, decrease the dissolved oxygen concentration and therefore its availability to consumers, and inhibit the growth of other phytoplankton species that serve as food for consumers (Echenique and Aguilera, 2009).

According to the National Fisheries Charter (Diario Oficial de la Federación, 2012), oysters are the most important resource because of their volume in the Gulf of Mexico (around 40 tons in 2012). The Gulf of Mexico contributes more than 93 % of the national oyster production. This production is mainly based on the exploitation of natural banks. Furthermore, it is one of the products with a lower price and wide acceptance because of its sanitary quality. The oyster banks in Términos Lagoon supply the local market and the areas adjacent to Ciudad del Carmen in the state of Campeche, Mexico, in the southeastern Gulf of Mexico.

The aim of this study was to identify potentially harmful cyanobacterial species in these oyster banks.

MATERIAL AND METHODS

The present study was conducted in the areas of extraction of bivalve molluscs in the Lagoon of San Carlos and Puerto Rico subsystem in Términos Lagoon (Figs. 1a, 1b), located 30 km west of Ciudad del Carmen, state of Campeche (18°33-38'N, 92°01'-14'W). The area is characterized by three meteorological seasons: a dry season from February to May, a rainy season from June to September and a windy season from October to January (Yáñez-Arancibia and Day, 1982). Monthly sampling was performed in six oyster banks at up to 2-m depth, from August 2012 through September 2013 (Figs. 1a, 1b).

At each site surface seawater samples were collected with a plastic 500-ml bottle; an aliquot of 100 ml was used to analyze cell abundances of phytoplankton taxa (Lindahl, 1986). Samples were fixed *in situ* with an alkaline solution of iodine (Utermöhl, 1958) and subsequently preserved by adding 4 % neutralized formalin (Throndsen, 1978). Additionally, circular horizontal tows were performed for five minutes with a conical hand net, with a 20-µm mesh size, at each sampling site. The collected material was placed into glass vials and fixed using the same procedure as for the quantitative analysis to identify the cyanobacterial taxa. *In situ* water temperature (°C), salinity, pH and oxygen saturation (%) were measured on-board using a HANNA multiparameter probe, model HI9828, with HI769828 sensor and a HACH multiparameter probe, model HQ40d (HANNA Instruments Inc., Woonsocket, Rhode Island, USA). Orthophosphate (P-PO_4^{3-}), ammonium (N-NH_4^+), nitrite (N-NO_2^-) and nitrate (N-NO_3^-) analyses were performed according to Strickland and Parsons (1972).

Quantification of planktonic cyanobacterial cells was determined following the Utermöhl technique (Utermöhl, 1958), taking 10 cm^3 of each sample and using an inverted Carl Zeiss Axio Observer.A1 microscope equipped with phase contrast 10x/0.25 Ph1 ADL and LD 20x/0.30 Ph1 objectives. Cyanobacterial cells (<20 µm), due to their small size, were not identified to species level. Abundance values were expressed as cells L^{-1}. Observation and identification of cyanobacteria species were performed on fixed samples with a Motic BA310 compound microscope equipped with the planachromatic objectives 4x/0.10, 10x/0.25, 20x/0.40, 40x/0.65 and 100x/1.25, using specialized taxonomic literature.

The hypothesis of differences between months and between sampling stations was tested by analysis of variance, and Tukey TSD (Truly Significant Difference) was applied with a significance level of 0.05 (Daniel, 2002). The normality of the recorded data was assessed by the Kolmogorov-Smirnov test and homoscedasticity with the Bartlett's test (Garson, 2012). The calculation routine was performed with Statgraphics Centurion XV program, version 18.2.06.

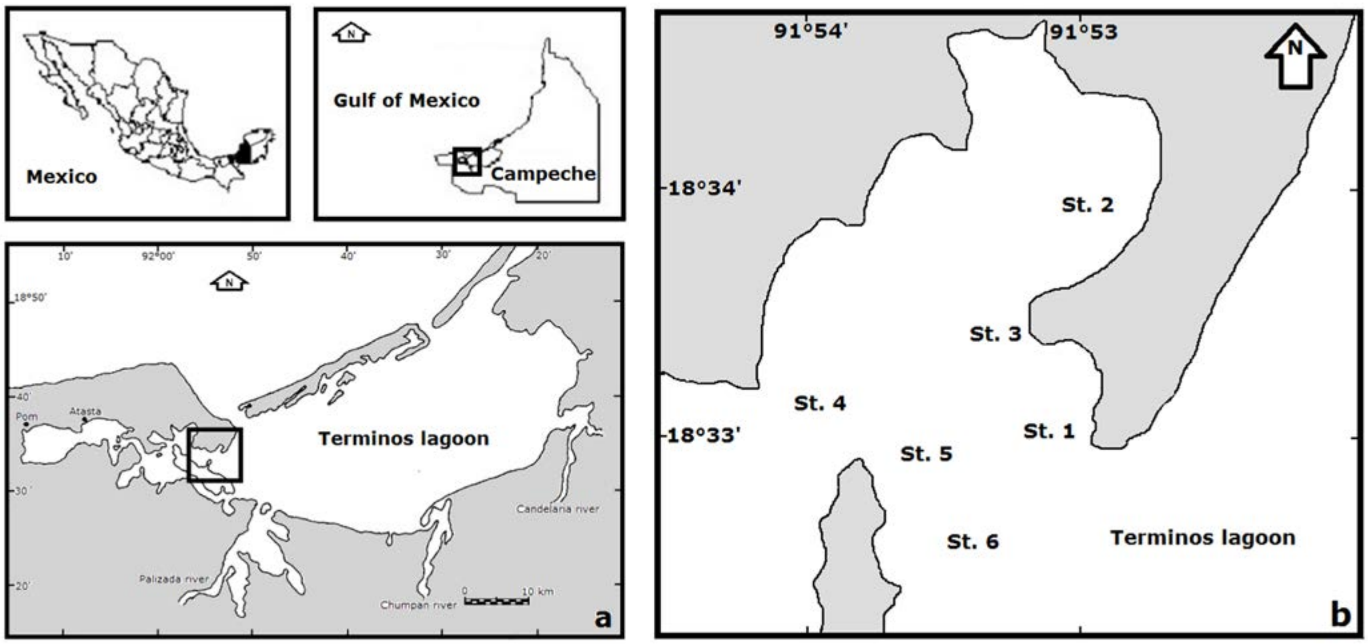

Figure 1. Study area: (a) the state of Campeche (shadowed), Mexico, and (a, b) location of sampling stations in oyster banks (square).

The presence and intensity of bloom of colonial or filamentous cyanobacteria were analyzed based on the ECOFRAME proposal (as described below) regarding the proportion of cyanobacteria and other major phytoplankton groups (Moss *et al*., 2003). The classification scheme for its application was modified for Términos Lagoon.

a) no aggregates are observed and there is no dominance (<95 %) of filaments or colonies of cyanobacteria;

b) the same situation as a), but surface formation of cyanobacteria is detected in a punctual or intermittent way;

c) dominance of filaments or colonies of cyanobacteria (>95 %) is evident and/or frequent blooms occur;

d) they do not show aggregates; however, when blooms occur, observed by cell counting and are infrequent in time, there is a dominance of ≈40 % of filaments or colonies of cyanobacteria.

RESULTS

Physical and chemical factors

Water temperature showed a range of temporal variability of ± 3 °C. Minimum average values (26.8-27.3 °C) were recorded during the windy season and maximum average values (29.7-30.6 °C) during the rainy season (Table 1). Salinity minimum average values were measured in the windy

Table 1. Summary statistics (by meteorological season) of environmental variables and nutrients at six sampling sites in the oyster banks of Términos Lagoon, Campeche, Mexico, in 2012-2013 (mean, range and standard deviation).

	Environmental variables				Nutrients (µmol L-1)			
Season	T°C	Salinity	pH	D. O (%)	Nitrite	Nitrate	Ammonium	Phosphate
	30.2	13.2	7.6	93.2	0.97	2.20	2.10	0.30
Rainy	29.7-30.6	11.6-14.3	7.4-7.8	88.4-96.3	0.30-3.40	1.31-3.42	1.15-3.79	0.14-0.60
	±0.37	±0.15	±0.15	±2.82	±1.26	±0.83	±1.12	±0.18
	27.0	7.4	8.2	104.6	0.58	1.09	3.16	0.38
Windy	26.8-27.3	6.1-8.7	7.9-8.4	98.9-111.8	0.04-3.39	0.47-2.26	2.80-3.64	0.20-0.63
	±0.19	±1.12	±0.18	±4.73	±1.24	±0.70	±0.31	±0.17
	28.0	18.4	7.1	112.2	0.17	0.78	1.80	1.05
Dry	27.8-28.4	17.4-19.5	6.9-7.3	106.2-118.2	0.07-0.35	0.54-1.28	1.62-2.05	0.73-1.52
	±0.25	±0.13	±0.13	±5.03	±0.09	±0.28	±0.19	±0.30
SD ÷ season	F= 260.1*	F= 225.1*	F= 64.8*	F= 44.5*	F= 53.3*	F= 11.9*	F= 6.5*	F= 15.2*

*Significant differences SD (p< 0.05, ANOVA nonparametric one-way) between the seasons.

season (6.1-8.7), and maximum average values during the dry season, with a range of 17.4-19.5 (Table 1). Minimum mean pH values were observed during the dry season (6.9-7.3), while the maximum mean was registered during the windy season, with a range of 7.9-8.4 (Table 1). Minimum average oxygen saturation values were recorded in the rainy season (88.4-96.3 %), while maximum average values (106.2-118.2 %) were observed in the dry season (Table 1). All the variables mentioned above showed significant differences between seasons ($p< 0.05$).

Nutrients

Variations in the concentrations of the inorganic nutrients were relatively wide (Table 1). Average concentrations of nitrite ($N\text{-}NO_2^-$) and nitrate ($N\text{-}NO_3^-$) were low throughout the study compared to ammonium. However, nitrite concentrations showed maximum values, ranging from 0.30 to 3.40 µmol L^{-1}, in the rainy season (Table 1). Nitrate concentrations showed maximum values ranging from 1.31 to 3.42 µmol L^{-1} in the rainy season and minimum values (0.54-1.28 µmol L^{-1}) in the dry season (Table 1). Minimum ammonium ($N\text{-}NH_4^+$) concentrations were observed in the dry season (1.62-2.05 µmol L^{-1}), while maximum concentrations (2.80-3.64 µmol L^{-1}) were observed in the windy season (Table 1). Orthophosphate ($P\text{-}PO_4^{3-}$) concentrations showed minimum average values (0.14-0.60 µmol L^{-1}) during the rainy season, while maximum average values (0.73-1.52 µmol L^{-1}) were registered during the dry season (Table 1). All the variables mentioned above showed significant differences between seasons ($p< 0.05$).

Cyanobacterial abundance

Fourteen taxa were identified, of which *Anabaena* sp., *Merismopedia* sp., *Oscillatoria* sp. and *Cylindrospermopsis cuspis* formed blooms. Cyanobacterial abundance increased by orders of magnitude to 10^6 cells L^{-1} in the windy season (October), and small peaks were also observed in the dry and rainy seasons (Fig. 2). Among cyanobacteria, the genus *Anabaena* Bory de Saint-Vincent was present throughout the study period, with a maximum abundance of $1.9x10^6$ cells L^{-1} and a minimum abundance of $3.6x10^4$ cells L^{-1} (Table 2, Fig. 4g). *Merismopedia* sp. and *Oscillatoria* sp. showed maximum abundances of $1.0x10^5$ and $2.5x10^5$ cells L^{-1}, respectively, in the windy season (Table 2, Figs. 4d, 4e). During the windy season, a bloom of *Cylindrospermopsis cuspis* was recorded with $1.3x10^6$ cells L^{-1}, and it lasted throughout the season with an abundance of up to $2.4x10^3$ cells L^{-1} (Table 2, Fig. 4a, c).

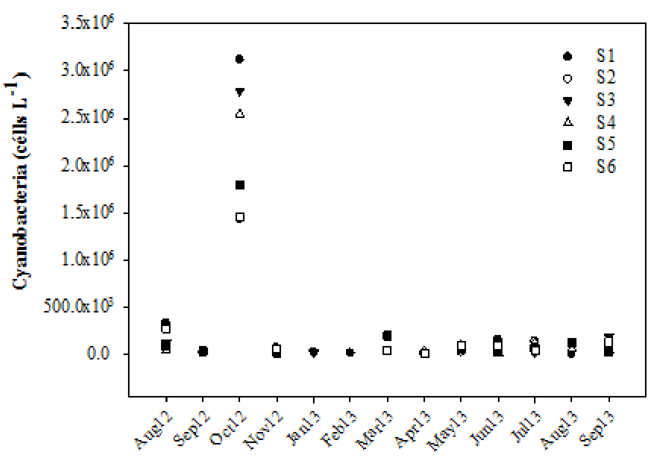

Figure 2. Temporal variation of cell abundances of cyanobacteria in the oyster banks of Términos Lagoon, Campeche, Mexico, in 2012-2013.

Percentage of cyanobacteria

The percentages of cyanobacteria with respect to the rest of phytoplankton were based on cell abundances (Fig. 3). At station S6 located at the entrance of the lagoon, a lower percentage of cyanobacteria (28 %) was observed (Fig. 3). Phytoplankton microalgal major groups showed the greatest contribution when cyanobacteria were not abundant. It should be noted that cyanobacteria were more frequent and with high abundances with only 14 species corresponding to 17 % of the total number of phytoplankton species.

Table 2. List of potentially harmful cyanobacteria observed at six sampling sites during three seasons in the oyster banks of Términos Lagoon, Campeche, Mexico, in 2012-2013.

Species	Rainy	Windy	Dry
	Maximum abundances (cells L-1).		
Human potentially toxic species*			
Merismopedia sp.	33,000	106,000	-
Oscillatoria sp.	83,000	258,000	195,000
Anabaena sp.	1,941,000	36,000	485,000
Cylindrospermopsis cuspis Komárek et Kling	-	13,248,000	-

*Species known as potentially toxic: microcystin LR, lipopolysaccharide (LPS), microcystin, anatoxin-a, anatoxin-a (S), saxitoxin, cylindrospermopsin, neusaxitoxin and neosaxitoxin (Bonilla, 2009).

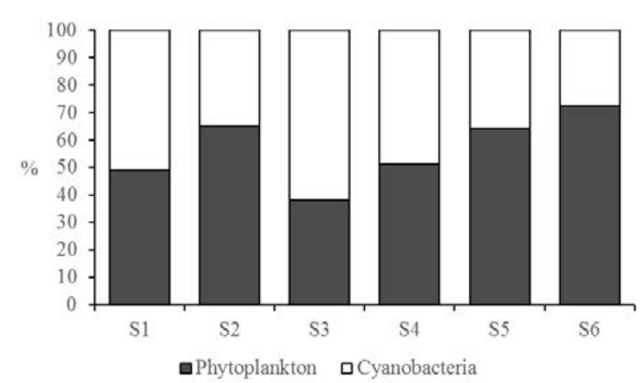

Figure 3. Contribution of cyanobacteria (%) to the total microalgae abundance at stations S1-S6 in the oyster banks of Términos Lagoon, Campeche, Mexico, in 2012-2013.

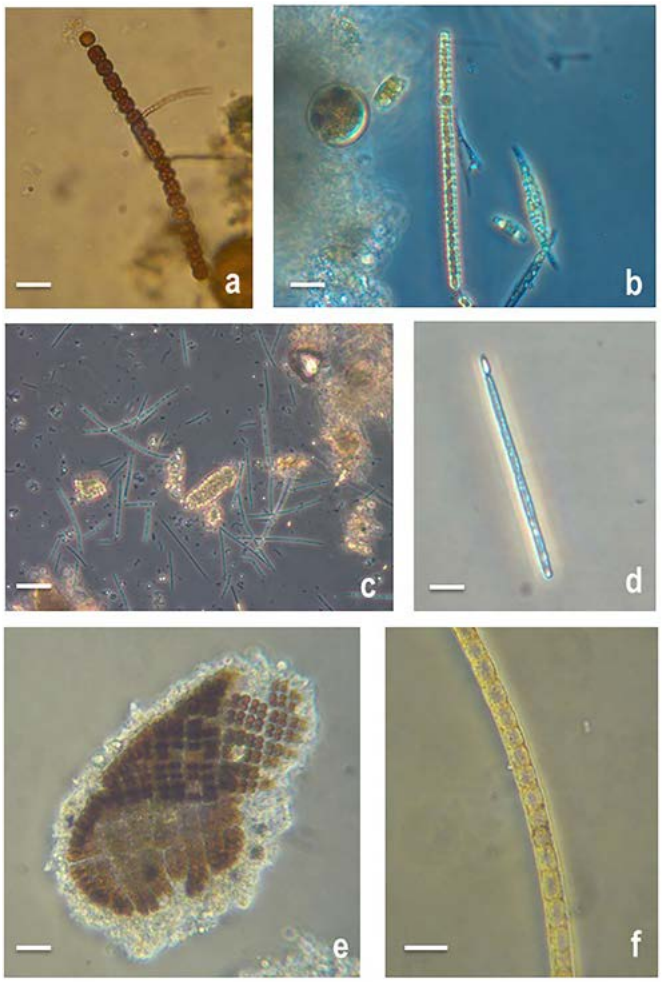

Figure 4. Micrographs of potentially harmful cyanobacteria in the oyster banks of Términos Lagoon, Campeche, Mexico: a, b) *Anabaena* spp.; c, d) *Cylindrospermopsis cuspis*; e) *Merismopedia* sp.; g) *Anabaena* sp. Scale bar = 10 µm

DISCUSSION

The water temperature variation during the study period is in accordance with the values reported by Robadue *et al.* (2004) and Yáñez-Arancibia and Day (2005) for Términos Lagoon during at least 50 years, and, in general, it is characteristic of a subtropical marine environment. The salinity range, which varied seasonally, was highest during the rainy season. According to Ramos-Miranda *et al*. (2006), this reflects the seasonal change in insolation, which results in greater evaporation and hence in a higher concentration of salts in the dry months. During the rainy season, the high freshwater input from rainfall and/or water discharges inundates the entire lagoon, inducing a marked salinity gradient (Hernández-Guevara *et al*., 2008). Only the Palizada River discharges approximately 75 % of the total fresh water that reaches the lagoon increasing by 20 % in the elevation of the water surface of the lagoon (Bach *et al*., 2005; Kuc-Castilla *et al*., 2015). This behavior was coinciding with that observed during the study period.

The values of pH and oxygen saturation suggested high activity of primary producers in the water column, resulting in changes in water quality (especially in pH and dissolved oxygen) as reported by Martínez-López *et al*. (2006), Hakspiel-Segura (2009) and Escobedo-Urías (2010) for coastal lagoons of the northwestern Mexican Pacific. The average values of nitrite, nitrate and ammonium recorded in different seasons were well below the values reported by Contreras-Espinoza *et al*. (1996), Herrera-Silveira *et al*. (2002) and Ramos-Miranda *et al*. (2006) for the southern Gulf of Mexico. However, the same pattern of high values in the rainy season, coupled with the proximity of river mouths with strong freshwater influence, can be seen (Yáñez-Arancibia and Day, 2005). High values of orthophosphate and silicate are associated with the period of increased river discharge determined by circulation and biogeochemical processes (Ramos-Miranda *et al*., 2006).

The tides, the discharge of fresh water and the local winds are linked to the period of high river discharge by rainfall enriching the waters at the entrance with new nutrients and causing high turbidity and low salinity (Yáñez-Arancibia and Day, 2005).

In the study area the presence of cyanobacteria is an anthropogenic pressure indicator. This feature is reinforced where salinity values are low and the relative proportion of cyanobacteria in biomass can be used as an indicator of eutrophication, as shown in Figure 3, where the highest percentages with respect to abundances were found at stations S1, S3 and S4, classified as brackish (salinity ≈14). This indicator has been reinforced because of the increase in terrestrial temperature, leading to an increase in cyanobacterial proliferations in continental ecosystems (Jöhnk *et al*., 2008), closely associated with eutrophication (Anderson *et al*., 2012). In general, cyanobacteria exhibit a cosmopolitan distribution, inhabiting hypersaline, marine and freshwater environments, and therefore have a wider range of habitats than phototrophic eukaryotes (Mur *et al*., 1999; Graham and Wilcox, 2000; Whitton and Potts, 2002; Ludeña-Hinojosa, 2007). Estuaries produce variations in salinity depending on the quantities of fresh water entering, and these changes give cyanobacteria an advantage over other competitors (Domingues *et al*., 2005, Lanzarot-Freudenthal, 2007).

In the study area cyanobacteria were present on the order of magnitude of 10^6 cells L^{-1}, mainly due to *Anabaena* sp. and *Cylindrospermopsis cuspis* with abundances of $1.9x10^6$ and $1.3x10^6$ cells L^{-1}, respectively. In October 2012, *C. cuspis* caused the temporary closure for 15 days of oyster (*Crassostrea virginica* Gmelin) harvesting (Poot-Delgado *et al*., 2016), decreed by Mexican governmental agencies based on the number of cells. Both species of cyanobacteria have been recorded in the fluvial-lagoon subsystems Pom-Atasta and Palizada del Este, adjacent to the study area, with abundances of $1.5x10^3$ and 0.5 to $8.2x10^3$ cells L^{-1}, respectively (Muciño-Márquez *et al.*, 2014; Muciño-Márquez *et al.*, 2015). *Cylindrospermopsis cuspis* was reported in the region of Los Tuxtlas in the southeastern part of the state of Veracruz (Komárek and Komárková-Legnerová, 2002; Komárek, 2003), with no apparent toxic episodes.

Cahuich-Sánchez (2016) performed a prospective study of lipophilic and hydrophilic marine toxins in *Crassostrea virginica* collected monthly during the period from 2012 to 2015 in the oyster field Playazo (station 6 in this study). He records the presence of diarrheic and other lipophilic toxins known as emerging toxins, due to the clinical signs observed in the mouse bioassay model, probably attributable to cyanobacteria. However, this must be confirmed by an analytical method (*e.g.*, HPLC-UV).

Cyanobacterial blooms have been recurring in various water bodies in the state of Veracruz, such as Catemaco Lake, where the mass proliferation of *Cylindrospermopsis raciborskii* (Wołosz.) Seenayya et Subba Raju was recorded, causing a high degree of bioaccumulation of the toxin cylindrospermopsin produced by this species throughout the food chain (Berry and Lind, 2010). In the coastal lagoon of Alvarado, *Dolichospermum flos-aquae* (Bréb. ex Bornet et Flahault) P. Wacklin, L. Hoffmann et J. Komárek recorded a bloom of $91x10^6$ cells L^{-1} in October 2013 (Aké-Castillo and Campos-Bautista, 2014).

Another species of cyanobacteria was *Merismopedia* sp. at the end of the rainy season (June-September) and dry season (February-May), with abundances of approximately 10^5 cells L^{-1}. According to Moreno-Ruíz (2000) and John *et al*. (2002), this is a genus indicative of water with moderate pollution. Some species of the genera of *Anabaena*, *Aphanizomenon* Morren ex Bornet et Flahault, *Lyngbya* C. Agardh ex Gomont, *Microcystis* Lemmerm., *Oscillatoria* Vaucher ex Gomont, *Phormidium* Kütz. ex Gomont and *Schizothrix* Kütz. ex Gomont produce compounds with an earthy and moldy odor (geosmin), representing a nuisance in the drinking water industry, growing areas and recreational lakes (Chorus and Bartram, 1999; Landsberg, 2002).

In recent decades cyanobacteria proliferations have increased markedly in continental freshwater and coastal marine ecosystems throughout the world, mainly due to increased eutrophication of water bodies (Chorus and Bartram, 1999; Acevedo-Torrano, 2012). However, despite their intrinsic importance, in Mexico the toxins produced by cyanobacteria have been reported during the last 15 years only from freshwater environments (Pica-Granados and Ramírez-Romero, 2012). Studies of cyanobacteria and their toxins in brackish and marine waters are therefore urgently needed.

CONCLUSIONS

Temperature and salinity were characterized by marked seasonal differences. The values and oxygen saturation suggested a predominance of photosynthetic activity in the windy season (October-February). At stations S1-S6 cyanobacteria were present on orders of magnitude of 10^6 cells L^{-1} in October 2012; however, they were notably absent in the windy season. *Anabaena* sp. and *Cylindrospermopsis cuspis* reached abundances of $1.9x10^6$ and $1.3x10^6$ L^{-1} cells, respectively. *Cylindrospermopsis cuspis* caused a 15-day temporary closure of the oyster *Crassostrea virginica* extraction activity in October 2012.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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