SEASONAL ABUNDANCE OF *GAMBIERDISCUS TOXICUS* AND *OSTREOPSIS* SP. IN COASTAL WATERS OF SOUTHWEST PUERTO RICO

ABONDANCE SAISONNIERE DE *GAMBIERDISCUS TOXICUS* ET D'UNE ESPECE D'*OSTREOPSIS* DANS LES EAUX COTIERES DU SUD-OUEST DE PUERTO RICO

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ABSTRACT

Population levels of the benthic epiphytic dinoflagellate thought to be the cause of ciguatera, Gambierdiscus toxicus, as well as an ecologically associated dinoflagellate, tentatively identified as Ostreopsis sp., were monitored for a 14 month period during 1983 and 1984. The area studied was a shallow inshore coral reef habitat located on the southwest coast of Puerto Rico (Caribbean Sea). Both dinoflagellate genera were found on a variety of macroalgal hosts as well as on other substrata. Regular counts of dinoflagellates on Dictyota spp. (Phaeophyta) at 1.0 m depth in the backreef area of Caracoles Reef, showed that neither species demonstrated an obvious seasonality with respect to population numbers. Populations of Gambierdiscus toxicus on Dictyota spp. were normally in the range of 100 to 300 cells/ g algal wet weight with a high of 8,000 cells/ g Dictyota counted at one sampling. Ostreopsis sp. was extremely variable in its population levels through the course of the study, with abundances generally exceeding 3,000 cells/ g Dictyota. A maximum of 43,000 Ostreopsis cells/ g Dictyota was recorded on one occasion.

RESUME

L'abondance de Gambierdiscus toxicus, l'épiphyte dinoflagellé benthique suspecté d'être la cause de la "ciguatera", ainsi que celle d'un dinoflagellé associé, identifié comme une espèce probable d'Ostreopsis, ont été suivis pendant 14 mois en 1983 et 1984. L'habitat étudié se trouve en eau peu profonde sur un platier interne, situé sur la côte sud-ouest de Puerto Rico (Mer des Caraïbes). Les deux genres de dinoflagellés ont été trouvés sur de nombreuses de macroalgues ainsi que sur d'autres profondeur à l'arrière du récif de Caracoles n'indiquent aucun changement saisonnier des niveaux des populations. Les populations de G. toxicus, épiphyte des espèces de Dictyota, étaient de l'ordre de 100 à 300 cellules/g de poids humide d'algues, avec un maximum de 8.000 cellules/g de Dictyota pour un échantillon. Tout au long de l'étude, le niveau de la population était extrâmement variable avec une abondance généralement supérieure à 3.000 cellules/g de Dictyota. Un maximum de 4.300 cellules d'Ostreopsis/g de Dictyota a été observé en une seule occasion.

INTRODUCTION

Ciguatera fish poisoning is a circumtropical phenomena (Banner, 1974) which has long been recognized (Randall, 1958; Ragelis, 1984). Randall (1958) first speculated that the primary vector of ciguatera was a benthic microorganism, either bacterial or algal. It wasn't until 2 decades later that Yasumoto (1977, 1979a, 1979b, 1979c) suggested that the causal agent was a benthic dinoflagellate, which he tentatively identified as a new species of the genus *Diplopsalis*. This supposition was based on observations of herbivorous fish feeding on detritus and the subsequent discovery of dinoflagellates comprising a portion of that material. The dinoflagellate was later described as a new genus and species, *Gambierdiscus toxicus*, by Adachi and Fukuyo (1979) based on collections from the Gambier Islands, French Polynesia. *Gambierdiscus toxicus* is also known in the Pacific from Hawaii (Withers, 1981; Shimizu et al., 1982), the Ryukyu Islands (Fukuyo, 1981), New Caledonia (Fukuyo, 1981; Yasumoto et al., (1984), as well as Guam, Fiji, and Okinawa (Yasumoto et al., 1984). *G. toxicus* was initially reported from Caribbean islands including Puerto Rico by Bagnis (1979). It is also known in the western Atlantic from the Florida Keys (Bergman, 1981; Besada et al., 1982), Ile St. Barthelemy in the leeward Antillean Islands (Besada et al., 1982), and from the British and American Virgin Islands (Tindall et al., 1984; Carlson et al., 1984). These studies have all been of short duration and with the exception of the Carlson et al. (1984) report, information on natural populations of *Gambierdiscus toxicus* is lacking. Tindall et al. (1984) has speculated that other species of dinoflagellates in addition to *G. toxicus* may be involved in ciguatera fish poisoning. Accordingly, populations of *G. toxicus* as well as its local associate, a dinoflagellate recently identified as *Ostreopsis* cf. *lenticularis*, were monitored at a shallow inshore coral reef habitat on the southwest coast of Puerto Rico over a 15 month period. Information concerning natural populations of *Ostreopsis lenticularis* has not previously been reported.

METHODS AND MATERIALS

Methods follow those of Yasumoto et al. (1980) as modified by Withers (personal communication). Benthic macroalgae or other living or inanimate substrates were collected and placed in plastic bags with as little disturbance as possible. Bags were closed underwater without additional exchange with surrounding seawater. On return to the laboratory, bags with substrate enclosed were shaken vigorously and contents passed through a 280 micron mesh screen. The filtrate was then passed through a 35 micron mesh screen. This size fraction, retaining the dinoflagellates, was suspended in a known volume of seawater. Two 0.5 ml aliquots of the above were counted in a gridded Sedgewick-Rafter chamber and numbers corrected to cells/g wet weight of algae or cells/cm^2 of substrate. Additional aliquots were taken in the event that counts were not in close agreement. Sampling was conducted from October 1983 through December 1984 with samples taken once or twice weekly. The sampling site was an inshore coral reef known locally as Cayo Caracoles and is located 1 km offshore from La Parguera on the southwest coast of Puerto Rico, Caribbean Sea.

At Caracoles Reef, sampling included substrates across the entire reef profile but was concentrated in the back reef at 1 m depth. Surface temperatures were recorded at each sampling at the back reef of Caracoles and are shown in Figure 1. During the course of study, 219 samples of algae (12 species), seagrasses, and both live and inanimate benthic substrates were examined.

RESULTS

Gambierdiscus toxicus and *Ostreopsis* cf. *lenticularis* were found at least occassionally on most algal and seagrass species monitored, see Table 1. Only *Bryopsis plumosa* lacked dinoflagellates completely. *Ostreopsis* was almost always more abundant than *Gambierdiscus* on all other substrates examined. *Microcoleus lyngbyaceus, Ceramium nitens*, and *Syringodium filiforme* lacked epiphytic *Gambierdiscus* when sampled but supported variable population densities of *Ostreopsis*. Siphonaceous green algae including *Caulerpa racemosa, C. sertulariodes, Halimeda opuntia, H. simulans, Penicillus capitatus*, and *Udotea flabellum* generally supported low populations, an average of 22 *G. toxicus* cells/g and 107 *O. lenticularis* cells/g. *Acanthophora spicifera* supported intermediate populations of both dinoflagellates while another rhodophyte, *Spyridia filamentosa*, supported generally few *Gambierdiscus* but large populations of *Ostreopsis*. On dead mangrove and dead seagrass leaves, *Ostreopsis* was the only dinoflagellate present. *Gambierdiscus toxicus* was more abundant on *Sargassum natans* than was *Ostreopsis lenticularis;* however this host was only sampled once during the course of study.

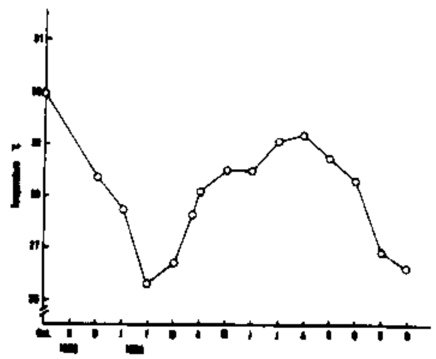

Figure 1: Surface temperatures measured at the back reef of Cayo Caracoles, October 1983 - December 1984.

Consistenly highest dinoflagellate populations were found on the seagrass *Thalassia testudinum* and the phaeophyte *Dictyota* spp. Population levels for *Gambierdiscus* and *Ostreopsis* on these macrophyte hosts at 1.0 m depth from the back reef of Caracoles are shown in Figures 2 and 3. *Dictyota* species are not specified separately as more than one species commonly grew entangled in loose mats. This multispecies assemblage included one or more species of *Dictyota dichotoma, D. divaricata, D. bartayresii*, and *D. cervicornis*.

Average *Gambierdiscus* population densities were at a maxiumum of 2,400 cells/g *Dictyota* at

Table 1. Population levels of *Gambierdiscus toxicus* and *Ostreopsis lenticularis* counted on various substrates at Cayo Caracolès, October 1983 - December 1984.

Substrate	Depths Collected (M)	N	*Gambierdiscus toxicus*	*Ostreopsis lenticularis*
			FORE REEF	
			cells/g	cells/g
Bryopsis plumosa (Chlorophyta)	0.9 - 1.2	2	0	0
Caulerpa racemosa (Chlorophyta)	0.6 - 0.8	2	0 - 18	0 - 4
Ceramium nitens (Rhodophyta)	7.6 - 9.1	2	0	20 - 289
Dictyota spp. (Phaeophyta)	6.1	1	0	16
Halimeda opuntia (Chlorophyta)	1.2 - 6.1	4	0 - 21	0 - 5
Halimeda simulans (Chlorophyta)	15.2	1	0	0
Udotea flabellum (Chlorophyta)	15.2	2	0 - 16	0 - 38
			cells/cm^2	cells/cm^2
Acropora palmata (coral)	0.4 - 0.8	2	0	0
Gorgonia ventilana (gorgonian)	2.4 - 6.1	2	0	0
Millepora complanata (coral)	0.6	1	0	0
Coral rubble with algal turf	0.9 - 1.2	3	0 - 16	0 - 12
			REEF FLAT AND REEF CREST	
			cells/g	cells/g
Acanthophora spicifera (Rhodophyta)	0.1 - 0.4	4	48 - 221	0 - 378
Dictyota spp. (Phaeophyta)	0.3 - 0.4	3	14 - 2,210	42 - 3,210
Halimeda opuntia (Chlorophyta)	0.4	1	97	0
Microcoleus lyngbyaceus (Cyanophyta)	0.1 - 0.3	2	0	0 - 156
Thalassia testudinum (seagrass)	0.2 - 0.4	8	0 - 8	11 - 2,814
			cells/cm^2	cells/cm^2
Millepora complanata (coral)	0.3	1	0	0
Porites porites (coral)	0.3 - 0.4	2	0	0 - 50
Zooanthus-Thalassia mat (zooanthid + seagrass)	0.2	2	0 - 2	0 - 3
Coral rubble with algal turf	0.3 - 0.4	3	21 - 54	0 - 58
			BACK REEF	
			cells/g	cells/g
Acanthophora spicifera (Rhodophyta)	0.7 - 1.0	6	38 - 138	57 - 6,234
Caulerpa sertulariodes (Chlorophyta)	1.0 - 1.2	5	0 - 189	136 - 791
Dictyota spp. (Phaeophyta)	0.5 - 2.5	86	0 - 7,788	85 - 43,359
Halimeda opuntia (Chlorophyta)	0.4 - 1.5	10	0 - 57	0 - 318
Halimeda simulans (Chlorophyta)	0.9 - 1.0	2	0 - 13	0 - 41
Penicillus capitatus (Chlorophyta)	1.2 - 1.5	2	0 - 17	0 - 434

Table 1. (cont.)

Substrate	Depths Collected (M)	N	*Gambierdiscus toxicus*	*Ostreopsis lenticularis*
			cells/g	cells/g
Sargassum natans (Phaeophyta)	floating	1	29	15
Spyridia filamentosa (Rhodophyta)	2.4 - 2.5	2	0 - 31	1,934 - 2,154
Syringodium filiforme (seagrass)	2.5	2	0	218 - 1,563
Thalassia testudinum (seagrass)	0.5 - 2.8	49	0 - 1,463	0 - 10,059
Dead *Thalassia testudinum* leaves (seagrass)	1.3	1	0	635
Dead *Rhizophora mangle* leaves (mangrove)	0.6 - 1.0	2	0	7 - 405
			cells/cm^2	cells/cm^2
Bare sand	1.0	1	0	19
Coral rubble with algal turf	1.0	1	0	35
Coral rubble without algal turf	0.9	1	2	15

the beginning of the study. Thereafter population densities decreased through February and March, after which they fluctuated between 50 and 500 cells/g *Dictyota* through the remainder of the study (Fig. 2). A high of 7,788 cells/g *Dictyota* was counted at one sampling. Averages of *G. toxicus* populations on *Thalassia* showed a similar decline (from a peak of 700 cells/g) from October to February 1984. Low population densities (< 50 cells/g *Thalassia*) were subsequently found through the remainder of the study with the exception of a small peak (145 cells/g *Thalassia)* in October 1984. *Dictyota* was consistently a better host for *Gambierdiscus* than was *Thalassia*.

Populations of *Ostreopsis* on both *Dictyota* spp. and *Thalassia* were highly variable with average densities generally between 2,000 and

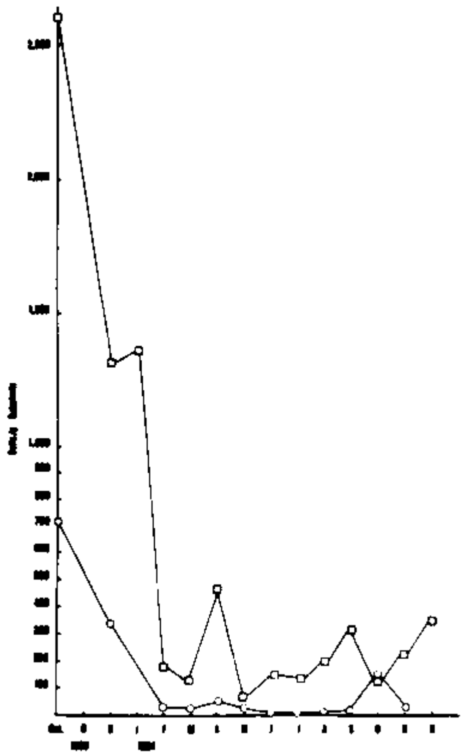

Figure 2: Population levels of *Gambierdiscus toxicus* epiphytic on *Thalassia* and *Dictyota* at 1.0 m at the back reef of Cayo Caracoles, October 1983 - December 1984 (squares represent *Dictyota* spp. and circles represent *Thalassia testudinum*).

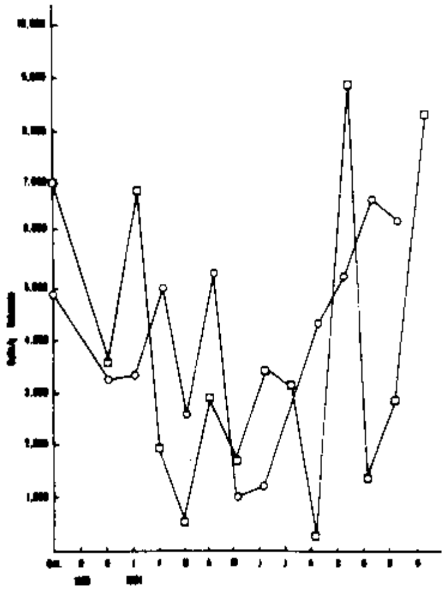

Figure 3: Population levels of *Ostreopsis lenticularis* epiphytic on *Thalassia* and *Dictyota* at 1.0 m at the back reef of Cayo Caracoles, October 1983 - December 1984 (squares represent *Dictyota* spp. and circles represent *Thalassia testudinum*).

7,000 cells/g host (Fig. 3). *Ostreopsis* did not demonstrate greater preference for either host although its greatest abundance was found on a sample of *Dictyota* (43,359 cells/g).

Population densities of *Gambierdiscus toxicus* and *Ostreopsis lenticularis* were found to be highly variable even within short distances and on the same substrate. *Dictyota* plants collected within a 3 m radius varied as much as an order of magnitude in difference during one sampling (80 cells/g to 887 cells/g *Dictyota*; 6-27-84). At the same sampling, *Ostreopsis* varied nearly as greatly (from 81 to 748 cells/g *Dictyota*). During other sampling periods, *Gambierdiscus* and *Ostreopsis* varied by at least a factor of 3. Coefficents of variation ($\frac{SD}{\bar{X}}$ X 100) ranged from 33% to 112% calculated for *Gambierdiscus* and from 25% to 65% for *Ostreopsis*. This high population variability is further reflected by the large ranges of dinoflagellate numbers given in Table 1.

In general, greater numbers of both dinoflagellates were found on the back reef side of Cayo Caracoles than in the shallow fore reef at comparable depths. Coral dominated cover in the fore reef and reef crest areas. Both live coral and gorgonian species were unsuitable for dinoflagellate colonization, although approximately 50 *Ostreopsis* sp. cells/cm^2 were found on *Porites porites* on one occasion. Low algal turfs growing on dead coral and coral rubble comprises the major algal cover on an area basis on the fore reef of local inshore reefs. These turfs also appeared to support fewer dinoflagellates/cm^2 than on the reef flat and back reef.

DISCUSSION

Gambierdiscus toxicus and *Ostreopsis lenticularis* colonized most algal hosts present at Caracoles Reef, and the latter species was almost always present in greater abundance than the former. Among all surfaces examined, both dinoflagellates demonstrated clear preference for *Dictyota* spp. and *Thalassia testudinum*. High population densities of *Ostreopsis* were also found on *Acanthophora spicifera* and *Spyridia filamentosa*.

Populations of *Gambierdiscus toxicus* on *Dictyota* and *Thalassia* demonstrated fluctuations not related to temperature, thus there does not appear to be a marked seasonality in *Gambierdiscus* abundance. The initial large peak in *G. toxicus* abundance was not repeated during the following period of greater than one year. Such bloom conditions are probably related to factors other than or in addition to temperature such as nutrient input with runoff due to rain. Bagnis (1981) similarly did not find correlation between abundance of Tahitian *G. toxicus* and physical factors. The highly fluctuating population densities of *Ostreopsis lenticularis* throughout the study period further do not indicate seasonality. Large variability in population densities from samples taken from the same site and host implies a high degree of patchiness in the small scale distribution of the two dinoflagellate species.

Population densities of *Gambierdiscus toxicus* at Caracoles Reef appear to be occasionally higher but otherwise comparable to reports of Hawaiian (Taylor, 1979; Withers, 1980; Shimizu, 1982) and Tahitian (Yasumoto, 1979a, 1979b, 1980) populations. Puerto Rican populations, however, do not approach the densities(to 318,000 cells/g *Jania*) reported from the Gambier Islands (Yasumoto et al., 1979a, 1980). In the only previous report of *G. toxicus* populations in the Caribbean, Carlson et al. (1984) presented maximum dinoflagellate densities on three algal species. As average population densities were not reported, it is difficult to make comparison of that work with the present study. Puerto Rican *Gambierdiscus toxicus* and *Ostreopsis lenticularis* populations have been shown to be highly variable both temporally and spatially. Caution is therefore required in formulating conclusions concerning status of benthic dinoflagellate populations based on short term studies.

A Puerto Rican *Ostreopsis lenticularis* clone has recently been shown to have considerably greater toxicity than a Puerto Rican *Gambierdiscus toxicus* clone (Tosteson et al., 1985). Greater toxicity in *O. lenticularis* coupled with its higher population densities than *G. toxicus*, probably indicate that the former species is a more important contributor to the phenomena of ciguatera than is the latter in southwest Puerto Rico.

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