

# Seasonal changes of benthic and epiphytic dinoflagellates in the Veracruz reef zone, Gulf of Mexico

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**ABSTRACT:** Benthic/epiphytic dinoflagellates were monitored every 2 wk from May to December 2005 in the Veracruz reef zone, Gulf of Mexico. This assemblage was characterized by low species diversity (at least 17 species from 11 genera) and high abundance: *Amphidinium* cf. *carterae* (41 172 cells g<sup>-1</sup> substrate wet weight), *Prorocentrum lima* (29 756 cells g<sup>-1</sup>), *Coolia monotis* (2724 cells g<sup>-1</sup>), *Ostreopsis heptagona* (1202 cells g<sup>-1</sup>); *P. lima* was the dominant species. Clear seasonal population dynamics occurred, with the highest abundance in May–June, and the dynamics differed at 2 neighbouring sampling sites. In August to December dinoflagellate abundance decreased considerably. Most of the epiphytic dinoflagellates did not show any significant preference for a macrophyte substrate (23 macroalgal and 2 seagrass species) or for any species or major algal group, although the seagrass *Thalassia testudinum* appeared to be the most abundant and permanent host species. The highest abundance of *P. lima* occurred on the chlorophyte *Ulva fasciata* (30 879 cells g<sup>-1</sup>) and *T. testudinum* (31 467). Dinoflagellates were usually scarce on dead coral (*Acropora* sp. and *Millepora alcicornis*) fragments (0 to 5039 cells g<sup>-1</sup>) and surface bottom sediments (2 to 84), and were slightly more abundant on living *Diploria strigosa* (329 to 1830) and more numerous in a fringing reef zone compared to a reef lagoon near an offshore island. Dinoflagellate abundance was not correlated with any measured physical or chemical parameter (temperature, salinity, pH, dissolved oxygen, nitrates, nitrites, ammonium, phosphates, precipitation or wind speed and direction). There is a high potential for outbreaks of ciguatera in the studied area.

**KEY WORDS:** Dinophyceae · Benthic · Epiphytic · Seasonal changes · Coral reefs · Ciguatera · Gulf of Mexico · Mexico

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## INTRODUCTION

Benthic and epiphytic dinoflagellates (Dinophyceae) in the southern Gulf of Mexico have been outside the scope of studies or monitoring programs. Two reports are known from the Mexican Caribbean (Almazán-

Becerril 2000, Hernández-Becerril & Almazán-Becerril 2004). Ciguatera fish poisoning in the coastal waters of the state of Baja California Sur in the NW Mexican Pacific and the states of Quintana Roo (Mexican Caribbean) and Yucatan (southeastern Gulf of Mexico) was reported 291 times over 16 yr (Parrilla-Cerrillo et

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al. 1993, Núñez-Vázquez et al. 2000). Some benthic species such as *Coolia monotis*, *Gambierdiscus toxicus*, *Prorocentrum lima*, *P. belizeaum* M. A. Faust, *Sinophysis canaliculata* and *Ostreopsis* spp. are known from the Mexican Pacific (for detailed information, see Okolodkov & Gárate-Lizárraga 2006). In Cuban waters, an extensive multi-year epidemiological study of ciguatera was performed in Ciego de Ávila province (Suárez-Hernández et al. 2001). In the Caribbean basin in general, sampling has been mainly in the eastern part of the region (Besada et al. 1982, Carlson & Tindall 1985, Ballantine et al. 1988, Lobel et al. 1988, Tindall et al. 1990, Heil et al. 1993, Bourdeaux et al. 1995, Tosteson et al. 1998).

In the tropical zone, especially in coral reef environments, benthic and epiphytic dinoflagellates have been found to be associated with seagrasses, brown and red algae, dead corals and sediments (Taylor 1979, Fukuyo 1981, Carlson & Tindall 1985, Ballantine et al. 1988, Bagnis et al. 1990, Faust 1990, 1995a,b, Tindall et al. 1990, Heil et al. 1993, 1998, Bourdeaux et al. 1995, Morton & Faust 1997, Tosteson et al. 1998, Turquet et al. 1998, Pearce et al. 2001, Delgado et al. 2002, 2005). Sand-dwelling dinoflagellates are known from tropical and subtropical areas (e.g. Horiguchi & Chihara 1987, Faust 1990, Faust & Balech 1993, Horiguchi et al. 2000, Ten-Hage et al. 2001, Horiguchi & Sukigara 2005, Tamura et al. 2005, Murray et al. 2006a,b) but are better known from temperate regions (e.g. Herdman 1921, Balech 1956, Baillie 1971, Larsen 1985, Dodge & Lewis 1986, Faust 1995b, Horiguchi 1995, Hoppenrath 2000a,b,c,d, Hoppenrath et al. 2003, 2004).

Seasonal/annual and interannual dynamics of benthic and epiphytic dinoflagellate assemblages in a reef zone have been studied in the Virgin Islands (Carlson & Tindall 1985), in French Polynesia (Bagnis et al. 1985), Puerto Rico (Ballantine et al. 1988), on Singapore reefs (Holmes et al. 1998), on Réunion Island (Turquet et al. 1998), and in the NW Mediterranean (Vila et al. 2001).

Species richness in benthic and epiphytic dinoflagellate assemblages is usually lower than that in planktonic ones. In the most comprehensive taxonomic-ecological study to date, 39 species from 12 genera, including unidentified taxa, were found (Turquet et al. 1998). Epiphytic dinoflagellates may show distinct substrate preferences (Shimizu et al. 1982, Carlson & Tindall 1985, Lobel et al. 1988, Heil et al. 1998).

In the present study, our objectives were: (1) to determine the species composition of benthic and epiphytic dinoflagellates, with an emphasis on numerically dominant species, inhabiting the port of Veracruz area, Mexico; (2) to determine the range of habitats for benthic and epiphytic dinoflagellates; (3) to relate the presence and abundance of dinoflagellates

with various macrophyte substrates; (4) to evaluate seasonal changes of benthic and epiphytic dinoflagellates; (5) to determine the physical-chemical variables and their relation to the abundance of dinoflagellates; and (6) to evaluate the risk of benthic and epiphytic dinoflagellates to the ecosystem and the human community.

## MATERIALS AND METHODS

Sampling of benthic and epiphytic dinoflagellates was a part of a monitoring program of harmful algal blooms launched on 10 May 2005 by the Aquarium of Veracruz and the Center of Ecology and Fisheries of the University of Veracruz (CEF-UV). Dinoflagellates were taken every 2 wk from 10 May through 13 December 2005, between 11:30 and 14:00 h, at 2 permanent stations (16 surveys in total) in the National Park Sistema Arrecifal Veracruzano (NPSAV), state of Veracruz, Mexico, located in the coastal zone of the southern Gulf of Mexico: Stn 3, a reef lagoon always open to the sea, ca. 150 to 200 m from Isla de Sacrificios (also classified as shallow, slightly subtidal, permanently submerged reef flats extending into a lagoon, thus belonging to the type II system according to Tindall & Morton 1998) was at 19° 10' 32.7" N, 96° 05' 40.9" W; and Stn 5, a semi-protected shallow area with a sandy bottom covered with coral rubble, the remnants of a fringing reef without live corals, ca. 50 m from the coastline, was at 19° 10' 37.4" N, 96° 07' 10.9" W (Fig. 1). It is difficult to ascribe our sampling sites to a particular type of system due to the lack of geomorphological studies. However, both sites clearly belong to the type I or II system because of their nearly constant, moderate to strong water flow, depth range (usually more than 0.5 m, sometimes down to 0.3 m at low tide), and oligotrophic conditions (Tindall & Morton 1998). In accordance with Schuhmacher's (1978) classification of principal reef zones, we consider our sampling sites as reef moats. Samples were collected by snorkeling. At permanent stations the depth was 0.5 to 1.2 m. Seagrass (50 to 170 g) was sampled manually from an area of less than 10 m<sup>2</sup> (usually from <1 m<sup>2</sup>); only blades were taken and were placed immediately into 500 ml plastic bottles. The seasonally dominant species of macroalgae (20 to 100 g, depending on their availability) were detached gently from the substrate manually or with a knife and sorted according to species; these were placed into plastic bottles underwater. Dead coral fragments (predominantly those of *Millepora alcicornis* L. and *Acropora* sp.; 300 to 1000 g) covering the sea bottom were collected manually and placed into plastic bottles of 500 to 1500 ml capacity. Sediments (200 to 700 g) were col-

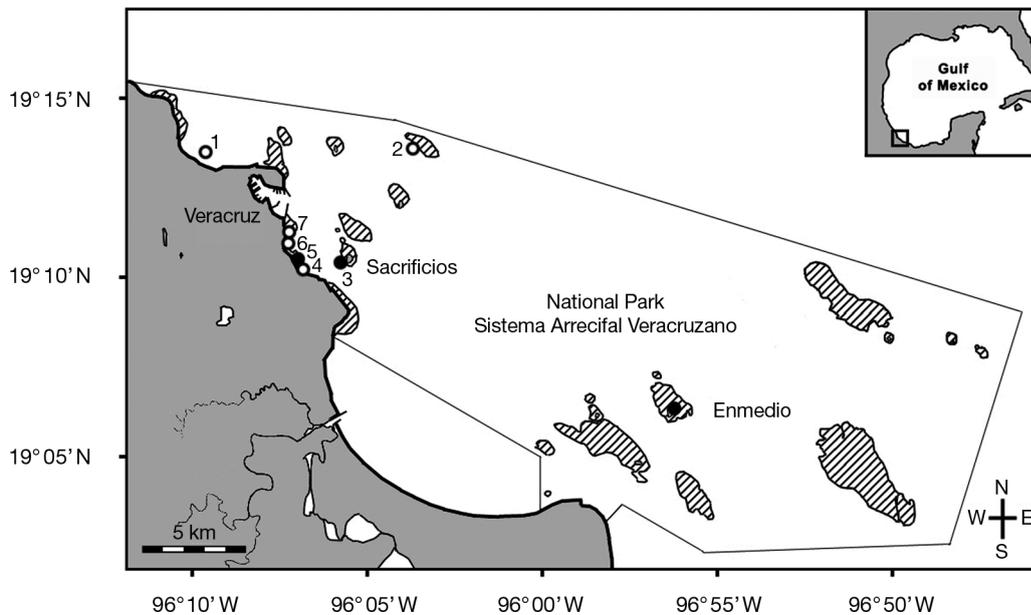


Fig. 1. Sampling sites in the National Park Sistema Arrecifal Veracruzano. ●: locations where planktonic, benthic and epiphytic dinoflagellates were sampled. ○: phytoplankton sampling sites. Hatched areas are coral reefs; the line indicates the park boundary

lected using a plastic bottle directly from the sea bottom. In 2 cases, sediments associated with turf macroalgae growing on top of large colonies of *Diploria strigosa* (Dana) were collected with a knife and carefully placed into a plastic bottle. All the samples were taken together with the surrounding seawater and transported to the boat, where 37% formalin was added to the bottles to a final concentration of 5%. On some occasions, the macroalgae attached to buoys and drifting *Sargassum* spp. were sampled and treated similarly. The buoys were taken from the water into the boat, and the macroalgae attached to the plastic globe and the rope (down to about 5 m below the globe) were separated with a knife and transferred to a plastic bottle. Two buoys were sampled on 10 January and 23 March 2006 at Stn 2 (15 samples in total). Drifting *Sargassum* spp. were taken on 13 December 2005, and 10 and 21 February 2006 en route between Stns 1 and 2. In total, 221 samples were analyzed quantitatively: 104 samples of macroalgae, 38 of seagrasses, 36 of surface sediments, 23 of dead corals, 2 of sediment from live corals, 11 from buoys, and 7 of drifting macroalgae.

In the laboratory, each sample was thoroughly mixed to remove epiphytes. Each sample was placed into a 1 l beaker, and macrophytes were washed manually to better separate dinoflagellate cells. Macrophytes were squeezed by hand to avoid excessive liquid. When *Thalassia testudinum* blades contained macroalgal epiphytes, they were scrubbed with a plastic or steel knife and mixed. Macrophytes were then weighed

with an Ohaus ES6R balance. The volume of liquid was measured in a glass or plastic graduated cylinder. The procedure for dead corals or sediments was simpler: they were separated from liquid and weighed, and the volume of liquid was measured. When a macrophyte sample contained sediment (e.g. *Caulerpa* spp.), it was considered a part of the substrate for dinoflagellates; the weight of sediment was added to that of the macrophyte, and the total wet weight was calculated.

A 1 ml aliquot of the liquid containing benthic and epiphytic dinoflagellates was taken and transferred to a Sedgwick-Rafter counting chamber. For sediment samples, an aliquot was taken 30 to 40 s after shaking to allow heavy inorganic particles to settle. One or 2 drops of 0.2% Trypan Blue solution were added to the counting chamber to facilitate counting of thecate species (Lebour 1925, Taylor 1978). Depending on the quantity of the larger cells, the whole chamber or only a part of it was scanned, and the cells were identified and counted using a Nikon Eclipse TS100 inverted microscope equipped with phase contrast objectives (10×/1.25 Ph1 ADL (Apodized Dark Low) WD 6.2 and LWD (Long Working Distance) 20×/0.40 Ph1 ADL WD 3.1). Smaller cells (e.g. those of *Amphidinium* cf. *carterae*) were counted in a bright field using a LWD 40×/0.55 Ph1 ADL WD 3.1 objective in 3 to 10 transversal bands depending on the number of cells found. The number of cells of different species was recalculated for the whole chamber and then per gram of host macrophyte, coral or sediment substrate wet weight (Bagnis et al. 1985, Bomber et al. 1985, 1988, 1989, Del-

gado et al. 2005), taking into account the total volume of the liquid fraction in the sample (the volume of liquid corresponding to 1 g of the substrate's wet weight). Cells were measured using a 10× eyepiece containing a micrometer disc and a 40× objective. Photographs for further identification were taken using a Nikon Coolpix S4 digital camera adapted to the microscope.

Temperature, pH and dissolved oxygen (DO) of the surface layer of water were recorded on board using a multiparameter meter Multi 197i (WTW Measurement Systems). Salinity was measured with a refractometer (Aquatic Ecosystem). Samples of DO, dissolved inorganic nitrogen (DIN) and dissolved phosphate ( $\text{NO}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$ ,  $\text{NH}_4\text{-N}$  and  $\text{PO}_4\text{-P}$ ) were collected in 8 l plastic tanks from the surface layer. Determinations of DO and nutrients were made in the laboratory using a portable data logging spectrophotometer Hach DR/2010. Precipitation, wind speed and direction graphs were averaged from the daily data obtained by the Meteorological Observatory of the National Commission of Water (Centro de Previsión del Golfo de México, Subgerencia Técnica, Gerencia Regional Golfo – Centro, Comisión Nacional del Agua, Veracruz, México; 19° 08' 34.4" N, 96° 06' 39.2" W).

Factor analysis was used to clarify relationships between the species composition of benthic/epiphytic dinoflagellates and physical-chemical characteristics. The abundance of each species and the values of different variables were incorporated into an agglomerative classification module. In the analysis, an *r* and *q* matrix was computed separately with the variables using a varimax rotation. Factor analysis was performed using STATISTICA software (Version 6, 1994 to 2001).

Although our studies of benthic and epiphytic dinoflagellates cover the period from May through December 2005, we present the annual dynamics of environmental conditions throughout the year from May 2005 through May 2006 for a better understanding of the seasonal changes of dinoflagellate taxocenosis in relation to physical-chemical factors.

## RESULTS

### Physical-chemical conditions

Physical-chemical conditions differed slightly between the 2 main sampling sites. The pH of the surface layer of the water in May to July fluctuated a little more at Stn 5 than at Stn 3, while in September to October fluctuations were much more pronounced at Stn 3 (Fig. 2).

From May through October 2005, easterly and northerly winds predominated (Figs. 3 & 4). Strictly northerly winds prevailed in October (for 17 d). From November to December, northwesterly winds blew for 22 d (according to the average values shown in Fig. 3, southerly winds prevailed; however, it does not hold true in this case due to the inadequacy of applying mean values expressed on a 360° scale). In March to May 2006, easterly and northeasterly winds took over. Average wind speed remained between 2.8 and 4.0  $\text{m s}^{-1}$  throughout the year, and minimal monthly values varied between 1.0 and 2.5  $\text{m s}^{-1}$  (Fig. 3). The maximal average monthly wind speed peaked in July and November 2005 (during the sampling period at Stns 3 and 5) and in January and February 2006, reaching 8.5, 10.7, 11.9 and 10.6  $\text{m s}^{-1}$ , respectively. The minimal average monthly wind speed values were recorded in August and December 2005.

The diurnal tidal range near Veracruz reaches 95 to 105 cm (calculated from González et al. 2006 [Oceanografía Física, CICESE], available at: <http://oceanografia.cicese.mx/predmar/>). Temperature and salinity curves from both Stns 3 and 5 are similar (Fig. 5A,B), with mean maximum temperatures in June and October 2005 and a slightly decreased salinity in July (<29). Average daily precipitation steadily increased from May and peaked in October, abruptly decreased in October to November (with the lowest daily precipitation of 277.3 mm in October 4), reached

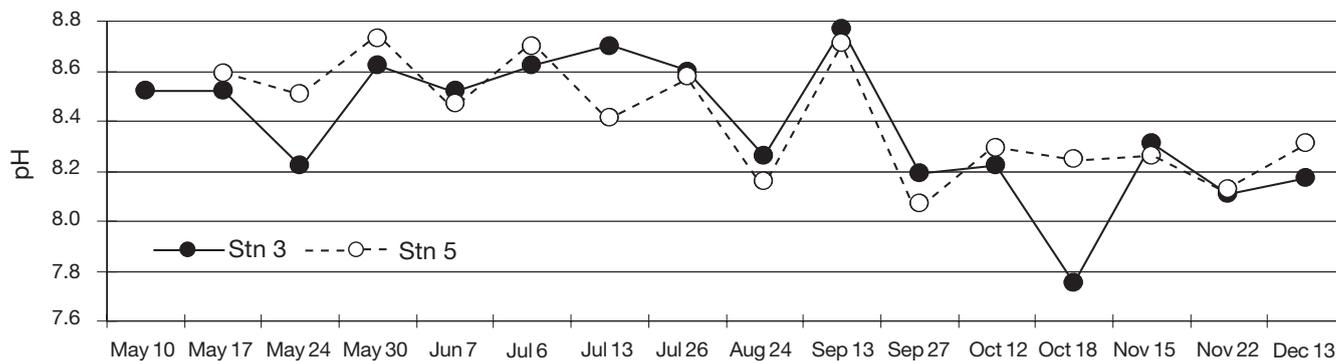


Fig. 2. Annual variability of pH in the water column at Stns 3 and 5 in 2005 in the National Park Sistema Arrecifal Veracruzano. See Fig. 1 for station locations

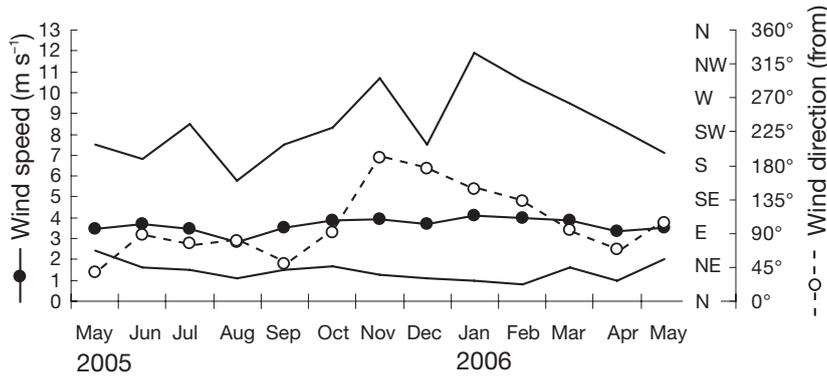


Fig. 3. Annual variability of average monthly wind speed and direction in the National Park Sistema Arrecifal Veracruzano. Solid lines (without symbols) indicate maximum and minimum wind speed

its minimal values in December 2005, and maintained very low levels until May 2006 (Fig. 5C,D). DO concentrations usually correspond with the percentage of oxygen saturation at both Stns 3 and 5 throughout the year except in October (Fig. 5E,F). DIN varied slightly throughout the year and peaked in October 2005 and February 2006, almost reaching 1.5 mg l<sup>-1</sup> (Fig. 5G,H). PO<sub>4</sub>-P concentrations for the most part also varied slightly; however, a high peak of 2.2 mg l<sup>-1</sup> was registered at Stn 3 in July 2005.

**Species composition of benthic/epiphytic dinoflagellates and associated macrophytes**

The number of species of benthic and epiphytic dinoflagellates at Stns 3 and 5 was comparatively low; 17 species were identified to specific and generic level (Fig. 6, Table 1). Few cells of *Gambierdiscus* were found, and the identification of its 2 species remains preliminary. The identification of athecate dinoflagellate taxa is problematic when dealing with fixed cells. The cell shape can be changed, and the chloroplast morphology is difficult to determine. Therefore, and because a very similar species exists but has not been recorded for the area yet, we hesitate to assign the *Amphidinium* species unambiguously and have recorded it as *A. cf. carterae*. Twenty-three macroalgal and 2 seagrass species were found associated with epiphytic dinoflagellates (Table 2). Some macroalgal species remain unidenti-

fied. The seagrass *Syringodium filiforme* was found only near Isla de Enmedio on 9 April 2005 (19° 06' 10.7" N, 95° 56' 18.7" W) as a band located closer to the coastline (between 10 and 20 m from it) than the extensive zone of *Thalassia testudinum*.

**Abundance of benthic/epiphytic dinoflagellates**

A minimal range of abundance of benthic dinoflagellates occurred in sediments on dead corals and in bottom sediments, and maximal values of abundance occurred on *Thalassia testudinum* and *Ulva fasciata* (Table 1). Sediments on top of the live coral *Diploria strigosa* contained more dinoflagellate cells than sediments on dead corals spread over the bottom.

On comparing Stns 3 and 5, one can see that (1) cell abundances in bottom sediments are low and almost equal, (2) cell abundance on dead corals at Stn 3 is considerably higher than at Stn 5, (3) dinoflagellate cell abundance found with the macroalgal species *Caulerpa sertularioides* and *C. cupressoides* is higher at Stn 5, and (4) while *Halimeda opuntia* was typical of Stn 3, *Acanthophora spicifera* and *Ulva fasciata* were characteristic of Stn 5 (Table 1).

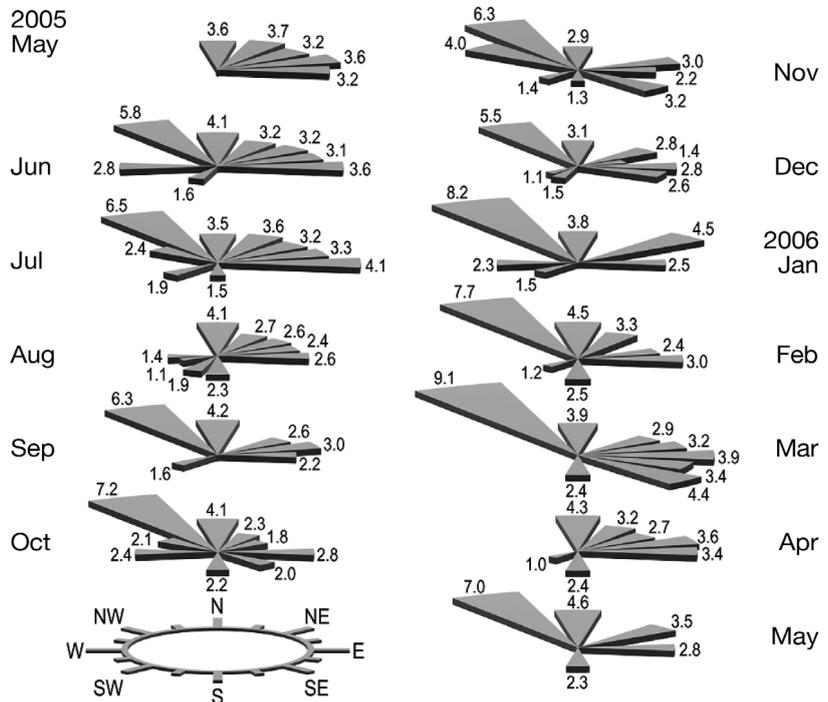


Fig. 4. Monthly wind speed (m s<sup>-1</sup>) averaged according to direction in the National Park Sistema Arrecifal Veracruzano

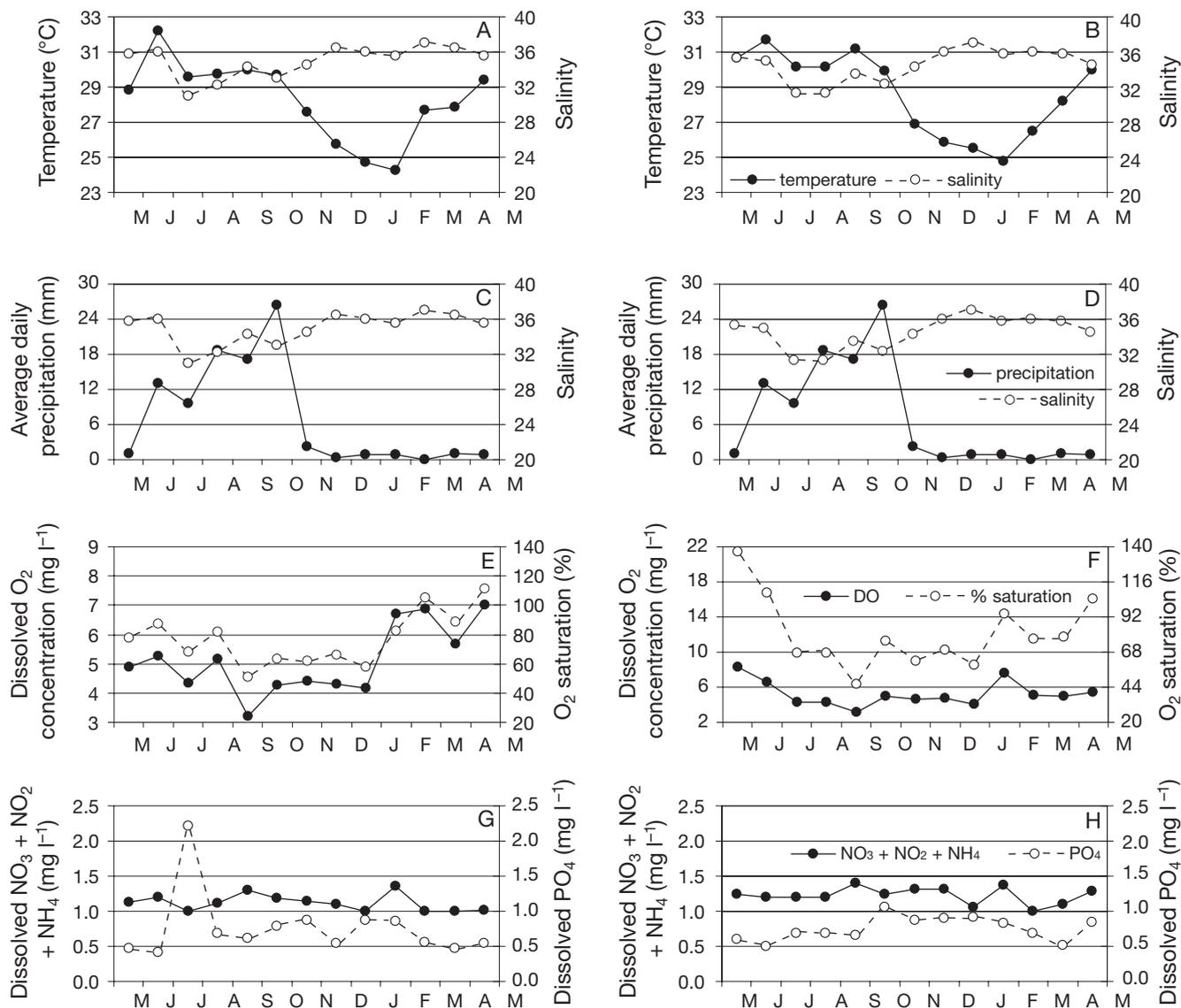


Fig. 5. Annual variability of some physical and chemical characteristics of the water column at (A,C,E,G) Stn 3 and (B,D,F,H) Stn 5 in the National Park Sistema Arrecifal Veracruzano. (A,B) Water temperature and salinity; (C,D) precipitation and salinity; (E,F) dissolved oxygen (DO) concentration and percentage of oxygen saturation; (G,H) dissolved inorganic nitrogen ( $\text{NO}_3\text{-N} + \text{NO}_2\text{-N} + \text{NH}_4\text{-N}$ ) and phosphate ( $\text{PO}_4\text{-P}$ ) concentrations. See Fig. 1 for station locations

Factor analysis results are not shown because no significant relationship between benthic/epiphytic dinoflagellates and the physical–chemical characteristics was observed.

#### Seasonal changes of benthic/epiphytic dinoflagellates

Macrophytobenthos, dead corals and sediments were collected every 2 wk; however, a continuous record was obtained only for the *Thalassia testudinum* association (Fig. 7). Differences between Stns 3 and 5

were: (1) the average number and maximal values of epiphytic dinoflagellates associated with it were much higher at Stn 5; (2) at Stn 3 the peak of abundance of epiphytic dinoflagellates occurred in late July, while at Stn 5 it occurred in late May (note that data before May 24 are lacking); (3) mean peak values of dinoflagellate abundance occurred in mid-October at Stn 3 and in late July at Stn 5; (4) in May to December at Stn 3 the epiphytic dinoflagellates taxocoenosis was dominated by *Prorocentrum lima*, *Coolia monotis* and *Amphidinium cf. carterae*, while at Stn 5 during all the surveys only *P. lima* was the predominant species and *A. cf. carterae* prevailed numerically only once; and

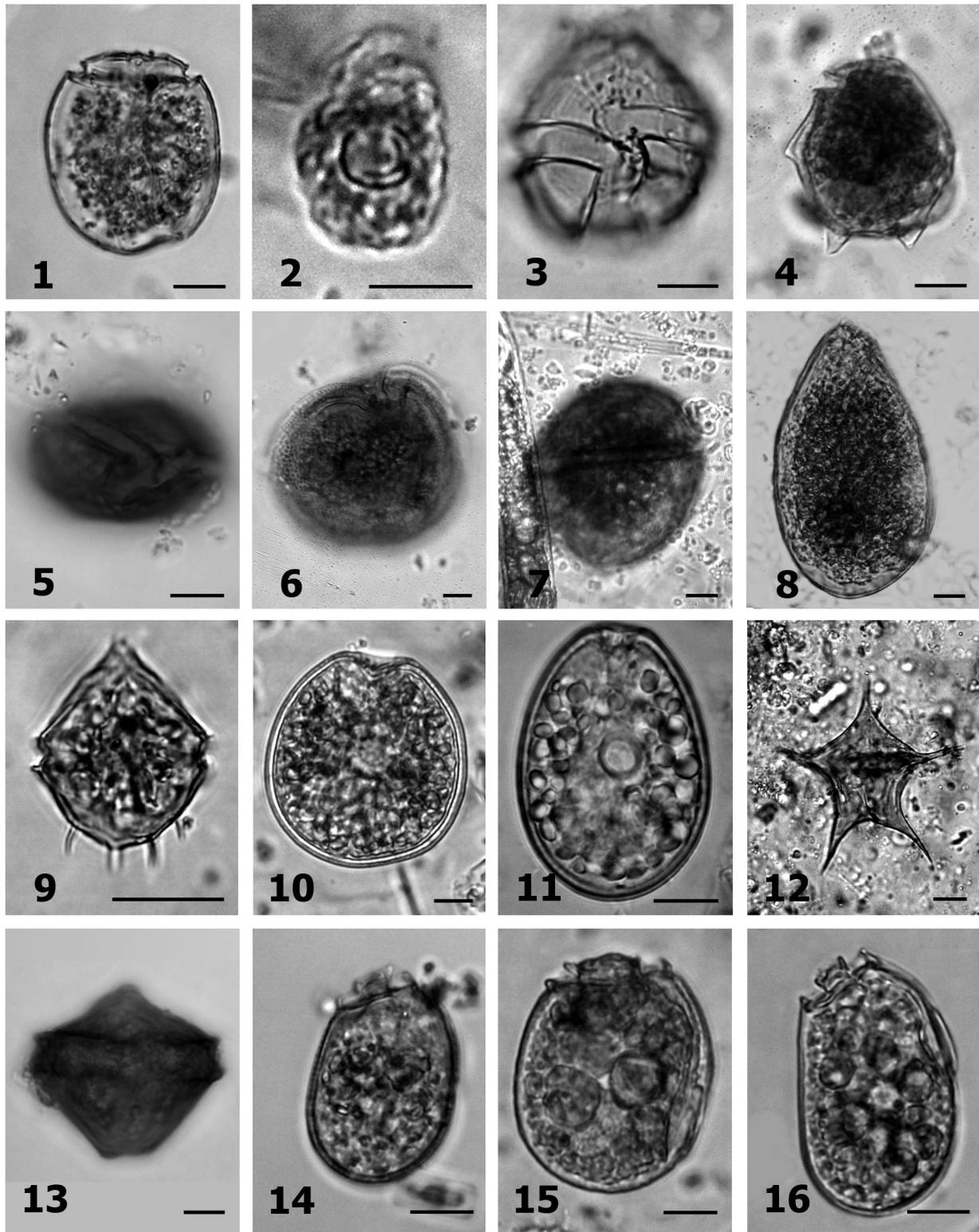


Fig. 6. Benthic and epiphytic dinoflagellates reported from the National Park Sistema Arrecifal Veracruzano: 1, *Amphidiniopsis* sp.; 2, *Amphidinium* cf. *carterae*; 3, (?) *Bysmatrum* sp., theca; 4, (?) *Cabra matta*; 5, *Coolia monotis*; 6, *Gambierdiscus* cf. *toxicus*; 7, (?) *Gambierdiscus yasumotoi*; 8, *Ostreopsis heptagona*; 9, *Peridinium quinquecorne*; 10, *Prorocentrum concavum*; 11, *Prorocentrum lima*; 12, *Protoperidinium compressum*, resting cyst; 13, *Protoperidinium subinermis*, resting cyst; 14, *Sinophysis ebriola*; 15, *Sinophysis microcephala*; 16, *Sinophysis stenosoma*. Scale bars = 10  $\mu$ m

Table 1. Benthic and epiphytic dinoflagellates found in the National Park Sistema Arrecifal Veracruzano in 2005–2006 (see Fig. 6). \*: dominant species; data on the toxicity were taken from published literature. CFP: ciguatera fish poisoning; DSP: diarrhetic shellfish poisoning; a question mark indicates doubtful identification

Species	Toxicity
<i>Amphidiniopsis</i> sp.	Not toxic
<i>Amphidinium</i> cf. <i>carterae</i> Hulburt*	Produces hemolysins and ichthyotoxins
(?) <i>Bysmatrum</i> sp.	Not toxic
(?) <i>Cabra matta</i> Murray et Patterson	Not toxic
<i>Coolia monotis</i> Meunier*	Produces cooliatoxin which is ichthyotoxic
<i>Gambierdiscus</i> cf. <i>toxicus</i> Adachi et Fukuyo	Produces ciguatoxins and maitotoxins; the main causative agent of CFP in the tropical zone
(?) <i>Gambierdiscus yasumotoi</i> Holmes	Produces a toxic substance similar to maitotoxins
<i>Ostreopsis heptagona</i> Norris, Bomber et Balech*	Toxic to mice
<i>Peridinium quinquecorne</i> T. H. Abé*	Not toxic
<i>Prorocentrum concavum</i> Fukuyo	Produces okadaic acid and causes DSP
<i>Prorocentrum lima</i> (Ehrenb.) F. Stein*	Produces diarrhetic toxins: okadaic acid and proro-centrolides; cause CFP and DSP
<i>Prorocentrum</i> spp., benthic	Some of them produce okadaic acid and proro-centrolides; cause DSP
<i>Protoperidinium compressum</i> (T. H. Abé) Balech (cysts)	Not toxic
<i>Protoperidinium subinermis</i> (Paulsen) A. R. Loebel. (cysts)	Not toxic
<i>Sinophysis ebriola</i> (Herdman) Balech	Not toxic
<i>Sinophysis microcephala</i> Nie et Wang	Not toxic
<i>Sinophysis stenosoma</i> Hoppenrath	Not toxic

(5) *Ostreopsis heptagona* was a component of the dominant complex only at Stn 3 in early May, and it was observed in low numbers at Stn 5 in May 2005. Due to the seasonal occurrence of the majority of the dominant macroalgal species (Table 2), the records of epiphytic dinoflagellates associated with them are discontinuous and sporadic.

### Fish mortality event

On 21 March 2006, when a strong northerly wind blew with a velocity of 8.7 m s<sup>-1</sup>, we observed many dead specimens of the sergeant major *Abudefduf sax-*

Table 2. Macrophytes associated with benthic and epiphytic dinoflagellates found in the National Park Sistema Arrecifal Veracruzano in 2005–2006. \*: dominant species. Chlo: Chlorophyta; Phaeo: Phaeophyta; Rho: Rhodophyta; Ang: Angiospermatophyta. Surveys—2005: 1, May 10; 2, May 17; 3, May 24; 4, May 31; 5, June 7; 6, July 6; 7, July 13; 8, July 26; 9, August 24; 10, September 13; 11, September 27; 12, October 12; 13, October 18; 14, November 15; 15, November 22; 16, December 13; 2006: 17, January 10 (buoys); 18, February 10 (drifting *Sargassum* spp.); 19, February 21 (drifting *Sargassum* spp.); 20, March 3 (buoys)

Species	Division	Surveys
<i>Acanthophora spicifera</i> (Vahl) Børgesen*	Rho	Stn 3: 1, 2, 9, 14; Stn 5: 5, 8–11, 13
<i>Caulerpa cupressoides</i> (Vahl) C. Agardh	Chlo	Stn 3: 10, 16; Stn 5: 15
<i>Caulerpa racemosa</i> (Forssk.) J. Agardh var. <i>uvifera</i> (C. Agardh) J. Agardh	Chlo	Stn 5: 6, 7, 10
<i>Caulerpa sertularioides</i> (J. F. Gmel.) Howe*	Chlo	Stn 3: 6, 9, 13; Stn 5: 5–12
<i>Cladophora</i> sp. (epiphyte on <i>Thalassia</i> )	Chlo	Stn 5: 6, 12, 13
<i>Codium isthmocladum</i> Vickers	Chlo	Stn 5: 2
<i>Dictyota cervicornis</i> Kütz.	Phaeo	Stn 3: 1, 2, 4, 5, 7, 15
<i>Galaxaura obtusata</i> (Ellis et Solander) J. V. Lamour.	Rho	Stn 3: 6, 7; Stn 5: 10
<i>Galaxaura squalida</i> Kjellm.*	Rho	Stn 3: 5, 6; Stn 5: 10
<i>Gracillaria</i> sp.	Rho	Stn 3: 1, 12; Stn 5: 5, 6, 8–11, 13, 16
<i>Grateloupia</i> cf. <i>dichotoma</i> J. Agardh	Rho	Stn 5: 6, 9, 10, 12
<i>Halimeda opuntia</i> (L.) J. V. Lamour.*	Chlo	Stn 3: 2–7, 9, 10, 12–16; Stn 5: 3
<i>Hypnea cervicornis</i> J. Agardh	Rho	Stn 5: 15
<i>Hypnea musciformis</i> (Wulfen) J. V. Lamour.	Rho	Stn 3: 15; Stn 5: 3
<i>Hypnea spinella</i> (C. Agardh) Kütz.	Rho	Stn 3: 10
<i>Laurencia</i> sp.	Rho	Stn 3: 5–15
<i>Padina gymnospora</i> (Kütz.) Sonder (attached to buoy cables)	Phaeo	Stn 2: 20
<i>Polysiphonia</i> sp.	Rho	Stn 5: 2
<i>Rhizoclonium</i> sp. (epiphyte on <i>Thalassia</i> )	Chlo	Stn 5: 6
<i>Sargassum filiopendula</i> C. Agardh (drifting in masses)	Phaeo	Between Stns 1 and 2: 19
<i>Sargassum vulgare</i> C. Agardh (drifting in masses)	Phaeo	Between Stns 1 and 2: 18, 19
<i>Ulva fasciata</i> Delile	Chlo	Stn 5: 3, 4, 6
<i>Ulva lactuca</i> L.	Chlo	Stn 5: 2
<i>Syringodium filiforme</i> Kütz.	Ang	Isla de Enmedio, 9 April 2005
<i>Thalassia testudinum</i> Banks ex König*	Ang	Stn 3: 1–16; Stn 5: 1–11, 13–16

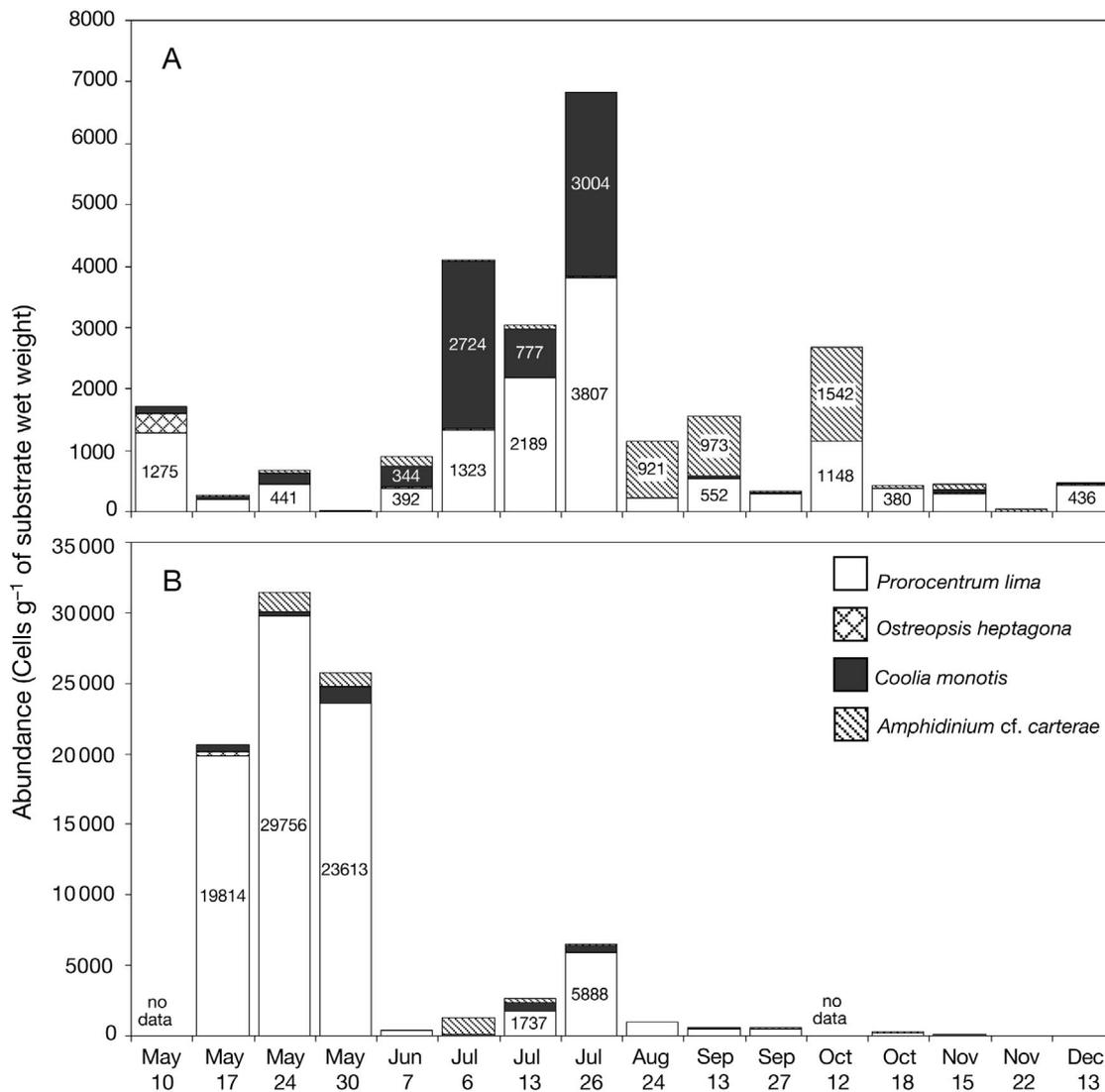


Fig. 7. Seasonal dynamics of the abundance of epiphytic dinoflagellates living on the seagrass *Thalassia testudinum* in the National Park Sistema Arrecifal Veracruzano at (A) Stn 3 and (B) Stn 5. See Fig. 1 for station locations

*atilis* (L.), the parrotfish *Sparisoma* sp. and the surgeonfish *Acanthurus* sp. tangled in *Thalassia testudinum* blades on a beach near Stn 5. Gut content analysis is necessary to prove the presence of toxic dinoflagellates as the main cause of fish mortalities.

## DISCUSSION

### Species composition and abundance of dinoflagellates

Dinoflagellate species richness in the NPSAV can be characterized as relatively low, which is in accordance with that described by Tindall & Morton (1998) for the

type I and II systems. During the sampling period, *Prorocentrum lima* was almost always dominant. At times, *Amphidinium cf. carterae*, *Coolia monotis* and *Ostreopsis heptagona* also comprised the dominant species complex. The majority of the dinoflagellate population peaks were due mainly to *P. lima*. At Stn 3, the dominance of *P. lima* and *C. monotis* in May to July 2005 was followed by the prevalence of *A. cf. carterae* in August to October (Fig. 7A). Similarly, in the Florida Keys a decrease in population density of *Gambierdiscus toxicus* was followed by maximum abundance of *P. lima* (Bomber et al. 1985). In French Polynesia, seasonal changes in a benthic dinoflagellate taxocoenosis could be traced by both the changes in species composition and cell abundances. During a temporal succes-

sion a niche vacated by *G. toxicus* was filled by *Ostreopsis lenticularis* Fukuyo (Bagnis et al. 1990). In addition, *Gambierdiscus* spp. showed a seasonal cycle in cell densities (Chinain et al. 1999). In east Tasmanian waters during winter, *Ostreopsis siamensis* Schmidt appears to be replaced by *C. cf. monotis* (Pearce et al. 2001). As regards the main dominant species, our data are in accordance with those of Popowski (1994) for the NW Cuban waters, and, surprisingly, are more similar to the dominant dinoflagellate assemblage found in the regions with the 'Mediterranean' (dry summer/wet winter subtropical) or temperate maritime climate than to that in tropical areas (Pearce et al. 2001, Vila et al. 2001). One should note that the climate of the east Tasmania coast (a temperate region), from which the dinoflagellate assemblage was described (Pearce et al. 2001), differs from the rest of the island, which is more similar to that in West and South Australia with its 'Mediterranean' climate. Because only rare cells of *Gambierdiscus cf. toxicus* were observed, toxicity studies should concentrate on the most abundant potentially toxic species (*P. lima*, *C. monotis*, *O. hep- tagona* and *A. cf. carterae*) and their seasonal population dynamics to verify our hypothesis on the risk of ciguatera in the NPSAV.

Species identification of potentially toxic species is crucial. Identification of species of the genus *Amphidinium* has long been considered problematic, but the genus has been redefined recently (Flø Jørgensen et al. 2004). *Amphidinium carterae* is extremely similar to *A. massartii* Biecheler, and because the size range of the 2 species overlaps completely and both species possess a central ring-shaped starch-sheathed pyrenoid, it is difficult to distinguish between them with certainty based on light microscopic observations (Murray et al. 2004). The morphological characteristics distinguishing the 2 species are the shape of the plastid and the slightly different epicone to body length ratio. These differences are not reliably preserved in fixed samples, and therefore we did not identify the species with certainty.

Our data on dinoflagellate abundance associated with macrophytes (at least with *Thalassia testudinum* and *Caulerpa sertularioides* + *C. cupressoides* sampled at both stations) support the conclusion of Carlson & Tindall (1985), who found that protected inshore habitats in the Virgin Islands sustain much greater toxic dinoflagellate cell numbers than coral reefs and related this to the high influence of wave activity which markedly reduces the benthic dinoflagellate population. The drastic decrease in dinoflagellate populations in early June at Stn 5 is hard to explain. In late May to early June, large changes in precipitation (but not in surface water salinity) and in the percentage of DO saturation occurred (Fig. 5D,F).

At Stn 5, *Thalassia testudinum* reached a larger size than at Stn 3 and on average there were much higher densities of the epiphytic macroalgae *Cladophora* spp. and *Rhizoclonium* spp., which predominantly cover the upper parts of *T. testudinum* leaves. These epiphytic macroalgae with their numerous filamentous thalli and high surface:volume ratio, appear to be responsible for greater epiphytic dinoflagellate populations. The highest population density of *Prorocentrum lima* was observed on 17, 24 and 31 May 2005 at Stn 5 (Fig. 7B), and it seems to be related to *T. testudinum* leaves with their upper parts densely covered with the macroalgal epiphytes. In the more northern areas, at 41° 20' to 47° 35' N, maximal *P. lima* abundance was observed in late spring–summer and in July to August (Vila et al. 2001, Levasseur et al. 2003).

Few studies have been made of the substrate specificity of benthic/epiphytic dinoflagellates. Bomber et al. (1985) showed that percent ash content and mean *Prorocentrum lima* abundance were negatively correlated ( $r = -0.3$ ,  $p < 0.05$ ). They hypothesized that host macrophytes with low ash weights are less rigid, allowing more movement in the water column, and that macrophyte surface area should be positively correlated with epiphytic dinoflagellate population density because more space is available for cell division and attachment on high surface area macrophytes. Bomber et al. (1989) also showed that the abundance of *Gambierdiscus toxicus* was positively correlated ( $r = 0.88$ ,  $p < 0.01$ ) with macroalgal host surface area. In the NW Cuban waters, epiphytic dinoflagellates showed no preference for any specific substrate (Popowski 1994). However, determination of the epiphytic dinoflagellate population density related to macroalgal surface area may introduce an additional 20 to 45 % error to each sample (Bomber 1985, Lobel et al. 1988, Morton & Faust 1997). An increased dinoflagellate biomass was shown to be associated with brown and red macroalgae, with low biomass associated with green algae and seagrasses (Morton & Faust 1997). In the present study, this holds true only for *Halimeda opuntia*. Other green algae do not show this trend; moreover, *Ulva* spp. and *Thalassia testudinum* had the highest abundance among all studied substrates. Our results are in agreement with the data presented by Bomber et al. (1985), who found *P. lima* to be the most abundant species on the green algae *Halimeda opuntia*, *Penicillus capitatus* Lam. and *Avrainvillea nigricans* Decne among 16 species of Chlorophyta, Phaeophyta and Rhodophyta, and on the seagrass *T. testudinum*. Vila et al. (2001) monitored 4 macroalgal species and found that the macroalgal substrate was not significant with respect to association with a certain species of macroalga for all 3 dominant dinoflagellate species.

Table 3. Abundance of benthic and epiphytic dinoflagellates on various substrates in the National Park Sistema Arrecifal Veracruzano in May to December 2005 (buoys and drifting *Sargassum* spp. were sampled in January and March 2006); abundance is given in cells per gram of wet substrate weight. See Fig. 1 for station locations

Substrate/species	Stn	Abundance range (cells g <sup>-1</sup> )	Avg. no. (cells g <sup>-1</sup> )	No. of samples
<i>Acanthophora spicifera</i>	5	2–1372	549	6
<i>Caulerpa sertularioides</i>	3	0–464	378	6
and <i>C. cupressoides</i>	5	9–2957	742	8
<i>Gracilaria</i> sp.	5	0–198	85	5
<i>Halimeda opuntia</i>	3	6–365	76	12
<i>Laurencia</i> sp.	3	143–3360	2380	4
<i>Ulva fasciata</i>	5	2089–30879	12276	3
<i>Thalassia testudinum</i>	3	16–6826	1528	14
	5	0–31467	4852	12
Bottom sediment	3	3–84	35	13
	5	2–77	16	9
Sediment on dead corals	3	0–5039	638	8
	5	0–26	16	6
Sediment on live corals	3	329–1830	1080	2
Macroalgae attached to buoys	2	476–11172	5338	11
<i>Sargassum vulgare</i> and <i>S. filipendula</i> (drifting)	1–3	4–459	91	7

In some samples of macrophytes at Stn 5, *Peridinium quinquecorne* was abundant. However, because macrophytes were placed into jars underwater and surrounding water was therefore included, we cannot be sure of the origin of the *P. quinquecorne* cells. They appear to be a part of epiphytic assemblages and could be a causative agent of recurrent red tides observed on 24 May, 4 and 12 August, 12 to 14, 18 and 25 October 2005, and on 20 June, 25 July to 5 August, and 15 August 2006, at Stns 4 and 5, and also northward within the study area, always very close to the coastline. This species, which is characterized by high swimming velocity, has been shown to be able to move out of the water column and to attach to solid objects away from the light and seems to follow intrinsic tide-dependent oscillations (Horstmann 1980). Additional studies are needed to show the relevance of *P. quinquecorne* to benthic/epiphytic assemblages in the NPSAV.

#### Seasonality and relationships between abiotic factors and abundance of dinoflagellates

Due to the length of our sampling period (8 mo), we cannot draw any conclusions about the number of dinoflagellate abundance peaks exhibited at Stns 3 and 5 during a calendar year (Fig. 7). However, it is evident that, despite the proximity of these 2 sampling sites,

the number of peaks and the peak periods may vary and are subject to local conditions that might depend on nutrients or sediment load rather than the direct influence of factors such as precipitation, salinity, temperature, wind speed and direction that were similar at the 2 stations. Additionally, the presence and seasonality of epiphytic macroalgae (*Cladophora* spp. and *Rhizoclonium* spp. in the case of *Thalassia testudinum*), which can provide a habitat for dinoflagellates, may be involved. If so, hydrographic and meteorological conditions and bathymetry may be indirectly responsible for the currently inexplicable pronounced differences between Stns 3 and 5.

Our results differ from what was observed in the Virgin Islands, where toxic epiphytic dinoflagellates demonstrated a bimodal pattern of seasonal abundance with population maxima significantly correlated with regional precipitation and corresponding to the peak periods of rainfall: maximal numbers

of most of the studied species usually followed periods of heavy rainfall (Carlson & Tindall 1985). In addition, in the Virgin Islands, all 7 dinoflagellate species studied were positively or negatively correlated with water temperature, and 2 species were positively correlated with NO<sub>3</sub>-N, NH<sub>4</sub>-N, dissolved PO<sub>4</sub>-P and total dissolved phosphorus. In east Tasmanian waters, a decrease in temperature was associated with a decline in dinoflagellate numbers through winter (Pearce et al. 2001). In contrast, in southwestern Puerto Rico, 2 dominant epiphytic dinoflagellate species were not strongly correlated with temperature or rainfall, although they displayed seasonal fluctuations over a 3 yr period (Ballantine et al. 1988). In Japan, no correlation between the abundance of *Gambierdiscus toxicus* and nutrients was demonstrated (Yasumoto et al. 1980). In Queensland, Australia, periodicity in the *G. toxicus* abundance does not seem to be related directly to temperature, and complex substrate interactions and other unknown factors appear to be involved (Gillespie et al. 1985). Summing up, seasonality of *G. toxicus* is observed sometimes, but not always, and the underlying factors that control population increases of this species remain unknown (Cruz-Rivera & Villareal 2006). In the NW Mediterranean, no significant correlations were observed between epiphytic dinoflagellates and water temperature or nutrients; however, the epiphytic dinoflagellate assemblage demonstrated a clear seasonality (Vila et al. 2001).

Regarding the relationship of the benthic/epiphytic dinoflagellate abundance and winds, only a few observations are available. For example, during the warmest months in the Virgin Islands, on windless days the abundance of many species diminished (Carlson & Tindall 1985). According to our factor analysis, no evident relationship occurred between dinoflagellates and physical–chemical factors in the NPSAV. Nevertheless, in October 2005, northerly winds could have contributed to the decrease in the epiphytic dinoflagellate population density, although even stronger winds (up to 10.6 to 11.9 m s<sup>-1</sup>) were recorded after the sampling period, in November 2005, January and February 2006. Most of the epiphytic dinoflagellates found in the NPSAV did not show any particular preference for macrophyte species. However, the seagrass *Thalassia testudinum* appears to be the most abundant and permanent host.

Other studies have concluded that the availability of macroalgal substrates, light intensity and water movements affected the spatial distribution of epiphytic dinoflagellates while precipitation, temperature and nutrients from macroalgae are important temporary factors (Carlson & Tindall 1985, Tindall & Morton 1998). Increases in cell abundance may result from the colonization of the substrate by free-living cells and/or *in situ* growth of already attached cells, while decreases in abundance may result from detachment or mortality of attached cells and/or loss of substrate. Therefore, the temporal patterns should be interpreted with respect to the succession of host macroalgae (Levasseur et al. 2003). In addition, macroalgal surface area and ash content appear to be regulating factors of dinoflagellate population density (Bomber et al. 1985). Depending on the geographic region, *Gambierdiscus toxicus* has been shown to prefer different macroalgal host species and has been found with more than 50 algal genera (Shimizu et al. 1982, Carlson & Tindall 1985, Lobel et al. 1988, Cruz-Rivera & Villareal 2006).

### Potential risk of ciguatera

The present study, as well as others in the Caribbean and elsewhere, showed that not only *Gambierdiscus toxicus* but also other toxic benthic/epiphytic dinoflagellate species can be a major component of the assemblages, and that additional ecological studies of ciguatera endemic areas should focus on species in addition to *G. toxicus*, especially those of the genera *Prorocentrum*, *Ostreopsis*, *Coolia* and *Amphidinium* (Besada et al. 1982, Bagnis et al. 1985, Bomber et al. 1985, 1988, Carlson & Tindall 1985, Ballantine et al. 1988, Popowski 1994, Bourdeaux et al. 1995, Faust 1995a, Morton & Faust 1997, Heil et al. 1998, Tindall & Morton 1998, Tosteson et al. 1998, Turquet et al. 1998).

Among the dinoflagellate species of the NPSAV, *Prorocentrum lima*, *Coolia monotis*, *Ostreopsis heptagona*, and *Gambierdiscus* cf. *toxicus* can be considered potentially ciguateric species because they have been reported as toxic elsewhere (Nakajima et al. 1981, Norris et al. 1985, Yasumoto et al. 1987). *Amphidinium* cf. *carterae* requires a more thorough identification.

Seventeen fish species have been reported from the reef lagoon of Isla de Sacrificios. The following taxa were the most common: among herbivorous grazers, the dusky damsel *Stegastes fuscus* (Cuvier in Cuvier et Valenciennes), the Cocoa damselfish *S. variabilis* (Castelnaud) and spotfin butterfly *Chaetodon ocellatus* Bloch; among benthic and demersal predators and planktivorous species, the slippery dick wrasse *Halichoeres bivittatus* (Bloch), the flagfin mojarra *Eucinostomus melanopterus* (Bleeker), the grey snapper *Lutjanus griseus* (L.), the silver jenny *E. gula* (Quoy et Gaimard), the sergeant major *Abudefduf saxatilis*, the yellowtail snapper *Ocyurus chrysurus* (Bloch) and the rock hind *Epinephelus adscensionis* (Osbeck) (H. Pérez-España pers. comm., August 2006). The great barracuda *Sphyrnaea barracuda* (Walbaum) was the only pelagic predator reported. *L. griseus*, *O. chrysurus*, *E. adscensionis*, *S. barracuda*, *Sparisoma* sp. and *Acanthurus chirurgus* (Bloch) contribute to the commercial catch in the study area (Jiménez-Badillo et al. 2006).

The presence of a typical ciguateric food chain in the NPSAV indicates the potential involvement of at least some of the recorded fish species in the transfer of ciguatera. In the Mexican Caribbean, the southern Gulf of Mexico, the Gulf of California and the west coast of the State of Baja California Sur, all ciguatera intoxications are ascribed to the carnivorous fish from the genera *Lutjanus* Bloch, *Epinephelus* Bloch, *Myceteroperca* Gill, *Semicossyphus* Günther, *Spherooides* Anonymus [Lacepède], *Arothron* Müller and *Canthigaster* Swainson, as well as to *Sphyrnaea barracuda* (Parrilla-Cerrillo et al. 1993, Barton et al. 1995, Lechuga-Devéze & Sierra-Beltrán 1995, Núñez-Vázquez et al. 2000, Núñez-Vázquez 2005); 3 of these taxa have been recorded in the NPSAV. In Puerto Rico, the transmission of the dinoflagellate toxins through herbivorous reef fishes to their barracuda predators must take place in a relatively short period of time, and barracudas showed the highest ciguatoxicity after several months of exposure to sustained, elevated sea surface temperatures (Tosteson et al. 1998).

Growth rates of toxic epiphytic dinoflagellates from the tropical and temperate zones range from 0.05 to 0.53 divisions d<sup>-1</sup>, and even a rate of >0.6 divisions d<sup>-1</sup> could be sustained under optimal conditions of light, salinity and temperature (Bomber et al. 1988, 1989, Morton & Norris, 1990, Heil et al. 1993, Jackson et al. 1993, Pearce et al. 2001, Levasseur et al. 2003). The

high toxin-producing epiphytic dinoflagellate densities found and their short generation time known from literature, the shallowness of numerous reef zones inhabited by seagrasses and macroalgae, and the presence of a typical coral reef ichthyocoenosis indicate a high potential for outbreaks of ciguatera in the study area.

## CONCLUSIONS

Although many important aspects of the studied benthic/epiphytic dinoflagellate assemblages (e.g. toxicity, light and depth preference, small-scale distribution, correct identification of *Prorocentrum* spp. and some rare species from other genera, and methodological problems of sampling and counting) remained beyond the scope of the present study, we can draw several conclusions. (1) Low species diversity and high abundance characterized the assemblage of benthic/epiphytic dinoflagellates in the NPSAV; *Prorocentrum lima* was the main dominant species. (2) In the study area benthic and epiphytic dinoflagellates occurred in a wide range of habitats and associated with various sessile and drifting macrophytes, dead and live corals, and sediments. (3) Most of the epiphytic dinoflagellates did not show any significant preference for macrophyte species, although the seagrass *Thalassia testudinum* appears to be the most abundant and permanent host species. (4) The assemblage demonstrated clear seasonal dynamics, being more abundant in May to June. The assemblage differed at 2 neighbouring sampling sites. (5) The population density of benthic/epiphytic dinoflagellates was not found to be correlated with any measured physical-chemical parameter. Finally, there is a high potential for outbreaks of ciguatera in the NPSAV, and the implementation of a monitoring program for early detection of non-pelagic harmful algal blooms, especially in the ciguatera endemic regions, is therefore necessary.

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