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# Examining the dynamic nature of epiphytic microalgae in the Florida Keys: What factors influence community composition?

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ABSTRACT

The factors that influence the composition of marine epiphytic microalgal assemblages are poorly-understood. To address this short-coming, 93 samples were collected from four distinct regions in the Florida Keys National Marine Sanctuary (FKNMS) during winter and summer months to test the model that epiphytic microalgal communities are influenced by environmental gradients related to different sites, seasons, and host macrophyte species. One hundred and eighty-three morphotypes from 13 classes (7 phyla) were identified, dominated by 106 Bacillariophyta (77 identified to species equivalent or below), 37 Cyanophyta (13 identified to species equivalent or below), and 30 Dinophyta (21 identified to species equivalent or below). The largest proportion of variability in epiphytic communities was related to physico-chemical parameters (37%), followed by site location (ocean- versus bayside; 15%), seasonal differences (11%), and host macrophyte species (10%). Four physico-chemical variables were found to be most influential: wave height, temperature, ammonium concentration, and salinity. Only six out of 616 epiphyte – host comparisons exhibited significant differences in individual epiphyte taxon abundance between different host species (within site and season), further demonstrating that host-specificity was not strongly evident in this study. Overall, the results of this (sub)tropical study indicate that changing environmental characteristics between sites and seasons were the primary drivers influencing epiphyte community composition. Similar findings were found in an accompanying study of phytoplankton and other studies from temperate and (sub)polar regions, suggesting that common, underlying processes exist among these disparate environments.

## 1. Introduction

Epiphytism is an important ecological component of marine benthic environments. Epiphytic algae, for example, often account for more primary production than their macrophyte (macroalgae or seagrass) hosts (Macreadie et al., 2014), including up to 60% of total benthic productivity (Moncreiff and Sullivan, 2001). There are notable negative impacts of these fouling organisms on host macrophytes, however, including hindrance of light penetration (Tew et al., 2017), increase in hydrodynamic drag (Hansen and Reidenbach, 2017), and competition for nutrients (Nelson, 2017).

One persistent topic of study in epiphyte ecology (with conflicting results), has been the relative influence of environmental factors versus substrate (macrophyte host) preferences. In terms of nutrients, epiphytic algae were evaluated as possible indicators of system response to nutrient loading, with mixed results (Nelson, 2017). Armitage et al. (2006) reported conflicting results to N and P additions on *Thalassia testudinum* epiphytic communities in Florida Bay (i.e., lack of epiphytic response in some cases), also observed by Green et al. (2015) in a similar study in the region.

Other environmental factors have been found to exert strong influences on epiphytic assemblages, including light intensity (Blake and Duffy, 2016), small-scale hydrodynamics (Quintano et al., 2016), and temperature (Gauna et al., 2016). Mabrouk et al. (2011) reported that wave motion, light availability, temperature, and motility of epiphytic species influenced temporal and bathymetric variations in epiphytic communities on *Posidonia oceanica* in coastal Tunisia. Orth et al. (1982) suggested that epiphytes may benefit from higher water movement (i.e., host swaying in response to wave motion or currents), creating a steeper nutrient gradient or facilitating removal of allelochemicals. Some epiphytes may be negatively impacted by water motion, however. Gauna et al. (2016) observed that epiphyte biomass and diversity was lower in

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exposed coastal environments versus more sheltered locations.

Seasonal differences in epiphytic communities have been documented, including a study by Ruesink (1998), who observed that colonization of *Isthmia nervosa* (Bacillariophyceae) on the red algae *Odonthalia floccosa* occurred in late summer in coastal waters of the US Pacific Northwest (Washington), after host growth ceased. Similarly, Lepoint et al. (1999) found that epiphytic biomass was higher in summer months on *P. oceanica* in coastal Tunisia, likely in response to increasing light and temperature. Conversely, Reyes-Vasquez (1970) reported little seasonal difference in diatom composition on *T. testudinum* in Biscayne Bay, Florida. El-Din et al. (2015) also did not observe any seasonal variation in epiphytic biomass or composition (Alexandria Harbor, Egypt), and there were minimal correlations with physico-chemical parameters.

Environmental factors, therefore, appear to have influential roles in epiphyte community dynamics in some (but not all) cases. Similarly, substrate specificity has been found to be influential, but not consistently so across taxa or regions. The "Neutral Substrate Hypothesis" states that macrophytes are generally neutral, neither stimulating nor impeding the growth of epiphytes. Early advocates of this hypothesis include Shelford (1918), who stated, "One could probably remove all the larger plants and substitute glass structures of the same form without greatly affecting the immediate food relations" (p. 47). The topic has been contested over time, with Cattaneo and Kalff (1979) concluding that there was no significant difference in epiphytic productivity among different hosts, whereas Gough and Gough (1981) challenged this generalized conclusion by stating that some hosts may be neutral, but others can significantly influence the epiphytic community. This conclusion is supported by Al-Handal and Wulff (2008) and Sutherland (2008), who found that epiphytic composition differed among host macrophytes, and Dhib et al. (2015), who reported that epiphytic biomass was most correlated with seagrass host (*Ruppia cirrhosa*) biomass in Tunisian waters (specificity), coupled with a general lack of correlation with environmental variables. Conversely, Snoeijs (1994) attributed differences in epiphytic diatom community composition between three macroalgal hosts in the Baltic to environmental factors (i.e., season and salinity) rather than host preference. More recently, Fricke et al. (2016) concluded that substrate preferences masked the epiphytic response to nutrient loading, demonstrating that the various factors influencing epiphytic community responses are interactive.

This brief review of the epiphyte literature reveals that there is no clear consensus on the over-riding importance of environmental factors or host specificity in shaping epiphytic community structure. This fact, coupled with the dearth of epiphytic microalgal community dynamic studies in (sub)tropical coral reef-dominated environments like the Florida Keys, has led to this study, the purpose of which was to examine the variation in microalgal epiphytic community structure in relation to changing environmental conditions and host macrophyte species across space and time. We are testing the model that epiphyte communities will differ by location, and that these differences could be interpreted in terms of key distinguishing features of each site, including host macrophyte, wave energy, temperature variation, salinity variation and nutrient concentrations.

## 2. Methods

### 2.1. Study site description

The study was based on data collected from four sites in the vicinity of Long Key in the Florida Keys (Fig. 1). Two sites, Heine Grassbed (HGB) and Tomato Patch Hardbottom (TPH), are located in Florida Bay, and the other two, Long Key Hardbottom (LKH) and Tennessee Reef Lighthouse (TRL), on the Atlantic Ocean side of the Keys. Detailed site descriptions are provided in an earlier publication (Parsons et al., 2017). Briefly, HGB is a nearshore *Thalassia* seagrass bed consisting of a silty sediment matrix in approximately 2 m water depth. TPH is a nearshore hardbottom site (approx. 1.5 m depth) consisting of Pleistocene-era reef matrix (reef crest and back reef) covered in a sand veneer colonized by soft corals, sponges, and macroalgae. LKH is an offshore hardbottom site (approx. 5 m depth; Pleistocene forereef) consisting of a sand veneer colonized by soft corals, sponges, and macroalgae. TRL reef is a modern reef flat/crest site (approx. 7 m depth) consisting of hard and soft corals, sponges, macroalgae, interspersed with sandy bottom areas.

### 2.2. Sampling field and laboratory methods

Macrophyte samples (hosts) were collected in summer 2014 (June and July) and winter 2014–2015 (December and January) at each site. A

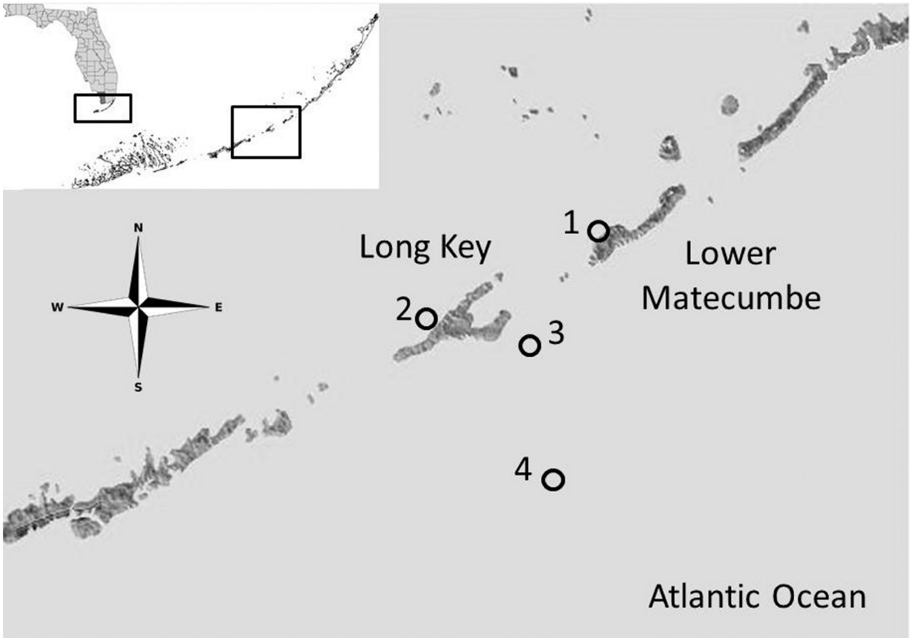

**Fig. 1.** Study area. 1) Heine Grassbed (HGB) on the bayside of Lower Matecumbe Key; 2) Tomato Patch Hardbottom (TPH) on the bayside of Long Key; 3) Long Key Hardbottom (LKH) on the Atlantic side of Long Key and 4) Tennessee Reef Lighthouse (TRL) on Tennessee Reef.

total of 93 samples were collected and analyzed for this study: three replicate samples for the following species were collected each of the four months at each site: *Thalassia testudinum* and *Halimeda incrassata* at HGB; *Dictyota cervicornis* and *H. incrassata* at TPH; *D. cervicornis* (not present in December) and *H. gracilis* at TPH; and *D. menstrualis* and *H. gracilis* at TRL.

Epiphyte sample collection, processing (including sieving), and analysis followed procedures provided in Parsons et al. (2017). It should be noted that collected epiphytes were limited to those that could be dislodged via shaking. Those species that tend to be firmly attached to the host substrate (e.g., members of the diatom genus *Cocconeis*), were likely under-represented using this methodology. Acknowledging that such understory species may be biased against, it was determined that scraping, freshwater immersion, and acid digestion procedures normally used to collect these individuals were unsuitable for this study as 1) delicate host macrophytes such as *Dictyota* could not be effectively scraped without total destruction of the thallus; 2) freshwater immersion would lyse epiphytes with delicate cell walls; and 3) acid digestion would eliminate the ability to enumerate only live cells. Rigorous quality assurance and quality control (QA/QC) procedures demonstrated that recovery of other epiphytes (e.g., *Gambierdiscus* spp.) was >95% (Parsons et al., 2017), validating the methods utilized for the majority of epiphytes living on the host macrophytes.

Water samples for nutrient analysis were collected carefully in triplicate at each site within 0.5 m of the bottom in acid-washed, 250 mL PFTE bottles, via SCUBA diving to visually ensure sediments were not disturbed prior to and during collection. Samples were then filtered through acid-washed Whatman GF/F glass fiber filters into clean 250 mL glass amber bottles, and frozen until analysis. Nutrient concentrations (nitrate, nitrite, ammonium, and phosphate) were determined in accordance with standard laboratory methods on a Bran+Luebbe® AutoAnalyzer 3 (www.seal-analytical.com/Methods).

Bottom water temperature and benthic ambient light conditions were recorded at each site every 15 min each month using an Onset® HOBO® Pendant® Temperature/Light 64 K data logger (UA-002-64). Salinity (bottom water) was measured using a refractometer on grab samples. Wave data (simulated) were obtained from Wind Guru (http://windguru.cz/int/; GFS 27 km daily archive; Islamorada, FL) and corrected for fetch using wind data retrieved from the National Climatic Data Center (http://www.ncdc.noaa.gov) for the Marathon Airport (KMTH) using the Daily Summaries dataset. Wind corrections were applied as weights multiplied to the wave data as outlined in Stanca and Parsons (2017). Temperature, light, and wave data were averaged at 1-day (1d), 3-day (3d), 1-week (1w), 2-week (2w), and 1-month (1 m) intervals (relative to sampling date) to account for immediate (1d), short-term (3d and 1w) and long-term (2w and 1 m) influences of these variables on epiphytic populations.

### 2.3. Epiphyte analysis

Epiphyte composition was determined by transferring 3 mL of each shaken and sieved epiphyte sample (15 mL) into one well of a six well tissue culture plate (Corning™ Costar™), left to settle for several hours, and thereafter analyzed on an Olympus IX71 phase contrast inverted microscope at powers of 200× and 400×. A minimum of 400 epiphyte cells was enumerated and identified to the lowest taxonomic level (morphotype) possible in each sample examined. Bright-field light microscopy was supplemented with other techniques to confirm the identification of certain key dinoflagellates and diatoms, including epifluorescence microscopy using Uvitex® staining (similar to calcofluor; Polysciences, Ltd., cat. #19517–10; for armored dinoflagellates) and acid-digestion of samples followed by analysis using differential interference contrast (DIC) microscopy (diatoms).

The list of texts and journal articles used most frequently to aid in taxonomic identification are provided in Stanca and Parsons (2017). The "cf." qualifier was used to indicate specimen that were similar to (or may actually be) the nominate species. The "acf." qualifier was used for taxa that were similar to (but not) the nominate genera (e.g., acf. *Gloeotheca* spp.). In some cases, it was not possible to identify the organism to the species level, although characteristics indicative of a genus were evident. In such cases, the organism was reported with the name of the genus followed by numbered "sp." (e.g., *Oscillatoria* sp. 1, *O.* sp. 2, *O.* sp. 3, etc.). Morphotypes which contained the "undet." (undetermined) identifier were likely to be algal entities, but could not be identified as any known genus. In some cases, species were classified into separate morphotypes based on size (e.g., Dinophyceae undet. >20 μm). The term "Other" is referred to the group consisting of small phytoflagellates and other undetermined microalgae. While these methods undoubtedly reduce the taxonomic resolution of some epiphytic groups (particularly diatoms), we believe that the described methods represented the best compromise for counting both live cells and the variety of groups (fragile and robust; large and small) encountered in these samples.

Cell biovolumes ($\mu m^3$) were estimated according to the specimen/genus/class-specific shape association and using the formulas recorded on "Atlas of shape" (http://phytobioimaging.unisalento.it/en-us/products/AtlasOfShapes.aspx?ID_Tipo= 0). Required cellular dimensions were measured for each single cell using a calibrated eyepiece reticle for input into the applicable formula.

Sample cell abundance was standardized to cells $cm^{-2}$ host macrophyte by multiplying the sum of each morphotype biovolume by the subsample proportion factor (e.g., proportion of sample counted to reach 400 cells divided by sample volume (15 mL) and the inverse of macrophyte surface area ($cm^2$) to give cell abundances as $\mu m^3\ cm^{-2}$ host macrophyte. Macrophyte surface area was calculated using image analysis of photographs taken of the algae (flattened under glass) using the software, Image J (http://imagej.nih.gov/ij; Parsons et al., 2017).

### 2.4. Statistical analysis

Analysis was limited to those epiphytic morphotypes occurring in at least 10% of the samples (i.e., present in at least 10 samples). Biovolume data ($\mu m^3$) were log-transformed (ln(cells+1)) prior to analysis. A resemblance matrix was computed on these data using Bray-Curtis similarity permutations (Bray and Curtis, 1957) to determine how similar each sample was to another based on the epiphyte assemblages. PERMANOVA tests were conducted on the epiphyte resemblance matrix to determine if there were differences in the epiphytic assemblages between seasons (summer vs winter), host (*Thalassia* vs *Dictyota* vs *Halimeda*) and location (bayside versus oceanside). For those results indicating differences, SIMPER (Similarity Percentage; Clarke, 1993) analysis was applied to the log-transformed abundance data to look at morphotype-specific differences between the categories. Further analyses of potential spatio-temporal differences in the environmental data (versus site, host and season) and taxon data (host only) were performed by one-way and two-way ANOVA, without replications (Sokal and Rohlf, 2001).

Distance-based linear model analysis (DISTLM) was used to determine the proportional relationships between the epiphytic resemblance matrix and environmental, site, host, and seasonal factors, respectively. These proportions, in turn, indicate the variation within the epiphyte similarity matrix potentially explained by each factor, thereby allowing for the potential influence each factor has in shaping epiphytic assemblage composition. A second DISTLM was conducted on the environmental variables specifically (18 tested in all). The most influential environmental parameters were identified using the "Best" selection procedure with the adjusted $R^2$ criterion. The environmental data were normalized (percentage about the mean) for this and the subsequent procedures (see below) to satisfy the assumptions of normality and homogeneity of variance, as well as to equalize the scaling of the variables.

Canonical Analysis of Principal Coordinates (CAP) was used to determine how the epiphyte assemblage composition differed among samples in relation to the environmental variables, as well as seasonal (summer vs winter) and location (bay versus ocean) factors. The environmental data were normalized by subtracting each variable by the mean value and dividing by the standard deviation prior to analysis. Significance of the CAP was determined using the trace statistic (similar to Pillai's trace in MANOVA; Anderson et al., 2008) and first squared canonical correlation permutations (similar to Roy's greatest root in MANOVA). All statistical analyses were done using PRIMER 7 (Clarke and Gorley, 2015) except for the ANOVA which used SPSS 26.

## 3. Results

### 3.1. Epiphyte composition

Overall, 37,200 epiphytic microalgae were counted, measured and classified from the 93 samples examined. A total of 183 morphotypes were identified from seven phyla (Table S1). There were 106 morphotypes of Bacillariophyta encountered in this study (77 identified to species equivalent or below), with 37 Cyanophyta (13 identified to species equivalent or below), 30 Dinophyta (21 identified to species equivalent or below), 7 Chlorophyta (2 identified to species equivalent or below), 1 Haptophyta, 1 Cryptophyta, and 1 miscellaneous morphotype (Other Phytoplankton) comprising the remainder. The highest species richness values recorded for the diatom genera were for *Amphora* (7 species), *Synedra* (8 species), and *Nitzschia* (7 species). The genus, *Prorocentrum*, was the most representative among Dinophyta (13 species), with several genera represented by two morphotypes. *Oscillatoria* was most diverse for Cyanophyta (6 species). In terms of cell abundance (by total biovolume), the epiphytic microalgal community was almost exclusively dominated by Bacillariophyta (83%), followed by Cyanophyta (10%) and Dinophyta (7%). Chlorophyta, Cryptophyta, Haptophyta and Other Phytoplankton represented <1% of total abundance. In summary, epiphyte communities examined in this study were dominated, in terms of abundance and species richness, by Bacillariophyta. Cyanophyta and Dinophyta were the two other important phyla contributing to the epiphyte composition.

### 3.2. Discriminating epiphytic assemblages

The PERMANOVA results indicated that the epiphytic assemblages differed between seasons ($p = 0.001$) and sites ($p = 0.001$), but not by host (*Thalassia* vs *Halimeda*: $p = 0.09$; *Halimeda* vs *Dictyota*: $p = 0.13$; *Thalassia* vs *Dictyota* did not co-occur). ANOVA results corroborate these findings, in which only six epiphyte morphotypes were more abundant on one host species versus another collected and analyzed from the same site and season (out of 616 possibilities; Table 1).

SIMPER analysis ranked morphotypes in terms of how each contributed to the dissimilarity among the epiphytes by location (Table 2) and season (Table 3). There were 26 morphotypes that cumulatively accounted for the 50% of the dissimilarity between locations; 15 were more abundant at the bayside sites (composed of 7 diatoms, 4 dinoflagellates, and 4 cyanobacteria morphotypes); 11 were more abundant oceanside (composed of 10 diatoms and 1 cyanobacteria morphotypes). There were also 26 morphotypes that cumulatively accounted for the 50% of the dissimilarity between seasons; 13 were more abundant in winter (composed of 11 diatoms and 2 dinoflagellates morphotypes) and 13 in summer (composed of 6 diatoms, 2 dinoflagellates, and 5 cyanobacteria morphotypes).

### 3.3. Environmental factors

The DISTLM results indicated that environmental variables explained most of the variability in the epiphytic assemblages, followed by site, season, and host (Table 4). Four environmental variables (3d wave, 3d temperature, salinity, and ammonium) represented the combination of parameters that best explained the variability in the epiphytic assemblage data in terms of parsimony and model improvement with the addition of additional terms.

**Table 1**

ANOVA results for epiphytes. Host 1 is the macrophyte that the epiphytes were significantly more abundant on; Host 2 is the macrophyte they were less abundant on. Only macrophytes from the same site and season were compared to isolate possible evidence of host specificity (616 comparisons). L = LKH; P = TPH; D = *Dictyota*; H = *Halimeda*; W = Winter (December and January); and S = Summer (June and July). ** = $p \le 0.01$; *** = $p \le 0.001$. There were 3 degrees of freedom for treatment (season x host) for both LKH and TPH. Total degrees of freedom were 24 and 20 for LKH and TPH, respectively. Numbers in parentheses represent average epiphyte abundance ($\ln(\mu m^3\ cm^{-2} + 1)$) ± 1 standard deviation.

| Epiphyte Morphotypes | Host 1 | Host 2 | *p*-value |
|---|---|---|---|
| *Licmophora* sp. 1 | PHW<br>(10.32 ± 0.26) | PDW<br>(0 ± 0) | *** |
| *Gloeotheca* spp. | PDS<br>(12.26 ± 0.35) | PHS<br>(3.37 ± 0.92) | *** |
| *Chaetoceros wighamii* | LDW<br>(4.18 ± 0.79) | LHW<br>(0 ± 0) | *** |
| *Pseudo-nitzschia* spp. | LDS<br>(6.47 ± 0.85) | LHS<br>(0 ± 0) | ** |
| *Cocconeis* spp. | PHS<br>(7.31 ± 0.97) | PDS<br>(0 ± 0) | ** |
| *Bleakeleya notata* | LDS<br>(11.21 ± 0.92) | LHS<br>(0 ± 0) | ** |

These four variables exhibit differences between seasons and among sites (Fig. 2). Ammonium concentrations were higher in the summer at all sites and typically higher at the bayside sites (HGB and TPH) versus the oceanside sites (LKH and TRL). Salinity was most similar between sites and seasons, with slightly elevated salinities during the winter (dry season) at three of the four sites (except HGB). Temperatures were typically higher in the summer versus winter, with the bayside sites exhibiting a greater range (i.e., warmer in the summer and colder in the winter). Relative wave heights were larger at the bayside sites during winter, likely in response to more northerly winds and longer fetches creating conditions of greater exposure. Wave heights were more consistent between seasons at the oceanside sites.

### 3.4. Epiphytic assemblages and environmental variability

The CAP results revealed that there were significant correlations between the four selected environmental variables and epiphyte assemblage data with correlations of 0.93 and 0.88 for the first two eigenvalues, respectively. The trace statistic and first squared canonical correlation were both significant ($p = 0.001$ after 999 permutations). The four sites separated out along the bay – ocean plane, with LKH and TRL samples in the upper right quadrant of the plot and HGB and TPH in the lower portion (Fig. 3). Seasonal separation (summer samples to the upper left; winter to the lower right) was particularly evident for HGB and TPH, slightly attenuated for LKH, and not evident for TRL. The sample distributions demonstrate that the bay sites (HGB and TPH) fluctuated between higher temperature and lower wave energy conditions in the summer, to cooler temperatures and higher wave conditions in the winter. The ocean sites (LKH and TRL) did not exhibit such large changes in temperatures and wave heights, particularly TRL which was the most stable site year-round. HGB and TPH samples also grouped with higher ammonium and salinity levels, likely reflecting a higher degree of benthic coupling in these shallow water environments (i.e., more recycled nitrogen), and the hyper-saline conditions that beleaguer Florida Bay, particularly during dry season (winter).

## 4. Discussion

Diatoms dominated the epiphytic community in this study, followed by cyanobacteria, and dinoflagellates, as has been documented in studies from other regions (e.g., the Red Sea (Al-Harbi, 2017); North

**Table 2**

The results of a SIMPER (similarity percentage) analysis displaying the average abundance of the taxa contributing to 50% of the cumulative difference between bayside and oceanside epiphytes. The abundance values are given as $\ln(\mu\text{m}^3\ \text{cm}^{-2}+1)$. The average dissimilarity is based on Bray-Curtis similarity, and is computed by calculating the dissimilarity between bayside sites (HGB and TPH) and the oceanside sites (LKH and TRL). The overall average dissimilarity between the two regions was 59.9%. The % contribution values indicate how much each taxon contributes to the overall dissimilarities between the two regions, with the cumulative % value summing these values to demonstrate how the overall dissimilarity is built by the contributing species.

| Species | Average bayside abundance | Average oceanside abundance | Average dissimilarity | % contribution | Cumulative % |
|---|---|---|---|---|---|
| *Synedra* cf. *fulgens* var. *gigantea* | 4.71 | 7.35 | 1.36 | 2.27 | 2.27 |
| *Licmophora* spp. | 7.39 | 10.82 | 1.35 | 2.25 | 4.52 |
| *Striatella unipunctata* | 7.46 | 7.73 | 1.34 | 2.24 | 6.76 |
| *Tabellaria* cf. *fenestrata* | 7.85 | 8.91 | 1.13 | 2.23 | 8.99 |
| *Licmophora remulus* | 6.49 | 5.19 | 1.31 | 2.19 | 11.18 |
| *Oscillatoria* spp. | 6.03 | 5.48 | 1.26 | 2.10 | 13.28 |
| *Synedra crotonensis* var. *prolongata* | 6.83 | 7.14 | 1.24 | 2.07 | 15.35 |
| *Gomphosphaeria aponina* | 6.77 | 1.89 | 1.22 | 2.03 | 17.38 |
| *Prorocentrum belizeanum* | 11.75 | 7.28 | 1.21 | 2.01 | 19.40 |
| *Bleakeleya notata* | 2.25 | 6.51 | 1.20 | 2.01 | 21.41 |
| *Eunotia* cf. *lunaris* | 4.13 | 7.11 | 1.19 | 1.99 | 23.40 |
| *Thalassiophysa hyalina* | 5.37 | 4.29 | 1.16 | 1.94 | 25.34 |
| *Synedra* spp. | 5.34 | 4.32 | 1.16 | 1.93 | 27.27 |
| Cyanophyceae (undetermined) 2f | 8.13 | 6.11 | 1.15 | 1.91 | 29.18 |
| *Thalassionema* spp. | 8.53 | 9.42 | 1.14 | 1.90 | 31.08 |
| *Licmophora flabellata* | 5.59 | 1.48 | 1.13 | 1.89 | 32.96 |
| *Merismopedia* spp. | 6.22 | 1.11 | 1.09 | 1.82 | 34.78 |
| *Coolia* spp. | 5.55 | 1.38 | 1.09 | 1.81 | 36.59 |
| *Ostreopsis* cf. *heptagona* | 4.55 | 3.30 | 1.08 | 1.80 | 38.39 |
| *Bacillaria paxillifera* | 0.24 | 5.81 | 1.08 | 1.79 | 40.19 |
| *Gambierdiscus* spp. | 5.06 | 2.46 | 1.02 | 1.71 | 41.90 |
| *Anabaena* spp. | 3.76 | 4.70 | 1.02 | 1.71 | 43.60 |
| *Rhabdonema adriaticum* | 5.18 | 1.29 | 1.01 | 1.69 | 45.29 |
| *Climacosphenia moniligera* | 2.98 | 4.39 | 1.00 | 1.68 | 46.97 |
| *Mastogloia fimbriata* | 5.12 | 2.89 | 1.00 | 1.67 | 48.64 |
| Bacillariophyta centrales (undetermined) | 4.37 | 2.08 | 0.96 | 1.60 | 50.24 |

**Table 3**

The results of a SIMPER (similarity percentage) analysis displaying the average abundance of the taxa contributing to 50% of the cumulative difference between winter and summer epiphytes. The abundance values are given as $\ln(\mu\text{m}^3\ \text{cm}^{-2}+1)$. The average dissimilarity is based on Bray-Curtis similarity, and is computed by calculating the dissimilarity between summer months (June and July) and the winter months (December and January). The overall average dissimilarity between the two locations was 60.9%. The % contribution values indicate how much each taxon contributes to the overall dissimilarities between the two seasons, with the cumulative % value summing these values to demonstrate how the overall dissimilarity is built by the contributing species.

| Species | Average winter abundance | Average summer abundance | Average dissimilarity | % contribution | Cumulative % |
|---|---|---|---|---|---|
| *Thalassiophysa hyalina* | 0.88 | 8.50 | 1.51 | 2.49 | 2.49 |
| *Gomphosphaeria aponina* | 0.51 | 7.76 | 1.41 | 2.31 | 4.80 |
| *Licmophora remulus* | 8.19 | 3.60 | 1.39 | 2.28 | 7.08 |
| *Striatella unipunctata* | 7.95 | 7.27 | 1.34 | 2.21 | 9.29 |
| *Tabellaria* cf. *fenestrata* | 8.21 | 8.57 | 1.34 | 2.21 | 11.50 |
| *Synedra* cf. *fulgens* var. *gigantea* | 6.56 | 5.61 | 1.34 | 2.20 | 13.70 |
| *Synedra crotonensis* var. *prolongata* | 8.67 | 5.41 | 1.32 | 2.18 | 15.87 |
| *Eunotia* cf. *lunaris* | 8.24 | 3.26 | 1.30 | 2.14 | 18.01 |
| *Licmophora* spp. | 8.26 | 10.01 | 1.29 | 2.12 | 20.13 |
| *Oscillatoria* spp. | 5.29 | 6.17 | 1.27 | 2.09 | 22.22 |
| *Licmophora flabellata* | 7.17 | 0.00 | 1.27 | 2.09 | 24.31 |
| *Bleakeleya notata* | 2.23 | 6.52 | 1.27 | 2.07 | 26.38 |
| Cyanophyceae (undetermined) 2f | 4.93 | 9.11 | 1.25 | 2.05 | 28.43 |
| *Synedra* spp. | 5.46 | 4.21 | 1.16 | 1.91 | 30.34 |
| *Thalassionema* spp. | 9.19 | 8.80 | 1.15 | 1.88 | 32.22 |
| *Ostreopsis* cf. *heptagona* | 6.01 | 1.94 | 1.14 | 1.87 | 34.09 |
| *Anabaena* spp. | 2.64 | 5.75 | 1.10 | 1.81 | 35.90 |
| *Prorocentrum belizeanum* | 8.94 | 9.91 | 1.10 | 1.80 | 37.70 |
| *Coolia* spp. | 5.85 | 1.10 | 1.06 | 1.74 | 39.44 |
| *Cyclotella* spp. | 0.86 | 5.65 | 1.06 | 1.74 | 41.18 |
| *Merismopedia* spp. | 1.27 | 5.75 | 1.02 | 1.68 | 42.86 |
| *Mastogloia fimbriata* | 2.63 | 5.22 | 1.02 | 1.68 | 44.54 |
| *Climacosphenia moniligera* | 4.51 | 2.95 | 1.02 | 1.67 | 46.21 |
| *Licmophora* sp. 1 | 5.55 | 0.00 | 1.00 | 1.64 | 47.85 |
| *Navicula transitans* | 7.05 | 5.20 | 0.98 | 1.62 | 49.47 |
| *Gambierdiscus* spp. | 3.25 | 4.15 | 0.98 | 1.61 | 51.08 |

Carolina coastal waters (Coleman and Burkholder, 1994); Argentina (Fricke et al., 2016); Antarctica (Majewska et al., 2016)). This commonality indicates that the epiphytic community structure appears to be similar (at least at the class level) across disparate regions, possibly reflecting interactions between these microalgae, or some form of niche separation. Geographic (location) and temporal (seasonal) differences played a much larger role in determining epiphytic assemblages on host macrophytes than the macrophyte species themselves (Table 4; Fig. 3). Frankovich et al. (2009) reported similar findings in their Florida Bay study; epiphytic diatom community structure was primarily influenced

**Table 4**

Results of the Distance-based linear model (DISTLM) analysis indicating the proportion of variation within the epiphytic similarity matrix explain by each factor alone (marginal tests) or in sequential order (variance explained after factors earlier in the sequence are already included). SS (trace) = the total sum of squares computed as the sum of the diagonal values of the centered matrix. The pseudo-*F* statistic (Pseudo-F) is an analog of Fisher's *F* ratio, but the distribution of this statistic is unknown when using DISTLM, requiring additional randomized permutations to build a distribution (known as $F^{\pi}$) from which an exact P-value can be calculated; hence, the "pseudo-" designation. Proportion = proportion of the variation in the epiphyte similarity matrix explained by each factor. Cumulative = cumulative proportion of the variation in the epiphyte similarity matrix explained by the factors (added in sequence). Res. df = residual degrees of freedom. Regr. df = regression degrees of freedom. *** = $p \leq 0.001$.

| Factor | SS (trace) | Pseudo-F | p-value | Proportion | Cumulative | Res. df | Regr. df |
|---|---|---|---|---|---|---|---|
| Marginal tests | | | | | | | |
| Environ | 71,206 | 6.53 | *** | 0.37 | – | 99 | 10 |
| Season | 20,313 | 12.73 | *** | 0.11 | – | 107 | 2 |
| Site | 28,965 | 6.25 | *** | 0.15 | – | 105 | 4 |
| Host | 18,822 | 3.82 | *** | 0.10 | – | 105 | 4 |
| Sequential tests | | | | | | | |
| Environ | 71,206 | 6.53 | *** | 0.37 | 0.37 | 99 | 10 |
| + Season | 6575 | 5.69 | *** | 0.03 | 0.41 | 98 | 11 |
| + Site | 22,569 | 7.87 | *** | 0.12 | 0.52 | 95 | 14 |
| + Host | 14,848 | 6.00 | *** | 0.08 | 0.60 | 92 | 17 |

by spatial and temporal effects. Rodriguez et al. (2010) also reported evidence of site-specific epiphytic assemblages in their Colombian coastal water study. In this study, diatom taxa were more common at the oceanside sites than the bayside sites, whereas dinoflagellate and cyanobacteria taxa were more common at the bayside sites (Table 2). This difference is possibly a result of diatoms being more competitive in more turbulent environments (Smayda and Reynolds, 2001) coupled with the preference of cyanobacteria and dinoflagellates for lower energy environments (Margalef, 1978; Badylak and Phlips, 2004).

Several studies have shown a general increase in dissimilarity of epiphyte assemblages with increasing distance, possibly due to patchiness of macrophyte or epiphyte populations (e.g., Vanderklift and Lavery (2000) observed that epiphytic patchiness occurred on the scale of meters). In their Florida Bay study, Frankovich et al. (2009) found that site differences in diatom composition were greater than within-site treatment effects, suggesting that future studies should focus on relative changes within sites rather than between sites. The significance of location in this study, therefore, could reflect such spatial scaling.

Seasonal differences in epiphytic assemblages were reported in other studies including Dhib et al. (2015), who observed that dinoflagellates exhibited a winter-spring maximum, while diatom abundance peaked in the summer in Tunisian coastal waters. Seasonal differences were not always evident, however. For example, El-Din et al. (2015) found no evidence of seasonality in epiphytic assemblages in Alexandria Harbor, Egypt. Reyes-Vasquez (1970) also reported little seasonal difference in diatom composition on *Thalassia* in Biscayne Bay, Florida. In this study, diatom taxa were more common in winter months (Table 3), again possibly due to increased turbulence. The five most dominant cyanobacteria morphotypes were most common in summer months (Table 3), possibly reflecting growth stimulation provided by higher temperatures (Watermann et al., 1999). High temperatures also have a direct effect on optimizing $N_2$ fixation by enhancing the rate of gas diffusion into the heterocyst (Bauersachs et al., 2014; Mantzouki et al., 2016).

In the current study, variations in physico-chemical characteristics of the overlying water across seasons and sites appear to be most related to epiphyte composition. This statement is supported by the fact that four variables (3d waves, 3d temperature, ammonium, and salinity) accounted for 37% of the variation observed in the epiphyte composition; more than the other three factors combined (site, season and host; Table 4). These findings are also supported by other researchers. Kendrick and Burt (1997) determined that water motion was an important factor in epiphyte composition on *Posidonia oceanica* blades in coastal waters of Western Australia. A similar influence may be reflected in the 3d wave relationship observed in this study. Pinckney and Micheli (1998) observed that diatom biomass was higher on substrates from low wave energy environments, whereas cyanobacteria biomass was higher on substrates from high energy habitats in Pamlico Sound, North Carolina. Mabrouk et al. (2011) reported that wave motion and temperature influenced epiphyte community composition (along with light intensity) in their coastal Tunisia study. Richlen and Lobel (2011) documented that the densities of several epiphytic dinoflagellates (*Gambierdiscus*, *Prorocentrum* and *Amphidinium*) were negatively correlated with water motion, whereas *Ostreopsis* was positively correlated. El-Din et al. (2015) suggested that wave exposure and water motion were likely to be influential factors in shaping epiphyte community composition. Interestingly, given the importance that water motion has received over the years in influencing epiphyte communities (e.g., Szemes, 1948), specific measurements have been challenging (e.g., boundary layers; Koch, 1994). Recent advances in the field, however, have improved the precision and accuracy of these measurements, which should lead to better assessment of the effects of water motion on epiphytes (Noisette et al., 2020).

Many authors have reported on the importance of temperature (e.g., Okolodkov et al., 2014), nutrients (e.g., Fricke et al., 2016), and salinity (e.g., Okolodkov et al., 2014) in influencing epiphyte composition. Other studies, however, found that environmental differences did not appear to affect epiphyte composition. Dhib et al. (2015) found that environmental variables did not correlate with epiphyte biomass on *Ruppia* in a Tunisian study. El-Din et al. (2015) also reported minimal correlation with physico-chemical parameters.

In this study, there were no significant differences in overall epiphyte composition among the different host macrophytes, with only six species being significantly more abundant on one particular host versus another within a given site and season; <1% of the pertinent comparisons (Table 1). Heil et al. (1998), however, documented that each dinoflagellate species encountered in their Australian study displayed distinct substrate preferences. Additionally, Al-Handal and Wulff (2008) concluded that substrate was a more influential factor than site in determining epiphytic diatom composition in an Antarctica study. Harlin (1980) argued that while some host and epiphyte associations appear to be specific, the specificity was speculated to be based on the seagrass habitat rather than the host surface. Similarly, Tindall and Morton (1998) stated that host preference may be evident within a site, but not across sites. Koike et al. (1991) suggested that epiphyte assemblage variation within a single host species population at a given site demonstrated the role of pioneering epiphytic species in influencing subsequent succession. In particular, early settlers play a crucial role as they settle under certain environmental conditions (Callow et al., 2002) and either facilitate or inhibit the settlement of later species (e.g., Raimondi, 1988). Another factor to consider is macrophyte host age. Mabrouk et al. (2014) observed that epiphyte assemblages appeared to be influenced by the lifespan of seagrass blades; short-lived species hosts (e.g., the seagrass, *Cymodocea nodosa*) were dominated by fast growing epiphytes (*Oscillatoria*), whereas the slower growing *P. oceanica* blades

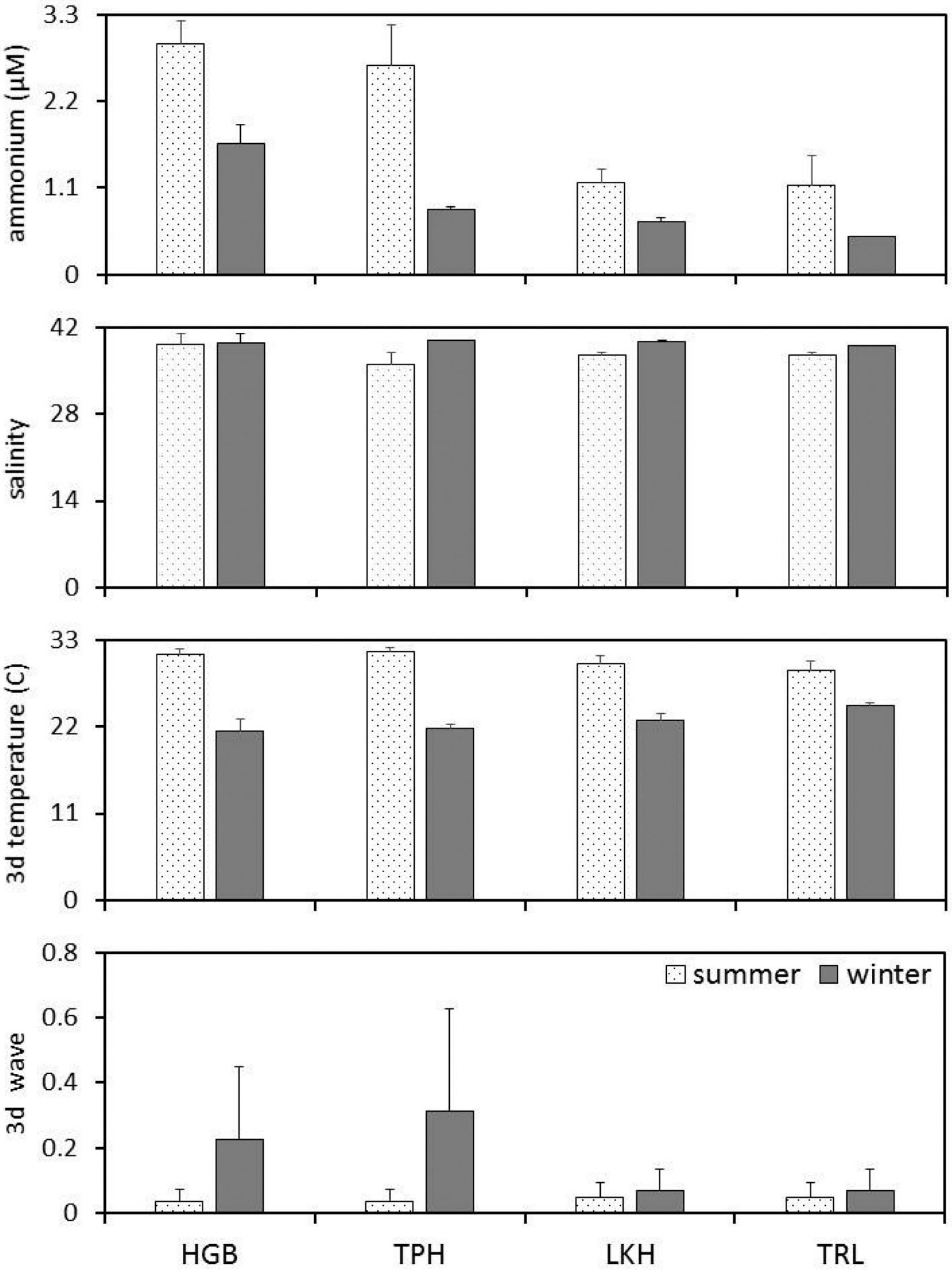

**Fig. 2.** Seasonal averages (± standard error) of the four environmental variables most related to the differences in epiphyte community composition between sites and seasons according to DISTLM analysis. 3d wave height values are standardized and therefore unitless.

hosted slower growing species, like *Prorocentrum*. It is clear that further research is needed to better understand the dynamics of the relationship between host macrophytes and their epiphytic communities.

The differences in the epiphyte communities documented at the four sites of this study ultimately reflect the differences between the environments of western Florida Bay and the Florida Keys barrier reef system in the Atlantic Ocean. Although these regions border each other, they are relatively isolated by the island keys themselves. For example, while *Halimeda* was collected at all four sites, *H. incrassata* was dominant (and most collected) at the bay sites (HGB and TPH), whereas *H. gracilis* was dominant (and most collected) at the ocean sites (LKH and TRL). Seasonal variations in temperature and wave heights were more amplified at the bay versus ocean sites (Fig. 3) reflecting how the lower surface to area ratio of Florida Bay leads to greater seasonal temperature changes (Boyer et al., 1999), and the significant influence of winter cold fronts in creating disruptive waves in the shallow waters of Florida Bay. The differences observed in the epiphytic assemblages among sites and between seasons were generally limited to specific epiphyte morphotypes. Thirty seven out of the 77 morphotypes (48%) included in the DISTLM and SIMPER analyses were responsible for 50% of the differences observed in the epiphytic species between sites and seasons (Tables 2 and 3).

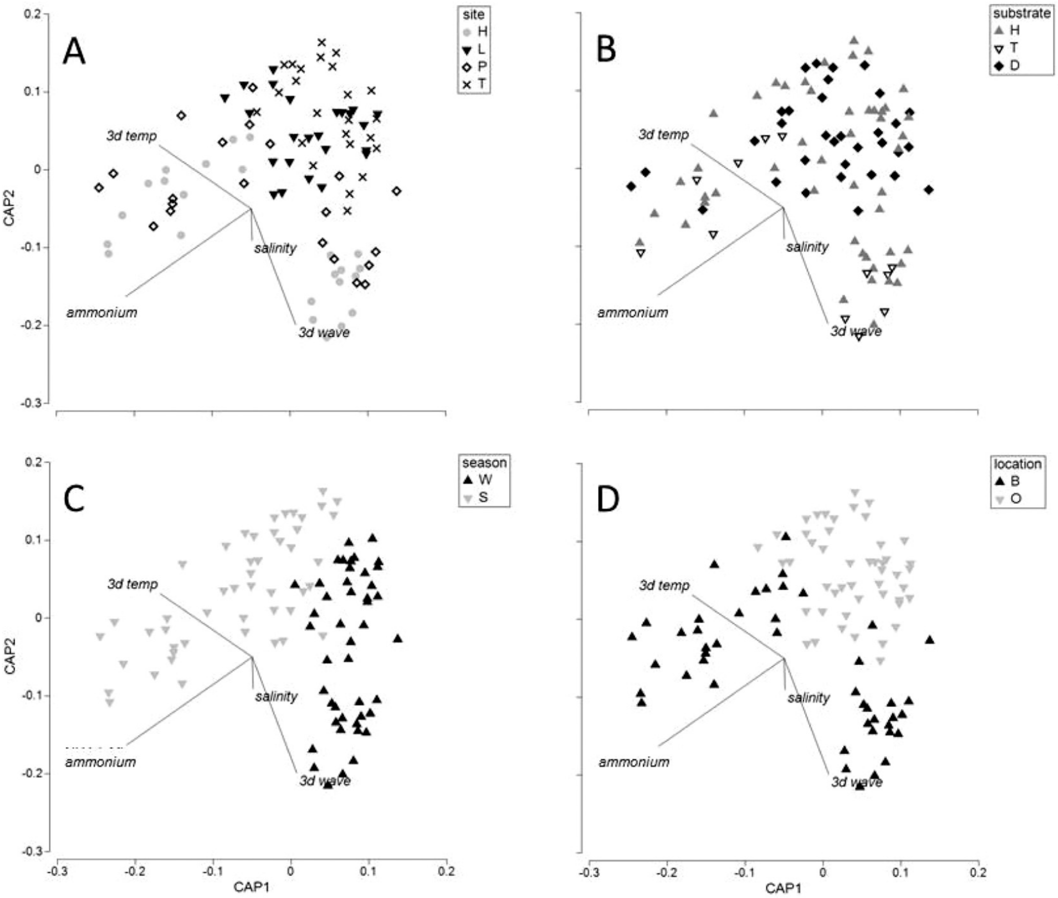

**Fig. 3.** Canonical Analysis of Principal Coordinates (CAP) demonstrating how the epiphyte assemblage composition among samples differed in relation to the environmental variables by: A) site (H = HGB; L = LKH; P = TPH; T = TRL); B) substrate (H = *Halimeda*; T = *Thalassia*; D = *Dictyota*); C) season (W = winter; S = summer); and D) location (B = bayside; O = oceanside).

## 5. Conclusions

The results of this study are similar to a related study on phytoplankton reported in Stanca and Parsons (2017) in the region; there are seasonal and location differences, with common influences of waves and temperature. These similarities suggest that common drivers are influencing the composition of phytoplankton and epiphytic microalgae, which is interesting given the different habitats (water column versus benthos). Most of the identified epiphytes appear to be permanent (perennial) members of the epiphytic community in the region, with fewer than one-third exhibiting seasonal or transient characteristics. Only 60% of the variability in epiphyte composition could be explained by the four factors tested in this study (environmental factors, season, site, and host; Tables 2 and 3). This result indicates that other factors that were not accounted for in this study may be important, including age (or life cycle) of the macrophyte host, epiphyte colonization and succession, and grazing. It is recommended, therefore, that such factors be considered in future studies of epiphytic flora in coastal environments.

Host specificity was not evident for the vast majority of epiphytic species encountered in this study. Rather, environmental factors were most influential, and were primarily expressed through site and seasonal differences sampled herein. A limited number of studies have been conducted globally in which species-level resolution was provided for multiple classes of micro-epiphytes (less than ten publications were identified by the authors), indicating that this study will provide valuable information to the field of epiphyte ecology in general. Additionally, these findings demonstrate that common environmental drivers exist across disparate environments.

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## Author declaration

Both authors (Stanca and Parsons) have seen and approved the final version of the manuscript being submitted. We warrant that the article is the authors' original work, has not received prior publication and is not under consideration for publication elsewhere.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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