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Identification and Monitoring of Microalgal Genera Potentially Capable of Forming Harmful Algal Blooms in Punta Galeta, Panama

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ABSTRACT: Coastal areas are attractive for human settlements because they allow easy access to benefits like food, security, and fishing. These aquatic ecosystems are supported by photosynthetic organisms that constitute the base of the food web. The term “Harmful Algal Bloom” (HAB) refers to the excessive proliferation of some taxa of these microorganisms reaching harmful levels to humans and other organisms. Biotoxins produced by these HABs could be transferred to the food chain and are the best-documented impact that HABs have on humans. The location and abundance of the HAB species producing the toxin is a good indicator of a possible human health hazard. The aim of this study was to monitor potentially harmful benthic/epibenthic microalgae in Punta Galeta, Panama over a 15-month period. The 3 main microalgae found were 2 dinoflagellates from the genera *Prorocentrum* and *Ostreopsis* and 1 diatom from the genus *Coscinodiscus*. Sampling made with both natural and artificial substrates yielded similar overall abundance patterns; however, for the macroalgae samples, there appeared to be significant host preferences for *Ostreopsis* and *Coscinodiscus*. Physicochemical measures taken at the site were found to fall within previously reported growth parameters for the microalgae found in the study.

KEYWORDS: Harmful Algal Bloom species, microalgae, dinoflagellates, vectors, coastal areas, food-borne disease

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Introduction

For generations, humans have established their cities near coastal areas due to the benefits offered by these ecosystems.¹ Fishing is one of the most important economic activities developed in these areas.

According to Food and Agriculture Organization (FAO)² data, fish supply has increased at an average annual rate of 3.2% in the last 50 years, outpacing world population growth (1.6%). This production is supported by aquatic ecosystems in which photosynthetic organisms (eg macrophytes, benthic and planktonic microalgae, and cyanobacteria) conform the base for the food web.³ However, the abundance of some taxa can reach harmful levels to humans and other organisms. These proliferations are known as Harmful Algal Blooms (HABs) and are formed by a variety of microalgae species including dinoflagellates (eg *Gambierdiscus*, *Prorocentrum*, *Ostreopsis*, *Coolia*, etc) and diatoms (eg *Coscinodiscus*).

Harmful Algal Blooms are a natural phenomenon and occur in all aquatic environments (eg freshwater, brackish, and marine) and at all latitudes. However, since the 1960s, impacts on the economy and public health seem to have increased in intensity, frequency, and geographical distribution.⁴

About 300 harmful microalgae species have been described. Of those, more than 100 are able to produce natural toxins harmful or even lethal to humans and animals, due to their strength and persistency.^{3,5,6}

There are several classes of marine biotoxins, such as saxitoxin (STX), domoic acid (DA), ciguatera toxin (CTX), brevetoxin (BTX), tetrodotoxin (TTX), okadaic acid (OA), azaspiracid (AZA), and palytoxin (PLTX).⁷ Many of these toxins represent a threat to human health not only in the form of food-borne illness but also by skin contact with contaminated water or by inhalation of toxic aerosols.⁷

Growth, distribution, and abundance of some HAB species are largely temperature driven and expected to shift in response to climate-induced changes as ocean temperatures rise.^{8,9} Nevertheless, conditions such as salinity, irradiance, turbulence, and substrate availability also should be considered.¹⁰

The Greater Caribbean Region (GCR) has been an area with high incidence of Ciguatera Fish Poisoning (CFP) produced by dinoflagellates in the genera *Gambierdiscus* and *Fukuyoa*.¹¹ Ciguatera Fish Poisoning occurs upon consumption of fish containing relatively high concentration of CTX. This toxin tends to be highest in the Greater Caribbean and Lesser Antilles, Bahamas, and Southern Florida, with occasional outbreaks in the Gulf of Mexico, the Yucatan Peninsula, and Central America.¹¹ Another dinoflagellate found in the Caribbean is the genus *Ostreopsis*. Toxins produced by *Ostreopsis* have been suspected to play a role in CFP by some researchers.¹² Other toxins associated with food-borne poisoning and respiratory and cutaneous irritation in Mediterranean beaches are PLTX, Ostreocin, and Ovatoxins.¹³



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Figure 1. Aerial view of the Punta Galeta location in the Province of Colon in Panama. The red star indicates the sampling area.¹⁴

In an effort to establish an observation network for ocean acidification and its impact on HABs, the International Atomic Energy Agency (IAEA) carried out the Regional Project RLA/7/020 along with 10 countries in the Caribbean area, including Panama to evaluate using nuclear and isotopic techniques for this objective. The work presented in this article was developed within the scope of this IAEA project. Therefore, this research is aimed to evaluate the influence of different substrata (macroalgae and screens) and environmental factors on growth and distribution of potential HAB organisms. We expect that the data collected will contribute to increase information for the Great Caribbean Region, which does not include much information of the Southern part of the Caribbean.

Material and Methods

Study area

This research was carried out at the Protected Landscape of Galeta Island, Province of Colon, located at the Panamanian Caribbean coast. The Protected Landscape of Galeta Island is an archipelago integrated by Punta Galeta and the following islands: Galeta, Palma Media, Milla, Peña Guapa, and Cocoli. It is located near the entrance of the Panama Canal at the Caribbean Sea. The sampling area was limited to Punta Galeta (Figure 1).

Sampling sites

Five sampling points were selected and distributed along the seagrass field and nearby mangrove forest: PG1 (9° 24' 6.88" N, 79° 51' 39.72" W), PG2 (9° 24' 7.08" N, 79° 51' 39.65" W), PG3 (9° 24' 7.47" N, 79° 51' 39.52" W), PG4 (9° 24' 7.63" N, 79° 51' 39.65" W), and PG5 (9° 24' 7.70" N, 79° 51' 39.81" W).

W). These coordinates were measured with a Garmin GPS model MAP 60CSx.

Field measurements

Meteorological conditions (atmospheric pressure, air temperature, and relative humidity) were measured using a portable meteorological station (Oregon Scientific model BTHR968) at the beginning and at the end of each sampling campaign. A Beaufort scale was used to determine the theoretical wind speed and a Forel-Ule scale to determine the apparent color of water.¹⁵⁻¹⁷ Physicochemical parameters (pH, water temperature, salinity, dissolved oxygen) were measured using a multi-parameter HACH equipment, model HQ40d.

Collection of microalgae samples

Monthly samples of microalgae were collected using 2 types of substrates (natural and artificial) from August 2016 until November 2017. The artificial substrate consisted of a piece of green nylon screen cut into rectangles of 10.2 cm × 15.2 cm. Each screen was attached to a monofilament fishing line and was suspended within the water column at depths larger than 20 cm from the sea floor using a pyramidal lead weight of 5 kg and a buoy.¹⁸ Submerged buoys were used to limit the length of the monofilament line and avoid disturbances in the screen. In each georeferenced site, a screen was placed per sampling campaign. After placement, the screens were deployed for at least 24 hours before being recovered. To recover the samples, 1-gallon transparent plastic bags with hermetic seals were used, placing the screens inside the bags with a little seawater after being removed. This process was carried out underwater. Natural substrates (macrophytes) were collected individually in transparent plastic bags.¹⁸ Five different macrophytes were collected: *Thalassia testudinum*, *Halimeda monile*, *Halimeda tuna*, *Dictyota sp.*, and *Acanthophora spicifera*.

Processing of HAB samples

Approximately 20% of the seawater from each plastic bag was poured through a 300- μ m pore metal sieve into a 1 L graduated cylinder with a variation of ± 5 mL. The bag was closed and vigorously shaken between 5 and 10 seconds to dislodge the microalgae bound to the artificial or natural substrate. The remaining homogenate was poured through the sieve into a test tube, and the total volume contained in each bag was recorded. The specimens of macrophytes were reserved to determine the weight with a scale; the screens used in the sampling were discarded and new ones were used for each experiment.¹⁸

The sieved seawater sample was re-homogenized and the rest of the seawater was filtered by gravity through a piece of 25 mm diameter nylon with 20 μ m pore size to collect the microalgae cells. Any particulate material left in the specimen was rinsed on the screen with filtered seawater. Then, the 20 μ m mesh was transferred to a conical 15 mL screw cap centrifuge

tube containing 10 mL of filtered seawater in the 300 μm mesh and the sample was preserved with 2 drops of a neutral iodine lugol solution.¹⁹

Cellular abundances of potential HAB (*Ostreopsis*, *Prorocentrum*, *Coscinodiscus*, and others) in each sample in screens or macrophytes were determined using microscopy. A semi motorized vertical fluorescence microscope was used, observing the samples in the objectives with magnifications of 10 \times and 40 \times . A 1.3 megapixel Moticam 1000 camera was also used, which allows the display of the lens samples on the computer screen. These 2 devices, together with the Motic Image Plus software, allowed the images to be projected on a computer and made captures of images and videos in microscopy.

Samples were shaken to suspend the cells of the microalgae and screens, then an aliquot of 0.1 mL was transferred to a slide and each cell found was counted and photographed.²⁰ For the screen samples, microalgae concentrations were expressed as cells per 100 cm^2 , calculated using equation (1), surface area of each screen (166 cm^2).

$$\frac{\text{Cell}}{100 \text{ cm}^2} = \left[\left(\frac{\left(\frac{\text{Cell counted}}{\text{Vol. counted}} \right) \left(\frac{\text{Tube volume}}{\text{Filtered out volume}} \right)}{\left(\frac{\text{Sampling volume}}{\text{Screen area}} \right)} \right) \right] 100$$

For macrophyte samples, microalgae concentrations were determined using a similar method, except that the concentrations were normalized to wet weight of the macrophyte sample and expressed as cells g^{-1} of algae or seagrass.¹⁸

Characterization

A taxonomic classification was done to the genus level by employing an Olympus fluorescent semi motorized vertical microscope model BX53, with 10 \times and 40 \times magnification objectives. For the last 2 months of sampling, a KONUS trinocular biological microscope model BIOREX-3 was used. Microalgae characterization was made by employing images from a variety of researches.^{4,21-24}

Statistics

One-way variance analysis (analysis of variance [ANOVA]) was used to assess environmental variability among sampling points, substrata, and monthly measurements. We also evaluated HAB preference in macrophytes. The abundance data of HAB for both substrates were normalized before the analysis to obtain an absolute value. The software used was GraphPad Prism 6.01 (2012; GraphPad Software, San Diego, CA, USA).

Results

Table 1 shows the descriptive statistics for physicochemical parameters measured for water in the field (pH, salinity,

Table 1. Descriptive statistics for pH, salinity (ppt), dissolved oxygen (mg L^{-1}), and temperature ($^{\circ}\text{C}$) monthly values measured in situ at the Punta Galeta site from June 2016 to November 2017.

POINT	PH				SALINITY (PPT)				DISSOLVED OXYGEN (MG L^{-1})				TEMPERATURE ($^{\circ}\text{C}$)			
	MEAN	MAX	MIN	SD	MEAN	MAX	MIN	SD	MEAN	MAX	MIN	SD	MEAN	MAX	MIN	SD
PG1	8.30	8.58	8.03	0.13	32.86	37.54	27.94	2.550	10.48	12.96	9.54	1.059	30.4	32.7	29.1	0.962
PG2	8.34	8.62	8.11	0.14	32.84	37.55	27.95	2.539	10.53	13.71	9.44	1.265	30.4	32.8	29.1	0.966
PG3	8.31	8.54	8.09	0.12	32.90	37.59	28.01	2.546	10.47	13.36	9.33	1.343	30.5	32.8	29.1	0.981
PG4	8.33	8.59	8.18	0.11	32.79	37.49	27.91	2.506	10.52	13.28	9.41	1.255	30.5	32.5	29.1	0.929
PG5	8.33	8.63	8.06	0.13	32.75	37.50	27.96	2.515	10.48	12.93	9.07	1.246	30.4	32.4	29.1	0.967

Abbreviations: SD, standard deviation of the mean.

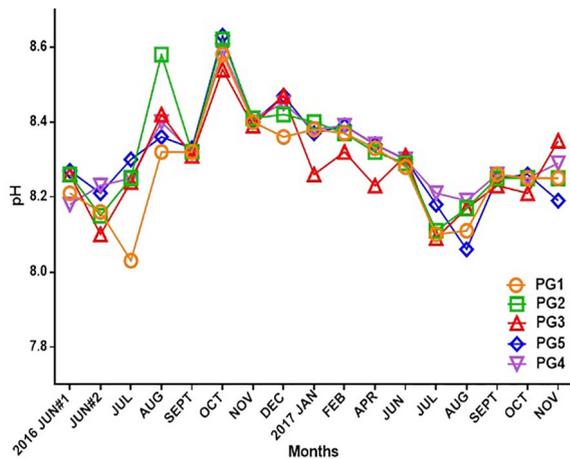


Figure 2. Field pH values corresponding to sampling points at Punta Galeta from June 2016 to November 2017.

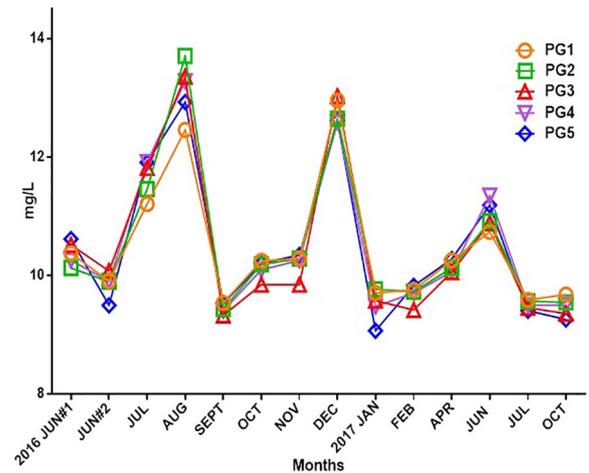


Figure 4. Field dissolved oxygen (mg L^{-1}) results corresponding to sampling points in Punta Galeta from June 2016 to November 2017.

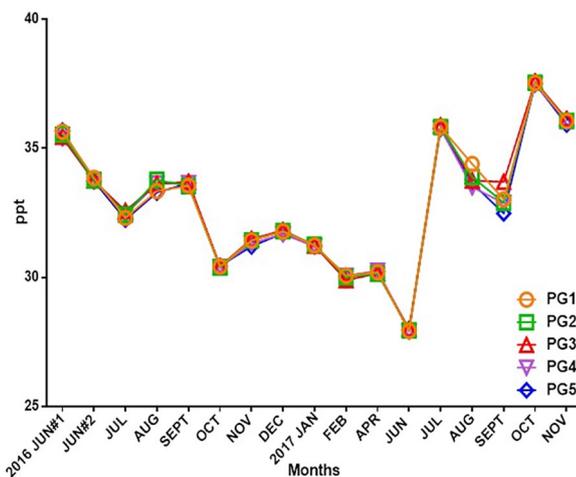


Figure 3. Field salinity (ppt) results corresponding to the sampling points at Punta Galeta from June 2016 to November 2017.

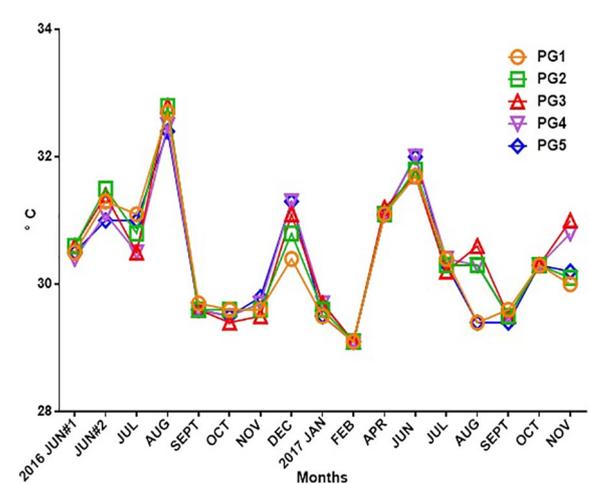


Figure 5. Field temperature ($^{\circ}\text{C}$) results corresponding to sampling points in Punta Galeta from June 2016 to November 2017.

dissolved oxygen, and temperature). Monthly variations in these parameters are shown in Figures 2 to 5. Salinity data were obtained from transforming conductivity results. All parameters seem to behave without much apparent variation.

For artificial substrates, organisms from genera *Prorocentrum*, *Ostreopsis*, and other microalgae were found, with the genus *Coscinodiscus* (other microalgae) being the more abundant (Table 2). Abundance of *Prorocentrum* ranged from 0 to -271.1 cells 100 cm^{-2} , while *Ostreopsis* ranged from 0 to 813.3 cells 100 cm^{-2} . Counts for other microalgae, including *Coscinodiscus*, ranged from 3524 to 9217 cells cm^{-2} (Figure 6A). For samples taken from macrophytes, *Prorocentrum* counts ranged from 0 to 818 cells g^{-1} , while *Ostreopsis* and other microalgae were observed at 0 to 25 912 cells g^{-1} and 0 to 6314 cells g^{-1} , respectively (Figure 6B).

One-way ANOVA results using monthly values as a factor showed a significant difference between the microalgae groups for each month, $F(13) = 2.172$, $P \text{ value} = .012$. *Prorocentrum*, *Ostreopsis*, and other microalgae were also found in samples

from the macrophytes employed (see Table 3). Here, one-way ANOVA analysis was also applied, to count for differences among macrophytes in the sampling points. Basically, no significant differences between the macrophytes specimens in the 5 sampling points were found, except for February 2017, $F(4) = 4$, $P \text{ value} = .0343$.

Regarding the host preference of the microalgae groups in the macrophytes, the total values of the cells counted for each group (*Prorocentrum*, *Ostreopsis*, and other microalgae) were used in all the macrophytes, emphasizing the genera that showed the most cells of each group. In this way, the cells of the genus *Prorocentrum* showed a preference of 81.6% in *H tuna*, while the genus *Ostreopsis* had greater affinity for *Dictyota sp.* with 85.3%. Other microalgae maintained an affinity of 42.7% with *T testudinum* (Figure 7). For the host preference of microalgae in macrophytes, there were no significant differences between the groups ($F = 1.408$, degree of freedom [DF] = 2), as the data in *A spicifera* were not taken into account due to the absence of organisms in the samples.

Table 2. Microalgae genera (P: *Prorocentrum*, O: *Ostreopsis*, OM: other microalgae) found on screens during the period of study at each sampling point.

SCREENS																
MONTH/YEAR	SAMPLING POINTS															
	PG1			PG2			PG3			PG4			PG5			
	P	O	OM	P	O	OM	P	O	OM	P	O	OM	P	O	OM	
AUG 2016			X			X			X			X	X		X	
SEP 2016			X							X			X			
OCT 2016		X	X			X				X	X	X			X	
NOV 2016	X	X	X			X			X			X			X	
DEC 2016						X			X						X	
JAN 2017						X			X			X				
FEB 2017					X	X										
APR 2017						X						X			X	
JUN 2017			X			X			X			X			X	
JUL 2017		X	X	X	X	X						X			X	
AUG 2017					X	X						X			X	
SEP 2017			X		X	X			X			X			X	
OCT 2017			X			X	X									
NOV 2017			X		X	X			X		X	X		X		

Normalized data from natural and artificial substrates based on results shown in Figure 6A and B were compared to determine possible differences among microalgae host preferences, abundances, and substrates. From this analysis, clear differences between artificial and natural substrates, especially for *Coscinodiscus*, were obtained for the months of November 2016 and June 2017. Also, a 2-way ANOVA was applied, using as factors the type of microalgae and substrates. From this exercise, significant differences were found between the microalgae, $F(2) = 5.351$, P value = .0113, and type substrates, $F(1) = 8.106$, P value = .0085, for November 2016. Significant differences were also found between the microalgae, $F(2) = 9.951$, P value = .0006, and type substrates, $F(1) = 5.444$, P value = .028, for June 2017.

Discussion

This study is the first to detect the presence of potentially harmful dinoflagellates from the genera *Prorocentrum* and *Ostreopsis* and a diatom from the genus *Coscinodiscus* in Punta Galeta, Panama. These findings are important as the OA and derivatives produced by *Prorocentrum lima* can cause Diarrhetic Shellfish Poisoning (DSP). Also, this genus is present worldwide, affecting seafood and fisheries activities.²⁵⁻²⁹ For the case of the *Coscinodiscus*, this genus was the most abundant found in Punta Galeta throughout the present study. According to

toxicology, benthic organisms can be damaged by mucilage produced by *Coscinodiscus*, which can aggregate, sink, and cover the sea bed. Bloom decay is also likely to cause anoxic conditions, whereas fisheries and aquaculture plants can be impacted by clogging of fishing nets and cages.⁴

The greatest abundance for the genus *Prorocentrum* in the Caribbean Sea was observed in the dry season at 27.4°C and salinity at 35 ppt³⁰ in a study conducted in 2 coastal sites in Guadalupe Island to evaluate the abundance of dinoflagellates on an invasive macrophyte *Halophila stipulacea*. Other researchers indicate that the abundance of *Prorocentrum* is very low in temperate cold-water environments; although a direct comparison with warmer waters cannot be made as there have not been many samplings in cold waters.³¹⁻³⁴ The results in Punta Galeta contrast with the findings of Boisnoir et al,³⁰ as we found that this genus had greater abundance in the rainy season, at an average temperature of 30°C and a variable salinity in each month of sampling.

Muciño-Márquez et al,³⁵ in a case study in Mexico, indicated that the genus *Prorocentrum* is abundant in high salinities finding maximum abundance of cells at 30 ppt and presenting a decrease when the salinity reached a value of 20 ppt. It is also known that these species are sensitive to sudden changes in salinity.³⁶ Different species of phytoplankton with specific requirements respond differently to changing environmental

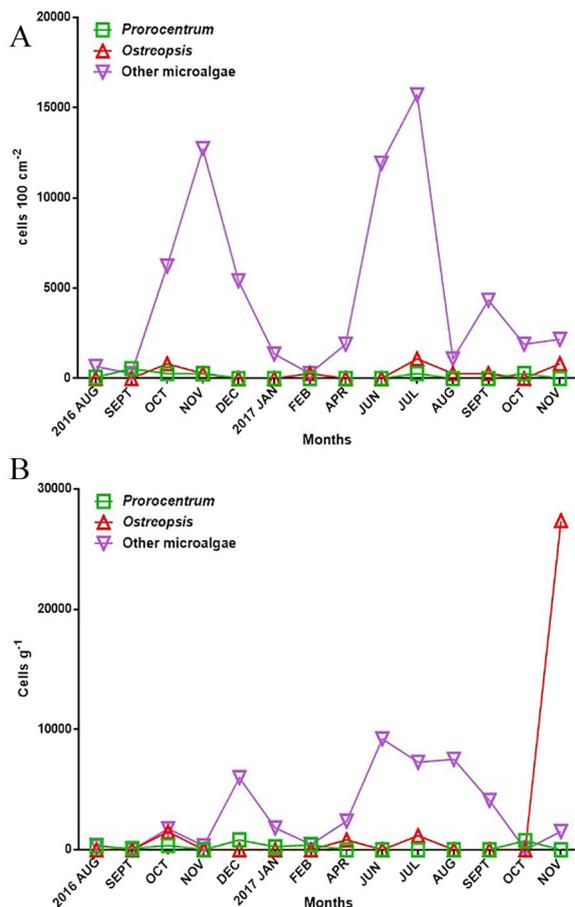


Figure 6. (A) Artificial substrates. (B) Microalgae collected on natural substrates (macrophytes) from August 2016 to November 2017. Green boxes represent the genus *Prorocentrum*, red triangles the genus *Ostreopsis*, and inverted orange triangles other microalgae.

conditions.³⁷⁻⁴⁰ The constant changes in the environment (natural and anthropic) can condition environments that favor the development of certain species. In Punta Galeta, the cell density in the genus *Prorocentrum* was correlated with high salinity (around 32.83 ppt), in accordance with the studies carried out in Mexico and Guadalupe mentioned above.

Marasigan et al⁴¹ point out that for the genus *Prorocentrum* and the dinoflagellates in general, a preference and better adaptation to seagrasses can be observed. Cells of this genus also have been found in detritus of floating mangroves.^{42,43} Contrary to previous investigations, our results show a preference for *H tuna* (81.58%) by this genus, followed by *T testudinum* (9.85%) and finally *Dyctiota sp.* (8.57%).

The genus *Ostreopsis* is usually found on hard substrates, such as rocks and mussel shells and plankton.⁴⁴ Most of the organisms of this genus (9 species) have been observed on macro algae samples, except for *Ostreopsis belizeanus* and *Ostreopsis caribbeanus*, which have been observed in plankton. Aligizaki and Nikolaidis⁴⁵ identified this genus in macro algae, sediments, and water column. In New Zealand, *Ostreopsis* has been observed in plankton and in drifting macroalgae.⁴⁶ In the Virgin Islands, *Ostreopsis* has been reported in samples of

Dictyota, which grows in dead coral.⁴⁷ Monti et al⁴⁸ showed that the genus *Ostreopsis* prefers to stay in brown and red algae in the northern Adriatic.

In Tasmania, *Ostreopsis* has been described as a common group in the seagrass.⁴⁹ The 9 species of *Ostreopsis* presented differences in preferences. For example, in Belize, *Ostreopsis lenticularis* was abundant in *A spicifera* and was less prevalent in *T testudinum*, *Dictyota dichotoma*, and *Halimeda opuntia*. *Ostreopsis hexagona* was abundant in *T testudinum*, but also was present in *A spicifera*, *H opuntia*, and *D Dichotoma*.⁵⁰ In Hawaii, *Ostreopsis* was abundant in *Martensia fragilis* and *A spicifera*.⁵¹ The results presented in other investigations are different from those found in Punta Galeta, with the exception of the one by Kohler and Kohler,⁴⁷ as they had a greater preference for *Dictyota sp.* (85.30%), followed by *T testudinum* (12.13%), *H tuna* (2.33%), and finally *H monile* (0.24%).

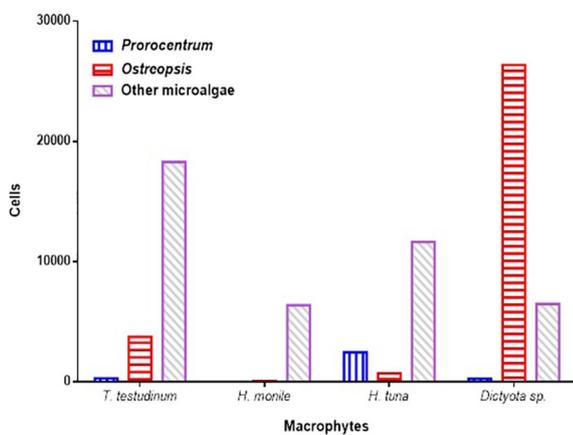
Boisnoir et al³⁰ indicate that for Gosier in Guadalupe Island, the highest abundances of *Ostreopsis spp.* occur during the rainy season with a temperature between 31.3°C and 31.4°C, but in Rivière Sens the highest abundance is given at 26.8°C. It also reached great abundance during the dry season with a salinity of 36 ppt. Similarly, our results showed greater abundance during the rainy season at an approximate temperature of 30°C. The changes in salinity at Punta Galeta were not associated with changes in abundance during the whole sampling period. Another important characteristic found in Punta Galeta is the high Dissolved Oxygen (9.07-13.71 mg L⁻¹), this appears to be related to the proliferation of *Prorocentrum*, which is probably in direct relation with the rapid growth of many macrophyte.

Some factors that condition the occurrence and intensity of dinoflagellate blooms are the specific nutritional needs of each species, water temperature, solar radiation, meteorological phenomena that lead to movements of water masses, mixing by the action of rising currents, tides, and development of a thermocline.^{52,53}

The genus *Coscinodiscus* has been reported as a marine planktonic group distributed in all waters of the world. Von Stosch⁵⁴ indicates that the species related to warm waters of the tropics are between 18°C and 30°C and that they die below 15°C and above 33°C. Our results agree as this diatom type was found within these temperature ranges. Santiago et al,⁵⁵ in a study conducted in the port of Recife (Brazil), indicate that for sites associated with estuarine waters there is a dominance of diatomaceous groups as they are favored by their Euryhaline characteristics. This is consistent with our results as the sampling sites were close to an estuary and the *Coscinodiscus* genus remained the dominant group during all cell counts. *Coscinodiscus* is considered an invasive species probably introduced with ballast water discharge⁴ from the ships at the entrance of the Panama Canal. This fact could be one of the reasons why this genus is found year round in the waters of Punta Galeta. Another hypothesis to explain the abundance of this genus could be that environmental conditions such as

Table 3. Microalgae genera (P: *Prorocentrum*, O: *Ostreopsis*, OM: other microalgae) found on macrophytes during the sampling period at Punta Galeta.

MONTH/YEAR	MACROPHYTES														
	THALASSIA TESTUDINUM			HALIMEDA MONILE			HALIMEDA TUNA			DICTYOTA SP.			ACANTHOPHORA SPICIFERA		
	P	O	OM	P	O	OM	P	O	OM	P	O	OM	P	O	OM
AUG 2016	X		X						X						
SEP 2016							X								
OCT 2016		X					X				X	X			
NOV 2016						X									
DEC 2016			X			X	X		X						
JAN 2017			X						X	X		X			
FEB 2017			X				X								
APR 2017		X	X		X	X			X				X		
JUN 2017			X						X						
JUL 2017		X	X			X			X				X		
AUG 2017			X			X									
SEP 2017			X						X						
OCT 2017							X								
NOV 2017		X	X						X	X		X			

**Figure 7.** Preference of microalgae in macrophytes. Cells were found in 4 macrophytes (*Thalassia testudinum*, *Halimeda monile*, *Halimeda tuna*, and *Dictyota sp.*). The genus *Prorocentrum* and the genus *Ostreopsis* are shown in blue and red, respectively. Other microalgae are shown in yellow.

temperature, salinity, dissolved oxygen, and nutrients present in Punta Galeta favor its growth, as also the gender competition.

It is important to notice that *Gambierdiscus* was not observed in this study. Nowadays, the rise of ocean temperature has led to increases in Ciguatera Fish Poisoning (CFP) at higher latitudes due to a broadening of the distribution of *Gambierdiscus* and *Fukuyoa* species poleward.¹⁰ However, these CFP-associated

genera still present high cell densities in warm, shallow bays and where temperatures are high and relatively stable throughout the year.⁵⁶ Although the sea surface temperature is higher and more stable in the Eastern Caribbean Sea (approximately 24°C–29°C), the temperature-CFP relationship weakens at temperatures >30°C, suggesting that an upper thermal limit may restrict *Gambierdiscus* occurrence.⁵⁶ An upper threshold is supported by experimental data showing a precipitous decline in *Gambierdiscus* and *Fukuyoa* growth rates at temperatures approaching ~31°C,⁹ but more substantial warming may cause cell mortality. The GCR shows optimal growth conditions for *Gambierdiscus* and *Fukuyoa*¹⁰ supporting the hypothesis that CFP occurrence is associated with these conditions; however, the environmental conditions presented in Punta Galeta (Caribbean Sea) do not favor the growth of these genera due to its high temperature (around 32°C).

Conversely, the climate at both sites of the Isthmus of Panama presents marked seasonal variations, related to the Intertropical Convergence Zone. Winds from the north move the intertropical convergence zone away from the isthmus, while the winds from the south generally push it toward the isthmus.⁵⁷ The effect of the winds is more intense at the Caribbean Sea (in Panama), resulting in bigger waves than in the Pacific Ocean. *Gambierdiscus* inhabits certain substrates (macroalgae, sea grass, sand, and death corals) and requires special conditions for its survival, such as zones without waves and

away from contributions of continental waters.^{18,58-60} As we mentioned before, the Panamanian Caribbean coast presents large waves with irregular tides (1 m amplitude) influenced by prevailing weather conditions. As for the waters of Panama, the superficial marine currents at the Caribbean have a predominant direction from coast to coast to the East and 500 km off-shore to the West shaping a counter clockwise ring of circulation during all seasons of the year. This constant dynamic does not favor the presence of this genus.

Another hypothesis could be that the allelopathic effect produced by *Prorocentrum* and *Ostreopsis* may lead to a competition among them and the production of chemical compounds inhibiting the proliferation of *Gambierdiscus* or another microalgae.⁶¹

Conclusions

In Punta Galeta, dinoflagellates of the genera *Prorocentrum* and *Ostreopsis* were found. Both are considered potential bloom-forming genera worldwide. In addition, the taxonomic genus *Coscinodiscus* which is known to cause damage to marine life was also found, showing the highest cellular abundance among the microalgae studied. The field sample collections indicate that Punta Galeta microalgae differ in their preference to adhere to natural substrates (macroalgal) or artificial substrates (screens). However, the physicochemical characteristics of the waters in Punta Galeta present favorable conditions to sustain the life of the place. However, some parameters as water temperature, salinity, marine currents, the effect of the wind, and the contribution of continental waters neither favor the growth of certain genera vectors of marine toxins nor the blooms of these genera at Punta Galeta.

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Author Contributions

Conceived and designed the experiments: AG, JF and KB. Analysed the data: AG, KB, JF and NT. Wrote the first draft of the manuscript: AG. Contributed to the writing of the manuscript: AG, KB, JF, KY. Agree with manuscript results and conclusions: AG, KB, JF, NT, KY. Jointly developed the structure and arguments for the paper: AG, KB and JF. Made critical revisions and approved final version: JF, KB and NT. All authors reviewed and approved of the final manuscript.

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