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SURVEY OF BENTHIC DINOFLAGELLATES ASSOCIATED TO BEDS OF *Thalassia testudinum* IN SAN ANDRÉS ISLAND, SEAFLOWER BIOSPHERE RESERVE, CARIBBEAN COLOMBIA.

Evaluación de dinoflagelados bénticos asociados a praderas de *Thalassia testudinum* en San Andrés Isla, Reserva Internacional *Seaflower*, Caribe colombiano.

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ABSTRACT

In order to determine the occurrence of epiphytic toxic dinoflagellates in the coastal waters of San Andrés Island, Caribbean Colombia, we analyzed the seagrass beds on the northern and eastern sides of the island. We found seven species of toxicogenic dinoflagellates, belonging to the genera *Prorocentrum* and *Ostreopsis*. The cell densities were generally low if compared with previous studies in other Caribbean sites, ranging from 0 to 836 cells/dry weight. The species encountered are known to produce toxins causing the Diarrehic Shellfish Poisoning and Ciguatera, poisonings which have been documented in the island.

Key words: Epiphytes; macroalgae; *Ostreopsis*; *Prorocentrum*; Toxic dinoflagellates.

RESUMEN

Con el objetivo de determinar la presencia de dinoflagelados tóxicos epifitos en aguas costeras de la isla de San Andrés, Caribe colombiano, se analizaron las praderas de pastos marinos de los sectores norte y oriente de la isla. Se encontraron siete especies toxinogénicas de dinoflagelados pertenecientes a los géneros *Prorocentrum* y *Ostreopsis*. Las densidades celulares fueron bajas comparadas con estudios en otros sitios del Caribe, con rangos entre 0 y 836 cel./peso seco. Las especies encontradas son conocidas por producir toxinas que causan diarrea (PSP) y Ciguatera, intoxicaciones que ya han sido documentadas en la isla.

Palabras clave: epifitos; macroalgas; *Ostreopsis*; *Prorocentrum*; dinoflagelados tóxicos.

INTRODUCTION

Harmful algal blooms (HABs), an overgrowth of microscopic algae with deleterious effects on plants, animals and/or humans, are a natural occurring phenomenon known for centuries, if not millennia (Freer and Vargas, 2003; Hallegraeff *et al*., 2003), typical of tropical and subtropical oceans. However, in the past two decades these events seem to have increased in frequency, intensity and geographical distribution (Hallegraeff *et al*., 2003), partially because of eutrophication, change in the hydrodynamics of the water bodies and ballast water transport to new locations (UNEP/FAO, 1996; Hallegraeff, 1998; Heisler *et al*. 2008; Sellner and Doucette 2003; Masó and Garcés, 2006; Parson and Preskitt, 2007; Smayda,2007).

Actually there are more than 135 recognized species of microalgae (cyanobacteria, dinoflagellates and diatoms) known to cause HABs, with deleterious impacts on marine environment, human health, aquaculture and tourism (Hoagland and Scatasta, 2006). These species may be planktonic or tychoplanktonic (benthic/epiphytic) (Sierra-Beltrán *et al*., 2005), and many of them produce toxins, which accumulate through the food web and, if consumed by humans, may cause several types of intoxications even when their cell number in the water is relatively low (Masó and Garcés, 2006).

Benthic dinoflagellates are among the organisms involved in these events. Several studies have been addressed to determine the species composition of benthic dinoflagellates in tropical marine waters. Since earlier studies have shown that these organisms preferentially attach to macroalgae, most studies have been directed to analyze these substrates (thoroughly reviewed in Cruz-Rivera and Villareal, 2006). Few studies (e.g. Ballantine *et al*., 1985; Foden *et al*., 2005; Corlett and Jones, 2007; Maranda *et al*., 2007) have been addressed in evaluating seagrass beds as a possible source of toxic dinoflagellates.

San Andrés, a small island off the coast of Nicaragua, is part of the Colombian Biosphere Reserve "Seaflower". It harbors about 5'062.400 m^2 of seagrass beds, mainly composed of the species *Thalassia testudinum* Banks ex König and *Syringodium filiforme* Kützing in Hohenacker. The beds are quite heterogenic, with great variability in density, composition and conservation state (Ángel and Polania, 2001).

Since HABs events represent a potential natural hazard to the tourism, the main income to San Andrés, as it has been reported recently (Carreño and Mera, 2008), and taking into account the extension of these beds, we decided to survey the epiphytic dinoflagellate community present in the seagrass beds in the island of San Andrés, to determine their species composition, if there are potentially toxic taxa and if they show any host preference. This is the first study on benthic dinoflagellates in the island.

MATERIALS AND METHODS

STUDY AREA

The island of San Andrés is part of the archipelago of San Andrés, Old Providence and Santa Catalina, declared International Biosphere Reserve SEAFLOWER in 2000 by UNESCO. The island has an area of 25 km^2 (Fig. 1), and is situated in the Caribbean

Sea, between 12°28' and 12°36' N and 81°40' and 81°44' W, 240 km off the coast of Nicaragua (IGAC, 1986).
The island, and the rest of the archipelago, is volcanic in origin. The subsidence of the volcanic base, and its simultaneous cover with calcareous deposits, biogenic in origin, during the Tertiary and Quaternary, gave rise to the present island (Diaz *et al*., 1996).
The marine platform on the oriental side is shallow, and gets to the coral reefs in the open ocean, which mitigate the waves. On this submarine terrace there are deposits of corals, coralline algae, urchins and other animals, which produce calcareous sand (Geister, 1975).

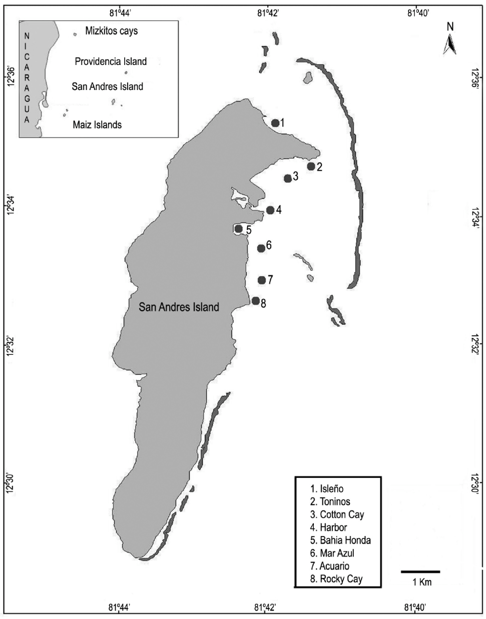

Figure 1. Island of San Andrés in the Caribbean Sea showing sample sites along the Northern and Eastern coast of the Island.

On the west side of the island, the transition is much more abrupt, with cliffs indicating strong marine erosion (IGAC, 1986).
The island lies in the transition zone between tropical dry and tropical wet climate. The influence of trade winds mitigates the dry and warm climate. The annual mean temperature is 27.4 °C, with maximum values between 29 and 30 °C (May-June) and minimum between 25.5 y 26.0 °C (December-February). The annual mean precipitation is 1797.8 mm, unevenly distributed in a dry season (January-April), with stronger winds, and a wet season (October -December) when 80% of the annual rain falls. During the period May-July the rains are moderate in intensity (IDEAM, 1995).
Along the northeast coast of the island the currents are slow, with the maximum speed (10 m/min) close to the reef. In the protected areas the current velocity does not exceed 4-5 m/min, with the same wind conditions (Garay *et al*., 1988).
The Archipelago is influenced by the Caribbean current which originates temporal convergent whirlpools affecting the distribution and concentration of plankton and associated nutrients (Garay *et al*., 1988; Garay and Castro, 1993).

The seagrass beds object of the present study are situated in the area confined by the coral reefs, in a large shallow lagoon. The water dynamics in the lagoon is quite slow, so the elimination and decomposition of the organic material is slow, favoring bioaccumulation processes in the sediments.

Experimental Design and Site Selection

Sample collection was undertaken in June and July 2007. We chose two sites inshore and six offshore (Fig. 1). Three of the offshore sites (Cotton Cay, Isleño, Mar Azul) are CARICOMP sites, while the other sites were chosen to cover all kinds of seagrass beds of the island. The main characteristics of the sites are the following:

1. Rocky Cay. 34 m from the coast, the major anthropogenic impact on this bed is due to hotel activity. According to Ángel and Polania, 2001, the beds of this area were the least disturbed (at least for the year of their study).
2. Bahía Honda. 27 m offshore, the bay is surrounded by mangroves. Until few years ago, the power plant of the island was here.
3. Isleño. 240 m offshore, in front of the most popular beach of the island. However, the bed is not in the area of main boat traffic, and sits aside the zone used by the tourists.
4. Toninos. 507 m offshore, there are some docks for small boats.
5. Cotton Cay. 648 m offshore, very shallow area with major boat traffic. Ángel and Polania, 2001, found that the leaves of *T. testudinum* were often discolored, and had a minor density if compared to other sites.
6. Harbor. 298 m offshore, site near to the harbor built in 1966, where boat traffic is quite heavy.
7. Mar Azul, 481 m offshore, there is heavy traffic of small boats to transport tourist to Haynes Cay (the Aquarium), one of the most visited places in the island. In this part of the island there are not many houses due to the presence of the Army. There is an ample bed, mainly of *T. testudinum* and *S. filiforme*, forming monospecific or mixed beds up to 7 m deep (Bolaños and Abril, 2007).
8. Acuario. 536 m offshore, is the closest point to Haynes Cay, where tourists may snorkel.

Sample Collection

In the two inshore sites (Rocky Cay and Bahía Honda), we set two 10 m transects perpendicular to the coast; along each transect, we placed three 20 cm x 25 cm quadrants at 0, 5 and 10 m from the coast. All the material inside the quadrant was collected. In the offshore stations, no transect was placed. Three quadrants were haphazardly placed in the bed and all the material was extracted as in the inshore stations.

During sampling the site coordinate were registered (GPS Garmin 12XL), as well as depth (SpeedTech - Instruments 400 kHz), transparency (Secchi disk), salinity and superficial temperature (SCHOTT LF1 conductimeter).

The plant cover was evaluated with 20 x 25 cm quadrants, and from this area all the plant material above ground was carefully extracted. Seagrass and macroalgae were separated in situ, each macroalga was identified when possible in the field and placed in a separate bag. Identification was confirmed in the lab with the use of available literature (Littler and Littler, 2000). All material was placed in ziploc bags and refrigerate until the laboratory. In each station, a sample of the superficial sediment was also taken to carry out organic matter content and the granulometric analysis. To compare the inshore and offshore stations, we used the mean of the points in each transect which, according to the non-parametric test of Mann Withney, were homogeneous.

Sample Processing

Samples of seagrass and macroalgae were carefully rinsed with filtered seawater to eliminate excess of sediment. Each species was placed in a separate bag with 250 mL of a solution composed of 50% filtered seawater and 50% Transeau (6 parts of water, 3 parts of 90% ethanol, 1 part of 100% formalin). The bags were agitated vigorously for 5 minutes to guarantee that all epiphytic microalgae left the substrate. The water of each bag was filtered through a 50 µm net; the filtered water was let standing for several days, alter which we collected all the deposit and enough supernatant to fill a 10 mL bottle.

Once the epiphytes were removed from the seagrasses and the macroalgae, these substrates were dried at 60 °C in an oven until constant weight. For *Thalassia testudinum*, we additionally determined the epiphytic content for gravimetry and measured the leaf area before drying.

For the granulometric analysis of the sediment, we used the quality range USDA. To determine the organic content, 100 g of sediment from each site was dried at 80 °C until constant weight. The organic content was determined burning 5 g of sediment at 550 °C for four hours.

Dinoflagellates

All the dinoflagellates in the samples were quantified with an optical microscope (OLYMPUS 9E 03069), at 10X. Species determination was carried out at 100X. When the cellular content was not distorting the image, we used an optical microscope with phase contrast (Nikon Eclipse E 600). The preliminary identifications were done using the available literature (Faust, 1990; Faust, 1993a; Faust, 1994; Faust, 1995; Faust and Gulledge, 2002; Faust *et al*., 1999; Morton *et al*., 2002). Our identifications were confirmed by the dinoflagellate taxonomist Dr. Arturo Sierra-Beltran from CIBNOR, Mexico.

The total number of dinoflagellates was calculated multiplying the number of microalgae counted in 0,2 mL for the total volume of water previously concentrated. The obtained value was then divided by the grams of dry weight of vegetal material of each quadrant. For each site, the reported number is the mean value of the quadrants with its standard deviation.

Statistical Analysis

To determine the dinoflagellates distribution pattern and the relationships with the environmental variables in each site, we used the non-metric multivariant analysis (NMS), with the Sorensen distance measure (PC-ORD version 3.17). To evaluate the homogeneity among substrates and their dinoflagellates content, we used the non parametric test of Kruskal Wallis (Statgraphics plus 5.1).

RESULTS

Characteristics of the Seagrass Beds

The beds under study were composed predominantly by *Thalassia testudinum* and

Halimeda spp., as dominant associated macroalga. Only in the site Isleño we did not find Halimeda (Fig. 2). This site differed also in having a substrate with fine sand, less plant cover and more homogeneity in the substrate evaluated (Table 1).

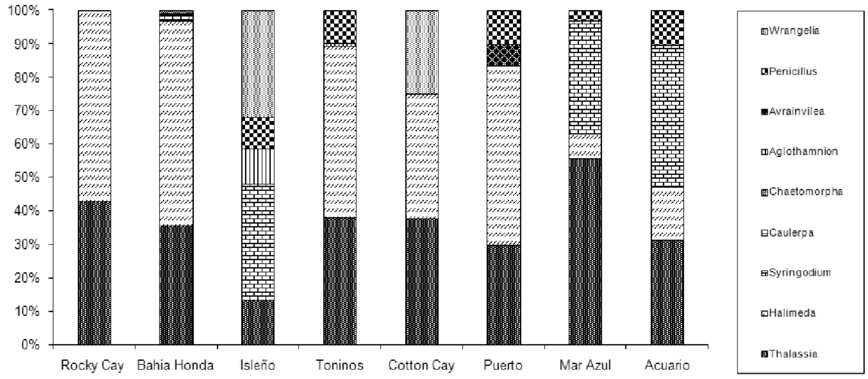

Figure 2. Biotic substrate composition in each of the sampled sites in San Andrés island.

Site	Geographic coordinates	Salinity (‰)	Depth (m)	Transparency (m)	Leaf area of *T. testudinum* (cm^2)	Organic matter (%) in the sediment	Sediment texture	Plant coverage (%)
Rocky Cay	12°32'36.40"N 81°42'16.20"W	37	0.97	0.81	17.77±8.18	6.4	sandy loam	75
Bahía Honda	12°33'42.00"N 81°42'26.22"W	37	0.47	0.41	22.7±14.25	27.29	sandy clay	100
Isleño	12°35'37.44"N 81°41'49.38"W	33	7.32	7.32	14.56±6.59	5.17	sandy clay loam	25
Toninos	12°34'38.44"N 81°41'24.68"W	33	2.13	2.13	12.88±5.53	5.06	sandy loam	80
Cotton Cay	12°33'34.56"N 81°41'58.56"W	35	3.96	3.96	16.57±8.87	5.64	sandy loam	95
Harbor	12°34'00.22"N 82°41'59.40"W	33	3.66	3.66	13.85±5.55	3.97	sandy loam	65
Mar Azul	12°34'29.44"N 81°41'36.06"W	35	7.92	7.92	16.38±7.4	4.17	sandy loam	55
Acuario	12°33'0.80" N 81°41'59.76"W	35	2.44	2.44	19.25±7.71	3.93	sandy loam	65

Table 1. Geographic coordinates and characteristics of the sampled sites.

The other sites had sediments composed of medium sized slime sand with the exception of Bahía Honda, where the sand is fine.
The inshore sites (Bahía Honda and Rocky Cay) were shallower and the water was more turbid, compared with the offshore sites. Moreover, the macroalgal cover was inferior to the offshore sites. Bahía Honda had the highest values of organic material content in the sediments, as well as the biggest leaf area in *T. testudinum* (Table 1).

Dinoflagellates Analysis

In the eight sites under study, we found 10 morphospecies of dinoflagellates, distributed in three genera and eight species (Fig. 3). Seven of these species are toxicogenic, the only species for which, to date, no toxin has been reported is *Sinophysis microcephala* Nie and C. Wang .

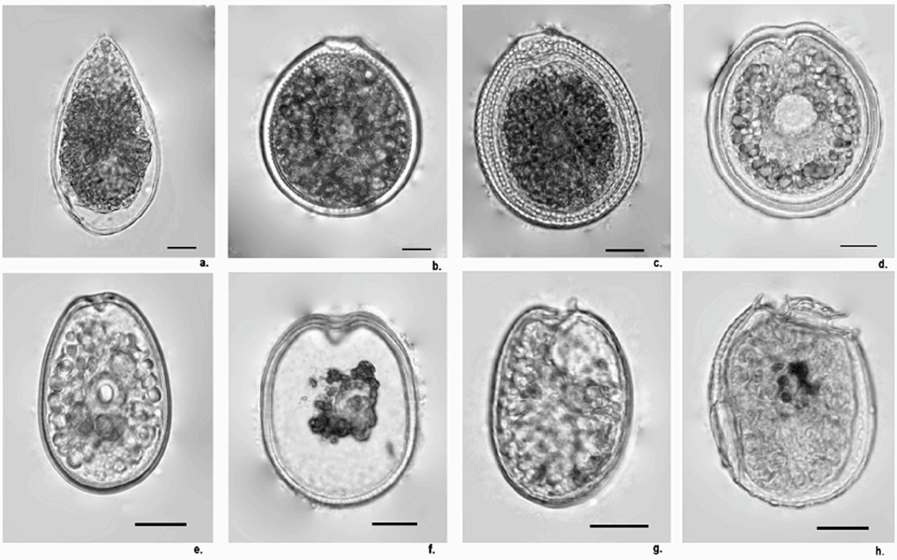

Figure 3. Dinoflagellates species found in this study associated to beds of *Thalassia testudinum* in San Andres Island. All scale bars=10 µm. a. *Ostreopsis ovata*, b. *Prorocentrum belizeanum*, c. *P. ermarginatum*, d. *P. hoffmannianum*, e. *P. lima*, f. *P. maculosum*, g. *P. rathymum*, h. *Sinophysis microcephala.*

All species, with exception of *Ostreopsis ovata* Fukuyo, absent in the Harbor, and *Prorocentrum emarginatum* Fukuyo, found only in Rocky Cay, were present at all sites, with abundances varying from 0 to 168 cells/dry weight (Table 2). The site where the dinoflagellates had their lowest abundance was Rocky Cay, where the most abundant species, *Ostreopsis ovata*, had a density of 23 cells/dry weight. On the other side, Bahía Honda generally had the highest values for all species, ranging from 23 to 168 cells/dry weight. The only exception to this pattern is the species *Prorocentrum lima* (Ehrenberg) Stein, which was found at densities of 5 cells/dry weight. At the other sites, *P. lima* was more abundant, ranging between 6 and 59 cells/dry weight (Table 2).

The microalgal flora epiphytic in the seagrass beds we evaluated was dominated by diatoms, with the exception of Bahía Honda and Harbor, where cyanophytes predominated. The dinoflagellates were the less abundant group at all sites, never exceeding the 15% of the total microalgal flora (Table 3). For the non-dinoflagellate taxa, the genus *Achnantes* was predominant in Rocky Cay and Mar Azul; Toninos and Harbor; Diploneis in Cotton Cay, *Synedra* in Acuario and *Oscillatoria* in Bahía Honda (Table 3). We also found, although less abundant, the genera *Licmophora* and *Fragilaria*. These two genera, together with *Achnantes* have been previously reported as *Prorocentrum lima* companion (Maranda *et al*., 2007).

The NMDS analysis shows a differential distribution of dinoflagellates, where we can divide the 8 sites in four groups, the first formed by Bahía Honda (Group A), with the

	Rocky Cay	Bahía Honda	Isleño	Toninos	Cotton Cay	Harbor	Mar Azul	Acuario
Ostreopsis ovata	23±41	110±177	13±5	1	8±8	-	2	5±4
Prorocentrum belizeanum	13±8	50±55	15±11	29±21	22±10	12±6	19±6	18±6
P. emarginatum	3±1	-	-	-	-	-	-	-
P. hoffmannianum	9±6	157±110	13±2	9±5	7±5	3±1	11±9	4±1
P. lima	5±3	5±6	59±39	17±15	45±19	6±4	20±4	27±14
P. maculosum	5±3	168±102	36±35	36±26	33±15	21±11	31±21	24±7
P. rathymum	2±2	130±106	17	9±3	11±10	4±3	2±1	14±6
Sinophysis microcephala	4±2	23±27	2±1	4±2	12±2	4±1	3±3	6±4
Total	65±6	624±69	171±19	102±8	125±13	49±4	88±8	104±8

Table 2. Mean and standard deviation of the of dinoflagellate species density (cells /grams dry weight) in each sampled sites.

greatest abundance of dinoflagellates, Harbor (Group C) and Rocky Cay (Group D) with the least concentration, and the other sites forming an intermediate group (B) (Fig. 4). We quantify the ordination total utility according with the stress values. The stress value we obtained was lower than 0.05 and the explained 77% of r2 between the original sample and the similarity matrix.

Host Preference

We found 12 biotic substrates, two species of seagrass and 10 specie of macroalgae belonging to 7 genera (Table 4). The species encountered only in one site were not included in the statistical analysis.

The number of dinoflagellates found on *Caulerpa sertularioides* (Gmelin) Howe (2223 cells/g) (Table 4) is at least of an order of magnitude higher than on the other substrates. Nevertheless, since this species was only encountered in one site, we did not include it in the Kruskal Wallis analysis.

In general terms, there is no evident host preference by any species of dinoflagellate. *Prorocentrum belizeanum* Faust was the only species to use *Avrainvillea longicaulis* (Kützing) Murray and Boodle as a host, and *P. emarginatum* was found exclusively on *Thalassia testudinum*. For the other species, although they were more abundant on some species

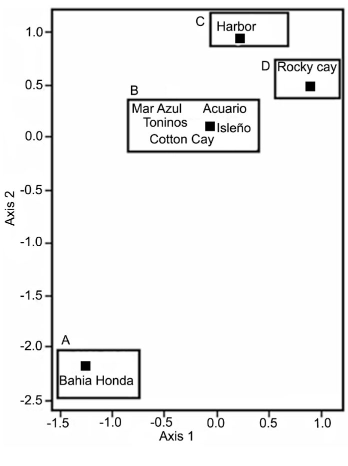

Figure 4. Graphic representation in two dimensions of the association between species distribution and sampling sites.

	Rocky Cay	Bahía Honda	Isleño	Toninos	Cotton Cay	Harbor	Mar Azul	Acuario
DIVISIÓN								
Bacillariophyta	27856±9115	69425±42736	1269456±940402	257377±302662	110648±73758	57314±24514	162241±91307	164660±134157
Cyanophyta	1111±939	303333±116851	369139±145223	104823±79607	46289±34464	249355±81301	79313±14153	46586±50573
Dinophyta	5183±4593	42680±18718	12765±12491	12431±3883	17804±3195	9373±3849	13882±4892	7098±5384
GÉNEROS								
Achnantes	10875±3551						10568±12160	
Climacosphenia			863198±570320					
Diploneis					16640±8962			
Oscillatoria		227790±157368		93020±82384		139493±128926		
Synedra								12471±4385

Table 3. Mean and standard deviation of the epiphytic assemblage density (cells/m^2) along the site sampled.

Biotic substrate	Sites	Total	*O. ovata*	*P. belizeanum*	*P. emarginatum*	*P. hoffmannianum*	*P. lima*	*P. maculosum*	*P. rathymum*	*S. microcephala*
Aglaothamnion sp. (2)	Isleño, Toninos	261±49	7±3	40±19	0	40±15	55±20	142±26	0	2±1
Avrainvillea longicaulis (1)	Harbor	4	0	4	0	0	0	0	0	0
Caulerpa sertularioides (1)	Bahia Honda	2223	594	0	0	792	0	0	836	0
Chaetomorpha sp. (1)	Bahia Honda	282	48	0	0	124	0	0	110	0
Halimeda incrassata (15)	Rocky Cay	52±102	42±91	14±15	0	3±3	7±3	5±3	3±1	2±1
H. monile (7)	Toninos, Harbor, Mar Azul, Acuario	20±19	1±2	10±10	0	3±2	2±2	10±7	2±2	2±1
H. opuntia f. *trilobata* (9)	Bahia Honda	189±226	56±50	16±22	0	62±74	12±16	0	71±102	1±1
Penicillus capitatus (4)	Harbor, Acuario	82±79	4±6	22±15	0	3±4	16±15	30±38	13±7	3±1
P. lamourouxii (6)	Isleño, Toninos, Harbor,									
	Mar Azul	120±102	5±5	36±37	0	5±3	86±73	41±24	8±12	0
Syringodium filiforme (11)	Bahia Honda, Isleño, Mar Azul, Acuario	133±207	24±11	57±80	0	13±12	5±7	72±136	18±13	51±7
Thalassia testudinum (51)	Todas las estaciones	189±231	9±9	14±11	3±1	95±98	12±15	95±101	84±99	9±10
Wrangelia sp. (5)	Cotton Cay, Isleño	144±66	12±16	24±17	0	9±9	53±32	45±23	22±22	6±4

Table 4. Mean and standard deviation of dinoflagellates density (cells /grams dry weight) on the different biotic substrate. The parentesis number after the substrate species is the number of sampled examined.

(*Ostreopsis ovata*, *Prorocentrum hoffmannianum* Faust and *P. rathymum* Loeblich, Shirley and Schmidt on *Caulerpa sertularioides, P. belizeanum* on *Syringodium filiforme*), the difference is not significant.

DISCUSSION

Along all the sites visited, the dinoflagellates were not the most abundant group of benthic microalgae. The benthic microflora was dominated by diatoms and cyanobacteria. Only in Bahía Honda, the dinoflagellates constituted a little more than 10% of all benthic phytoplankton (Table 3). The number of benthic dinoflagellate species we encountered is comparable to values reported by other studies (Faust, 1995; Landsberg *et al*., 2005; Parson and Preskitt, 2007). Considering that we sampled only during two months, the richness of the microflora in the seagrass habitats under study is undoubtedly underestimated. However, it gives us a good approximation of the species present in this period of the year. Six of the eight species encountered belong to *Prorocentrum*, a large genus containing <50 described species (Steidinger and Tangen, 1997). Although most species are planktonic, the majority of toxic taxa are tychoplanktonic (Morton *et al*., 2002). *Prorocentrum belizeanum* is a pantropical species (Faust and Gulledge, 2002), reported in the Caribbean Sea (Faust 1993a; Delgado *et al*., 2002), Pacific (Okolodkov and Garate-Lizarraga, 2006; Parson and Preskitt, 2007) and Indian Ocean (Turquet *et al*., 1998). Usually is associated with floating detritus (Faust, 1993a). *P. emarginatum* is an epibenthic species on macroalgae, common in the Pacific Ocean (Taylor *et al*., 2003; Okolodkov and Garate-Lizarraga, 2006; Parson and Preskitt, 2007), and Caribbean (Faust, 2004). *P. hoffmannianum* and *P. maculosum* Faust were originally described from mangrove habitats in Belize (Faust, 1990; Faust, 1993a), and are considered common species in the Caribbean (Faust and Gulledge, 2002). *P. hoffmannianum* has been reported in Hawaii as well (Parson and Preskitt, 2007). *P. lima* is a cosmopolitan species, found from boreal to tropical waters around the world (Faust and Gulledge, 2002; Foden *et al*., 2005; Okolodkov, 2005; Heredia-Tapia *et al*., 2002; Maranda *et al*., 2007; Parson and Preskitt, 2007; Taylor *et al*., 2003). *P. rathymum* has long been considered a synonim of *P. mexicanum*, a common species found in tropical and subtropical waters in the Atlantic and Pacific Ocean (Faust and Gulledge, 2002; Levasseur *et al*., 2003). However, Cortés-Altamirano and Sierra Beltrán, 2003, offered evidence that these taxa are different species. Since true *P. mexicanum* is planktonic, the records of benthic *P. mexicanum* should be considered as *P. rathymum*.

The other two species of dinoflagellates reported in the present paper are *Ostreopsis ovata*, a species found in Pacific and Indian Oceans as well as in Caribbean waters (Faust and Gulledge, 2002; Okolodkov and Garate-Lizarraga, 2006, Parson and Preskitt, 2007), in the Mediterranean Sea (Aligizaki and Nikolaidis, 2006; Monti *et al*., 2007; Tognetto *et al*., 1995; Aligizaki *et al*., 2008) and, more recently, along the subtropical coast of New Zealand (Chang *et al*., 2000); and *Sinophysis microcephala*, a tropical species in Pacific and Caribbean waters (Faust 1993b; Selina and Hoppenrath, 2004; Okolodkov and Garate-Lizarraga, 2006).

All species reported here, therefore, fall well in their known range of distribution. Nevertheless, they are new records for the Archipelago, since there are no previous studies for the region.

Seven of the eight encountered species are (potentially) toxicogenic. All species of *Prorocentrum* we encountered are reported as toxicogenic (Table 3), known to produce okadaic acid and its derivatives, responsible of diarrheic shellfish poisoning (Marr *et al*., 1992; Koike *et al*., 1998; Pavela-Vrancic *et al*., 2002; Masó and Garcés, 2006) and maybe involved in ciguatera fish poisoning (Morton *et al*., 2002). *Ostreopsis ovata* produces palytoxin-equivalent (Riobó *et al*., 2006; Ciminiello *et al*., 2007), involved in ciguatera-like fish poisoning (Katircio lu *et al*., 2001), and causing respiratory and skin irritations (Simoni *et al*., 2004).

The dinoflagellates were found epiphytic on all the collected substrates (Table 4), although some species were absent from some algae. *P. emarginatum* was found exclusively on *Thalassia testudinum*. *O. ovata, P. hoffmannianum* and *P. rathymum* were found in greatest abundance on *Caulerpa sertularioides*, while the other species were absent from this alga. These three species were found associated also on *Chaetomorpha* cf. *crassa*, where they were present in great density as well, if compared to the other substrates. The association of these three taxa has not been reported before, whilst in several studies *Ostreopsis ovata* has been found associated with *Prorocentrum lima*, *P. compressum* and *Coolia monotis*, (Chang *et al*., 2000), with *Gambierdiscus toxicus* and *Ostreopsis lenticularis* (Ballantine *et al*., 1985), or with *P. lima*, *C. monotis* and *Coscinodiscus* sp. (Vila *et al*., 2001). *P. maculosum* showed its greatest abundance on *Aglaothamnion* sp., followed for *Thalassia testudinum* and *Syringodium filiforme*. *P. belizeanum* preferred the seagrass *Syringodium filiforme*, and was the only species to use *Avrainvillea longicaulis* as a host. In general term, there was not a clear pattern in host preference, although *P. lima* was most abundant on *Penicillus*, followed for the filamentous genera *Aglaothamnion* and *Wrangelia*. Maranda *et al*., 2007, have reported for *P. lima* a preference for filamentous hosts, and our results seem to support this observation. However, this was not true for *Ostreopsis ovata*. Although Aligizaki and Nikolaidis, 2006, claimed a similar host preference for *Ostreopsis* spp., in our findings *O. ovata* had the greatest abundance on *Caulerpa sertularioides*, a moderately leafy macroalga, and low densities on more ramified taxa.

The densities of dinoflagellates we encountered are generally lower than values reported in the literature. For example, Aligizaki and Nikolaidis (Aligizaki and Nikolaidis, 2006) reported up to 4.05×10^5 cells/g fresh weight of algae for *Ostreopsis ovata* + *O. siamensis*. Ballantine *et al*., 1985, registered highly variable abundances of *Ostreopsis* sp., with peaks of >43000 cells/g fresh weight. Lawrence *et al*., 2000, found *P. lima* in concentrations in the order of 103-104 cells/dry weight of algae in a mussel farm in Canada.

Recently, Parson and Preskitt, 2007, in Hawaii, reported densities which are more similar to our findings: they encountered densities between 0 and 69 cells/g wet weight of algae for *P. emarginatum*, between 3 and 224 cells/g wet weight for *P. lima* and between 0 and 48 cells/g wet weight for *Sinophysis microcephala*. They also state that their values are low when compared with previous published studies.

The NMDS analysis shows a differential distribution of dinoflagellates. The eight sites sampled can be assembled in four groups, characterized as follow:

Group A (Bahía Honda). Stable water body, with the highest inorganic nutrient concentration and organic matter in the sediment. Fine sand, beds where *T. testudinum* and *Halimeda* spp. predominate. The site has the greatest concentration of the following species: *P. hoffmannianum, P. maculosum* and *P. rathymum*. *P. lima* and *P*. cf. *maculosum* (2) were the species less abundant, and their concentration were low if compared to the other groups.

Group B (Isleño, Toninos, Cotton Cay, Mar Azul, Acuario). Stable water body, less organic matter in the sediment and records of less nutrientes disolved in the water column; sandy loam sediment texture, diverse biotic substrate. Highest concentrations of the species *P. maculosum*, *P. lima* and *P*. cf. *maculosum* (2).

Group C (Harbor). Instable water body, one of the lowest organic matter content in the sediment; sandy loam sediment texture, predominates *Halimeda* spp. and *T. testudinum*, the predominant species is *P. maculosum*.

Group D (Rocky Cay). Instable water body, low organic matter content in the sediment and low nutrient records in the water column; sandy loam sediment texture, predominates *T. testudinum* and *Halimeda* spp., the predominant species is *O. ovata* and exclusive presence of *P. emarginatum*.

The two sites inshore (Bahía Honda and Rocky Cay), stand alone, very different from each other and the rest (Fig. 4). All the other sites were sampled offshore. With the exception of the Harbor, which also stands apart from the rest, all the others group together, showing homogeneity of characteristics not found in the inshore sites.

The fact that the Harbor, despite being an offshore sample site, does not group with the rest, is probably due to the anthropogenic impact is constantly exposed to.

There is an on-going line of research trying to determine the relationship between water nutrification and harmful algal blooms. Even though most scientists believe in a positive correlation between coastal enrichment and increase of HABs, this has not been thoroughly proven (Masó and Garcés, 2006). Instead, the relationship has revealed ambiguous in various cases (e.g. Yasumoto *et al*., 1979; Parson and Preskitt, 2007).

In our case, the greatest abundance of dinoflagellates was found in Bahía Honda, an enclosed embayment which has been under heavy anthropogenic pressure for various decades (Lonin and Mendoza, 1997). The historical records of nutrients closed to the bay (Palmer-Cantillo, 2007) show high values of nitrogen and phosphorous when compared to the other sites.

Bahía Honda is a small bay, surrounded by mangroves, with low turbulence and limited exchange of water with the open ocean. As mentioned before, the Bay was heavily impacted in the past (Lonin and Mendoza, 1997). In 1961, a power plant was installed in the nearby Bahía Hooker, and the water used to refrigerate the plant was directly discharged in the bay, together with oil and combustible of the same plant. In 1974, a district of approximately 75 houses was built close by, and all the sewage discharge were (and still are) directly released in the bay. In 1996, the harbor was built north to the bay, and the communication of the bay with the open water was reduced to a shallow and narrow channel, reducing even more its hydrodynamics and consequent water exchange. The bay is therefore highly contaminated with coliforms and has a high input of nutrients, mainly from sewage discharge (Palmer-Cantillo, 2007). Although in Bahía Honda the dinoflagellates were not the most abundant group of epiphytic microalgae we found, they were present in higher percentage than in all other sites. Unfortunately our data cannot be compared with studies previous to the anthropogenic impact on the bay, since there are none. However, we consider worthed to continue monitoring the bay, to determine if this pattern is persistent in the other seasons, and if it could be related to the nutrient discharge in the bay.

Although the densities presented here are relatively low, if compared to other sites, we are not immune to health risk. Diarrheic Shellfish Poisoning (DSP) is associated to low cellular concentration (Blanco *et al*., 1998, cited in Masó and Garcés, 2006). Indeed, most toxic species found in the seagrass beds monitored, produce toxins responsible of DSP, and various cases of intoxications have occurred, although the authorities are not incline to publicly admit them, fairing a negative impact on the tourism, the main economic activity in the island (Mancera, personal observation).
Further investigations are required, to determine if other dinoflagellates are seasonally part of the benthic flora, and to monitor the abundance of toxic taxa, to determine if they will pose a health risk to the island population.

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