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A comparison of toxic dinoflagellate densities along a gradient of human disturbance in the North Line Islands

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Abstract

Understanding the ecology influencing the toxic dinoflagellate populations implicated in ciguatera or ciguatera-like sea-food poisoning in humans is a problem of great importance to small-scale fisheries and island communities in the tropical Pacific and Caribbean waters. This study attempted to ascertain the effects of long term human disturbance on the densities of toxic dinoflagellates of the genera *Gambierdiscus, Ostreopsis,* and *Prorocentrum* and the corresponding percent algal coverage in reef environments in theNorth Line Islands. Toxic dinoflagellate densities measured in cells/g macroalgae and the total percent algal cover were recorded at three sites of varying levels of human impact. The most heavily impacted site was a lagoonal area near the town London at Christmas Island, and this site was found to have both the highest densities of toxic dinoflagellates as well as the highest macroalgal cover. The second most impacted site was located at Cook Island, a protected area on Christmas Island. The least impacted site was located on the back reef at Palmyra Atoll, a National Wildlife Preserve, and was found to have the lowest densities of toxic dinoflagellates. Statistically significant dinoflagellate preference for areas of higher human disturbance was found for *Ostreopsis* between the first and second sites ($p= 0.007$) and for *Prorocentrum* between the second and third sites ($p= 0.003$).

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Introduction

Ciguatera poisoning is believed to be one of the most common forms of seafood-derived illnesses in humans, with estimates of as many as 50,000 cases a year. Ciguatera is attributed to a lipid-soluble toxin produced by several genera of benthically-associated dinoflagellates, including those of the *Ostreopsis spp.*, *Prorocentrum spp.*, and *Gambierdiscus spp.*, especially *G. toxicus* (Bagnis and Yasumoto 1977). Poisonings often lead to gastrointestinal inflammation, with associated symptoms of vomiting and diarrhea, and more severe cases can lead to neurological damage that can be sporadically recurrent for many years after the initial exposure(Anderson & Lobel, 1987). Although this tropical reef-fish associated illness is rarely fatal, it is of special concern to small-scale fisheries in poor or densely populated communities in the Pacific and Caribbean. This is because these communities are entirely dependent on the fisheries for their protein intake, as well as because ciguatera is considered to be one of the most important constraints to fisheries resource development in these areas (Lewis, 1984; Olsen *et al*, 1984).

Unfortunately, attempts to elucidate the prevalence of ciguatera poisoning and the ecological factors governing its outbreaks are confounded by several factors. Epidemiologically, its impact is not known with great accuracy due to the fact that its occurrence is highly underreported by many of the isolated island countries affected (Anderson & Lobel, 1987). Additionally, experimentation to determine environmental and habitat preferences of the dinoflagellates associated have been conflicting across many variables.

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Gambierdiscus toxicus was first identified as the dinoflagellate responsible for producing the toxins associated with ciguatera in the Gambier Islands of French Polynesia by Yasumoto *et al* in 1977 (Yasumoto *et al*, 1977b). Initially, *G. toxicus* was believed to be unique in its production of ciguatera-related toxins. However, as the testing of benthic dinoflagellates for toxicity progressed, it became apparent that many benthic dinoflagellates were elaborators of toxins. Some of these toxins have ichthyotoxic or hemolytic effect, but the toxins of main concern remain those maito- and ciguatoxins produced by select dinoflagellate species of the *Gambierdiscus*, *Ostreopsis*, and *Prorocentrum* genera, which are toxic to mice. The water-soluble maitotoxin is the form of toxin usually found concentrated in the lower trophic level herbivores (Anderson & Lobel, 1987). Interest in isolating each toxin to accurately catalogue physiological effects, especially on neurons, and for the creation of monoclonal antibodies for use in accurate, convenient and rapid identification fueled extensive research throughout the late 70's and 80's. The ciguatoxin-specific membrane immunobead assay (MIA) used in this experiment was reviewed for commercial use in 1998, and is intended to serve as a simple presence/absence assay to assist fishermen in determining the safety of their catch (Hokama *et al*, 1998).

Ecologically, *Gambierdiscus toxicus* and the other toxic dinoflagellates have been very difficult to characterize. *Gambierdiscus* is epiphytic, growing on benthic macroalgal mats where light intensity is less than 10%, and limited to shallow waters (Anderson & Lobel, 1987; Carlson & Tindall, 1985). *G. toxicus* has also been observed to be negatively affected by low salinity, and thus is not found in river mouths or areas of high runoff (Yasumoto *et al*, 1980; Carlson & Tindall, 1985). Oceanographically, toxic

benthic dinoflagellates have been associated with oceanic rather than neritic waters, leading to speculation that trace metal concentrations play a significant role in the ecology of these dinoflagellates (Anderson & Lobel, 1987).

Temperature, nutrient, and macroalgal host preferences and interactions for these dinoflagellates have been less clear. Although most of the experiments that have been carried out over a long time period (>1 yr) have found that there is a seasonal cycle for abundance, analysis has not been able to determine what component of seasonal change is responsible for these blooms. Considering these results in relation to temperature data has not been illuminating, as abundance of certain populations of *Ostreopsis* and *Gambierdiscus* have been found to be negatively correlated with temperature, while others have been found to be positively correlated (Bomber *et al*, 1985; Carlson & Tindall *et al*, 1985; Gillespie, 1985). Data correlating nutrient concentrations to abundance is similarly contrary (Wolk, 2004; Yasumoto, 1980;Carlson *et al*, 1984; Carlson & Tindall, 1985; Yasumoto *et al*, 1984; Durand-Clement, 1987). However, there is a key distinction between those experiments finding correlation and those not; those that found correlation took water samples from very near to the host macroalgal surface, while those that did not took samples from water in the general vicinity of the macroalgae. This would suggest that these epiphytic dinoflagellates use as a resource the nutrients and sugars leaked by macroalgae, and use concentration of leaked nutrients as a selection criteria in choosing host habitat (with higher nutrient leakage positively correlating to higher toxic dinoflagellate abundance/ preference

Gambierdiscus may also show sensitivity to water flow. It was first noted by Yasumoto that the highest abundance of *G. toxicus* was on macroalgae that lined

channels (Yasumoto *et al*, 1980). Additionally, Bomber determined that *G. toxicus* abundances are positively correlated with the flexibility and negatively correlated with the percent of stiffening structures in host macroalgae and hypothesized that this was due to the ability for these macroalgae to sway in the water and increase flow/ available water masses for their epiphytes (Bomber *et al*, 1985). However, this effect was negated at areas of highest water energy where abundance drops under the wave crash zone, and decreases during storm events (Wolk, 2004; Carlson & Tindall, 1985; Anderson & Lobel, 1987; Bomber *et al*, 1985).

Lastly, one popular conception throughout ciguatera research has been the idea that outbreaks are more common after an area of coral reef habitat has been disturbed or destroyed (by a hurricane, trawling, dredging, etc.). Evidence has been found both in favor of and against this hypothesis (Anderson & Lobel, 1987). It has been noted that the freshly denuded surfaces created after these disturbance events are easily colonized by the delicate and opportunistic macroalgae preferred by herbivores as a food source, and that these macroalgae, due to their temporal scarcity, have not been sampled to determine toxic dinoflagellate abundance (Anderson & Lobel, 1987). It is possible, then, that these denuded surfaces and the algae they harbor serve as sporadic suppliers of large amounts of ciguatoxin or maitotoxin, and may be related to later outbreaks.

Although toxic dinoflagellate blooms have been studied in relation to such single event disturbances as described above, there are very few studies comparing the effect of cumulative, long term disturbance (specifically human disturbance) on dinoflagellate density. Such disturbance could include destructive fishing practices that rearrange the seafloor, nutrient or sediment deposition that could kill corals and support the growth of

macroalgal communities, or overfishing that leads to a change in herbivory. The Line Islands provide an ideal locale for carrying out such an experiment, as they generally experience a decreasing gradient of human pressure moving northwest along the chain (as estimated by number of people/ miles of coastline). This study aims to determine if dinoflagellate densities are significantly effected by such pressures, and how closely the mechanism of density change matches that associated with single-event disturbances.

We expect to find that with more human impact and disturbance, we will see an increase in toxic dinoflagellate density. We expect that the mechanism of increasing density will be similar to that occurring during a single-event disturbance, in that an increase in density of dinoflagellates will be correlated to an increase in macroalgal cover. Better understanding of the dynamics of toxic dinoflagellate populations in areas of close proximity to human settlement will allow for better prediction of disease risk in areas that are used regularly by humans, as well as help to identify steps to take to control ciguatera outbreaks and illness.

Methods

Environmental Characterization/ Macroalgal Coverage:

Samples were collected at Christmas Island and Palmyra Atoll, to approximate a gradient of decreasing disturbance as human populations decreased from approximately 8,000 at Christmas, to approximately 20 or fewer scientists and staff at the National Wildlife Refuge, Palmyra. At Christmas Island, two sites were chosen for analysis (Fig.1). The first site was a backreef, located in a marine protected area and bird refuge near Cook Island. The second site was located at the mouth of the lagoon near the town London. These sites were chosen for their inherent differences in human impact—the

greater impact being near London, which was easily accessible and used by local fishermen, and the least impact being at Cook Island, where fishing prohibited. Palmyra served as a comparison site of minimal human disturbance due to its protected status in all areas. A backreef area at Penguine Spit was selected in Palmyra for comparison with the Christmas backreef site (Fig 2). However, no similar lagoonal site was found on Palmyra to be accessible during the available field time

At each area chosen for study, a 30m transect was laid down parallel to the closest shore area in a depth of water approximating 4m, with a 0.5m x 0.5m quadrat placed every 5m. The percent cover of macroalgae in the quadrat was visually estimated, categorizing the coverage by the most abundant individual genera present, , ,and the total algal coverage was later calculated using photographs taken of each quadrat.

Samples of the most abundant algal genera with masses ranging from approximately 100-200g were collected in ziplock bags from individual clumps of macroalgae in the area nearby the transect tape for later cell counts of *Gambierdiscus, Ostreopsis,* and *Prorocentrum*. The cell counts along with the weighed macroalgae (taken as dry weight) were used to calculate the density of cells per gram of algae for each sample.

Dinoflagellate Density:

To measure the dinoflagellate densities, the macrophyte samples were shaken in their bags for five minutes to loosen the dinoflagellates, and poured into a stack of seives (125 µm on top of 38 µm). The bag and the algae collected on top of the sieve mesh were rinsed three times each with seawater filtered through a 20 µm sieve. Next, the macroalgae was removed from the sieve, pressed three times with a paper towel to

remove excess water, and then massed with a hanging balance. The filtrate and small amount of excess water on top of the 38 µm sieve was collected with a pipette and placed into a 15 ml falcon tube, which was then centrifuged to help separate cells from detritus and sediment. The thin layer of greenish or light brown organic material which collected on top of the denser sediment was collected and resuspended in a new tube with 10 ml of filtered seawater. To make the cell count sample, 1.5 ml of the suspended cell/water mixture was drawn off the tube and preserved with 10 µl of formalin, while the rest was refrigerated and saved for toxicity tests.

The cell counts were performed on 20 µl of the preserved sample, scanning on a Zeiss Axiostar compound microscope at 100x and using 400x to confirm identifications (see Fig 3 &4). The cell counts per slide of each genus allowed us to calclute their densities in cells per gram of macroalgae.

Ciguatoxin Immunobead Assay:

To test the collected dinoflagellates for the production of ciguatoxin, a membrane immunobead assay (created by Hokama *et. al* 1998*)* was used. Plastic support sticks laminated with polyvinylidene fluoride membrane were placed into test tubes containing 4 ml of cell suspension and 2 ml of methanol for 20 minutes. The sticks were then taken out and air dried for a minimum of 20 minutes. Once the sticks were completely dry, they were immersed in 0.5 mL latex immunobead suspension for 15 minutes, rinsed in deionized water, and air dried. Color changes on the membranes were recorded, with a blue to purple color indicating the presence of the toxin, while no change indicated that there was no toxin present in high enough concentrations to be detected by the test. Color intensity of the membrane on the stick served as an indicator of the concentration

of ciguatoxin. A negative control of a blank membrane stick directly soaked in methanol, dried, and placed in the immunobead solution was also made for comparison with the sample assays.

Results

The density comparisons of *Gambierdiscus, Ostreopsis,* and *Prorocentrum* between the lagoonal site of Christmas Island and the marine protected area by Cook Island showed higher densities of all three dinoflagellates at the lagoonal site (Fig. 5). *Ostreopsis* was the most abundant genus present in the lagoonal site, with a mean density of 55.14 cells/g (SD=62.72), compared to average densities of 2.04 cells/g (SD=4.56) and 10.44 cells/g (SD=12.73) for *Gambierdiscus* and *Prorocentrum* respectively. The Christmas backreef site had mean densities of 1.17 cell/g (SD=2.61), 0 cells/g and 2.33b cells/g (SD=5.21)for *Ostreopsis, Gambierdiscus,* and *Prorocentrum* (Table 1). A significant difference between mean densities between sites was only found for the *Ostreopsis* populations (Mann-Whitney U test, p= 0.007). Comparing the densities of *Gambierdiscus* and *Prorocentrum* between sites found no significant difference between means (p-values 0.317 and 0.290, respectively).

Dinoflagellate densities at the backreef sites of Palmyra and Christmas Island were very low or absent. No dinoflagellates of any of the three genera were found in Palmyra, and no *Gambierdiscus* were observed at either site. The mean densities of *Prorocentrum* and *Ostreopsis* at the Christmas Island backreef site were 2.33 cells/g (SD=5.21) and 1.17 cells/g (SD=2.61) (Table 1). Only *Prorocentrum* had a significantly

higher density at Christmas, with a p-value of 0.003. Testing for significant differences in *Ostreopsis* populations gave a p-value of only 0.317.

The highest total macroalgal coverage was recorded in the lagoonal site near London (53.67%). Palmyra and Cook Island showed similar percent cover (1.67% and 0.83% respectively) (Table 2).

At the lagoonal site comparisons were able to be made between the dinoflagellate densities on the two collected macroalgaes, *Halimeda* and *Padina.* Higher densities were associated with *Padina* for all three dinoflagellate genera (Fig 7). *Gambierdiscus* and *Ostreopsis* showed significant preference, with p-values of 0.034 and 0.047. The *Prorocentrum* populations were not found to be significantly different by the Mann-Whitney U test (p= 0.332), however a comparison of the 95% confidence intervals between the two populations showed no overlap (Table 3).

The toxicity tests showed weakly positive results for all samples except the control. *Gambierdiscus* cell counts/gram for the samples tested varied from 0 cells/gram to 400 cells/g.

Discussion:

Disturbance and Toxic Dinoflagellate Density: Intrasite Variation

The overall trends in the data tended to support the hypothesis that there should be higher toxic dinoflagellate densities in areas of higher disturbance and greater human impact. On Christmas Island, the human impact and implications were much more pronounced at the London lagoonal site than at Cook Island. This location was a major fishing point for the area, as well as in the immediate proximity of London, the largest

town on Christmas. Conversely, Cook Island is a protected area approximately 2.2 km from London, from which fishing is restricted. The higher mean densities of toxic dinoflagellates found at London for all three genera support the hypothesis that some aspect of the heavy human presence promotes a higher density of toxic dinoflagellates. However, a statistically significant difference between London and Cook Island dinoflagellate densities was only found for the *Ostreopsis* populations ($p= 0.007$). The significance for the comparisons of *Gambierdiscus* and *Prorocentrum* populations were $p= 0.317$ and 0.290, respectively.

Disturbance and Toxic Dinoflagellate Density: Intersite Variation

The mean dinoflagellate densities observed between the Christmas and Palmyra backreef sites also suggest a correlation between areas of higher disturbance and higher dinoflagellate densities. The Palmyra backreef site, representing the site with the least human impact in the whole study, had no observed counts of any of the 3 dinoflagellates of interest. In the Christmas Island site, *Prorocentrum* was the dominant genus, and the only dinoflagellate population to show a statistically significant preference for Cook Island, which is in closer proximity to human disturbance..

Percent Algal Cover and Density:

The initial hypothesis that increasing human disturbance would correlate to an increase in dinoflagellate densities was based on the understanding that disturbance might promote an increase in macroalgal growth in an area, and thus increase the habitat available for benthic dinoflagellate colonization. Although this study did not attempt to clarify the exact mechanisms by which such macroalgal expansion may occur, the

transects measuring macroalgal cover were intended to provide insight into the relationship between macroalgal cover and the measured dinoflagellate densities at these sites. The finding that the site with highest macroalgal cover (London lagoon) also corresponded to the site with highest dinoflagellate density, while the two sites with similarly low percent algal cover (Palmyra and Christmas backreef) had comparably small counts suggests that macroalgal cover has an important effect in supporting toxic dinoflagellate densities (see Fig 8).

The implication here is that controlling the macroalgal population in marine areas exposed to continual human disturbance will be critical in maintaining lower base densities of toxic dinoflagellates. Higher background levels of toxin due to a larger base population of dinoflagellates could lead to a higher likelihood that individuals will reach their toxicity tolerance threshold as the toxin bioaccumulates in their bodies and will develop ciguateric symptoms over the course of their lives. A higher proportion of the population in these areas would suffer from symptoms, and have to take continual precautionary measures concerning fish consumption in order to avoid illness. Personal correspondence has confirmed that cases of ciguatera poisoning, although not outbreaks, continue to be of concern in the area of London and affect the desirability of consuming certain species of fish (Kim Anderson, 2007). This background baseline of poisoning cases differs from the ciguatera outbreaks associated with single-event disturbances.

Algal Host Preferences:

The comparison of dinoflagellate densities associated with *Halimeda* vs. *Padina* at Christmas Island showed a preference for *Padina* by all three of the dinoflagellate genera, with a significant preference by *Gambierdiscus* and *Ostreopsis.* The 95 %

confidence intervals for *Prorocentrum* showed very little overlap, meaning the mean density values for the two sites were likely different. This result is not surprising as earlier studies have shown a preference by these dinoflagellates for brown and red algae, which matches the higher densities associated with *Padina*, the brown algae, compared to *Halimeda*, the green algae.

However, , it may be a more accurate to show the relationship between cell number vs. the surface area of the associated macrophyte. Lobel et al stated that algal mass was suitable for intraspecific comparisons of host alga, but cells/ surface area was a better measurement for interspecific comparisons (Lobel, et al 1988). Density could misrepresent dinoflagellate preference for a macroalgal host, because one host could provide much greater surface area, and thus habitat, for the dinoflagellates to associate per standard weight. The *Halimeda* and *Padina* results could be an example of this effect. Since *Halimeda* is much more structurally dense than *Padina*, a similarly sized sample of *Padina* would have a much lower sample weight, and thus inflated density, compared to *Halimeda.*

This result also highlights the importance that different types of macroalgae can have in affecting the overall abundance of toxic dinoflagellates in a reef community, with some species providing more desirable habitat and thus contributing to higher abundances. Comparing London and Cook Island further in this light, we would expect higher baseline toxicity levels at London not only from the higher density of dinoflagellates on *Halimeda*, but also from the presence of dinoflagellate-dense *Padina* in this community. Consequently, to get a full understanding of the profile of toxicity of an

area, it will be important to consider not only relative densities of dinoflagellates, but also the community composition of macroalgal hosts.

Toxicity Test Results:

With all of the samples yielding similar intensity light blue, or weak positive, results for ciguatoxin except for the control, including several samples that had zero cell counts for *Gambierdiscus*, the toxicity tests remain inconclusive. It is likely that these results include several, if not all, false positive results. There are many potential explanations for this which need to be explored further. One idea is that since the assays are normally used with fish flesh samples and not cell isolates from macroalgae, there were higher levels of contaminants released from the loose cells that could adhere to the membrane stick and cause immunobeads to attach, even without matching the specificity of the antibodies.

Issues Affecting Data Analyses:

Though statistically significant results could not be deduced from some data comparisons, probable general trends can be inferred from them, indicating potentially significant connections between variables, which need further study with larger sample sizes in order to clearly elucidate their relationships. Several issues clouded the conclusiveness of our results:

The Assumption That All Algal Hosts are Created Equal:

Halimeda was used as the macroalgal host to count densities across all sites because of its presence and ease of collection at all sites. The underlying assumption made in this collection technique was that density trend on *Halimeda* would be representative of density trends on the whole macroalgal community. Collection of one

or a few types of macroalgae to determine the ecological preferences of toxic dinoflagellates was a standard technique used in previous research (Chinain, *et al*, 1999; Yasumoto, *et al*, 1980; McCaffrey, *et al*, 1989), and no mention was made of finding conflicting trends when analyzing different macroalgal hosts. In order to confirm the creditability of using dinoflagellate densities on *Halimeda* as indicative of community densities of these dinoflagellates at our particular study sites, it would have been useful to have been able to collect and process more macroalgae from different genera for comparison

High Data Variance Due to Small Sample Sizes:

For this study, time limitations at each site and during processing prevented more samples from being collected and analyzed. Because n=5 for each site, there were instances in which there was not enough resolution of the data to differentiate between the means being compared. Other studies of benthic dinoflagellate densities have found that only after n=10 does variance of data "reach acceptable levels" (Lobel *et a,* 1988).

Accuracy Due to Low Cell Densities:

In addition to the effects of small samples sizes on the precision and ability to make accurate comparisons of the data on a fine scale, the frequency of low cell densities for multiple samples made the accuracy of their counts less certain. Cell counts of phytoplankton are recognized to demonstrate accuracy as a function of the square root of the number of cells counted (Lund *et al,* 1958), so the low numbers of these dinoflagellates mean that their population sizes could be lower than the level at which field data can resolve their algal host or site preferences with high confidence.

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Conclusion

In looking at the densities of *Gambierdiscus, Ostreopsis,* and *Prorocentrum* associated with areas of varying human disturbance on the reef systems at Christmas Island and Palmyra Atoll, it was expected that there would be greater densities of all genera on the sites most heavily impacted by humans. This hypothesis was confirmed at Christmas Island, as trends showed higher densities of dinoflagellates at the lagoonal site near the town London compared to the backreef protected area near Cook Island. In the inter-island comparison between the backreef sites at Christmas and Palmyra there was a similar trend of lower densities at the least impacted site, in this case, Palmyra. The site with the highest densities of dinoflagellates, London lagoon, also had the highest percent cover of macroalgae. This implies that both human disturbance and percent macroalgal cover positively correlate with toxic dinoflagellate density, and implicates human presence as a potential influence on the macroalgal abundance. Further study of the relationship between macroalgal cover and toxic dinoflagellate densities is needed to confirm this observed trend. Examining the effect of macroalgal community composition on density and abundance of dinoflagellates would also assist in accurately ascertaining the overall toxicity of an environment of human interest. Lastly, further experimentation focusing on removal of contaminants must be done in order to create a reliable method for using current MIA technologies to test for toxicity in *Gambierdiscus toxicus* cells.

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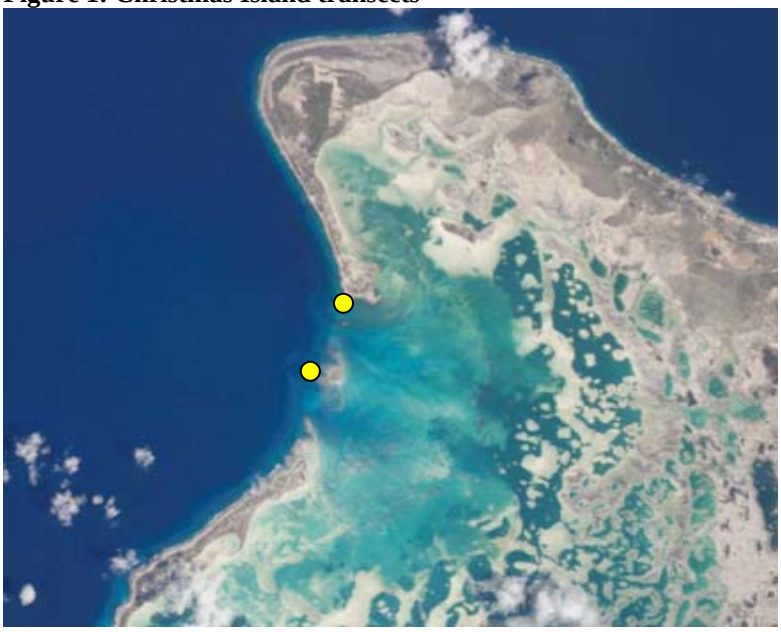
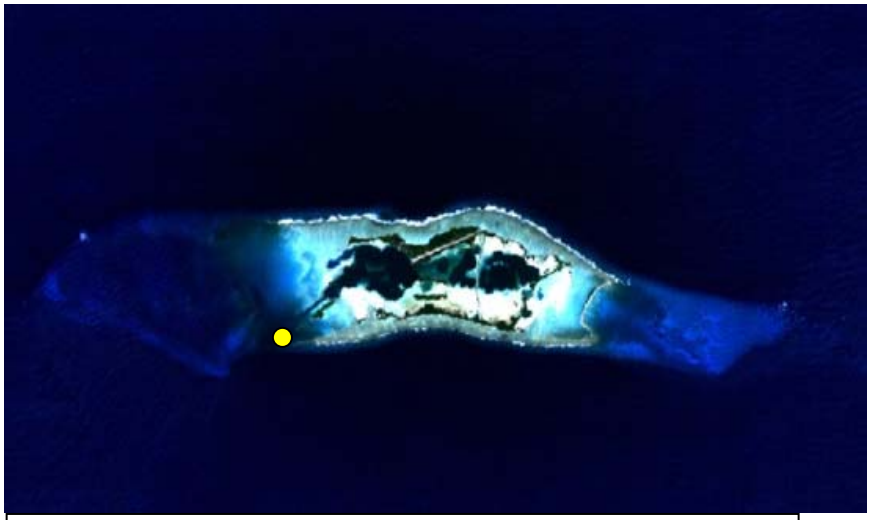
Appendix

Figure 1: Christmas Island transects



Christmas Island transects: one lagoonal site in the north located near town of London, one backreef site located near Cook Island.

Figure 2: Palmyra transect



Palmyra Atoll transect site: located at Penguin Spit in backreef area

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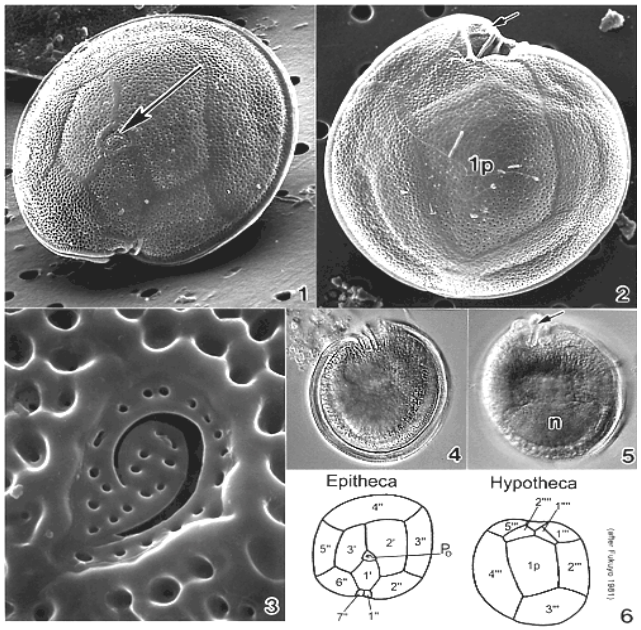
Figure 3. *Gambierdiscus* Morphology



Chinain, Faust, Pauillac, Morphology and Molecular Analyses..., *Journal of Phycol*,1999

Fig 4. *Ostreopsis*

Osteopsis cell at 40x magnification from London Lagoon

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Figure 5

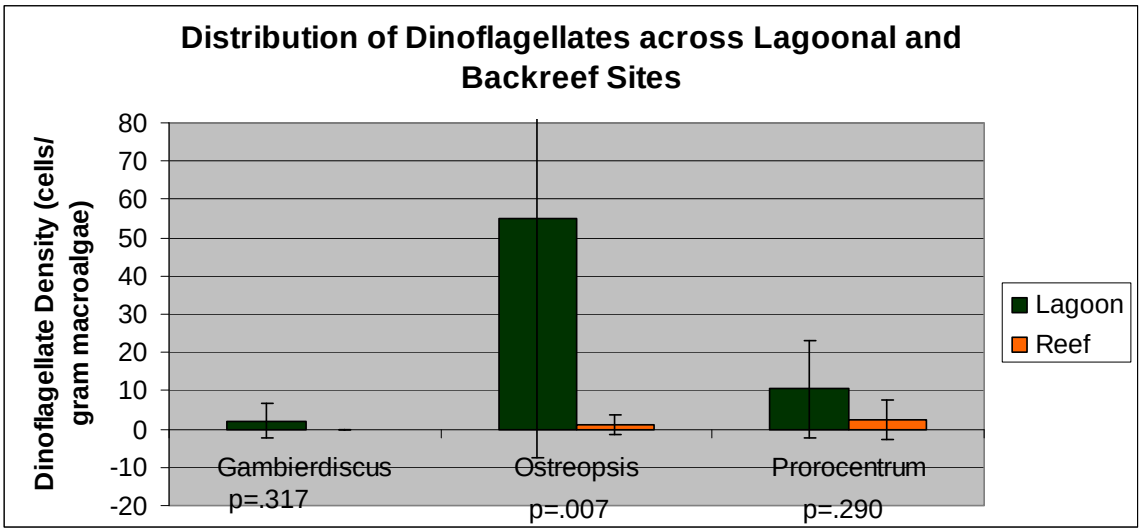

Table 1:Comparisons of dinoflagellate density between London and Cook Island

G. distribution: Lagoon v Reef				O. distribution: Lagoon v Reef				P. distribution: Lagoon v Reef			
Lagoon:	10.21	avg:	SD	Lagoon:	17.01	avg:	SD	Lagoon:	10.21	avg:	SD
	0.00	2.04	4.56		160.32	55.14	62.72		10.83	10.44	12.73
	0.00				22.00				0.00		
	0.00				65.99				0.00		
	0.00				10.39				31.18		
Reef:	0.00	avg:	SD	Reef:	0.00	avg:	SD	Reef:	0.00	avg:	SD
	0.00	0.00	0.00		0.00	1.17	2.61		0.00	2.33	5.22
	0.00				0.00				0.00		
	0.00				0.00				0.00		
	0.00				5.83				11.66		

Fig 6

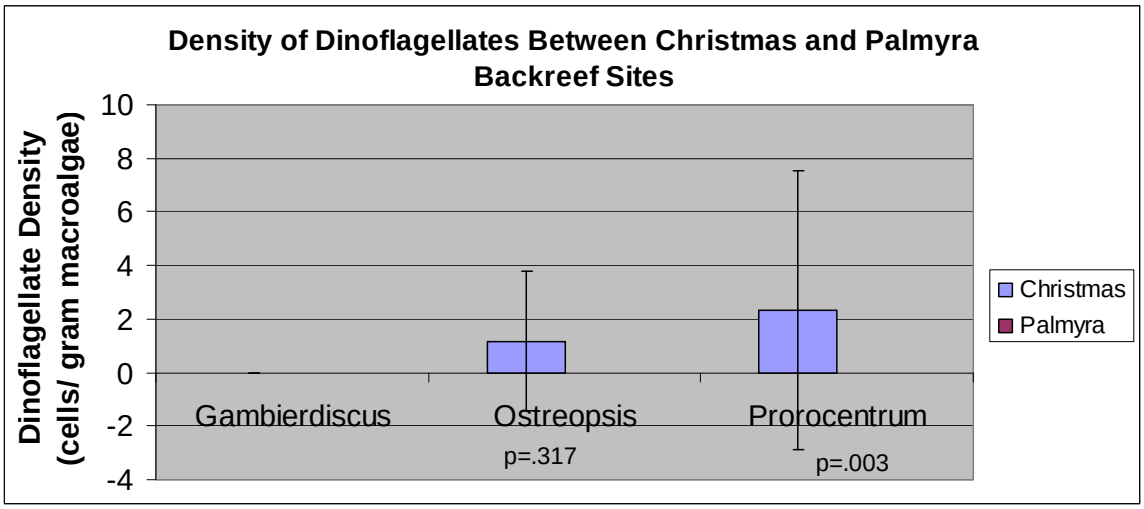

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Table 2: Macroalgal cover by type at 3 sites

Transect 1- Cook Island

Quad	% ulva	% Padina	% Halimeda	% filamentous	% cover total
1	0	0	0	4	4
2	0	0	<1	0	<1
3	0	0	0	0	0
4	0	0	0	0	1
5	0	0	0	0	0
6	0	0	0	0	0
average	0	0	0	0.666666667	0.833333333

Site 2- London

Quad	% ulva	% Padina	% Halimeda	% filamentous	% cover total
1	9	1	0	0	10
2	0	0	0	21.3	21.3
3	0	8	0	91	99
4	0	6	0	91	97
5	0	70	0	26	96
6	<1	8	0	12	20
average	1.5	15.5	0	40.21666667	53.66666667

Palmyra

Quad	% ulva	% Padina	% Halimeda	% filamentous	% cover total
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	6	0	6
4	0	0	0	0	0
5	0	0	1	0	1
6	0	0	3	0	3
average	0	0	1.6666667	0	1.666666667

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Fig 7

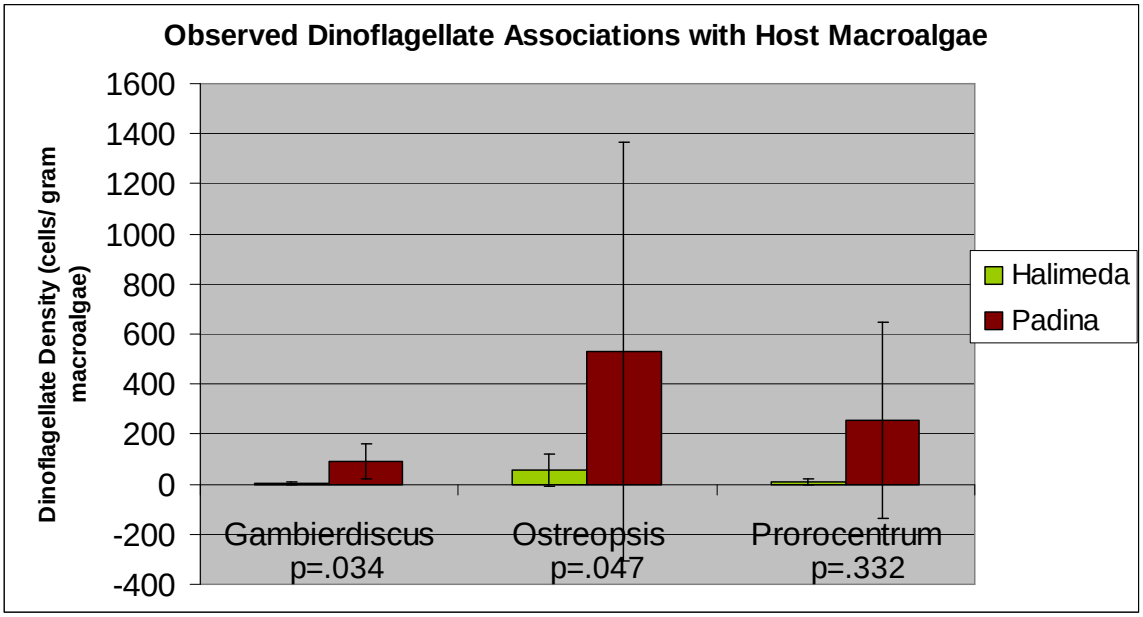

Table 3: Dinoflagellate densities on two different macroalgal hosts

G. preference: *H* v *P*				**O. preference: H v P**				**P. preference: H v P**			
Halimeda:	10.21	avg:	SD	Halimeda:	17.01	avg:	SD	Halimeda:	10.21	avg:	SD
	0.00	2.04	4.56		160.32	55.14	62.72		10.83	10.44	12.73
	0.00				22.00				0.00		
	0.00				65.99				0.00		
	0.00				10.39				31.18		
Padina:	164.07	avg:	SD	Padina:	82.03	avg:	SD	Padina:	246.10	avg:	SD
	154.92	91.25	71.33		2010.20	530.29	834.03		927.64	254.21	389.69
	0.00				342.03				0.00		
	39.95				119.86				0.00		
	97.31				97.31				97.31		

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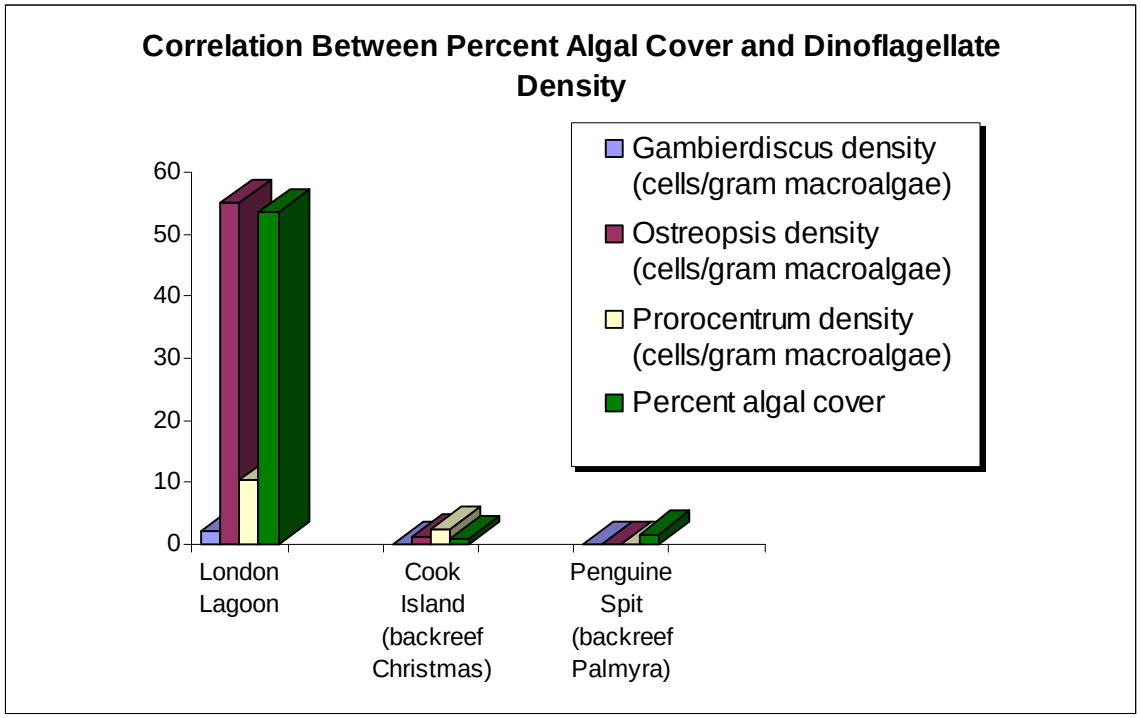
Figure 8.



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