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# *Gambierdiscus* species exhibit different epiphytic behaviors toward a variety of macroalgal hosts

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ABSTRACT

Ciguatera fish poisoning is a common form of seafood poisoning caused by toxins (ciguatoxins) that accumulate in demersal (reef) food webs. The precursors of ciguatoxins are produced by dinoflagellates of the genus *Gambierdiscus*, and enter the food web via herbivory and detritivory. The *Gambierdiscus* genus was recently revised and new research on the physiology and ecology of the revised species is needed. While it has been demonstrated that *Gambierdiscus* spp. are predominately epiphytic, the variability in epiphytic behavior among the various *Gambierdiscus* species is not known. Five *Gambierdiscus* species isolated from the Greater Caribbean Region were the focus of this study (*G. belizeanus*, *G. caribaeus*, *G. carolinianus*, *G. carpenteri*, and *G. yasumotoi*). Cells of *Gambierdiscus* were grown in wells with algae fragments from eight different macroalgal host genera (*Acanthophora*, *Caulerpa*, *Dasya*, *Derbesia*, *Dictyota*, *Laurencia*, *Polysiphonia*, and *Ulva*) where the epiphytic behavior and growth of the different *Gambierdiscus* species were monitored over 29 days. The results of this experiment demonstrate that epiphytic behavior (growth and attachment) differs among the *Gambierdiscus* species toward the various macroalgal hosts. Results tended to be specific to *Gambierdiscus* – host pairings with few commonalities in the way a particular *Gambierdiscus* species interacted across hosts or how the various *Gambierdiscus* species responded to a particular host. The *Gambierdiscus* – host pairings that resulted in the highest growth and attachment combinations were examined in terms of known cellular toxicity and host palatability to determine which pairings could represent the most likely vectors for the transfer of ciguatoxins (or precursors) into the demersal food web. Two pairings, *Gambierdiscus belizeanus* – *Polysiphonia* and *G. belizeanus* – *Dictyota*, best met these criteria, providing a hypothetical approach to better focus sampling and monitoring efforts on such potential vectors in the benthic environment.



## 1. Introduction

Ciguatera fish poisoning (CFP) is the most common phycotoxin-related seafood poisoning worldwide (Ragelis, 1984; Fleming et al., 1998). CFP affects people who have consumed fishes containing ciguatoxins (CTX), the precursors of which (gambiertoxins) are produced by some dinoflagellate species of the genus, *Gambierdiscus*. Epiphytic in nature, *Gambierdiscus* cells are consumed by herbivorous fish and invertebrates grazing upon the macroalgae that host them, thereby transferring gambiertoxins into the food web. Once introduced into the food web, the gambiertoxins are biotransformed into ciguatoxins, which then bioaccumulate and biomagnify into higher trophic levels via predation by larger fish.

For years, researchers have reported that CFP flare-ups oftentimes follow disturbances to coral reefs, such as hurricanes, dredging, and shipwrecks (Cooper, 1964; Bagnis, 1994; de Sylva, 1994). The general consensus on this supposition is that coral degradation results in dead coral surfaces that can be colonized by macroalgae, therefore providing more substrate for *Gambierdiscus* populations. As coral reef degradation has been increasingly documented on reefs worldwide (Bruno et al., 2009), there is concern that CFP outbreaks may become more common as a result.

Although much work has focused on the ecological and environmental factors that affect *Gambierdiscus* populations (reviewed in Parsons et al., 2012), very little work has considered the role that the macroalgal hosts play. The epiphytic relationship between *Gambierdiscus* cells and their host macroalgae may be advantageous for the dinoflagellates in various ways. For example, Villareal and Morton (2002) demonstrated that *Gambierdiscus* may utilize the three-dimensional structure of an algal host to minimize

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light exposure, allowing them to thrive in shallow, well-lit tropical locations, despite their intolerance to high light levels. It has been demonstrated that cells actively swim during daylight hours and rapidly attach to hosts upon darkness (Nakahara et al., 1996). Nakahara et al. (1996) also suggest that cells normally swim around near the macroalgae thalli, but quickly attach to the surface of the algae when a sudden disturbance or strong water motion occurs, so as not to be dispersed. Another possible advantage of epiphytism is the nutritional value that algal hosts may offer to *Gambierdiscus* cells (Grzebyk et al., 1994), especially those living in an otherwise oligotrophic environment, such as a coral reef.

Some researchers have observed *Gambierdiscus* form a mucilaginous matrix over the thallus of the host macroalgae and aggregate within it (Yasumoto et al., 1980; Ballantine et al., 1988). Other studies have reported that the cells attach to their host by a mucus thread, tethering themselves to the algae, sometimes with a rotating motion (Besada et al., 1982; Nakahara et al., 1996; personal obs.). Additionally, Bomber et al. (1988) demonstrated that *Gambierdiscus* may utilize drift algae as a means for attachment and transport, a likely dispersal mechanism that has resulted in their circumtropical distribution.

Early experiments investigating *Gambierdiscus* host preferences were conducted by Saint Martin et al. (1988), who determined that *Gambierdiscus* cells preferred to affix themselves on algae rather than inorganic substrates, although cells did colonize dead parts of the experimental macroalgae. Their results also suggested that the preference of *Gambierdiscus* for macroalgae is independent of macroalgal phylum. The authors concluded that the mechanism behind the attraction to the macroalgae is unknown, but suggested that it may be associated with the production or diffusion of one or more substances produced by the macroalgae which may be necessary for the *Gambierdiscus* growth. Parsons et al. (2011) examined how the epiphytic relationship between *Gambierdiscus toxicus* (BIG 12) varied among twenty-four different macroalgal species from Hawaii. Their results indicated that *G. toxicus* will attach to some prospective host species, while completely avoiding others, suggesting that *Gambierdiscus* may not be obligate epiphytes. In addition, some host species allowed for proliferation of *G. toxicus* cells, while others appeared to inhibit growth.

Cells of *Gambierdiscus* have been found on various types of substrates, although the highest abundances have been reported for highly foliose rhodophytes and phaeophytes (Gillespie et al., 1985; Bomber and Aikman, 1989; Cruz-Rivera and Villareal, 2006). The review by Parsons et al. (2012), however, presented numerous examples of conflicting substrate preferences of *Gambierdiscus*. For example, while the genus *Halimeda* has been reported to commonly host *Gambierdiscus* from some locations (e.g., Florida Keys, Bomber et al., 1988; French Polynesia, Chinain et al., 2010; Cook Islands, Rhodes et al., 2010), others reported finding no cells on *Halimeda* from other locations (e.g., the Great Barrier Reef, Heil et al., 1998). In Cuban coastal waters, *Acanthophora* was found to host no epiphytic dinoflagellates (Delgado et al., 2006), whereas it was reported to host high abundances elsewhere (e.g., British Virgin Islands, Carlson, 1984; Belize, Morton and Faust, 1997). Differences were also reported for preferences of *Dictyota* as a host, supporting both dense populations of *Gambierdiscus* (e.g., Cuba, Delgado et al., 2006; Caribbean, Ballantine et al., 1988; Carlson and Tindall, 1985), versus no populations at all (e.g., Tahiti, Nakahara et al., 1996). Nakahara et al. (1996) noted that although *Gambierdiscus* cells were detected on a variety of coral reef macroalgae species, their preference for associating with a particular species varied across different geological areas.

Parsons et al. (2012) suggested that much of the contradiction in host preferences of *Gambierdiscus* can be attributed to the fact that the earlier studies assumed all encountered *Gambierdiscus* cells were *Gambierdiscus toxicus*, before many other species were yet to be discovered and described. With a total of 13 species within the genera now described (see Litaker et al., 2009; Fraga et al., 2011; Fraga and Rodriguez, 2014; Nishimura et al., 2014), the physiological and ecological differences among these species (including epiphytic preferences and behavior) remains unknown. The purpose of this study, therefore, was to ascertain whether differences exist in host preferences and epiphytic behavior among *Gambierdiscus* species; important factors when considering pathways of CTX flux in the food web that play a role in ciguatera flare-ups. Specifically, the objectives of this study were twofold: (1) to determine if growth rates vary among *Gambierdiscus* species in the presence of different potential macroalgal hosts and (2) to determine if *Gambierdiscus* species have different attachment attributes (i.e., vs. non-attachment) in the presence of different potential macroalgal hosts.

## 2. Methods

### 2.1. Gambierdiscus *culturing*

Five species of *Gambierdiscus* known to be present in the Greater Caribbean Region were tested. Three of the species, *G. carolinianus, G. carpenteri*, and *G. yasumotoi*, were isolated from the Florida Keys and genotyped at Woods Hole Oceanographic Institution (WHOI; D.M. Anderson and M.L. Richlen). The other two species used, *G. caribaeus* and *G. belizeanus*, were provided by WHOI (M.L. Richlen), who isolated them from St. Thomas, USVI. All cultures were maintained in 0.7 μm-filtered Florida Keys seawater (salinity of 35) with modified Keller's medium (without TRIS, Cu, or Si; with $GeO_2$ to inhibit diatom growth), at 23–24 °C on a 12/12 light/dark cycle at ~75 μmol photons $m^2$ $s^{-1}$.

### 2.2. Algae collection

In the months prior to the start of experimentation, large fragments (~20 g wet weight) of macroalgae from single or neighboring thalli were collected by scuba diving and snorkeling near Long Key in the Florida Keys (Fig. 1). Macroalgae species collected were chosen based on either their common presence in the Florida Keys, particularly those growing in habitats known to harbor *Gambierdiscus* populations, and/or by their previously known associations with *Gambierdiscus* populations in past studies. Once the algae were transported back to the lab, they were shaken vigorously to remove epiphytes, and placed under the same growth conditions as the *Gambierdiscus* cultures (as described above) with air flow and weekly water changes. Algae were identified to species level, to the best of our ability using taxonomic keys and descriptions by Dawes and Mathieson (2008)

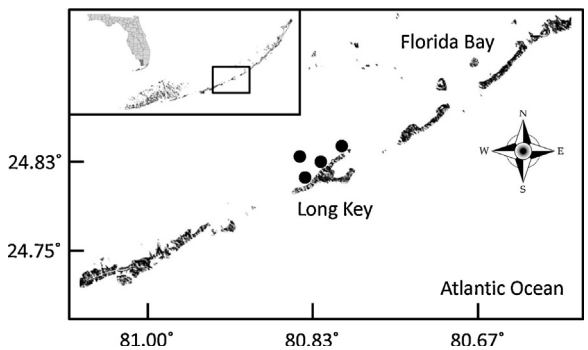


**Fig. 1.** A map of the Florida Keys emphasizing the location of Long Key. The sites where macroalgae were collected for the experiment are indicated by the black circles.

and Littler and Littler (2000). Species were tentatively identified as *Laurencia intricata*, *Dasya crouaniana*, *Polysiphonia ramantacea*, *Ulva fasciata*, *Dictyota cervicornis*, *Acanthophora spicifera*, *Derbesia marina*, and *Caulerpa mexicana*. The eight species chosen represent all three major phyla of macroalgae and include various morphological characteristics (Table 1).

Two weeks prior to the start of the experiment, a portion of each of the algae (approximately 200 mg wet weight) growing in the conditions described above was transferred into 250 ml beakers containing 100 ml of ambient filtered seawater from the Florida Keys (i.e., without Keller's medium). The purpose of this procedure was to ensure the macroalgae were growing prior to the experiment (not stressed or dying which could influence the results) using the enriched media initially, followed by the two week ambient water incubation (and daily water changes) to reduce/remove the influence that the prior enrichment would have on the experiment.

### 2.3. Experimental design and setup

The basic experimental design was to place *Gambierdiscus* cells in close living association with the various host macroalgae species in order to monitor growth and behavior in response to the hosts. This experiment included five treatments of *Gambierdiscus* species, eight treatments of macroalgae host species, and a control (*Gambierdiscus* cells living with no host algae).

Five days prior to Day 0 (start of experiment), macroalgae fragments of ~25 mg were placed individually into separate wells of a Corning six well polystyrene culture plate (#3516) containing 8 ml of ambient Florida Keys seawater for each treatment to be used in the experiment in triplicate (8 host algae + 1 control * 5 *Gambierdiscus* species * triplicates = 135 wells). The fragments were acclimated in the ambient seawater under the experimental conditions for five days, with frequent (almost daily) water changes, to allow for any harmful exudates resulting from the cutting of algal thalli (to make the 25 mg fragments) to dissipate and be removed before the *Gambierdiscus* cells were added.

On Day 0, new wells of 8 ml ambient Florida Keys seawater were prepared for all treatments. Twenty-five cells of the appropriate species of *Gambierdiscus* were added to each well (individual cells were transferred via micropipette from cultures that were in stationary phase), and then the appropriate acclimated algae fragments were added. Triplicate controls for each *Gambierdiscus* species (containing no algae) were also prepared under the same conditions. Cells were counted on Days 1, 8, 15, 22, and 28, the frequency of which was intended to capture both the growth and interaction dynamics over a full growth cycle of *Gambierdiscus*. Preliminary experiments, conducted in which cells were counted daily for the first five days, followed by counts every three days thereafter, demonstrated that exponential growth always occurred between Days 8 and 22, and that weekly counts would provide sufficient data for analysis. Cell counts were conducted using an Olympus SZX-71 stereoscope followed by examination using an Olympus IX-71 inverted microscope to count cells underneath the algal fragment. All counts used 100× magnification. Each cell was categorized as dead, alive and unattached to host, or alive and attached to (or in contact with) the host. Water changes were performed 2–3 times weekly by slowly removing 4 ml of the water by transfer pipet (with a loss of <1% of cells when done carefully), and replaced with 4 ml filtered ambient seawater. Water changes were done on different days than cells counts to ensure that the changes did not disturb the cells. At the end of the experiment, after all counts were completed, macroalgae fragments were reweighed and recorded (g wet weight).

### 2.4. Data analysis

Prior to statistical analysis, the cell counts from the five *Gambierdiscus* species were converted to relative abundance values (% of total cells dead, % attached and alive, % unattached and alive). Cell count data gathered over the course of the experiment were used to calculate average growth rates and the average proportion of attached (versus total) cells. Averages were computed from three intervals of the growth cycle: Day 0–8; Day 8–15; and Day 15–22. These three intervals were used to account for cell mortality (cells in some treatments died within the first interval) and to provide an overall value for growth and attachment at the initiation and sustenance of exponential growth, thereby removing potential bias of different growth responses in the intervals among species.

In order to determine how the different *Gambierdiscus* species interacted with the different host algal species, the average growth rates and the average proportion of attached cells were statistically analyzed using SPSS version 22. As much of the data was not normal and could not be transformed to meet normality, we utilized a Kruskal–Wallis test with pairwise comparisons (post hoc tests) for the analysis. A Spearman rank correlation analysis was conducted on the overall average growth rate versus average attachment (for all *Gambierdiscus* – host combinations) to test if *Gambierdiscus* growth was related to (and possibly a function of) attachment.

Results of the *Gambierdiscus* – host algae post hoc (pairwise comparison) analyses were also ranked separately according to their growth and attachment comparisons with the other pairings (i.e., highest statistically-valued pairing would be ranked with a one; pairs that were not statistically different would be given the same rank). The growth and attached ranks were then averaged for each pairing to provide scores to determine which *Gambierdiscus* – host pairings had the highest growth – attachment combinations. Host palatability was determined using references cited in Table 1, as well as data presented in Randall (1967). In the latter case, 48 reef fish species examined in Randall (1967) contained identifiable algal biomass, and those species containing any of

**Table 1**
General description of the host macroalgae used in this study including genus, phyla, palatability, and functional group descriptions.

| Algal host genus | Phyla | Palatability | Functional-form Group |
|---|---|---|---|
| *Dictyota* | Phaeophyta | Chemically defended, consumed by some herbivores[2,7] | Sheet[1] |
| *Acanthophora* | Rhodophyta | Palatable[2] | Coarsely branched[1] |
| *Laurencia* | Rhodophyta | Chemically defended, but can be highly palatable[2] | Coarsely branched[1] |
| *Dasya* | Rhodophyta | Chemically defended[5], low palatability[6] | Coarsely branched |
| *Polysiphonia* | Rhodophyta | Highly palatable, especially to some damselfish[4] | Filamentous |
| *Ulva* | Chlorophyta | Chemically defended, still palatable[5] | Sheet[1] |
| *Derbesia* | Chlorophyta | | Filamentous |
| *Caulerpa* | Chlorophyta | Chemically defended[3] | Coarsely branched[1] |

1 – Littler et al. (1983); 2 – Cruz-Rivera and Villareal (2006); 3 – Norris and Fenical (1982); 4 – Hata et al. (2010); 5 – De Lara-Isassi et al. (2000); 6 – Gilbert (2005); 7 – Bolser and Hay (1996).

the eight host algae used in this experiment were tallied, and these values were divided by 48 to provide a general frequency of consumption for each host algae (i.e., "x" species out of 48 consumed *Acanthophora*).

Host algae end wet weights were compared to beginning wet weights in order to determine % biomass either gained or lost throughout the duration of the experiment to determine if this measure of host health affected the *Gambierdiscus* data. To this end, Pearson correlation analyses were used to determine if any correlations existed among changes in biomass weights and growth rates, end cells, or attachment rates (data were normal).

## 3. Results

### 3.1. Do macroalgal hosts affect the growth of the different Gambierdiscus species?

All five *Gambierdiscus* species showed significantly higher growth rates in some algae treatments versus others, although results were not consistent across host treatments (Fig. 2; Table 2). Three species (*G. caribaeus*, *G. carpenteri*, and *G. yasumotoi*) exhibited higher growth rates in at least one of the host treatments versus the controls, suggesting a stimulatory effect of some of the hosts for these species. In all other cases, host treatments were either no different from the control, or resulted in a lower growth rate, which would suggest suppression of growth in *Gambierdiscus*. There were no statistical differences among the hosts in terms of overall *Gambierdiscus* growth rates (i.e., averaged across all *Gambierdiscus* species; $p = 0.112$), demonstrating the variability in host–*Gambierdiscus* interactions discussed in more detail below (i.e., an individual host treatment did not have the same effect on growth rates across the various *Gambierdiscus* species).

Individually, the *Gambierdiscus* species grew better on some hosts versus others and results varied from species to species (Fig. 2; Table 2). For example, *G. belizeanus* growth rates in the *Caulerpa* treatment were among the higher values for this species, whereas growth rates were in the lower range in *Caulerpa* treatments for *G. carpenteri* and *G. yasumotoi*. Three *Gambierdiscus* species exhibited negative growth and cell mortalities in some host treatments: *G. carolinianus* – *Laurencia*; *G. carpenteri* – *Ulva*; and *G. yasumotoi* – *Derbesia*, *Dictyota*, and *Laurencia*, but not others (Fig. 2; Table 2). Only one host (*Laurencia*) treatment resulted in cell mortality for more than one *Gambierdiscus* species, and all other cases were unique. Additionally, all three replicates in these treatments exhibited mortality, so results were consistent. The *G. carpenteri* – *Ulva* treatment was particularly notable, with very quick mortalities in all three replicates resulting in an average negative growth rate of −0.425.

The various *Gambierdiscus* species grew at different rates on the hosts in all treatments (Fig. 3; Table 2). The slowest (or non-

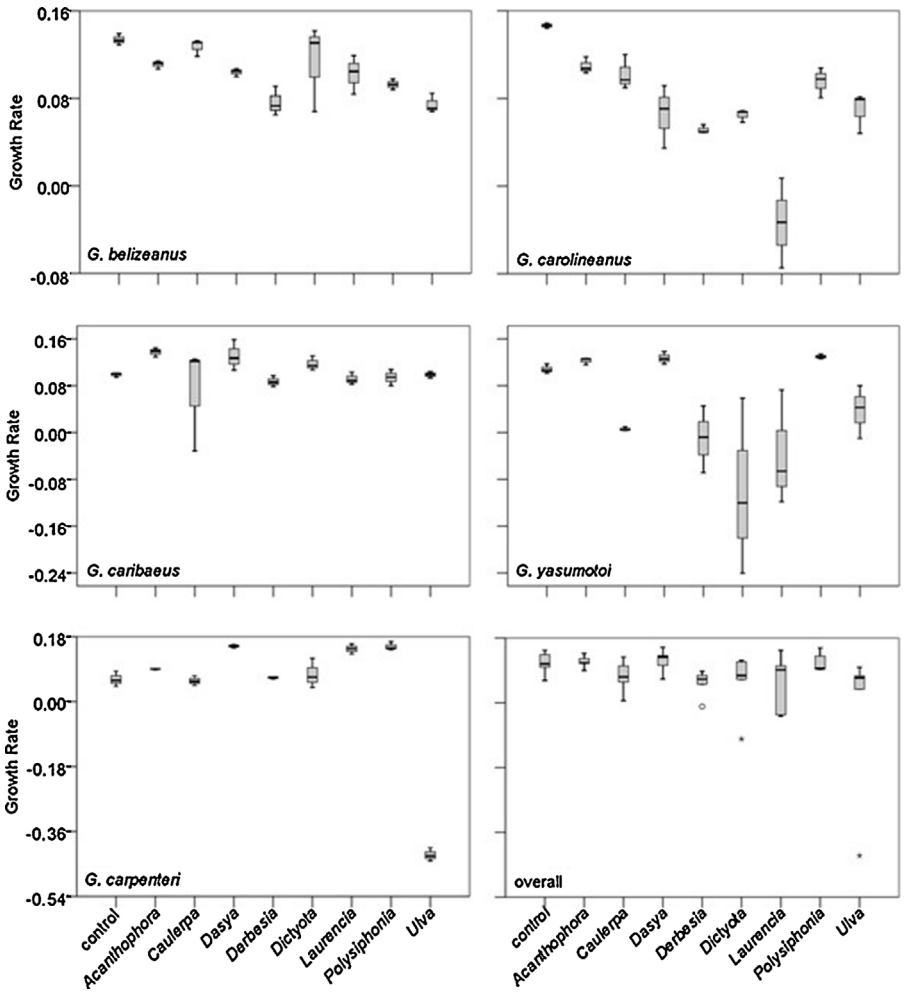


**Fig. 2.** Box plots of growth rate ($\Delta$ cells day$^{-1}$) for each *Gambierdiscus* species across control and host treatments displaying minimum and maximum values (upper and lower whiskers), first and third quartiles (top and bottom of boxes), and the median values. Points that are outliers (>1.5 times height of boxes) are depicted by circles. Extreme outliers (>3 times height of boxes) are depicted by asterisks.

**Table 2**
Growth rates of the various *Gambierdiscus* species for each of the host treatments. Values shown are averages ± standard deviation. The letters under each value indicate Kruskal–Wallis pairwise comparison groupings within each column, those in parentheses for each row. *p*-values for comparisons made in each row are provided under the host treatments. *p*-values for comparisons made within each column are provided under the *Gambierdiscus* species.

| | *G. belizeanus* ($p = 0.015$) | *G. caribaeus* ($p = 0.015$) | *G. carolinianus* ($p = 0.015$) | *G. carpenteri* ($p = 0.002$) | *G. yasumotoi* ($p = 0.002$) |
|---|---|---|---|---|---|
| Control | 0.134 ± 0.005 | 0.099 ± 0.004 | 0.146 ± 0.002 | 0.062 ± 0.021 | 0.108 ± 0.008 |
| ($p = 0.015$) | A(B) | C(D) | A(A) | C(E) | B(C) |
| *Acanthophora* | 0.111 ± 0.004 | 0.138 ± 0.008 | 0.109 ± 0.008 | 0.090 ± 0.002 | 0.123 ± 0.006 |
| ($p = 0.015$) | C(C) | A(A) | B(BC) | B(D) | AB(B) |
| *Caulerpa* | 0.127 ± 0.008 | 0.072 ± 0.089 | 0.102 ± 0.016 | 0.058 ± 0.013 | 0.006 ± 0.003 |
| ($p = 0.047$) | AB(A) | BC(ABC) | BC(A) | C(B) | C(C) |
| *Dasya* | 0.104 ± 0.004 | 0.131 ± 0.026 | 0.066 ± 0.029 | 0.154 ± 0.004 | 0.127 ± 0.011 |
| ($p = 0.047$) | C(C) | AB(ABC) | CDEF(D) | A(A) | A(B) |
| *Derbesia* | 0.076 ± 0.013 | 0.087 ± 0.010 | 0.052 ±0.004 | 0.066 ± 0.003 | −0.010 ± 0.057 |
| ($p = 0.047$) | DE(AB) | C(A) | F(C) | C(B) | C(D) |
| *Dictyota* | 0.114 ± 0.040 | 0.118 ± 0.012 | 0.065 ± 0.006 | 0.076 ± 0.041 | −0.101 ± 0.151 |
| ($p = 0.138$) | ABCD(A) | AB(A) | E(A) | BC(A) | C(A) |
| *Laurencia* | 0.103 ± 0.018 | 0.092 ± 0.010 | −0.034 ± 0.041 | 0.146 ± 0.014 | −0.037 ± 0.099 |
| ($p = 0.047$) | BCDE(B) | C(B) | G(C) | A(A) | C(C) |
| *Polysiphonia* | 0.093 ± 0.005 | 0.094 ± 0.014 | 0.095 ± 0.014 | 0.153 ± 0.012 | 0.130 ± 0.004 |
| ($p = 0.015$) | D(C) | BC(C) | BCD(C) | A(A) | A(B) |
| *Ulva* | 0.075 ± 0.009 | 0.099 ± 0.005 | 0.069 ± 0.019 | −0.425 ± 0.019 | 0.038 ± 0.045 |
| ($p = 0.138$) | E(A) | C(A) | DEF(A) | D(A) | C(A) |

)growing species in the treatments containing *Caulerpa*, *Derbesia*, and *Laurencia* was *Gambierdiscus yasumotoi*. Additionally, *Gambierdiscus carolinianus* also had the lowest growth rate in the *Dasya* treatment, whereas *Gambierdiscus carpenteri* was lowest in the *Acanthophora* treatment. As noted earlier, *G. carpenteri* exhibited rapid mortality (and a large negative growth rate) in the *Ulva* treatment, and also exhibited the lowest growth rates in the control, as opposed to *G. carolinianus* had the highest growth rates in the control. As was similarly presented in Fig. 2, there were no overall differences in growth among the *Gambierdiscus* species averaged across the host treatments, likely due to the high variability exhibited in the data (particularly for *G. carpenteri* and *G. yasumotoi*; Fig. 3). Overall, the growth rate results suggest that the individual *Gambierdiscus* species respond to, and interact differently with, different host macroalgae, and that there are no consistent results across hosts or species.

### 3.2. *Do* Gambierdiscus *species have different epiphytic behaviors (attachment vs. non-attachment) in the presence of different macroalgal hosts?*

The *Gambierdiscus* species did exhibit different attachment behaviors among the host treatments (Fig. 4; Table 3). Attachment was highest for *Gambierdiscus belizeanus* in the *Dasya* and *Polysiphonia* treatments, and was lowest in the *Acanthophora*, *Caulerpa*, and *Laurencia* treatments. For *Gambierdiscus caribaeus*, attachment was lowest on *Acanthophora* and *Caulerpa*, and highest in the *Dasya*, *Derbesia* and *Polysiphonia* treatments. Similar results were observed for *Gambierdiscus carolinianus*. *Derbesia* also harbored the highest proportion of cells for *G. carpenteri*, while *Caulerpa* and *Ulva* harbored no *Gambierdiscus carpenteri* cells. Due to high variability in the results, there were no differences in the proportion of attached *Gambierdiscus yasumotoi* cells among the hosts. On average, *Derbesia* and *Polysiphonia* harbored the highest proportion of *Gambierdiscus* cells ($p = 0.008$), whereas *Acanthophora*, *Caulerpa*, *Dictyota*, and *Laurencia* harbored the fewest.

From the host perspective, *Acanthophora* harbored a lower proportion of *Gambierdiscus caribaeus* and *Gambierdiscus yasumotoi* cells than the other *Gambierdiscus* species (Fig. 5; Table 3). Two species, *Gambierdiscus belizeanus* and *Gambierdiscus carolinianus*, attached more onto *Caulerpa* than the other *Gambierdiscus*. Low proportions of *G. yasumotoi* cells attached to the *Dasya*, *Derbesia* (along with *G. belizeanus* and *G. carolinianus*), and *Polysiphonia* treatments versus the other *Gambierdiscus* species. The high mortality of *Gambierdiscus carpenteri* in the *Ulva* treatment is again exhibited by the lack of attached cells. Overall, no *Gambierdiscus* species had a significantly higher proportion of attached cells versus the other species when averaged across the host treatments ($p = 0.199$), due in part to the high variability in the data caused by different interactions across the hosts (e.g., Fig. 4). As was the case for growth, the results tend to be specific for each host – *Gambierdiscus* treatment with few generalizations that can be made across hosts or *Gambierdiscus* species. Lastly, there was no significant correlation ($p = 0.870$) between attachment and growth rate across all of the *Gambierdiscus* species (i.e., growth was not related to attachment).

The top ten *Gambierdiscus* – host pairings in terms of highest growth and attachment combinations are presented in Table 4. Three species, *Gambierdiscus belizeanus*, *Gambierdiscus caribaeus*, and *Gambierdiscus carpenteri*, each tallied three of the top ten rankings, while *Polysiphonia* and *Dasya* were the most common hosts in the top ten rankings (three each). The review of Randall (1967) indicated that *Polysiphonia* was present in the most fish stomach contents (21 out of 48 species examined; 44%), followed by *Dictyota* (40%), and *Laurencia* (31%). The *G. belizeanus* – *Polysiphonia* and *G. belizeanus* – *Dictyota* pairings were considered to be the likely vectors for the trophic transfer of ciguatoxins (or precursors) into the food web based on the high growth-attachment rankings coupled with known cellular toxicity and host palatability (see Section 4).

### 3.3. *Changes in wet weight of host algae*

Four host algae gained biomass over the course of the experiment (*Dictyota*, *Laurencia*, *Polysiphonia*, and *Ulva*), and four algae lost biomass (*Acanthophora*, *Caulerpa*, *Dasya*, and *Derbesia*; Table 5). The change in weight (%) was not correlated to either *Gambierdiscus* growth rates ($p = 0.250$) or proportion of cells attached ($p = 0.661$).

## 4. Discussion

The results of this experiment demonstrate that not only does the epiphytic behavior of *Gambierdiscus* vary among multiple host

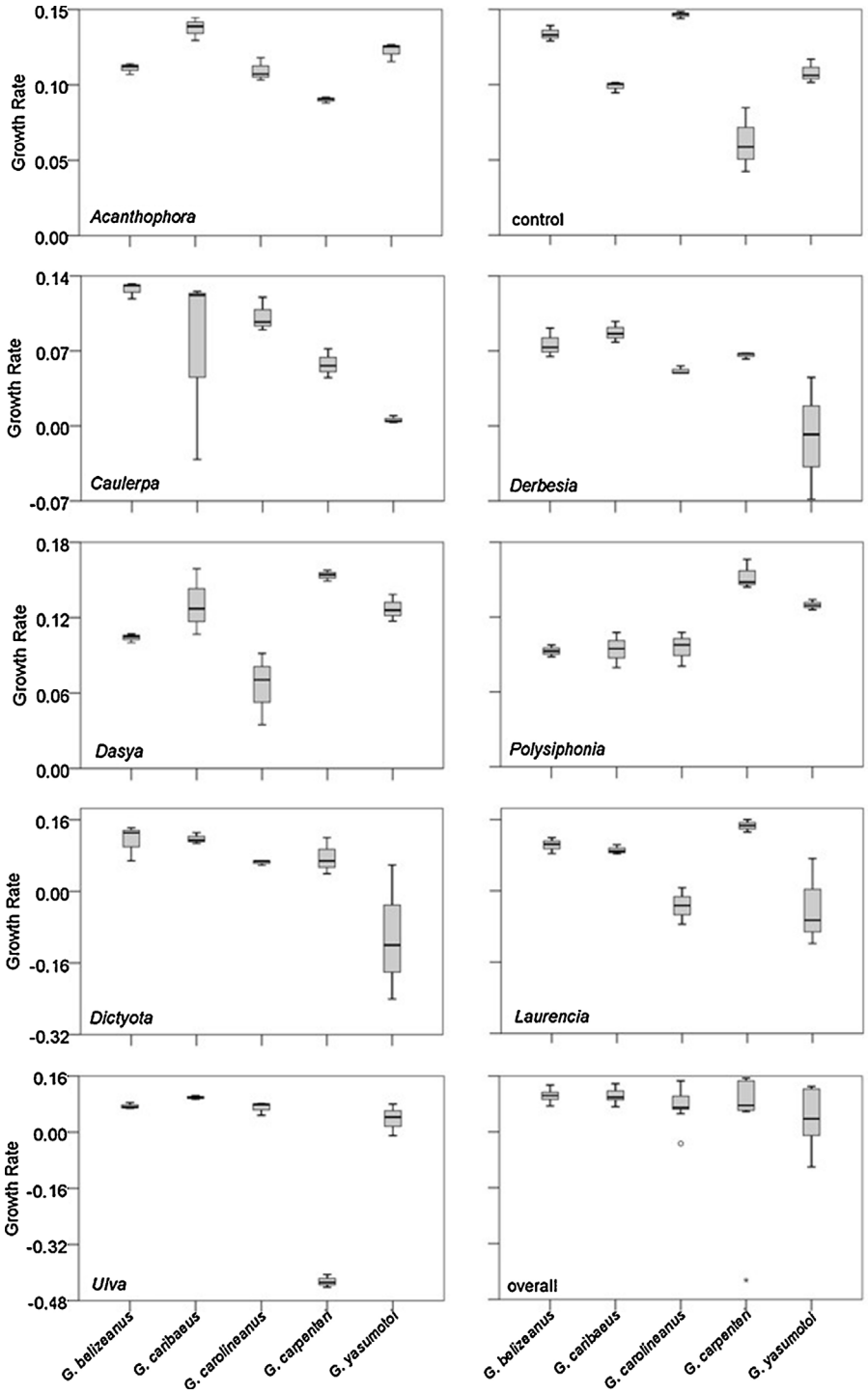


**Fig. 3.** Box plots of growth rate ($\Delta$ cells day$^{-1}$) for *Gambierdiscus* species within each control and host treatment. Box plot descriptions provided in the caption of Fig. 2.

species, but it also differs among the *Gambierdiscus* species themselves. While previous research has speculated that variability seen in *Gambierdiscus*–host interactions was due in part to strain (species) differences within the *Gambierdiscus* genus (e.g., Bomber et al., 1989; Grzebyk et al., 1994; Parsons et al., 2011), this is the first study to demonstrate that such species differences do, in fact, exist.

One interesting finding was that *Gambierdiscus caribaeus*, *Gambierdiscus carpenteri*, and *Gambierdiscus yasumotoi* were the only species that exhibited evidence of possible growth stimulation in the presence of certain host species (Fig. 2; Table 2). In all other cases, the various *Gambierdiscus* species exhibited growth rates that were lower or no different in the host treatments versus the control. Previous studies (Withers, 1981; Carlson et al., 1984; Grzebyk et al., 1994) have reported that extracts from host species stimulated *Gambierdiscus* growth in some cases (e.g., *Acanthophora spicifera*, *Portieria hornemannii*, *Chaetomorpha linum*), but inhibited *Gambierdiscus* growth in others (e.g., *Halymenia floresia*). While this study did not examine algal extracts, it does provide data demonstrating that not only does *Gambierdiscus* growth vary

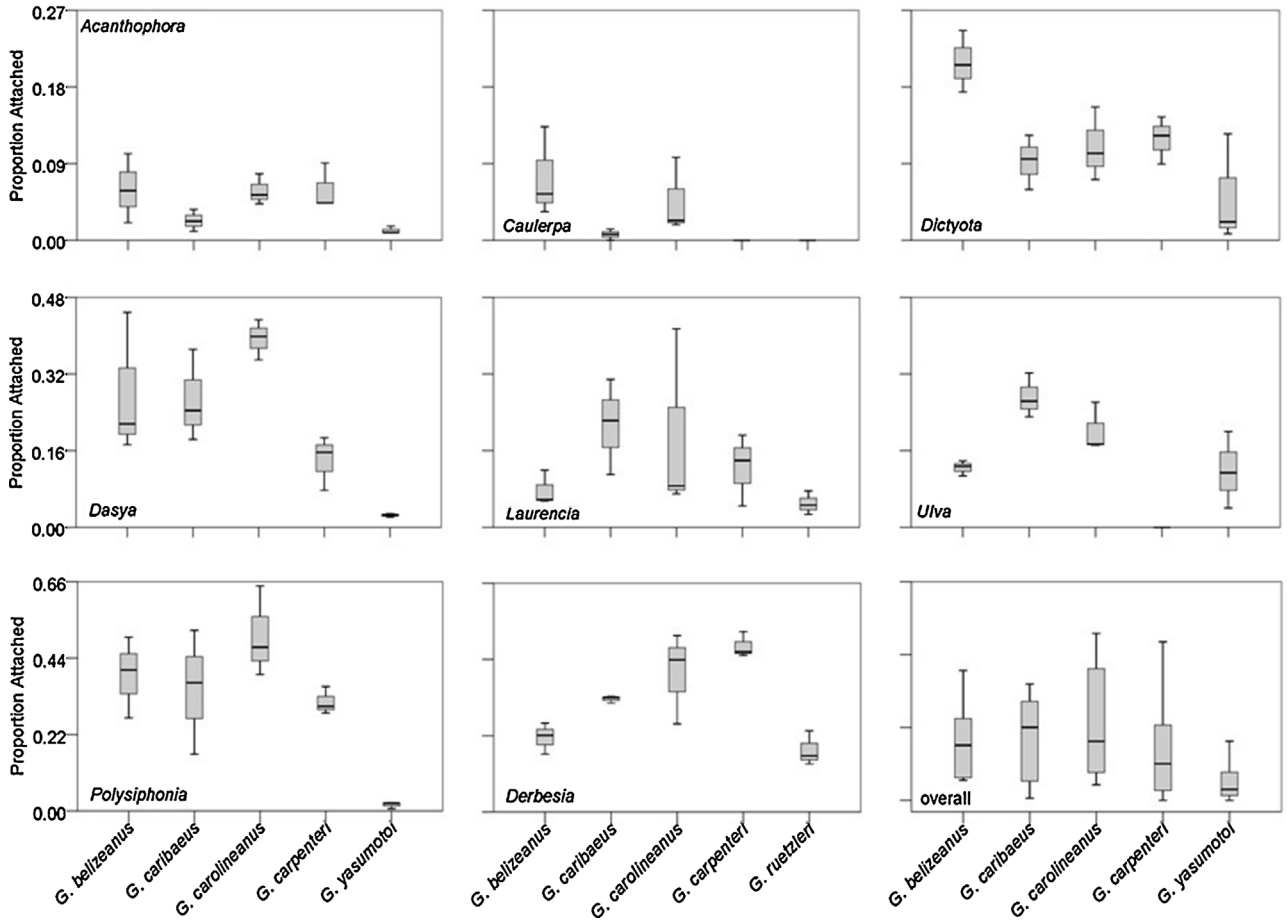


**Fig. 4.** Box plots of the proportion of attached cells of *Gambierdiscus* species plotted in reference to each host algae. Box plot descriptions provided in the caption of Fig. 2.

among potential host algae, but within the *Gambierdiscus* genus itself.

Many researchers have commented on *Gambierdiscus* attachment to host algae in previous studies. For example, Yasumoto et al. (1980) reported that *Gambierdiscus* attached itself to *Jania* via envelopment in a mucus membrane. Ballantine et al. (1988) similarly reported attachment on *Dictyota* in the Caribbean. Bomber et al. (1988) observed both mobile cells of *Gambierdiscus* and cells embedded within a mucilaginous sheath on the surface of host drift algae. Some cells would detach and swim when disturbed. Nakahara et al. (1996) demonstrated that *Gambierdiscus* cells do not simply attach to a host, but detach and swim about the host under various cues (low turbulence, presence of light). Additionally, behavior varied among host species. For example,

**Table 3**
Proportion of cells of the various *Gambierdiscus* species attached to each of the host treatments. Values shown are averages ± standard deviation. The letters under each value indicate Kruskal–Wallis pairwise comparison groupings within each column, those in parentheses for each row. *p*-values for comparisons made in each row are provided under the host treatments. *p*-values for comparisons made within each column are provided under the *Gambierdiscus* species.

| | *G. belizeanus* ($p = 0.001$) | *G. caribaeus* ($p = 0.009$) | *G. carolinianus* ($p = 0.009$) | *G. carpenteri* ($p = 0.025$) | *G. yasumotoi* ($p = 0.064$) |
|---|---|---|---|---|---|
| *Acanthophora* | 0.060 ± 0.041 | 0.023 ± 0.013 | 0.058 ± 0.018 | 0.059 ± 0.027 | 0.011 ± 0.004 |
| ($p = 0.047$) | D(AB) | D(B) | C(A) | C(A) | A(B) |
| *Caulerpa* | 0.074 ± 0.053 | 0.007 ± 0.007 | 0.046 ± 0.044 | 0.000 ± 0.000 | 0.000 ± 0.000 |
| ($p = 0.015$) | D(A) | D(B) | C(A) | D(B) | A(B) |
| *Dasya* | 0.279 ± 0.149 | 0.266 ± 0.096 | 0.394 ± 0.042 | 0.140 ± 0.057 | 0.025 ± 0.004 |
| ($p = 0.047$) | AB(AB) | AB(AB) | A(A) | C(B) | A(C) |
| *Derbesia* | 0.215 ± 0.045 | 0.326 ± 0.010 | 0.401 ±0.131 | 0.478 ± 0.037 | 0.178 ± 0.050 |
| ($p = 0.047$) | B(C) | A(B) | AB(ABC) | A(A) | A(C) |
| *Dictyota* | 0.209 ± 0.036 | 0.093 ± 0.032 | 0.110 ± 0.043 | 0.119 ± 0.028 | 0.051 ± 0.064 |
| ($p = 0.369$) | B(A) | C(A) | C(A) | C(A) | A(A) |
| *Laurencia* | 0.063 ± 0.029 | 0.174 ± 0.081 | 0.154 ± 0.158 | 0.102 ± 0.061 | 0.040 ± 0.020 |
| ($p = 0.138$) | D(A) | BC(A) | BC(A) | C(A) | A(A) |
| *Polysiphonia* | 0.391 ± 0.116 | 0.351 ± 0.179 | 0.504 ± 0.131 | 0.314 ± 0.039 | 0.018 ± 0.009 |
| ($p = 0.047$) | A(AB) | AB(AB) | A(A) | B(B) | A(C) |
| *Ulva* | 0.124 ± 0.016 | 0.272 ± 0.046 | 0.202 ± 0.052 | 0.000 ± 0.000 | 0.118 ± 0.080 |
| ($p = 0.015$) | C(C) | AB(A) | B(AB) | D(D) | A(BC) |

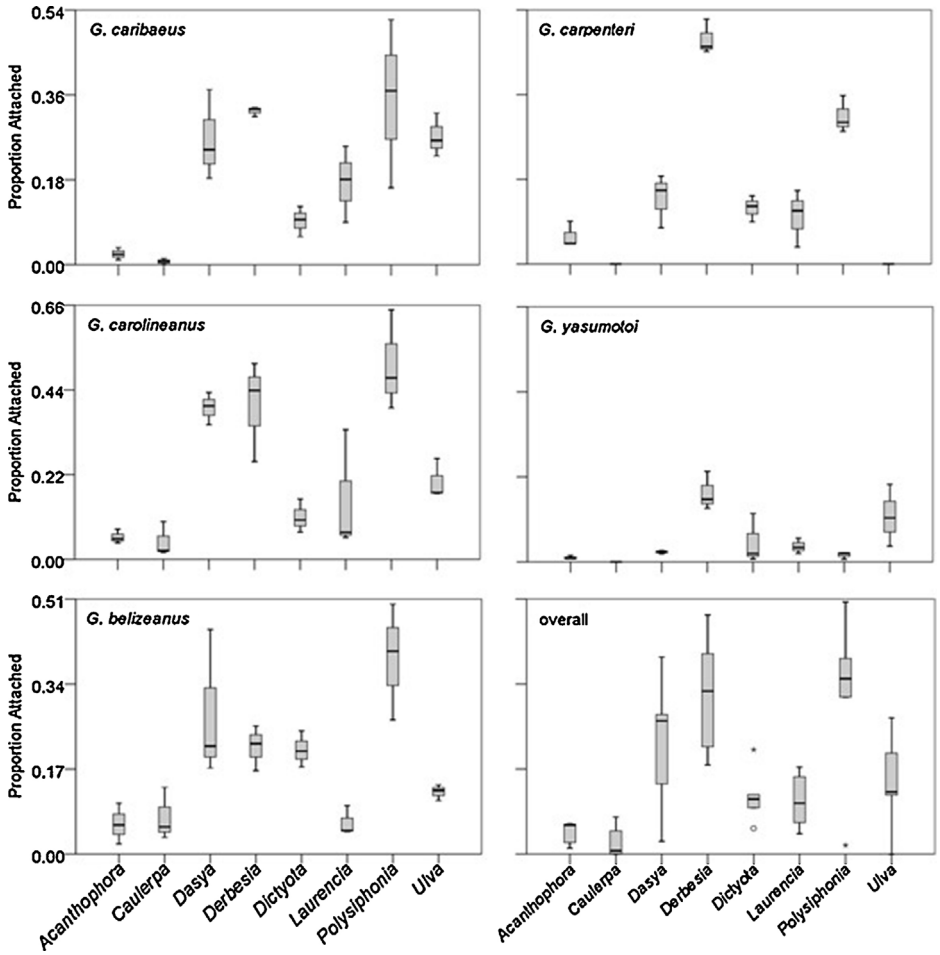


**Fig. 5.** Box plots of the proportion of attached cells on host algae plotted in reference to each *Gambierdiscus* species. Box plot descriptions provided in the caption of Fig. 2.

**Table 4**
Ranks of the *Gambierdiscus*–host combinations based on averages of pairwise comparison ranks (Kruskal–Wallis) of growth rate and proportion attached cells, published *Gambierdiscus* toxicity data, host palatability, and hypothetical vector threat for the most likely transfer of toxins into the food web based on the available data. The bold text indicates the two combinations representing the biggest vector threat.

| Rank | *Gambierdiscus* – host combination | *Gambierdiscus* ciguatoxicity | Host palatability[1,2] | Vector threat |
|---|---|---|---|---|
| 1 | *G. carpenteri* – *Polysiphonia* | 0[3] | High (44%) | Low |
| 2 | *G. caribaeus* – *Dasya* | 0.0087[4] | Low (0%) | Low |
| 3 | *G. carpenteri* – *Dasya* | 0[2] | Low (0%) | Low |
| 4 | *G. belizeanus* – *Dasya* | 0.123[5] | Low (0%) | Low |
| 5 | *G. carpenteri* – *Laurencia* | 0[3] | High (31%) | Low |
| 6 | *G. carolinianus* – *Polysiphonia* | Unknown | High (44%) | Unknown |
| 7 | *G. belizeanus* – *Polysiphonia* | 0.123 | High (44%) | **High** |
| 8 | *G. caribaeus* – *Ulva* | 0.0087 | Moderate–High (15%) | Low |
| 9 | *G. belizeanus* – *Dictyota* | 0.123 | Moderate–High (40%) | **Moderate–High** |
| 10 | *G. caribaeus* – *Derbesia* | 0.0087 | Unknown (0%) | Low |

1 – see Table 1; 2 – percentage of fish species containing algae in their gut contents in the Randall (1967) study (out of 48 species); 3 – field samples, LC–MS/MS (Kohli et al., 2014); 4 – C-CTX-1 equivalents (Lartigue et al., 2009); 5 – P-CTX-3C equivalents (Chinain et al., 2010).

*Gambierdiscus* swam in the presence of *Jania* sp., *Amphiroa* sp., and *Galaxaura* sp., but remained on the bottom of the petri dish in the presence of other species such as *Turbinaria ornata*, *Laurencia* sp., and several others (Nakahara et al., 1996). Additionally, *Gambierdiscus* would attach to live *Jania* via a mucus thread, but would not attach to dried *Jania*. Such behavior suggests that chemical cues may be present, influencing the epiphytic behavior of *Gambierdiscus*. Alternatively, differences in the physical structure of live versus dried *Jania* may have been a factor.

The results of this study are similar; *Gambierdiscus* cells attached to some hosts, but not others (e.g., *G. yasumotoi* on *Derbesia* versus *Caulerpa*; Fig. 4). Additionally, attachment behavior differed among the five *Gambierdiscus* species examined. For example, *G. yasumotoi* had the lowest attachment on *Dasya* and *Polysiphonia* versus the other *Gambierdiscus* species (Fig. 5). Some species of *Gambierdiscus* may naturally be more or less epiphytic than others, a suggestion which is supported by the data presented here. It is important to remember, however, that this experiment was conducted under static water conditions (except for the water exchanges), thereby preventing any testing of water motion effects on *Gambierdiscus* attachment. As mentioned previously, Nakahara et al. (1996) observed that *Gambierdiscus* cells were more likely to attach when disturbed. Future studies should examine the role of water motion of attachment behavior among the *Gambierdiscus* species.

Of the host algae used in this experiment, *Dictyota* and *Ulva* gained the most biomass (Table 5). Growth rates of *Gambierdiscus* populations exposed to these hosts, however, did not differ overall

**Table 5**
Average change in biomass (based on percentage different from initial wet weight) for each host algae. The percent change in biomass was averaged across all treatments.

| Host algae | % change in biomass |
|---|---|
| *Acanthophora* | −12.33 |
| *Caulerpa* | −36.69 |
| *Dasya* | −18.94 |
| *Derbesia* | −43.98 |
| *Dictyota* | +81.53 |
| *Laurencia* | +8.42 |
| *Polysiphonia* | +14.32 |
| *Ulva* | +77.39 |

versus other hosts (Figs. 2 and 3, Table 2). Conversely, other macroalgae species lost biomass over the course of the study (e.g., *Caulerpa* and *Dasya*), suggesting a decline in health. Once again, however, there was no difference in overall growth rates or attachment these hosts versus others (Figs. 2 and 3; Table 2). Overall, algal host weight gain or loss was not correlated with *Gambierdiscus* growth rates or attachment, indicating that general host health did not appear to influence *Gambierdiscus* responses to the hosts; rather, it was host specific.

The most likely vector for ciguatoxins (or precursors) to move up into demersal (reef) food webs is via herbivory on the host macroalgae harboring *Gambierdiscus* cells. This vector was proposed decades ago by Dawson et al. (1955) and Randall (1958), and confirmed by Yasumoto et al. (1977a, 1977b) who found numerous cells of what are now known to be *Gambierdiscus* in detrital samples and the stomach contents of fish. Cruz-Rivera and Villareal (2006) proposed two factors that likely influence the transfer of gambiertoxins to the food web: (1) the density of *Gambierdiscus* cells on a host macrophyte and (2) the palatability (targeting) of the host by herbivores. The density of *Gambierdiscus* cells on a host will be a function of *Gambierdiscus* growth rates (influenced by both host and environmental conditions such as temperature), surface area of the host (discussed in Lobel et al., 1988; Parsons et al., 2011), and cell attachment to the host (i.e., cells have to be attached to the host in order to be consumed by the herbivore). As *Gambierdiscus* growth rates were not correlated with cell attachment, however, these factors do not necessarily align at all times.

The highest densities of *Gambierdiscus* cells on a host alga would be expected to occur in a *Gambierdiscus* – host pairing resulting in the highest growth rates for *Gambierdiscus* coupled with the highest proportion of attached cells. The candidates that best meet these requirements from this study are presented in Table 4. The next parameter required for the consumption of *Gambierdiscus* cells by an herbivore is palatability – will an herbivore target the host algae for consumption?

Palatability of host macroalgae plays an important role in ciguatoxin transfer within a food web (i.e., all macroalgae species are not equally consumed by herbivores; Cruz-Rivera and Villareal, 2006). Some macroalgae have mechanisms to defend themselves against grazing, including chemical defenses, low nutritional values, and structural defenses. For example, some calcified algae such as *Halimeda* have tough, leathery exteriors and are subjected to reduced grazing pressures because they are less susceptible to grazers with weak mouthparts (Cruz-Rivera and Villareal, 2006). Many algal species produce chemicals to deter grazers while other algae are of very low nutritional quality and therefore too costly energy-wise for herbivores to consume. Algae with low nutritional value are oftentimes subjected to lower grazing pressures when more nutritional algae are available (Atsatt and O'Dowd, 1976). Results presented in Table 4 suggest that *Dictyota* and *Polysiphonia* are the most palatable algae of the species used in this study. While other studies have demonstrated that *Dictyota* is not palatable in some cases (e.g., for urchins; Bolser and Hay, 1996), fish do consume it, as demonstrated by the Randall (1967) study, and observations in the field of parrotfish eating *Dictyota* in the Florida Keys (Beach and Walters, 2000). Additionally, *Polysiphonia* is not only known to be consumed by fish (Randall, 1967), but damselfish (*Stegastes nigricans*) cultivate this algae for their consumption (Hata and Kato, 2006). Therefore, based on these studies, *Polysiphonia* and *Dictyota* are the most palatable of the algae used in this study, and as good hosts for *Gambierdiscus* cells, would be likely candidate vectors for the trophic transfer of ciguatoxin into the food web in this hypothetical scenario (Table 4). This scenario, of course, depends on the ability of the *Gambierdiscus* cells to produce gambiertoxins.

Earlier studies have postulated that ciguatera outbreaks were a result of intense bursts of ciguatoxin production (Helfrich and Banner, 1968), likely due to the presence of "super-producing" strains of *Gambierdiscus* (Holmes et al., 1991; Legrand, 1998). Recently, toxin production has been revisited following the reclassification of the genus and description of new species. Chinain et al. (1999, 2010) found that *Gambierdiscus polynesiensis* was more ciguatoxic than other *Gambierdiscus* species tested. Rhodes et al. (2014) similarly reported that *G. polynesiensis* was more ciguatoxic than other *Gambierdiscus* isolates from the Cook Islands (*G. australes* and *G. pacificus*). Cultures now known to be *Gambierdiscus* ribotype 2 were more ciguatoxic than *G. caribaeus* in a study conducted by Lartigue et al. (2009). A 1000-fold range in ciguatoxicity was exhibited in the isolates tested (i.e., *G. polynesiensis* versus *G. caribaeus*). This range assumes that the toxin quantification methods in the studies are quasi-comparable; i.e., the N2A assay used in Lartigue et al. (2009; standardized to C-CTX-1 equivalents) gives similar results to the receptor-binding assay used by Chinain et al. (2010); standardized to P-CTX-3C equivalents, acknowledging that P-CTX-3C is approximately twice as potent as C-CTX-1 (Lewis, 2001). Based on these assumptions, of the Caribbean isolates tested, *G. belizeanus* (STB-1 tested by Chinain et al., 2010) is approximately three-times more ciguatoxic than *G.* ribotype 2 (CCMP 1655), and 10–100 times more toxic than *G. caribaeus* (CCMP 1651). Therefore, while *G. polynesiensis* appears to be the primary toxin producer putatively responsible for Pacific cases of ciguatera (based on published data), the current front-runner for the "super-producing" Caribbean species is *G. belizeanus*, albeit at levels of toxin production >10 times lower than *G. polynesiensis*. Therefore, the *Gambierdiscus* – host pairings tested in this study that present the most likely vectors for toxin transfer into the food web are *G. belizeanus* – *Polysiphonia* and *G. belizeanus* – *Dictyota* (Table 4). As the annual number of ciguatera cases is similar in the Caribbean and Pacific (12 and 23 cases per 10,000 people, respectively; Tester et al., 2010), it is likely that a more potent strain or species of *Gambierdiscus* is present in the Caribbean, but has yet to be discovered (or tested). For example, *G. carolinianus* is genetically similar to *G. polynesiensis* (Fraga and Rodriguez, 2014), which may translate into a high toxicity for this species, but it has yet to be tested.

In conclusion, while the basic pathway for ciguatoxin introduction into benthic food webs is known, many critical, specific aspects remain unresolved. The results of this study demonstrate that epiphytic behavior (growth and attachment) varies among *Gambierdiscus* species. Theoretically, in order for gambiertoxins to be introduced into the food web, there must be a palatable host (such as *Polysiphonia* or *Dictyota* in this study) that harbors a significant density (>1000 cells $g^{-1}$ wet weight host; Litaker et al., 2010) of toxic *Gambierdiscus* cells (possibly *G. belizeanus* based on the results of this study and known toxicity of Caribbean isolates). As other studies have demonstrated that multiple *Gambierdiscus*

species may co-exist on an algal host (Vandersea et al., 2012), and that toxicity varies among *Gambierdiscus* species (discussed above), teasing out the scenarios that allow for toxic *Gambierdiscus* cells to reach threshold densities on a palatable host remains elusive. Future efforts to better understand these factors may clarify the role of the macroalgae community in the production and transfer of gambiertoxins to higher trophic levels, the pulse/burst of which can lead to flare-ups of CFP.

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## References

Atsatt, P.R., O'Dowd, D.J., 1976. Plant defense guilds. Science 193, 24–29.
Bagnis, R., 1994. Natural versus anthropogenic disturbances to coral reefs: comparison in epidemiological patterns of ciguatera. Mem. Queensl. Mus. 34, 455–460.
Ballantine, D.L., Tosteson, T.R., Bardales, A.T., 1988. Population dynamics and toxicity of natural populations of benthic dinoflagellates in southwestern Puerto Rico. J. Exp. Mar. Biol. Ecol. 119, 201–212.
Beach, K.S., Walters, L.J., 2000. Dictyota bloom in Florida keys national marine sanctuary: fragments and fouling. In: AAUS 20th Symposium Proceedings. pp. 61–63.
Besada, E., Loeblich, L., Loeblich, I., 1982. Observations on tropical, benthic dinoflagellates from ciguatera-endemic areas: *Coolia*, *Gambierdiscus*, and *Ostreopsis*. Bull. Mar. Sci. 32, 723–735.
Bolser, R.C., Hay, M.E., 1996. Are tropical plants better defended? Palatability and defenses of temperature vs. tropical seaweeds. Ecology 77, 2269–2286.
Bomber, J.W., Aikman, K.E., 1989. The ciguatera dinoflagellates. Biol. Oceanogr. 6, 291–311.
Bomber, J.W., Tindall, D.R., Miller, D.M., 1989. Genetic variability in toxin potencies among seventeen clones of *Gambierdiscus toxicus* (Dinophyceae). J. Phycol. 25, 617–625.
Bomber, J.W., Morton, S.L., Babinchak, J.A., Norris, D.R., Morton, J.G., 1988. Epiphytic dinoflagellates of drift algae: another toxigenic community in the ciguatera food chain. Bull. Mar. Sci. 43, 204–214.
Bruno, J.F., Sweatman, H., Precht, W.F., Selig, E.R., Schutte, V.W.G., 2009. Assessing evidence of phase shifts from coral to macroalgal dominance on coral reefs. Ecology 90, 1478–1484.
Carlson, R.D., 1984. Distribution, Periodicity, and Culture of Benthic/Epiphytic Dinoflagellates in a Ciguatera Endemic Region of the Caribbean. (Ph.D. Thesis)-Southern Illinois University, Carbondale, pp. 308.
Carlson, R.D., Morey-Gaines, G., Tindall, D.R., Dickey, R.W., 1984. Ecology of toxic dinoflagellates from the Caribbean Sea: effects of macroalgal extracts on growth in culture. In: Ragelis, E.P. (Ed.), Seafood Toxins. American Chemical Society, Washington, D.C., pp. 271–287.
Carlson, R.D., Tindall, D.R., 1985. Distribution and periodicity of toxic dinoflagellates in the Virgin Islands. In: Anderson, D.M., White, A.W., Baden, D.G. (Eds.), Toxic Dinoflagellates. Elsevier Publishers B.V., New York, pp. 171–176.
Chinain, M., Darius, H.T., Ung, A., Cruchet, P., Wang, Z., Ponton, D., Laurent, D., Pauillac, S., 2010. Growth and toxin production in the ciguatera-causing dinoflagellate *Gambierdiscus polynesiensis* (Dinophyceae) in culture. Toxicon 56, 739–750.
Chinain, M., Faust, M.A., Pauillac, S., 1999. Morphology and molecular analyses of three toxic species of *Gambierdiscus* (Dinophyceae): *G. pacificus*, sp. nov., *G. australes*, sp. nov., and *G. polynesiensis*, sp. nov. J. Phycol. 35, 1282–1296.
Cooper, M., 1964. Ciguatera and other marine poisoning in the Gilbert Islands. Pac. Sci. 18, 411–440.
Cruz-Rivera, E., Villareal, T.A., 2006. Macroalgal palatability and the flux of ciguatera toxins through marine food webs. Harmful Algae 5, 497–525.
Dawes, C.J., Mathieson, A.C., 2008. The Seaweeds of Florida. University Press of Florida.
Dawson, E.Y., Aleem, A.A., Halstead, B.W., 1955. Marine algae from Palmyra Island with special reference to the feeding habits and toxicology of reef fishes. Allan Hancock Foundation Occasional Paper, the University of Southern California, , pp. 17.
De Lara-Isassi, G., Álvarez-Hernández, S., Collado-Vides, L., 2000. Ichtyotoxic activity of extracts from Mexican marine macroalgae. J. Appl. Phycol. 12, 45–52.
de Sylva, D.P., 1994. Distribution and ecology of ciguatera fish poisoning in Florida, with emphasis on the Florida Keys. Bull. Mar. Sci. 54, 944–954.
Delgado, G., Lechuga-Devéze, C.H., Popowski, G., Troccoli, L., Salinas, C.A., 2006. Epiphytic dinoflagellates associated with ciguatera in the northwestern coast of Cuba. Rev. Biol. Trop. 54, 299–310.
Fleming, L.E., Baden, D.G., Bean, J.A., Weisman, R., Blythe, D.G., 1998. Seafood toxin diseases: issues in epidemiology and community outreach. In: Reguera, B., Blanco, J., Fernández, M.L., Wyatt, T. (Eds.), Harmful Algae. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, Paris, pp. 245–248.
Fraga, S., Rodriguez, F., 2014. Genus Gambierdiscus in the Canary Islands (NE Atlantic Ocean) with description of *Gambierdiscus silvae* sp. nov., a new potentially toxic epiphytic benthic dinoflagellate. Protist 165, 839–853.
Fraga, S., Rodriguez, F., Caillaud, A., Diogene, J., Raho, N., Zapata, M., 2011. *Gambierdiscus excentricus* sp. nov. Dinophyceae, a benthic toxic dinoflagellate from the Canary Islands (NE Atlantic Ocean). Harmful Algae 11, 10–22.
Gilbert, E.I., 2005. Juvenile Green Turtle (*Chelonia mydas*) Foraging Ecology: Feeding Selectivity and Forage Nutrient Analysis. (M.S. Thesis)University of Central Florida, Orlando, FL, pp. 47.
Gillespie, N.C., Holmes, M.J., Burke, J.B., Doley, J., 1985. Distribution and periodicity of *Gambierdiscus toxicus* in Queensland, Australia. In: Anderson, D.M., White, A.W., Baden, D.G. (Eds.), Toxic Dinoflagellates. Elsevier Science Publishing Co., Inc., pp. 183–188.
Grzebyk, D., Berland, B., Thomassin, B.A., Bosi, C., Arnoux, A., 1994. Ecology of ciguateric dinoflagellates in the coral reef complex of Mayotte Island (SW Indian Ocean). J. Exp. Mar. Biol. Ecol. 178, 51–66.
Hata, H., Watanabe, K., Kato, M., 2010. Geographic variation in the damselfish-red alga cultivation mutualism in the Indo-West Pacific. BMC Evol. Biol. 10, 185.
Hata, H., Kato, M., 2006. A novel obligate cultivation mutualism between damselfish and Polysiphonia algae. Biol. Lett. 2, 593–596.
Heil, C.A., Bird, P., Dennison, W.C., 1998. Macroalgal habitat presence of ciguatera dinoflagellates at Heron Island, a coral cay in the southeastern Great Barrier Reef, Australia. In: Reguera, B., Blanco, J., Fernández, M.L., Wyatt, T. (Eds.), Harmful Algae. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, Paris, pp. 52–53.
Helfrich, P., Banner, A.H., 1968. Ciguatera fish poisoning. 2. General patterns of development in the Pacific. Occasional Papers of the *Bernice P. Bishop Mus.* 23, 371–382.
Holmes, M.J., Lewis, R.J., Poli, M.A., Gillespie, N.C., 1991. Strain dependent production of ciguatoxin precursors (gambiertoxins) by *Gambierdiscus toxicus* (Dinophyceae) in culture. Toxicon 29, 761–775.
Kohli, G.S., Murray, S.A., Neilan, B.A., Rhodes, L.L., Harwood, D.T., Smith, K.F., Meyer, L., Capper, A., Brett, S., Hallegraeff, G.M., 2014. High abundance of the potentially maitotoxic dinoflagellate *Gambierdiscus carpenteri* in temperate waters of New South Wales, Australia. Harmful Algae 39, 134–145.
Lartigue, J., Jester, E.L., Dickey, R.W., Villareal, T.A., 2009. Nitrogen source effects on the growth and toxicity of two strains of the ciguatera-causing dinoflagellate *Gambierdiscus toxicus*. Harmful Algae 8, 781–791.
Legrand, A.M., 1998. Ciguatera toxins: origin, transfer through the food chain and toxicity to humans. In: Reguera, B., Blanco, J., Fernández, M.L., Wyatt, T. (Eds.), Harmful Algae. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, Paris, pp. 39–43.
Lewis, R.J., 2001. The changing face of ciguatera. Toxicon 39, 97–106.
Litaker, R.W., Vandersea, M.W., Faust, M.A., Kibler, S.R., Chinain, M., Holmes, M.J., Holland, W.C., Tester, P.A., 2009. Taxonomy of *Gambierdiscus* including four new species, *Gambierdiscus caribaeus*, *Gambierdiscus carolinianus*, *Gambierdiscus carpenteri* and *Gambierdiscus ruetzleri* (Gonyaulacales, Dinophyceae). Phycologia 48, 334–390.
Litaker, R.W., Vandersea, M.W., Faust, M.A., Kibler, S.R., Nau, A.W., Holland, W.C., Chinain, M., Holmes, M.J., Tester, P.A., 2010. Global distribution of ciguatera causing dinoflagellates in the genus *Gambierdiscus*. Toxicon 56, 711–730.
Littler, D.S., Littler, M.M., 2000. Caribbean Reef Plants. Offshore Graphics, Inc., Washington, D.C.
Littler, M.M., Littler, D.S., Taylor, P.R., 1983. Evolutionary strategies in a tropical barrier reef system: functional-form groups of marine macroalgae. J. Phycol. 19, 229–237.
Lobel, P.S., Anderson, D.M., Durand-Clement, M., 1988. Assessment of ciguatera dinoflagellate populations: sample variability and algal substrate selection. Biol. Bull. 175, 94–101.
Morton, S.L., Faust, M.A., 1997. Survey of toxic epiphytic dinoflagellates from the Belizean barrier reef ecosystem. Bull. Mar. Sci. 61, 899–906.
Nakahara, H., Sakami, T., Chinain, M., Ishida, Y., 1996. The role of macroalgae in epiphytism of the toxic dinoflagellate *Gambierdiscus toxicus* (Dinophyceae). Phycol. Res. 44, 113–117.
Nishimura, T., Sato, S., Tawong, W., Sakanari, H., Yamaguchi, H., Adachi, M., 2014. Morphology of *Gambierdiscus scabrosus* sp. nov. (Gonyaulacales): a new epiphytic toxic dinoflagellate from coastal areas of Japan. J. Phycol. 50, 506–514.
Norris, J.N., Fenical, W., 1982. Chemical defenses in tropical marine algae. Smithson. Contrib. Mar. Sci. 12, 417–431.
Parsons, M.L., Aligizaki, K., Bottein, M.Y.D., Fraga, S., Morton, S.L., Penna, A., Rhodes, L., 2012. Gambierdiscus and *Ostreopsis*: reassessment of the state of knowledge of their taxonomy, geography, ecophysiology, and toxicology. Harmful Algae 14, 107–129.
Parsons, M.L., Settlemier, C.J., Ballauer, J.M., 2011. An examination of the epiphytic nature of *Gambierdiscus toxicus*, a dinoflagellate involved in ciguatera fish poisoning. Harmful Algae 10, 598–605.
Ragelis, E.P., 1984. Ciguatera seafood poisoning: overview. In: Ragelis, E.P (Ed.), Seafood Toxins. American Chemical Society, Washington, D.C., pp. 22–36.
Randall, J.E., 1958. A review of ciguatera, tropical fish poisoning with a tentative explanation of its cause. Bull. Mar. Sci. Gulf Carib. 8, 236–267.
Randall, J.E., 1967. Food Habits of Reef Fishes of the West Indies. University of Miami, Institute of Marine Science, , pp. 183.

Rhodes, L., Harwood, T., Smith, K., Argyle, P., Munday, R., 2014. Production of ciguatoxin and maitotoxin by strains of *Gambierdiscus australes*, *G. pacificus* and *G. polynesiensis* (Dinophyceae) isolated from Rarotonga, Cook Islands. Harmful Algae 39, 185–190.

Rhodes, L.L., Smith, K.F., Munday, R., Selwood, A.I., McNabb, P.S., Holland, P.T., Bottein, M.Y., 2010. Toxic dinoflagellates (Dinophyceae) from Rarotonga, Cook Islands. Toxicon 56, 751–758.

Saint Martin, K., Durand-Clement, M., Bourdeau, P., 1988. Contribution to the study of the relations between macroalgae and *Gambierdiscus toxicus* (Dinophyceae), responsible for ciguatera. Cryptogam. Algol. 9, 195–202.

Tester, P.A., Feldman, R.L., Nau, A.W., Kibler, S.R., Litaker, R.W., 2010. Ciguatera fish poisoning and sea surface temperatures in the Caribbean Sea and the West Indies. Toxicon 56, 698–710.

Vandersea, M.W., Kibler, S.R., Holland, W.C., Tester, P.A., Schultz, T.F., Faust, M.A., Holmes, M.J., Chinain, M., Litaker, R.W., 2012. Development of semi-quantitative PCR assays for the detection and enumeration of *Gambierdiscus* species (Gonyaulacales, Dinophyceae). J. Phycol. 48, 902–915.

Villareal, T.A., Morton, S.L., 2002. Use of cell-specific PAM-fluorometry to characterize host shading in the epiphytic dinoflagellate *Gambierdiscus toxicus*. PSZNI Mar. Ecol 23, 127–140.

Withers, N.W., 1981. Toxin production, nutrition, and distribution of *Gambierdiscus toxicus* (Hawaiian strain). In: Gomez, E.D. (Ed.), Fourth International Coral Reef Symposium. Manila, Philippines, pp. 449–451.

Yasumoto, T., Bagnis, R., Thevenin, S., Garcon, M., 1977a. A survey of comparative toxicity in the food chain of ciguatera. Bull. Jpn. Soc. Sci. Fish. 43, 1015–1019.

Yasumoto, T., Nakajima, I., Bagnis, R., Adachi, R., 1977b. Finding of a dinoflagellate as a likely culprit of ciguatera. Bull. Jpn. Soc. Sci. Fish. 43, 1021–1026.

Yasumoto, T., Oshima, Y., Murakami, Y., Nakajima, I., Bagnis, R., Fukuyo, Y., 1980. Toxicity of benthic dinoflagellates. Bull. Jpn. Soc. Sci. Fish. 46, 327–331.