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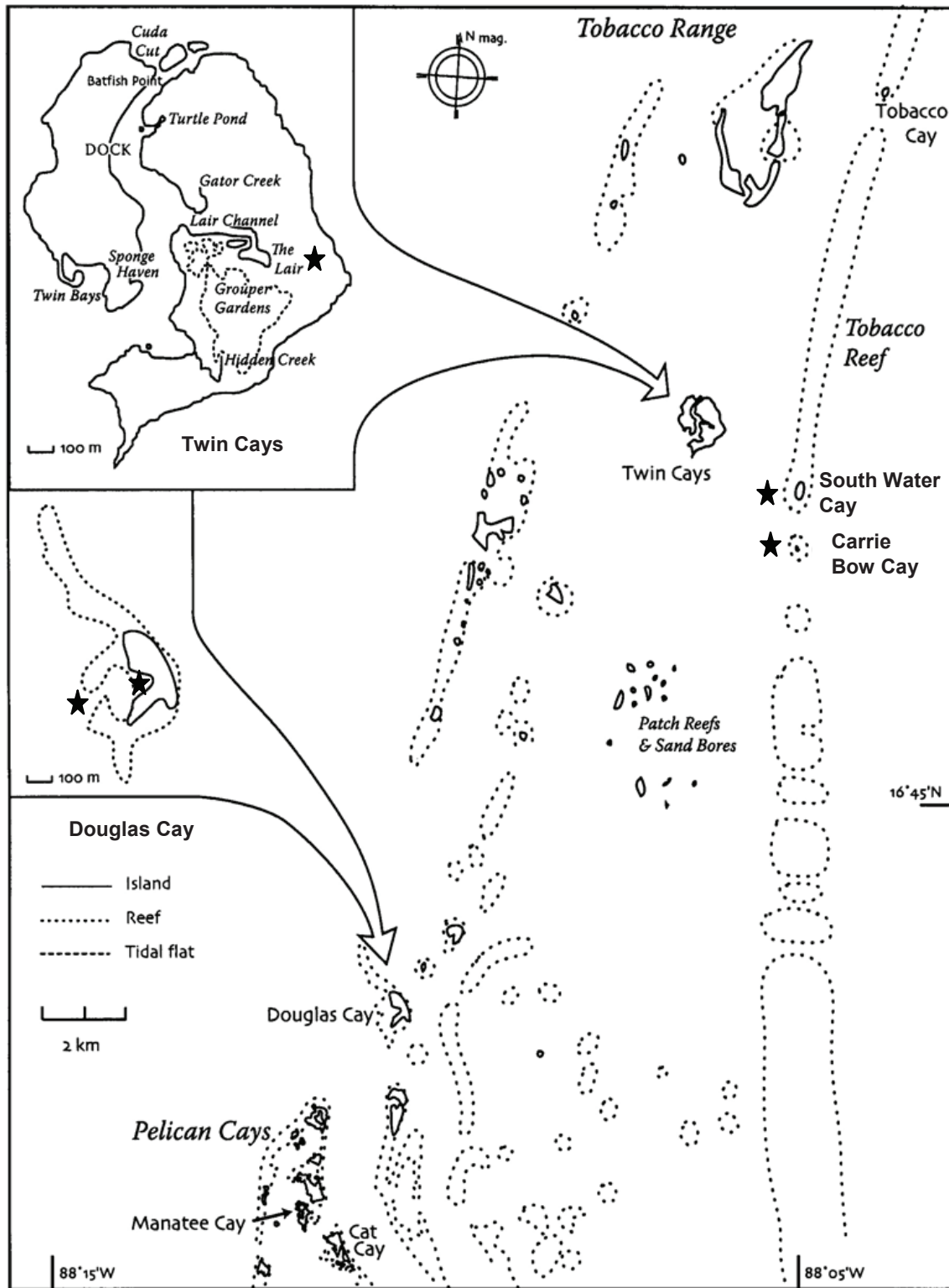
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**CIGUATERA-CAUSING DINOFLAGELLATES IN A CORAL-REEF-  
MANGROVE ECOSYSTEM, BELIZE**

**BY**

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**Figure 1.** Map illustrates the Belizean Central Lagoon. Locations of sampling sites are: 1) Douglas Cay, 2) Twin Cays, 3) South Water Cay, and 4) Carrie Bow Cay located on the Belizean Barrier Reef. Sampling sites designated with a star (★). Image adapted from a map by Molly K. Ryan, Smithsonian Institution

# CIGUATERA-CAUSING DINOFLAGELLATES IN A CORAL-REEF MANGROVE ECOSYSTEM, BELIZE

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## ABSTRACT

The purpose of this study is to examine the reported distribution of known toxin producing dinoflagellates associated with ciguatera fish poisoning (CFP) in both the Caribbean and the Pacific. Historically, the ciguatoxins are produced by *Gambierdiscus toxicus* have been assumed to be the primary cause of CFP world wide. Other species produce a variety of toxins, including maitotoxins, saxitoxins or okadaic acid, which are suspected of contributing to CFP, but not yet definitively confirmed. These include *Coolia monotis*, *Gambierdiscus belizeanus*, *Prorocentrum belizeanum*, *P. lima*, *P. mexicanum*, *P. hoffmannianum*, and *Ostreopsis lenticularis*, *O. siamensis* in the Caribbean; and *Amphidinium carterae*, *A. klebsii*, *Coolia monotis*, *G. polynesiensis*, *G. australes*, *G. pacificus*, *G. yasumotoi*, *Ostreopsis lenticularis*, *O. heptagona*, *O. ovata*, *O. mascarenensis*, *O. siamensis*, *P. lima* and *P. minimum* in the Pacific. Knowing the distribution of CFP-dinoflagellates as producers of toxins is important and yet has to be fully characterized in tropical waters. This study examined epiphytic and benthic CFP-dinoflagellate associations on macrophytes, in sand, on floating detritus and forming blooms in the Belizean barrier coral-reef system. The degree of overlap between these CFP-species in the Caribbean is compared with studies from the Pacific, Mediterranean, New Zealand and Australian tropical waters.

## INTRODUCTION

Dinoflagellate species in the genus *Gambierdiscus* Adachi et Fukuyo have a pantropical distribution between 34°S and 35°N and found throughout the Caribbean, the Hawaiian Islands, French Polynesia, Australia and Indian Ocean (Anderson and Lobel, 1987). Recently, *Gambierdiscus* has been found on oil production platforms along the west Texas coast (Villareal et al., 2007) and off Cape Fear North Carolina (Litaker et al., 2009). Benthic dinoflagellates including *Gambierdiscus* in coral-reefs often are found on the surface of corals and macrophytes, and are food source of herbivore fishes. Some dinoflagellates are producers of toxins that biologically accumulate within the food chain. Yasumoto et al., (1977) and Adachi and Fukuyo (1979) identified *Gambierdiscus toxicus*

dinoflagellate causing ciguatera. Ciguatera, or ciguatera fish poisoning (CFP), is a human disease caused by the ingestion of contaminated marine fish in tropical and subtropical waters. Ciguatera causes gastrointestinal and neurological disorders and sometime human death. Benthic dinoflagellates, *Coolia*, *Ostreopsis*, *Prorocentrum*, have been reported in ciguatera endemic areas associated with *Gambierdiscus toxicus* (Ballantine et al., 1985; Carlson and Tindall, 1985; Bomber and Aikman, 1989). Information is limited on the ecology and distribution of these dinoflagellates in coral-reef associated mangroves.

A long term study of coral-reef mangrove embayments in Belize has identified abundant CFP-dinoflagellate species (Faust et al., 2005 and references therein). Field observations support the findings that marine embayments that have low turbulence protected from wind have restricted water exchange from the lagoon and high nutrients, would favor dinoflagellates. Limited exchange of water favors retention of dinoflagellates and trapping nutrient rich organic material which rapidly recycles and providing relatively high nutrient environment (Margalef, R., 1978; Margalef, R. et al., 1979). The congruence between Margalef's prediction and the distribution of dinoflagellates in naturally eutrophic systems suggests that increased nutrient inputs in the oligotrophic Belizean central lagoon will favor a shift towards benthic dinoflagellate species. The effect will be most pronounced in bays where turbulence is likely to be reduced. This species shift may have consequences for food web dynamics and the prevalence of toxin-producing dinoflagellates in the food chain.

Little is known about the diversity and distribution of *Gambierdiscus* species, the degree to which individual species vary in toxicity, and the role each plays in causing CFP. As a result of the lack of detailed observations, few generalizations have been made about CFP-dinoflagellates distribution. To date, six new *Gambierdiscus* species have been described from the Belizean coral-reef mangroves: *Gambierdiscus belizeanus* (Faust, 1995b), *G. caribaeus* sp. nov., *G. carpenteri* sp. nov., *G. ruetzleri* sp. nov., *Gambierdiscus* ribotype 1 and *G. ribotype 2* (Litaker et al., 2009). Four *Gambierdiscus* species have been identified from the French Polynesia: *G. toxicus*, *G. australes*, *G. pacificus*, and *G. polynesiensis* (Chinain et al., 1999b); and *G. yasumotoi* species from southeastern Asia (Holms, 1998); and an Unique Ribotype from Australia (Litaker et al., 2009). *Gambierdiscus carolinianus* identified off shore at Cape Fear, North Carolina, United States of America.

The purpose of this study was to examine the abundance and species distribution of benthic harmful and CFP-dinoflagellates in shallow coral-reef mangrove ecosystem in Belize. Namely, to assess dinoflagellate species on four macroalgae at South Water Cay, Twin Cays, and Carrie Bow Cay; to identify dinoflagellates present in sand over four days at South Water Cay; to illustrate dinoflagellate species associated on floating detritus at Carrie Bow Cay; to document blooms of dinoflagellate species in protected embayments at the Lair and Douglas Cay; to compare dinoflagellates in high energy lagoon water at the South Water Cay; and to report the distribution of CFP-dinoflagellate assemblages in above habitats from surveys conducted during two weeks in May from 1991 to 2008.

## METHODS

### Study Area

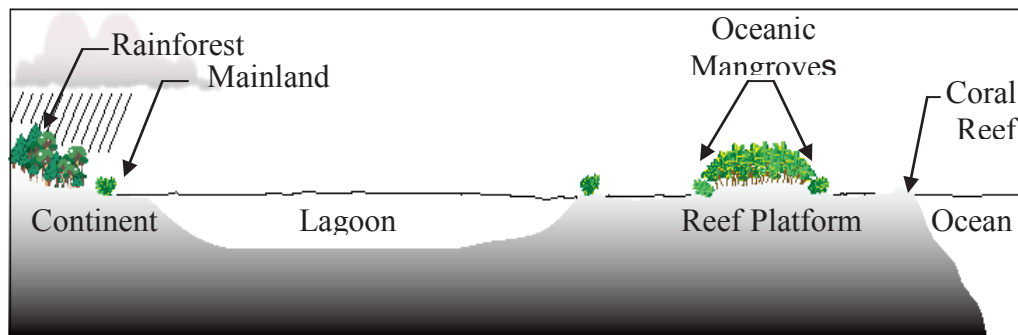
The Belizean barrier coral-reef mangroves exhibit an uninterrupted (250 km long) coral-reef platform separated by a wide, shallow lagoon from the continent (Rützler and Macintyre, 1982); see Fig.1. Twin Cays, and Douglas Cay are situated in the lagoon among a network of channels fringed by red mangroves, and South Water and Carrie Bow Cays are located on the outer edge of the reef platform (Fig. 2). Douglas Cay (16°42.5'N, 88°10.3'W) is a small mangrove island that is part of the Pelican Archipelago, chain of islands in the south-central lagoon in Belize, known the largest barrier reef mangrove ecosystem in the new world (Macintyre and Rützler, 2000a). The reefs in this system are characterized by ridges that form a number of shelf atolls (James and Ginsburg, 1979), both submerged, exposed that was formed during the Holocene (Macintyre et al., 2000b). Douglas Cay is colonized by red mangroves, *Rhizophora mangle* Linnaeus, a dense network of prop roots with no dry land (Purdy, 1994). It is located in a remote region of the central lagoon relatively free of human disturbance. The embayments of some lagoon atolls are surrounded by mangroves (> 4 m high) on shallow (< 1m) ridges, which isolate the water inside. As a result, these embayments are considered separate water masses and are warmer and more saline than the open waters of the surrounding central lagoon (Villareal et al., 2000).

Twin Cays (16°49.4'N, 88°6.1'W) are located approximately 14 km NE of Pelican Cays (Fig. 1). They are characterized by two shallow intertidal mangrove islands divided by a relatively deep Main Channel (3 to 8 m) and contain a series of lagoons, channels, mud flats and ponds, covered with red mangroves. The Lair study site is a shallow cove located at the end of a long narrow channel (0.5 to 3 m deep), and has a shallow sill in transition of water. The Lair is protected from winds, and the surrounding lagoon which cause restricted water exchange. Water exchange is greater in the Lair than observed for Douglas Cay (Kibler et al., 2005). The Lair is high in organic matter originating from mangroves trees and benthic production associated with the soft sediments. The Lair harbors egrets and schools of fishes and was sampled as a comparison site with Douglas Cay in May 2003.

Water samples of dinoflagellate were collected at the center of Douglas Cay embayment (Fig. 1). The hydrographic isolation of the embayment and low tidal amplitude (<20 cm) allows retention of nutrients and carbon-rich detrital material supplied by the surrounding mangrove trees (Kibler et al., 2005). Brown pelicans (*Pelecanus occidentalis*) frequent this embayment and feed on dense schools of small planctivorous fishes. The outer portion of the embayment and the sill harbor corals and macroalgae while the surrounding shallows are dominated by *Thalassia testudinum* Koenig seagrass. A fringe reef 150 m beyond the sill restrict water exchange and retain dinoflagellates in Douglas Cay from incoming waves. Dinoflagellates collected outside the fringe reef served as lagoonal control. This station was eight meter deep and exposed to the prevailing winds and waves of the central lagoon (Fig. 1).

Carrie Bow Cay (16°48'N, 88°5'W) and South Water Cay (16°49'N, 88°05'W) both, are located on the Belizean barrier reef and lie 22 km SE of Dangriga, Belize (Fig. 1). The central lagoon in Belize is about 20 km wide and separates the continent

from an uninterrupted reef platform (Fig. 2). The area around both cays is representative of the Belizean central lagoon, with shallow embayments and a network of highly variable coral ridges, seagrass beds and small islands, colonized primarily by oceanic red mangroves. Carrie Bow Cay is a small island, having few permanent residents, few coconut trees, and shoreline waters that receive some nutrient enrichment from human activities. South Water Cay is a larger island than Carrie Bow Cay, having permanent residences, groves of coconut trees, and shoreline waters that receive steady nutrient enrichments from human activities. The offshore habitats are composed of sand, *Thalassia*-dominated seagrass meadow, corals, and macroalgae. Sampling was conducted on the western (landward) side of both islands in shallow seagrass beds (> 2 m). This open lagoon is dominated by wind mixing, high turbulence and low nutrients (Faust et al., 2005). The present study describes CFP-dinoflagellate assemblages in shallow lagoonal water as a comparison site for Douglas Cay and Twin Cays embayments.



**Figure 2:** Cross section of the Atlantic Barrier Reef-Mangroves situated in the Belizean Central Lagoon. Image is after Rützel and Feller (1996), *Scientific American*, 278:94-99.

### Physical Parameters, Nutrient Enrichments, and Chlorophyll *a* Biomass

Temperature, salinity, dissolved oxygen, light, and irradiance were estimated in the water column at three depths with the following instruments: a Yellow Spring Instrument (YSI) 33-S-C-T M (model 6600) meter with a YSI oxygen analyzer Model 57, YSI 5739 oxygen probe, and YSI 5795A portable, battery operated submersible stirrer. Irradiance was estimated by the integrating quantum scalar irradiance meter, Biospherical Instruments # QSI-140 meter. Environmental variables of temperature, salinity, dissolved oxygen, and light intensity measured in Douglas Cay, the Lair, Twin Cays, and Carrie Bow Cay during two weeks period in May 2004 (Table 1 and Table 2).

Dissolved ammonium concentrations in the water samples, were measured fluorometrically using the method of Holmes et al., (1999), and dissolved inorganic phosphorus estimated by the method of Ambler (1991) over two weeks period in May from 2002 to 2006 (Table 1 and Table 2).

Water samples for chlorophyll *a* analysis were vacuum-filtered through 25mm GF/F filters (< 10 cm Hg) and were immediately frozen in liquid nitrogen. Samples were subsequently extracted with 7.5 ml 90% acetone and were macerated with a tissue grinder before being analyzed using the acidification method for chlorophyll *a* described by Parsons et al. (1984).

Table 1. Environmental variables, ammonium, temperature, salinity, dissolved oxygen, light, and chlorophyll *a* measured in Douglas Cay, The Lair, Twin Cays, and lagoon at Carrie Bow Cay, field station. Data represent the mean concentrations of six parameters estimated daily for two weeks in May 2004.

Study Sites	NH <sub>4</sub> <sup>+</sup> μmole L <sup>-1</sup>	Temp. °C	Salinity psu <sup>1</sup>	Dissolved O <sub>2</sub> mg L <sup>-1</sup>	Light μE m <sup>-2</sup> s <sup>-1</sup>	Chl <sub>a</sub> μg L <sup>-1</sup>
Lagoon	0.1-0.2	28.5-30.6	34.8-35.7	3.5-4.0	1600-2000	0.5-0.8
Douglas	0.6-6.0	28.3-29.3	35.6-36.1	2.2-5.0	1500-2000	5.0-15.0
The Lair	0.1-0.8	29.1-31.2	37.5-38.0	2.0-5.0	1200-2000	0.8-5.0

<sup>1</sup> Percent salinity unit.

Table 2. Mean concentrations for ammonium, phosphate, and chlorophyll *a* ± 1 standard deviation measured in the lagoon, embayment-fringe waters, and in the transition zones between the two. N=66 to 4238 for chl *a*. N=averaged 33 and 32 for ammonium and phosphate, respectively, in years when they were measured from 2002 to 2006. No data (-).

<b>Mangrove Embayment Nutrients</b>						
Year	NH <sub>4</sub> <sup>+</sup> (μM)		PO <sub>4</sub> <sup>-</sup> (nM)		Chl <i>a</i> (μg L <sup>-1</sup> )	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
<b>Embayment Water</b>						
2002	5.5	4.0	-	-	-	-
2003	0.9	0.7	-	-	4.8	4.2
2004	1.7	2.0	0.4	0.5	3.3	7.0
2005	0.5	0.2	23.3	22.6	3.7	3.4
2006	2.8	1.8	182.5	260.2	6.1	1.9
<b>Transition waters</b>						
2002	-	-	-	-	0.6	0.4
2004	0.2	0.2	0.02	0.02	1.2	2.1
2005	2.9	2.1	54.5	36.5	1.4	3.3
<b>Lagoon Waters</b>						
2002	0.4	0.4	-	-	-	-
2003	0.2	0.05	0.01	0.01	0.2	0.2
2004	0.09	0.1	30.6	13.2	0.8	0.5
2005	0.3	0.1	-	-	0.4	0.8
2006	0.9	0.5	-	0.3	0.1	0.1

## Sampling Location and Methods

Dinoflagellate species were examined from four benthic habitats: 1) Epiphytic populations of dinoflagellate collected from four species of macrophytes, *Halimeda opuntia* (Linnaeus) Lamouroux, *Dictyota dichotoma* (Hudson) Lamouroux, *Acanthophora spicifera* (Vahl) Boergesen, and *Thalassia testudinum* Koenig. These macrophytes were chosen because of their presence at every site and their wide-spread distribution throughout the Caribbean (Morton and Faust, 1997). 2) Colored sand populations of dinoflagellates that were submerged and washed gently by incoming waves, during low tides offshore at South Water Cay. 3) Dinoflagellates from floating detritus attached to nylon rope fibers (artificial surface), suspended in the water column at Carrie Bow Cay and examined over two weeks. 4) Bloom-forming dinoflagellates discovered in nutrient enriched mangrove embayments at the Lair and Douglas Cay, and at the open lagoon of South Water Cay.

1). At South Water Cay, Twin Cays and Carrie Bow Cay, four macrophytes species were selected; samples of each species (4 to 6 replicates) collected by hand in plastic bags from 0.5-2 m depth in near-shore water. Macrophytes in the plastic bags were shaken vigorously for 1 min to dislodge epiphytic organisms. Cells in the sample supernatant were used to count cells and identify dinoflagellate species. Macrophytes removed from the bag and fresh weight measured. 2). At South Water Cay, golden-brown colored surface sand at the edge of the shore was sampled with a spoon and placed into a test tube of 20 ml prefiltered seawater (filtered through a 0.45  $\mu\text{m}$ -pore-size nucleopore filter). Sand samples containing dinoflagellates was shaken vigorously in a test tube by hand for 1 min to remove attached populations and processed for cell counts and species identification as described above. 3). At Carrie Bow Cay, nylon rope of many fibers (1 inch wide) floated on the shallow (1 m) deep water surface, attached to the dock for 24 hours. This rope was then washed and dinoflagellates attached to the rope rinsed into a beaker of 100 ml filtered seawater and treated as above. Similarly, plastic screens (20x20 cm size) were placed hanging from a PVC pipe, and incubated in 1 m deep water at the dock for collecting dinoflagellates and treated as described. 4). At South Water Cay and Carrie Bow Cay lagoon, net tows using a 20  $\mu\text{m}$ -mesh net fitted with a calibrated flow meter was used to collect plankton samples. To reduce error caused by net clogging, only short tows of less than 2 to 5 minutes were taken. Net samples were concentrated to a volume of 100 ml and placed in a plastic bag, stored in a cooler and taken to the laboratory.

## Sample Preparations

*Light microscopy.* Dinoflagellate specimens were fixed in final 1% glutaraldehyde solution (Faust, 1990). Cells of dinoflagellates in the filtrate from colored sand or macroalgal surfaces were enumerated using a Palmer-Maloney counting chamber (Stein, 1973). Cell counts were expressed as cells  $\times 10^6 \text{ g}^{-1}$  wet sand; or as cells  $\text{g}^{-1}$  fresh weight. Cell numbers were calculated using 3-6 sub-samples; values given represented the means of cell concentration estimated with the Carl Zeiss light microscope.



*Scanning electron microscopy.* Two to 5 ml of fixed sample was used to isolate dinoflagellates with a glass capillary pipette under a Carl Zeiss dissecting microscope for scanning electron microscopy (SEM). Cells were post fixed with OsO<sub>4</sub> for 30 minutes at room temperature and filtered through 13 mm diameter and 0.8 µm pore size Nucleopore polycarbonate membrane filter. Specimens were rinsed 5 to 8 times with de-ionized water and dehydrated in a graded ethanol series (30, 50, 75, 95, and 100%). The filter was then mounted on a stub and critical point dried. This preparation was coated with elemental carbon. The cells were imaged using a XL-30 Environmental Scanning Electron Microscope (Philips, New York, N.Y.).

The Kofoid nomenclature was used for identifying dinoflagellate species (Kofoid, 1909). Dinoflagellate samples of this investigation are deposited in the United States of America Dinoflagellate Research Collection, Smithsonian Institution Washington, D.C.

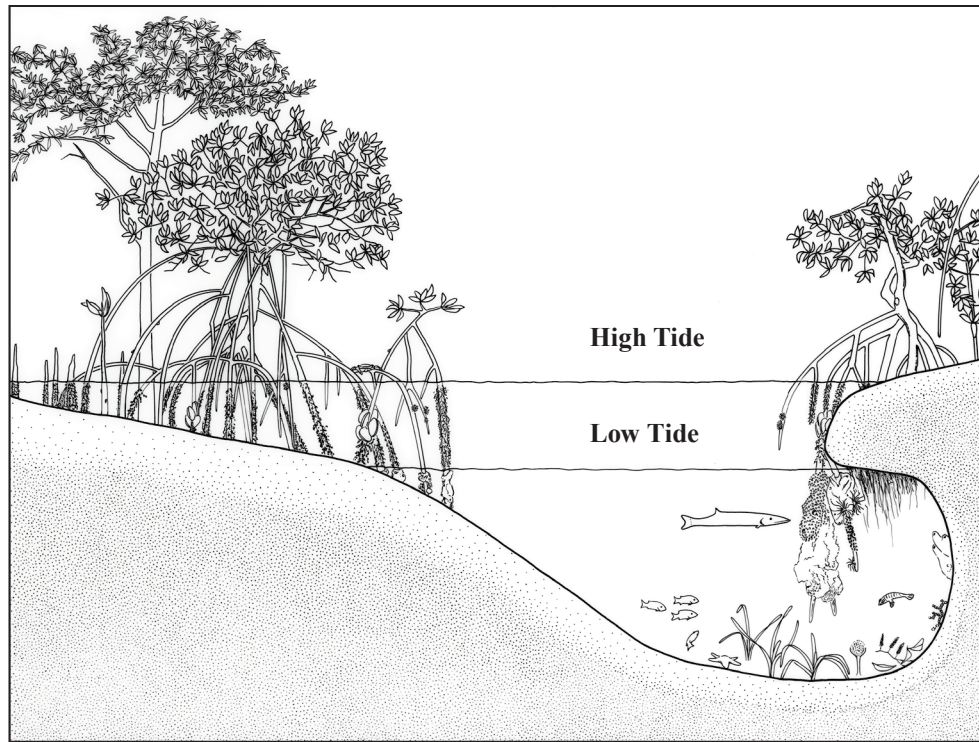
## RESULTS

### Atlantic Barrier-reef Mangroves

The mangroves in the Belizean Central Lagoon provide an ideal setting for conducting studies on the distribution of benthic dinoflagellates in four ecologically variable habitats (Fig. 1). Twin Cays and Douglas Cay are enclosed islands (Fig. 2). In a cross section of a tidal channel in Twin Cays, sponges and other sessile organisms are attached to prop roots of mangroves and on undercut peat bank surface covered by sponges, turtle grass and macroalgae (Fig. 3). The channel has restricted water exchange (< 20 cm tide) and protection from wind in the lagoon. In these mangroves, nutrient input from detritus and natural sources enhance development of dinoflagellates (Faust, 1995a). In comparison, South Waters Cay and Carrie Bow Cay are surrounded by fast moving open lagoon currents. Patches of macroalgae and seagrass beds and coral communities thrive on the sediment surface and endure stable salinities during the dry season (Faust et al., 2005).

### CFP-dinoflagellates Morphology

The morphology of the four genera, *Gambierdiscus*, *Ostreopsis*, *Prorocentrum* and *Coolia*, in this study is illustrated in scanning electron micrographs (Fig. 4). Each genus differs in cell size, shape, and arrangement of the thecal plates. A major difficulty identifying dinoflagellates with the light microscope is recognizing morphological details and distinguishing small differences among miniscule thecal plates. This caused problems in earlier publications; for example, *Gambierdiscus* species have been identified either as *Gambierdiscus toxicus* and *Gambierdiscus* sp., or as *Gambierdiscus* multispecies complex with overlapping geographic distributions (i.e. Holmes, 1998; Chinain et al., 1999b). Four genera are illustrated in high resolution image (Fig. 4). The morphology and type locality *Gambierdiscus*, *Ostreopsis*, *Coolia* and *Prorocentrum* of these known harmful and CFP-dinoflagellate species are described.



**Figure 3.** Twin Cays are enclosed tidal islands. In a cross section of the Lair Channel, sponges and other sessile organisms are attached to prop roots of mangroves and on undercut peat bank surface covered by sponges, turtle seagrass, macroalgae and juvenile fishes. Coral communities and benthic dinoflagellates thrive on the sediment surface. Image after Rützler and Feller (1988), *Oceanus*, 30:16-24.

*The genus Gambierdiscus.* The type species of the genus is *Gambierdiscus toxicus* Adachi et Fukuyo, 1979. Originally, specimens of *Gambierdiscus* were collected from numerous locations at Gambier Island, French Polynesia, in May 25, 1975; however, the collection site (type locality) of *G. toxicus* is currently unknown. Adachi and Fukuyo (1979) illustrated the thecal plate morphology of *G. toxicus* in detailed line drawings. These drawings are accepted for identifying *Gambierdiscus* thecal morphology. No type material of *G. toxicus* is currently known (Tester et al., 2008). Later Chinain et al., (1999b) used living cultures of the original *G. toxicus* isolate and illustrated the species morphology in scanning electron micrographs; unfortunately no culture is available today. Holmes (1998) had frozen specimens *G. toxicus* GTT-91 which represent similar cell size, shape and morphology of thecal plates of *G. toxicus* Adachi et Fukuyo (1979) which is the epitype of the genus *Gambierdiscus* (Tester et al., 2008).

The morphology of *Gambierdiscus* in apical view is illustrated in scanning electron micrograph (Fig. 4). Cells are large to medium, round anterior-posteriorly compressed and sub-lenticular in ventral view. The apical pore plate (Po) is oval to ellipsoidal, a characteristic fish-hook shaped apical pore that has species-specific morphology. The plate formula of the genus *Gambierdiscus* is: Po, 3', 7'', 6c, 8s, 5''', 1p, 2'''' according to Chinain et al., (1999b). The thecal plates are named according to Kofoid (1909). The epitheca and hypotheca are usually not noticeably different in size.

The cell surface is usually smooth laced by numerous deep and small pores (Steidinger and Tangen, 1996). Ten species of *Gambierdiscus* are known. Morphology of *G. toxicus* GTT-91 epitype Chinain et al., (1999b), is compared with nine known species described in scanning electron micrographs in the following publications: *G. belizeanus* Faust (Faust, 1995b, Hernández-Becerril, 2004); *G. yasumotoi* Holmes (Holmes, 1998); and *G. pacificus* Chinain et Faust, *G. australes* Faust et Chinain, *G. polynesiensis* Chinain et Faust in Chinain et al., (1999b); and *G. caribaeus* Vandersea et al., sp. nov., *G. carolinianus* Litaker et al., sp. nov., *G. carpenteri* Kibler et al., sp. nov., and *G. ruetzleri* Faust et al., sp. nov. (Litaker et al., 2009).

The type locality of ten known *Gambierdiscus* species are: *G. toxicus* GTT-91 Teahupoo, Tahiti, Society Archipelago, French Polynesia; *G. pacificus* Otepa in Hao Island, Tuamotu Archipelago, Pacific Ocean; *G. polynesiensis* Mataura in Tubuai Island Australes Archipelago, Pacific Ocean; and *G. australes* Rurua, Raivavae Island, Australes Archipelago, Pacific Ocean (Chinain et al., 1999b); *Gambierdiscus yasumotoi* Palau Hantu Island, East China Sea (Holmes, 1998). Species identified in Belize are: *G. belizeanum* South Water Cay (Faust, 1995b). Type locality of recently identified new species at Belize is: *G. caribaeus* sp.1 at Carrie Bow Cay; *G. carpenteri* sp. 2 at South Water Cay; and *G. ruetzleri* sp. 3 at South Water Cay. The last new species identified is *G. carolinianus* sp. 4 Cape Fear, North Carolina, United States of America in Litaker et al., (2009).

*The genus Ostreopsis.* The genus *Ostreopsis* was identified in the plankton by Schmidt 1902 in The Gulf of Siam, Thailand. The type species of the genus is *O. siamensis* (Schmidt, 1902) and illustrated in hypothecal view in Figure 4.

The morphology of *Ostreopsis* species are medium to large cells. *Ostreopsis lenticularis* is lenticular in shape anterior-posteriorly compressed, tear shaped in apical view. (Fig. 4). The apical pore plate (Po) is characteristic with a curved, narrow apical plate and is species-specific in morphology (not shown). The plate formula of the genus *Ostreopsis* is: Po, 3', 7'', 6C, s, Vp, Rp, 5''', 2'''' (Steidinger and Tangen, 1996). The epitheca and hypotheca are similar in size. Cell surface is smooth and covered with randomly spaced small pores. The morphology of *Ostreopsis* species in scanning electron micrographs are illustrated in the following publications: *O. ovata* Fukuyo and *O. lenticularis* Fukuyo (1981) and (Faust et al., 1996a); *O. heptagona* Norris, Bomber et Balech (1985) and (Faust et al., 1996a); *O. mascarenensis* Quod (1994), (Faust et al., 1996a), and Lenoir et al., (2004); *O. labens* Faust and Morton (1995); and *O. siamensis* Schmidt (Faust et al., 1996a); *O. marinus* Faust, *O. belizeanus* Faust, and *O. caribbeanus* Faust (1999).

Type locality of the eight known *Ostreopsis* are: *O. siamensis* Schmidt 1902 The Gulf of Siam, Thailand; *O. lenticularis* Fukuyo 1981, The Gambier Islands of French Polynesia; *O. ovata*, Fukuyo, 1981, Ryukyu Island, Pacific Ocean; *O. heptagona* Norris et al., 1985, Florida Keys, United States of America; *O. mascarenensis* Quod, 1994, Saint Leu, Reunion Island, Mascareignes Archipelago, West Indian Ocean; *O. labens* Faust and Morton, 1995, Man of War Cay, Belize, Central America; *O. belizeanus* Faust 1999, Main Channel, Twin Cays, Belize, Central America; *O. marinus* Faust 1999, The Main Channel, Twin Cays, Belize, Central America, and *O. caribbeanus* Faust 1999, Laurel Reef, Puerto Rico, Caribbean.

*The genus Coolia.* The genus *Coolia* was identified by Meunier (1919). The type species in the genus is *Coolia monotis* Meunier. The species illustrated in ventral view on Figure 4.

The morphology. *Coolia monotis* is relatively small, slightly compressed anterior-posteriorly and are oval in ventral view. Epitheca is slightly smaller than the rounded hypotheca and nearly oblique (Faust, 1992). A distinct feature is the shape and size of the thecal plates including the apical pore (Po) plate. The apical pore plate (Po) is elongated and its shape species-specific. Cell surface smooth or areolated and covered with scattered large pores (Steidinger and Tangen, 1996). The plate formula of the genus *Coolia* is: Po, 3', 7'', 7c, s, 5''', 2'''''. The following publications illustrate the morphology *Coolia* species in scanning electron micrographs: *C. monotis* (Faust, 1992, and Faust and Gullledge, 2002); *C. tropicalis* Faust (1995b); *C. areolata* Ten-Hage et al., (2000); *Coolia* sp. Mohammad-Noor et al., (2005); and *C. canariensis* Fraga et al., (2008).

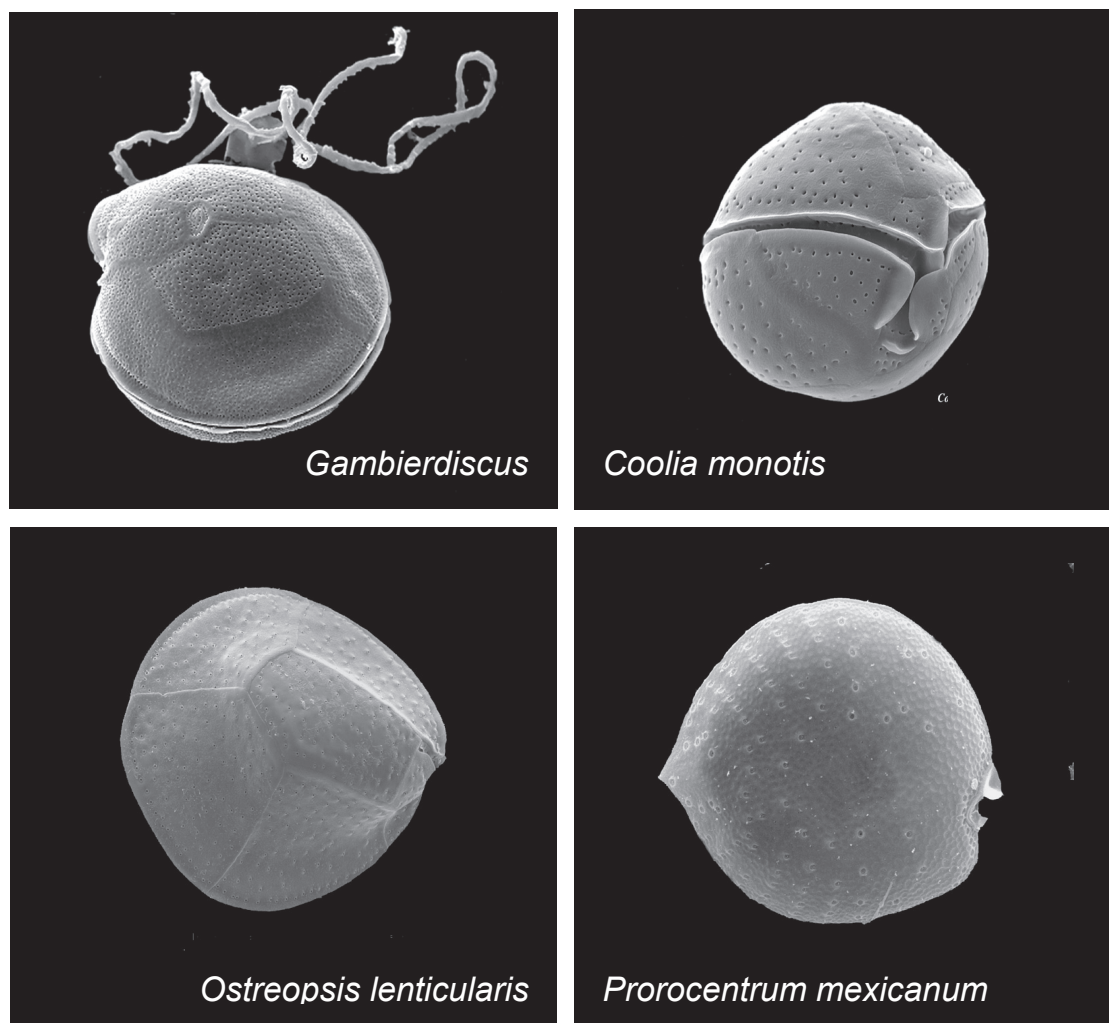
Type locality of five known *Coolia* species are: *Coolia monotis* Meunier, 1919, North Sea, Deswartes, Nieuport, Belgium; *C. tropicalis* Carrie Bow Cay, Belize, Central America (Faust, 1995a), both species present in Belize; *Coolia areolata* identified at La Possession Bay, La Reunion Island, S.W. Indian Ocean, (Ten-Hage et al., 2000); *Coolia* sp. at Port Dickson, Sepanggar Bay, Bangi Island, Malaysia (Mohammad-Noor et al., (2005); and *C. canariensis* at Punta Hidalgo, Tenerife, Canary Islands, NE Atlantic Ocean (Fraga et al., (2008).

*The genus Prorocentrum.* The genus *Prorocentrum* was described by Ehrenberg (1834). The type species of the genus is *P. micans* Ehrenberg 1833. *Prorocentrum* species in the genus are numerous (<70 species).

The morphology. Species in the genus *Prorocentrum* are small to medium size, and cells vary in shape from spheroid to pyriform in valve view. Valves can be convex to concave in lateral view (Table 4). Species in the genus *Prorocentrum* possess left and right laterally compressed valves, and 5-14 periflagellar pore-plates. Cells possess two anterior dissimilar flagella (Steidinger and Tangen 1996). Some species have anterior winged spines or short anterior plate; an example illustrates the morphology of *P. mexicanum* Tafall (Cortés-Altamirano and Sierra-Beltrán, 2003) as a rugose cell surface with rows of minute trichocyst pores shown in scanning electron micrograph (Fig. 4). The morphology of seven *Prorocentrum* species identified in this study are found in the following references: *P. arenarium* Faust (Faust 1994); *P. belizeanum* Faust (Faust 1993); *P. hoffmannianum* Faust (Faust 1990); *P. lima* (Ehrenberg) Dodge (Dodge 1975) and Faust and Gullledge 2002, and references therein); *P. emarginatum* Fukuyo (Fukuyo 1981); and *P. sculptile* Faust (Faust 1994). Specimens of *P. micans* were rarely seen in the collections gathered in the coral reef-mangroves Belize.

Type locality of seven *Prorocentrum* species identified in this study are: *P. hoffmannianum* Twin Cays, Belize, Central America (Faust, 1990); *P. belizeanum* Twin Cays, Belize, Central America (Faust, 1993); *P. arenarium* Carrie Bow Cay, Belize, Central America, and *P. sculptile* South Water Cay, Belize, Central America (Faust

1994); *P. mexicanum* (Faust, 1996b) and *P. rhathymum* Bahia Mazatlan, North Pacific Ocean, Mexico (Cortés-Altamirano and Sierra-Beltrán, 2003); and *P. emarginatum* The Ryukyu Islands, Pacific Ocean (Fukuyo, 1981; Faust, 1990). The Type locality of *P. lima* (Ehrenberg) Dodge 1975 is unknown, since several other *Prorocentrum* species with a triangular periflagellar area and ovoid shape have similar morphology and have been illustrated in line drawings (Faust and Gullledge, 2002).



**Figure 4.** Morphology of *Gambierdiscus*, *Coolia*, *Ostreopsis* and *Prorocentrum* is illustrated in scanning electron micrographs. Cell dimension are estimated by the length and width of the species: *Gambierdiscus* 53-85  $\mu\text{m}$  x 44-58  $\mu\text{m}$ ; *Coolia monotis* 23-49  $\mu\text{m}$  x 23-38  $\mu\text{m}$ ; *Ostreopsis lenticularis* 65-75  $\mu\text{m}$  x 57-63  $\mu\text{m}$ ; and *Prorocentrum mexicanum* 32-40  $\mu\text{m}$  x 26-30  $\mu\text{m}$ .

## Temperature, Salinity, and Nutrient Measurements

The water temperature, salinity and nutrients estimated during two weeks study periods from 2002 to 2006 are shown in Table 1 and Table 2. The mean value of water temperature was the highest in the Lair, median in the lagoon, and the lowest in Douglas Cay. Salinity was the highest in the Lair and lowest in lagoonal waters. Both, Douglas Cay inner lagoon and the Lair, are protected coral reef-mangrove embayments, divorced from surrounding open lagoonal waters, protected from prevailing winds by being surrounded by mangrove trees. In contrast, South Water Cay and Carrie Bow Cay are exposed to high turbulence in the open oligotrophic ocean lagoon (Kibler et al., 2005). Dissolved oxygen concentrations in the water column at Douglas Cay and the Lair, Twin Cays varied from hypoxic to supersaturate depending on the time of the day. Fluctuation is due to the balance between respiration and photosynthetic oxygen production. In these shallow systems, midday photosynthetically active radiation is sufficient to saturate photosynthesis in the water column and at sediment surface. Mean values of  $\text{NH}_{4+}$  concentrations were higher in Douglas Cay than in the Lair and approximately 60 times greater than  $\text{NH}_{4+}$  concentrations found in the surrounding oligotrophic lagoon (Table 1). Mean values of  $\text{NH}_{4+}$  and  $\text{PO}_4$ , and chlorophyll *a* concentrations in the water are shown in Table 2. The location of three open lagoon water collection sites were: at the entrance to Douglas Cay, The Lair, Twin Cays, and Carrie Bow Cay (Fig. 1). Dissolved  $\text{NH}_{4+}$ ,  $\text{PO}_4$  and chlorophyll *a* concentrations were higher in the embayments of Douglas Cay and The Lair, in between on the fringe reef transition water sites, and nutrients much lower in oligotrophic central lagoon waters estimated in May from 2002 to 2006 (Table 2).

## CFP-dinoflagellates from Macrophytes

Natural populations of epiphytic dinoflagellates included six CFP-dinoflagellate species on four macrophytes algae collected from South Water Cay, Twin Cays and Carrie Bow Cay. Highest densities of dinoflagellates were on *Acanthophora spicifera*, median on *Dictyota dichotoma* and *Halimeda opuntia*, and lowest on *Thalassia testudinum*. *Gambierdiscus toxicus* on four macrophytes displayed highest cell densities ranging from 3000 to 280 cells  $\text{g}^{-1}$  tissue at South Water Cay and lower cell densities ranging from 1500 to 35 cells  $\text{g}^{-1}$  tissue at Carrie Bow Cay and 800 to 20 cells  $\text{g}^{-1}$  tissue at Twin Cays (Table 3). *Ostreopsis lenticularis* was dominant epiphyte on the four macrophytes species collected at three locations. Highest cell densities of *O. lenticularis* estimated 1500 cells  $\text{g}^{-1}$  tissues on *A. spicifera*, median on *T. testudinum* and *D. dichotoma* 350 to 300 cells  $\text{g}^{-1}$  tissues and lowest 200 cells  $\text{g}^{-1}$  tissues on *H. opuntia* at South Water Cay. *Ostreopsis lenticularis* cell density declined from 75 to 15 cells  $\text{g}^{-1}$  tissues on the other macrophytes hosts at Twin Cays and Carrie Bow Cay.

*Ostreopsis heptagona* cell density 85 to 5 cells  $\text{g}^{-1}$  tissues were the lowest on *A. spicifera*, *H. opuntia* and *T. testudinum* and a minor part of *D. dichotoma* and varied per collection sites (Table 3). *Prorocentrum lima* on macrophytes displayed densities ranging from 480 to 15 cells  $\text{g}^{-1}$  tissue at South Water Cay, Twin Cays, and Carrie Bow

Cay. Highest densities of *P. lima* were associated with *A. spicifera*, *D. dichotoma* and *H. opuntia* macroalgae, while cell densities were lower on *T. testudium*. Cell density of *P. lima* was high on the macroalgae, but density of *P. hoffmannianum* and *P. mexicanum* were three to four orders lower. Cell density of *P. hoffmannianum* species ranged from 170 to 15 cells g<sup>-1</sup> of tissue, whereas, *P. mexicanum* cells ranged from 40 to 7 cells g<sup>-1</sup> of tissue on the macroalgae. Cell density of *P. mexicanum* was high on *Dictyota* and *Halimeda* while low on *A. spicifera*, and *T. testudinum* (Table 3).

Table 3. Distribution of epiphytic dinoflagellates on four species of macrophytes collected at three sites from South Water Cay (SW), Twin Cays (TC), and Carrie Bow Cay (CB). Dinoflagellate concentrations expressed as cells g<sup>-1</sup> fresh weight.

Location	<i>G. toxicus</i>	<i>P. lima</i>	<i>P. hoffmannianum</i>	<i>P. mexicanum</i>	<i>O. lenticularis</i>	<i>O. heptagona</i>
<b>Host macrophyte: <i>Acanthophora spicifera</i></b>						
SW	3000	37	80	0	1500	15
TC	800	375	89	18	70	80
CB	1500	480	35	20	15	75
<b>Host macrophyte: <i>Dictyota dichotoma</i></b>						
SW	1000	400	70	25	300	5
TC	60	380	89	15	15	0
CB	80	420	93	20	30	0
<b>Host macrophyte: <i>Halimeda opuntia</i></b>						
SW	300	150	60	20	200	35
TC	50	280	75	40	55	15
CB	35	300	120	30	40	20
<b>Host macrophyte: <i>Thalassia testudinum</i></b>						
SW	280	15	15	7	350	85
TC	20	180	150	18	35	0
CB	52	275	170	15	75	35

#### Sand-welling CFP-dinoflagellates

A sand patch approximately 2 m<sup>2</sup> in size exhibited a golden-brown color caused by photosynthetic benthic dinoflagellates along the western shore at South Water Cay in 2001. Colored sand was present only on the top 1 to 2 mm sand surface. The sand below was dark gray, indicating reduced oxygen levels. Dinoflagellates in colored sand were examined over 4 days (Table 4). After this period a storm washed away the colored sand. The only other organisms in the samples were photosynthetic cyanobacteria, ciliates and

nematodes. No diatoms were present. These benthic assemblages flourished at water temperatures ranged from 28.5 °C to 30.1 °C and predicted salinities ranged from 34.5 to 35 percent salinity unit (psu). Live specimens under the microscope were swimming slowly within the interstitial spaces of the sand grains or attached to the grains surface.

A total of 16 dinoflagellate species were identified: eight known toxic species, three potentially toxic species, and additional five benthic bloom forming species. Toxic species included *G. toxicus* and *G. belizeanus*, *C. monotis*, *O. lenticularis*, *P. lima*, and *P. mexicanum*, *Amphidinium carterae* and *A. klebsii*. The potentially toxic species were *O. labens*, and bloom-forming *Bysmatrum subsalsum*, and *Protoceratium grindleyi*. Benthic species included *Prorocentrum sculptile*, *Scrippsiella* sp., and three *Gymnodinium* species (sp. 1, 2, and 3). In the sand, total dinoflagellate concentrations ranged from 1.36 to 1.85 cells x 10 g<sup>-1</sup> sand. CFP and potentially toxic species represented 35.8% to 59.7% of total cell populations in the sand at South Water Cay. *Ostreopsis labens* and *O. lenticularis* populations were most abundant, *P. mexicanum* and *P. lima* intermediate, and *C. tropicalis*, *G. toxicus* and *G. belizeanus* the lowest. Among the benthic species, *B. subsalsum* was the highest, *Gymnodinium* sp. 1, 2, and 3 species intermediate, and *Scrippsiella* sp. the lowest (Table 4).

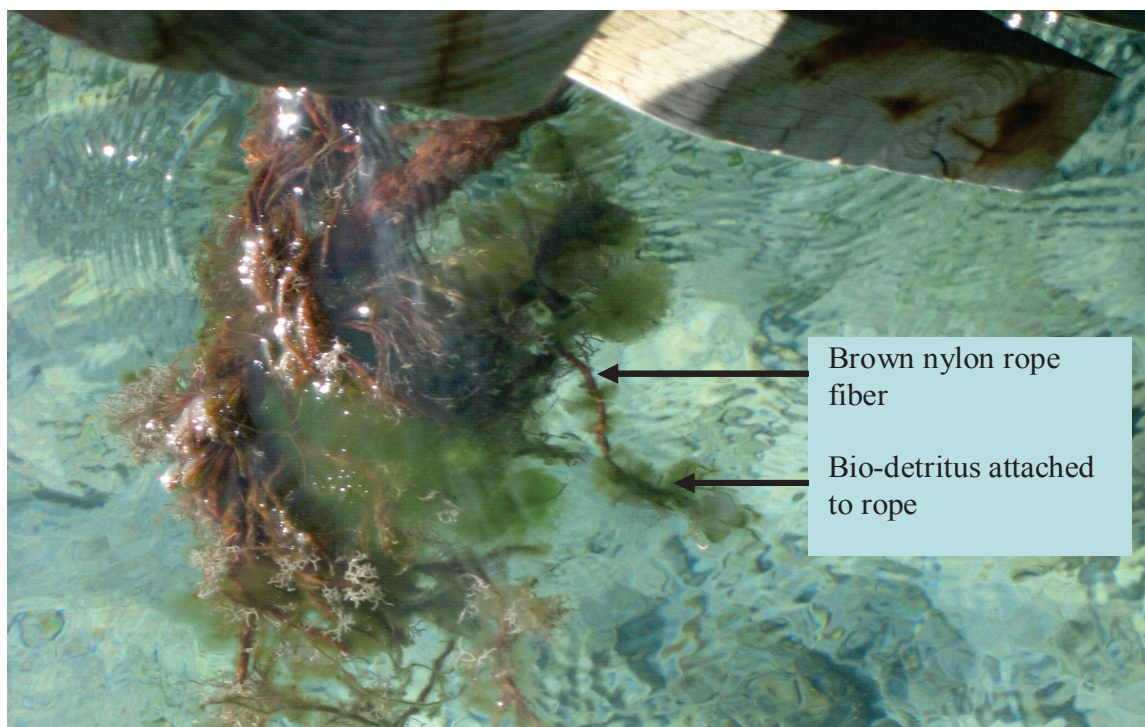
Table 4. Sand-dwelling toxic dinoflagellate populations in colored sand collected from South Water Cay over 4 days. Dinoflagellate concentrations cells x10 g<sup>-1</sup> sand, and expressed as the percent of total dinoflagellate populations per species. No data (-).

Taxa	Day 1	%	Day 2	%	Day 3	%	Day 4	%
<i>Coolia tropicalis</i>	49.9	3.6	44.1	3.2	93.6	5.8	85.9	5.8
<i>Gambierdiscus toxicus</i>	118.8	8.7	88.2	6.5	97.9	6.1	79.0	4.5
<i>Gambierdiscus belizeanus</i>	37.3	2.7	44.1	3.2	102.1	6.3	79.0	3.4
<i>Ostreopsis labens</i>	137.2	10.3	123.3	8.9	195.3	12.1	289.8	15.6
<i>Ostreopsis lenticularis</i>	56.2	4.1	52.6	3.8	195.7	12.1	69.7	3.7
<i>Prorocentrum lima</i>	87.3	6.4	52.6	3.8	-	-	-	-
<i>Prorocentrum mexicanum</i>	112.5	8.2	70.6	5.1	130.0	8.1	145.8	7.8
<i>Prorocentrum sculptile</i>	90.4	6.6	44.1	3.2	21.6	1.3	108.8	5.8
<i>Bysmatrum subsalsum</i>	180.9	13.2	247.0	17.8	162.9	10.1	257.4	13.8
<i>Scrippsiella</i> sp.	18.4	1.3	70.2	5.1	65.2	4.1	113.8	6.1
<i>Amphidinium carterae</i>	37.3	2.7	-	-	76.0	4.7	75.6	4.1
<i>Amphidinium klebsii</i>	31	2.2	17.5	1.3	76.6	4.7	103.9	5.6
<i>Gymnodinium</i> sp. 1	118.3	8.7	182.1	13.2	152.1	9.4	103.7	5.6
<i>Gymnodinium</i> sp. 2	106.2	7.7	199.9	14.4	97.6	6.1	185.7	10
<i>Gymnodinium</i> sp. 3	149.8	10.9	93.9	6.8	152.1	9.4	173.9	9.4
<i>Protoceratium grindleyi</i>	37.3	2.7	52.6	3.8	-	-	-	-
Total cells x 10 g <sup>-1</sup> sand	1369.1		1382.6		1617.4		1856.7	
% Toxic species		48.9		35.8		59.7		49.3
% Non-toxic species		51.1		64.2		40.3		50.7



### CFP-dinoflagellates on Floating-detritus

Dinoflagellates attached to nylon rope at Carrie Bow Cay were assemblages of benthic/epiphytic species of photosynthetic dinoflagellates with a high proportion of harmful, potentially toxic species present. A detritus in mucilaginous matrix was situated between free floating nylon rope fibers (Fig. 5). Detritus samples on each day were collected at 8 am. Floating detritus was ideal way to quantify species composition of dinoflagellates from 2000 to 2008 (Table 5). These populations existed at water temperature that ranged from 27° to 29°C in 2000 to 2006 and at higher temperatures 30° to 32 °C in 2007 and 2008. Salinity ranged from 34 to 35 psu from 2000 to 2008.



**Figure 5.** In this image, CFP -dinoflagellates presence in detritus mucilaginous matrix and free-floating nylon fibers is shown. The nylon rope hangs off the dock at Carrie Bow Cay. The detritus is green to light brown in color caused by the photosynthetic pigments of dinoflagellates. The nylon rope has a cinnamon color. The water is clear to blue-green in the lagoon.

Sixteen CFP and possibly toxic species in genera *Gambierdiscus*, *Ostreopsis*, *Gambierdiscus* and *Prorocentrum* were identified on floating detritus (Table 5). Total cell concentrations in the samples ranged from 224 to 624 cells x ml<sup>-1</sup> detritus. Density of *Prorocentrum* species ranged from 123 to 305 cells x ml<sup>-1</sup> detritus; *Ostreopsis* species ranged from 40 to 457 cells x ml<sup>-1</sup> detritus; and *Gambierdiscus* species ranged from 22 to 50 cells x ml<sup>-1</sup> detritus. Density of cell was the highest for *P. belizeanum*, *P. lima* and *P. hoffmannianum*; mediate for *O. marinus*, *O. belizeanum*, *O. lenticularis* and *O. ovata*, and lowest for *G. toxicus* and *G. belizeanus*. Cell concentration of *Gambierdiscus* species appeared least variable during the study. Data illustrates that concentration of *Ostreopsis*

species was highest in 2008 lowest in 2000; whereas, *Prorocentrum* cells concentrations were highest in 2000 and lowest in 2008. Data suggest that elevated water temperatures (29° to 32°C) enhanced abundance of seven *Ostreopsis* species, at the same time assemblages of *Gambierdiscus* and *Prorocentrum* declined (Table 5).

Table 5. Density of dinoflagellates associated with cells x ml<sup>-1</sup> detritus attached to rope at Carrie Bow Cay from 2000 to 2008. No data (-).

Taxa	2000	2003	2004	2005	2006	2007	2008
<i>Ostreopsis marinus</i>	15	13	13	-	12	35	55
<i>Ostreopsis siamensis</i>	27	7	8	-	14	16	72
<i>Ostreopsis ovata</i>	37	27	11	22	25	37	83
<i>Ostreopsis lenticularis</i>	19	-	7	-	7	27	58
<i>Ostreopsis caribbeanus</i>	19	1	8	5	6	14	64
<i>Ostreopsis labens</i>	15	-	8	13	13	22	61
<i>Ostreopsis belizeanus</i>	22	11	16	-	16	29	64
<i>Gambierdiscus toxicus</i>	22	27	31	38	50	13	19
<i>Gambierdiscus belizeanum</i>	-	25	23	23	29	9	17
<i>Prorocentrum hoffmannianum</i>	79	41	40	41	74	35	10
<i>Prorocentrum belizeanum</i>	84	30	32	27	47	22	15
<i>Prorocentrum lima</i>	42	18	23	18	29	24	11
<i>Prorocentrum arenarium</i>	30	23	7	10	-	18	7
<i>Prorocentrum emarginatum</i>	43	13	13	12	7	11	8
<i>Prorocentrum rhathymum</i>	8	-	-	8	42	-	-
<i>Prorocentrum mexicanum</i>	19	25	10	7	13	-	-
Total cells ml <sup>-1</sup>	481	261	250	224	384	624	595
<i>Ostreopsis</i> cells ml <sup>-1</sup>	154	59	71	40	93	180	457
<i>Gambierdiscus</i> cells .ml <sup>-1</sup>	22	52	54	61	79	22	36
<i>Prorocentrum</i> cells ml <sup>-1</sup>	305	150	125	123	212	220	102

#### Bloom-forming CFP-dinoflagellates

Ten species of dinoflagellates formed blooms in the Lair, Douglas Cay and South Water Cay. Often these blooms were dominated by one or more dinoflagellate species in both the naturally enriched embayment and the open lagoon (Table 6). Bloom cell concentration in the Lair was the highest of *P. mexicanum* reaching 7.60 x 10<sup>4</sup> cells L<sup>-1</sup> in 2005 and cells of *P. rhathymum* 2.78 x 10<sup>4</sup> cells L<sup>-1</sup> to 1.05 x 10<sup>3</sup> cells L<sup>-1</sup> in 1994 and 1996 respectively. Other species includes *C. monotis* 4.25 x 10<sup>3</sup> cells L<sup>-1</sup>; *G. toxicus* 4.00 x 10<sup>3</sup> cells L<sup>-1</sup>; *P. belizeanum* 1.53 x 10<sup>3</sup> cells L<sup>-1</sup>; and *P. hoffmannianum* 1.99 x 10<sup>3</sup> cells L<sup>-1</sup>. Bloom cell concentration in Douglas Cay ranged of *G. toxicus* 1.14 x 10<sup>3</sup> cells L<sup>-1</sup>; *O. labens* 0.98 x 10<sup>3</sup> cells L<sup>-1</sup>; *G. australes* 4.39 x 10<sup>3</sup> cells L<sup>-1</sup>; *O. lenticularis* 1.50 x 10<sup>3</sup> cells L<sup>-1</sup>; and *P. hoffmannianum* 1.12 x 10<sup>3</sup> cells L<sup>-1</sup>. Bloom cell concentrations at South Water Cay estimated are of *O. labens* 2.46 x 10<sup>3</sup> cells L<sup>-1</sup>; *O. lenticularis* 1.53 x 10<sup>3</sup> cell L<sup>-1</sup> to 1.50 x 10<sup>3</sup> cells L<sup>-1</sup>; and *O. siamensis* 1.35 x 10<sup>3</sup> cells L<sup>-1</sup>. Number of bloom-forming dinoflagellate species varied at three localities: eight species in the Lair, *C. monotis*,

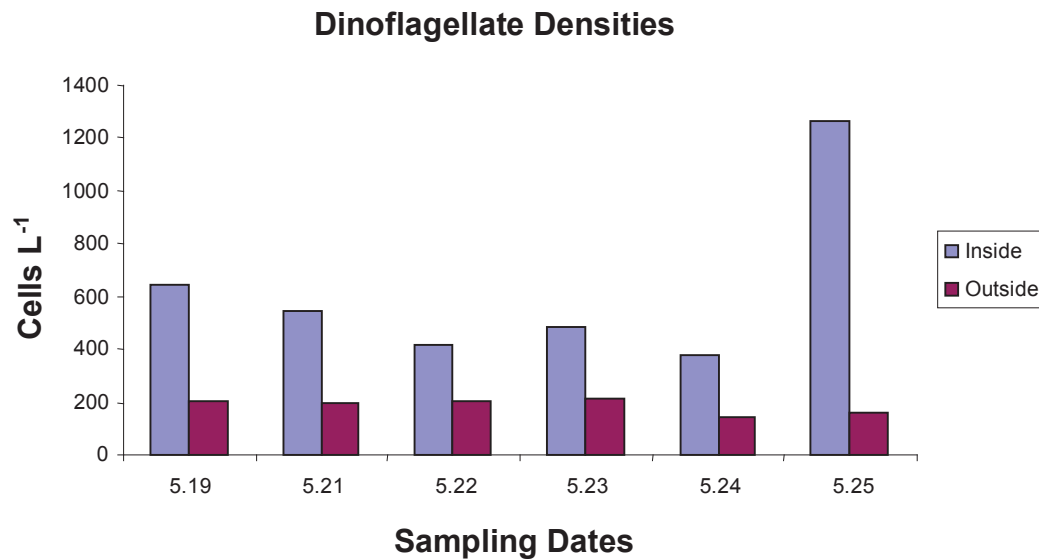
*G. toxicus*, *P. hoffmannianum*, *P. belizeanum*, *P. rhathymum* and *P. mexicanum*; five species in Douglas Cay, *G. toxicus*, *G. australes*, *P. hoffmannianum*, and *O. lenticularis*, *O. labens*; and three species in South Water Cay, *O. labens*, *O. lenticularis* and *O. siamensis*. Bloom-forming dinoflagellates species in high nutrient waters thrived in The Lair and Douglas Cay. Whereas, three *Ostreopsis* species formed blooms in the South Water Cay low nutrient lagoon habitat. This finding suggests that harmful dinoflagellate species appear to exhibit habitat preferences (Table 6).

Table 6. Bloom-forming dinoflagellates present at The Lair. Twin Cays (TC) No. 1, Douglas Cay (DC) No. 2, and South Water Cay (SW) No. 3. No data (-).

Taxa	Concentration Cells L <sup>-1</sup>	Localities (number)			Year
		TC	DC	SW	
<i>Coolia monotis</i>	4.25 x 10 <sup>3</sup>	1	-	-	1991
<i>Gambierdiscus toxicus</i>	4.00 x 10 <sup>3</sup>	1	-	-	1991
<i>Gambierdiscus toxicus</i>	1.14 x 10 <sup>3</sup>	-	2	-	1994
<i>Gambierdiscus toxicus</i>	1.00 x 10 <sup>3</sup>	-	-	-	1994
<i>Gambierdiscus australes</i>	4.39 x 10 <sup>2</sup>	-	2	-	2005
<i>Ostreopsis labens</i>	2.46 x 10 <sup>3</sup>	-	-	3	1994
<i>Ostreopsis labens</i>	0.98 x 10 <sup>3</sup>	-	2	3	1995
<i>Ostreopsis lenticularis</i>	1.53 x 10 <sup>3</sup>	-	-	3	1994
<i>Ostreopsis lenticularis</i>	1.50 x 10 <sup>3</sup>	-	2	3	1994
<i>Ostreopsis siamensis</i>	1.35 x 10 <sup>3</sup>	-	-	3	1996
<i>Prorocentrum hoffmannianum</i>	1.99 x 10 <sup>3</sup>	1	-	-	1991
<i>Prorocentrum hoffmannianum</i>	1.12 x 10 <sup>3</sup>	-	2	-	1991
<i>Prorocentrum belizeanum</i>	1.30 x 10 <sup>3</sup>	1	-	-	1991
<i>Prorocentrum belizeanum</i>	1.53 x 10 <sup>3</sup>	1	-	-	1995
<i>Prorocentrum rhathymum</i>	2.78 x 10 <sup>4</sup>	1	-	-	1994
<i>Prorocentrum rhathymum</i>	1.05 x 10 <sup>3</sup>	1	-	-	1996
<i>Prorocentrum mexicanum</i>	7.60 x 10 <sup>4</sup>	1	-	-	2005

#### CFP-dinoflagellates, Comparing Inside and Outside of Douglas Cay

Comparison of dinoflagellate cell densities in the plankton are illustrated for the Douglas Cay protected embayment and the outside fringe reef transition waters over six days in 2002. The investigation revealed that dinoflagellates maintained five fold higher cell populations inside Douglas Cay compared to cell populations present in outside lagoon waters (Fig. 6). Cell density inside the embayment ranged from 376 to 1263 cells L<sup>-1</sup>, and in the outside lagoon dinoflagellate cells ranged from 145 to 208 cells L<sup>-1</sup>. Density of CFP-dinoflagellates inside Douglas Cay represented approximately 50% of the total dinoflagellate assemblage. The dominance of dinoflagellates is attributable to high ambient nutrients inside DC mangrove embayment and low nutrient in the lagoon (Tables 1 and 2). Comparative distribution and abundance CDP-dinoflagellate taxa appear to fall into two functional groups, high nutrients in protected embayment and low nutrient in open lagoon waters.



**Figure 6.** Comparison of cell densities of CFP-dinoflagellates in plankton inside and outside waters from Douglas Cay estimated in 2002. Cell concentrations are (Cells L<sup>-1</sup>).

## DISCUSSION

### Epiphytic and Benthic Dinoflagellate Community

From this study, it appears that harmful and potentially toxin producing and CFP-dinoflagellates in the genera *Gambierdiscus*, *Ostreopsis*, *Coolia* and *Prorocentrum* are commonly found in coral-reef mangrove marine environments in Belize. Dinoflagellates exhibited variable abundance on macrophytes, sand, floating detritus, and during blooms. Field observations support the findings that marine embayments that are protected from wind have low water turbulence and restricted water exchange from the lagoon and high nutrients, would favor dinoflagellates as predicted by Margalef, R. (1978), and Margalef, R. et al., (1979). The congruence between Margalef's prediction and the distribution of dinoflagellates in naturally eutrophic systems suggest that increased nutrient inputs in the oligotrophic Belizean central lagoon will favor a shift toward CFP-dinoflagellate species. The effect will be most pronounced in bays where turbulence is likely to be reduced. This species shift may have consequences for food web dynamics and the prevalence of toxins producing dinoflagellate population in the food chain (Faust et al. 2005 and references therein).

In comparison, CFP dinoflagellates on macrophytes were much lower in Belize than reported in ecological surveys conducted at the Caribbean (Carlson and Tindall, 1985; Ballantine et al., 1988). In contrast, on macrophytes bloom populations of *G. toxicus* were high at the fringing reefs in Tahiti, Yasumoto et al., (1979), Papara reef (Nakahara et al., 1996), and Hitiaa reef (Bagnis et al., 1990). Yasumoto et al., (1980); and Ballantine et al., (1988) illustrated that *G. toxicus* cells formed a mucilaginous matrix over the macroalgal surface. Nakahara et al., (1996) observed *G. toxicus* cells attached or cells swimming around on the thallus of *Janina* sp.; and *Coolia canariensis* species attached to epiphytes on rocks at the tidal pools in the Canary Islands (Fraga et al., 2008).

In this study, surveys of harmful dinoflagellates assemblages on macrophytes indicate low cell density and low potential for outbreaks of ciguatera in Belize. The association of CFP-dinoflagellates with a specific macroalgae indicated substrate specificity, as recognized by Yasumoto et al., (1979), but in other areas numerous dinoflagellate species occupied red, brown, and green algae (Bomber et al., 1988a). The flexibility of the thallus which provides nutrients for dinoflagellates may be another regulatory factor (Nakahara et al., 1996). Populations of *G. toxicus* have a distinct seasonal pattern in Florida (Bomber et al., 1988b) and are most abundant in the warm season. Furthermore, Bagnis (1987) hypothesized that CFP outbreaks and high densities of toxic dinoflagellate are an indication of coral reef stress or other disturbances as reported at Hao Atoll, French Polynesia.

#### Microbial Associations with CFP-dinoflagellates in Sand

CFP-Dinoflagellate assemblages were found attached loosely to sand particles. These organisms also did freely swim within the water column, entangled in detritus aggregates, and glided within interstitial spaces of sediment particles (Faust, 1996a). In South Water Cay during the study period, days were relatively calm and the number of benthic species flourished. Sixteen benthic harmful and CFP-dinoflagellate species were identified representing a significant proportion (36-60%) of the total dinoflagellate cell populations. Half of the 16 sand-dwelling species found at South Water Cay are known toxin producers and 8 species potential bloom-former (Table 4). It is not currently known if these potentially toxic species in sand are actually producing toxin. There is growing evidence that saxitoxins can serve as an anti-grazing factor (Teegarden et al., 2001). The development of these assemblages appears to require a balance of multiple factors, including nitrogen and phosphorus enrichments, and nutrients from rainfall, to allow their development (Yasumoto et al., 1980; Carlson, 1984; Carlson and Tindall, 1985; Chinain et al., 1999a). The availability of excess nutrients in runoff from highly populated South Water Cay may affect the development of dinoflagellate populations.

Harmful blooms were observed of *O. lenticularis*, *C. monotis*, and *G. toxicus* on associated macroalgae (Ballantine et al., 1988; Fukuyo, 1981; Nakahara et al., 1996), and on dead corals (Carlson and Tindall, 1985). This study supports the finding that inshore localized area of sand at South Water may serve as a nursery for harmful-dinoflagellates. The highest cell densities of *G. toxicus*, *G. belizeanus*, *O. labens*, *P. mexicanum*, *B. subsalsum*, and *Gymnodinium* spp. were observed in sand. The density of dinoflagellate species varied daily over four days (Table 4). Vila et al. (2001) identified *Ostreopsis* spp. in shallow sandy protected rocky habitats, and Tognetto et al. (1995) found *O. ovata* on the rocks along the seashore. Since sand is a favored habitat of benthic dinoflagellate species, knowing species distribution of CFP-dinoflagellates is necessary, to understand the source of toxic outbreaks.

The inshore sand at South Water Cay provides an ideal habitat where benthic dinoflagellates divide among sand grains and proliferate in nutrient enriched benthic environment (Fenchel, 1988). The interstitial spaces between sand grains are a shelter for the dinoflagellates from meiofauna predators (Faust 1996b). Sand grains are associated

with biodetritus, benthic debris, and mangrove detritus (Faust 1990). Dinoflagellates are particle associated forms loosely attached to detrital particles. Dinoflagellates in detritus aggregates move upward through the shallow water column with sunrise and downward at sunset observed in the Lair, Twin Cays (Faust and Gullede, 1996b). During this journey dividing microalgae (dinoflagellates, diatoms, and cyanobacteria) are food source of meiofauna (nematode, ciliate, copepod), leading to the establishment of a dynamic microbial food web. Additionally, microalgae produce dissolved oxygen for the meiofauna residing in the water column. Detritus aggregates facilitate the transport of dinoflagellates daily in to the plankton, where they become an actively dividing population; and cause the redistribution of benthic dinoflagellates (Bomber et al., 1989). This phenomenon is similar to the vertical migration of *Scrippsiella arenicola* in tide pools (Horiguchi and Pienaar, 1988), *B. subsalsa* in The Lair channel (Faust, 1996a), and assemblage of cells in floating and sinking detritus in shallow costal waters (Sherr et al., 1986).

#### Floating Detritus-specialized Environment of Harmful and CFP-dinoflagellates

Nylon rope off Carrie Bow Cay dock indicated that biodetritus is a specialized environment that provides surface area of dinoflagellates to attach and flourish (Figure 5). These organisms form a mucilaginous matrix between nylon fibers. Dinoflagellate assemblages found in shallow open waters habitats included benthic species associated with biodetritus at Carrie Bow Cay. Sixteen benthic dinoflagellate species identified are: seven *Ostreopsis*, two *Gambierdiscus* and seven *Prorocentrum* are reported the first time from a fringe coral-reef mangrove habitat (Table 5). As many as twelve dinoflagellate species in the collections included toxin producers (e.i. Steidinger, 1983). CFP-dinoflagellates presence in the plankton was considered unusual, since the sediment-detritus habitat represent high energy, shallow depth and a low nutrient environment. This study illustrates that shallow sediments are habitats for potentially toxin producing dinoflagellates, *Ostreopsis*, *Gambierdiscus* and *Prorocentrum* species. So far, no toxic outbreaks have been documented at Carrie Bow Cay.

CFP-dinoflagellates associated with the sediment-detritus-water column system appear to be a new phenomenon and a specialized environment for dinoflagellates. Benthic dinoflagellates introduced by biodetritus swept into the water column as free-floating cells, can result in the establishment new benthic microbial communities by the currents. Natural transport of dinoflagellates within open lagoon water appears to be of importance in near-shore sandy areas in coral reefs. This report is the first to provide empirical data on this topic. Floating detritus appears to facilitate dinoflagellate aggregation comparable with planktonic diatom aggregation (Gotschalk and Alldredge, 1989) or as an adaptation by organisms promoting sedimentation in nutrient depleted waters (Smetacek, 1985). This study illustrates this for benthic harmful dinoflagellate assemblages which re-enter the water column on floating detritus and establish new dinoflagellate populations. This floating platform of mangrove detritus creates microhabitats for a variety of toxic benthic organisms within the water column. This aggregated platform has its own diverse microbial assemblages, nutrient gradients and solid-liquid interfaces in low nutrient micro-environments (Alongi, 1994).

Globally *Ostreopsis* species are associated with *Gambierdiscus*, and *Coolia* species. *Ostreopsis* genus did not receive major attention until the taxonomical study of Fukuyo (1981), who re-described two new species, *O. lenticularis* Fukuyo and *O. ovata* Fukuyo. Since then, six new species of *Ostreopsis* was added: *O. heptagona* Norris et al., (1985), *O. mascarenensis* Quod (1994), *O. labens* Faust and Morton (1995), *O. marinus* Faust (1999), *O. belizeanus* Faust (1999), and *O. caribbeanus* Faust (1999). *Ostreopsis ovata* and *O. lenticularis* were detected in the Catalan coast (northwest Mediterranean) with the highest density of epiphytic species ( $6.2 \times 10^6$  cells  $g^{-1}$  dry weight macroalgae) ever reported (Vila et al., 2001, and Penna et al., 2005). *Ostreopsis* was present both in the water column and sand concomitant with maximal cell densities on macroalgae in warm waters. In the Belizean central lagoon, *Ostreopsis* species preferred warm, swift moving low nutrient lagoon water to thrive in sand (Table 4), nylon rope (Table 5), and forms blooms (Table 6). Dinoflagellate species exhibited habitat preferences in high turbulence and low nutrient waters at South Water Cay and Carrie Bow Cay (Table 6). Smayda and Reynolds (2002) suggested that algal blooms are natural events and that elevated cell concentrations relate to local nutrient anthropogenic enrichment.

*Coolia*, *Gambierdiscus*, *Ostreopsis*, and *Prorocentrum* species co-exist in the Belize coral reef mangroves and may play a role in CFP-dinoflagellates distribution (Bomber and Aikman, 1989). Dinoflagellate species in Belize within these genera are attached to macroalgae (Morton and Faust, 1997), seagrass, coral and coral rubble, detritus (Faust and Gullidge, 1996b) and sediment surfaces (Yasumoto et al., 1979, Ballantine et al., 1988, and Vila et al., 2001). Cells-adhering surface relationship may involve excreted organic substances from the macroalgae, physical, chemical, structural and surface interactions (Bomber et al., 1989). The detritus-dinoflagellate association supplies nutrients and provides surface for colonization of benthic dinoflagellates. Because *in situ* floating-detritus is a delicate biofilm surface, upon disruption by water currents, benthic organisms can become free floating assemblages (Carlson and Tindall, 1985). In this situation, floating detritus provide a nursery for harmful dinoflagellates and for dispersing dinoflagellates and associated microorganisms (Faust, 2004).

Knowing the ecology and habitat preference of CFP-dinoflagellates in floating detritus is of ecological importance. Bomber et al., (1988b) reported an opportunistic pattern with regard to the roles of temperature, salinity, and light in seasonality, growth, and toxicity of ciguatera-causing *Gambierdiscus toxicus*. This study confirms rising water temperature of just 2 °C to 3 °C may regulate the distribution of *Ostreopsis* species in shallow fringe reef open high energy, and exposed sandy areas. Here I used a simple method for assessing the abundance of ciguatera-associated dinoflagellates *in situ* using an artificial substrate of a nylon rope. The rope provides surface for dinoflagellates to attach and colonize. Data of cell abundance was estimated from cells rinsed from the rope and used to enumerate dinoflagellate populations for short or long-term investigation. Results indicate that high water temperatures stimulated the growth rate of seven *Ostreopsis* species in 2007 to 2008, whereas, it depressed growth of *Prorocentrum* while density of *Gambierdiscus* remained unchanged (Table 5). *Ostreopsis* spp. is common epiphytic species in northern New Zealand (Rhodes et al., 2000). Density of *O. ovata*,

*O. siamensis*, and *O. lenticularis* was substantially higher when sea surface temperatures exceeded 31°C in November 1995 and October 1996 (Chang et al., 2000). Dinoflagellate culture isolates of *Ostreopsis* exhibited rapid growth rates after reaching 31 °C (Tindall and Morton, 1998).

#### Bloom-forming CFP-dinoflagellates

Dinoflagellates formed blooms in Twin Cays, Douglas Cay and South Water Cay. It is also clear that in terms of species variations among localities; in The Lair eight blooms, in Douglas Cay five blooms, and in South Water Cay five blooms were observed (Table 6). The highest cell concentrations observed was in protected high nutrients low energy habitat in The Lair and Douglas Cay; whereas to those in the low nutrients, high energy open lagoon habitat in South Water Cay (Tindall and Morton, 1998). These blooms occurred during the dry season and persisted in time and space from 1991 to 2006. Dinoflagellate species-specific variability and abundance during blooms differed at the three localities studied. Blooms typically varied in intensity and duration, lasting 3 to 5 days. Frequent blooms can therefore be considered a persistent feature of coral-reef mangrove systems during the dry season.

Bomber et al. (1988a) illustrated the distribution of *G. toxicus* in drift algae samples collected in the Florida Straits and Bahamian waters. Seven other harmful species also occurred in the drift samples. The same species were found at up to 14 of 16 benthic stations. The distribution of these dinoflagellates from drift algae help to explain their circumtropical distribution and their presence in Bermuda. In the South Pacific region harmful dinoflagellates not only account for the majority of species but appear to cause most ciguatera seafood poisoning in French Polynesia, Caledonia and Tuvalu (Chang, 2004). Seafood poisoning is a major problem, causative organisms are epiphytic/benthic dinoflagellates *G. toxicus*, and possibly *O. siamensis*, *O. lenticularis*, *O. ovata*, *C. monotis*, *Prorocentrum lima*, *P. concavum*, *P. mexicanum*, *Amphidinium carterae*, and *A. klebsii* (Anderson & Lobel 1987). Harmful-blooms of *Gambierdiscus* were endemic in Papara, Tahiti (Chinain et al., 1999a), intermediate in Australia (Hallegraeff, 1987), and highest in New Zealand (Rhodes et al., 2000). In the summer at northern New Zealand, in summer, *O. siamensis*, *O. lenticularis* and *O. ovata* cell concentrations were the highest. Other less abundant species included *P. lima*, *P. compressum* and *C. monotis*. There was also clear regional difference in their distribution and cell concentrations of the dominant species (Chang et al., 2000).

In shallow mangrove embayments at Twin Cays, Douglas Cay, and South Water Cay, physical relationships exist between pelagic and benthic environments. *Ostreopsis*, *Gambierdiscus*, *Coolia*, and *Prorocentrum* species, found attached to macroalgae (Table 3), sand (Table 4), and floating detritus (Table 5). Cells formed blooms in the water column by floating upward from the sand surface attached to detritus or from the surface of macrophytes. Epiphytic dinoflagellates of drift algae and their circumtropical distribution recognized (i.e. Bomber et al., 1988a). Present work suggests that the ecology of these dinoflagellates is complex in coral-reef mangrove habitats.



Belizean mangrove embayments function as nutrient oases with high nutrient levels and low turbulence environments, and ideal habitat for benthic dinoflagellates. Twin Cays and Douglas Cay which are protected from wind mixing, show high degree of stratification and have restricted water exchange with surrounding oligotrophic waters of the open Atlantic barrier reef system. Limited water exchange favors retention of *C. monotis*, *G. toxicus*, *G. australes*, *P. belizeanum*, *P. mexicanum*, *P. rhathymum*, *P. hoffmannianum*. *Ostreopsis* harmful and non-harmful dinoflagellates often thrive in the Mediterranean semi-enclosed coastal lagoons (Penna et al., 2005; Vila et al., 2001). Dinoflagellate species are highly variable both spatially and temporally in protected Douglas Cay and The Lair embayments and in the open lagoon areas characterized by high turbulence at South Water Cay and Carrie Bow Cay, as well as in Mediterranean coastal lagoons. These specialized habitats enhance development of benthic populations of harmful dinoflagellates. Note that *O. labens*, *O. lenticularis* and *O. siamensis* were thriving in warm water temperatures and enter the microscopic food web (Table 6).

## CONCLUSION

This review was undertaken with the intention to clarify the role of benthic and epiphytic marine dinoflagellates and their varied distribution and habitat preferences in a coral-reef mangrove ecosystem in Belize. Progress in this field has been slow, but great strides have been made within the last few years in the ciguatera-causing dinoflagellates. Although, in tropical systems, the biodiversity of CFP-dinoflagellates has yet to be fully characterized. The ecology of these dinoflagellates is complex and is affected by light, currents, nutrients, temperature, and habitat preferences. Since the dynamics of an individual dinoflagellate species is directly affected by benthic, planktonic and epiphytic associations within the aquatic community, it is most essential that field studies include and assess all dinoflagellate species. Coral-reefs are nurseries for harmful dinoflagellates and possible sites for ciguatera outbreak world-wide. Because CFP events are highly variable, both temporally and spatially, understanding species diversity, as well as nutrient and habitat requirements may, prove crucial in determining the conditions and species suites most predictive of CFP event occurrences.

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