

Assessment of Ciguatera Dinoflagellate Populations: Sample Variability and Algal Substrate Selection

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Abstract. Preliminary assessment is made of two key components in ciguatera ecology. First, we examined the numerical variability of *Gambierdiscus toxicus* as an epiphyte on the macroalgae, *Dictyota* and *Galaxaura*. Variability was examined by a statistical bootstrap technique to determine the minimum number of samples required to adequately estimate the abundance of *G. toxicus* at one station and to test for statistically significant differences between two stations. A minimum of 10 replicates were needed at the relatively low *G. toxicus* abundance found at our study site. Second, we demonstrated the feasibility of conducting controlled laboratory experiments to assess the short-term colonization behavior of *G. toxicus* on selected macroalgae offered in varying mass and surface area ratios. To assess *G. toxicus* abundance and distribution, the number of cells per unit of host alga must be standardized. We show that contradictory conclusions can be reached depending on whether the number of dinoflagellate cells is normalized to algal biomass or surface area.

Introduction

The continuing enigma of ciguatera, namely the stochastic variability of fish toxicity in time and space, must have its basis in the ecology and population dynamics of the benthic dinoflagellates that produce ciguatoxin and maitotoxin. Several dinoflagellate species have been implicated as producing chemical compounds that likely result in fish toxicity (Yasumoto *et al.*, 1987). One of these *Gambierdiscus toxicus*, appears to be most significant in ciguatera ecology. Despite the identification over a decade ago of the most likely progenitors of the toxins

and numerous field and laboratory studies throughout the tropics (reviewed in Withers, 1982; Ragelis, 1984; Salvat, 1985; Anderson *et al.*, 1985; Anderson and Lobel, 1987), the state of knowledge about ciguatera and *G. toxicus* physiology and ecology remains "very primitive indeed" (Scheuer and Bagnis, 1985).

Clearly, one reason for this situation is the complex and gradual manner in which small quantities of the highly potent ciguatoxin and related toxins move through the food chain to the higher predators. We believe that a contributing factor has been the lack of a standardized, statistically rigorous methodology for examining the distribution and abundance of the ciguatera dinoflagellates in time and space. In this preliminary study, we addressed the question: "How many samples of a macroalgal species must be collected at one time and at one site and depth to adequately quantify the abundance of *G. toxicus*?" Strictly speaking, the results of our data analysis are valid only for one study site at one point in time and for the two macroalgal hosts we examined, but these data and methodologies document the large errors associated with inadequate sample size and provide a means to determine the minimum number of samples needed to test for statistically significant differences between stations. In addition, we tested the feasibility of conducting laboratory studies of the preference of *G. toxicus* for a particular macroalgal host. Analysis of these data demonstrate the misleading nature of dinoflagellate abundance expressed per gram of host alga and provide a good argument for yet another change to common enumeration methodologies—namely the normalization of dinoflagellate abundance to host surface area rather than biomass (Bomber *et al.*, 1985). Ciguatera is a complex ecological phenomenon that is poorly understood. It seems appropriate to re-evaluate commonly accepted

methodologies and assumptions and to test the feasibility of addressing certain fundamentally important behavioral and physiological issues in the laboratory.

Materials and Methods

Study site

This field study was conducted at St. Barthelemy (17°54'N, 62°50'W) in the Caribbean (French West Indies) from 8 to 20 August 1986. Extensive collections were made at the "Pointe de Negre" site located on the south side of the island, about 3 km from the port of Gustavia. Laboratory facilities and logistics on the island were provided by the New England Biolabs Foundation.

Macroalgal collection and dinoflagellate enumeration

Two macroalgal species were selected for intensive study, *Dictyota* sp. (probably *D. bartayresii* and maybe mixed with *D. dichotoma* and *D. divaricata*) and *Galaxaura* sp. (probably *G. corneum*). Both species were epiphytized by *G. toxicus*. *Dictyota* is a brown alga and *Galaxaura* is a red articulated coralline alga. The growth form of each is a complex 3-D structure of branches. Both algae were growing abundantly in close proximity.

All specimens were collected between 5–6.5 m depth at the Pointe de Negre study site on 17 and 19 August 1986. Specimens were picked, placed in individual plastic bags that were sealed underwater, and transported to the laboratory in the dark at ambient water temperature. The bags were then vigorously shaken and kneaded to dislodge *G. toxicus*. The 20–250 μm size fraction was collected by pouring the contents through Nitex sieves. The adequacy of this method was confirmed by successive shaking and washing, which produced few additional dinoflagellates.

The collected material was backwashed into a vial with approximately 10 ml of filtered seawater. *G. toxicus* individuals were enumerated with a compound microscope at 100 \times total magnification. A minimum of one ml of the concentrated suspension was scanned for each sample. Macroalgae were blot-dried and weighed. Masses ranged between 1 and 20 grams. Dinoflagellate abundance was initially expressed as cells per gram blot dry weight of host algae. Subsequent measurements of algal surface area per gram blot dry weight allow the data to be normalized to surface area as well.

Preference studies

Two laboratory experiments were conducted. One used equal biomass amounts of macroalgae and the other combined two algae in different proportions. In all treatments, algae were rinsed several times to remove most epiphytes (determined by visual inspection of algae

under a dissecting microscope) and placed in pairs in 125-ml beakers with filtered seawater. A known number of *G. toxicus* cells were then added (final concentration approximately 80 ml^{-1}). The samples were maintained for 24 hours in an incubator at 26°C on a 12:12 L:D cycle at approximately 50 $\mu\text{Einst m}^{-2} \text{s}^{-1}$. Beakers were mildly swirled every 4 h (except overnight) to evenly distribute those dinoflagellates which had not yet settled. When the experiments were terminated, the number of *G. toxicus* cells on each alga and those remaining unattached in the beaker were counted separately. Because of the differences in algal morphology, the results are expressed as the percentage of all *G. toxicus* attached to the algae, as cells per unit mass and as cells per surface area. Two *G. toxicus* isolates were used—strain T3 isolated from Gambier Islands by R. Bagnis and strain SB01 isolated from Lorient, St. Barthelemy, by M. Durand-Clement in July 1986. They were grown in modified ES medium (Durand, 1984).

Macroalgae measurements

Surface area, wet weight, and displacement volume were assessed for the macroalgae *Galaxaura* and *Dictyota*. Surface area is the most difficult measurement to obtain accurately. We used three methods for surface area calculations. One technique consisted of dipping a dried macroalgal sample in a detergent solution that is supposed to adhere to the algal surface in a layer of consistent thickness (Harrod and Hall, 1962). By knowing the weight of solution that coats a standard area of plastic sheeting, the surface area of an alga can be calculated. We encountered numerous problems with this method and did not obtain consistent or reliable data. Clearly, this concept has potential, and modifications made by Bomber (1985, pers. comm.) using wet specimens and full strength detergent might be necessary, but problems exist with how the solution coats and is adsorbed by different algae. Other techniques involved morphological measurements of surface area. Enlarged silhouettes of *Dictyota* were analyzed using a computer and a digitizer graphics unit since that species' shape is essentially two-dimensional. *Galaxaura* was measured in pieces under a microscope with a micrometer. *Galaxaura*'s shape is tubular, and painstaking measurements were made of all individual pieces in a sample. Surface area by these latter two methods provided consistent data.

Results

Algal mass measurement

The primary problem we encountered was the measurement of algal characteristics for valid interspecific comparison of host-alga selection by *G. toxicus*. The

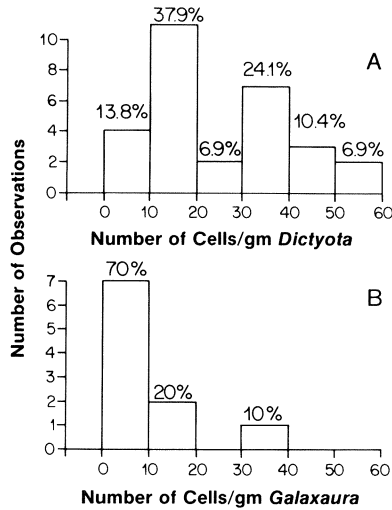


Figure 1. Frequency distribution of the number of *Gambierdiscus toxicus* cells on (A) *Dictyota* and (B) *Galaxaura*. Percentage relative frequency is specified above each column.

common measure of 'cells per gram blot-dry weight' is suitable for *intraspecific* comparison of *G. toxicus* on a host alga. This measurement had the advantage of being easy and rapid in the field. However, the best measurement for *interspecific* host algal comparisons is 'cells per surface area.'

The surface area of *Dictyota* was 105 ± 31 (range 67–151) $\text{cm}^2 \text{gm}^{-1}$ ($n = 13$). The surface area of *Galaxaura* was 31 ± 8 (range 25–42) $\text{cm}^2 \text{gm}^{-1}$ ($n = 4$). Conversion of the number of *G. toxicus* cells per gm alga to cells per cm^2 was obtained by dividing by 105 for *Dictyota* and 31 for *Galaxaura*. The variance in surface areas is probably due in large part to measurement error, but the possibility of allometric variation in these algae also needs to be examined.

Field abundance

Gambierdiscus toxicus was present at the study site, but in relatively low abundance. Every *Dictyota* sample ($n = 29$) hosted at least 5 cells g^{-1} blot-dry weight. Eighty-six percent of the collection had greater than 10 cells g^{-1} (Fig. 1A). The mean (\pm S.D.) number of *G. toxicus* on *Dictyota* was 24 ± 14 (range 5–56) cells g^{-1} or 23 cells per 100 cm^2 . Only half of the *Galaxaura* samples ($n = 10$) were epiphytized by *G. toxicus* and another 20% had fewer than 10 cells g^{-1} (Fig. 1B). The mean (\pm S.D.) abundance of *G. toxicus* on *Galaxaura* was 6 ± 10 (range 0–30) cells g^{-1} or 19 cells per 100 cm^2 . The number of *G. toxicus* per gram of host alga was not a function of the size of individual *Dictyota* (Fig. 2) or *Galaxaura* samples, based on linear, exponential, log, or power function regression tests.

Sampling statistics

We collected many ($n = 29$) specimens of *Dictyota* to evaluate the sample size necessary for statistical analysis of the distribution and abundance of *G. toxicus*. We also examined this for *Galaxaura* but with, as it turned out, too few samples.

The analysis used was a statistical "bootstrapping" routine (Diaconis and Efron, 1983). The graphs (Figs. 3, 4) show 25 random combinations (with replacement) from the sample set for each sample size. The sample size range for *Dictyota* was 2 to 29 and for *Galaxaura* was 2 to 10. Thus, for each incremental sample size to $n = 25$, calculations were made based on the total sample pool.

The analysis is presented in three plots each for *Dictyota* (Fig. 3) and *Galaxaura* (Fig. 4): (A) the mean number of *G. toxicus* cells per gram of alga, (B) standard error of these means, and (C) the percentage change in the standard error as sample size increases. The first plot shows the spread in possible averages among 25 sample sets for a given sample size. It illustrates the wide variability obtainable at small sample sizes, especially $n < 10$ drawn from the same sample pool. The second plot quantifies this variability as the standard error of the means. It decreases substantially with increasing sample size. The third plot defines the degree to which results fluctuate as the percentage change in the standard error with the incremental addition of samples.

At small sample sizes the variability in the means is due more to random sample selection than actual differences in the density of *G. toxicus* at a site. This variability is greater for *Galaxaura*, since 50% of the samples lacked *G. toxicus* cells. Thus, fewer samples of *Dictyota* than *Galaxaura* would more accurately assess *G. toxicus* abundance. A sample size of $n = 10$ to 15 *Dictyota* sam-

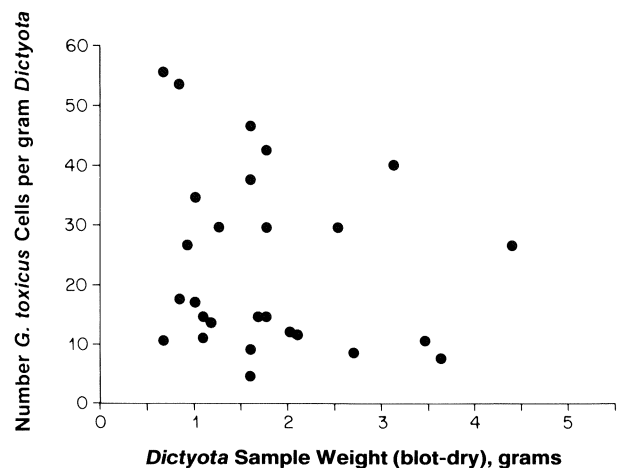


Figure 2. Relationship between the number of *Gambierdiscus toxicus* cells per gm of *Dictyota* as a function of the sample weight of individual *Dictyota* masses ($n = 29$). No statistical correlation was found.

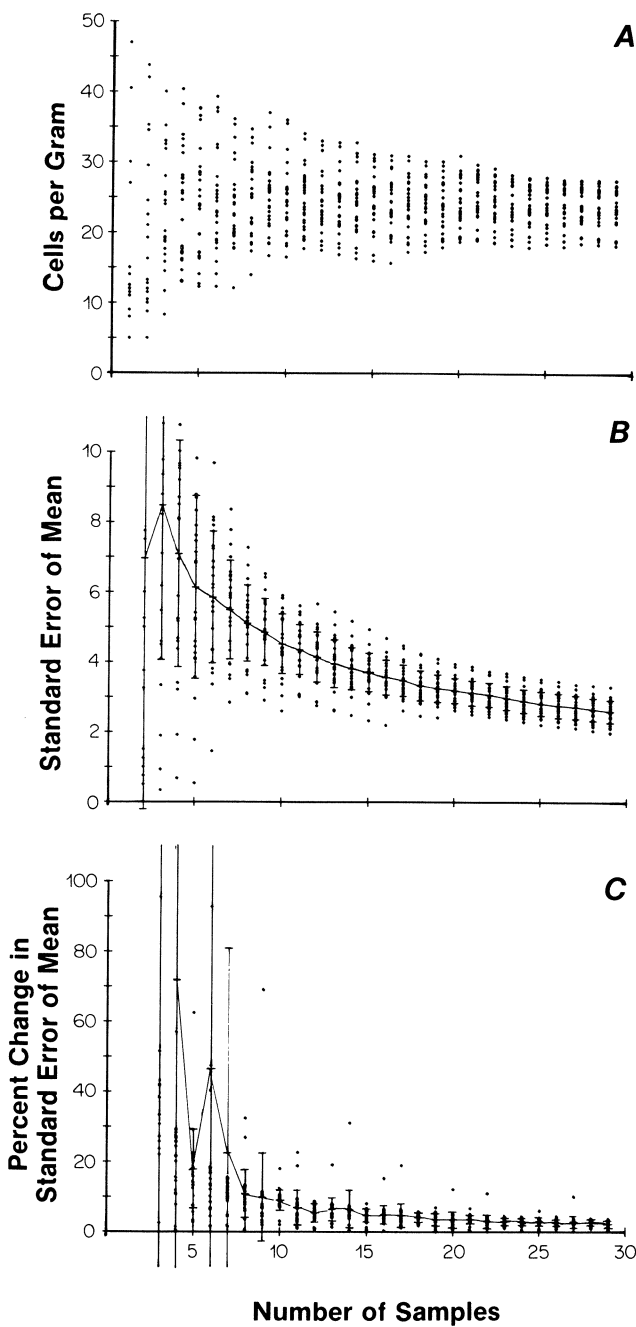


Figure 3. *Dictyota*. (A) Variations in means for given sample sizes drawn from the total pool of 29 samples calculated by the “bootstrap” method. The mean was calculated 25 times for each sample size. At sample sizes less than $n = 5$, some points exceed the limit of the ordinate. (B) Standard error of the means in Figure 5 calculated 25 times with the overall average connected by a solid line across all sample sizes. This indicates the variance associated with a mean calculated for a given sample size. As sample size increases, this variance decreases. (C) Percent change in the standard error of the means from n to $n + 1$ calculated 25 times for each n . The overall average is connected by a solid line across all n 's. In this case, as sample size increased, the percentage change in possible means decreased substantially up to about $n = 15$ after which additional samples did not significantly affect the mean.

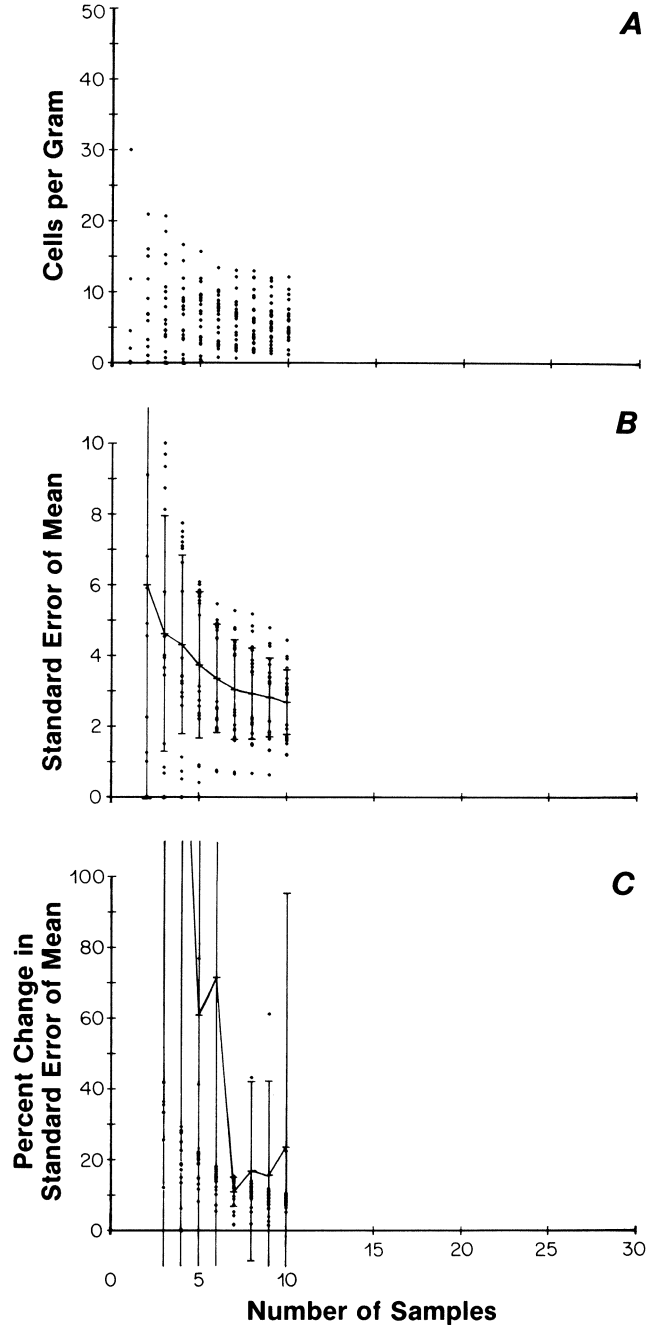


Figure 4. *Galaxaura*. (A) Variations in means. (B) Standard error of the means calculated in A. (C) Percent change in the standard error of the means. In this case, $n = 10$ was insufficient to determine the point at which additional samples would not significantly affect the results. See Figure 3 for details.

ples would be statistically adequate using parametric analyses to estimate the abundance of *G. toxicus* (Fig. 3). For comparison to other collections, these analyses can be used to determine the variance values that would be significantly different for specified sample sizes. Given the means and standard errors calculated above, the val-

ues of sample means, which would be significantly different at $P < .05$ and $P < .01$ assuming the same degree of standard error, can be simply specified by a t -test. It would be necessary to generate a new set of plots in other studies with different *G. toxicus* abundance and distribution.

Substrate selection

Several experiments were designed to evaluate the feasibility of determining if the *G. toxicus* abundance on different macroalgae is the result of stochastic processes or a demonstration of active substrate preference and selection. Preference was evaluated by counting the number of dinoflagellate cells on each of the macroalgal choices (which were offered alone or in pairs in several different mass ratios) after 24 hours. Although in some cases the mass of the algal choices were the same, comparisons are complicated by the different morphologies. For example, *Dictyota* is flat and *Galaxaura* is round, but both have equivalent mass to volume ratios (*i.e.*, 1.05 and 0.96, respectively), although *Dictyota* has more than triple the surface area compared to the same mass of *Galaxaura*. In control trials where only one macroalga was available, approximately the same fraction of the introduced *G. toxicus* population chose to settle on *Dictyota* and *Galaxaura* (55 and 51%, respectively) after 24 h.

A detailed breakdown of the *Dictyota:Galaxaura* selectivity is given in Table I, which shows the effects of differential availability of the competing host algae. The data are expressed in three ways. When the number of attached cells on each host species was expressed as a percentage of the total attached cells, the *Dictyota* portion varied systematically between 24 and 74% as the *Dictyota:Galaxaura* mass ratio changed from 0.25 to 2.0 between treatments. Stated differently, when *Dictyota* only represented 20% of the macroalgal biomass, 24% of the cells selected it as a host. At the other extreme, when *Dictyota* represented 67% of the biomass, 74% of the cells selected it. Alternatively, if the *G. toxicus* abundance was normalized to the mass of the host species, the number on *Dictyota* was between 1.3 and 2.0 times greater than that on *Galaxaura* for the four treatments.

Comparative analysis of the number of *G. toxicus* cells per unit surface area on different algae suggests a different picture. *Dictyota* has approximately three-times greater surface area per gram wet weight than does *Galaxaura* (ratio D:G cm^2/gm is 3.4). On a per unit surface area basis, the number of cells on *Dictyota* was between 1.7 and 2.7 times less than on *Galaxaura*. The number of *G. toxicus* per gram and per cm^2 of *Dictyota* or *Galaxaura* remained remarkably consistent even when the ratio of the macroalgal masses varied eight-fold.

At Pointe de Negre, the number of *G. toxicus* cells per

gram of *Dictyota* was significantly greater (a ratio of 4:1) than on *Galaxaura* (t -test, $t = 3.6449$, $DF = 37$, $P < 0.01$). The two macroalgal species were common and present in approximately equal abundance at the study site.

Discussion

Numerous investigators world-wide have conducted field surveys to elucidate the *in situ* population biology of *G. toxicus* and the other ciguatera dinoflagellates. To date, reports on the distribution and abundance of these dinoflagellates have been uniformly based on small sample sizes ($n < 10$), with the dinoflagellate numbers being variously described in terms of: (A) the maximum number of cells per gram of host algae (genera or species not always specified; Carlson *et al.*, 1984; Ballantine *et al.*, 1985; Carlson and Tindall, 1985; Taylor, 1985; Taylor and Gustavson, 1983); (B) cells per gram of a specified alga (Shimizu *et al.*, 1982; Bagnis *et al.*, 1985a; Ballantine *et al.*, 1985; Taylor, 1985); (C) cells per gram of multiple unspecified algae (Bagnis *et al.*, 1985b; Caire *et al.*, 1985; Gillespie *et al.*, 1985a, b; Taylor and Gustavson, 1983); and (D) cell counts per algal surface area (Bomber *et al.*, 1985). The purposes of these surveys varied, as did the macroalgal species distributions, but the lack of a coherent picture of *G. toxicus* ecology (Scheuer and Bagnis, 1985; Anderson and Lobel, 1987) nevertheless argues that a re-examination of commonly used methodologies and assumptions is warranted. A desirable initial goal should be the standardization of sampling and enumeration procedures.

One of the first questions facing any field survey is the number of macroalgal species to sample and the number of replicates of each species to include. The major logistical constraints of time, money, and distance to cover often have led researchers to sample multiple species of macroalgae, each collected with few if any replicates at a given site. The statistical analysis of our Pointe de Negre data makes it clear that small sample sizes are inherently misleading, and that only when the *Dictyota* sample size exceeded $n = 10$ did the variance reach acceptable levels. Of course, a sample size of 10 is minimal for any parametric statistical test. Given the high variance that we observed for small sample sizes, non-parametric tests would have been of dubious merit. The issue of sample variance is further complicated by the level of absolute numerical abundance of cells per sample. Phytoplankton ecologists recognized early that the accuracy of a count varies as a function of the square root of the number counted (Lund *et al.*, 1958). To obtain twice the accuracy, four times the number of organisms must be counted. Consequently, there is a critical population level below which field survey data will not resolve substrate preference or biogeography with certainty.

Table I

Gambierdiscus toxicus substrate preference with differing amounts of macroalgal hosts

	<i>Dictyota</i> (=D)	<i>Galaxaura</i> (=G)	Ratio D:G # cells g ⁻¹	Ratio D:G # cells/cm ²	Ratio G:D # cells/cm ²
Treatment 1: Mass ratio 0.25 D:G					
blot weight, g	0.2	0.8			
% of total macroalgal biomass	20%	80%			
% of total macroalgal surface area	46%	54%			
% attached cells*	24%	76%			
# cells per g	2750	2167	1.3		
# cells per cm ²	26	69		0.25	2.7
Treatment 2: Mass ratio 0.5 D:G					
blot weight, g	0.4	0.8			
% total macroalgal biomass	33%	67%			
% of total macroalgal surface area	63%	37%			
% attached cells*	49%	51%			
# cells per gram	3168	1625	2.0		
# cells per cm ²	30	52		0.58	1.7
Treatment 3: Mass ratio 1.0 D:G					
blot weight, g	0.4	0.4			
% of total macroalgal biomass	50%	50%			
% of total macroalgal surface area	75%	25%			
% attached cells*	60%	40%			
# cells per gram	3250	2168	1.5		
# cells per cm ²	31	70		0.44	2.3
Treatment 4: Mass ratio 2.0 D:G					
blot weight, g	0.8	0.4			
% of total macroalgal biomass	67%	33%			
% of total macroalgal surface area	87%	13%			
% attached cells*	74%	26%			
# cells per g	2188	1500	1.5		
# cells per cm ²	21	48		0.44	2.3

* Attached cells on each species as a percent of the total cells attached to macroalgae.

Another problem encountered in attempts to compare field distributional data for *G. toxicus* is that the species of host macroalgae collected for the various surveys differ so dramatically. This is not only a reflection of the difficulty in finding one or two macroalgal species distributed throughout all coastal marine habitats, but it also indicates that little is known about the differential abundance of *G. toxicus* and other benthic dinoflagellates on particular macroalgal species. If researchers could go to a site and know that they could obtain meaningful data on *G. toxicus* abundance by sampling only one or two algal species, sampling statistics could be improved and ecological issues more easily resolved. Given that it seems desirable to designate key macroalgae that are significantly associated with ciguatera dinoflagellates, we argue that an experimentally derived hierarchy of host species is needed. A laboratory procedure to determine this hierarchy is described here. Algae to be examined in the laboratory should include representatives of the flora found in each of the coastal marine habitats (e.g., tidepool, forereef, backreef, lagoon, etc.).

Adhering to our belief that more can be learned from

a statistically relevant number of replicates of one or two key host species rather than an equal number of samples split between the various macroalgae present in a study area, we focused our attention on *Dictyota* and *Galaxaura* during our field study. We chose these species because of their circumtropical distribution, because they are abundant at most shallow and deep reef environments, and because they have been cited as supporting high *G. toxicus* populations (especially *Dictyota*; Carlson, 1984; Carlson *et al.*, 1984; Ballantine *et al.*, 1985; Carlson and Tindall, 1985; Gillespie *et al.*, 1985a; Taylor, 1985; Taylor and Gustavson, 1983). Ballantine *et al.* (1985) studied the seasonal abundance of *G. toxicus* on *Dictyota* at Puerto Rico and found typical densities ranging between 100–300 cells g⁻¹ with a maximum of 8000. They recognized the huge variability in cell counts among samples collected close to one another, and noted that *Dictyota* appeared to be preferred as a substrate over the seagrass *Thalassia testudinum*. Elsewhere in the Caribbean, *G. toxicus* was also found to be significantly more abundant in association with *Dictyota* spp. than sympatric *Spyridea filamentosa* and *Cladophora hetero-*

nema (Carlson, 1984; Carlson and Tindall, 1985). *Spyrroidea* in turn was considered a preferred host for *G. toxicus* based on field distributions in Hawaii (Shimizu *et al.*, 1982).

These data are discussed here because they emphasize the value in having comparable macroalgal species (or an established hierarchy of species) as substrates to be collected in field surveys, but they are also examples of how preference has been inferred without suitable background information. The relatively obvious source of error that, with one exception (Bomber *et al.*, 1985), has largely been ignored in field studies to date is that cell counts normalized to mass are only comparable between macroalgal hosts if these hosts have the same surface area per unit mass. The value of this concept was first recognized by Bomber *et al.* (1985) who saw no correlation between the field abundance of another epiphytic dinoflagellate, *Prorocentrum lima*, and the mass of macroalgal species. The dinoflagellate distribution was best explained on the basis of available surface area. On first inspection, our laboratory data might be seen as evidence for active preference of *G. toxicus* for *Dictyota*, since the dinoflagellate abundance per gram of host alga was always higher on *Dictyota* than on *Galaxaura*. In fact, when the dinoflagellate abundance is normalized to host surface area, the opposite conclusion is reached—namely that the preference is for *Galaxaura*. As seen in Table I, the percentage of all attached cells that selected *Galaxaura* was always higher than the percentage of available macroalgal surface area represented by *Galaxaura*, typically 1.5–2 times higher. If attachment were simply surface area dependent, a 1:1 correspondence would be expected. This apparent preference is also seen in the ratio of cells cm^{-2} on *Galaxaura* versus *Dictyota*, which varies between 1.7 and 2.7. A simple surface area dependence with no preference would again be evidenced by values closer to 1.0.

In this context, it is noteworthy that we typically saw four times as many *G. toxicus* cells (per gram) on *Dictyota* than on *Galaxaura* at Pointe de Negre (Figs. 3A, 4A). This corresponds to a nearly equal dinoflagellate abundance per cm^2 (*i.e.*, a ratio near 1.0 as discussed above), so active preference seems unlikely. However, these data might be the end result of an initial colonization based on preference, as seen in our short-term laboratory experiments, followed by differential growth or mortality of the dinoflagellate on each macroalga. The separation and quantification of these two processes clearly requires further study that is beyond the scope of this paper. Our intent is to emphasize the difficulties associated with comparisons between different macroalgal hosts and the ease with which incorrect interpretations can be made if data are expressed in commonly accepted units of cells g^{-1} of host algae. Bomber (1985) reports that macroalgal

species can be divided into three general groups on the basis of surface area g^{-1} , with differences spanning a factor of four between species. Until surface area mass $^{-1}$ relationships are determined for other important macroalgae, we argue that *G. toxicus* abundance data cannot be interpreted either in terms of substrate preference or geographic distribution patterns. Only data for the same host species would be comparable, and then only if the number of replicate samples is sufficient.

Another fascinating and unexpected result from the preference experiments is that a relatively constant number of *G. toxicus* cells attached to each gram or cm^2 of our host algae, even when each host species' fractional biomass varied eight-fold. One possible interpretation is that there is a "carrying capacity" for each species. Given reports of much higher numbers of *G. toxicus* per gram of *Dictyota* by Ballantine *et al.* (1985), it seems more likely that we are seeing colonization that was still in progress when the experiment was terminated after 24 h. This consistency is reassuring and argues that the studies of the dynamics of *G. toxicus* substrate attachment and preference are feasible in the laboratory. We have shown that valuable information can be obtained by comparing the short-term colonization behavior of *G. toxicus* when offered different macroalgal hosts. These results suggest that there is preference expressed in the early stages of colonization. Our next step is to extend these experiments in time so as to evaluate other factors that will affect the final abundance of dinoflagellates, namely host chemistry, dinoflagellate growth, water turbulence effects, light effects, and so forth.

In summary, we have initiated a ciguatera research program that we hope will generate field data that are not only statistically sound but that also will allow comparisons to be drawn with results from other researchers throughout the world. Central to this approach is a focus on one or two key macroalgal host species, as well as the collection of sufficient replicates for our abundance estimates to be a valid representation of the real *G. toxicus* distribution. Normalization of these data to host surface area would be more informative and less subject to misinterpretation than the more common units of cells g^{-1} . Finally, we have demonstrated the ease with which substrate preference studies can be conducted in the laboratory. We recognize that the natural abundance and distribution of *G. toxicus* in the field is a reflection of both substrate attachment and the resulting growth and mortality of the established dinoflagellate population. This complex phenomenon must first be separated into discrete components, however, each to be studied in isolation if we are ever to fully comprehend the spatial and temporal dynamics of ciguatera.

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