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Effects of depth, habitat, and water motion on the abundance and distribution of ciguatera dinoflagellates at Johnston Atoll, Pacific Ocean

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ABSTRACT: A major impediment to understanding the seemingly random occurrence of ciguatera toxicity is uncertainty regarding the field ecology of benthic dinoflagellates that introduce toxins into the coral reef food web. Although broad generalizations have been made, past studies have often yielded contradictory results, particularly between ecological patterns documented in the Pacific versus the Caribbean. This study employed standardized methodology to investigate the distribution and abundance of toxigenic benthic dinoflagellates from the genera *Gambierdiscus, Prorocentrum, Ostreopsis*, and *Amphidinium* at Johnston Atoll, Pacific Ocean, to determine how water motion, depth and habitat type influence patterns of biodiversity. Sampling stations located in lagoon and channel habitats in the atoll supported the highest total dinoflagellate abundance, while dinoflagellate numbers were lower at sampling stations in back reef and reef crest habitats. Total dinoflagellate abundance was primarily determined by the degree of water motion; however, this effect varied among genera. Of the 4 genera surveyed, 3 (*Gambierdiscus*, *Prorocentrum*, *Amphidinium*) were negatively correlated with water motion; conversely, *Ostreopsis* had a positive correlation. Habitat separation was observed between *Ostreopsis* and *Prorocentrum* spp., which were negatively correlated. *Gambierdiscus* was present at all sampling stations and at all habitats, even though it was rarely the dominant genus. This study provides a characterization of the ciguatera dinoflagellate community across a variety of coral reef habitats, contributing to an accurate and coherent characterization of the population dynamics of this important dinoflagellate community.

KEY WORDS: Ciguatera fish poisoning · *Gambierdiscus* · *Prorocentrum* · *Ostreopsis* · Johnston Atoll · Epiphytic · Coral reefs

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INTRODUCTION

Ciguatera fish poisoning (CFP) is a widespread form of food poisoning caused by the consumption of finfish contaminated by lipid-soluble toxins, which originate in an assemblage of epiphytic dinoflagellates found in tropical and sub-tropical ecosystems. Numerous studies (e.g. Ragelis 1984, Lange 1994) rank CFP as the most common illness related to finfish consumption and potentially the most common of all marine food poisonings. Globally, Fleming et al. (1998) estimates that there are 50 000 to 500 000 poisonings per year. The symptoms of CFP have been well-described and include a variety of gastrointestinal, neurological and cardiovascular disturbances (Bagnis et al. 1979, Lewis et al. 1988, Lehane & Lewis 2000, Palafox & Buenconsejo-Lum 2001). These symptoms vary from individual to individual; moreover, marked differences in symptomology have been observed among geographic regions (Lewis et al. 1988, Pottier et al. 2001).

CFP is caused by exposure to an assemblage of chemically related natural toxins known as ciguatoxins, which differ in chemical structure and potency (reviewed in Lehane & Lewis 2000). These are piscine

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metabolites resulting from biotransformation of precursor toxins (gambiertoxins) produced by the benthic dinoflagellates in the genus *Gambierdiscus*. However, in addition to ciguatoxins, the coral reef food web may also contain toxins produced by epiphytic dinoflagellates from several genera, including *Prorocentrum* (okadaic acid-producing species), *Ostreopsis*, and *Amphidinium.* Certain species/strains from these genera produce potent toxins that kill mice and therefore have the potential to contribute to ciguatera toxicity (Nakajima et al. 1981, Yasumoto et al. 1987).

Several *Prorocentrum* species produce a polyether toxin known as okadaic acid (OA) (e.g. Murakami et al. 1982, Dickey et al. 1990, Morton et al. 1998), which is commonly associated with diarrhetic shellfish poisoning (DSP) in humans. DSP is caused by the consumption of toxic shellfish that have accumulated OA and dinophysistoxins (DTX) produced by planktonic dinoflagellates in the genus *Dinophysis* (Yasumoto et al. 1985). *Prorocentrum* spp. have a cosmopolitan distribution that includes regions affected by ciguatera and DSP, also produce OA and DTX, and are therefore suspected of contributing to DSP and possibly ciguatera. Although few studies have investigated the occurrence of OA in ciguatoxic fish, it has been isolated from *Sphyraena barracuda*, which is responsible for the vast majority of ciguatera poisonings in the Caribbean (Gamboa et al. 1992). However, the role of these toxins in ciguatera has not been conclusively demonstrated either experimentally or through the systematic survey of toxic fish.

A second, powerful toxin potentially involved in CFP is palytoxin. Palytoxin was first isolated from the zoanthid *Palythoa toxica* (Moore & Scheuer 1971) and is also produced by certain *Ostreopsis* species (Usami et al. 1995, Lenoir et al. 2004, Ciminiello et al. 2006). Palytoxin has been detected in crustaceans, soft corals, and mussels as well as fish from several families, including Chaetodontidae, Scaridae, and Balistidae (Fukui et al. 1987, Gleibs & Mebs 1999, Taniyama et al. 2003). Recently, blooms of *Ostreopsis* spp. caused respiratory illness and skin irritation in tourists and workers at beaches along the Tyrrhenian Sea (Sansoni et al. 2003, Penna et al. 2005, Ciminiello et al. 2006), with palytoxin exposure as the putative cause of illness (Ciminiello et al. 2006). These reports indicate that *Ostreopsis* spp. apparently can affect human health through marine aerosols in addition to their presence in the food chain, adding a second vector of exposure to toxins produced by these species. Palytoxin has also been implicated in cases of poisoning from the consumption of mackerel (Kodama et al. 1989), parrotfishes (Noguchi et al. 1987, Taniyama et al. 2003), and grouper (Taniyama et al. 2002); thus, given its ubiquity in the food chain and remarkable toxicity, palytoxin may indeed contribute to ciguatera.

Additional toxic compounds have been isolated from fish, the origins of which have yet to be determined. Several researchers have detected the presence of fast-acting toxins (FAT) in ciguateric fish, which were demonstrated to cause neurological symptoms similar to brevetoxins (Vernoux & Talha 1989, Pottier et al. 2003). Although ciguatoxin congeners have been suspected as playing possible roles as FAT, currently the origin of these compounds is unknown.

Although the contribution of these additional toxins to human illness is unknown, the heterogeneity observed in the CFP symptoms among different locales and even individual cases suggests the involvement of multiple toxins. Research on the trophic pathways of ciguatoxin has provided considerable insight into how the uptake and movement of toxins produced by *Gambierdiscus* spp. occurs; therefore, it seems likely that other toxins from the benthic community may enter the food chain in a similar manner.

The field ecology and population dynamics of these dinoflagellates are not well characterized, particularly with respect to habitat preference and the influence of environmental parameters. This is partly attributable to a lack of sustained scientific research in this area, compounded by conflicting and sometimes contradictory results yielded by past ecological studies of toxic benthic dinoflagellates. Field surveys indicate that *Gambierdiscus* dinoflagellates appear to have a poor tolerance for strong light intensity and land runoff (Yasumoto et al. 1980, Carlson & Tindall 1985, Grzebyk et al. 1994), and a preference for sheltered habitats (Carlson & Tindall 1985, Taylor 1985, Grzebyk et al. 1994, Tindall & Morton 1998); however, in the Pacific, highest abundances were observed in areas subject to strong currents (Yasumoto et al. 1979, 1980). While differences in the physical habitat conditions specific to the study area likely contribute to these discrepancies, differences in sampling methodologies and microscopic analyses may also help explain inconsistencies, and are the focus of discussion here.

Biological sampling protocols used in studies of ciguatera dinoflagellates vary widely in collection, filtration and processing methods. Although survey results are often expressed as 'cells g^{-1} macroalgae', the quantity of the 20 µm fraction analyzed under the microscope differs from study to study and occasionally is not specified. Furthermore, a method used in many studies for enumerating ciguatera dinoflagellates involves normalizing cell counts to the weight of the macroalgal host, but comparisons made among different host algae species with different surface area to mass ratios introduces a significant source of error due to differences in algal morphology and consequently, surface area among species.

Lobel et al. (1988) demonstrated that contradictory conclusions may be reached regarding dinoflagellate

abundance depending on whether the number of cells is normalized to algal biomass or surface area. Hence, the common biomass measurement used in dinoflagellate ecology studies 'cells g^{-1} wet wt' is only appropriate for enumerations from the same host macroalga, while 'cells per surface area' should be used in interalgal comparisons (Lobel et al. 1988). With few exceptions (Bomber et al. 1985, Ballantine et al. 1988), most field surveys examining the distribution and abundance of ciguatera dinoflagellates have been based on samples collected from more than one macroalgal species with substantial differences in morphology, and enumerations were normalized to biomass instead of surface area.

Here we describe surveys conducted at Johnston Atoll, Pacific Ocean, to characterize the ecology of toxic benthic dinoflagellates in habitats in which herbivorous fishes typically forage. In contrast to planktonic dinoflagellates where toxin production is rare, a surprising number of benthic dinoflagellates are toxin producers (Nakajima et al. 1981, Anderson & Lobel 1987), necessitating the study of an assemblage rather than a single bloom-forming species. Patterns of species abundance, distribution and spatial heterogeneity were examined to describe the spatial distribution and community composition of toxigenic benthic dinoflagellate populations. These surveys employed standardized sampling methodology (Lobel et al. 1988) to evaluate the environmental factors correlated with dinoflagellate biodiversity: specifically, physical parameters such as water flow and depth, as well as habitat type (e.g. lagoon, back reef, reef crest).

Understanding how the population dynamics and biodiversity of ciguatera dinoflagellates relate to the occurrence of fish toxicity has been a major goal of ciguatera researchers since the discovery of the toxin progenitor. Bagnis et al. (1990) showed that herbivorous fish rapidly acquire toxicity following a bloom of *Gambierdiscus toxicus* on a reef; hence, regular monitoring would help to identify a certain 'threshold' level of abundance that may indicate an increase in the level of toxin entering the food chain, signaling the precipitation of an outbreak. Ultimately, a clear characterization of the patterns of distribution of these dinoflagellates as well as the biotic and abiotic factors that contribute to their proliferation would greatly contribute to the identification of particular patterns that might be predictive of toxicity, which in turn would benefit monitoring protocols in ciguatera endemic areas.

MATERIALS AND METHODS

Description of study area. Johnston Atoll, Pacific Ocean, is a remote atoll located 800 miles (1287 km) southwest of Hawaii and 900 miles (1448 km) north of the Line Islands of Kiribati (Fig. 1). The island is ~46 acres (0.18 km^2) and encompasses 50 square miles (80.5 km^2) of reef habitat; a barrier reef wraps around the north and western edges of the atoll platform. Johnston Atoll comprises 4 islands, 2 of which were artificially created by the military through dredging of the surrounding coral reefs.

Algae sample collection. A total of 173 macroalgae samples was collected using SCUBA and analyzed from 14 sites comprising representative reef habitat throughout the atoll (Fig. 1). Sampling occurred from May to August 2003 during the calm summer months. Prior to commencing a survey, the following information was recorded at each site: date and time, station number, global positioning system (GPS) coordinates, location, weather conditions and cloud cover, habitat type, and sea state.

One species of macroalgae, *Caulerpa serrulata*, was selected for intensive sampling to avoid errors associated with differences in surface area when normalizing dinoflagellate abundance to host alga biomass (Lobel et al. 1988). *C. serrulata* is a toothed, spiral shaped green alga that is distributed circumtropically and is ubiquitous throughout the atoll. At least 10 samples were collected at each site; each of which was placed in a 50 ml polypropylene conical centrifuge tube (Fisher Scientific), sealed underwater and preserved in 4 to 5% formalin following the dive. The depth and substrate type of each sampling site was recorded.

Sample processing. Samples were filtered using a 202 µm nitex sieve stacked above a second 20 µm sieve. A small amount (~20 ml) of the sample was decanted into this filtration unit. The tube was resealed and shaken vigorously for 1 min. The remaining liquid was then poured into the filters and the tube refilled about halfway with pre-filtered seawater. The shaking procedure was repeated and then the entire contents of the tube were decanted into the 202 µm sieve. The material collected on the 20 µm sieve was backwashed in the tube and brought up to 10 ml with filtered seawater. The macroalgae retained in the 202 µm filter was removed, blotted dry with a paper towel, and immediately weighed.

Sample analysis. Processed samples were gently shaken and 1 ml was analyzed for benthic dinoflagellate abundance in a Sedgewick Rafter counting cell slide using an Olympus BH-2 light microscope at 100× magnification. The 10 most abundant benthic dinoflagellate taxa were counted, and counts of additional species were recorded and included in genera totals for each site. Cells were identified using line drawings and photomicrographs by Fukuyo (1981), Faust & Gulledge (2002), Tomas (1996), D. M. Anderson & P. S.

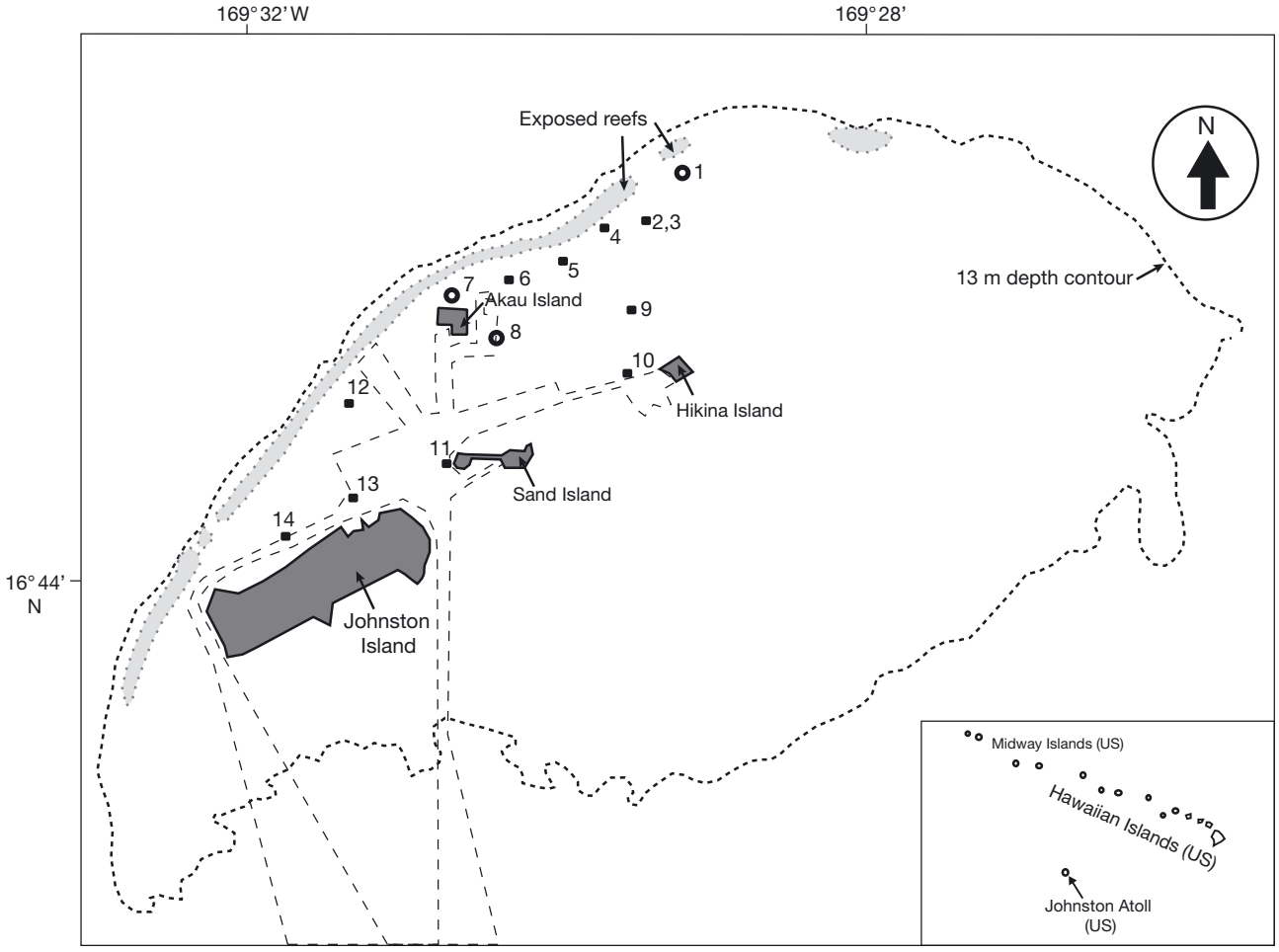

Fig. 1. Sampling stations at Johnston Atoll, Pacific Ocean. Macroalgae samples were collected from 14 sites (■, ○) to analyze dinoflagellate community abundance and distribution: (1) JA95B, (2/3) WP201 A/B, (4) WP202, (5) B11, (6) S09, (7) S09B, (8) JA51, (9) WP203, (10) WP204, (11) JA86, (12) JA100, (13) 94P, (14) JA101. Sampling habitats included reef crest, back reef, lagoon, and dredged channel sites. Light grey: coral reefs; dark grey: land. Thin dashed lines: dredged channels. Clod cards were deployed at 11 sites (■) to measure water motion

Lobel (unpubl. data), and G. Usup (unpubl. data). The number of cells g^{-1} macroalgae wet wt was calculated for each sample.

Water motion measurements. Water motion was measured at sampling sites using the 'clod-cards' technique developed by Doty (1971) to test algal growth relative to water motion. The method uses the dissolution rate of blocks of dried plaster of Paris (clods) affixed to pieces of plastic, tile, or other appropriate surface (cards) to describe water motion experienced by benthic organisms. The clod cards are deployed for a 24 h period, after which the dissolution rate is quantified for each card using a parameter termed the diffusion index factor (DF). The DF is calculated by dividing the weight loss experienced by the control clod cards into the weight loss experienced by cards deployed in the field. Expressing water motion using the DF allows comparisons between different clod card lots that may have subtle differences in their rate of diffusion, and permits comparisons among DF values reported from other studies.

To create the clod cards, 2 lots of approximately eighty 30 g plaster of Paris blocks were cast in plastic ice cube trays following the instructions outlined by Doty (1971). After drying for 48 h, the bases were filed so that each block was within <0.2 g of a pre-selected weight. Each block was then glued to a small tile measuring ~2 × 5 inches and allowed to dry for 24 h. After drying, the cards were secured to bricks using duck tape and deployed in groups of 10. Control blocks were placed in a bucket, submerged, and the bucket was covered to create still water conditions. The clod cards were deployed during calm conditions at 5 macroalgae

sampling stations in July 2003 and 5 additional sites in August 2003 (Fig. 1). At each station, the set of 10 clod cards was deployed in close proximity to the area where algal samples were collected. Following each deployment, the cards were collected and allowed to dry for 24 h prior to weighing. DF values were obtained by dividing the still-water calibration value into the weight lost by each block during its period of field use.

Measure of spatial dispersion. Patterns of spatial heterogeneity in which a population of individuals exhibits 'contagiousness' in their distribution has been well-documented in the phytoplankton community (Bainbridge 1957, Platt & Denman 1980, Harri 1988, Kuosa 1988, Martin 2003). In a contagious distribution, the population is not uniformly or randomly distributed; rather, there are always patches of high density (clumps) distributed on a general background of low density (Elliott 1977). This particular distribution presents difficulties in data analysis and statistical testing, since the variance is frequently greater than the mean (Elliott 1977). To test for contagiousness in the distribution of dinoflagellates at Johnston Atoll, Morisita's index (I_δ) was calculated for each species (Morisita 1962, Elliott 1977). I_δ is a measure of dispersion that describes whether the distribution is random, contagious, or uniform, and is independent of the sample mean and total numbers in the sample. The standardized I_δ was calculated for each species at each site in Microsoft Excel using Poptools version 3.1.0 (Hood 2002). The index is defined as:

$$I_\delta = n\frac{\sum x_i(x_i-1)}{(\sum x_i)[(\sum x_i)-1]} \quad (1)$$

where x_i is the number of individuals in the ith sample and n is the number of samples. This standardized index ranges from −1 to +1 with 95% confidence limits at +0.5 and −0.5. When the distribution of individuals follows a random distribution, $I_\delta = 0$. Indices of $I_\delta > 0$ indicate a patchy distribution while indices of $I_\delta < 0$ are indicative of uniform distributions.

Statistical analyses. Statistical analyses were performed using JMP software (SAS Institute, Cary, NC, USA) and SPSS 15.0. Data were first tested for equality of variances using Levene's test. Differences in mean species abundance among sampling sites and for the environmental parameters of depth and water motion were tested using Welch's variance-weighted ANOVA (Zar 1996). If significant differences were detected, then a Games-Howell test was used to analyze pairwise differences. Spearman's rank correlation coefficient was used to examine the statistical significance of species–species, species–depth, and species–water motion correlations.

RESULTS

Ciguatera dinoflagellate community

The 10 most abundant benthic dinoflagellate taxa observed in the samples collected from Johnston Atoll were enumerated: *Gambierdiscus* spp., *Ostreopsis siamensis*, *O. ovata*, *O. lenticularis*, *Prorocentrum lima*, *P. concavum*, *Prorocentrum* cf. *rhathymum*, *P. emarginatum*, *Amphidinium* cf. *klebsii*, and *Amphidinium* cf. *carterae* (Fig. 2). Species identifications were made solely using light microscopy, and as detailed morphological studies

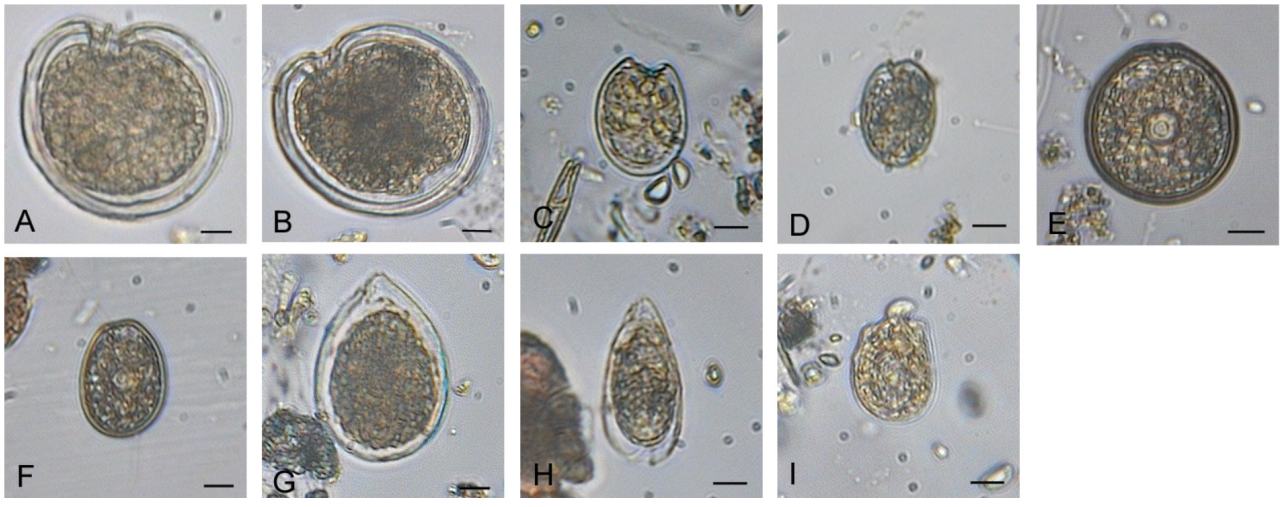

Fig. 2. *Gambierdiscus* spp., *Ostreopsis* spp., *Prorocentrum* spp., and *Amphidinium* spp. Light micrographs of epiphytic dinoflagellates from Johnston Atoll, Pacific Ocean: (A,B) *Gambierdiscus* spp.; (C) *P. emarginatum*; (D) *P.* cf. *rhathymum*; (E) *P. concavum*; (F) *P. lima*; (G) *O. siamensis*; (H) *O. ovata*; (I) *A.* cf. *carterae*. Scale bar: 10 µm

were not conducted for this study, in some instances we were not able to conclusively discern species; thus, certain identifications were tentative. *Gambierdiscus* species are largely distinguished by differences in thecal plate architecture that are not visible using light microscopy; thus, counts recorded were generic. *Prorocentrum* species were identified based on cell size and shape; presence/absence of a central pyrenoid, areolae, and apical spine; and the shape of the periflagellar area. *P. concavum* were either round or broadly oval; cells featured areolae, a central pyrenoid and a deep, v-shaped excavation of the periflagellar area. *P. lima* cells were narrower and oval in shape, usually straight-sided, with a central pyrenoid and narrow, v-shaped periflagellar excavation. *P. emarginatum* cells were broadly oval or round, sometimes asymmetrical, with a central pyrenoid, and featured a deep, asymmetrical, v-shaped, periflagellar excavation. *P.* cf. *rhathymum* cells were identified based on their narrow oval shape, an off-center, asymmetrical depression in the apical area, and an obvious apical spine. Cells were relatively straight-sided but asymmetrical in shape. Without using scanning electron microscopy, the identification of these cells as *P. mexicanum* or *P. rhathymum* using solely morphological criteria is ambiguous; thus, cells described in this study are designated as *P.* cf. *rhathymum* based on differences in habitat and distribution: while *P. rhathymum* is regarded as an epibenthic, cosmopolitan species, *P. mexicanum* is primarily described as planktonic and from northern latitudes of the American Pacific (Cortés-Altamirano & Sierra-Beltrán 2003). *Ostreopsis* cells were largely distinguished by cell size and shape. *O. siamensis* were large, broadly ovoid and pointed at the sulcal area, and also featured slight but distinct cingulum undulation. *O. lenticularis* cells were also large, lenticular and symmetrical in shape, and lacked cell undulation. *O. ovata* cells were small in size, with a very distinctive narrow, tear-drop shape. *Amphidinium* cells were seldom observed and specific identifications are tentative designations due to similarity or plasticity in the morphological features and the necessity for complementary genetic data to conclusively identify species (e.g. Murray et al. 2004, Dolapsakis & Economou-Amilli 2009).

Water motion measurements

Water motion measurements were significantly different among the deployment sites ($p < 0.001$). The highest mean DF (23.2) was measured at the reef crest and the lowest mean DF (6.4) was measured at a station in the lagoon, which was also the deepest deployment site (Table 1). These values are similar to DF values measured at Johnston Atoll by Jokiel & Morrissey (1993), who deployed a series of clod cards in a transect that ran from the reef crest (DF = 25) to the lagoon (DF = 5). Dissolution rates were higher at the reef crest sites, which experienced considerable surge, and the back reef sites. Back reef locations were subject to strong unidirectional current that ran from the reef crest across the back reef. Channel and lagoon sites were generally calmer, with no surge and little current.

Benthic dinoflagellate distribution and abundance

Epiphytic dinoflagellates surveyed at Johnston Atoll were not distributed randomly in a site; rather, the distribution of all dinoflagellate species was patchy at more than one sampling site (Table 2). The frequent occurrence of a patchy or contagious distribution necessitated the use of appropriate statistical methods for analyzing non-normal, heteroscedastic data, and demonstrates the necessity of collecting multiple replicates to adequately account for patchiness within a site when characterizing patterns of distribution at the ecosystem level.

Overall, lagoon and channel sites supported substantially larger numbers of dinoflagellates than back reef and reef crest habitats. The sampling station with the highest overall dinoflagellate abundance was JA51, followed by JA100 and WP204. JA51 and WP204 were located in dredged channels, and JA100 in the lagoon. At all 3 sites, *Prorocentrum* spp. comprised the majority of the sample (Fig. 3). These sites were

Table 1. Sample collection sites at Johnston Atoll, Pacific Ocean. Sampling station, habitat type, deployment depth, number of clod cards deployed (N), maximum and mean diffusion index factor (DF), and SD. na: not available

Sampling station	Habitat type	N	Depth (m)	Max DF	Mean DF	SD
JA51	Channel	10	13	na	na	na
JA100	Lagoon	10	11	8.0	6.4	0.98
JA101	Channel	10	5	8.9	6.8	1.80
JA94P	Channel	10	5	10.6	6.8	1.95
JA86	Channel	10	4	12.1	8.7	1.53
WP203	Back reef	10	5	11.7	9.4	1.49
WP204	Channel	10	6	14.0	10.5	2.13
WP201A	Back reef	9	5	19.0	13.8	2.21
B11	Back reef	10	3	18.4	16.1	1.36
S09	Back reef	10	2	19.1	18.1	0.70
WP201B	Back reef	10	4	23.1	19.2	3.07
WP202	Reef crest	10	4	27.7	23.3	2.94
JA95B	Back reef	13	3	na	na	na
S09B	Back reef	10	3	na	na	na

Table 2. Morisita's index of dispersion for each genus or species at each sampling station at Johnston Atoll. *Gamb* spp. = total *Gambierdiscus* spp.; *O. siam* = *Ostreopsis siamensis*; *O. lent* = *O. lenticularis*; *O. ovat* = *O. ovata*; *P. rha* = *Prorocentrum* cf. *rhathymum*; *P. conc* = *P. concavum*; *P. emar* = *P. emarginatum*; *A. kleb* = *Amphidinium* cf. *klebsii*; *A. cart* = *A.* cf. *carterae*; *Proro* spp. = total *Prorocentrum* spp.; *Ostreo* spp.= total *Ostreopsis* spp.; *Amph* spp.= total *Amphidinium* spp. ND = not detected. *p < 0.05

Sampling station	*Gamb* spp.	*O. siam*	*O. lent*	*O. ovat*	*P. rha*	*P. conc*	*P. lima*	*P. emar*	*A. kleb*	*A. cart*	*Proro* spp.	*Ostreo* spp.	*Amph* spp.	Total
JA51	0.56*	1.00*	1.00*	0.64*	0.52*	0.53*	0.56*	0.56*	0.61*	0.78*	0.52*	0.60*	0.61*	0.51*
JA100	0.54*	1.00*	1.00*	0.54*	0.53*	0.56*	0.56*	0.56*	0.55*	1.00*	0.52*	0.55*	0.53*	0.53*
JA101	0.54*	0.57*	ND	0.52*	0.52*	0.52*	0.55*	0.51*	0.67*	0.54*	0.51*	0.53*	0.58*	0.51*
JA94P	0.54*	0.55*	0.69*	0.52*	0.52*	0.54*	0.56*	0.51*	0.55*	0.61*	0.51*	0.10	0.59*	0.50*
JA86	0.61*	0.54*	ND	0.57*	0,53*	0.52*	0.58*	0.56*	0.53*	0.54*	0.52*	0.54*	0.53*	0.52*
WP203	0.55*	0.58*	ND	0.56*	0.59*	0.54*	0.52*	0.53*	0.53*	0.54*	0.53*	0.58*	0.55*	0.53*
WP204	0.58*	0.87*	0.72*	0.63*	0.56*	0.53*	0.58*	0.52*	0.57*	0.66*	0.54*	0.70*	0.54*	0.54*
WP201A	0.59*	0.52*	0.60*	0.59*	0.73*	0.59*	1.00*	0.73*	1.00*	ND	0.55*	0.53*	1.00*	0.53*
B11	0.53*	0.53*	0.66*	0.59*	0.58*	0.52*	0.58*	0.60*	0.54*	0.59*	0.53*	0.56*	0.52*	0.53*
S09	0.52*	0.51*	0.57*	0.53*	0.76*	0.61*	0.52*	0.53*	0.57*	0.62*	0.52*	0.52*	0.54*	0.51*
WP201B	0.63*	0.52*	0.59*	0.59*	1.00*	0.56*	0.70*	0.55*	1.00*	1.00*	0.57*	0.53*	1.00*	0.53*
WP202	0.53*	0.59*	0.71*	0.71*	0.56*	0.72*	0.57*	0.55*	0.67*	0.66*	0.52*	0.61*	0.55*	0.54*
JA95B	0.53*	0.66*	1.00*	0.66*	1.00*	0.54*	0.56*	0.55*	0.59*	0.59*	0.53*	0.59*	0.54*	0.52*
S09B	0.53*	0.60*	0.55*	0.62*	0.56*	ND	0.55*	0.59*	1.00*	1.00*	0.55*	0.57*	1.00*	0.56*

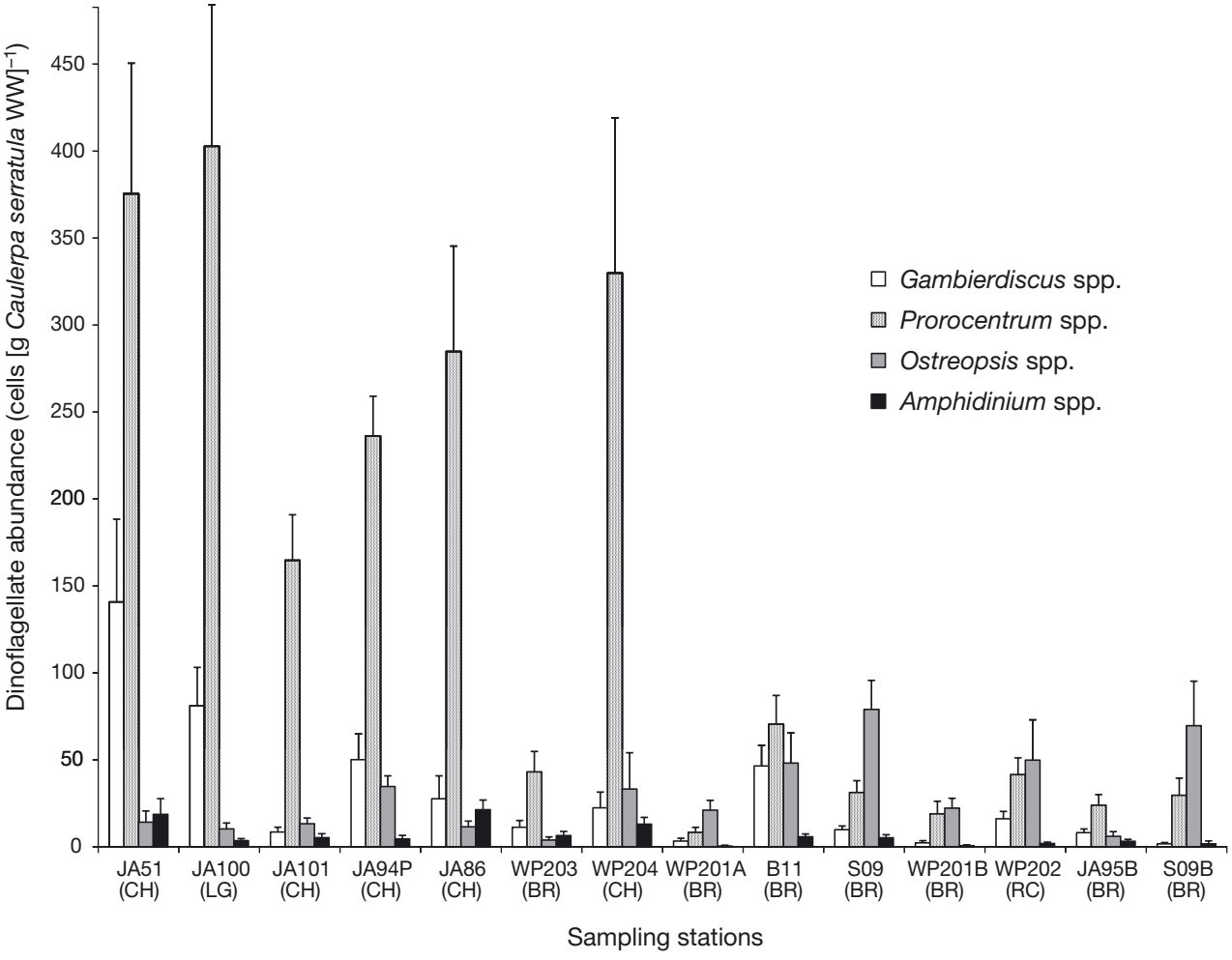

Fig. 3. Total abundance of each genus of benthic dinoflagellates at Johnston Atoll, Pacific Ocean. Habitat type of collection location displayed next to each sampling station (LG: lagoon, CH: channel, BR: back reef, RC: reef crest). Bars: mean ± 1 SE

located in relatively calm areas that experienced minimal current; 2 of them were also deeper (>10 m) than the other sampling sites (Table 1). Statistically significant differences in total dinoflagellate abundance were found among sampling stations (Table 3); pairwise comparisons were significant between sampling station JA51 (channel) and all other stations except JA86, JA 94P, WP204, which were also located in dredged channels (see Appendix 1).

In addition to changes in abundance, the biodiversity of each sampling station also changed dramatically among habitat types (Fig. 3). While *Prorocentrum* spp. comprised the highest proportion of dinoflagellates in the channel/lagoon sites, abundances were diminished in reef crest/back reef sites. Conversely, at several of these high energy reef crest/back reef sites, *Ostreopsis* spp. comprised the largest proportion of the dinoflagellate density.

Gambierdiscus spp. were found in 78% of the samples and were present at every sampling site, regardless of depth, water motion, or habitat (Fig. 3), but generally comprised a minor component of the phytoplankton assemblage. In general, however, the lagoon and channel sites supported higher populations than the back reef/reef crest sites. The highest mean abundance of *Gambierdiscus* spp. was observed at a sampling site located in a dredged channel (JA51); this site also supported the highest mean abundance of total dinoflagellates. Although Welch's ANOVA indicated significant differences among sampling sites, none of the pairwise comparisons were significant (see Appendix 1). The discrepancy between the results of these tests may relate to a more complex contrast in the data, such as differences between groups of means, or perhaps a linear trend in the data. A second explanation may relate to technical differences between the tests; the Games–Howell post hoc test is designed for unequal variances and unequal sample sizes, and consequently is more conservative. *Gambierdiscus* spp. persisted in a wide variety of habitats throughout the atoll, including the shallow reef crest sites exposed to the brunt of wave action. *Prorocentrum* spp. abundance was highest at sampling stations located in lagoon or channel habitats (Fig. 3), while populations at reef crest/back reef sites were substantially lower and never exceeded 100 cells g^{-1} macroalgae. *P.* cf. *rhathymum* consistently comprised the largest proportion of total *Prorocentrum* spp. abundance in the channel/lagoon sites; however, at the reef crest/back reef sites, *P. emarginatum* was generally most abundant (Fig. 4). Differences in total *Prorocentrum* spp. abundance were statistically significant between the reef crest/back reef sites and the lagoon/channel sites (Appendix 1).

Table 3. *Gambierdiscus* spp., *Ostreopsis* spp., *Prorocentrum* spp., and *Amphidinium* spp. Comparisons of species abundance at sampling stations based on Welch's ANOVA. NS = not significant ($p > 0.05$)

Dinoflagellate taxon	*F* ratio	p
Gambierdiscus spp.	5.74	<0.0001
O. siamensis	5.55	<0.0001
O. lenticularis	NS	
O. ovata	2.79	0.0042
P. cf. *rhathymum*	8.36	<0.0001
P. concavum	NS	
P. lima	4.95	<0.0001
P. emarginatum	5.79	<0.0001
A. cf. *klebsii*	2.72	0.0051
A. cf. *carterae*	NS	
Total *Prorocentrum* spp.	15.50	<0.0001
Total *Ostreopsis* spp.	4.78	<0.0001
Total *Amphidinium* spp.	3.75	0.0003
Total dinoflagellate abundance	15.52	<0.0001

The highest total abundance of *Ostreopsis* spp. was recorded at shallow (~3 m) stations located in back reef habitats, which experienced higher levels of water motion. *O. ovata* comprised the largest proportion of total *Ostreopsis* spp. abundance at all but one of the sampling sites, followed by *O. siamensis* and then *O. lenticularis*. Significant differences in abundance were found between sites S09 and WP203/95B; and 94P and WP203/95B (Appendix 1).

Amphidinium spp. were a minor component of the dinoflagellate community throughout the atoll. Sampling stations with the highest densities of *Amphidinium* spp. included sites located in dredged channels (Fig. 3). All of the stations located on or near the reef crest had cell densities of <100 cells g^{-1} macroalgae. Although Welch's ANOVA indicated significant differences among sampling sites, none of the pairwise comparisons were significant (Appendix 1).

Influence of water motion on dinoflagellate distribution and abundance

Total dinoflagellate abundance was significantly negatively correlated with water motion (Table 4); however, the responses to water motion differed among the genera surveyed. All of the *Prorocentrum* species surveyed were significantly negatively correlated with water motion ($p < 0.001$); the correlation was strongest for *P.* cf. *rhathymum* and *P. concavum.* The abundances of all *Prorocentrum* spp. were substantially reduced at sampling stations with a DF > 10. *Gambierdiscus* spp. were also negatively correlated with water motion ($p < 0.01$); moreover, the lagoon site supporting the highest abundance was also the site

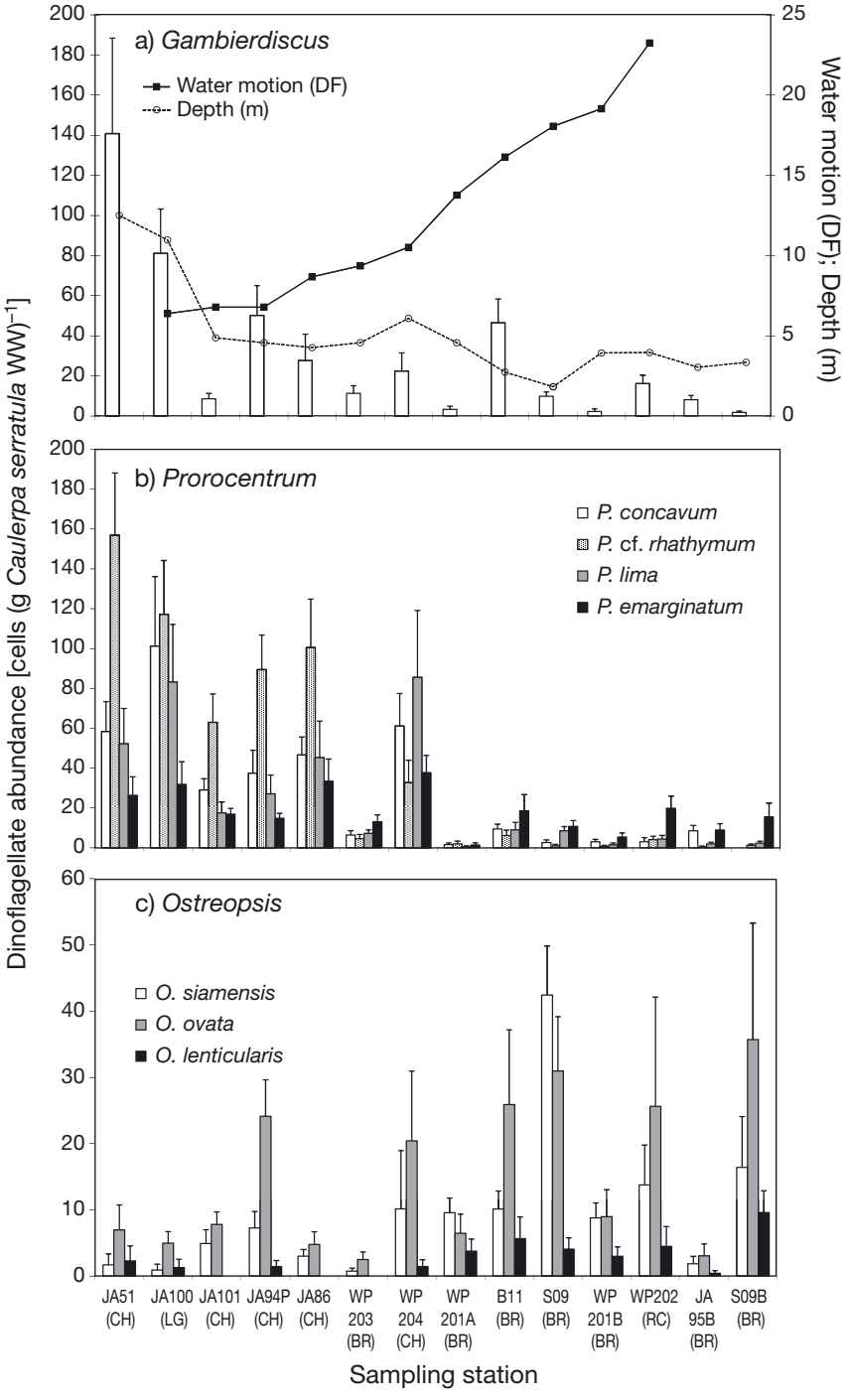

Fig. 4. Water motion, sample collection depth, and benthic dinoflagellate abundance at Johnston Atoll, Pacific Ocean. Abundance of genera (a) *Gambierdiscus*, (b) *Prorocentrum*, and (c) *Ostreopsis*; note differences in scale among left-hand *y*-axes. In (a), water motion (mean diffusion index factor [DF]) and depth (m) are shown on the right-hand *y*-axis. Habitat type of collection location displayed next to each sampling station (LG = lagoon, CH = channel, BR = back reef, RC = reef crest). Bars: mean ± 1 SE

with the lowest diffusion index value (Fig. 4). Both *Amphidinium* species were also negatively correlated with water motion, but this correlation was significant only for *A.* cf. *carterae* ($p < 0.05$). Conversely, both *Ostreopsis siamensis* and *O. lenticularis* were positively correlated with water motion ($p < 0.001$) and higher abundances of each were observed in back reef and reef crest habitats (Fig. 4).

Influence of depth on dinoflagellate distribution and abundance

Samples were collected from varying depths to examine the effect of depth on dinoflagellate abundance and distribution. Sampling depths at Johnston Atoll ranged from 2 m at a back reef site (S09) to 13 m in a channel site (JA51). Overall, total dinoflagellate abundance was significantly positively correlated with depth ($p < 0.001$; Table 4). The highest numbers of both *Gambierdiscus* spp. and *Prorocentrum* spp. were recorded at the deepest site (JA51) followed by the second deepest site (JA100), which were located in channel and lagoon habitats, respectively. The abundance of *Gambierdiscus* spp. and *Prorocentrum* spp. were significantly positively correlated with depth while *Ostreopsis* spp. were significantly negatively correlated (Table 4), likely due to the near absence of this genus in samples collected from the deeper channel and lagoon habitats.

Correlations between dinoflagellate species

Several statistically significant correlations were observed between species in this community (Table 5). The abundance of *Gambierdiscus* spp. and both *Amphidinium* spp. were positively correlated with each of the *Prorocentrum* species. Statistically significant negative correlations were observed between *P* cf. *rhathymum*/*P. concavum* and *Ostreopsis siamensis*/*O. lenticularis*. This is likely due to apparent habitat separation between *Ostreopsis* and *Prorocentrum*. The abundance of *G. toxicus* was negatively correlated with *O. siamensis* and *O. lenticularis;* however, this correlation was not statistically significant.

Table 4. *Gambierdiscus* spp., *Ostreopsis* spp., *Prorocentrum* spp., and *Amphidinium* spp. Spearman rank correlation coefficients (r_s) of dinoflagellate abundance vs. water motion and depth. Data for species, genera and total dinoflagellate abundance. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

Dinoflagellate taxon	Water motion	Depth
Gambierdiscus spp.	−0.310**	0.296**
O. siamensis	0.414***	−0.396***
O. lenticularis	0.305**	−0.257*
O. ovata	0.077	−0.176*
P. cf. *rhathymum*	−0.740***	0.668***
P. concavum	−0.675***	0.548***
P. lima	−0.535***	0.421***
P. emarginatum	−0.258**	0.236*
A. cf. *klebsii*	−0.155	0.056
A. cf. *carterae*	−0.190*	0.107
Total *Prorocentrum* spp.	−0.681***	0.576***
Total *Ostreopsis* spp.	0.286**	−0.287**
Total *Amphidinium* spp.	−0.206*	0.077
Total dinoflagellate abundance	−0.537***	0.461***

DISCUSSION

Influence of environmental parameters

This study examined the population dynamics of the ciguatera dinoflagellate community at several spatial levels, from patterns of dispersion encountered at a single site to the abundance and distribution throughout the coral reef ecosystem. In past ecological studies on ciguatera dinoflagellates, wide variations in cell densities within a site were reported (Yasumoto et al. 1979, 1980, Carlson 1984, Carlson et al. 1984, Ballantine et al. 1985, Taylor 1985). In the earliest environmental studies of ciguatera dinoflagellates, Yasumoto et al. (1980) observed 'micro-regionality' in which the population of *Gambierdiscus toxicus* was highly variable within a small area. Taylor (1985) noted considerable small scale variability in the distribution of *G. toxicus* in the Caribbean, even in beds of the same macroalgae. Carlson (1984) similarly observed spatial heterogeneity, and clumping in ciguatera dinoflagellates in the Virgin Islands suggested that the distribution of tropical benthic dinoflagellates should be described as 'contagious', which was confirmed here (Table 2). In general, the cell counts at all of our sampling sites were low compared to other studies, likely due to the number of sampling stations located in back reef/reef crest habitats, where cell density was reduced. A second reason may be potential differences in morphology (surface area) of our substrate compared to substrates examined in other studies, and/or possibly that *Caulerpa serrulata* is not a highly preferred host.

These surveys, however, demonstrated that habitat type, depth and water motion significantly affect both dinoflagellate abundance and community composition

Table 5. *Gambierdiscus* spp., *Ostreopsis* spp., *Prorocentrum* spp., and *Amphidinium* spp. Spearman rank correlation coefficients (r_s) between dinoflagellate species. *Gamb* spp. = total *Gambierdiscus* spp.; *O. siam* = *O. siamensis*; *O. lent* = *O. lenticularis*; *O. ovat* = *O. ovata*; *P. rha* = *P.* cf. *rhathymum*; *P. conc* = *P. concavum*; *P. emar* = *P. emarginatum*; *A. kleb* = *A.* cf. *klebsii*; *A. cart* = *A.* cf. *carterae*; *Proro* spp. = total *Prorocentrum* spp.; *Ostreo* spp. = total *Ostreopsis* spp.; *Amph* spp. = total *Amphidinium* spp. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

	Gamb spp.	*O. siam*	*O. lent*	*O. ovat*	*P. rha*	*P. conc*	*P. lima*	*P. emar*	*A. kleb*	*A. cart*	*Ostreo* spp.	*Proro* spp.	*Amph* spp.
Gamb spp.	x												
O. siam	−0.093	x											
O. lent	−0.078	0.419***	x										
O. ovat	0.145	0.437***	0.305***	x									
P. rha	0.494***	−0.208**	−0.191*	0.12	x								
P. conc	0.491***	−0.229**	−0.193*	0.036	0.698***	x							
P. lima	0.428***	−0.105	−0.144	0.205*	0.627***	0.609***	x						
P. emar	0.341***	−0.023	−0.008	0.254**	0.449***	0.435***	0.550***	x					
A. kleb	0.251**	−0.038	−0.059	0.109	0.254**	0.37***	0.370***	0.169*	x				
A. cart	0.042	−0.128	−0.122	0.028	0.228**	0.253**	0.380***	0.158**	0.258**	x			
Ostreo spp.	0.09	x	x	x	0.009	−0.122	0.094	0.17*	0.024	−0.076	x		
Proro spp.	0.576***	−0.199*	−0.18*	0.182*	x	x	x	x	0.378***	0.332***	0.042	x	
Amph spp.	0.223**	−0.065	−0.087	0.117	0.301**	0.422***	0.466***	0.332***	x	x	0	0.454***	x

among sampling stations. We found that the calm lagoon/channel sampling stations supported significantly higher total dinoflagellate abundance; conversely, dinoflagellate abundance was lowest at reef crest/back reef sites that were subject to wave activity (Fig. 3). These patterns agree with ecological studies of ciguatera dinoflagellates in the Caribbean that documented the highest abundance of this community in sheltered habitats (Carlson & Tindall 1985, Taylor 1985). We also observed distinct patterns of species distribution among these reef habitats. At the calm lagoon/channel sites, *Prorocentrum* was the dominant genus, with *P.* cf. *rhathymum* generally comprising nearly one-third or more of the total, followed by *P. concavum* and *Gambierdiscus* spp. or *P. lima*. Again, this pattern agrees with Caribbean studies in which protected inshore sampling stations were consistently dominated by *P. mexicanum*, *P. concavum* and *G. toxicus* (Carlson & Tindall 1985, Taylor 1985). The abundance of *Prorocentrum* species, particularly *P.* cf. *rhathymum*, was diminished in the reef crest/ back reef sites that experienced current and surge; all members of this genus were negatively correlated with water motion (Fig. 4, Table 4).

With respect to surveys of *Gambierdiscus toxicus* in the Pacific, maximum abundances were observed in high energy areas (Yasumoto et al. 1979, 1980); however, at Johnston Atoll *Gambierdiscus* spp. were negatively correlated with water motion (Fig. 4, Table 4). Although the calm lagoon and channel sites supported higher abundances of *Gambierdiscus*, low levels were documented across a variety of habitats. In this manner, *Gambierdiscus* spp. appear to have a 'weedy' quality that allows it to withstand a wide range of environmental conditions, with the exception of regions subject to significant land runoff (Taylor 1985, Grzebyk et al. 1994). *Gambierdiscus* spp. have been observed on many different types of substrate, including a variety of algal species and dead coral (Besada et al. 1982, Taylor 1985, Anderson & Lobel 1987, Kohler & Kohler 1992, Parsons & Preskitt 2007), although experimental studies have showed that algal exudates can either be stimulatory or inhibitory to *Gambierdiscus* growth (Carlson et al. 1984, Taylor 1985, Bomber et al. 1989, Grzebyk et al. 1994). Gillespie et al. (1985) described *Gambierdiscus* as 'opportunistic' with respect to substrate; in our surveys *Gambierdiscus* appeared to be opportunistic with respect to habitat as well, a quality that may help explain the inconsistencies in habitat preferences among past ecological studies.

In contrast with *Gambierdiscus* and *Prorocentrum, Ostreopsis* spp. was positively correlated with water motion (Table 4). *Ostreopsis* and *Amphidinium* spp. were consistently low in the lagoon/channel habitats, with each genus often comprising <10% of the total dinoflagellate count. Although *Ostreopsis* spp. abundances were low in all habitats surveyed, all 3 species were present in greater proportions at high energy reef crest/back reef sites (Fig. 4), similar to observations of the spatial variability of *Ostreopsis* spp. in the Mediterranean, where populations were more abundant in 'shaken' and 'slightly shaken' areas (Vila et al. 2001). These data also support observations by Carlson (1984) that documented highest abundances of *Ostreopsis* spp. in turbulent coral reef habitats.

It is not known why certain dinoflagellate taxa such as *Ostreopsis* are more resistant to the effects of wave action, whereas others appear to be highly susceptible. Benthic dinoflagellates in this community are known to produce mucus, which they use to tightly adhere to surfaces (Heil et al. 1993, Babinchak et al. 1994). Besada et al. (1982) reported that *Ostreopsis* and *Gambierdiscus toxicus* secrete enormous amounts of mucilage to which they are attached and/or enmeshed by means of a short thread. Microscopic observation of *G. toxicus* revealed that cells living on the macroalgal surface were covered by a mucous layer or aggregated within a mucilaginous matrix (Yasumoto et al. 1980, Fukuyo 1981, Besada et al. 1982, Ballantine et al. 1988). Nakahara et al. (1996) found that *G. toxicus* cells attached themselves to macroalgae thalli using a short thread; notably, disturbance appeared to provoke substrate attachment. Similarly, cells of *Ostreopsis* species have been observed in a mucilaginous matrix (Besada et al. 1982, Vila et al. 2001). The ability of *Ostreopsis* and *Gambierdiscus* spp. to tightly adhere to a substrate through the secretion of a mucilaginous matrix may help to explain why these species successfully inhabit inhospitable habitats in which they are not forced to compete with other benthic dinoflagellates for space. Mucus production has also been observed in *Prorocentrum* spp. (Loeblich et al. 1979, Carlson et al. 1984, Heil et al. 1993); hence, if *Prorocentrum* spp. are capable of producing mucilage in a similar manner to *Ostreopsis,* it is puzzling as to why these species appear to be highly susceptible to water motion. Nonetheless, the ability of *Ostreopsis* and *Gambierdiscus* spp. to produce a highly effective holdfast appears to allow them to persist in turbulent habitats at Johnston Atoll and may confer an advantage to these species in the continual competition for space in the benthic microbial community.

The results of our surveys show that water motion significantly reduces dinoflagellate abundance and are in agreement with the Caribbean studies that observed maximum dinoflagellate abundances in calm habitats (Carlson & Tindall 1985, Taylor 1985). The effect of water motion on the benthic dinoflagellate community relates to the concept of the carrying capacity of a macroalgal species, which is in turn

determined by the algal surface area and morphology (e.g. branched, tufted, coralline) (Lobel et al. 1988). Tindall & Morton (1998) suggested that the carrying capacity of a particular algal species is variable, dependent on the velocity of water flow as well as other physical and chemical factors. They described a model in which there is a progressive increase in the carrying capacity of macroalgae and consequently, dinoflagellate biomass, as flow velocity nears zero. In calm habitats the formation of a 3-dimensional benthic canopy may be possible, consisting of the macroalgal substrate, filamentous epiphytes, sediment, and the benthic microalgal community. Benthic dinoflagellates that inhabit this canopy occupy space on a particular substrate in several ways, and the manner in which they associate with the substrate likely changes with the intensity of water motion. Dinoflagellates have alternately been observed directly adhering to the macroalgal surface in a mucoid matrix (Yasumoto et al. 1980, Fukuyo 1981, Besada et al. 1982, Ballantine et al. 1988), inhabiting surface sediments and detritus (Kohler & Kohler 1992), suspended from the substrate using mucus threads (Besada et al. 1982, Nakahara et al. 1996), and some species also remain motile, swimming freely around the macroalgae thalli (Nakahara et al. 1996). In turbulent conditions, the complexity of the benthic canopy is greatly curtailed and available substrate would be restricted to space available on the macroalgae thallus for direct adherence by the dinoflagellates (Vila et al. 2001). In this manner, the means by which the dinoflagellates associate with the substrate, which is in turn influenced by intensity of water motion, substantially influences the carrying capacity of macroalgae in different habitats.

With respect to depth, these genera have been found in samples collected at depths of up to 30 m; although, the highest cell concentrations of ciguatera dinoflagellates have been documented at 0.5 to 3 m (Bomber et al. 1985, Carlson & Tindall 1985, Taylor 1985). Therefore, it is surprising that both *Prorocentrum* and *Gambierdiscus* spp. were positively correlated with depth (Table 4). Laboratory unialgal culture experiments determined that the average light intensity optimum for *G. toxicus* is ~10% sunlight and the most efficient growth was achieved under blue light (Bomber et al. 1988a). Under conditions in the field, Bomber et al. (1988a) characterizes the optimum depth of *Gambierdiscus* spp. in the range of 1 to 4 m but *Gambierdiscus* and *Prorocentrum* spp. have also been frequently documented at very shallow depths and under high light intensities that seemingly exceed their tolerances (Carlson & Tindall 1985), including floating algae in surface waters (Bomber et al. 1988b). In culture, these dinoflagellates were able to acclimate quickly to different light intensities (Bomber et al. 1988b) and may also be able to adapt to growth under lower light levels.

The deepest sampling stations in our surveys were generally located in lagoon or channel habitats. Conversely, many of the shallower sampling stations were in reef crest/back reef habitats. Given the strong effect of water motion on the dinoflagellate community observed in this study, it is likely that the correlation between dinoflagellate abundance and depth is influenced by water motion. A more effective means of examining the distribution of these dinoflagellates at depth could be accomplished by conducting a vertical transect in an area unaffected by water motion to specifically examine population abundance at varying depths. However, given the disconnection observed between the light tolerance limits identified in culture experiments and dinoflagellate distribution in the field, this community seems to be well-suited to exploiting a variety of habitats and tolerating environmental conditions seemingly unfavorable to growth.

Community dynamics

Several significant correlations were found among the species examined in this study: *Gambierdiscus* spp. were positively correlated with all 4 *Prorocentrum* species, abundances of *Ostreopsis siamensis* and *O. lenticularis* were positively correlated and each was negatively correlated with *P.* cf. *rhathymum* and *P. concavum* (Table 5). Several studies observed habitat separation between *G. toxicus* and *Ostreopsis* spp. (Besada et al. 1982, Bomber et al. 1985, Taylor 1985, Grzebyk et al. 1994, Tindall & Morton 1998); however, our data instead suggest habitat separation between *Ostreopsis* and *Prorocentrum* species.

In this study, all 4 *Prorocentrum* spp. were positively correlated; this correlation was strongest between *P.* cf. *rhathymum* and *P. concavum* (Table 5), which were consistently the dominant species at the calm lagoon/channel sites (Fig. 4). This is similar to studies documenting reciprocal codominance between *P. mexicanum*/ *P. concavum* and *Gambierdiscus toxicus* species at protected inshore stations, and dominance of turbulent coral reef habitats by *Ostreopsis siamensis* (Carlson 1984, Carlson & Tindall 1985). These observations confirm the characterization of the community dynamics of ciguatera dinoflagellates in the Caribbean and demonstrates that remarkably similar population dynamics also exist in the Pacific.

The means by which *Prorocentrum* spp. seem to dominate preferred protected habitats is unknown; however, laboratory experiments have shown that these species are capable of rapid growth compared to the other genera in this community, although growth

rates vary among genetic strains (Heil et al. 1993). This higher growth rate may allow *Prorocentrum* spp. to rapidly proliferate and occupy new substrate when it is made available, effectively excluding other species. Additionally, there is evidence that these species may also use allelopathic activity to compete for space in the benthic canopy (Carlson 1984). Although allelopathic activity has been identified in phytoplankton (Legrand et al. 2003), few studies have been conducted on benthic dinoflagellates. Exudates from *P. lima* have been found to inhibit the growth of *Amphidinium klebsii*, *Gambierdiscus toxicus*, *Ostreopsis lenticularis*, and *Caulerpa monotis* (Sugg & Van Dolah 1999). Additionally, laboratory culture experiments by Carlson (1984) found that *P. concavum* produced ectocrines that were allelopathic to *G. toxicus* but enhanced *P. mexicanum* growth. In the competition for newly created space, the higher growth rate of *Prorocentrum* species may also confer an advantage, allowing for rapid colonization. The production of ectocrines remains a largely unexplored but potentially important factor in the pattern of succession on substrates and in other interactions among these species.

Since the disturbance and destruction of coral reefs have precipitated numerous ciguatera outbreaks (Randall 1958, Ruff 1989, Lehane & Lewis 2000), the process by which colonization of this habitat occurs is important to understanding the subsequent manifestation of toxicity in reef fishes. Clearly there are numerous complex interactions that determine this community's composition and distribution. Studying this process of succession at the microbial scale under different environmental conditions and in different coral reef habitats may help explain observed patterns of ciguatera dinoflagellate ecology and provide insight into how and why toxicity occurs, how ciguatera persists in endemic areas, and why ciguatera is a highly localized phenomenon.

CONCLUSIONS

This study employed standardized sampling methodology to examine the ecology of toxic benthic dinoflagellates at Johnston Atoll and determine how habitat, depth, and water motion correlate with dinoflagellate biodiversity. These data show that total abundance of toxic dinoflagellates and community composition is significantly different among habitats, and that the ciguatera dinoflagellate community structure is primarily determined by the degree of water motion. The negative correlation observed between *Ostreopsis* and *Prorocentrum* spp. suggests habitat separation between these genera. *Gambierdiscus* spp. were widely distributed in all habitats but under normal conditions comprise a minor component of the benthic dinoflagellate assemblage. This study has established a quantitative baseline of Pacific ciguatera dinoflagellates in a variety of habitats and under normal environmental conditions, and contributes to an accurate and coherent characterization of the population dynamics of this important community.

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Appendix 1. *Ostreopsis* spp. and *Prorocentrum* spp. Pairwise comparisons of dinoflagellate abundance between the 14 sampling sites at Johnston Atoll, Pacific Ocean. Habitat type of collection location is displayed next to each sampling station: LG = lagoon, CH = channel, BR = back reef, RC = reef crest. Dinoflagellate taxa abbreviations indicate significant differences between sampling sites ($p < 0.05$, Games-Howell multiple pairwise comparisons). Osi = *O. siamensis*; Prh = *P.* cf. *rhathymum*; Pem = *P. emarginatum*; Pcn = *P. concavum*; TlPr = total *Prorocentrum* spp.; TlOp = total *Ostreopsis* spp.

Collection site	JA51 (LG)	JA100 (CH)	JA101 (CH)	JA94P (CH)	JA86 (BR)	WP203 (CH)	WP204 (BR)	WP201A (BR)	B11 (BR)	S09 (BR)	WP201B (RC)	WP202 (BR)	JA95B (BR)	S09B
JA51 (CH)	x													
JA100 (LG)		x												
JA101 (CH)	TlDn		x											
JA94P (CH)			TlPr	x										
JA86 (CH)					x									
WP203 (BR)	TlPr, TlDn, Prh	TlPr	TlPr	TlPr, TlOp, TlDn, Prh	Pcn	x								
WP204 (CH)					Pcn		x							
WP201A (BR)	TlPr, TlDn, Prh	TlPr	TlPr, TlDn, Prh, Pcn, Pem	TlDn, TlPr, Prh, Pem	TlPr, TlDn, Prh, Pcn			x						
B11 (BR)	TlDn, Prh			TlPr, Prh					x					
S09 (BR)	TlDn, TlPr, Osi, Prh	TlPr, TlOp, Osi	TlPr, Osi, Prh, Pcn	TlPr, TlDn, Osi, Prh	TlPr, Osi, Prh, Pcn	TlOp, Osi		TlDn, Osi	Osi	x				
WP201B (BR)	TlPr, TlDn, Prh	TlPr	TlPr, TlDn, Prh, Pcn	TlPr, TlDn, Prh	TlPr, TlDn, Prh, Pcn					TlDn, Osi	x			
WP202 (RC)	TlPr, TlDn, Prh	TlPr	TlPr, Pcn	TlPr, TlDn, Prh	Pcn							x		
JA95B (BR)	TlDn, TlPr, Prh	TlPr	TlPr, TlDn, Prh	TlPr, TlDn, TlOp, Prh	TlPr, TlDn, Prh, Pcn					TlDn, TlOp, Osi			x	
S09B (BR)	TlDn, TlPr, Prh	TlPr	TlPr, Prh, Pcn	TlPr, TlDn, Prh	TlPr, Pmx, Pcn									x

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