



Population Dynamics of Benthic-Epiphytic Dinoflagellates on Two Macroalgae From Coral Reef Systems of the Northern Mexican Caribbean

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In the present study the abundance of epiphytic dinoflagellates was evaluated at two coral reef sites of the natural protected areas, Arrecife de Puerto Morelos and Isla Contoy, located in the northern Mesoamerican Reef System of the Mexican Caribbean. Abundances were monitored from April to December, 2015 on two genera of macroalgae belonging to different functional groups: *Dictyota* and *Amphiroa*. In general, the total abundance of dinoflagellates was higher in Puerto Morelos on both macroalgae. *Ostreopsis* cf. *marina* and *O. heptagona* were the dominant species. Relative abundance of these species varied from 8 to 99% of total epiphytic dinoflagellates. Maximum abundances at Puerto Morelos were registered in April, with 33,801 cells·g⁻¹ on *Dictyota* and 6,264 cells·g⁻¹ on *Amphiroa*. In Isla Contoy the maximum abundance was 16,006 cells·g⁻¹ and it was detected on *Dictyota* during December. Other dinoflagellate genera were more abundant during the warmer period (May–September) in both locations. *Prorocentrum* was the second most abundant genus and was represented by six species (*P. hoffmannianum*, *P. lima*, *P. belizeanum*, *P. elegans*, *P. emarginatum*, and *P. rathymum*). The maximum pooled abundance of *Prorocentrum* species was 4,144 cells·g⁻¹ on *Dictyota* in August. *Coolia* spp. did not reach abundances higher than 1,000 cells·g⁻¹ and *Gambierdiscus* spp. only exceeded 100 cells·g⁻¹ during August. Mean water temperature in Isla Contoy was significantly lower than that of Puerto Morelos during the entire study period. There was a negative correlation between the water temperature and the abundance of *O. cf. marina*. The dominance of *Ostreopsis* in the Caribbean is significant because of its capability to produce palytoxin analogs and its potential role in ciguatera fish poisoning outbreaks in the region. This is the first study that reports blooms of *Ostreopsis* in Mexican Caribbean coral reefs, a fact that emphasizes the significance of this genus at global scale.

Keywords: *Ostreopsis*, *Dictyota*, ciguatera, Caribbean Sea, coral reef

INTRODUCTION

There is the general assumption that ciguatera fish poisoning (CFP) outbreaks are linked to benthic dinoflagellate blooms, especially of toxic species of *Gambierdiscus* genus (Chinain et al., 1999; Turquet et al., 2001). However, the ecological processes that occur between the benthic harmful algae blooms and intoxication in humans are not as clear as planktonic harmful algae blooms (HABs), which can be more easily detected, especially those that color the water. For a better understanding of the ciguatera phenomenon, it is necessary to increase the knowledge of the factors that control the abundance of toxin-producing epiphytic dinoflagellates, such as the influence of environmental variability, host-epiphytic dynamics, herbivory patterns on the macroalgae substrates, trophic relationships between vectors and even the preferences of human consumption on top predator fishes.

Most research has focused on the environmental variability and host-epiphytic dynamics through the analysis of the experimental responses of benthic dinoflagellates to variations of temperature, irradiance, nutrient concentration and salinity (Bomber et al., 1988; Chinain et al., 1999; Hales et al., 1999; Anderson et al., 2008; Lartigue et al., 2009; Kibler et al., 2015), as well as the substrate preference of some dinoflagellate species (Lobel et al., 1988; Bomber et al., 1989; Nakahara et al., 1996; Parsons and Preskitt, 2007; Parsons et al., 2011; Rains and Parsons, 2015), and the monitoring of dinoflagellate abundance on several substrates and macroalgae (Morton and Faust, 1997; Vila et al., 2001; Okolodkov et al., 2007, 2014; Moreira et al., 2012; Cohu et al., 2013). Also, other features such as depth, the water motion and habitat structure have been studied in relation to abundance and occurrence of the benthic dinoflagellates (Richlen and Lobel, 2011; Cohu et al., 2013; Boisnoir et al., 2018; Yong et al., 2018).

These investigations demonstrate that in many cases, benthic dinoflagellate abundances are influenced by increased temperature (Tester et al., 2010; Kibler et al., 2015). On this basis, seasonal variability of water temperature has received special attention as a predictive variable of dinoflagellate abundance and ciguatera outbreaks (Chinain et al., 1999; Tosteson, 2004; Chateau-Degat et al., 2005; Gingold et al., 2014). Although temperature could be the most important variable that explains changes in the abundance of benthic dinoflagellates, other factors could account for the dominance of a particular species. Particularly, macroalgal species could control the population dynamics of associated dinoflagellates. Macroalgae could promote or inhibit dinoflagellate growth through providing shelter or surface for attachment (Bomber et al., 1989; Parsons et al., 2011; Rains and Parsons, 2015).

The historical relationship between dinoflagellates and macroalgae may be facing a nonreversible change due to the replacement of macroalgae coastal communities as consequence of eutrophication and climate change (McCook, 1999). The impact of these phenomena on benthic communities is especially evident in tropical coral reefs ecosystems (Hoegh-Guldberg et al., 2007). These ecosystems constitute a common habitat for toxin-producing dinoflagellates (Yasumoto et al., 1980; Chinain et al., 1999). Hard corals are key species in these

ecosystems because they function as shelter for a variety of species. Coralline macroalgae are associated with the coral in healthy coral reefs (Björk et al., 1995). However, coral cover is decreasing dramatically and their skeletons are being colonized by fleshy opportunistic macroalgae (Kohler and Kohler, 1992). This implies that macroalgae cover can reach high cover percentages and, in turn, constitute an extensive substratum for epiphytic dinoflagellates. Therefore, it is necessary to understand if the replacement of coralline macroalgae communities with fleshy macroalgae communities also changes the epiphytic dinoflagellate assemblage inhabiting them.

In the Mexican Caribbean coast, the CFP is a syndrome with a relatively high incidence. The main vector of ciguatera toxins is the great barracuda (*Sphyraena barracuda*) (Arcila-Herrera et al., 1998), but other fishes have also been involved in outbreaks. The presence of several potentially toxic species of the genera *Gambierdiscus*, *Prorocentrum*, *Ostreopsis*, *Coolia*, and *Amphidinium* has been registered in the Mexican Caribbean (Hernandez-Becerril and Almazan-Becerril, 2004; Almazán-Becerril et al., 2015, 2016).

In the coral reefs of the Mexican Caribbean fleshy macroalgae cover is higher than 50% and may reach 90% at some sites (Delgado-Pech, 2016). *Dictyota* is one of the dominant genera and may account for the 80% of the total macroalgae cover at some sites and periods. This implies that, as dominant macroalgae, *Dictyota* could increase the density of dinoflagellates in absolute terms (cells·m²).

The aim of this work is to characterize taxonomic composition and abundance of dinoflagellate community in two functionally distinct algae: *Dictyota* and *Amphiroa* at two sites located at the northern portion of the Mesoamerican Coral Reef Barrier. Each genera of macroalgae represent different states of coral reef health. *Dictyota* represents an impacted coral reef and *Amphiroa* a healthy one.

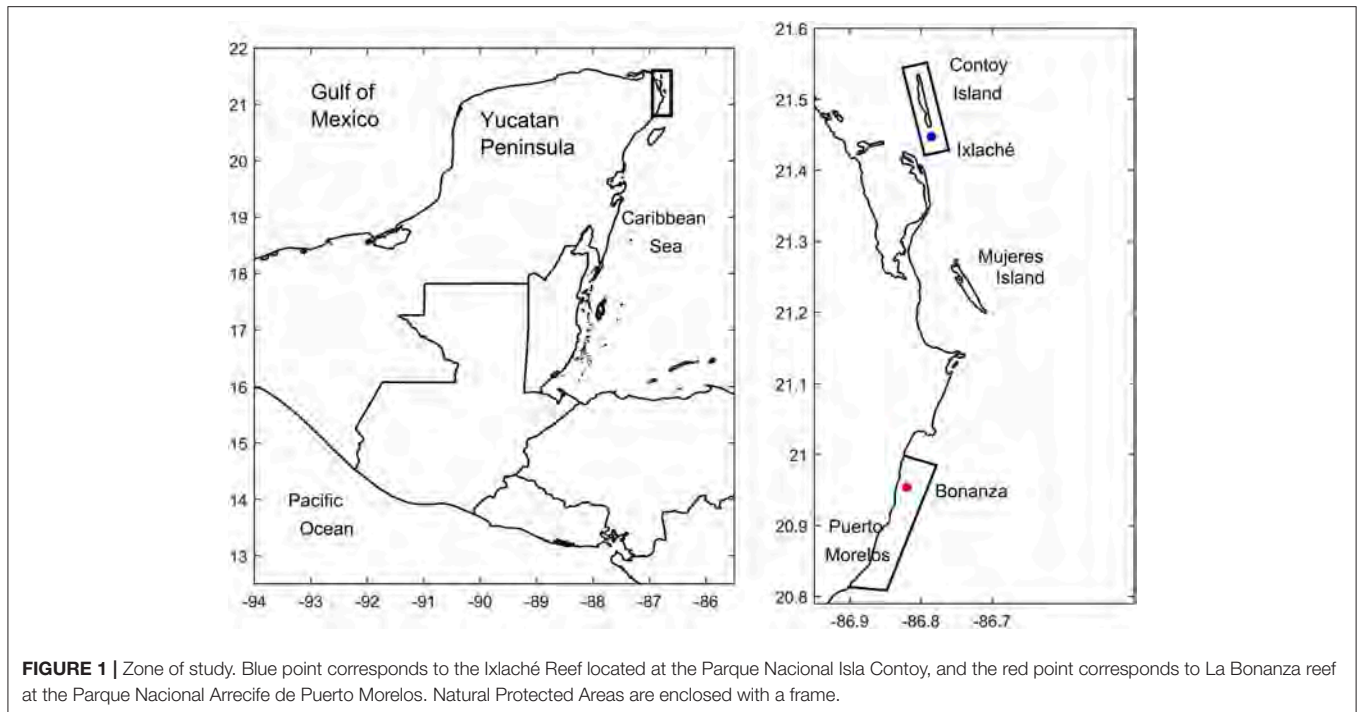
MATERIALS AND METHODS

Study Area

This study was carried out from April to December 2015 at two reef sites located in the northern Mexican Caribbean: Isla Contoy (IC) and Puerto Morelos (PM). Both sites are protected natural areas of the Mesoamerican Reef Barrier System (**Figure 1**). Ixlaché reef is located 1 km south of IC. It is a small reef 800 m long and 60 m wide. Depth varies between 0.5 and 3 m, macroalgae cover is 60%, and is influenced by coastal currents, waves, tides, and winds predominantly from the southeast and polar fronts. On the other hand, the Bonanza reef site is part of the PM reef; it is a shallow area of <2 m depth and macroalgae cover is higher than 75%.

Substrate Selection

Two macroalgae species were selected for this study, each representing a distinct functional group (Littler et al., 1983; Steneck and Deither, 1994). *Amphiroa* is a slow-growing rate coralline articulated macroalgae present at relatively low cover in the sites of study. *Dictyota* is fast-growing rate fleshy, foliose macroalgae with a high cover. Both sites selected for this study



are macroalgae-dominated reefs where *Dictyota*, *Turbinaria*, *Lobophora*, and *Laurencia* are very common, although patches of the coralline algae *Amphiroa* still remain.

Collection of Macroalgae Samples

At each site, 10 specimens of each genus of macroalgae were collected. The distance between collected specimens was at least 10 m according to Lobel et al. (1988). Collected macroalgae were stored in plastic bags with seawater and placed into a cooler with water of the site to maintain the temperature. Algae were fixed with formaldehyde at 4% final concentration and stored until analysis.

In the laboratory, macroalgae samples were strongly shaken during 2 min to detach epiphytic dinoflagellates. Macroalgae were separated from the seawater and the excess of water was removed with absorbent paper. Macroalgae were weighed and fixed in formaldehyde. A total of 400 samples were processed.

Environmental Variables

Water temperature was recorded every 3 h during the study period. HOBO Pro-v2 sensors were placed at the reef sites anchored at the level of the macroalgae mats. For nutrient determinations, water samples were collected with a 50 ml syringe, previously washed with a 10% HCl solution, connected to a filter holder containing a Whatman® membrane filter of 0.22 μm size pore and 2.5 mm diameter. Filtered seawater was recovered into a 30 ml Nalgene® polypropylene bottles that were stored in a cooler with ice. Once in the laboratory the samples were stored at −80°C. The quantification of NO_x^- ($\text{NO}_3^- + \text{NO}_2^-$) and PO_4^{3-} was performed by using a Skalar® segmented flux

autoanalyzer following the techniques described by Strickland and Parsons (1972) and Grasshoff et al. (1983).

Abundance and Identification of Epiphytic Dinoflagellates

The abundance of epiphytic dinoflagellates was estimated with the help of a Carl Zeiss inverted microscope model Axiovert 40 CFL at 10X magnification using a Sedgewick-Rafter counting chamber. Abundance is expressed in $\text{cells}\cdot\text{g}^{-1}$ of wet weight macroalgae (Reguera et al., 2016).

For dinoflagellate species identification the following specialized literature was used: Fukuyo (1981), Faust (1990, 1993, 1994, 1995, 1997, 1999), Faust et al. (1996), Litaker et al. (2009), and Almazán-Becerril et al. (2015, 2016).

Dinoflagellate observations and measurements were made with an Axio Imager A2 Zeiss microscope at 40X magnification using the differential interference contrast and epifluorescence techniques. Photographs of the specimens were taken with an AxioCam ICc1 digital camera coupled to the microscope. The image processing was carried out with the software Axiovision®.

Statistic Analysis

The non-parametric Mann-Whitney *U*-test ($\alpha = 0.05$) was used to compare the dinoflagellate abundance at each sampling date between sites, and the non-parametric Wilcoxon rank test ($\alpha = 0.05$) was used to evaluate the potential differences between sites during the whole period of sampling by paired data. The Spearman rank correlation was applied to describe the community structure on basis of the associations between species in both macroalgae. The association of temperature and nutrient concentration was assessed using the same correlation test.

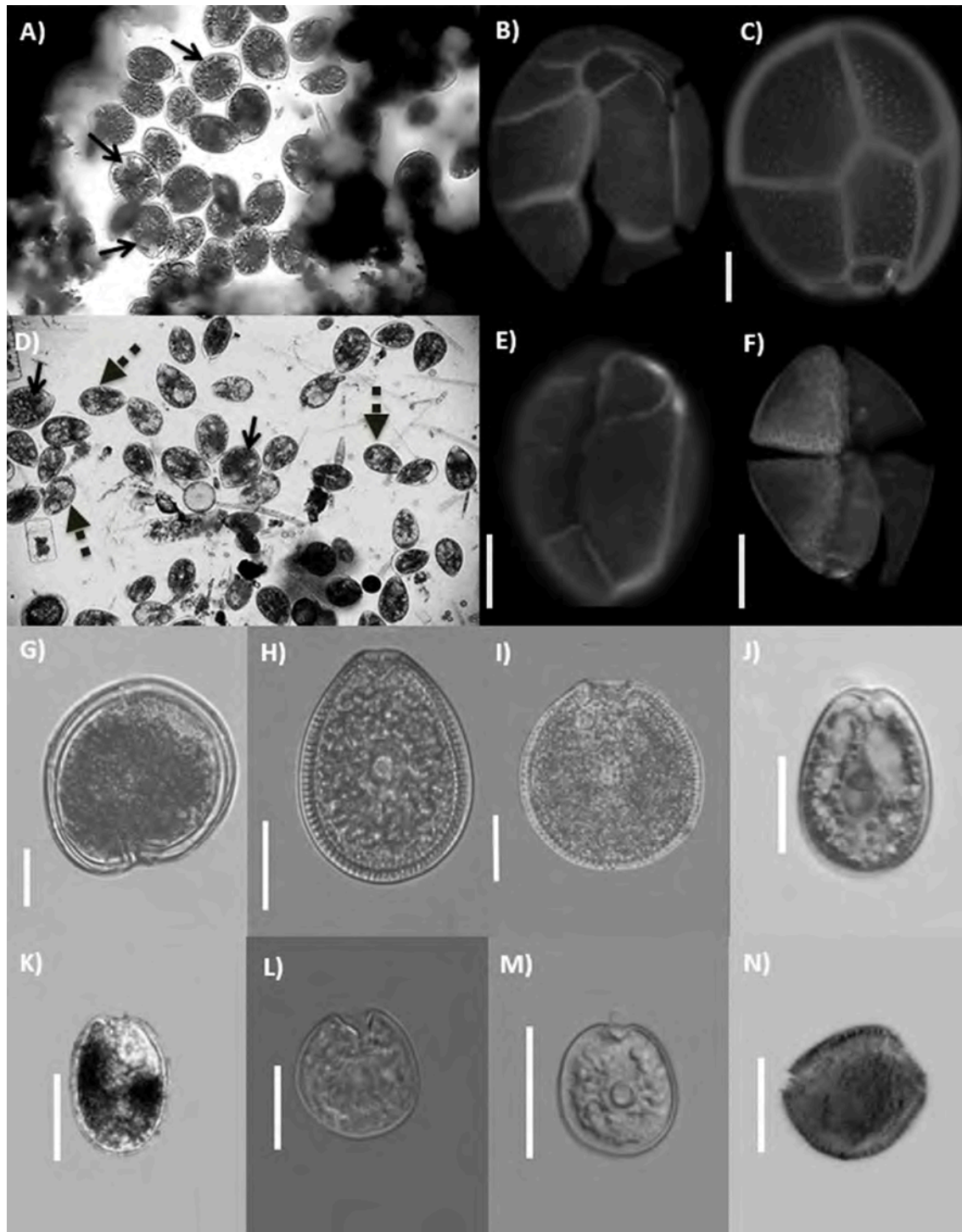


FIGURE 2 | Species of benthic dinoflagellates found in the study zone. **(A)** *Ostreopsis marina* bloom from IC. **(B)** Epifluorescence images of *O. marina* epitheca and **(C)** hypotheca. **(D)** *Ostreopsis heptagona* bloom from PM. Note the presence of some cells of *O. marina* (arrows). **(E)** Epifluorescence images of *O. heptagona* epitheca and **(F)** hypotheca. **(G)** *Gambierdiscus* sp. **(H)** *Prorocentrum hoffmannianum*. **(I)** *Prorocentrum belizeanum*. **(J)** *Prorocentrum lima*. **(K)** *Prorocentrum rathymum*. **(L)** *Prorocentrum emarginatum*. **(M)** *Prorocentrum cf. levis*. **(N)** *Coolia* sp. Scale bar = 20 μm.

TABLE 1 | Mean abundance (in cells·g⁻¹) by date of the main taxonomic groups of benthic dinoflagellates on the macroalgae *Dictyota*.

Date	Coolia	Gamb	Ohep	Omar	Pbel	Plev	Pemar	Phoff	Plim	Prhat	Total
PUERTO MORELOS											
Apr17	803 (186)	94 (29)	14,129 (4,677)	19,672 (6,192)	90 (30)	98 (32)	377 (88)	1,188 (323)	1,544 (492)	475 (97)	38,471 (11,420)
May08	263 (77)	23 (16)	20,606 (3,910)	8,132 (1,086)	64 (20)	5 (5)	73 (30)	683 (157)	1281 (327)	516 (82)	31,645 (4,852)
May 29	75 (27)	26 (21)	18,876 (4,876)	967 (343)	41 (18)	13 (9)	115 (49)	621 (127)	1595 (461)	74 (23)	22,403 (5,667)
Jun22	260 (142)	26 (18)	307 (99)	1,559 (537)	71 (23)	61 (54)	178 (85)	544 (172)	811 (338)	142 (32)	3,958 (1,442)
Jul09	369 (112)	50 (12)	179 (50)	922 (272)	51 (19)	20 (6)	63 (25)	373 (85)	1728 (743)	154 (44)	3910 (1,163)
Aug10	673 (136)	180 (59)	11,412 (1,656)	2,713 (643)	35 (13)	100 (30)	178 (48)	799 (245)	2,523 (848)	610 (94)	19,221 (3,081)
Aug28	260 (65)	90 (28)	8,318 (1,295)	172 (35)	146 (47)	77 (21)	136 (40)	724 (181)	1,527 (412)	609 (261)	12,059 (2,034)
Sep17	495 (130)	94 (23)	252 (74)	147 (12)	63 (25)	110 (30)	51 (18)	751 (137)	2405 (253)	479 (73)	4,846 (464)
Nov04	164 (56)	18 (13)	109 (31)	1,574 (365)	28 (8)	8 (6)	21 (11)	74 (20)	534 (124)	59 (18)	2,590 (480)
Dec02	42 (13)	15 (8)	14 (8)	1,917 (519)	13 (6)	3 (3)	9(4)	59 (13)	211 (52)	3 (3)	2,285 (546)
ISLA CONTOY											
Apr17	777 (215)	25 (6)	0	548 (161)	35 (12)	29 (10)	46 (15)	181 (59)	117 (33)	84 (20)	1,843 (511)
May08	279 (74)	3 (2)	0	7,446 (1681)	20 (7)	5 (3)	17 (7)	136 (29)	117 (20)	7 (4)	8,031 (1,721)
May29	60 (24)	10 (10)	0	220 (98)	31 (22)	0	80 (19)	66 (22)	59 (21)	0	528 (156)
Jun22	169 (41)	19 (5)	0	2,094 (399)	60 (14)	3 (2)	151 (25)	98 (21)	50 (10)	21 (8)	2,665 (417)
Jul09	79 (44)	3 (3)	0	937 (519)	22 (8)	5 (3)	56 (16)	194 (57)	130 (25)	19 (9)	1,445 (578)
Aug10	457 (149)	67 (15)	0	72 (24)	82 (17)	27 (14)	125 (23)	337 (33)	245 (46)	45 (11)	1,457 (202)
Aug28	648 (165)	70 (23)	7 (6)	144 (31)	45 (11)	34 (19)	199 (34)	252 (49)	180 (47)	39 (14)	1,619 (241)
Sep17	615 (124)	75 (20)	10 (5)	247 (75)	64 (17)	93 (23)	182 (44)	185 (34)	148 (28)	41 (20)	1,658 (250)
Nov04	184 (52)	30 (13)	6 (6)	581 (140)	7 (5)	0	28 (11)	75 (20)	76 (21)	10 (6)	997 (168)
Dec02	11 (6)	5 (3)	284 (84)	15,721 (3,677)	25 (11)	2 (2)	2 (2)	59 (20)	49 (16)	3 (3)	16,160 (3,770)

The number between parentheses represents the standard error.

TABLE 2 | Mean abundance (in cells·g⁻¹) by date of the main taxonomic groups of benthic dinoflagellates on the macroalgae *Amphiroa*.

Date	Coolia	Gamb	Ohep	Omar	Pbel	Plev	Pemar	Phoff	Plim	Prhat	Total
PUERTO MORELOS											
Apr17	160 (47)	4 (4)	4,625 (960)	1,639 (382)	9 (7)	16 (5)	16 (6)	92 (26)	192 (32)	28 (7)	6,781 (1,334)
May08	36 (12)	5 (4)	7,109 (832)	1,199 (208)	22 (5)	3 (3)	5 (4)	130 (22)	212 (110)	81 (30)	8,804 (964)
May29	6 (5)	3 (3)	849 (314)	9 (6)	3 (2)	2 (2)	8 (4)	27 (8)	63 (22)	8 (4)	978 (335)
Jun22	16 (4)	3 (2)	191 (47)	377 (65)	1 (1)	0	7 (4)	59 (16)	76 (14)	5 (3)	734 (114)
Jul09	49 (16)	7 (5)	122 (33)	428 (138)	24 (11)	0	25 (9)	159 (72)	422 (156)	9 (8)	1,243 (361)
Aug10	66 (22)	48 (35)	1,029 (237)	227 (62)	38 (19)	11 (7)	37 (19)	126 (24)	358 (51)	25 (9)	1,964 (372)
Aug28	54 (28)	11 (5)	3,150 (1,087)	196 (75)	16 (8)	16 (9)	0	163 (49)	361 (120)	44 (18)	4,010 (1,329)
Sep17	44 (14)	13 (5)	246 (70)	61 (30)	13 (5)	4 (3)	3 (3)	116 (31)	607 (122)	14 (6)	1,119 (230)
Nov04	39 (13)	28 (7)	69 (21)	533 (118)	13 (5)	5 (5)	5 (5)	55 (15)	202 (54)	8 (4)	956 (186)
Dec02	47 (20)	71 (35)	347 (139)	2,665 (864)	0	0	7 (5)	18 (10)	88 (30)	20 (20)	3,263 (1,020)
ISLA CONTOY											
Apr17	2 (2)	3 (2)	0	623 (303)	4 (1)	3 (2)	2 (1)	17 (3)	26 (8)	4 (2)	684 (315)
May08	123 (75)	0	0	4,425 (1,504)	24 (20)	2 (2)	13 (9)	157 (77)	213 (106)	8 (8)	4,965 (1,737)
May29	6 (2)	0	0	28 (10)	0	0	18 (4)	17 (7)	3 (2)	1 (1)	73 (11)
Jun22	21 (5)	7 (4)	0	144 (31)	5 (3)	3 (2)	44 (12)	38 (18)	27 (16)	1 (1)	290 (71)
Jul09	44 (12)	11 (6)	0	85 (37)	17 (6)	0	33 (9)	57 (12)	100 (42)	1 (1)	347 (85)
Aug10	111 (20)	13 (7)	0	85 (18)	47 (12)	4 (4)	98 (20)	131 (17)	76 (25)	0	565 (78)
Aug28	206 (44)	6 (5)	0	14 (14)	13 (7)	54 (22)	28 (13)	79 (16)	45 (9)	0	445 (78)
Sep17	276 (89)	14 (7)	5 (5)	221 (91)	12 (6)	43 (14)	46 (13)	82 (20)	41 (12)	3 (3)	743 (196)
Nov04	115 (44)	46 (24)	22 (10)	1,105 (261)	21 (11)	6 (6)	0	48 (31)	47 (17)	0	1,410 (326)
Dec02	18 (7)	0	24 (13)	793 (165)	0	0	3 (3)	28 (16)	31 (24)	0	897 (179)

The number between parentheses represents the standard error.

RESULTS

Dinoflagellate Species

We recorded species of the genera *Gambierdiscus*, *Coolia*, *Prorocentrum*, *Ostreopsis*, *Amphidinium*, *Bysmatrum*, and *Sinophysis*. Identification of the species of *Gambierdiscus* and *Coolia* is difficult based only on the characters observed (size and shape of the cells) during the evaluation of abundance. Therefore, hereafter the *Gambierdiscus* and *Coolia* specimens will be referred as *Gambierdiscus* spp. and *Coolia* spp. Five species of *Ostreopsis* were observed but only two of them accounted for more of the 95% of the abundance of this genus. These two morphotypes of *Ostreopsis*, were clearly differentiated during the counting and were designated as *O. cf. marina* and *O. heptagona*. Finally, six *Prorocentrum* species were found: *P. belizeanum*, *P. emarginatum*, *P. hoffmannianum*, *P. lima*, *P. cf. levis*, and *P. rathymum*. *Gambierdiscus* spp., *Coolia* spp., *Ostreopsis cf. marina*, *O. heptagona*, and the six *Prorocentrum* species were the most abundant groups found in the samples,

therefore, the analysis was constrained to these taxa. **Figure 2** shows the main species recorded in the zone of study.

Abundance Variability

Taking into account the entire sampling period, total abundance of epiphytic dinoflagellates was higher at PM on both algae (**Tables 1, 2; Figure 3**). However, when the total abundance and the abundance by group were compared at each sampling date, it became evident that the numeric differences were not always significant for all groups (**Table 3**). Total dinoflagellate abundances on *Amphiroa* were significantly higher at PM during all sampling dates ($p < 0.05$, Mann-Whitney test) except for Sep17 and Nov09 when no differences were detected. In *Dictyota* total abundances were higher in PM during the entire study period, except on Jun22 when no differences were detected.

In PM, the maximum mean abundance recorded in *Dictyota* was $38,471 \pm 11,420$ cells·g⁻¹ on Apr16. In the case of *Amphiroa*, the maximum abundance was $8,803 \pm 964$ cells·g⁻¹ and was recorded on May08. At IC the maximum density of

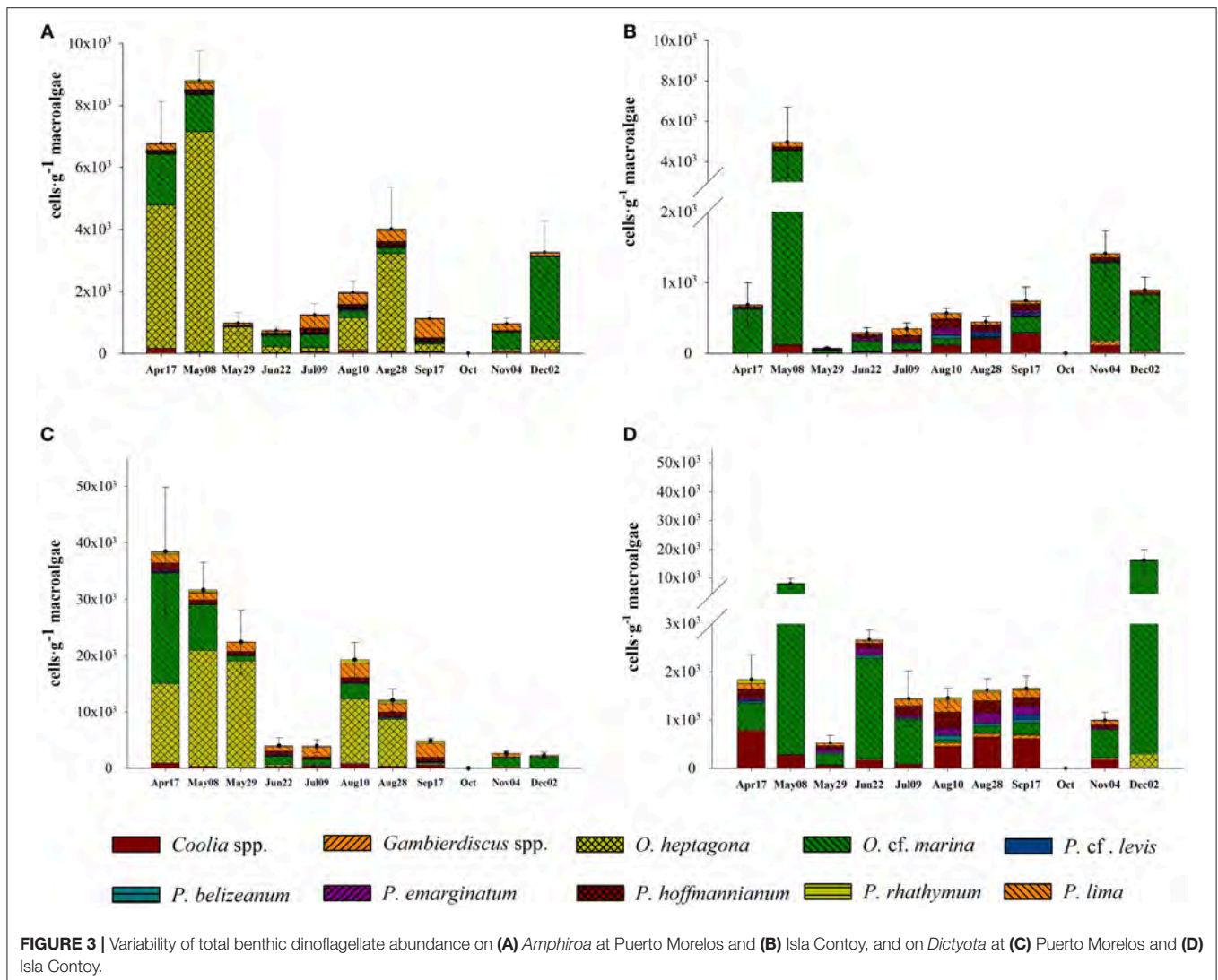


TABLE 3 | *P*-values of the abundances comparisons of the main taxonomic groups of epiphytic dinoflagellates at each sampling date between sites (Mann–Whitney test, $\alpha = 5\%$).

	Apr17	May08	May29	Jun22	Jul09	Aug10	Aug28	Sep17	Nov04	Dec02
Amphiroa										
Total	<0.001	<0.05	<0.001	<0.001	<0.01	<0.01	<0.001	ns	ns	<0.01
Omar	<0.05	ns	ns	<0.001	ns	<0.05	<0.001	ns	ns	<0.05
Ohep	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05	<0.05
Ptot	<0.001	ns	<0.05	ns	<0.05	ns	ns	<0.001	ns	ns
Gamb	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Coolia	<0.001	ns	ns	ns	ns	ns	<0.001	<0.01	ns	ns
Dictyota										
Total	<0.001	<0.002	<0.001	ns	<0.05	<0.001	<0.001	<0.001	<0.01	<0.001
Omar	<0.001	ns	ns	ns	ns	<0.001	ns	ns	0.02	<0.001
Ohep	<0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01
Ptot	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	ns
Gamb	ns	ns	ns	ns	<0.001	ns	ns	ns	ns	ns
Coolia	ns	ns	ns	ns	<0.05	ns	ns	ns	ns	ns

The abundances were higher at PM except in the case of the values indicated in bold.

dinoflagellates on *Dictyota* occurred on Dec02 ($16,160 \pm 3,770$ cells·g⁻¹), and on *Amphiroa* the highest abundance was $16,160 \pm 3,770$ cells·g⁻¹ in May08. In PM, the maximum individual abundance was registered on *Dictyota* during Apr16 ($112,293$ cells·g⁻¹).

Dominant species during most of the period were *Ostreopsis heptagona* and *O. cf. marina*. Therefore, the variability of total abundance of epiphytic dinoflagellates were due the variation in the contribution of these species at each site. In fact, the contribution of *Ostreopsis* species varied linearly with total abundance (Figures 4A,B).

The contribution of *O. cf. marina* was important at both sites, but *O. heptagona* was important only in PM because this species was absent at IC during almost the entire sampling period. *O. cf. marina* and *O. heptagona* reached maximum densities of $53,231$ and $\sim 46,462$ cells·g⁻¹ on a *Dictyota* sample during Apr16.

The contribution of *Gambierdiscus* spp. to total abundance was marginal on both macroalgae and at both sites: from 0 to 3.26% on *Amphiroa*, and from 0 to 4.6% on *Dictyota*. The highest abundance of this group on *Dictyota* was found at PM on Aug10 (178 ± 60 cells·g⁻¹); and on *Amphiroa* the maximum abundance was observed on Dec02 (71 ± 38 cells·g⁻¹).

The most abundant species of *Prorocentrum* were *P. hoffmannianum* and *P. lima* on both algae, representing the largest fraction of this genus (50–94%). *Prorocentrum* was important (65% of total abundance) when *Ostreopsis* abundances were low. Maximum abundance of all *Prorocentrum* species was registered at PM on *Dictyota* ($4,144$ cells·g⁻¹).

The contribution of *Coolia* spp. to total abundance was lowest at PM. This genus did not present a contribution higher than 10% on both algae. In contrast, in IC on some dates it reached more than 30% on both macroalgae. The abundance of this group was lower than $1,000$ cells·g⁻¹ on, and its abundance on *Amphiroa* was always lower than 300 cells·g⁻¹.

Associations Between Species

There were more correlations between species on *Dictyota* than on *Amphiroa* in both sites (Table 4). *Prorocentrum lima*, *P. belizeanum*, and *P. hoffmannianum* were correlated with each other on *Amphiroa* at both sites but not on *Dictyota*. Also *P. rathymum* was correlated with *O. heptagona* on *Amphiroa* at PM. Given that this species was almost absent at IC, the correlation did not exist at this site. At Isla Contoy, *P. hoffmannianum*, and *P. cf. levis* were correlated with *Coolia* spp. The association between *P. lima* and *P. hoffmannianum* was the only association detected in both algae at both sites. On *Dictyota*, the community structure was more complex because it included the correlations of *Coolia* with *Gambierdiscus*, and each of these genera with the *Prorocentrum* species. The negative correlations of *O. marina* (with *Gambierdiscus*, *P. emarginatum*, and *P. hoffmannianum*) were particularly important, but this only occurred at IC.

Temperature and Nutrients

The temperature varied from 24.04 to 30.20°C at IC and from 26.68 to 29.7°C at PM. Warmer temperatures were registered between August and September, and April was the coldest month in both sites (Figure 5). Over the entire sampling period, mean water temperature was slightly lower at IC (28.25°C) than at PM (28.74°C). Although small, this difference was significant ($p < 0.05$, Wilcoxon test). On Apr17, the temperature was 1.46°C higher at PM (27.90°C) than IC (26.44°C). Over the rest of the sampling period, the temperature differences between sites were lower than 1°C . After May29, the temperature decreased almost 1°C at both sites, followed by a gradual increase until the maximum value in August–September. This behavior is common in the zone and is related to the influence of the polar fronts coming from the north from December to February, although during the year of study, polar fronts occurred until July. For example, the event which occurred during June 12–19 was intense enough to decrease the temperature recorded on Jun22.

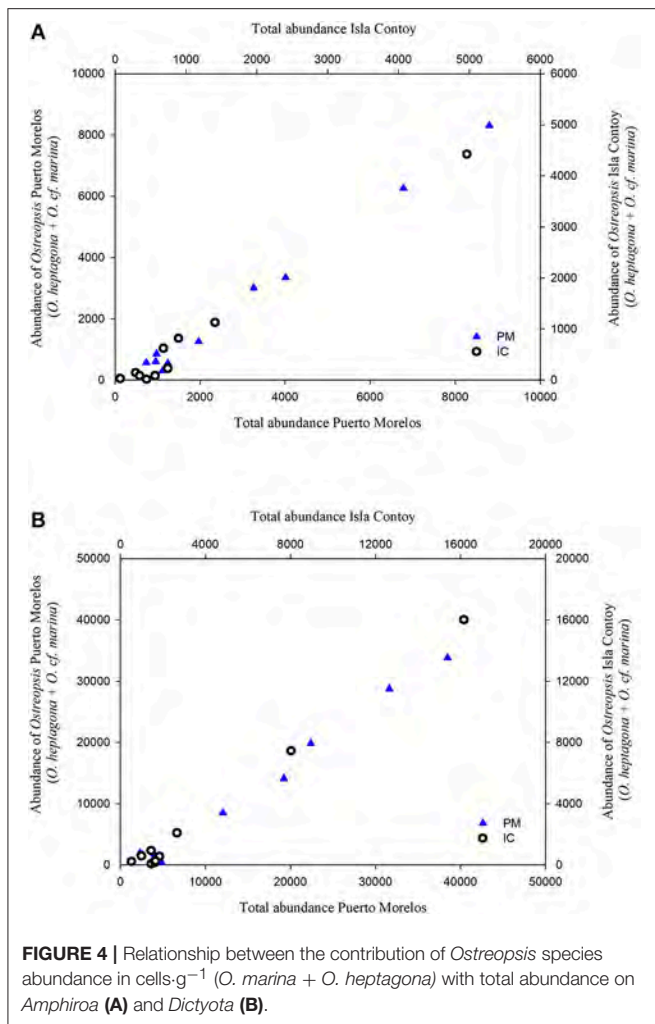


FIGURE 4 | Relationship between the contribution of *Ostreopsis* species abundance in cells·g⁻¹ (*O. marina* + *O. heptagona*) with total abundance of *Amphiroa* (A) and *Dictyota* (B).

High NO_x^- ($\text{NO}_3^- + \text{NO}_2^-$) concentrations were measured on both sites with mean values of 8.0 and 7.8 $\mu\text{mol}\cdot\text{l}^{-1}$ for PM and IC, respectively, but no significant differences were detected along the entire period of study. Conversely, PO_4^{-3} concentration was different between sites: 2.3 $\mu\text{mol}\cdot\text{l}^{-1}$ at IC and 2.4 $\mu\text{mol}\cdot\text{l}^{-1}$ in PM ($p < 0.05$, Wilcoxon test). NO_3^- and PO_4^{-3} showed a high correlation at PM ($r = 0.95$) but not for IC. Temperature did not correlate with nutrients at PM nor IC ($p > 0.05$, Spearman rank correlation).

Effect of Environmental on Dinoflagellate Abundances

The correlation between temperature and the abundance of *O. cf. marina* is shown in Figure 6. At both sites and on both algae this species showed a negative correlation with temperature ($p < 0.05$, Spearman rank correlation). Maximum abundances were detected at temperatures between 27 and 28°C, independent of the macroalgae substrate. In contrast, *Prorocentrum lima* and *P. emarginatum* were positively correlated with temperature at IC and only on *Dictyota*: $r = 0.66$ and $r = 0.79$, respectively. A negative correlation between *O. heptagona* and PO_4^{-3}

concentration was found at PM on both algae ($r = -0.67$ and $r = 0.7$ on *Amphiroa* and *Dictyota*, respectively). Correlations with NO_3^- only were observed at IC with the abundances of *P. emarginatum*, *P. rathymun*, and *Coolia* spp. on *Dictyota* (Table 5).

DISCUSSION

Taxonomic Composition of Epiphytic Dinoflagellates

Describing the taxonomic composition of epiphytic dinoflagellates is more complicated now than in the past because at present there are more described species for each of the benthic genera. Pioneer studies on benthic dinoflagellates considered all specimens with the typical shape of *Gambierdiscus* to be *G. toxicus*, or all those individuals similar to *Coolia* as *C. monotis*. However, Faust (1995) and Litaker et al. (2009) increased to four the richness of the genus *Gambierdiscus* in the Caribbean: *G. belizeanus*, *G. caribaeus*, *G. carolinianus*, and *G. carpenteri*. Also the number of *Coolia* species increased after reports of the presence of *C. tropicalis*, *C. santacroce*, *C. malayensis*, and *C. areolata* (Faust, 1995; Karafas et al., 2015; Almazán-Becerril et al., 2016). Since the morphological identification of each of these species involves the inspection and analysis of the thecal plates, which is not a practical procedure given the high number of samples to analyse, it was necessary to join the species of each genus and consider them as taxonomic groups, although the ecological behavior of individual species of the same genus remain unclear.

Prorocentrum has the highest richness among the benthic-epiphytic thecate dinoflagellates, and the Caribbean harbors a great fraction of these species (Faust, 1990, 1993, 1994, 1997), however, just some of them are common in the macroalgae samples which allow an easier identification of the specimens. *Prorocentrum lima*, *P. hofmannianum*, *P. belizeanum*, *P. rathymun*, and *P. emarginatum* are well-recognized under light microscope as different species.

Ostreopsis taxonomy represents a challenge due to the lack of specific morphological characters for species discrimination (Penna et al., 2005; Parsons et al., 2012), however, in this study two *Ostreopsis* species were identified as *O. heptagona* and *O. cf. marina*. In the first case, the contact between the plates 4' and 4'' (according to the interpretation of Besada et al., 1982) was an adequate characteristic to identify this species. The other species was referred as *O. cf. marina* because the original description of this species made by Faust (1999) matches with the characteristics of the specimens observed in this study. A closely related morphological species is *O. lenticularis*, also reported for the Caribbean (Ballantine et al., 1988; Moreira et al., 2012, 2017), which has nearly the same shape (Fukuyo, 1981; Faust, 1999). However, the size reported for this species in the literature is consistently lower than the size of the specimens observed in this study. Therefore, the discrimination of the specimens of these two species under microscope during counting was based on the size and shape of the specimens and was ratified by plate analysis of some cells in several samples. As consequence, the

TABLE 4 | Correlations between dinoflagellate species on the same macroalgae.

	<i>Amphiroa</i>		<i>Dictyota</i>	
	Puerto Morelos	Isla Contoy	Puerto Morelos	Isla Contoy
<i>P. belizeanum</i> - <i>P. lima</i>	0.74*	0.89***		
<i>P. belizeanum</i> - <i>P. hoffmannianum</i>	0.81**	0.84**		
<i>P. belizeanum</i> - <i>P. rathymum</i>				0.66*
<i>P. belizeanum</i> - <i>P. emarginatum</i>			0.67*	0.76*
<i>P. hoffmannianum</i> - <i>P. lima</i>	0.77**	0.84**	0.66*	0.95***
<i>P. hoffmannianum</i> - <i>P. emarginatum</i>			0.67*	
<i>P. hoffmannianum</i> - <i>P. rathymum</i>			0.77**	0.75*
<i>P. hoffmannianum</i> - <i>P. cf. levis</i>			0.79**	0.79**
<i>P. lima</i> - <i>P. cf. levis</i>			0.74*	0.77**
<i>P. rathymum</i> - <i>P. cf. levis</i>				0.79**
<i>P. rathymum</i> - <i>P. lima</i>				0.66*
<i>P. rathymum</i> - <i>O. heptagona</i>	0.84**			
<i>Coolia</i> spp.- <i>P. hoffmannianum</i>		0.84**	0.80**	0.66*
<i>Coolia</i> spp.- <i>P. cf. levis</i>		0.68*	0.75*	0.81**
<i>Coolia</i> spp.- <i>P. lima</i>			0.69*	0.69*
<i>Coolia</i> spp.- <i>P. rathymum</i>			0.65*	0.83**
<i>Coolia</i> spp.- <i>Gambierdiscus</i> spp.			0.83**	0.66*
<i>Gambierdiscus</i> spp.- <i>P. hoffmannianum</i>			0.87**	
<i>Gambierdiscus</i> spp.- <i>P. lima</i>			0.85**	
<i>Gambierdiscus</i> spp.- <i>P. belizeanum</i>				0.64*
<i>Gambierdiscus</i> spp.- <i>P. rathymum</i>			0.71*	0.63*
<i>Gambierdiscus</i> spp.- <i>P. cf. levis</i>			0.94***	
<i>Gambierdiscus</i> spp.- <i>P. emarginatum</i>				0.68*
<i>O. heptagona</i> - <i>P. emarginatum</i>			0.65*	
<i>O. heptagona</i> - <i>P. hoffmannianum</i>			0.65*	
<i>O. marina</i> - <i>Gambierdiscus</i> spp.				-0.71*
<i>O. marina</i> - <i>P. emarginatum</i>				-0.69*
<i>O. marina</i> - <i>P. lima</i>				-0.70*

Numbers indicate the Rho value of the Spearman rank correlation test.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

taxonomic composition of epiphytic dinoflagellates cannot be unequivocally determined by using the standard methodology (rough morphometric discrimination), and for purposes of practicality and comparability it is necessary to work with this “schematic” community.

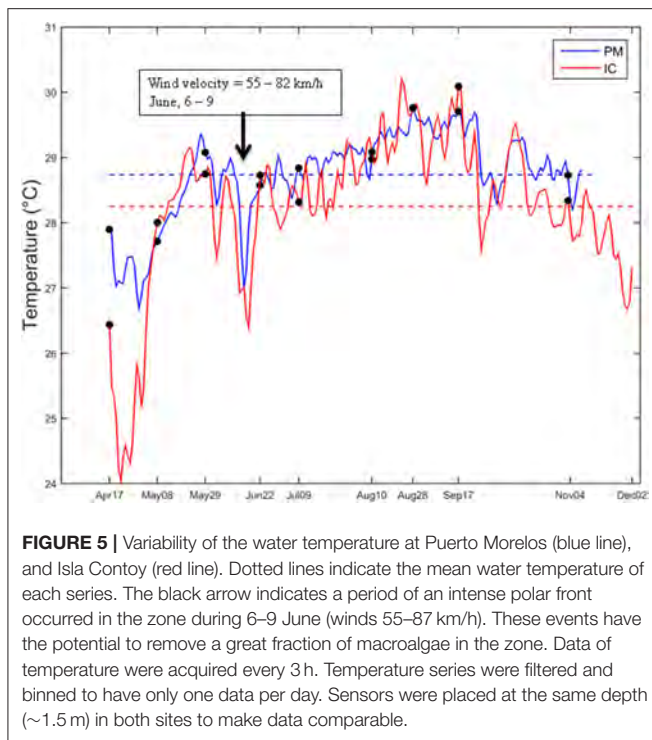
The taxonomic composition of the epiphytic dinoflagellate community on the surveyed algae of this study is quite similar to those reported in similar studies in the Caribbean (Morton and Faust, 1997; Delgado et al., 2006; Boisnoir et al., 2018) and other regions of the North Atlantic like the south of the Gulf of Mexico (Okolodkov et al., 2007, 2014) or Florida (Bomber et al., 1989) and even in the Pacific (Parsons and Preskitt, 2007; Richlen and Lobel, 2011) although each species (or genus) did not contribute in the same proportion to the total abundance. For example, Delgado et al. (2006) found that *P. lima* and *G. toxicus* were more abundant than *O. lenticularis* and *C. monotis*, whereas Boisnoir et al. (2018) reported that *Ostreopsis* spp. and *Prorocentrum* spp. were numerically more abundant than *Coolia* and *Gambierdiscus*. In the Gulf of Mexico, Okolodkov et al. (2007) found that depending of the site and the date *P. lima* or

C. monotis could dominate the community, and in the Yucatan coast, *Prorocentrum* species, particularly *P. rathymum* was the dominant species on seagrass meadows present in Yucatan coast (Okolodkov et al., 2014).

Abundance and Seasonal Patterns

The results of this study demonstrate that all the taxonomic groups were present on both algae at both localities, excepting *O. heptagona* which was almost absent at IC with only a few cells observed between September and December. As the distribution of this species comprises the Gulf of Mexico, Florida and the Caribbean (Bomber et al., 1989; Faust et al., 1996), its low abundance at IC during the period of study could be the effect of a local characteristic of the site, because at the same time a high number of specimens were detected at PM.

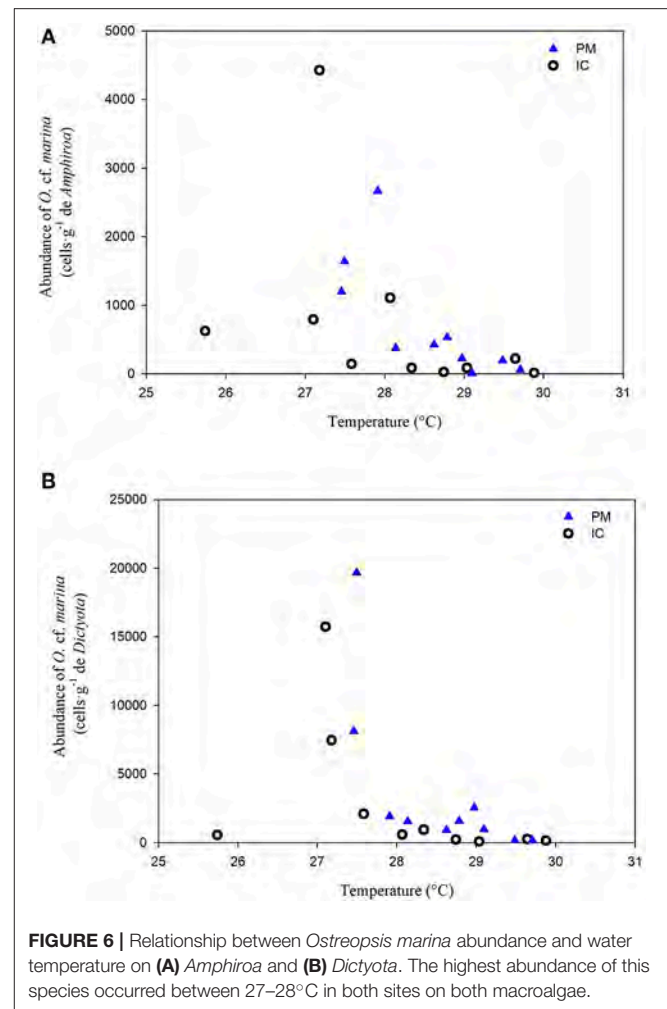
The short period of sampling of this study was not enough to determinate seasonal patterns for any species of dinoflagellate (although positive significant correlations were found between temperature and *P. lima* and *P. emarginatum*) and it appears that more time is necessary to characterize seasonal variability. For



example, Ballantine et al. (1988) found evidence of seasonality in *Gambierdiscus* and *O. lenticularis* in Puerto Rico in a 3-year study. Also, Chinain et al. (1999) used a 5-year time series to describe the seasonality of *Gambierdiscus* spp. at Tahiti, and Chateau-Degat et al. (2005) used an 8-year study in French Polynesia to describe the seasonal behavior of *Gambierdiscus*. Higher abundances were observed during April and May 08 on both algae in PM, and in May 08 for both algae at IC, additionally the higher abundance in *Dictyota* at IC was detected on Dec02.

These fluctuations were dominated by the contribution of *Ostreopsis* species which was largely variable and increased linearly with total abundance. The high abundance of *Ostreopsis* species was also reported at the Caribbean in Cuba (Moreira et al., 2012, 2017) and Puerto Rico (Ballantine et al., 1988) where *O. lenticularis* reached higher abundances than the reported in this study (Table 6). When the abundance of *Ostreopsis* species was removed from the data, the contribution of *Coolia* spp., *P. lima*, and *P. hoffmannianum* were more important, while the abundance of *Gambierdiscus* spp. and the rest of *Prorocentrum* species was almost marginal. *Prorocentrum lima* is a conspicuous member of benthic dinoflagellate communities in the Caribbean and Gulf of Mexico where it attaches to a variety of algae and seagrasses and at some sites could be the dominant species with maximum abundances above 10,000 cells·g⁻¹ on phaeophyte algae (Delgado et al., 2006), and up to 29,756 cells·g⁻¹ of *Thalassia testudinum* (Okolodkov et al., 2007). Also, at Florida Keys, Bomber et al. (1989) found that *P. lima* was more abundant on green algae *Penicillus capitatus* and *Avrainvillea nigricans* as compared to other brown or red algae species.

The case of *Gambierdiscus* abundance is remarkable because the species of this genus and those of *Fukuyoa* are thought to



be primarily responsible for the toxin production involved in ciguatera intoxications. Although there is an intrinsic difficulty in comparing abundances between different studies based on number of cells normalized by wet weight of macroalgae due to the high variability of weight to surface area ratios between the different groups of host macroalgae (Lobel et al., 1988), there are some reports of high abundances of *Gambierdiscus* spp. on *Dictyota* in the Caribbean. For example, Ballantine et al. (1988) reported a maximum abundance slightly higher than 2,000 cells·g⁻¹ in Puerto Rico and Morton and Faust (1997) found 400 cells·g⁻¹ in Belize. Also, Bomber et al. (1989) reported a maximum mean value of 8,191 ± 300 cells·g⁻¹ in Florida. In contrast, the *Gambierdiscus* abundances found on *Dictyota* in this study were similar to the values reported by Lobel et al. (1988) at the Caribbean Island St. Barthélemy (5–56 cells·g⁻¹). The factors that control the abundance of *Gambierdiscus* populations in the zone remain to be solved.

In the Caribbean high abundances of *Ostreopsis lenticularis* has been reported for Puerto Rico (Ballantine et al., 1988) and Cuba (Moreira et al., 2012, 2017), but no reports of high densities exist for *O. marina* and *O. heptagona* in this region. Table 5 shows that maximum densities recorded for *Ostreopsis* species in the

TABLE 5 | Correlation of epiphytic dinoflagellates with temperature and nutrients.

	<i>Amphiroa</i>						<i>Dictyota</i>					
	Isla Contoy			Puerto Morelos			Isla Contoy			Puerto Morelos		
	T	NO _x ⁻	PO ₄ ³⁻	T	NO _x ⁻	PO ₄ ³⁻	T	NO _x ⁻	PO ₄ ³⁻	T	NO _x ⁻	PO ₄ ³⁻
<i>Omar</i>	-0.69*			-0.87**			-0.74*			-0.79**		
<i>Pemar</i>	0.63*						0.79**	0.75*				
<i>Plim</i>							0.66*					
<i>Prhat</i>							0.82**					
<i>Ohep</i>						-0.67*						-0.70*
<i>Coolia</i>								0.64*				

* $p < 0.05$, ** $p < 0.01$.

TABLE 6 | Abundances from the literature of *Ostreopsis* species on the macroalgae *Dictyota* in the Caribbean basin.

Site	Species	Maximum abundance ^a	Date of maximum abundance	Number of replicates	Period of study	References
La Parguera, Puerto Rico	<i>O. lenticularis</i>	$1.6 \times 10^4/4.4 \times 10^4$	September 1985	4–5	October 1983–June 1986	Ballantine et al., 1988
Coral Reef off Belize	<i>O. lenticularis</i>	4×10^2	–	–	May–June 1994	Morton and Faust, 1997
Cienfuegos Bay, Cuba	<i>O. lenticularis</i>	5.3×10^5	February 2012	2	May 2011–March 2012	Moreira et al., 2017
Cienfuegos Bay, Cuba	<i>O. lenticularis</i>	7.9×10^4	June 2010	2	January–September 2010	Moreira et al., 2012
Puerto Morelos, México	<i>O. heptagona</i>	$2.1 \times 10^4/4.8 \times 10^4$	May 2015	10	April–December 2015	This study
Puerto Morelos, México	<i>O. cf. marina</i>	$2.0 \times 10^4/5.3 \times 10^4$	April 2015	10	April–December 2015	This study

^aMean maximum abundance values/^amaximum abundance in any sample.

Caribbean vary from 10^2 to 10^5 cells·g⁻¹ in *Dictyota*. Indeed, the highest abundance was recorded in Cuba ($531,000$ cel·g⁻¹) that is two times the maximum value reported by Ballantine et al. (1988) for the same species at Laurel Reef site, in Puerto Rico ($235,803$ cells·g⁻¹). High abundances have been reported for *O. siamensis* (Shears and Ross, 2009) at New Zealand coasts (1.4×10^6 cells·g⁻¹ of *Carpophyllum plumosum*) and for *O. cf. ovata* at the Mediterranean (Cohu et al., 2013) where the maximum abundance recorded was 8.54×10^6 cells·g⁻¹ of *Dictyota* spp. These results demonstrate that at temperate and tropical latitudes *Ostreopsis* species can reach high abundances, emphasizing the potential of *Ostreopsis* species to produce large benthic blooms globally (Rhodes, 2011).

Community Structure on *Dictyota* and *Amphiroa*

In terms of abundance, both algae are used as substrate by all species. However, in terms of community structure, correlation between species can be used to know if the substrate offers common advantages for different species, or if the increase of one species implies the decrease of another. The results of this study suggest that the structure of the epiphytic dinoflagellate community is different on each of the host algae *Amphiroa* and *Dictyota*, and there are even differences in the same alga between sites. These findings suggest that *Prorocentrum* species and *Coolia* constitute the basic structure on *Amphiroa*, and on *Dictyota* the structure is given by *Prorocentrum*, *Gambierdiscus* and *Coolia* that share the habitat. In addition, *O. marina* presents habitat

separation with *Prorocentrum* and *Gambierdiscus*. A negative correlation between *Ostreopsis* and *Prorocentrum* was reported by Richlen and Lobel (2011) in the Pacific, but Boisnoir et al. (2018) found the contrary in the Eastern Caribbean. Also, the habitat separation between *Gambierdiscus* and *Ostreopsis* has been reported by Bomber et al. (1989) in Florida, but in the Caribbean, Ballantine et al. (1988) did not find evidence of correlation between these genera. The dominance of *Ostreopsis* over the other genera of dinoflagellates could be related to its preference to sites with little water motion, as these are shallow sites (Richlen and Lobel, 2011; Cohu et al., 2013; Boisnoir et al., 2018).

The Effect of Environment on the Population Dynamics

The distance between study sites is nearly 50 km; both sites are shallow and located in the northern region of the Mesoamerican Barrier Reef System. Therefore it is reasonable to suppose that these sites also undergo the same climatological and oceanographic conditions, generating similar abundances and taxonomic compositions of dinoflagellate populations. However, the results showed some important differences. For example, the differences in temperature are low but significant and this could be explained by the climate patterns. The region presents three seasons: (1) the season influenced by polar fronts (locally called “nortes”) between December and February, (2) the dry season between March to May, and (3) the rainy season between June

and November. The presence of a polar front can occur year-round (Henry, 1979), but its intensity is higher during winter. During 2015 the cold fronts were intense even during July (2015). **Figure 5** shows the temperature series in the study sites. Both series have the same behavior but the magnitude of the variations is higher at IC than PM. The main effect of the polar fronts on the shallow aquatic ecosystems is the abrupt decrease of water temperature, but also the generation of strong movement of the surface layer of water. The energy of polar fronts decreases as they flow southward, therefore their effect on the coral reef systems should be higher at IC than PM. Since the energy of polar fronts removes host macroalgae, the turnover of dinoflagellate populations could be higher at IC, preventing their accumulation and resulting in higher abundances at PM. As the nutrient concentration remains high year-round, *Dictyota* can recover its biomass and abundance in short periods, providing surface for the attachment of *Ostreopsis*, which also grows rapidly (Ballantine et al., 1988). This implies that *Dictyota* and *Ostreopsis* are opportunistic genera exploiting an expanding habitat produced by the phase shift in the coral reefs.

Implications of *Ostreopsis* Blooms in the Caribbean Coral Reefs

The presence of *Ostreopsis* blooms on *Dictyota* along the Caribbean coasts has some ecological implications. First, toxicity has been reported in populations of *O. lenticularis* from Cuba and Puerto Rico (Ballantine et al., 1988; Moreira et al., 2012), as well as *O. heptagona* (Norris et al., 1985); in addition, some samples of *O. cf. marina* taken after the sampling period of this study showed toxicity on mouse bioassays and hemolytic activity (unpublished data). In fact, *Ostreopsis* species produce palytoxin-like compounds which are involved in human intoxications and possibly mass mortality of vertebrates and invertebrates (Ramos and Vasconcelos, 2010; Faimali et al., 2012; Patocka et al., 2017). Secondly, *Dictyota* is a macroalgae favored by the changes in the coral reef systems. In the sites studied these algae can form mats of several tens of square meters, at times reaching nearly 80% of cover (Delgado-Pech, 2016). However, the low palatability of *Dictyota* to larger benthic herbivores reduces grazing (Cruz-Rivera and Villareal, 2006) and prevents the flux of toxins to higher trophic levels. *Dictyota* mats are persistent, growing on other calcareous and coralline algae, corals and any other hard surface. Still, their attachment to the substrate is not firm enough to avoid detachment by the strong waves formed under the influence of polar fronts. During these events, the cover of *Dictyota* and the abundance of *Ostreopsis* decrease in the benthic environment, although, in the case of *Ostreopsis*, the cells (and their toxins) could remain in the water column and possibly enter the planktonic food web. If the coral-algal phase shift in the Caribbean results in dominance of *Dictyota* and other fleshy macroalgae, toxic *Ostreopsis* species could increase their abundance and distribution and cases of ciguatera and other syndrome of benthic origin could become a more serious public health problem in the region.

CONCLUSIONS

The taxonomic composition of epiphytic dinoflagellates inhabiting the macroalgae from the northern of the Mesoamerican Coral Reef Barrier was comprised of species of the genera *Ostreopsis*, *Prorocentrum*, *Coolia*, and *Gambierdiscus*.

Ostreopsis was the dominant genus in the sites studied. Particularly, *O. cf. marina* and *O. heptagona* reached maximum densities of 53,000 and 48,000 cells·g⁻¹ on *Dictyota*. However, the period of sampling was not enough to detect seasonal trends of the dinoflagellate species.

Total abundances were higher in PM on both algae. These differences could be attributed to the intensity of environmental variables. For example, the lower temperature and the higher effect of polar fronts in IC could prevent the accumulation of epiphytic dinoflagellates.

O. marina showed a negative correlation with temperature between 27 and 30°C. On the contrary *P. lima* and *P. emarginatum* showed a positive correlation with temperature at IC on both macroalgae.

Nutrient concentrations NO_x⁻ and PO₄³⁻ were higher in both sites during the whole period of study, but in the most of the cases their concentration was not correlated with the abundance of dinoflagellates, excepting *O. heptagona* which showed a negative correlation with PO₄³⁻ concentration. On the contrary, *P. emarginatum*, *P. rathymum*, and *Coolia* spp. were positively correlated with NO_x⁻ on *Dictyota* at IC.

The structure of epiphytic dinoflagellates was different between algae. There were more correlations between species on *Dictyota* than *Amphiroa*. The association between *P. lima* and *P. hoffmannianum* was the only one detected in both algae and in both sites.

The high cover of *Dictyota* in the study zone and the high abundance of *O. cf. marina* and *O. heptagona* on this macroalgae could represent a risk for human and marine fauna health if production of toxins is confirmed in this species.

The report of *Ostreopsis* blooms in the Mexican Caribbean coasts and the reports of blooms of this genus in other sites along the Caribbean basin emphasize the importance of this genus worldwide.

AUTHOR CONTRIBUTIONS

AA-B conceived and designed the study, wrote, and edited the document. EI-S and BD-P collected data, processed samples, wrote and edited the document, and built the figures and tables. EG-M and EN-V interpreted results, wrote, and edited the document. AO-O quantified the nutrient concentrations, interpreted the results, and wrote and edited the document.

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