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García-Hansen *et al.* 2004, Hernández-Becerril and Almazán Becerril 2004, La Barbera-Sánchez *et al.* 2004, Sierra-Beltrán *et al.* 2004, Tosteson 2004, Vargas-Montero and Freer 2004). Variability in noxious epiphytic dinoflagellates has been attributed to natural and anthropogenic changes affecting macroalgal assemblages, such as: temperature, turbulence, nutrients, changes in substrates, fresh water sources, waves, inputs of limestone on dead coral and seagrass, as well as effects of hurricanes and strong cold fronts (Taylor 1985, Diogène 1992, Grzebyk 1994).

In the inlet of the Jaimanitas River near Havana, the sediment is muddy at the river outlet, muddy to sandy further out, and sandy farther offshore. The macrophytic assemblages are composed of three functional-form groups: filamentous, foliaceous, and arborescent (Littler and Littler 1980). Each group is dependent on sediment type and belongs to the following genera: *Ceramium, Bryothammnion, Hypnea, Acanthophora, Gracillaria, Gelidium, Ectocarpus, Sphacelaria, Dictyota, Padina, Ulva, Cladophora, Codium,* and *Chaetomorpha*, all belonging to the Phaeophyta, Chlorophyta, or Rhodophyta group.

Even though ciguatera is one of the main causes of food poisoning from fish in northwestern Cuba, the first studies were performed in the 1990s (Valdés *et al*. 1992, Delgado *et al*. 2000, Popowski *et al*. 2001), which concluded that these organisms were more abundant on brown macroalgae during the summer.

This study describes abundance and spatial and temporal distribution of potentially harmful epiphytic dinoflagellates and their association with macroalgae substrate, according to factors influencing environmental variability.

MATERIAL AND METHODS

Sampling during two annual cycles (March to March of 1999-2000 and 2001-2002) was performed in two transects (5 sampling stations per transect) perpendicular to the shore in the sub tidal zone (0.50-4 m depth) of Jaimanitas Inlet (23°5'37.29" N, 82°29'20.86" W), located 6 km northwest of Havana, Cuba. The Jaimanitas River is surrounded by urban development. Salinity varies from 10 to 38 psu according to freshwater fluxes during rainy season.

Macroalgae samples were collected monthly by snorkelling, following the procedure proposed by Quod *et al*. (1995). Macroalgae were collected (20 to 100 g/m^2) and placed in plastic bags.

At the laboratory, the algal samples were vigorously shaken to remove epiphytic dinoflagellates. The suspension was passed through three successive 150, 100, 75, and 20-µm mesh sieves. The last fraction retained (20 µm) was preserved in 25 ml seawater containing 1 ml lugol acid. During the two annual cycles, 1340 samples were obtained. Macroalgae were dried on filter paper for 72 h before weighing (Sauter D-7470, EB Ingen, 0.01 mg precision).

Cell concentrations were calculated for all samples by counting 1 ml preserved sample on a Sedgwick-Rafter chamber with a light microscope (Olympus). Cells were expressed in cells/g algae (wet weight). Dinoflagellates were identified using Adachi and Fukuyo (1979), Fukuyo (1981), Faust (1991, 1996), and Tomas (1997).

Water samples near the bottom were collected to determine NH_4, NO_3, and NO_2 concentrations following the procedures reported by IOC (1993). Total inorganic nitrogen (TIN) was estimated adding known amounts of NH_4, NO_3 and NO_2.PO_4 and total (TP) concentrations were estimated as described in IOC/UNESCO (1983). Salinity and temperature were measured with a bucket thermometer and refractometer (ATAGO).

To determine significant differences between dinoflagellates abundance and chemical and physical variables, an ANOVA/MANOVA analysis was used, followed by a LSD analysis if significant differences were found. The relationship between the density of *G. toxicus* and *P. lima* and environmental variables were tested by Principal Component Analysis (PCA). All statistical analysis was performed with Statistica™ v. 4.5 software (StatSoft, Inc., Tulsa, OK).

RESULTS

Hydrological characteristics

Annual nutrient concentrations (µmol/l), temperature (°C), salinity (psu), and mean wind speed (m/s) maximum and minimum values are shown in Table 1. The NH_4 showed the higher concentration (>65% TIN) during the two annual cycles. Maximum and minimum temperatures were typical for the seasons (32.9°C during the summer and 25°C during the winter). Salinity values oscillated from brackish (10.3 psu) to seawater (38.3 psu) values and wind speeds from 0.5 to 10.5 m/s. During May-October, the highest concentrations of NO_3, NH_4, PO_4, TIN, and TP occurred. The lowest salinity occurred during the 1999-2000 cycle (Table 2).

Composition of epiphytic harmful dinoflagellates

Seven species in four genera of harmful epiphytic dinoflagellates were identified; of these, five are first reports for Cuban waters. Four species of *Prorocentrum* were the most abundant (Table 3).

Abundance and distribution of harmful dinoflagellates

Harmful dinoflagellates were found at all the stations during both annual cycles. There was an insignificant difference in cell numbers between transects (ANOVA/MANOVA $p<0.05$). Higher abundance occurred at the deeper sampling stations (5 and 6) with mean values of 1150 and 1120 cell g^{-1} for the first annual cycle and 1437 and 1409 cell g^{-1} for the second one. Low abundance of harmful dinoflagellates was found at Station 1, near the mouth of the river, during both

TABLE 1

Annual mean (min-max and SD) values of nutrient concentration (µmol), temperature (°C), salinity (psu), and wind speed (m/s) during two annual cycles

Parameters	Period 1999-2000				Period 20001-2002			
	Max	Min	Mean	S.D	Max	Min	Mean	S.D.
Temperature (ºC)	32.5	26.1	28.57	1.91	32.4	25	29.01	2.07
Salinity (psu)	38.3	10.3	31.09	7.95	35.8	30	33.07	1.84
Wind (m/s)	10.5	0	3.9	3.66	7.5	0.5	2.51	2.24
NO_3 (µmol/l)	12.15	0.2	4.57	3.24	8.93	1.09	5.05	2.3
NO_2 (µmol/l)	0.15	0	0.03	0.10	0.15	0	0.004	0.015
NH_4 (µmol/l)	39.88	0.8	10.68	9.16	18.83	3.07	9.99	4.33
TIN (µmol/l)	52.84	1.4	15.24	12.15	27.76	4.16	15.14	6.54
PO_4 (µmol/l)	4.28	0.005	1.09	1.16	1.83	0.007	0.88	0.56
TP (µmol/l)	7.87	0.07	1.97	1.84	2.38	0.003	1.42	0.57

TABLE 2

Monthly mean values of physico-chemical variability in two annual cycles

Parameters	Period 1999-2000												
	M	A	M	J	J	A	S	O	N	D	E	F	M
Temperature (ºC)	26.42	27.4	28.48	30.2	30.85	31.44	31.40	30.18	26.88	27.39	27.05	26.42	27.42
Salinity (psu)	34.28	35.34	0.5	31.75	31.34	19.52	11.61	33.3	36.03	35.17	37.83	37.97	37.81
Wind(m/s)	0.35	5.00	22.27	0.5	1.5	1.5	0.5	1.5	10.5	8.5	0.5	9.42	1.2
NO_3 (µmol/l)	3.53	2.24	4.23	5.39	3.83	8.98	11.25	4.26	3.81	3.36	2.66	2.54	3.40
NO_2 (µmol/l)	0.02	0.007	0.02	0.01	0.007	0.01	0.23	0.007	0.02	0.001	0.000	0.000	0.02
NH_4 (µmol/l)	7.29	5.61	12.79	13.42	6.64	16.06	37.95	8.07	6.91	6.63	5.37	5.21	6.88
TIN (µmol/l)	10.84	7.86	17.06	18.91	10.48	24.69	49.43	12.34	10.74	9.68	8.03	7.75	10.31
PO_4 (µmol/l)	0.45	0.22	1.32	1.45	0.96	2.45	4.13	0.96	0.79	0.46	0.21	0.22	0.56
TP (µmol/l)	1.1	0.48	1.80	2.04	1.53	4.34	7.07	1.53	1.47	1.19	1.02	0.88	1.15

Parameters	Period 2001-2002												
	M	A	M	J	J	A	S	O	N	D	E	F	M
Temperature (ºC)	27.52	27.35	29.41	30.35	30.85	31.68	31.5	30.33	30.5	28.59	26.52	25.26	27.44
Salinity (psu)	34.83	34.62	31.75	32.31	31.31	31.56	30.81	31.24	33.17	34.53	34.85	34.41	34.63
Wind(m/s)	0.35	5	0.5	0.5	0.85	1.5	0.5	2.85	1.35	2.5	5.44	7.5	3.5
NO_3 (µmol/l)	3.39	2.7	5.47	5.40	7.07	6.80	7.87	6.68	6.96	4.15	3.48	3.58	2.12
NO_2 (µmol/l)	0.01	0.009	0.003	0.01	0.002	0.007	0.002	0.001	0.003	0.003	0.004	0.000	0.000
NH_4 (µmol/l)	7.02	5.50	12.18	13.04	13.55	12.23	17.2	11.76	11.68	8.85	6.10	6.05	4.69
TIN (µmol/l)	10.43	8.22	17.66	18.53	20.62	18.96	25.07	18.45	18.65	13.00	9.58	9.64	6.81
PO_4 (µmol/l)	0.28	0.21	1.22	1.34	1.33	1.27	1.55	1.23	1.49	0.60	0.30	0.33	0.24
TP (µmol/l)	1.00	0.39	1.64	2.07	1.84	1.75	1.97	1.83	1.82	1.23	1.01	1.07	0.91

cycles, with mean concentrations of 175 and 348 cell g^{-1} (Fig. 1A).

P. lima was the dominant species (<50%) at all sampling stations followed by *G. toxicus* (8 to 33%). The remaining species were lower than 8% (Figs. 1B and 1C).

The average abundance in the two annual cycles during March-July was the highest in June (1012 and 1089 cell g^{-1}), respectively. The distribution pattern changes between August and November, reaching the lowest mean concentration (21 cell g^{-1}) in February (Fig. 2A). In 1999-2000, we observed a very abrupt decline in the amount of cells during August and September. During this month, no noxious dinoflagellate species were found. A second peak of abundance (609 cells g^{-1}) occurred during October (Fig. 2A).

P. lima was the most abundant species in both cycles, except during April and May

TABLE 3

Benthic harmful dinoflagellates identified in the study area, and relative abundance in two cycles

Species	Periods 1999-2000	Periods 2001-2002
Gambierdiscus toxicus Adachi & Fukuyo	++	+++
Prorocentrum lima (Ehrenberg) Dodge	+++	+++
P. belizeanum Faust*	+	++
P. mexicanum Osorio-Tafall*	+	+
P. concavum Fukuyo*	+	+
Coolia monotis Meunier*		+
Ostreopsis lenticularis Fukuyo*		+

+ < 10^3 cell.g^{-1}; ++ $10^3 > 10^4$ cell.g^{-1}; +++ $10^4 > 10^5$ cell.g^{-1}. * New report for Cuba.

1999, when *G. toxicus* represented 66.75 and 56.62%, respectively, and in May 2001, when this species represented 51% of the total dinoflagellates; the others species were present in low concentrations (Figs. 2B, 2C).

Harmful dinoflagellates occurred in greater numbers on Phaeophyta and Chlorophyta macroalgae during both study periods: 1999-2000 (39 to 57%) and 2001-2002 (36 to 62%). Abundance of *P. lima* was most important on *Padina* sp and *Dictyota dicotoma* (Phaeophyta, 82%), than on *Ulva lactuca* and *Chaetomorpha* spp. (Chlorophyta, 17%, Fig. 3A.). *G. toxicus* was mainly found covering *D. dicotoma* and *Padina* sp. (Phaeophyta, 78%), *Hypnea cervicornis* (Rhodophyta, 17%), and *Chaetomorpha* and *Ulva lactuca* (Chlorophyta, 5%, Fig. 3B). *Padina* sp. was the sole macroalgae where the seven species of noxious dinoflagellates were found.

ANOVA/MANOVA was applied to the abundance data for *P. lima* and *G. toxicus* between transects, sampling stations, and months, showing no significant differences (p>0.05) among transects. However, for *P. lima*, there are significant differences (p>0.05) between stations and months. LSD analysis showed that Sampling Station No. 5 differed of the other ones during May and October in the first study period and in June during the second cycle. *G. toxicus* showed significant differences (p>0.05) in the months of May and June (1999-2000) and May (2001-2002).

For 1999-2000, significant and positive correlations were observed in Component I of the variables (nutrients and temperature) and negative correlations with salinity; this suggests an effect related to the contribution of fresh water. In Component II, both dinoflagellates species have similar responses to environmental variables**.** Therefore, we suggest that the conditions facilitate development of both species (Fig. 4A). In 2001-2002, abundance and distribution was similar to the previous period. Nutrients are positively and significantly associated with Component I (except for nitrite) and negatively associated with salinity. The same was observed for the Component II, where the two dinoflagellate species are significantly associated (Fig. 4B).

DISCUSSION

Most Cuban industries and population settlements are located near rivers using it for disposal of wastewater. The rivers introduce elevated amounts of nitrogen and phosphorus

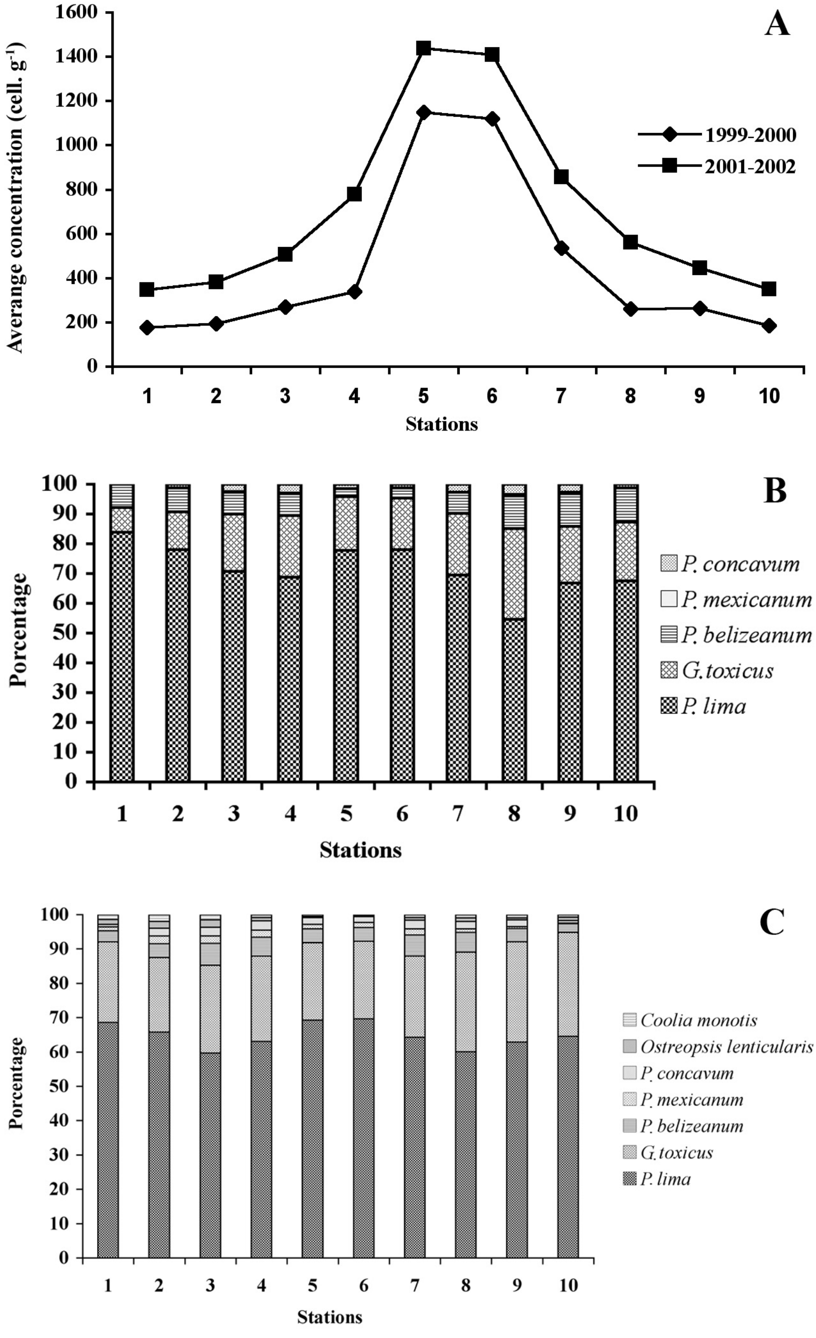

Fig. 1. A. Distribution and average abundance of harmful dinoflagellates found at sampling stations (March 1999-2000 to March 2001-2002). B. Percentage of harmful dinoflagellates at each station by species from March 1999 to March 2000. C. Percentage of harmful dinoflagellates in each station by species from March 2001 to March 2002.

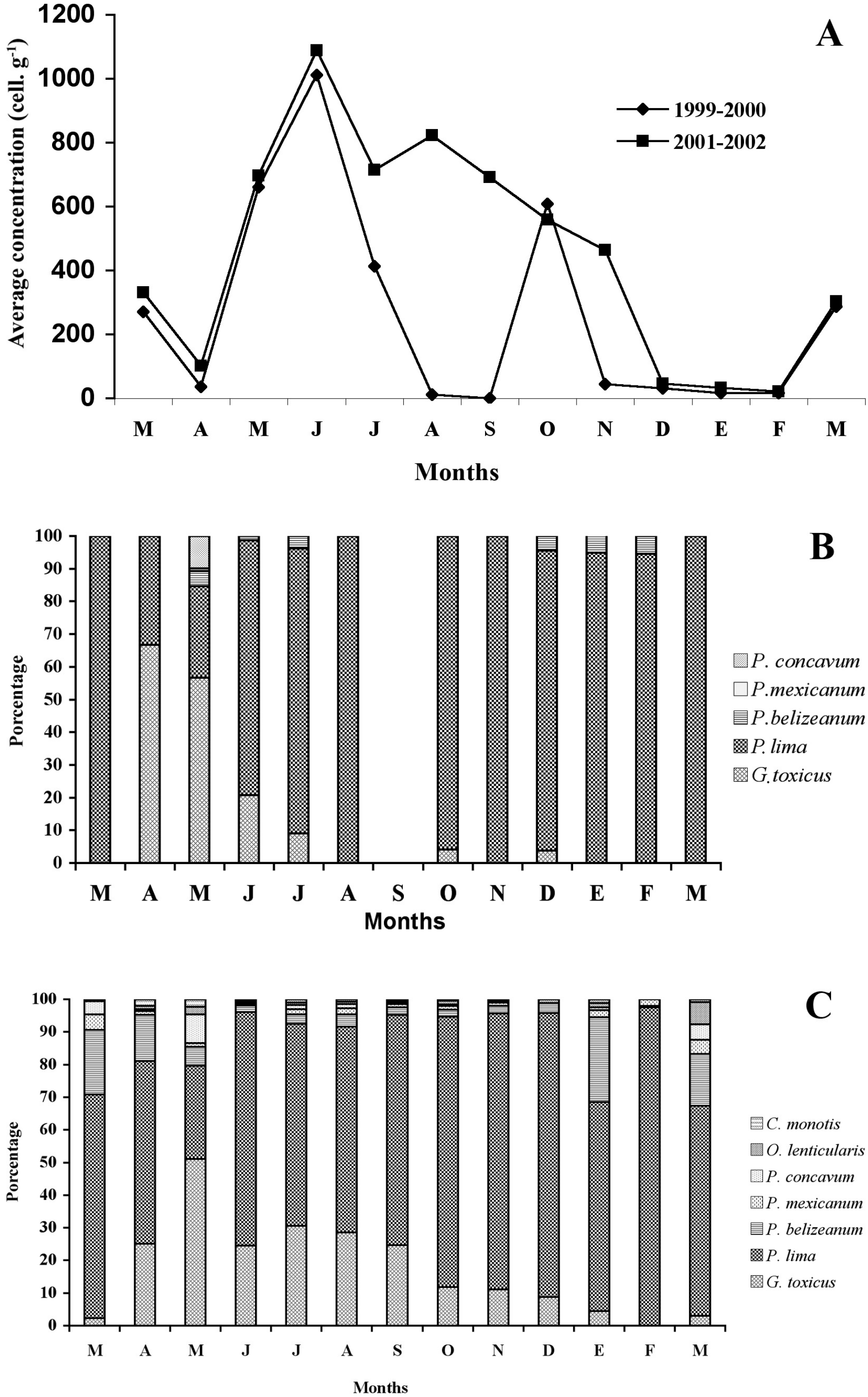

Fig. 2. A. Monthly average abundance of harmful dinoflagellates from March 1999-2000 to March 2001-2002. B. Percentage of harmful dinoflagellates species by month from March 1999 to March 2000. C. Percentage of harmful dinoflagellates species by month from March 2001 to March 2002.

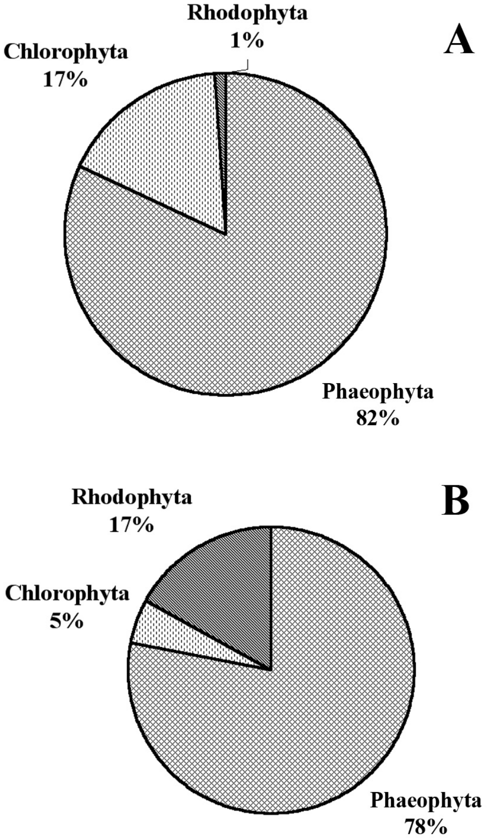

Fig. 3. A. Percentage of *P. lima* in each group of macroalgae (Phaeophyta, Chloropyta, and Rhodophyta). B. Percentage of *G. toxicus* in each group of macroalgae (Phaeophyta, Chloropyta, and Rhodophyta).

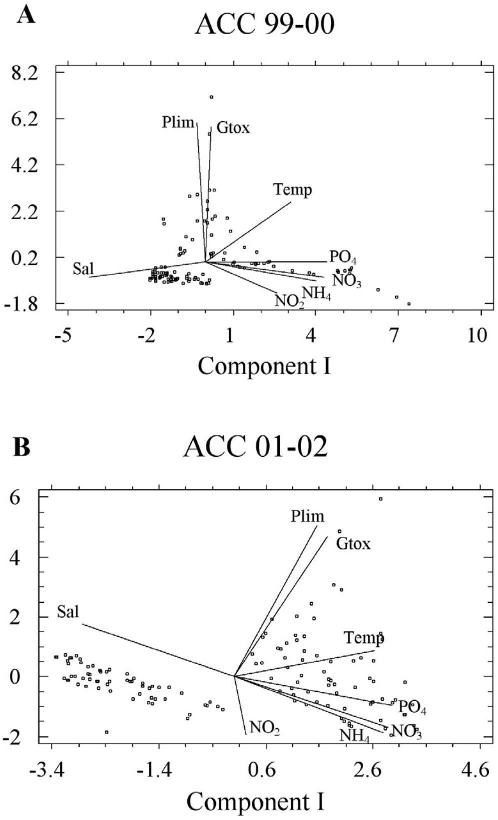

Fig. 4. A. Relationship between *P. lima* and *G. toxicus* abundance and physical-chemical variables obtained from Principal Component Analysis of data during 1999-2000. B. Relationship between *P. lima* and *G. toxicus* abundance and physical-chemical variables obtained from Principal Component Analysis of data during 2001-2002.

along the coast, creating a deleterious effect on bays and coastal lagoons (González 1990). The large quantities of nitrates and nitrates found in the Jaimanitas River has also been demonstrated by Montalvo *et al*. (2001), as was the case for large quantities of phosphorus, considered representative of eutrophic areas (D'Avanzo 1994). Similar examples of nitrogen compounds arise from human activity in many Caribbean coast (Schaffalke 1999).

All the dinoflagellates species found in this study have been reported by Morton and Faust (1997) as potentially toxic along the Belize coast, and probably associated with outbreaks of ciguatera. Abundance of these species is within the range reported by Gillespie *et al*. (1985), Bagnis *et al*. (1988), McCaffrey *et al*. (1990). For the Pacific coast, Taylor and Gustavson (1986), Carlson and Tindall (1985) have reported similar results; Morton and Faust (1997) reported on the Caribbean and Florida keys. Their abundance is not higher than those reported for the Gambier Islands in the southeast Pacific Ocean (Yasumoto *et al*. 1980, Bomber *et al*. 1989).

High harmful dinoflagellates concentrations in May and October are related to physicochemical conditions in this area, influenced by large quantities of nutrients introduced by the Jaimanitas River during the summer rainy season. Higher water temperatures, nutrient concentrations, and increased water transparency are factors promoting the growth of macroalgae and epiphytic dinoflagellates. In the western Caribbean, Taylor and Gustavson (1986) found wide distribution of *G. toxicus* except at those locations receiving significant freshwater, similar to what was found in this study, since *G. toxicus* was more abundant farther offshore. Lower abundance seems to be related to heavy rainfall (338 mm) in August and September 1999 from decreasing salinity and water transparency. The heavy rain disturbed the substrate; in the area near of the mouth of the Jaimanitas River, only filamentous algae (*Chaetomorpha*) remained, while at greater distance offshore, there were some patches of *Padina* sp. All macroalgae showed that sediment deposition probably limited the growth of dinoflagellates. *G. toxicus* cannot produce cysts under growth-limiting conditions, but *P. lima* does; hence, once the disturbance passed, *P. lima* is able to colonize the macroalgae, as occurred in October, when this species representing 96% of the dinoflagellates counted.

In the absence of heavy rain, *G. toxicus* was abundant under weak wind conditions, calm seas, and increased water transparency (Popowski *et al*. 2001) in the vicinity of Havana (May to August). Turket *et al*. (1998) found low abundance of *G. toxicus* in the Reunion Islands when strong winds produced heavy waves. Low concentrations seem to be characteristic when cold fronts reach the northern coast of Cuba from November to February. Bordeaux and Durant-Clement (1991) suggest that cold fronts, increase of water turbidity, and currents limit the growth of this species, as also shown in this study.

In our study, *G. toxicus* was more abundant in May during the first study year and May-June during the second year, both occurring with the beginning of the rainy season and the end of the winter season. Under the conditions in May, the increase of epiphytic dinoflagellates probably depends on several environmental factors acting at the same time (Carlson and Tindall 1985).

The higher abundance of potentially noxious dinoflagellates occur during the summer (Valdés *et al*. 1992), when, according to Cuban Public Health Ministry statistics, most cases of ciguatera poisoning occur, most of them in this region. Carrera and Castro (2002) also determined that, even though the disease is present throughout the year, its incidence increases from March through October.

Prorocentrum is one of the most important genera among dinoflagellates because many species are toxic or potentially toxic and are widespread. In this area, *P. lima* predominates in abundance and spatial-temporal distribution of toxic dinoflagellates and is perennially present. Bomber *et al*. (1985) reported this species during the entire year in the Florida Keys, being more abundant from November to May. Heil *et al*. (1998) reported that this species is the most abundant along Australian shores and is associated with ciguatera.

Yasumoto *et al*. (1980) and Grzebyk *et al*. (1994) hold the view that *G. toxicus* develops best on Rhodophyta; however in this area, *G. toxicus* and *P. lima* were found on three macroalgal groups, and were most abundant on Phaeophyta. Heil *et al*. (1998) reports that, in Australian waters, *P. lima* grows on brown algae. In an artificial bay in northwestern Cuba, Delgado *et al*. (2000) and Popowski *et al*. (2001) report the preference of this dinoflagellate for brown algae. Saint-Martin *et al*. (1988) suggest that the preference of *G. toxicus* for macroalgae is independent of macroalgal phylum.

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RESUMEN

Se estudió la abundancia espacial y temporal de dinoflagelados epífitos asociados a la ciguatera durante dos ciclos anuales (marzo 1999 a marzo 2000, y marzo 2001 a marzo 2002) en la costa del noroeste de Cuba. Recolectamos 14 especies de macroalgas (Phaeophyta, Chlorophyta y Rhodophyta) y obtuvimos 1340 muestras. Identificamos siete especies de dinoflagelados potencialmente nocivas, cinco de ellas nuevos registros para el fitobentos cubano: *Prorocentrum belizeanum* Faust, P. *concavum* Fukuyo*, P. mexicanum* Tafall*, Coolia monotis* Meunier, y *Ostreopsis lenticulares* Fukuyo. El análisis de ANOVA/MANOVA mostró diferencias espaciales significativas: la abundancia celular más baja se encontró cerca de la desembocadura del río y la más alta en el área más profunda. *Prorocentrum lima* (Ehrenberg) Dodge*,* se encontró principalmente sobre las Phaeophyta seguido de las Chlorophyta y Rhodophyta. *Gambierdiscus toxicus* se encontró principalmente sobre las Phaeophyta seguido de las Rhodophyta y Chlorophyta. Todas las especies halladas en el área del estudio estaban sobre *Padina* spp. (Phaeophyta). No se encontró ninguna especie de dinoflagelado sobre *Acanthophora spicifera* (Rhodophyta). Las condiciones ambientales en verano (temperatura, nutrientes, transparencia de agua y la baja velocidad del viento) favorecen el desarrollo de las macroalgas, siendo un substrato adecuado para el desarrollo de dinoflagelados potencialmente nocivos, y posiblemente el vector principal para la extensión de la ciguatera en la costa noroeste cubana.

Palabras clave: dinoflagelados bentónicos, abundancia, Cuba, ciguatera.

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