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Nyctemeral variation of the genus *Prorocentrum* (Dinophyceae) in the coastal lagoon Sontecomapan, Veracruz, Mexico

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ABSTRACT

The genus *Prorocentrum* Ehrenberg includes 62 marine species, of which 21 are considered harmful algal blooms (HABs), and approximately 10 species produce toxins. The objective of this study was to determine distribution and abundance of *Prorocentrum* species, and the influence of some environmental variables during a nyctemeral cycle at the mouth of the coastal lagoon Sontecomapan, Veracruz, Mexico, in October 27 and 28, 1999. Water samples were collected every two hours with a van Dorn bottle at the surface and in the middle of the water column to measure temperature, salinity, pH, dissolved oxygen, and composition and abundance of *Prorocentrum*. To understand the relationship between environmental variables and *Prorocentrum* abundance, we applied a linear regression analysis. The results showed a total of five species in the nyctemeral cycle: *Prorocentrum compressum*, *P. gracile*, *P. micans*, *P. mexicanum*, and *P. robustum*, of which the first four are blooms. *Prorocentrum gracile* was an abundant and frequent species that recorded a significant relationship with salinity (r^2 = 0.52); this variable was the environmental factor that determined the distribution and temporal abundance of *Prorocentrum*.

Keywords: Algal blooms, Phytoplankton, Salinity.

INTRODUCTION

The genus *Prorocentrum* Ehrenberg includes 62 marine species, both planktonic and benthic, which are oval-shaped single-celled organisms, round or pyriform in shape, and with a cell wall formed by two valves. The left valve is flat, while the right one is V-shaped. Each valve consists of a single simple plate; however, at the anterior end, the region where the flagella emerge has a set of plates (up to 9) that can be separated completely or linked together to form a mesh called "plate pores", where the two flagellar pores can be seen. One flagellum is longitudinally elongated and the second one is helical. The main morphological features used to identify species are: cell shape, size (cell width and length), position of apical spines, shape of the right theca, arrangement of pores on the surface of the theca, and number and arrangement of the periflagellar plates (Dodge 1982, Balech 1988, Steidinger and Tangen 1996, Faust and Gulledge 2002). Twenty one species of *Prorocentrum* are considered as bloom-forming species, such as *P. compressum*, *P. gracile*, *P. mexicanum*, *P. triestinium*, among others, of which about 10 species produce toxins, such as *Prorocentrum lima*, *P. minimum*, *P. rhathymum*, *P. shikokuense*, among others. These species pose health risks to aquatic organisms and humans and have a negative impact on local economy (Fott 1971, Steidinger and Tangen 1996, Cortés 1998, Faust *et al.* 1999). Due to the importance of *Prorocentrum*, this research shows the distribution and abundance of the genus *Prorocentrum* in a nyctemeral cycle at the mouth of the coastal lagoon in Sontecomapan, Veracruz.

MATERIAL AND METHODS

The Sontecomapan lagoon (Fig. 1) is located within the “Los Tuxtlas” Biosphere

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Reserve, in the region of the basin that forms Volcán de San Martin, part of the Tuxtla volcanic field, and the Sierra de Santa Martha, in the state of Veracruz, México. Its coordinates are 18° 30' and 18° 34' N, and 95° 00' and 95° 04' W (Contreras 1985). A phytoplankton sampling was performed at the mouth of the Sontecomapan lagoon (Fig. 1) along the nyctemeral cycle; samples were collected every two hours over a 36-hour period on 27 and 28 October 1999.

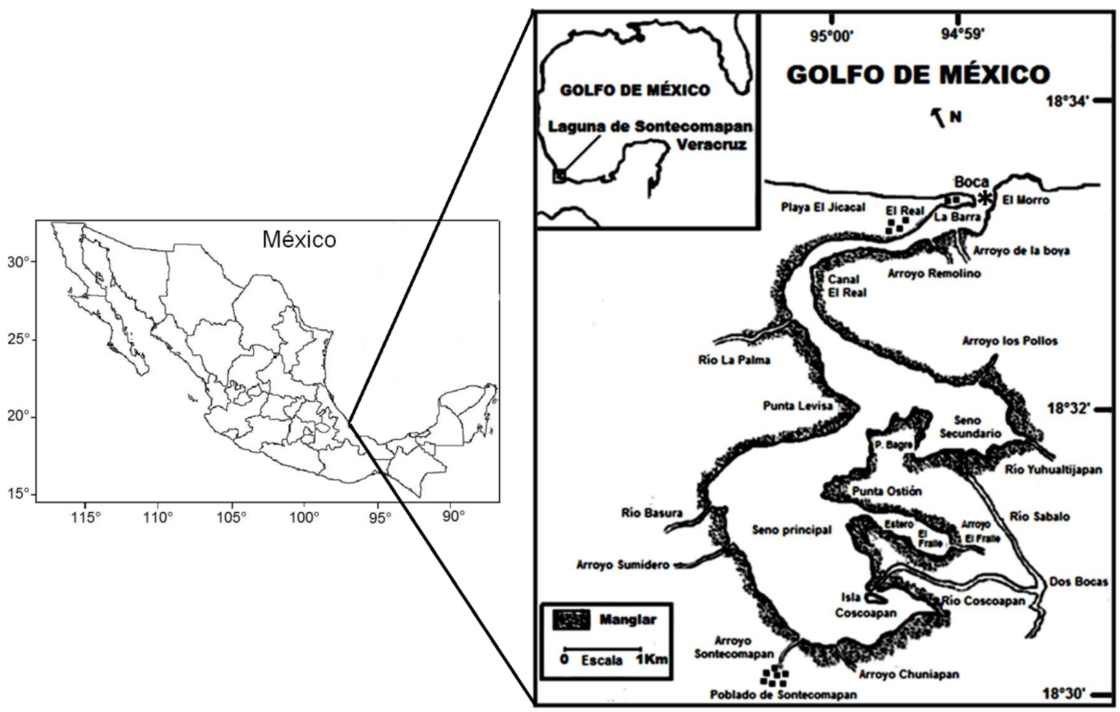

Fig. 1. Geographical localization of study area and sampling zone.

Phytoplankton samples were collected from 10-50 cm in depth with the use of a van Dorn bottle, providing a total of 36 samples, which were placed in bottles of 250 ml and fixed with acetate-lugol at a ratio of 100:1 with respect to the sample. In terms of environmental variables, temperature and pH were recorded using YSI-85 a thermo-haline-conductivity Model, and salinity with an RF20 ‰ refractometer.

For phytoplankton quantification, samples were homogenized, and 2 ml aliquots were taken and deposited in Utermöhl cameras (Hasle 1978) of the same volume for their observation in an inverted microscope (Olympus CK40). Phytoplankton taxa were identified by consulting the following works: Osorio (1943), Dodge (1982), Fukuyo *et al*. (1990), Licea *et al*. (1995), Faust *et al.* (1999), Faust and Gulledge (2002), among others.

Differences between environmental parameters and the abundances at the two depths in the nyctemeral cycle were defined by a variance analysis with Mann-Whitney's nonparametric tests using STATISTICA 99, Statsoft 1999 (Statistics notes 1994). Considering that the environmental factors did not show a significant variation ($p>0.05$), we combined the matrixes of 10 and 50 cm in depth to obtain the total behavior of *Prorocentrum* species in the nyctemeral cycle.

To analyze the influence of environmental variables on distribution and abundance of the genus *Prorocentrum* in nyctemeral cycle, we performed a linear regression analysis, using STATISTICA 99 software. This statistical technique is generally used to show the relationship between environmental variables and species to define the line that best fits the point cloud (Bland and Altman 1996).

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RESULTS AND DISCUSSION

Environmental variables, such as temperature and pH, showed no significant change ($p > 0.05$) throughout the nyctemeral cycle: temperature presented oscillations between 22.8 ºC and 26.2 ºC (Fig. 2a); pH was next to neutral, ranging from 7.86 (Fig. 2b) and varying from 7.86 at return; salinity showed significant changes ($p < 0.05$) with a steep variation with maximum values of 30.5 ups between 17:00 to 21:00 hours, and decreasing from 03:00 to 13:00 hours with values between 4 and 6 ups; this behavior was repeated on both days (Fig. 2c). The recorded salinity changes were similar to those reported for this water body for the rainy season in other years by Guerra and Lara (1995) and Figueroa and Weiss (1999), and for other coastal lagoons by Lacerda *et al*. (2004), Ferreira *et al*.(2005), and Noriega *et al*. (2009).

Five species of the genus *Prorocentrum* were recorded in the nyctemeral cycle: *P. compressum*, *P. gracile*, *P. micans*, *P. mexicanum*, and *P. robustum* (Fig. 3), of which the first four are considered as HAB-forming species. Abundance of these *Prorocentrum* species between the two sampled depths (10 and 50 cm) showed no significant changes ($p > 0.05$) during the nyctemeral cycle; thus both values were added to get the total sample abundance during the cycle.

This study shows the morphological characteristics of the species reported and their abundance and relationship to environmental variables in the nyctemeral cycle:

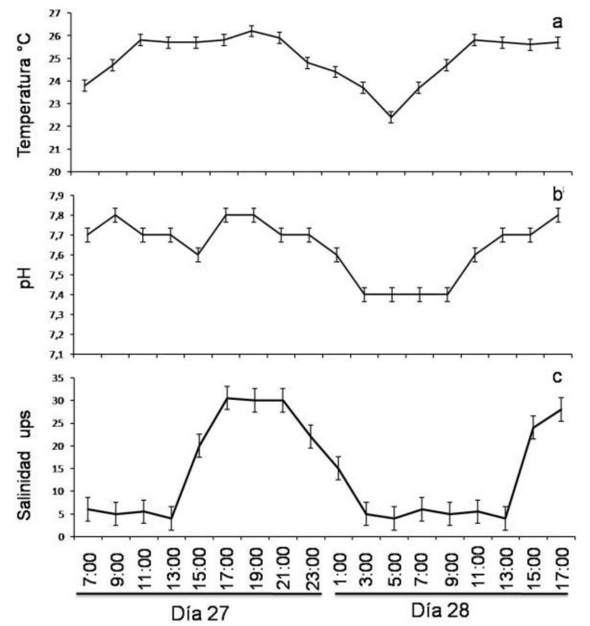

Fig. 2. Environmental variables recorded at the mouth of the Sontecomapan lagoon, Veracruz, during the nyctemeral cycle in October 27 and 28, 1999. a) Temperature, b) pH and c) Salinity.

Prorocentrum compressum
(Bailey 1850) Abé ex Dodge (1975).Fig. 3. (A and B).

Synonym: *Pyxidicula compressa* Baiyle 1950, Figs. 13 y 14. *Euxuviaella compressa* Ostenfeld 1899, p. 59; 1903, p. 579. *E. lenticulata* Matzenauer, *vide* Dodge 1982, Fig. 2I. *Prorocentrum bidens* Schiller 1928, Fig. 21. *P. lebourae* Schiller 1928, Figs. 6a-c.

Description: The elliptical-shaped cell is more or less wide in valvar view, little compressed in lateral view, and anterior view has a small depression, no spine. Cell wall is moderately thick. It shows a small pore, sometimes projecting a couple of small denticulations that can be asymmetric. The poroids are small, superficial, and dense.

Size: Length: 35-40 µm and width: 31-38 µm; these measurements are within the range reported by Licea *et al*. (1995), length and width: 21-47 µm.

Toxicity: While this species does not produce toxins, it is considered to form HABs (Cortés 1998, Barreda 2007); due to their proliferation, they can cause asphyxia in aquatic organisms such as fish and oysters. A bloom of *P. compressum* was reported in Campeche in 2007 with an abundance of 0.32 to 185 $\times$ 10^3 cél·L^{-1} (Barreda 2007). In this study *P. compressum* was a rare, infrequent, and abundant species (0.5 $\times$ 10^3 cél·L^{-1}) on day 27 at 21: 00 hours, and at 03:00, 09:00 and 17:00 hours on day 28 (Fig. 4) with a recorded temperature of 23.7 to 25.9 °C, salinity of 5 to 30 ups, and pH of 7.3 to 7.8; it did not show a significant relationship (r^2= 0.01) with environmental variables.

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Distribution in the Gulf of Mexico: The species has been reported in Nuevo Campechito, Campeche (Cruz 1968, Licea and Santoyo 1991, Barreda 2007), Bahía Apalachee, Florida (Menzel 1971), Gulf of Mexico (Taylor 1990), Tabasco (Licea *et al*. 2004), Sontecomapan, Tamiahua, The National Park Sistema Arrecifal, in Veracruz (Figueroa and Weiss 1999, Weiss 2001), and in the coral reefs of the Riviera Maya, Yucatán Peninsula (Licea *et al*. 2004, De la Lanza 2006, Okolodkov *et al*. 2011).

Prorocentrum gracile
Shütt 1895. Fig. 3. (C)

Synonym: *Prorocentrum macrurus* Athanassopoulos 1931, Fig. 15. *P. hentschelii* Schiller 1933, Figs. 38 a-b. *P. sigmoides* Bohm 1933, Fig. 1

Description: The cell is pyriform and pointed; more or less rounded forward and acuminate backward; sometimes slightly truncated at the posterior pole. The anterior tooth is well developed with a narrow spiniform axis and a narrow membrane. Poroids have a very fine and dense arrangement. Large pores in the valve margins form transverse oblique rows.

P. gracile could be confused with *P. micans*, but it is distinguished by the cell shape, which is oval; it has a small difference in the pore

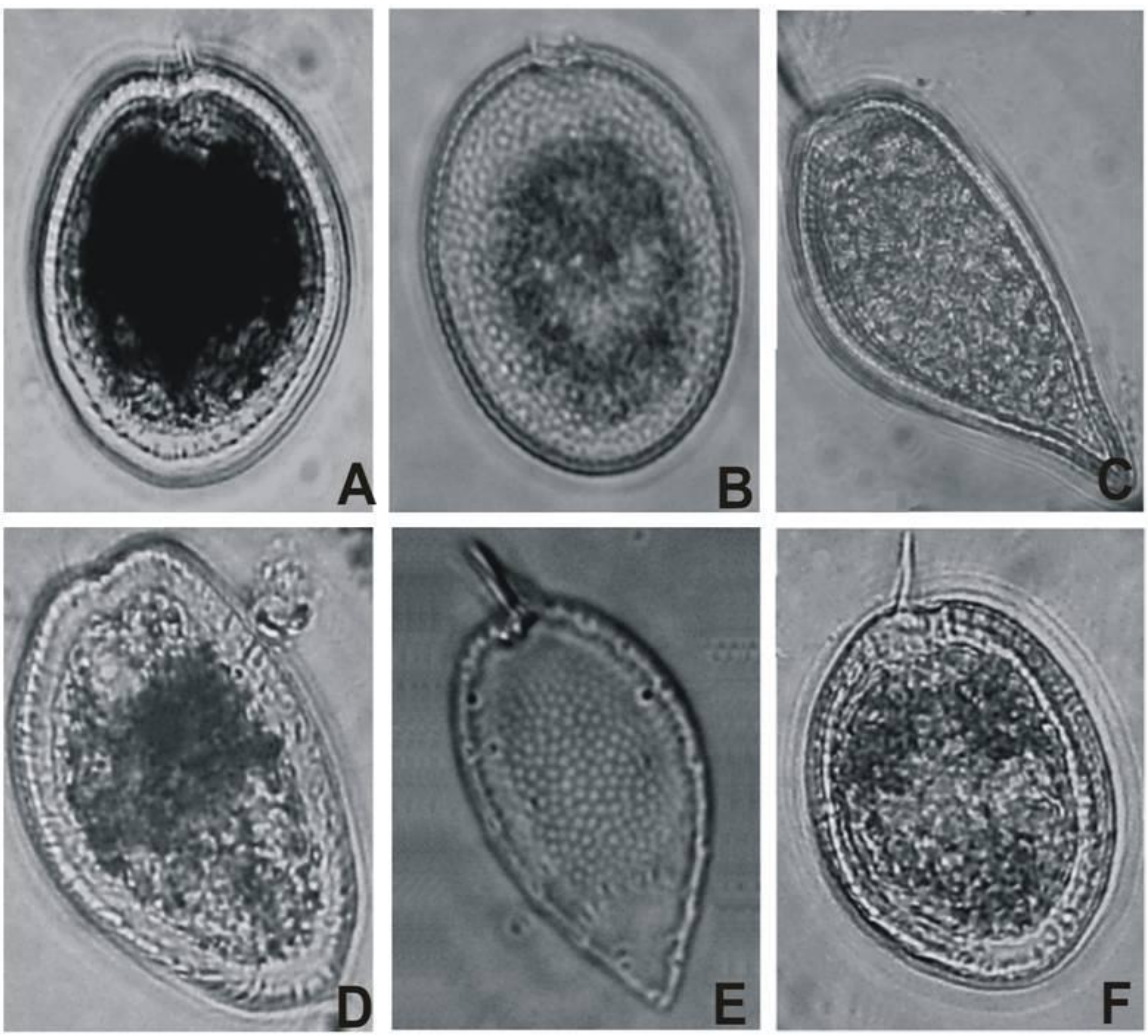

Fig. 3. A and B) *Prorocentrum compressum*, C) *Prorocentrum gracile,* D) *Prorocentrum mexicanum*, E) *Prorocentrum micans,* F) *Prorocentrum robustum.*

arrangement, which consists of large pores located mainly in the apical and antapical regions; *P. gracile* has a bigger spine with a fin and a posterior extremity almost always pointed (Licea *et al*. 1995, Faust and Gulledge 2002, Cohen *et al*., 2006). It should be noted that Cohen *et al.* (2006) mention *P. sigmoides* is synonymous with *P. gracile*. We agree with the authors, because these species are morphologically similar; the cells are elongated, spiky, and with large pores.

Size: Length: 37-61 µm, width: 15-37 µm, along the spine: 7-10.5 µm; these measurements are within the range reported by Dodge (1982), Licea *et al*. (1995), Hernández *et al*. (2000) and Cohen *et al.* (2006), and they are: 40-60 µm in length and 18-25 µm in width, 8-11 µm along the spine.

Toxicity: *P.gracile* does not produce toxins; it is considered as HAB-forming species (Cortés 1998, Terán *et al.* 2006). Alvial and Garcia (1986) reported a bloom of *P. gracile* in Bahia Iquique in Chile with an abundance of 20.5×10^3 cél·L^{-1} and a duration of 20 days, giving a water color from brown to reddish brown without affecting aquatic organisms. However, *P. gracile* was reported (23.3×10^3 cél·L^{-1}) associated with a toxic bloom of *Prorocentrum* sp. at the Embarcadero Cutter in Tabasco in April 2007, causing fish mortality (Secretaria de Salud, Tabasco 2007). *P. gracile* was abundant and frequent in the nyctemeral cycle on day 27 from 17:00 to 23:00 hours; an abundance of 0.7 to 24.5×10^3 cél·L^{-1} was reported (Fig. 4) with temperature ranging from 24.4 to 26.2°C; salinity from 22 to 30 ups with pH of 7.8; a significant relationship was recorded r^2= 0.52 with salinity (Fig. 5). On day 27 at 21:00 hours a peak (24.5×10^3 cél·L^{-1}) in abundance was reported when salinity was 30 ups; on the other hand, when salinity was lower (20 ups), abundance decreased to 0.7 cél·L^{-1}. However, on day 27 between 09:00 and 13:00 hours when salinity was between 4 or 5 ups, no organisms of this species were reported. We infer that *P. gracile* has a preference for salinities between 30 to 24 ups. Leal *et al.* (2001) reported *P. gracile* with low abundances in Cuban waters with a high salinity of 35 ups. If the conditions are optimal for salinity, temperature, nutrients, and light, among other factors, species could produce a harmful algal blooming.

Distribution in the Gulf of Mexico: The species has been reported in Bahía Campeche, the Grijalva-Usumacinta lagoon system, Laguna de Términos, Campeche (Cruz 1968, Licea and Santoyo 1991, Barreda 2007), Tampa Bay, Florida (Steidinger *et al.* 1967, Steidinger and Gardiner 1982), Gulf of Mexico (Taylor 1990, Avendaño and Sotomayor 1982), Embarcadero Cutter, Laguna del Carmen-Pajonal-Machona, Tabasco (Terán *et al.* 2006, Secretaria de Salud Tabasco 2007), Sontecomapan, Tamiahua, and The National Park Sistema Arrecifal in Veracruz (Figueroa and Weiss 1999, Weiss 2001, Aké and Vázquez 2008, Okolodkov *et al.* 2011), and the coral reefs of the Riviera Maya, Yucatán Peninsula (Licea et al. 2004, De la Lanza 2006).

Prorocentrum mexicanum
Osorio, 1942. Fig. 3. (D)

Synonym: *Prorocentrum maximum* Schiller 1937.

Description: The cells in this species are slightly compressed and oval shaped in valve position with the dorsal curvature much more pronounced than the ventral one. The dorsal edge is prolonged in outgoing rounded anterior form. Hernández *et al.* (2000) mention that the apical spine is short, fine, and slightly curved, provided with a delicate wing, and visible in sagittal position. In the sagittal view body contour is ellipsoidal with flat apical region; the right and left spines have a similar development between themselves.

*Prorocentrum mexicanu*m resembles *P. caribbaeum*; however, the latter is larger and heart-shaped (Faust 1993). Furthermore *P. caribbaeum* has more *valve* pores (145-203) than *P. mexicanum* (100) (Faust 1993). This species has also been confused with *rhathymum*, but its cells are ovoid to oblong, and no pyrenoids are presents. It has a simple apical spine. The thecal thrichocyst surface is smooth, adorned with numerous pores that in shallow depressions radiate from the central perpendicular region to the cell periphery. It lacks marginal pores. The number of trichocyst pores

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differs from the valve with 70 at the right side, including 6 or 7 that surround the periflagellar zone; the left side has about 90. It also shows a simple apical spine (Cortés and Sierra 2003, Aligizaki *et al*. 2009).

Size: Its average dimensions were: length: 28-30 µm, width: 22-29 µm; these measurements were found to be within the variation range reported by Osorio, (1942), Hernández *et al*. (2000) and Faust and Gulledge (2002): length: 20-39 µm, width: 12-29 µm.

Toxicity: *Prorocentrum mexicanum* was thought to produce a fast-acting toxin (Steidinger 1982, Carlson 1984, Faust 1995) and hemolytic toxins that are non-toxic to mice (Nakajima *et al.* 1981). *P. mexicanum* has been reported to be associated with other HAB-forming species in Cabo Catoche, Yucatán, reaching densities of 2,500 $\times$ 10^3 cél·L^{-1} that caused death to marine organisms and socio-economic damages in the region worth sixty million pesos (Herrera 2003). In the nyctemeral cycle *P. mexicanum* was a rare species, reporting an abundance of 0.5 $\times$ 10^3 cél·L^{-1} on days 27 and 28 at 15:00 hours (Fig. 4) with a temperature of 25.6 °C, salinity of 16 - 24 ups, and pH of 7.7 - 7.8; it showed no significant relationship (r^2= 0.01) with environmental variables.

Distribution in the Gulf of Mexico: The species was registered in The National Park System Arrecifal in Veracruz (Okolodkov *et al*. 2011), and in coral reefs of the Riviera Maya and Cabo Catoche in the Yucatán Peninsula (Herrera 2003, Licea *et al*. 2004, Álvarez and Herrera 2006, De la Lanza 2006).

Prorocentrum micans
Ehrenberg, 1833.Fig. 3. (E)

Synonym: *Prorocentrum schilleri* Böhm Schiller 1933, Figs. 40 a-e. *P. levantinoides* Bursa (1959), Figs. 125-127

Description: Its oval cells are asymmetric and somewhat angular, spiniform or heart shaped. Cells are flattened in side view; well-developed teeth with spiniform axis that argues a membrane; sculpture of very fine poroids with dense and larger pores that accumulate in the margins forming oblique transverse rows.

This species can be confused with *P. gracile* (Balech 1988, Cohen *et al*. 2006); it differentiates by being a less rounded cell with a bigger spine, a fin, and a posterior extremity that is almost always pointed.

Size: Length: 41-50 µm, width: 26-33 µm, along the spine: 7-8 µm; these measurements were found to be within the variation range reported by Osorio (1942), Balech (1988), Hernández *et al*. (2000), and Cohen *et al*. (2006): length: 15-80 µm, width: 15-50 µm, along the spine: 7-12 µm.

Toxicity: This species is considered as

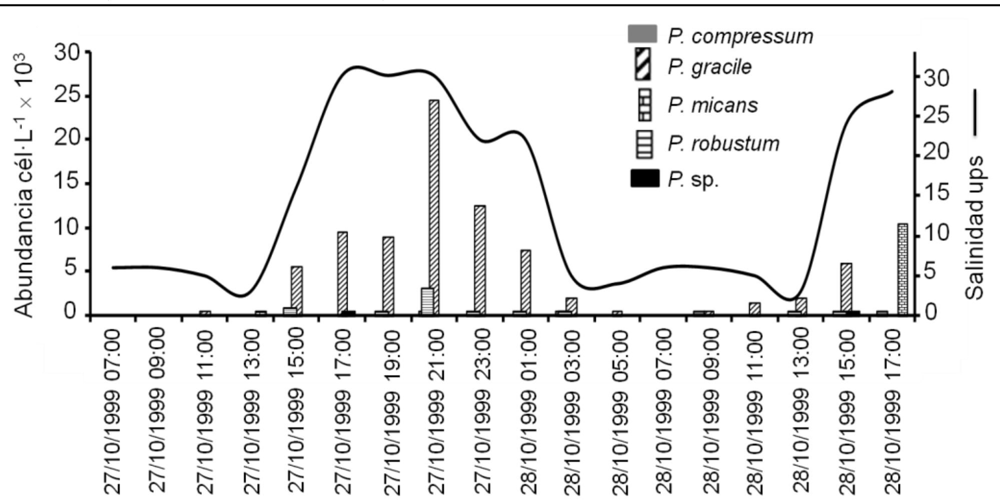

Fig. 4. Distribution of *Prorocentrum* species and salinity in the mouth of the Sontecomapan lagoon, Veracruz, during the nyctemeral cycle in October 27 and 28, 1999.

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HAB-forming (Cortés 1998); Lassus and Berthome (1988) mention *P. micans* produces a paralytic toxin (PSP) in crustaceans; however, it has not been verified if it produces toxins. It has been reported to cause anoxia in water bodies (Rodríguez 2006). In Chile (1993) a bloom of *P. micans* was reported to have caused mortality in salmon farms and economic losses (Avaria *et al*. 1999). In the Mexican Pacific Ocean, *P. micans* has been reported to form HABs with other phytoplankton species, and fish mortality by asphyxiation has also been reported (Cortés 1997, 1998, Gárate *et al*. 1990, 2007). In the nyctemeral cycle, *P. micans* was little frequent although abundant with the highest abundance of 10.5×10^3 cél·L^{-1} on day 28 at 17:00 hours (Fig. 4), and with a temperature of 25.7 °C, salinity of 28 ups, and pH of 7.8; it showed no significant relationship (r^2= 0.01) with environmental variables.

Distribution in the Gulf of Mexico: The species has been reported in Bahía Campeche, Grijalva-Usumacinta lagoon system, Laguna de Términos, Campeche (Cruz 1968, Licea and Santoyo 1991, Barreda 2007), Tampa Bay, Florida (Lackey and Hynes 1955, Dragovich and Kelly 1964, Steidinger *et al.* 1966, Steidinger *et al*. 1967, Steidinger and Gardiner 1982), Gulf of Mexico (Balech 1967, Taylor 1990), Laguna del Carmen-Pajonal-Machona and Mecoacán, Tabasco (Terán *et al*. 2006), Sontecomapan, Tamiahua, and The National Park Sistema Arrecifal, Veracruz (Figueroa and Weiss 1999, Weiss 2001, Aké and Vázquez 2008, Okolodkov *et al*. 2011), and the coral reefs of the Riviera Maya in Celestum, Dzilam, Sisal, Progreso in Yucatán (Licea *et al*. 2004, Álvarez and Herrera 2006, De la Lanza 2006).

Prorocentrum robustum
Osorio 1942. Fig. 2. (F)

Synonym: *Prorocentrum scutellum* Schröder 1900, Chart 1, Fig. 12. *Prorocentrum sphaeroideum* Schill 1928, Chart 61, Fig. 25.

Description: Cells are oval to circular, and both margins are round; wide flagellar slit located on the right valve; an embedded pointed thorn can be found behind the left valve with the base slightly widened and with a well-developed wing. This spine shows a clear inclination toward the dorsal region.

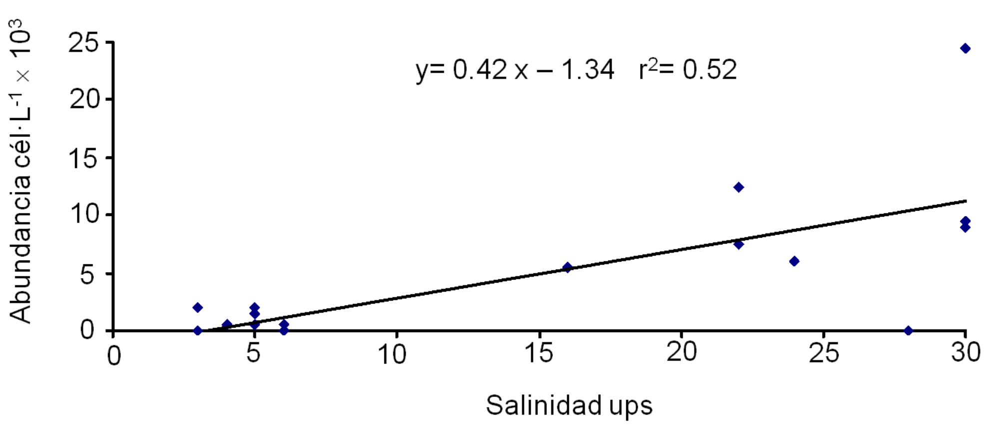

Fig. 5. Linear regression of salinity with the *Prorocentrum gracile* in the nyctemeral cycle.

P. robustum can be confused with *P. scutellum*, but it can be differentiated by its heart-shaped valve; it has an apex with a small fin with a notch and rounded or pointed posterior part. *P.*

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robustum form is oval to circular (Osorio 1942, Hernández *et al*. 2000).

Size: Length: 31-40.4 µm, width: 26-33 µm, along the spine: 5-6 µm; these measurements were found to be within the variation range reported by Osorio (1942) and Hernández *et al*. (2000), which are: length: 32-43 µm, width: 27-30.5 µm, and 5 µm along the spine.

Toxicity: *P. robustum* is not considered as HAB-forming species. In the nyctemeral cycle it was abundant with little frequency of appearance; its highest abundance of 3.5×10^3 cél·L^{-1} was reported on day 27 at 21:00 hours (Fig. 4) with a temperature of 25.9 °C, a salinity of 30, and pH of 7.8; it showed no significant relationship (r^2= 0.01) with environmental variables. *P. robustum* has been reported as a rare species in the Mexican Pacific Ocean (Osorio 1942, Hernández *et al*. 2000).

Distribution in the Gulf of Mexico: It is the first time *P. robustum* has been reported in the study area and for the Gulf of Mexico. *P. scutellum*, which is confused with *P. robustum*, has been reported in Banco, Campeche (Cruz 1968), Tampa Bay, Florida (Steidinger *et al.* 1966, 1967), and in the Yucatán Peninsula (Pérez de los Reyes *et al.* 1996). This study considered *P. robustum* a different species by the characteristics mentioned above.

CONCLUSIONS

In the nyctemeral cycle the five registered *Prorocentrum* species in the mouth of the coastal lagoon Sontecomapan, Veracruz were: *P. compressum*, *P. gracile*, *P. micans*, *P. mexicanum*, and *P. robustum*. They were regulated by salinity (ranging from 4 to 30.5 ups), seawater and continental water exchange, which favored changes in their distribution and temporal abundance, making them clearly marine stock. Different phytoplankton species with specific requirements respond differently to changing environmental conditions (Smayda 1980, Reynolds 1987, Verdugo 2004 and Ferreira *et al.* 2005).

In the case of *P. gracile*, it was abundant in the nyctemeral cycle when salinity was high and decreased when salinity concentrations were low; it has generally been reported that these species are sensitive to sudden changes in salinity and are resistant to osmotic shock cells (Madigan *et al.* 2004). Ferreira *et al.* (2005) mention that a decrease in continental water supply in coastal lagoons would increase salinity, and thus modify the structure and composition of the phytoplankton community. As a result, it could lead to favor opportunistic marine species to produce algal blooms, as could be *P. compressum*, *P. gracile*, *P. micans*, and *P. mexicanum* as HAB-forming species. They have also been reported as dominant species and associated with HABs in the Mexican Pacific (Cortés and Alonso 1997, Alonso and Ochoa 2004, Gárate *et al*. 2006). In Tabasco (at the Embarcadero Cutter), fish mortality related with a bloom of *P. gracile* has been reported (LESP 2005). In this paper *P. gracile* failed to exceed 1×10^9 cél L^{-1}. Gárate *et al.* (2007) reported a bloom of *P. micans* in Bahia Magdalena, Mexico in a diurnal cycle during flood tide, suggesting that the blooms of the genus *Prorocentrum* occur mainly in shallow stations and with a narrow temperature range. In this study, the temperature remained constant during the nyctemeral cycle, which is why the species reported in this study had no optimal temperature ranges to form a bloom. However, other factors like nutrient concentration should be assessed to gather more information, and thus be able to better explain its behavior.

It is important, therefore, to know the behavior of *Prorocentrum* species in nyctemeral cycles, because it would make possible to see which the hours of more affectation are and to monitor them.

Moreover, it was evident in the study area that *Prorocentrum* species are harmful in low abundances, given the characteristics of changes in physical and chemical factors in the system and the prompt response of species to these changes. Due to their short life span, it is possible that the necessary conditions can be given for these species to form HABs, mainly by the increase in the eutrophication processes of coastal ecosystems, thus affecting health and economy of the local population.

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