Assessing the use of artificial substrates to monitor *Gambierdiscus* populations in the Florida Keys

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Abstract

Four distinct coastal locations were sampled on a monthly basis near Long Key (Florida Keys, USA) over a 13-month period to study *Gambierdiscus* population dynamics on different substrates, including four macrophyte species (*Dictyota* spp., *Halimeda* spp., *Laurencia* spp., and *Thalassia testudinum*) and three artificial substrates (polyvinyl chloride (PVC) tiles, burlap, and fiberglass window screen). Cell densities of *Gambierdiscus* were generally lower on *Dictyota* versus *Halimeda* and *Laurencia.* Cell densities of *Gambierdiscus* were significantly correlated among macrophyte hosts in 54% of the comparisons, and between macrophyte hosts and artificial substrates in 72% of the comparisons. Predictive slopes determined from regression analyses between cell densities on artificial substrates and macrophyte hosts indicated that, on an areal basis, fewer cells were present on macrophytes versus artificial substrates (cells cm^{-2}) and that slope variation (error) among the different macrophytes and sites ranged from 5% to 200%, averaging 61% overall. As the data required log-transformation prior to analyses, this level of error translates into two-orders of magnitude in range of estimation of the overall average abundance of *Gambierdiscus* cells on macrophytes (135 cells g^{-1} wet weight); 20 to 2690 cells g^{-1} ww. The lack of consistent correlation among *Gambierdiscus* cell densities on macrophytes versus artificial substrates, coupled with the high level of error associated with the predictive slope estimations, indicates that extreme caution should be taken when interpreting the data garnered from artificial substrate deployments, and that such deployments should be thoroughly vetted prior to routine use for monitoring purposes.

1. Introduction

Although ciguatera fish poisoning (CFP) is the most common form of harmful algal bloom (HAB)-derived seafood poisoning (Fleming et al. 1998), effective monitoring and prevention protocols remain elusive to protect people from this detrimental and trying disease (Dickey and Plakas 2010). Since its discovery in the 1970s (Yasumoto et al. 1977), ongoing studies have focused on the abundance of the organisms ultimately responsible for this syndrome, members of the benthic dinoflagellate genus, *Gambierdiscus* (reviewed in Lewis 2001; Litaker et al. 2010; Parsons et al. 2012; and others). Researchers have traditionally linked ciguatera outbreaks to epibenthic "blooms" of *Gambierdiscus* (e.g., Withers 1982; Bagnis et al. 1990), where higher numbers of cells were thought to lead to higher ciguatoxin fluxes into the food web, resulting in CFP events. As such, threshold cell densities have been proposed cautioning for the potential of a ciguatera outbreak (e.g., 100 cells g^{-1} wet weight algae, Taylor and Gustavson 1983; 1,000 cells g^{-1} wet weight algae, Litaker et al. 2010).

One difficulty in quantifying such thresholds, however, is that due to the different morphologies and chemical composition of the various macroalgal hosting species, *Gambierdiscus* cell densities (calculated as cells per gram wet weight algae = cells g^{-1} ww) are not standardized across the different hosts (Bomber 1985). For example, those that are calcareous will have more mass than others with softer structure. Rather, the surface area of the algal host should be taken into consideration when making such computations. For example, Lobel et al. (1988) reported that *Gambierdiscus* densities were approximately1.5 times higher on the phaeophyte, *Dictyota*, versus the rhodophyte, *Galaxaura*, when calculated as cells g^{-1} ww, but were approximately 40% lower when calculated based on surface area (cells cm^{-2} algae). The authors concluded that

55 standardized methodologies should be implemented before *Gambierdiscus* population densities
56 can be accurately assessed. Bomber et al. (1989) also acknowledged the importance of surface
57 area in the study of *Gambierdiscus* epiphytism, and employed the "thin layer of liquid" method
58 of Harrod and Hall (1962) to determine the surface area of 15 different macrophytes collected at
59 Knight Key in the Florida Keys. The researchers found a significant positive correlation
60 between macrophyte surface area and *Gambierdiscus* cell densities. While subsequent studies
61 affirmed that *Gambierdiscus* cell densities on macrophyte hosts was likely driven by host surface
62 area, results continued to be presented as cells g^{-1} ww host, due in part to the difficulties in
63 determining surface area, and the better precision provided by wet weight determinations (e.g.,
64 Parsons and Preskitt 2007).
65
66 Another issue that further impedes the study of *Gambierdiscus* populations in benthic
67 environments is that the macrophyte community is often different from site-to-site and season-to-
68 season (e.g., Mathieson and Dawes 1975). This dynamic makes it difficult to compare
69 *Gambierdiscus* cell densities across sites and seasons (Parsons and Preskitt 2007), as the
70 experimental design becomes unbalanced due to missing samples when the target macrophyte is
71 not present (or in senescence). The need to both standardize *Gambierdiscus* cell density
72 estimates, and to have access to consistent substrate across time and space, has led researchers to
73 explore the use of artificial substrates to monitor *Gambierdiscus* population dynamics.
74
75 The earliest known assessment of artificial substrates to quantify *Gambierdiscus* cells was by
76 Caire et al. (1985), who deployed tape strips ("algae traps") on Mururoa atoll (French Polynesia)
77 for daily enumeration of *Gambierdiscus* cells over a 13-month period. The authors observed that

Gambierdiscus cells did not settle on the artificial substrate for four months, and were not frequently present until after eight months of deployment. The authors thought the delay was due (in part) to the requirement of a suitable host macroalgal species first becoming established (after 2 – 3 months), although the establishment of a new population of *Gambierdiscus* likely required both suitable hosts and the presence of a mobile population from which colonizing cells could be recruited. Faust (2009) deployed nylon rope fibers and plastic screens (20x20cm) to collect *Gambierdiscus* and other benthic dinoflagellates in coastal waters of Belize. Samples were analyzed independently of macrophyte samples, however, so no assessment beyond the success in harvesting dinoflagellates was reported. Tan et al. (2013) utilized fiberglass screens to assess the benthic dinoflagellate population at a fringing reef site off Sampadi Island, Malaysia. Once again, while the screens provided the means to examine the dinoflagellate population, no comparative study was done for dinoflagellates on the macrophyte hosts themselves to determine how representative the artificial substrate samples were. Jauzein et al. (2016) deployed screens using a modified design from Kibler et al. (2010) and included the testing of different mesh sizing (porosity) to determine which mesh was best suited to sample for *Ostreopsis* cf. *ovata* in Villefranche Bay on the French Mediterranean coast. Results indicated that mesh openings of 1-3 mm were optimal. Macroalgal samples were collected concurrently with the 24- and 48-hr deployments, but a comparative data analysis was not included in their study.

Tester et al. (2014) compared cell densities from screen deployments against those on macrophyte samples from sites in Belize and Malaysia. The authors concluded that screens should be immersed for at least 24 hours (to allow ample time for the recruitment of dinoflagellates onto the screen surface), and that there were statistically significant relationships

between *Gambierdiscus* cell densities on macroalgae (log cells g^{-1} ww) and screens (log cells 100 cm^{-2}) at the two Belizean sites, but not the Malaysian location. There was, however, a significant relationship when data from all three sites were pooled. While this study represents a step forward in standardizing monitoring methods for *Gambierdiscus* populations, more thorough study is needed across different habitats and regions to properly gauge the effectiveness of 24-hr screen deployments as an accurate proxy for *Gambierdiscus* cell densities in the environment.

The purpose of this study, therefore, is to assess the use of artificial substrates to monitor *Gambierdiscus* populations in the Florida Keys. The above concerns were addressed by utilizing monthly deployments, which are most compatible with the research team's sampling schedules. The study was also conducted over an annual cycle at four sites in order to address site and temporal variability.

2. Methods

2.1 Site descriptions.

Samples were collected monthly over a thirteen-month period between March 2012 and March 2013 at four study sites near Long Key in the Florida Keys (Fig. 1). Two sites, Heine Grass bed (HGB) and Tomato Patch Hardbottom (TPH), are located in Florida Bay, whereas the other two, Long Key Hardbottom (LKH) and Tennessee Reef Lighthouse (TRL), are located on the Atlantic Ocean side of the Keys. HGB is a nearshore *Thalassia* seagrass bed in approximately 2 m water depth. Siphonous chlorophytes are also present, including *Halimeda incrassata*, *Udotea* spp., and *Penicillis* spp. TPH is a nearshore hardbottom site (approx. 1.5 m depth) consisting of soft

corals, sponges, and macroalgae, including *Laurencia gemmifera, Dictyota cervicornis,* and *Halimeda incrassata*. LKH is an offshore hardbottom site (approx. 5 m depth) consisting of soft corals, sponges, and macroalgae, including *Laurencia intricata, Dictyota cervicornis,* and *Halimeda gracilis*. TRL is a reef flat/crest site (approx. 7 m depth) consisting of hard and soft corals, sponges, and macroalgae, including turf algae, *Dictyota menstrualis*, and *Halimeda gracilis*. At each site, three pairs of (semi)permanent pins were placed pairs approximately 20m apart, each pair separated by 10m (Fig. 2). Transect lines were deployed along each pair of pins, providing a geographic framework from which subsequent sampling took place.

2.2 Sample collection.

Macrophyte samples were chosen and collected based on their common abundance. The targeted species included *Dictyota cervicornis* (Kützing 1859), *D. menstrualis* ((Hoyt) Schnetter, Hörning & Weber-Peukert 1987), *Halimeda gracilis* (Harvey ex J. Agardh 1887), *H. incrassata* ((J. Ellis) J.V. Lamouroux 1816), *Laurencia gemmifera* (Harvey 1853), *L. intricata* (J.V. Lamouroux 1813), and *Thalassia testudinum* (K.D. Koenig 1805) (Fig. 3). The two or three most dominant macrophytes were sampled in triplicate at each site, at least 10 m apart from each other. Each triplicate was taken near (but not closer than 2m) one of the three deployed transect lines. The samples were collected via SCUBA by gently placing a screw-capped polypropylene 50 mL centrifuge tube over a macrophyte thallus or blade, cutting the thallus or blade at the insertion point, and capping the tube for transport back to the boat (the R.V. *Megalodon*; 27 foot Grady White 272 Sailfish).

Three types of artificial substrates were also deployed and collected: PVC tiles, burlap fabric, and fiberglass window screening (Fig. 4a). The burlap fabric and screening were mounted in wooden embroidery hoops (15cm diameter; Joann Fabric; item #12212403), and the PVC tiles were cut into 10cm x 10cm squares, 0.6 cm thick. The surface area of the burlap hoops was calculated as the sum of the area of the inner and outer burlap faces (radius = 6.8cm, including wooden rim of hoop), and the inner and outer faces of the hoop (outer circumference = 42.73cm; inner circumference = 34.56cm; width = 1 cm). Total area of each burlap hoop was calculated to be 376 cm^2. For the screens, the same calculations were used to calculate the areas of the various wooden hoop components, and the area comprised of the screen filaments was calculated according to Tester et al. (2014). The total area of each screen hoop was calculated to be 255 cm^2.

The artificial substrates were then mounted onto polyvinyl chloride (PVC) frames (46cm per side), centered approximately 20cm above the frame base corresponding to the sediment surface (Fig. 4b). Two of each substrate was affixed to each of two frames for a total of six artificial substrates per frame and four replicates for each substrate. Both frames were then anchored one meter out from either side of one of the center pins at each site (Fig. 2). The artificial substrates were deployed each month, and collected the next month to provide an approximate 30-day soak time. For collection, a 1-quart Ziploc freezer bag was carefully fitted over each artificial substrate, with care taken to not disturb the material settled/growing on each substrate. After the substrate was secured in the first Ziploc bag, it was inserted into a second bag for protection and to prevent leakage. All samples were then stowed in a mesh dive bag for transport back to the vessel.

Back onshore, the macrophyte and artificial substrate samples were shaken and then filtered through the 200 and 20 µm sieves (PVC; Nitex® mesh; 6.3 cm diameter), refilled with 20 µm-filtered ambient seawater, and shaken and filtered an additional four times. One macrophyte and one artificial substrate sample from each site, each month was shaken and filtered an additional five times through the cleaned 20 µm sieves to determine if any *Gambierdiscus* cells remained after the initial five rinse steps. These QA/QC samples were referred to as "percent recovery samples". The fourth replicate of each artificial substrate was set aside as a back-up sample as needed (e.g., leakage or substrate failure). The material collected on the 20 µm sieve was then washed into a 15 mL centrifuge tube using ambient filtered seawater and brought to a volume of 15 mL. The percent recovery samples were rinsed and washed into a separate 15 mL centrifuge tube using ambient filtered seawater and brought to a volume of 15 mL and labeled accordingly. All tubes were then preserved with 1% glutaraldehyde (by volume) and stored on ice for transport back to the laboratory and then in a 4˚ C refrigerator until analyzed. Macrophyte samples were stored back in their original 50 mL centrifuge tubes with ~35 mL of ambient filtered seawater and refrigerated until analyzed.

2.3 Macrophyte identification and sample size estimation.

Back at the laboratory, macrophyte samples were removed from the centrifuge tubes, blotted dry, and weighed (g wet weight) on a Mettler Toledo AL204 balance. The macrophytes were then identified, using keys as necessary (Littler and Littler 2000; Dawes and Mathieson 2008), and included microscopy and thallus cross-sectioning as needed. Selected macrophyte samples were flattened under glass on a photography light table and photographed using a Canon Rebel EIS

192 digital XTI camera alongside a 20 cm ruler with mm markings (Fig. 3). The photographs were
193 then imported into Image J software (http://imagej.nih.gov/ij/) for surface area determination,
194 where pixel counts were converted to cm^2 using a pixel calibration factor obtained by calculating
195 the pixel widths per mm on the ruler. As the surface area was determined for only one side of the
196 macrophyte using this method, the values obtained were multiplied by two to account for the
197 other side of the macrophyte. Regression equations were derived to convert algal biomass (g wet
198 weight) to surface area (cm^2), which were then used to calculate the surface areas of all algal
199 samples collected and analyzed. The surface area of *Thalassia* blades was calculated by
200 multiplying the blade width by the blade length, and multiplying by two to account of both sides
201 of the blade.
202
203 *2.4* Gambierdiscus *cell enumeration.*
204 The abundance of *Gambierdiscus* cells was determined by transferring 3 mL of the epiphyte
205 sample into each of three wells in a six well flat bottomed tissue culture plate (Corning™
206 Costar™), stained with Uvitex® (similar to calcofluor; Polysciences, Ltd., cat. #19517-10; for
207 armored dinoflagellates), and analyzed on an Olympus IX71 inverted microscope at powers of
208 200x and 400x using a DAPI filter. Sample cell densities were determined by multiplying the
209 summed cell counts from the three wells by a subsample proportion factor (i.e., 9 mL out of the
210 15 mL washed off of the 20 µm sieve = 9/15), and then dividing this value by either the
211 macrophyte wet weight or surface area to provide values for *Gambierdiscus* cells g ww^{-1} or cm^{-2},
212 respectively. Discrimination among *Gambierdiscus* species was not possible with this level of
213 microscopy, so counts in this study are given for total *Gambierdiscus* spp.
214

215 *2.5 Data analysis.*

216 The cell densities calculated for each replicate were averaged for each macrophyte and artificial
217 substrate each site for each month. This step was taken to acknowledge that 1) missing values
218 (i.e., where three replicates were not collected or analyzed) prevented the use of repeated
219 measures analysis; and 2) replicate #1 from one substrate does not necessarily equate to replicate
220 #1 from another substrate (critical for use of the paired t-test analysis explained below). Percent
221 recovery values were calculated by dividing the cell densities (cells g^{-1} ww) from the percent
222 recovery samples by the cell densities of the samples collected in the first five rinses.
223 Coefficients of variation were calculated from each triplicate set of samples for each macrophyte
224 and artificial substrate to assess the variability (i.e., patchiness) of *Gambierdiscus* cell densities
225 at each site and on each substrate by dividing the standard deviation among each triplicate set by
226 the average of each set.

227

228 In preparation for statistical analysis, Cell densities were tested for normality and homogeneous
229 variance using the EXPLORE procedure in SPSS 23. The data had to be log transformed to meet
230 these parametric requirements. Log transformation was conducted on the cell density data after
231 the values were multiplied by 100 to ensure the transformed variables were > 0; a step necessary
232 for the regression analyses outlined below which utilized a y-intercept value of zero.

233

234 In a few cases, identified outliers (outside of the 95% confidence intervals on boxplots) were
235 omitted to make the data normal (e.g., for the overall analysis - LKH *Laurencia* wet weight (ww)
236 from July 2010 (0 cells g^{-1} ww) and LKH screen from July 2012 (0 cells cm^{-2}); for the LKH-
237 specific analysis - *Dictyota* wet weight from March 2012 and August 2012 (0 cells g^{-1} ww,

238 respectively); and for the TRL-specific analysis - tile from April 2012 (0.99 cells cm^{-2})). In four
239 cases, however, no outliers were identified and the data were non-normal because of a high
240 frequency of zero values (i.e., overall *Dictyota* cells g^{-1} ww and cells cm^{-2} (20% were zeros) and
241 TPH *Dictyota* cells g^{-1} ww and cells cm^{-2} (50% were zeros)). In these cases, it was decided to
242 also analyze these data using parametric methods as the zeros were important in comparing cell
243 densities on *Dictyota* versus the other substrates. Care was taken in interpreting the results to
244 account for potential anomalies caused by the inclusion of these zero values. This approach was
245 justified by the need to directly compare and quantify *Gambierdiscus* cell densities on the
246 various substrates (rather than relying on ranking as would be the case with many of the non-
247 parametric tests), with a particular need to test for the predicative capability of the artificial
248 substrates (i.e., linear regression analysis).
249
250 The transformed cell densities for the macrophytes were compared using paired t-tests to
251 determine if *Gambierdiscus* cell densities were higher on one macrophyte versus another both in
252 terms of log cells 100g ww^{-1} and log cells $100cm^{-2}$ to account for different macrophyte
253 morphologies (i.e., degree of calcification). Pearson correlation analyses were also conducted to
254 test if the macrophytes carried similar cell densities over the course of the study. The artificial
255 substrate cell densities (log cells $100cm^{-2}$) were then compared to the macrophyte cell densities
256 (log cells 100g ww^{-1} and log cells $100cm^{-2}$) using a no-intercept regression model, which
257 allowed direct comparison of the slopes between the artificial substrate and macrophyte cell
258 densities to examine if they differed between substrates, macrophyte species, and/or sites – a
259 comparison made more difficult if y-intercepts were included. When using a no-intercept
260 regression model, the R^2 value provided explains the variability of the dependent variable about

the origin and is not, therefore, applicable for regression goodness-of-fit. This is due in part to the fact that the total sum of squares is not corrected for a constant, as the constant is zero in this case (SPSS 23). Pearson correlation analysis was therefore used to test how well the artificial substrates mirrored the macrophyte cell densities, as the Pearson correlation computations do not have these constraints. In cases where the Pearson correlation coefficient was not significant between an artificial substrate and macrophyte substrate, it was assumed that the artificial substrate cell densities could not be used to predict macrophyte cell densities, and the slope was therefore set to zero in such cases.

3. RESULTS

3.1 Sample Collection

Over the 13-month course of the study, 98 *Dictyota*, 155 *Halimeda*, 75 *Laurencia,* and 39 *Thalassia* samples were collected and processed. Additionally, 146 burlap hoops, 148 screen hoops, and 150 tiles (out of 160 possible) were collected and processed during this time frame. The remaining samples were either lost due to a broken hoop (burlap and screen) or lost frames (all three substrates). Additionally, the fourth replicate hoop for burlap and screen substrates was lost in other instances not accounted for in these values. The site-by-site collection was as follows: HGB (39 each of *Halimeda* and *Thalassia,* and 40 of each artificial substrate); LKH (36 *Dictyota*, 39 *Halimeda*, 37 *Laurencia*, and 40 of each artificial substrate); TPH (26 *Dictyota*, 38 *Halimeda*, 38 *Laurencia*, and 40 of each artificial substrate); and TRL (36 *Dictyota*, 39 *Halimeda*, and 40 of each artificial substrate). Each *Dictyota* sample averaged 0.75 g wet weight (ww); *Halimeda* – 2.2 g ww; *Laurencia* – 1.55 g ww; and *Thalassia* – 0.5 g ww). Overall, over 99% of the *Gambierdiscus* cells were collected in the first five rinses of processing the

macrophytes and artificial substrates, indicating that the cell harvesting method utilized was thorough. Coefficients of variation (CV) ranged from 0.06 to 1.73 for macrophyte samples and from 0 to 1.73 for the artificial substrate samples (Table 1). *Thalassia* samples had the lowest average CV (0.53), possibly due to being sampled at only one site (HGB). *Halimeda* was otherwise lowest (0.68) and *Laurencia* was highest (0.84). The artificial substrates had lower CV values in general, ranging from 0 to 1.73. Burlap had the lowest average value (0.49) whereas tile had the highest (0.67). The remaining analyses utilized all samples except for the omitted outliers identified earlier were used in subsequent analysis.

3.2 Macrophyte wet weight to surface area comparisons

Regression analysis comparisons between algae wet weights and surface area (determined from ImageJ analysis) demonstrated that wet weight data could be reasonably converted to algal surface area for the various species examined (Table 2), albeit with some degree of error given that 4 out of 6 R^2 values were below 0.7 (Table 2). The fit (R^2) of the regression equations varied from a low value of 0.450 (for *Dictyota menstrualis*) to a high value of 0.964 (for *Laurencia gemmifera*). The low R^2 value for *D. menstrualis* could be an artifact of a low sample number (15), or varying morphology over seasons (e.g., Yñiguez et al. 2010; Brandt 2016; pers. obs.).

3.3 Paired t-test results

The paired t-test results indicated that overall (i.e., pooled across all sites), *Laurencia* harbored more *Gambierdiscus* cells than *Dictyota* and *Halimeda* on a wet weight basis (Table 3; Fig. 5). Additionally, more cells were present on *Thalassia* than *Halimeda* at HGB (Fig. 5a). At LKH,

more cells were present on *Dictyota* than *Halimeda* (Fig. 5b), with the opposite relationship evident at TPH (Fig. 5c). More cells were present on *Laurencia* than *Dictyota* at TPH (Fig. 5c). No other host differences in cell densities were apparent on a wet weight basis, including TRL (Table 3; Fig. 5d). In terms of surface area, *Halimeda* hosted more cells than *Thalassia* at HGB (Fig. 6a), opposite of the wet weight comparison (Fig. 5a). Both *Laurencia* and *Halimeda* hosted more cells than *Dictyota* overall and at TPH (Fig. 6c), but not at LKH (Fig. 6b). No other host differences in cell densities were apparent on a surface area basis, including TRL (Table 4; Fig. 6d).

3.4 Macrophyte cell density correlations

In addition to the differences in cell densities exhibited among the host macrophytes, the patterns (correlations) of cell density also differed among the hosts (Table 3). On a wet weight basis, *Dictyota* cell densities correlated with *Laurencia* and *Halimeda* cell densities at LKH, but not overall, or at TPH or TRL, possibly reflecting the influence of the high number of zero values in *Dictyota* cell densities. Cell densities on *Halimeda* were correlated with densities on *Laurencia* overall, and at LKH and TPH. Cell densities on *Halimeda* were also correlated with *Thalassia* cell densities at HGB. Results were similar in terms of surface area, but with slightly better correlations (Table 4). Cell densities on *Dictyota* correlated with densities on *Laurencia* at LKH and TPH, but not overall. Cell densities on *Dictyota* and *Halimeda* were correlated at LKH, but not at TPH or TRL. Cell densities on *Halimeda* and *Laurencia* were correlated overall and at LKH, but not at TPH. As was the case with wet weight comparisons, cell densities on *Halimeda* and *Thalassia* were correlated on a surface area basis at HGB. Overall, 12 out of 22 (54%) of the macrophyte correlations were significant (Tables 3 and 4).

3.5 Correlation of Gambierdiscus *cell densities on macrophyte hosts (log cells 100g^{-1} ww) versus artificial substrates (log cells 100cm^{-2})*

Cell densities of *Gambierdiscus* on *Dictyota* (log cells 100g^{-1} ww) did not correlate with either screens or tiles when data were pooled, likely due to the insignificant correlations among the TPH and TRL samples (Table 5). Cell densities on *Dictyota* were significantly correlated with all three artificial substrates at LKH, however. Cell densities on *Halimeda* and *Laurencia* were strongly correlated with all three artificial substrates when samples were pooled, but the relationships were weaker on a site-by-site basis and insignificant for *Laurencia* at LKH (tiles) and TPH (burlap and screens), and for *Halimeda* at TRL (screens and tiles). The *Halimeda* – burlap correlations were best across sites (Fig. 7a), whereas *Dictyota* – tile correlations were worst (Fig. 7b). HGB had the highest correlations overall (macrophytes with burlap; Fig. 7c), whereas TRL displayed the worst (macrophytes with tiles; Fig. 7d).

3.6 Correlation of Gambierdiscus *cell densities on macrophyte hosts (log cells 100cm^{-2}) versus artificial substrates (log cells 100cm^{-2})*

Similar results were obtained when comparing *Gambierdiscus* cell densities on a per cm^2 basis (Table 6), in which cell densities on *Dictyota* did not correlate with cell densities on tiles when samples were pooled, but were weakly correlated with cell densities on screens, and strongly correlated with cell densities on burlap. Once again, cell densities on *Halimeda* and *Laurencia* were strongly correlated with those on the artificial substrates when data were pooled, but were weaker on a site-by-site basis. Insignificant correlations were computed for cell densities on *Laurencia* versus tiles at LKH, *Dictyota* versus burlap at TPH, *Halimeda* versus screens at TPH,

353 *Laurencia* versus burlap and screens at TPH, *Dictyota* versus all substrates at TRL, and
354 *Halimeda* versus screens and tiles at TRL. The *Halimeda* – screen correlations were best across
355 sites (Fig. 8a), whereas *Dictyota* – tile relationships were worst (Fig. 8b). HGB displayed the
356 best correlations between macrophytes and artificial substrates (screens; Fig. 8c), while TRL
357 displayed the worst (tiles; Fig. 8d). Overall, the results of the comparisons between cell densities
358 on macrophytes by surface area versus artificial substrates gave similar results (but slightly
359 higher correlations) than the wet weight comparisons. Additionally, no definitive patterns
360 between algae and artificial substrates were evident across sites. Overall, 78 out of 108 (72%) of
361 the macrophyte – artificial substrate correlations were significant (Tables 5 and 6).
362
363 *3.7 Comparison of slopes to assess validity of artificial substrate estimates of* Gambierdiscus
364 *cell densities on macrophyte hosts (log cells 100g^{-1} ww)*
365 Sixteen out of the 54 comparisons between cell densities on the macrophytes (log cells 100g^{-1}
366 ww) versus artificial substrates had non-significant correlations and therefore non-significant
367 regression results (Table 7). In these cases, therefore, the artificial substrates could not predict
368 cell densities on the macrophytes. The range of slope error (% error) between the macrophytes
369 and artificial substrates within sites varied from 3% (all comparisons at HGB; and *Halimeda* at
370 TPH) to 174% (*Dictyota* at TPH), the latter high error due to non-significant regressions at TPH.
371 Between-site variability was high, as depicted by the high % errors associated for each artificial
372 substrate overall (59%, 74%, and 92%) for tiles, burlap, and screens, respectively.
373
374 *3.8 Comparison of slopes to assess validity of artificial substrate estimates of* Gambierdiscus
375 *cell densities on macrophyte hosts (log cells 100cm^{-2})*

Thirteen out of the 54 comparisons between cell densities on the macrophytes (log cells $100cm^{-2}$) versus artificial substrates had non-significant correlations and therefore insignificant regression results (Table 8). Once again, as the artificial substrates could not predict cell densities on the macrophytes in these instances, the slope of each of these relationships was set to zero. Therefore, *Dictyota* cell densities could not be predicted using artificial substrates at TPH (burlap) or TRL (all substrates), nor across sites (tiles). Tiles could not predict cell densities on *Laurencia* at LKH, but other relationships were significant at this site. At TPH, screens were only effective for *Dictyota*, and burlap for *Halimeda* and pooled macrophyte samples. Similarly, at TRL screens only could predict cell densities on pooled macrophyte samples, and burlap was only effective for *Halimeda* and pooled samples. HGB produced the lowest error among substrates and macrophytes (3%), while TPH and TRL had error values ranging from 87-173%. Once again, between-site variability was high, as depicted by the high % errors associated for each artificial substrate overall (50%, 59%, and 75%) for burlap, tiles, and screens, respectively.

As the macrophyte versus artificial substrate cell densities were both calculated as log cells $100cm^{-2}$ in Table 8 (as opposed to log cells $100g^{-1}$ ww macrophyte versus log cells $100cm^{-2}$ artificial substrate in Table 7), the slopes can be used to compare cell densities on macrophytes versus artificial substrates. Average slopes (both within site and within each substrate), were < 1 in all cases except for the HGB comparisons (in which slopes were just above 1). These results indicate that generally speaking, the artificial substrates harbored more cells cm^{-2} than the macrophytes.

4. Discussion

4.1 Gambierdiscus *cell density differences on macrophyte hosts*

As has been reported in many previous studies (e.g., Parsons and Preskitt 2007), *Gambierdiscus* densities on macrophytes were variable, with some indication of substrate preference; e.g., *Laurencia* over *Halimeda* and *Dictyota* in terms of both cells ww^{-1} and cm^{-2} macrophyte for pooled samples (Tables 3 and 4). Such preferences were not consistent across sites, however; e.g., *Dictyota* harbored more cells than *Halimeda* at LKH (on a wet weight basis), whereas the opposite was documented at TPH (Table 3; Fig. 5), possibly reflecting the presence of different *Gambierdiscus* species at the two sites (Parsons et al. 2012). Overall, 6 out of 11 comparisons by wet weight (Table 3), and 5 out of 11 by surface area (Table 4), produced significant differences in *Gambierdiscus* cell densities between macrophyte species. Altogether, these results suggest that macrophyte preferences may be a factor in the epiphytic behavior of *Gambierdiscus*, but the presence of multiple species with different host preferences (Rains and Parsons 2015) may be masking such signals.

In a similar fashion, *Gambierdiscus* cell densities changed over time in a similar (correlated) way among the various macrophyte hosts in some, but not all cases (Tables 3 and 4). The lack of correlation in some cases, however, could reflect preferences and behaviors of the different *Gambierdiscus* species (Rains and Parsons 2015), or other, unaccounted for factors such as preferential grazing of herbivores (removing more cells from one macrophyte species versus another; Kopp et al. 2010). Importantly, these latter results indicate that *Gambierdiscus* cell densities do not change in a similar manner across the different macrophyte hosts over time, which means that exogenous, universal factors (such as temperature) do not solely dictate the dynamics of *Gambierdiscus* cell densities in the benthos, but rather that other, possibly

422 macrophyte-specific factors are also important (Bomber et al. 1989). These factors could include
423 grazing and host preferences as mentioned above, the production of nutrients or other beneficial
424 substances by the host (Bomber et al. 1989), the three-dimensional structure of the host
425 (providing more substrate and shading; Villareal and Morton 2002), as well as the influence of
426 other epibionts living on the macrophyte (e.g., Yasumoto et al. 1980; GEOHAB 2012).
427
428 *4.2 Algae – Artificial substrate correlations and regressions*
429 The results clearly demonstrated that none of the artificial substrates provided consistent proxy
430 estimates of *Gambierdiscus* cell densities (via regressions) or changes over time (via
431 correlations) on the macrophyte hosts from the four sites, although overall, tiles fared better than
432 burlap or screens (Tables 7 and 8). Surface area comparisons fared better than wet weight,
433 although 24% and 31% of these relationships were insignificant, respectively (Tables 7 and 8).
434 As such, monthly deployments of any of the substrates do not appear to be a reliable method to
435 monitor *Gambierdiscus* populations, at least in regards to the four Florida Keys sites sampled in
436 this study. There were some reliable results, however, such as strong relationships between
437 artificial substrate cell densities and those on *Halimeda* across the four sites (Tables 5 and 6;
438 Figs. 7a and 8a), and within sites (e.g., HGB; Tables 5 and 6; Figs. 7c and 8c), including low
439 errors between slopes among the artificial substrates (3%; Tables 7 and 8). These encouraging
440 results, however, were countered by poor relationships between cell densities on artificial
441 substrates versus macrophytes for *Dictyota* and at TRL, for example (Tables 5 and 6; Figs. 7b,d
442 and 8b,d), resulting in large errors in slope comparisons among artificial substrates (up to 200%;
443 Tables 7 and 8).
444

These stark differences could be due to variable behaviors exhibited by *Gambierdiscus* species towards different host macrophytes as mentioned previously (Parsons et al. 2011; Rains and Parsons 2015), or different swimming and attachment behaviors of *Gambierdiscus* cells in response to different hydrodynamic conditions at the four sites. For example, TRL is a reef crest site on the Florida Keys barrier reef complex approximately 11 km offshore, whereas HGB is a sheltered seagrass bed within 500 m of the shoreline. TRL is deeper (7 m versus 2 m) and subjected to stronger wave energy (pers. obs.; United States National Weather Service Marine Forecasts). Previous studies have reported that *Gambierdiscus* cells prefer calm, stable environments (Gillespie et al. 1985; Taylor 1985; Grzebyk et al. 1994), and that turbulence causes *Gambierdiscus* cells to attach to macrophyte hosts rather than swimming in close proximity to the host (Nakahara et al. 1996). Based on such observations, it appears *Gambierdiscus* cells will exhibit different behaviors under different hydrodynamic conditions, thereby causing different relationships between artificial and macrophyte substrates among disparate locations (e.g., Systems I-IV, Tindal and Morton 1998).

Interestingly, 19 out of the 28 individual regression analysis slopes (not pooled or averaged) were < 1 (Table 8), suggesting that cell densities were higher per unit area on artificial substrates than macrophytes. These findings may be explained by several factors including: 1) the effects of grazers on the macrophytes (removing *Gambierdiscus* cells; Loeffler et al. 2015); 2) possible inhibition of cell settlement and *Gambierdiscus* growth by the macrophyte hosts (Parsons et al. 2011); or 3) lack of a nutritional or commensal benefit provided by the macrophyte. These results suggest that *Gambierdiscus* cells may just settle on substrates regardless of chemical cues present, or may be influenced by chemical cues of other members of the epiphytic community.

468 The results of Caire et al. (1985), however, are not in agreement with this conclusion as
469 *Gambierdiscus* cells did not settle on their tape strips until an epibiota was established.
470
471 *4.3 Assessment of the usefulness of artificial substrates to monitor* Gambierdiscus
472 *populations*
473 While convenient, and likely to be employed in monitoring programs until better techniques are
474 developed and vetted (e.g., Berdalet et al. 2017), artificial substrates may not be providing an
475 accurate assessment of *Gambierdiscus* abundance in the benthos. Cell densities on artificial
476 substrates are not consistently correlated with those on macrophyte hosts, and the slopes of the
477 pairings that have significant correlations vary widely (up to 200%) limiting the usefulness of
478 artificial substrates for quantification purposes. This latter short-coming is further high-lighted
479 when considering that the regression relationships were calculated using log-transformed data
480 (needed for normalization), amplifying the error of estimation of cell densities on macrophytes.
481 For example, the overall average *Gambierdiscus* cell density for this study across all sites and
482 macrophytes was 135 cells g^{-1} ww (not log-transformed). The average slope of artificial substrate
483 cell densities to those on the macrophytes was 0.67, with a standard deviation of 0.41, resulting
484 in an overall error of 61%. This error translates into slope variation of 0.41 to 1.08 (rounded
485 figures), which would result in macrophyte cell density estimates of 20 to 2690 cells g^{-1} ww.
486 Such error on the order of two orders of magnitude will not be useful for effective monitoring
487 purposes.
488
489 An additional concern is the high degree of variability displayed in the triplicate samples
490 collected (Table 1). The coefficients of variation ranged from 0 to 1.73, with an average of 0.72

for the macrophyte samples and 0.59 for the artificial substrates. These values are similar to those reported by Tester et al. (2014), who reported values of 0.72 and 0.54 for macroalgae and screens, respectively, and are within the ranges reported in earlier studies (e.g., 0.86 – 1.7; Carlson 1984). Although these values are similar, they demonstrate a critical hurdle that must be addressed as ciguatera monitoring programs refine their methods; sample variability and *Gambierdiscus* patchiness (Bertalet et al. 2017). While larger amounts (e.g., Okolodkov et al. 2014; 100+ g ww collections) or numbers (e.g., Tester et al. 2014; >5 samples) of sample may lower variability, such added effort must be weighed against ease of sample collection, processing, and analysis.

While there is undoubtedly a need to develop and implement a standardized protocol to monitor *Gambierdiscus* populations across multiple regions and environments, monthly artificial substrate deployments appear to have inherent weaknesses that will prevent their effective use on such a large scale. Rather, the best course of action may be to evaluate one of the artificial substrates for a specific site to determine if a significant relationship is possible with one or several macrophytes. Clearly some sites may not be suitable for such monitoring techniques (e.g., System I sites like TRL; Tindall and Morton 1998), thereby requiring the utilization of different methodologies (e.g., the Benthic Dinoflagellate Integrator (BEDI); Mangialajo et al. 2017). If a significant result is obtained, it may be necessary to retest the approach in different years or different times of the same year. The results presented here suggest that tiles may be the best substrate to use for monthly deployments, as there was less risk of substrate failure (i.e., hoop breakage) and overall errors were lower (but still less than ideal) for tiles (Tables 7 and 8).

513 Until the time that better monitoring techniques can be developed and tested, extreme caution
514 should be taken when interpreting the data garnered from artificial substrate deployments.
515
516 Acknowledgements
517 We would like to thank Chris Lienhardt, Erin Rasnake, Kevin Tyre, and Shannon White for their
518 assistance in the field and in the laboratory. Funding for this work was provided by NOAA NOS
519 (Cooperative Agreements NA11NOS478-0060 and NA11NOS4780028). This is ECOHAB
520 publication number 890.

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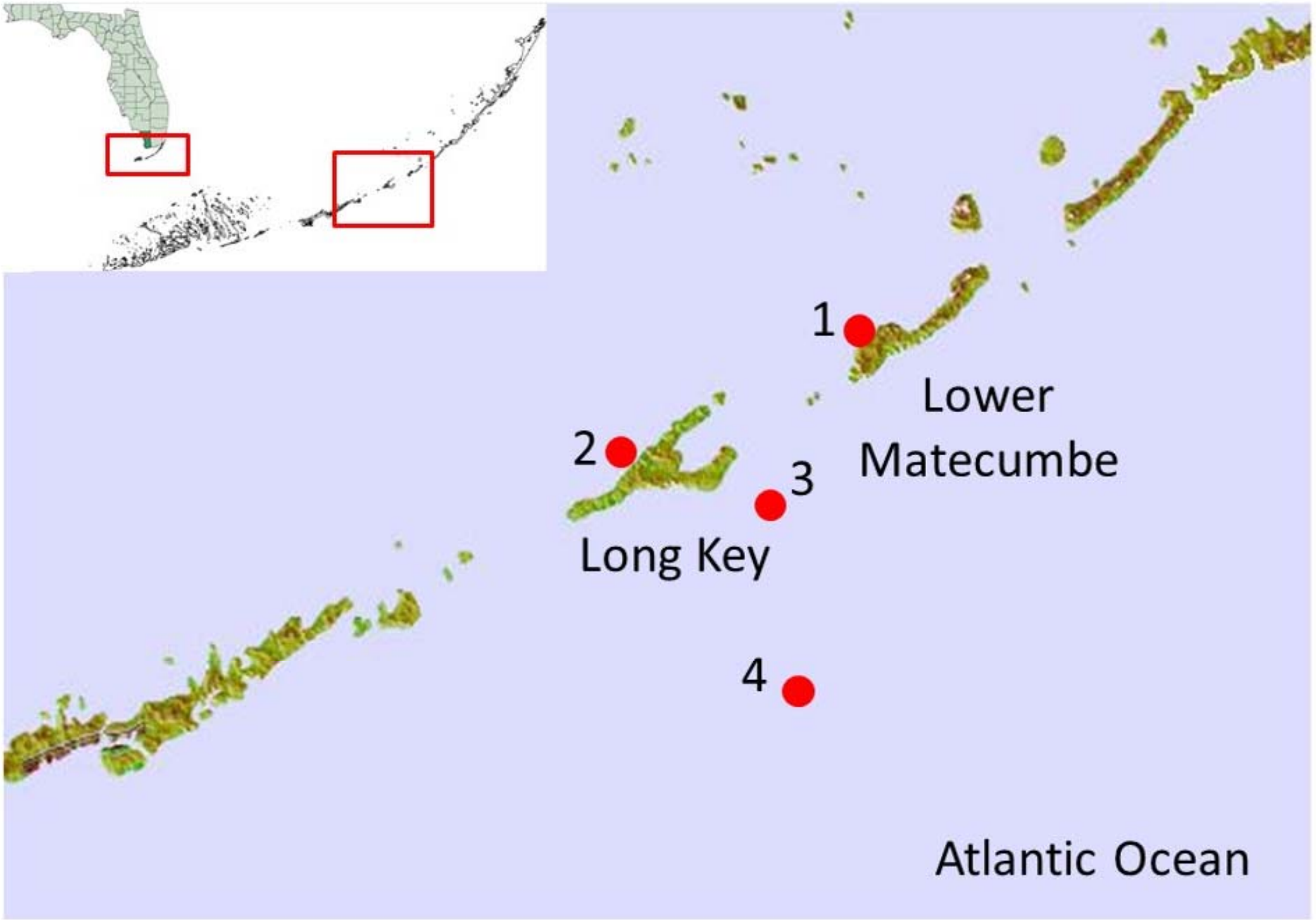
List of Figures

691 Figure 6. *Gambierdiscus* cell abundance (log cells 100cm^{-2}) on macrophytes present at A) HGB;
692 B) LKH; C) TPH; and D) TRL. Solid gray line: *Dictyota*; dashed line: *Laurencia*; solid black
693 line: *Halimeda*; dotted line: *Thalassia*.
694
695 Figure 7. *Gambierdiscus* cell abundance on macrophytes (log cells 100g^{-1} ww) versus artificial
696 substrates (log cells 100cm^{-2}) displaying A) the best relationships across sites (*Halimeda* versus
697 burlap); B) the worst relationships across sites (*Dictyota* versus tile); C) the site with the best
698 relationships (HGB; screen); and D) the site with the worst relationships (TRL; tile).
699
700 Figure 8. *Gambierdiscus* cell abundance on macrophytes (log cells 100cm^{-2}) versus artificial
701 substrates (log cells 100cm^{-2}) displaying A) the best relationships across sites (*Halimeda* versus
702 screen); B) the worst relationships across sites (*Dictyota* versus tile); C) the site with the best
703 relationships (HGB; screen); and D) the site with the worst relationships (TRL; tile).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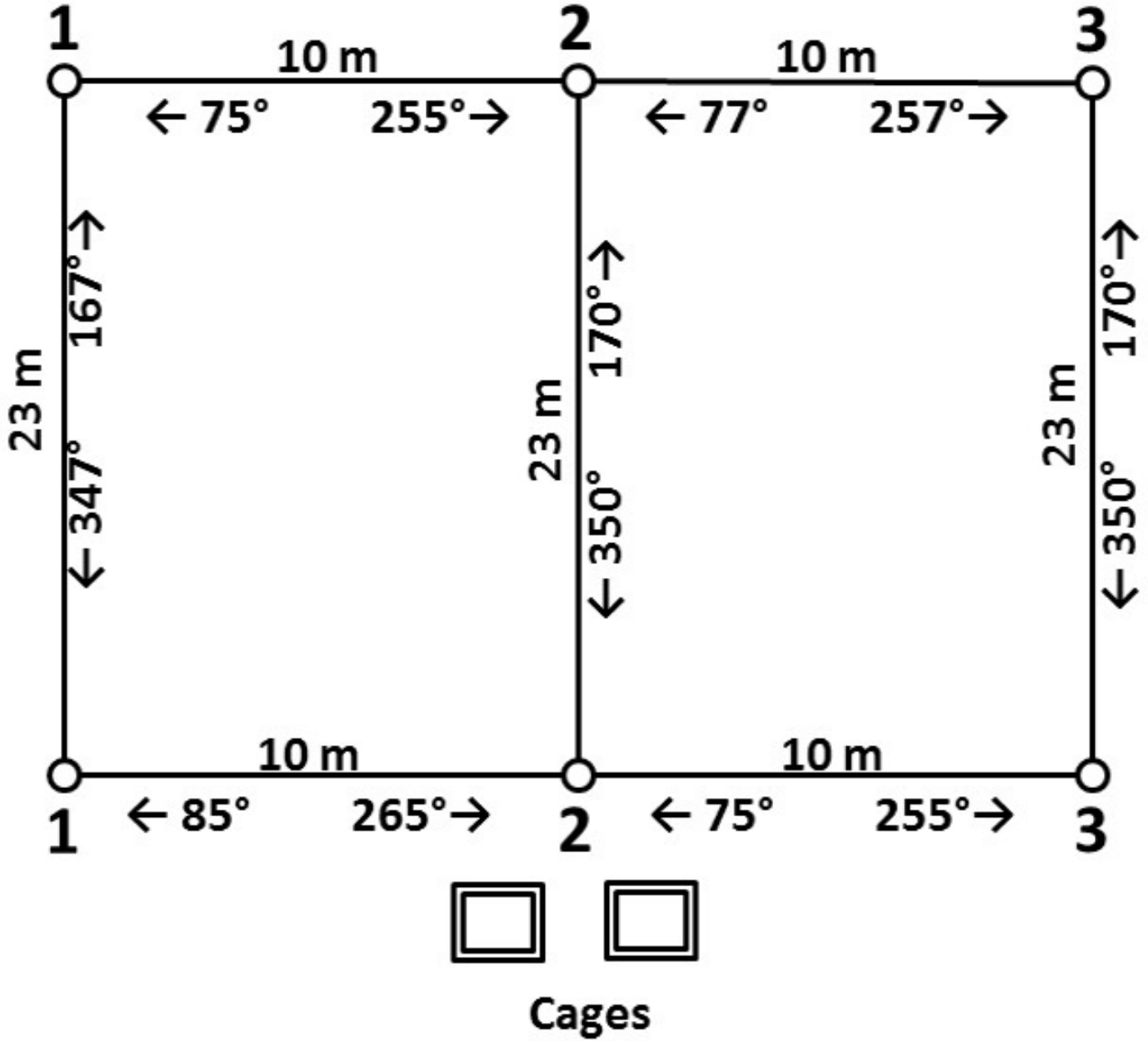
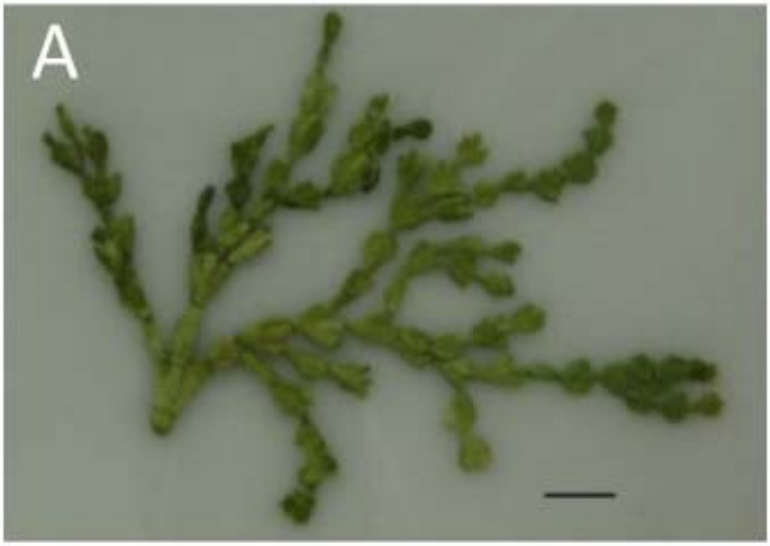
2
3
Long Key
4
Atlantic Ocean


1
10 m
2
10 m
3
← 75°
255°→
← 77°
257°→
23 m
167°→
← 347°
23 m
170°→
← 350°
23 m
170°→
← 350°
10 m
10 m
1
← 85°
265°→
2
← 75°
255°→
3
Cages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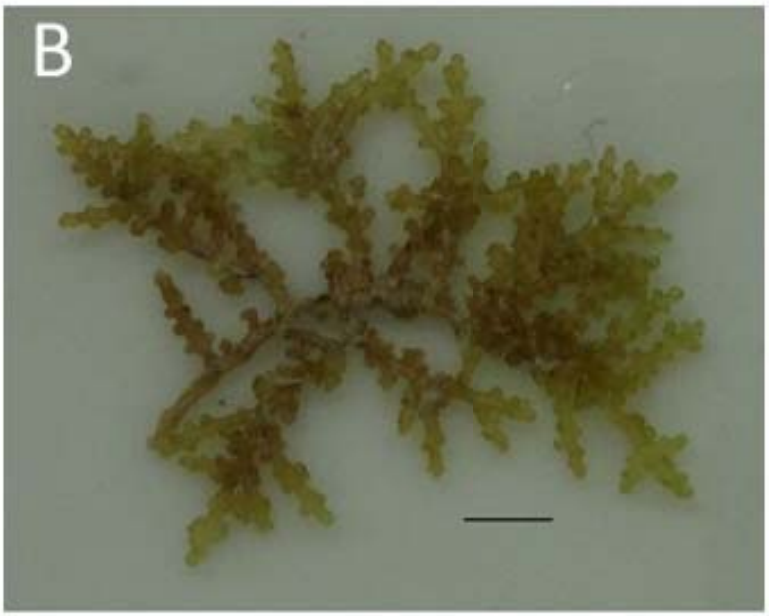
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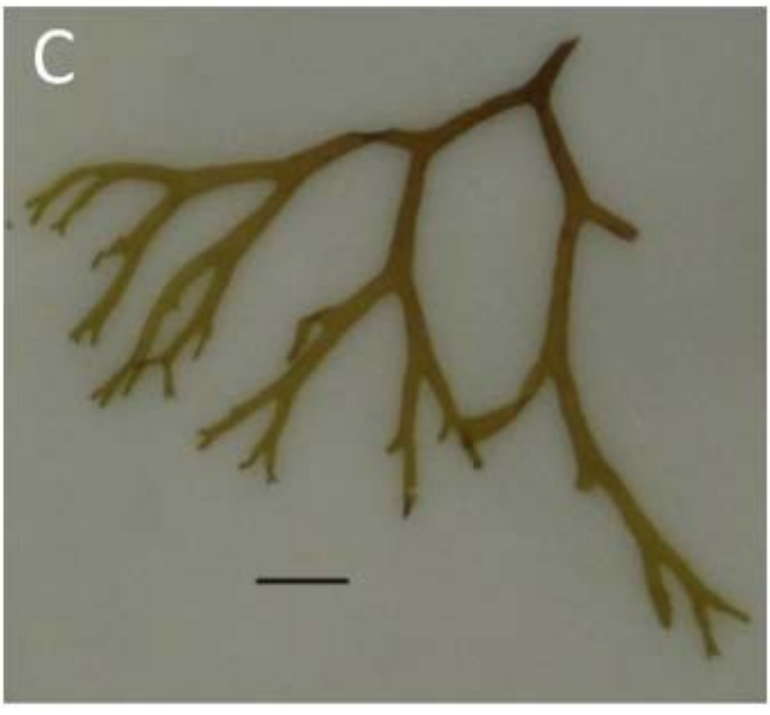
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D


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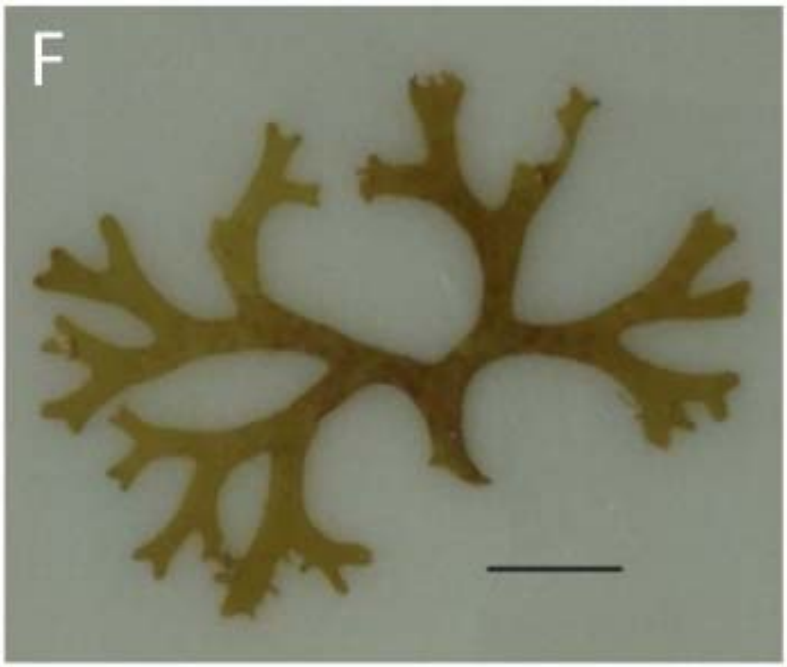
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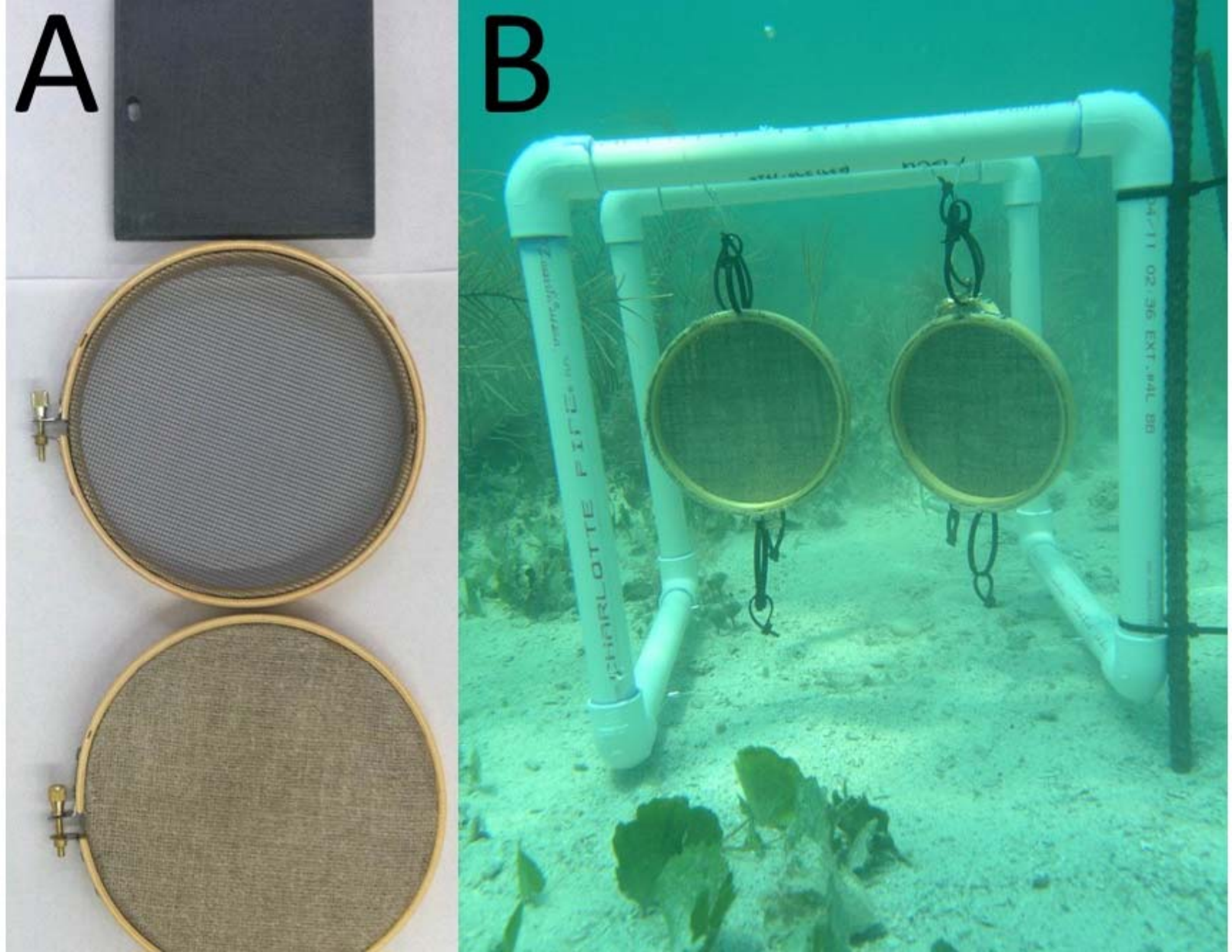
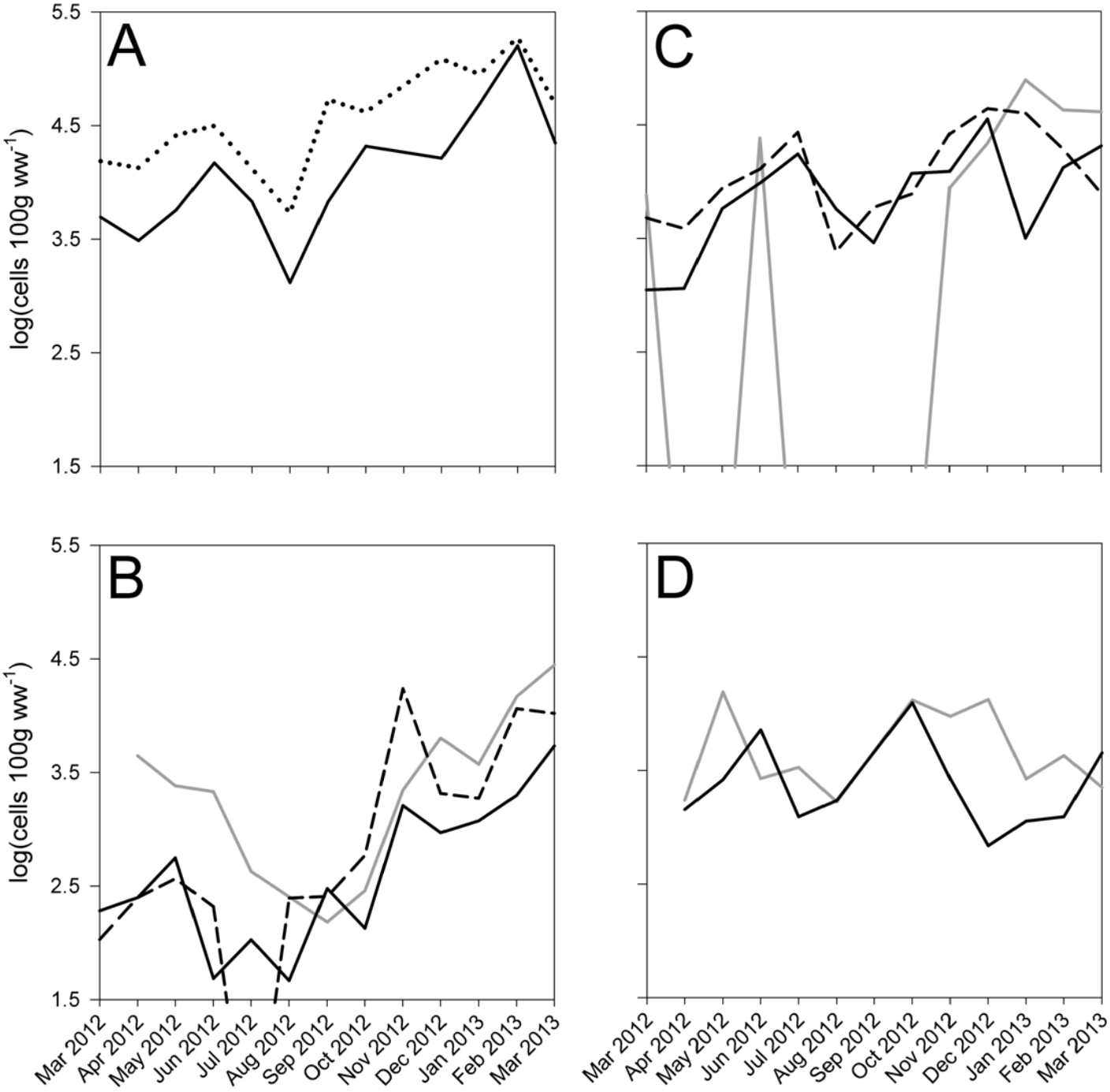
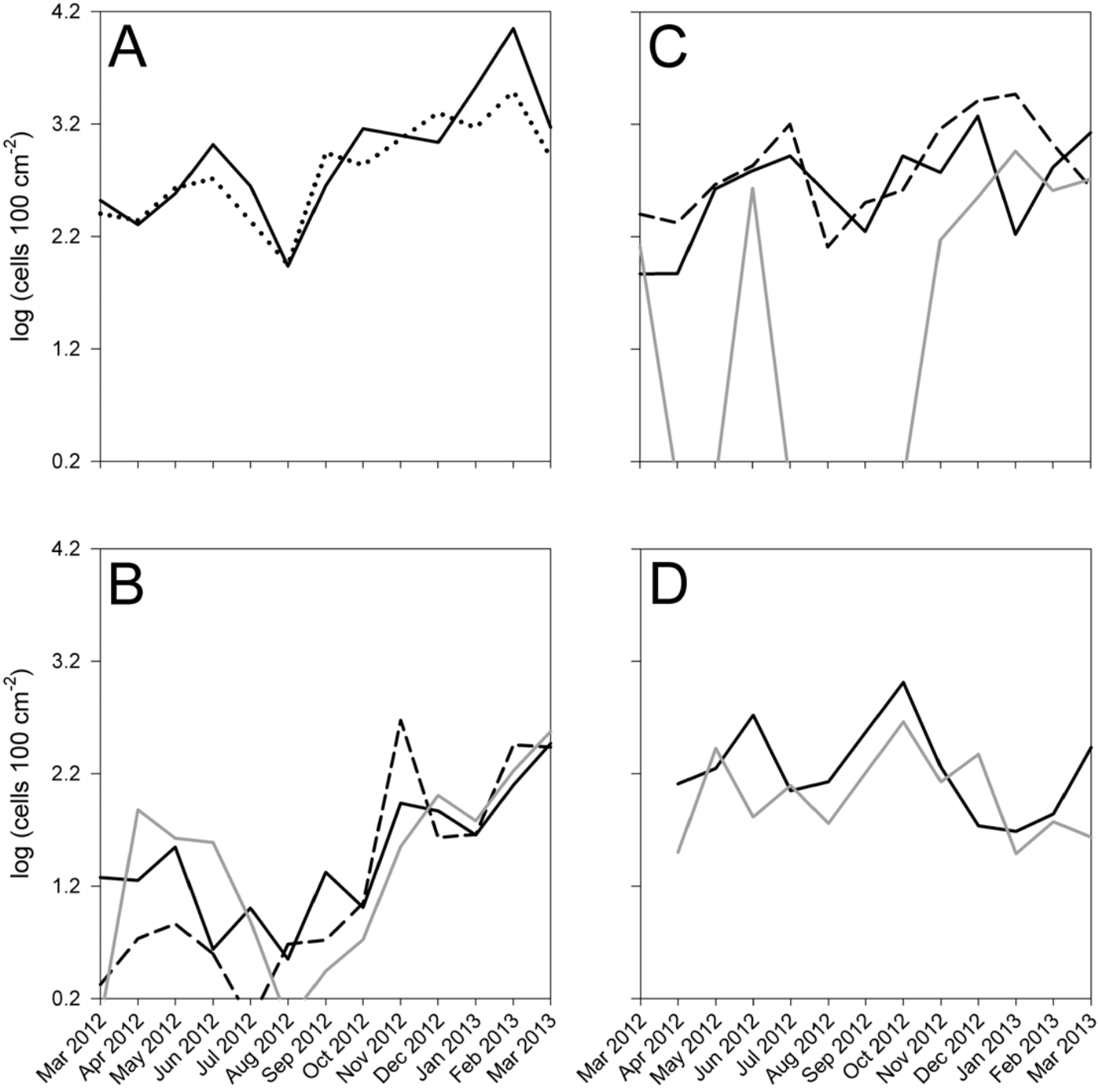
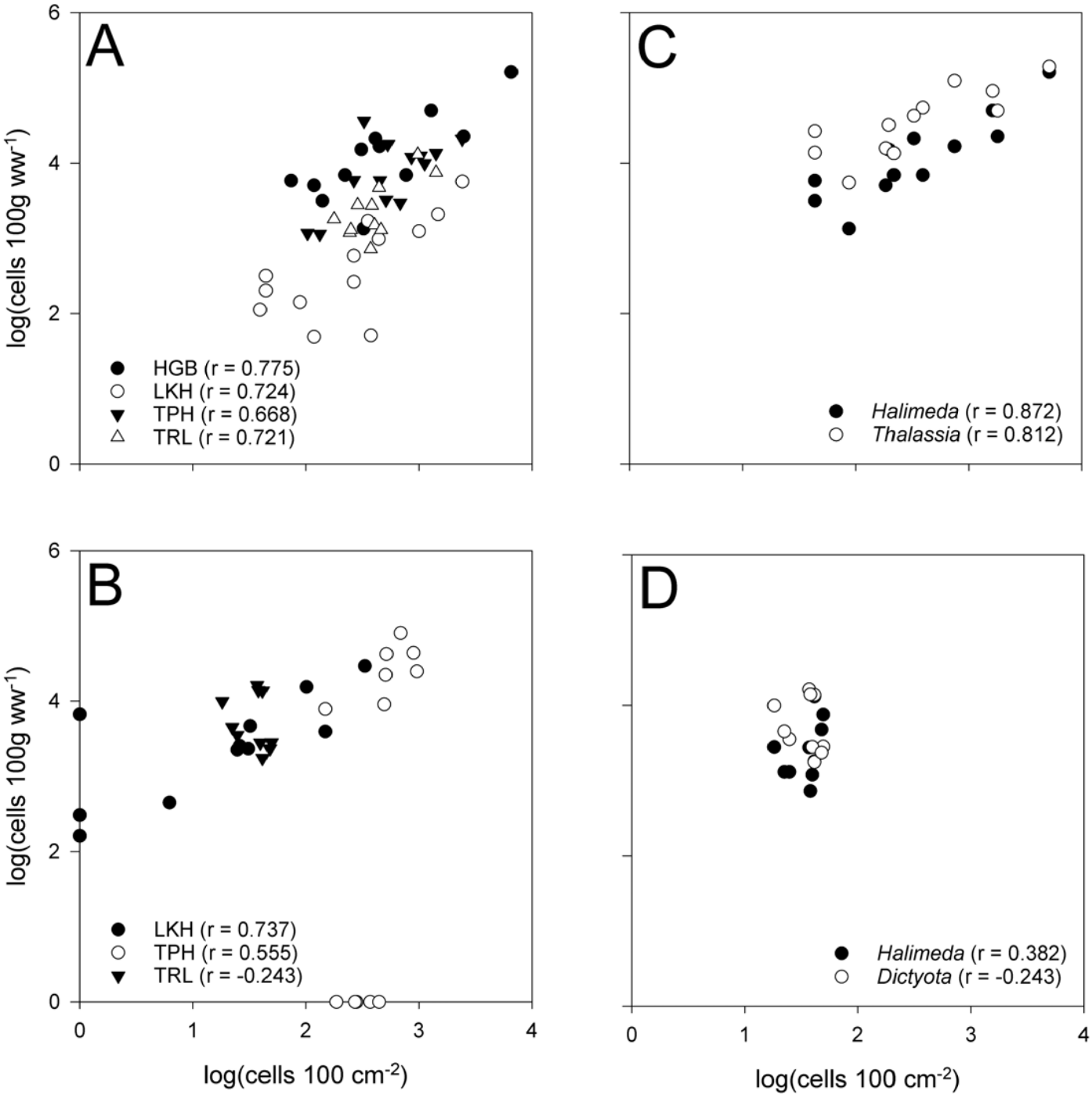

A
B
CHARLOTTE PIPE


A
B
C
D
log(cells 100g ww-1)
5.5
4.5
3.5
2.5
1.5
Mar 2012
Apr 2012
May 2012
Jun 2012
Jul 2012
Aug 2012
Sep 2012
Oct 2012
Nov 2012
Dec 2012
Jan 2013
Feb 2013
Mar 2013


A
B
C
D
4.2
3.2
2.2
1.2
0.2
log (cells 100 cm-2)
Mar 2012
Apr 2012
May 2012
Jun 2012
Jul 2012
Aug 2012
Sep 2012
Oct 2012
Nov 2012
Dec 2012
Jan 2013
Feb 2013
Mar 2013


A
B
C
D
log(cells 100g ww-1)
log(cells 100 cm-2)
HGB (r = 0.775)
LKH (r = 0.724)
TPH (r = 0.668)
TRL (r = 0.721)
LKH (r = 0.737)
TPH (r = 0.555)
TRL (r = -0.243)
Halimeda (r = 0.872)
Thalassia (r = 0.812)
Halimeda (r = 0.382)
Dictyota (r = -0.243)
0
2
4
6
0
1
2
3
4

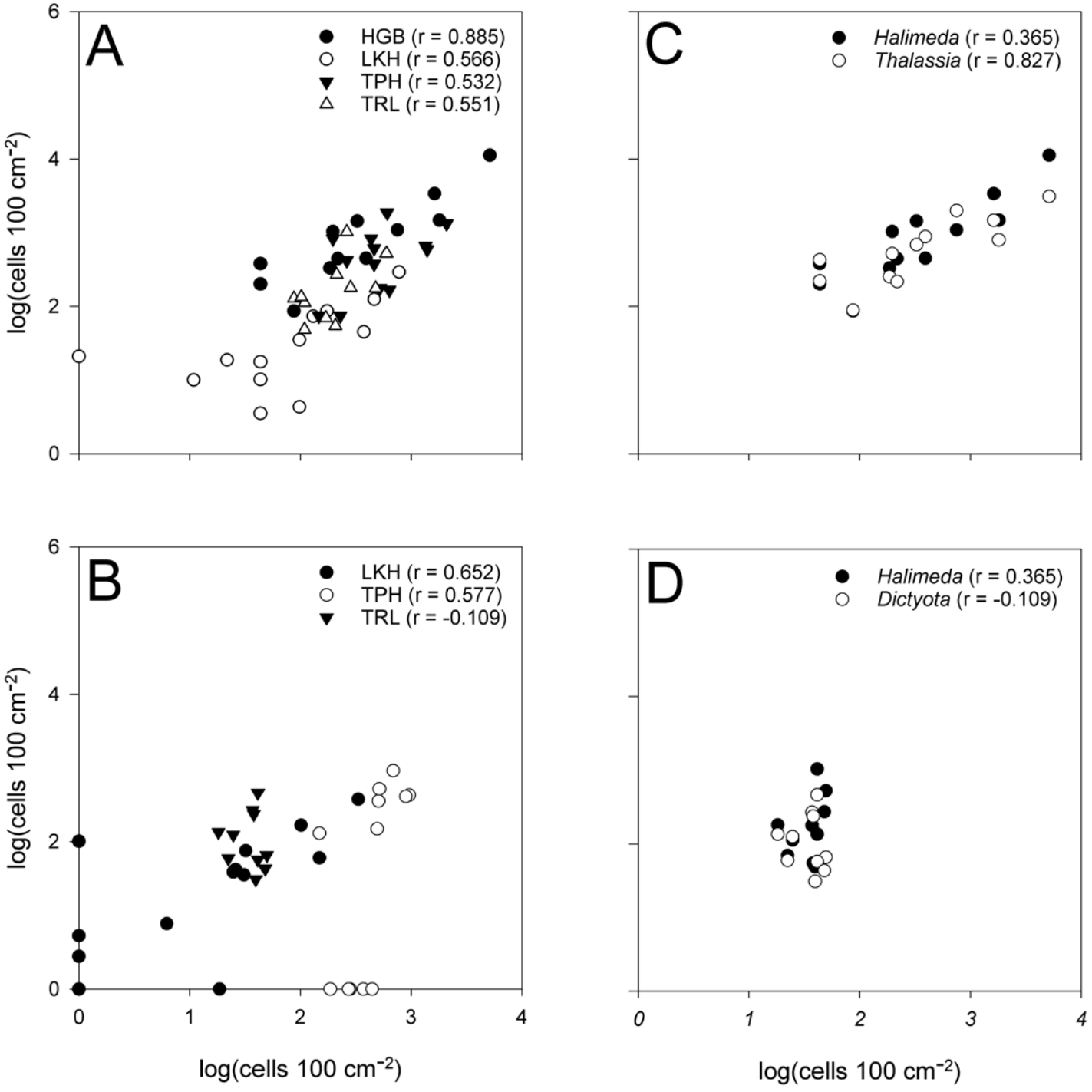

A
HGB (r = 0.885)
LKH (r = 0.566)
TPH (r = 0.532)
TRL (r = 0.551)
C
Halimeda (r = 0.365)
Thalassia (r = 0.827)
B
LKH (r = 0.652)
TPH (r = 0.577)
TRL (r = -0.109)
D
Halimeda (r = 0.365)
Dictyota (r = -0.109)
log(cells 100 cm-2)
log(cells 100 cm-2)
0
2
4
6
0
1
2
3
4

Table 1. Coefficients of variation for macrophyte and artificial substrate samples. The ranges are given (minimum to maximum), as well as the average value (in parentheses).

Substrate	HGB	LKH	TPH	TRL	Overall
Dictyota	-	0.22 – 1.73 (0.86)	0.30 – 0.96 (0.68)	0.21 – 1.58 (0.71)	0.21 – 1.73 (0.75)
Halimeda	0.20 – 1.14 (0.55)	0.39 – 1.73 (1.04)	0.06 – 1.73 (0.46)	0.19 – 1.23 (0.66)	0.06 – 1.73 (0.68)
Laurencia	-	0.17 – 1.73 (1.11)	0.20 – 1.00 (0.59)	-	0.17 – 1.73 (0.84)
Thalassia	0.18 – 1.12 (0.53)	-	-	-	0.18 – 1.12 (0.53)
Burlap	0.15 – 1.41 (0.46)	0.25 – 1.41 (0.58)	0 – 0.96 (0.45)	0.09 – 1.09 (0.44)	0 – 1.41 (0.49)
Screen	0 – 1.73 (0.63)	0.23 – 1.73 (0.93)	0.07 – 0.75 (0.40)	0.27 – 0.93 (0.54)	0 – 1.73 (0.61)
Tile	0.12 – 0.58 (0.34)	0.28 – 1.73 (1.05)	0.16 – 1.06 (0.49)	0.52 – 1.73 (0.94)	0.12 – 1.73 (0.67)

Table 2. Regression equations to convert algal biomass (g wet weight = w.w.) to surface area (cm^2 = s.a.), where n = number of samples used to compute the regression equation.

Species	Equation	R^2 value	p-value	n
Dictyota cervicornis	s.a. = 55.719(w.w.) + 2.556	0.603	0.000	30
Dictyota menstrualis	s.a. = 19.114(w.w.) +13.899	0.450	0.004	15
Halimeda gracilis	s.a. = 6.675(w.w.) + 14.725	0.495	0.000	37
Halimeda incrassata	s.a. = 10.920(w.w.) + 11.144	0.793	0.000	48
Laurencia gemmifera	s.a. = 19.857(w.w.) – 1.056	0.964	0.000	29
Laurencia intricata	s.a. = 57.182(w.w.) – 5.943	0.671	0.001	11

Table 3. Paired t-test and Pearson correlation analysis results comparing *Gambierdiscus* cell densities (log cells $100g^{-1}$ ww), among the macrophyte hosts pooled across sites, and within each site. The numbers in parentheses are the numbers of paired data used for each correlation computation.

Site	Host 1	Host 2	Host 1 paired average	Host 2 paired average	95% confidence interval of difference (1 - 2) lower	upper	paired t-test p-value	correlation (n)	correlation p-value
Pooled	*Dictyota*	*Halimeda*	2.92 ± 1.659	3.276 ± 0.728	-0.909	0.215	0.219	0.181 (37)	0.283
Pooled	*Dictyota*	*Laurencia*	2.61 ± 1.926	3.55 ± 0.803	-1.710	-0.173	0.003	0.287 (26)	0.164
Pooled	*Halimeda*	*Laurencia*	3.28 ± 0.818	3.55 ± 0.803	-0.440	-0.102	0.003	0.872 (25)	0.000
HGB	*Halimeda*	*Thalassia*	4.06 ± 0.558	4.54 ± 0.448	-0.644	-0.317	0.000	0.892 (12)	0.000

LKH	*Dictyota*	*Halimeda*	3.38 ± 0.698	2.73 ± 0.619	0.305	1.004	0.002	0.694 (11)	0.018
LKH	*Dictyota*	*Laurencia*	3.38 ± 0.698	2.87 ± 1.190	-0.121	1.142	0.102	0.612 (11)	0.045
LKH	*Halimeda*	*Laurencia*	2.61 ± 0.640	2.77 ± 1.114	-0.625	0.310	0.476	0.738 (12)	0.004
TPH	*Dictyota*	*Halimeda*	2.37 ± 2.295	3.85 ± 0.466	-2.829	-0.148	0.032	0.261 (13)	0.389
TPH	*Dictyota*	*Laurencia*	2.37 ± 2.295	4.06 ± 0.401	-2.967	-0.422	0.013	0.539 (13)	0.057
TPH	*Halimeda*	*Laurencia*	3.85 ± 0.466	4.06 ± 0.401	-0.453	0.041	0.094	0.826 (13)	0.000
TRL	*Dictyota*	*Halimeda*	3.68 ± 0.373	3.38 ± 0.380	-0.031	0.633	0.071	0.138 (11)	0.686

Table 4. Paired t-test and Pearson correlation analysis results comparing *Gambierdiscus* cell densities (log cells 100cm^{-2}), among the macrophyte hosts pooled across sites, and within each site. The numbers in parentheses are the numbers of paired data used for each correlation computation.

Site	Host 1	Host 2	Host 1 paired average	Host 2 paired average	95% confidence interval of difference (1 - 2) lower	upper	paired t-test p-value	correlation (n)	correlation p-value
Pooled	*Dictyota*	*Halimeda*	1.53 ± 0.976	2.08 ± 0.692	-0.882	-0.208	0.002	0.303 (37)	0.068
Pooled	*Dictyota*	*Laurencia*	1.34 ± 1.091	2.01 ± 1.049	-1.152	-0.167	0.011	0.351 (26)	0.079
Pooled	*Halimeda*	*Laurencia*	2.03 ± 0.784	2.01 ± 1.049	-0.191	0.227	0.860	0.880 (26)	0.000
HGB	*Halimeda*	*Thalassia*	2.80 ± 0.494	2.61 ± 0.482	0.073	0.312	0.004	0.926 (12)	0.000

LKH	*Dictyota*	*Halimeda*	1.33 ± 0.836	1.43 ± 0.567	-0.459	0.253	0.541	0.710 (13)	0.007
LKH	*Dictyota*	*Laurencia*	1.33 ± 0.836	1.22 ± 0.871	-0.305	0.530	0.569	0.672 (13)	0.012
LKH	*Halimeda*	*Laurencia*	1.43 ± 0.567	1.22 ± 0.871	-0.095	0.526	0.156	0.826 (13)	0.000
TPH	*Dictyota*	*Halimeda*	1.37 ± 1.333	2.62 ± 0.445	-2.042	-0.462	0.005	0.226 (13)	0.457
TPH	*Dictyota*	*Laurencia*	1.37 ± 1.333	2.80 ± 0.428	-2.128	-0.734	0.001	0.553 (13)	0.050
TPH	*Halimeda*	*Laurencia*	2.62 ± 0.445	2.80 ± 0.428	-0.457	0.098	0.185	0.445 (13)	0.127
TRL	*Dictyota*	*Halimeda*	1.97 ± 0.395	2.20 ± 0.404	-0.530	0.070	0.118	0.376 (11)	0.255

Table 5. Pearson correlations of *Gambierdiscus* cell densities on macrophytes (log cells $100g^{-1}$ ww) and artificial substrates (log cells 100 cm^{-2}) across all sites, and within each site. Pooled samples combine all macrophyte samples in each category for analysis. Significance values are indicated by *** $p \leq 0.001$; ** $p \leq 0.01$ * $p \leq 0.05$. The numbers in parentheses are the numbers of paired data used for each correlation computation. The Overall category correlation values are averaged for individual macrophyte species or artificial substrates across all categories. The Average Correlation values are averaged across macrophytes and artificial substrates for each site category.

Site	Algae	Burlap	Screen	Tile	Average Correlation
All	*Dictyota*	0.399*	0.297	0.074	
		(37)	(36)	(37)	
All	*Halimeda*	0.655***	0.786***	0.787***	
		(49)	(48)	(49)	
All	*Laurencia*	0.631***	0.805***	0.740***	
		(25)	(24)	(25)	
All	Pooled	0.650***	0.764***	0.841***	0.751
		(49)	(48)	(47)	
HGB	*Halimeda*	0.775**	0.872***	0.707**	
		(12)	(12)	(12)	
HGB	*Thalassia*	0.700*	0.812***	0.664*	
		(12)	(12)	(12)	
HGB	Pooled	0.736**	0.841***	0.687*	0.755

		(12)	(12)	(12)	
LKH	*Dictyota*	0.939***	0.866***	0.737**	
		(11)	(11)	(11)	
LKH	*Halimeda*	0.724**	0.597*	0.597*	
		(13)	(13)	(13)	
LKH	*Laurencia*	0.762**	0.666*	0.392	
		(13)	(13)	(13)	
LKH	Pooled	0.911***	0.788***	0.671*	0.790
		(13)	(13)	(13)	
TPH	*Dictyota*	0.369	0.550	0.555*	
		(13)	(13)	(13)	
TPH	*Halimeda*	0.668*	0.554*	0.666*	
		(13)	(13)	(13)	
TPH	*Laurencia*	0.296	0.316	0.652*	
		(13)	(13)	(13)	
TPH	Pooled	0.449	0.382	0.752**	0.528
		(13)	(13)	(13)	
TRL	*Dictyota*	0.171	0.552	-0.243	
		(11)	(11)	(10)	
TRL	*Halimeda*	0.721*	0.600	0.382	
		(11)	(11)	(10)	
TRL	Pooled	0.591	0.718*	0.095	0.468
		(11)	(11)	(10)	

Overall	Substrate	0.601	0.636	0.516	0.584
Overall	*Dictyota*	0.470	0.566	0.281	0.439
Overall	*Halimeda*	0.709	0.682	0.628	0.673
Overall	*Laurencia*	0.563	0.596	0.595	0.584

Table 6. Pearson correlations of *Gambierdiscus* cell densities on macrophytes (log cells 100cm^{-2}) and artificial substrates (log cells 100 cm^{-2}) across all sites, and within each site. Pooled samples combine all macrophyte samples in each category for analysis. Significance values are indicated by *** $p \leq 0.001$; ** $p \leq 0.01$ * $p \leq 0.05$. The numbers in parentheses are the numbers of paired data used for each correlation computation. The Overall category correlation values are averaged for individual macrophyte species or artificial substrates across all categories. The Average Correlation values are averaged across macrophytes and artificial substrates for each site category.

Site	Algae	Burlap	Screen	Tile	Average correlation
All	*Dictyota*	0.514***	0.413*	0.226	
		(37)	(36)	(37)	
All	*Halimeda*	0.642***	0.764***	0.772***	
		(49)	(48)	(49)	
All	*Laurencia*	0.658***	0.849***	0.792***	
		(26)	(25)	(26)	
All	Pooled	0.681***	0.810***	0.845***	0.776
		(49)	(48)	(47)	
HGB	*Halimeda*	0.786**	0.885***	0.736**	
		(12)	(12)	(12)	
HGB	*Thalassia*	0.724**	0.827***	0.683*	
		(12)	(12)	(12)	
HGB	Pooled	0.773**	0.876***	0.725**	0.802

		(12)	(12)	(12)	
LKH	*Dictyota*	0.880***	0.739**	0.652*	
		(13)	(13)	(13)	
LKH	*Halimeda*	0.684**	0.566*	0.566*	
		(13)	(13)	(13)	
LKH	*Laurencia*	0.797***	0.721**	0.485	
		(13)	(13)	(13)	
LKH	Pooled	0.856***	0.735**	0.675*	0.755
		(13)	(13)	(13)	
TPH	*Dictyota*	0.378	0.553*	0.577*	
		(13)	(13)	(13)	
TPH	*Halimeda*	0.662*	0.532	0.639*	
		(13)	(13)	(13)	
TPH	*Laurencia*	0.280	0.312	0.642*	
		(13)	(13)	(13)	
TPH	Pooled	0.552*	0.494	0.778**	0.608
		(13)	(13)	(13)	
TRL	*Dictyota*	0.336	0.496	-0.109	
		(11)	(11)	(10)	
TRL	*Halimeda*	0.770**	0.551	0.365	
		(11)	(11)	(10)	
TRL	Pooled	0.767**	0.672*	0.301	0.580
		(11)	(11)	(10)	

Overall	Substrate	0.624	0.631	0.540	0.599
Overall	*Dictyota*	0.527	0.550	0.336	0.471
Overall	*Halimeda*	0.709	0.660	0.616	0.661
Overall	*Laurencia*	0.578	0.627	0.640	0.615

Table 7. Slopes (± standard error) of the zero-intercept regression models to predict *Gambierdiscus* cell abundances on macrophytes (cells $100g^{-1}$ ww) from cell abundance on artificial substrates (cells $100cm^{-2}$). "n.s." denotes insignificant relationships as determined by Pearson correlation analysis (Table 4), resulting in a slope of zero. The numbers in parentheses are the numbers of paired data used for each regression computation. Pooled sample slopes were computed by combining all macrophyte samples in each site category for analysis for each artificial substrate. The Overall macrophyte slopes are the average of the slopes for each macrophyte across sites. The Pooled Overall category slopes are the average slopes of the Pooled slopes from each site category. The Artificial Substrate Overall slopes are computed from the average slopes for each macrophyte (across sites) and for each site (across macrophytes). The data in the Average Slope and Standard deviation columns were computed from the macrophyte and pooled slopes within each site category. The % error was computed by dividing the Average Slope by the Standard deviation values.

Site	Algae	Burlap	Screen	Tile	Average slope	Standard deviation	% error
All	*Dictyota*	1.146 ± 0.096 (37)	n.s. (36)	n.s. (37)	0.382	0.662	173%
	Halimeda	1.327 ± 0.032 (49)	1.447 ± 0.030 (48)	1.623 ± 0.066 (49)	1.466	0.149	10%
	Laurencia	1.355 ± 0.049	1.482 ± 0.040	1.602 ± 0.115	1.480	0.124	8%

		(25)	(24)	(25)			
	Pooled	1.410 ± 0.032 (49)	1.537 ± 0.032 (48)	1.723 ± 0.057 (47)	1.56	0.157	10%
	Average	1.310	1.116	1.237	1.221	0.097	8%
	Stdev	0.114	0.745	0.826	-	-	-
	% error	9%	67%	67%	-	-	-
HGB	*Halimeda*	1.498 ± 0.059 (12)	1.562 ± 0.067 (12)	1.596 ± 0.073 (12)	1.552	0.050	3%
	Thalassia	1.663 ± 0.076 (12)	1.730 ± 0.091 (12)	1.773 ± 0.089 (12)	1.722	0.055	3%
	Pooled	1.598 ± 0.070 (12)	1.663 ± 0.084 (12)	1.703 ± 0.084 (12)	1.655	0.053	3%
	Average	1.586	1.652	1.690	1.643	0.053	3%
	Stdev	0.083	0.085	0.089	-	-	-
	% error	5%	5%	5%	-	-	-
LKH	*Dictyota*	1.348 ± 0.032	1.636 ± 0.128	2.051 ± 0.345	1.678	0.353	21%

		(11)	(11)	(11)			
	Halimeda	1.077 ± 0.054 (13)	1.301 ± 0.118 (13)	1.635 ± 0.283 (13)	1.338	0.281	21%
	Laurencia	1.175 ± 0.084 (13)	1.441 ± 0.129 (13)	n.s. (13)	0.872	0.767	88%
	Pooled	1.228 ± 0.034 (13)	1.492 ± 0.107 (13)	1.865 ± 0.309 (13)	1.528	0.320	21%
	Average	1.207	1.468	1.388	1.354	0.133	10%
	Stdev	0.113	0.138	0.941	-	-	-
	% error	9%	9%	68%	-	-	-
TPH	*Dictyota*	n.s. (13)	n.s. (13)	0.939 ± 0.230 (13)	0.313	0.542	174%
	Halimeda	1.398 ± 0.042 (13)	1.417 ± 0.046 (13)	1.472 ± 0.037 (13)	1.429	0.038	3%
	Laurencia	n.s. (13)	n.s. (13)	1.549 ± 0.034 (13)	0.516	0.894	173%

	Pooled	n.s. (13)	n.s. (13)	1.547 ± 0.030 (13)	0.516	0.893	173%
	Average	0.350	0.354	1.377	0.694	0.592	85%
	Stdev	0.699	0.709	0.294	-	-	-
	% error	200%	200%	21%	-	-	-
TRL	*Dictyota*	n.s. (11)	n.s. (11)	n.s. (10)	0.000	0.000	n.a.
	Halimeda	1.291 ± 0.031 (11)	n.s. (11)	n.s. (10)	0.430	0.745	173%
	Pooled	n.s. (11)	1.489 ± 0.038 (11)	n.s. (10)	0.496	0.860	173%
	Average	0.430	0.496	n.s.	0.309	0.270	87%
	Stdev	0.745	0.860	-	-	-	-
	% error	173%	173%	-	-	-	-
Dictyota Overall	Average	0.624	0.409	0.748	0.593	0.171	29%

	Stdev	0.725	0.818	0.975	-	-	-
	% error	116%	200%	130%	-	-	-
Halimeda Overall	Average	1.318	1.145	1.265	1.243	0.088	7%
	Stdev	0.156	0.647	0.710	-	-	-
	% error	12%	56%	56%	-	-	-
Laurencia Overall	Average	0.843	0.974	1.050	1.148	0.092	8%
	Stdev	0.736	0.844	0.910	-	-	-
	% error	87%	87%	87%	-	-	-
Pooled Overall	Average	1.059	1.545	1.323	1.309	0.243	19%
	Stdev	0.784	0.694	0.773	-	-	-
	% error	74%	45%	58%	-	-	-
Artificial substrate	Average	0.992	1.039	1.140	-	-	-

Overall							
	Stdev	0.461	0.609	0.604	-	-	-
	% error	74%	92%	59%	-	-	-

Table 8. Slopes (± standard error) of the zero-intercept regression models to predict *Gambierdiscus* cell abundances on macrophytes (cells $100cm^{-2}$) from cell abundance on artificial substrates (cells $100cm^{-2}$). "n.s." denotes insignificant relationships as determined by Pearson correlation analysis (Table 5), resulting in a slope of zero. The numbers in parentheses are the numbers of paired data used for each regression computation. Pooled sample slopes were computed by combining all macrophyte samples in each site category for analysis for each artificial substrate. The Overall macrophyte slopes are the average of the slopes for each macrophyte across sites. The Pooled Overall category slopes are the average slopes of the Pooled slopes from each site category. The Artificial Substrate Overall slopes are computed from the average slopes for each macrophyte (across sites) and for each site (across macrophytes). The data in the Average Slope and Standard deviation columns were computed from the macrophyte and pooled slopes within each site category. The % error was computed by dividing the Average Slope by the Standard deviation values.

Site	Algae	Burlap	Screen	Tile	Average slope	Standard deviation	% error
All	*Dictyota*	0.610 ± 0.055 (37)	0.675 ± 0.062 (36)	n.s. (37)	0.428	0.372	87%
	Halimeda	0.871 ± 0.030 (49)	0.957 ± 0.027 (48)	1.085 ± 0.041 (49)	0.971	0.108	11%
	Laurencia	0.804 ± 0.063	0.910 ± 0.054	1.023 ± 0.063	0.912	0.110	12%

		(26)	(25)	(26)			
	Pooled	0.870 ± 0.028 (49)	0.957 ± 0.024 (48)	1.087 ± 0.031 (47)	0.971	0.109	11%
	Average	0.789	0.875	0.799	0.821	0.047	6%
	Stdev	0.123	0.135	0.533	-	-	-
	% error	16%	15%	67%	-	-	-
HGB	*Halimeda*	1.039 ± 0.039 (12)	1.086 ± 0.040 (12)	1.108 ± 0.048 (12)	1.078	0.035	3%
	Thalassia	0.967 ± 0.041 (12)	1.011 ± 0.042 (12)	1.031 ± 0.049 (12)	1.003	0.033	3%
	Pooled	1.011 ± 0.039 (12)	1.056 ± 0.039 (12)	1.077 ± 0.048 (12)	1.048	0.034	3%
	Average	1.006	1.051	1.072	1.043	0.034	3%
	Stdev	0.036	0.038	0.039	-	-	-
	% error	4%	4%	4%	-	-	-
LKH	*Dictyota*	0.593 ± 0.064	0.738 ± 0.080	0.976 ± 0.148	0.769	0.193	25%

		(13)	(13)	(13)			
	Halimeda	0.602 ± 0.047 (13)	0.733 ± 0.075 (13)	0.942 ± 0.157 (13)	0.759	0.171	23%
	Laurencia	0.544 ± 0.074 (13)	0.687 ± 0.086 (13)	n.s. (13)	0.410	0.362	88%
	Pooled	0.624 ± 0.042 (13)	0.769 ± 0.063 (13)	0.997 ± 0.146 (13)	0.797	0.188	24%
	Average	0.591	0.732	0.729	0.684	0.081	12%
	Stdev	0.034	0.034	0.486	-	-	-
	% error	6%	5%	67%	-	-	-
TPH	*Dictyota*	n.s. (13)	0.530 ± 0.127 (13)	0.543 ± 0.133 (13)	0.358	0.310	87%
	Halimeda	0.954 ± 0.034 (13)	n.s. (13)	1.002 ± 0.036 (13)	0.652	0.565	87%
	Laurencia	n.s. (13)	n.s. (13)	1.070 ± 0.035 (13)	0.357	0.618	173%

	Pooled	0.974 ± 0.036 (13)	n.s. (13)	1.028 ± 0.025 (13)	0.667	0.579	87%
	Average	0.482	0.133	0.911	0.508	0.390	77%
	Stdev	0.557	0.265	0.247	-	-	-
	% error	115%	200%	27%	-	-	-
TRL	*Dictyota*	n.s. (11)	n.s. (11)	n.s. (11)	0.000	0.000	n.a.
	Halimeda	0.846 ± 0.031 (11)	n.s. (11)	n.s. (11)	0.282	0.488	173%
	Pooled	0.823 ± 0.026 (11)	0.933 ± 0.033 (11)	n.s. (11)	0.585	0.510	87%
	Average	0.557	0.311	0	0.289	0.279	96
	Stdev	0.482	0.539	0	-	-	-
	% error	87%	173%	-	-	-	-
Dictyota	Average	0.301	0.486	0.380	0.389	0.093	24%
	Stdev	0.347	0.335	0.473	-	-	-

	% error	115%	69%	124%	-	-	-
Halimeda	Average	0.862	0.555	0.827	0.748	0.168	22%
	Stdev	0.164	0.522	0.467	-	-	-
	% error	19%	94%	56%	-	-	-
Laurencia	Average	0.579	0.652	0.781	0.670	0.102	15%
	Stdev	0.423	0.455	0.521	-	-	-
	% error	73%	70%	67%	-	-	-
Pooled	Average	0.832	0.929	0.790	0.850	0.071	8%
	Stdev	0.152	0.428	0.470	-	-	-
	% error	18%	46%	59%	-	-	-
Artificial substrate	Average	0.666	0.636	0.699	-	-	-
	Stdev	0.258	0.306	0.360	-	-	-
	% error	50%	75%	59%	-	-	-