100 YEARS Journal of Ecology

British Ecological Society

Journal of Ecology 2014, **102**, 730–737 doi: 10.1111/1365-2745.12233

Ocean acidification outweighs nutrient effects in structuring seagrass epiphyte communities

Justin E. Campbell[1]* and James W. Fourqurean[2]

[1]*Smithsonian Marine Station, Ft. Pierce, FL 34949, USA; and* [2]*Marine Science Program, Department of Biological Sciences and Southeast Environmental Research Center, Florida International University, 11200 SW 8th St., Miami, FL 33199, USA*

Summary

1. Developing a framework for assessing interactions between multiple anthropogenic stressors remains an important goal in environmental research. In coastal ecosystems, the relative effects of aspects of global climate change (e.g. CO_2 concentrations) and localized stressors (e.g. eutrophication), in combination, have received limited attention.

2. Using a long-term (11 month) field experiment, we examine how epiphyte assemblages in a tropical seagrass meadow respond to factorial manipulations of dissolved carbon dioxide ($CO_{2(aq)}$) and nutrient enrichment. *In situ* $CO_{2(aq)}$ manipulations were conducted using clear, open-top chambers, which replicated carbonate parameter forecasts for the year 2100. Nutrient enrichment consisted of monthly additions of slow-release fertilizer, nitrogen (N) and phosphorus (P), to the sediments at rates equivalent to theoretical maximum rates of anthropogenic loading within the region (1.54 g N m^{-2} d^{-1} and 0.24 g P m^{-2} d^{-1}).

3. Epiphyte community structure was assessed on a seasonal basis and revealed declines in the abundance of coralline algae, along with increases in filamentous algae under elevated $CO_{2(aq)}$. Surprisingly, nutrient enrichment had no effect on epiphyte community structure or overall epiphyte loading. Interactions between $CO_{2(aq)}$ and nutrient enrichment were not detected. Furthermore, $CO_{2(aq)}$-mediated responses in the epiphyte community displayed strong seasonality, suggesting that climate change studies in variable environments should be conducted over extended time-scales.

4. *Synthesis.* The observed responses indicate that for certain locations, global stressors such as ocean acidification may take precedence over local eutrophication in altering the community structure of seagrass epiphyte assemblages. Given that nutrient-driven algal overgrowth is commonly cited as a widespread cause of seagrass decline, our findings highlight that alternate climate change forces may exert proximate control over epiphyte community structure.

Key-words: carbon dioxide, CCA, climate change, CO_2, coralline algae, determinants of plant community diversity and structure, eutrophication, filamentous algae, plant–climate interactions, *Thalassia testudinum*

Introduction

Over the next century, it is anticipated that many coastal ecosystems will have to endure multiple anthropogenic stressors, both global and local in nature (Halpern *et al.* 2008). In addition to regional stressors such as eutrophication and associated declines in water quality, broader climate change stressors (e.g. ocean acidification) are now being realized and are gaining importance in terms of how they might alter ecosystem functionality over both short and long time spans (Hall-Spencer *et al.* 2008; Kroeker *et al.* 2011). While these various stressors tend to act in concert, we currently have a poor understanding of potential interactions between these perturbations that function across various spatiotemporal scales (Crain, Kroeker & Halpern 2008; Wernberg, Smale & Thomsen 2012).

At local and regional scales, coastal habitats are particularly susceptible to increases in nutrient loading due to their proximity to urban and agricultural developments, resulting in widespread declines in the health and integrity of these systems (Boesch 2002; Lotze *et al.* 2006; Orth *et al.* 2006; Waycott *et al.* 2009). In seagrass meadows, chronic eutrophication can degrade water quality by increasing the abundance of fast-growing algal taxa (phytoplankton and macroalgae) that can overgrow and shade benthic vegetation (Tomasko &

*Correspondence author: E-mail: campbellju@si.edu

Lapointe 1991; Duarte 1995; Valiela *et al.* 1997; Hauxwell *et al.* 2001; McGlathery 2001). Increases in epiphytic microalgae can also contribute to light attenuation at the leaf surface, further representing an important mechanism of seagrass decline in eutrophic waters (Tomasko & Lapointe 1991; Neckles, Wetzel & Orth 1993; Short, Burdick & Kaldy 1995). As local/regional nutrient loading may serve to accelerate algal growth, such factors rarely act in isolation, calling into question how this effect might be further modified by global climate change stressors.

Ocean acidification (OA) has been documented to have both stimulatory (Andersen & Andersen 2006; Russell *et al.* 2009) and inhibitory (Kuffner *et al.* 2008) effects on algal growth. As dissolved CO_2 concentrations rise, certain carbonate species increase in abundance (CO_2 and HCO_3^-), while others decrease (CO_3^{2-}). Associated with these shifts is a decline in seawater pH, with forecasts of nearly a 0.3–0.5 unit reduction by the year 2100 (Caldeira & Wickett 2003). Many algal groups produce skeletons of carbonate mineralogy that can be sensitive to external pH and adversely affected by seawater acidity, thus exerting an adverse effect on growth (Hall-Spencer *et al.* 2008; Martin *et al.* 2008; Porzio, Buia & Hall-Spencer 2011). In contrast, OA has also been shown to increase the dominance of some fleshy algal groups, either by enhancing photosynthetic carbon fixation or releasing them from competitive associations with calcareous taxa (Gao, Ji & Aruga 1999; Kubler, Johnston & Raven 1999; Russell *et al.* 2009). While OA can have consequences for the structure and functionality of algal assemblages, interactions with other localized stressors have received limited attention (but see Russell *et al.* 2009 and Falkenberg, Russell & Connell 2013b).

This study examines the interaction between nutrient enrichment and ocean acidification on the abundance and community structure of seagrass epiphytes. Epiphytes represent an important component of seagrass ecosystems, substantially contributing to both above-ground biomass and overall productivity (Morgan & Kitting 1984). They are comprised of a varied collection of calcified and fleshy organisms, many of which are sensitive to shifts in physicochemical parameters. In addition to supporting higher trophic levels (Kitting, Fry & Morgan 1984), epiphytes play an important role in biogenic sediment production (Frankovich & Zieman 1994) and can influence CO_2 fluxes in seagrass beds via the precipitation and dissolution of calcium carbonate (Barron *et al.* 2006). Given their productivity and ability to regulate seagrass health via direct shading, research is needed that documents how epiphyte communities respond to multiple environmental stressors.

Using *in situ* experiments (Campbell & Fourqurean 2011, 2013), we assess how epiphyte responses to local/regional stressors (eutrophication) might be modified by broader climate change forcings (ocean acidification). Our methodology further allows us to incorporate natural variation in epiphyte community structure over extended time periods. We test the hypothesis that nutrient enrichment and ocean acidification will both serve to alter epiphyte assemblages by increasing the abundance of fleshy, uncalcified taxa. Both stressors were manipulated in isolation and in combination, thus we further test the hypothesis that shifts towards uncalcified taxa will be most pronounced in treatments where both stressors are present.

Materials and methods

STUDY SITE

Manipulation of CO_2 concentrations and nutrient availability was conducted within a shallow (1 m depth) seagrass bed in the Florida Keys, Florida, USA (24.55° N, 81.75° W). The benthic community was dominated by the seagrass *Thalassia testudinum*, with sparse abundances of the seagrasses *Syringodium filiforme* and *Halodule wrightii*. Several species of calcareous macroalgae (*Halimeda* spp. and *Penicillus* spp.) were further present in a patchy distribution. The sediments were composed of roughly 9% organic matter, with the remaining mineral fraction consisting of fine-grained, biogenic calcium carbonates. Grazers within the seagrass canopy were predominantly comprised of amphipods (*Shoemakerella* sp.), with lower abundances of isopods and gastropods.

EXPERIMENTAL DESIGN

CO_2 and nutrients were manipulated for 11 months (5 August 2010 to 18 July 2011) in a balanced, 3 × 2 factorial design. The factor of CO_2 consisted of three levels (CO_2-enriched chambers, CO_2-ambient chambers and open unchambered plots). The factor of nutrient enrichment consisted of two levels (nitrogen/phosphorus addition, and control). Thirty experimental seagrass plots were arranged in a grid design (5 rows × 6 columns), and replicates ($n = 5$) for each treatment were randomly assigned within each column (see Fig. S1 in Supporting Information). Seagrass plots (0.17 m^2) were spaced at 1-m intervals throughout the grid.

CO_2 ENRICHMENT

Optically clear, open-top acrylic chambers were used to artificially enrich seagrass plots with CO_2 (see Campbell & Fourqurean 2011 for a detailed description). CO_2-enriched seawater was generated in the field by bubbling 100% CO_2 gas into the intake port of a series of submerged water pumps, which subsequently delivered enriched seawater into the acrylic chambers via an underwater PVC network. This technique has previously been demonstrated as an effective means of confining CO_2 enrichment to a target area of the benthos. As CO_2-enriched seawater is pumped into the chambers, it cycles within the seagrass canopy before being flushed out the top of the chamber. This design allows for long-term constraint of carbonate parameters within the chambers, while limiting reductions in light and water motion. The CO_2-ambient chambers received unenriched seawater from a separate series of water pumps connected to an independent PVC network. To serve as an additional control, fully open seagrass plots lacking chambers were also established. The level of CO_2 enrichment was carefully controlled, and set to approximate forecasts for the year 2100, roughly a 0.3 unit reduction in pH and pCO_2 of nearly 800 μatm (Caldeira & Wickett 2003, 2005). On a weekly basis, all chambers were scrubbed clear of any fouling, and pH measurements (NBS scale, relative accuracy ± 0.002) were taken within all chambers and open plots during the 12:00–15:00 time period. Replicate

water samples (40 mL) were collected periodically during the experiment to monitor salinity and total alkalinity. All water samples were immediately filtered through a 0.7-μm GFF filter and stored on ice until further processing. Total alkalinity was measured in the laboratory via automated, potentiometric titration with 0.1 N HCl. Salinity was measured with an Orion conductivity metre. Carbonate parameters (CO_2, HCO_3^-, CO_3^{2-} and calcite/aragonite saturation states) were calculated with the CO_2SYS Excel Macro (Lewis & Wallace 1998), using the dissociation constants of Mehrbach *et al.* (1973), refit by Dickson & Millero (1987).

NUTRIENT ENRICHMENT

Fertilizer was evenly distributed by hand over each designated chamber or open plot on a monthly basis. Nitrogen (N) was added in the form of urea-coated slow-release N fertilizer (Polyon, Pursell Technologies; 38-0-0, 94% nitrogen as urea), and phosphorus (P) was added as defluorinated granular phosphate rock [Multifos, IMC Phosphates] $Ca_3(PO_4)^2$, 18%P). Previous studies have effectively used these enrichment methods to increase nutrient availability to the above- and below-ground biomass of benthic macrophytes, as well as the epiphyte community in South Florida (Armitage & Fourqurean 2009; Armitage, Frankovich & Fourqurean 2011; Ferdie & Fourqurean 2004; Frankovich *et al.* 2009). Final loading rates were 1.54 g N m^{-2} d^{-1} and 0.24 g P m^{-2} d^{-1}, similar to prior fertilization studies within this region (Ferdie & Fourqurean 2004; Armitage *et al.* 2005; Armitage, Frankovich & Fourqurean 2011), and reflecting theoretical sewage loading as based upon regulatory estimates of potential maximum wastewater and stormwater discharges. Total estimates of anthropogenic N and P loading as reported by the Monroe County Stormwater Management Program (MCSM 2001) were adjusted for groundwater sorption, and then delivery rates were calculated by applying this amount to nearshore waters (adjacent 10 m) surrounding the Florida Keys. During the course of the experiment, the nutrient content of seagrass leaf material in all chambers and open plots was measured as a means of monitoring nutrient availability. In oligotrophic settings, added nutrients can be rapidly consumed via abiotic processes (binding to particulate matter) and biological uptake. Thus, examining the nutrient content of seagrass leaf material can provide an increasingly reliable indication of nutrient status (Gerloff & Krombholtz 1966; Fourqurean, Zieman & Powell 1992b). Seagrass leaf N and P content (% dry weight) was assessed every 2 months by drying and grinding seagrass material harvested within each chamber and open plot. Nitrogen content was analysed using a CHN analyser, and P content was determined via dry oxidation, acid hydrolysis extraction followed by colorimetric analysis (Fourqurean, Zieman & Powell 1992a).

EPIPHYTE SAMPLING

The epiphyte community was assessed seasonally (December 2010 and July 2011). During each sampling, six seagrass shoots were haphazardly harvested from each chamber and open plot, carefully bagged to prevent dislodging/loss of epiphytes and placed on ice for transport. To determine epiphyte percentage coverage, one shoot from each bag was randomly selected, leaves were detached from the short shoot with a razor blade, and both sides were scanned at high resolution (1200 dpi). Image files were analysed with point count software (CPCe, Kohler & Gill 2006), whereby 100 points were randomly superimposed across the surface of each shoot, and the epiphyte taxa underneath each point was recorded. Epiphyte load was quantified by analysing the chlorophyll *a* (Chl-*a*) content of the attached epiphytes on two randomly selected seagrass shoots from each plot. Leaves were removed from each short shoot, rinsed in deionized water and measured for length and width. Epiphytes were scraped into a single pre-weighed 20-mL glass scintillation vial, lyophilized to obtain a dry weight and then stored in 20 mL of 90% acetone for 72 h. The Chl-*a* content of the extract was measured fluorometrically (Strickland & Parsons, 1972) on a Shimadzu RF-5301 PC Spectrofluorometer (excitation = 435 nm, emission = 667 nm). Total epiphyte load was quantified as shoot-specific mass (epiphyte dry mass/shoot), leaf-specific mass (epiphyte dry mass/leaf area) and leaf-specific Chl-*a* load (ug Chl-*a*/leaf area). Epiphyte autotrophic index (ug Chl-*a*/g epiphyte dry mass) was additionally calculated. Epiphyte $CaCO_3$ load was assessed using a gravimetric-acidification technique. The remaining three seagrass shoots from each chamber and control plot were rinsed in deionized water, and all leaves were removed from the short shoot with a razor and dried at 70 °C for 48 h. Leaves were then weighed, acidified for 3 min in 5% HCl, DI rinsed, dried and reweighed. Epiphytic calcium carbonate load (g $CaCO_3$ g^{-1} dry plant mass) was determined by calculating weight loss after acidification.

STATISTICAL ANALYSES

All data were tested for normality and variance homogeneity. Seawater carbonate parameters were analysed by comparing the 95% confidence intervals of the mean carbonate measurements (recorded during the 12:00–15:00 time period throughout the experiment) within the chambers and control plots. Measurements of epiphyte abundance, epiphyte Chl-*a* characteristics, epiphyte carbonate load and seagrass nutrient content were analysed with a repeated measures, two-way ANOVA; with season as the within-subject factor, and CO_2 and nutrient enrichment as the between-subject factors. When significance was detected, *post hoc* analysis was performed with a Holm–Sidak test, whereby the overall significance level was adjusted to 0.05 to account for multiple comparisons. During each sampling event, all replicate measurements within each chamber and control plot were averaged to avoid pseudoreplication.

Results

SEAWATER PARAMETERS

Elevated CO_2 concentrations were effectively maintained within the experimental chambers, remaining near parameter forecasts for the year 2100 (Table 1 and Fig. S2). Mean pH within the CO_2-enriched chambers (for both levels of nutrient addition) was 7.88, while mean pH within the unenriched chambers and control plots was 8.20 and 8.19, respectively. CO_2 concentrations were 2.5 times higher, and CO_3^{2-} concentrations were 1.7 times lower within the CO_2-enriched chambers as compared to the control chambers and open plots. Total alkalinity was unaltered by CO_2 enrichment, averaged 2503 ± 57.50 (±1SE) μmol kg^{-1} across all treatments and ranged from 2743.84 in February to 2266.81 in April. Salinity averaged 35.5 ± 0.6 and ranged from 37.8 in May to 33.9 in November. Temperature averaged 29.1 ± 0.7 °C, with a maximum of 35.0 °C in August and a minimum of 19.6 °C in December. Prior studies using this chamber system further revealed diurnal variation in seawater carbonate chemistry at this experimental site, with pH fluctuations near 0.2 units over a 24-h period (Campbell & Fourqurean 2011). Such variation is

Table 1. Seawater carbonate parameters within the chambered and open control plots

Seawater parameter	Open plot	Open plot NP	Lo CO_2	Lo CO_2 NP	Hi CO_2	Hi CO_2 NP
pH (NBS scale)	8.19 (8.22–8.15)	8.19 (8.22–8.16)	8.20 (8.23–8.17)	8.19 (8.22–8.16)	7.86 (7.92–7.81)	7.90 (7.95–7.84)
DIC (μmol kg^{-1})	2140 (2191–2090)	2140 (2190–2089)	2132 (2180–2083)	2136 (2185–2087)	2319 (2374–2263)	2305 (2360–2250)
CO_2 (μmol kg^{-1})	11.6 (12.8–10.4)	11.5 (12.7–10.3)	11.1 (12.2–10.0)	11.3 (12.4–10.2)	29.9 (35.0–24.8)	27.4 (32.6–22.3)
HCO_3^- (μmol kg^{-1})	1866 (1924–1809)	1865 (1922–1808)	1853 (1907–1798)	1860 (1914–1805)	2138 (2200–2075)	2119 (2181–2057)
CO_3^{2-} (μmol kg^{-1})	262 (280–245)	263 (280–246)	268 (284–252)	265 (281–249)	151 (169.6–132.6)	159 (176–141)
pCO_2 (μatm)	447 (490–404)	445.4 (488.7–402.0)	429 (468–390)	437 (476–398)	1147 (1329–965)	1054 (1243–864)
Ω Calcite	6.3 (6.8–5.9)	6.4 (6.8–6.0)	6.5 (6.9–6.1)	6.4 (6.8–6.0)	3.7 (4.1–3.2)	3.8 (4.3–3.4)
Ω Aragonite	4.2 (4.5–4.0)	4.3 (4.5–4.0)	4.3 (4.6–4.1)	4.3 (4.6–4.0)	2.4 (2.8–2.1)	2.6 (2.9–2.3)

Values represent the averages of repeated measurements (n = 35) taken at the 12:00–15:00 time period during the course of enrichment.
Bracketed values represent 95% confidence intervals.
Across all treatments, total alkalinity averaged 2503 μmol kg^{-1}, while temperature and salinity averaged 29.1 °C and 35.5, respectively.

likely the result of biological activity (photosynthesis and respiration) that serve to increase pH during the day and reduce pH at night (Yates *et al.* 2007). These diurnal trends in seawater chemistry were further evident in all chambered treatments.

NUTRIENT ENRICHMENT

Nutrient addition increased the N content of seagrass leaf material (repeated measures ANOVA, $F_{1,22} = 31.50$, $P < 0.001$), indicating an increase in the availability of nitrogen within the nutrient-enriched plots. Nitrogen content (mean ± 1SE) was 2.28 ± 0.09%N in the unenriched treatments, and 2.47 ± 0.1%N in the nutrient-enriched treatments. Despite phosphorus addition, P leaf content was unaltered over the course of the experiment, with unenriched and enriched plots averaging 0.16 ± 0.004%P and 0.15 ± 0.004%P, respectively. This lack of a response suggests that P availability might not be limiting to primary producers within this region, as previously documented for seagrass communities at nearby locations in Florida Bay (Armitage *et al.* 2005; Armitage, Frankovich & Fourqurean 2006). Seasonal trends in %N and %P content followed patterns characteristic of seagrass beds within this region, with increases in the winter and declines during the following spring (Fourqurean *et al.* 2005). Such trends were evident across all treatments and indicate a general increase in N and P availability during the winter when growth rates are minimal, and decline in availability during the summer when growth rates are maximal (Fourqurean *et al.* 2005).

EPIPHYTE ABUNDANCE AND $CACO_3$ LOAD

The epiphyte community was dominated by a collection of crustose coralline algae (*Melobesia membranacea* and *Hydrolithon farinosum*) and filamentous algae (*Ceramium brevizonatum* and *Polysiphonia binneyi*.). The serpulid worm *Spirorbis* sp. and an unidentified foraminiferan were also present. Thus, epiphyte categories were collapsed into broad groupings of coralline algae (CCA), filamentous algae, serpulid worms or foraminifera. Other unidentified epiphytes represented <1% of the taxa on a percentage cover basis.

Percentage coverage of CCA was altered by both CO_2 enrichment (repeated measures ANOVA, $F_{2,24} = 22.32$, $P < 0.001$) and season ($F_{1,24} = 6.09$, $P = 0.021$) (Figs 1a and S3, Table S1). The interaction between these factors was additionally significant ($F_{2,24} = 5.63$, $P < 0.001$), as CO_2-induced reductions in CCA coverage were greatest during the winter sampling (approximately a 10-fold decline). Within the CO_2-ambient chambers and control plots, CCA abundance was highest in the winter. Epiphytic $CaCO_3$ load followed similar trends to CO_2-induced and seasonal shifts in CCA percentage coverage (Fig. 1b). Nutrient enrichment had no effect on CCA coverage in either season ($F_{1,24} = 1.24$, $P = 0.277$).

The abundance of the serpulid worm (*Spirorbis* sp.) was reduced with CO_2 enrichment ($F_{2,24} = 6.56$, $P = 0.005$, Fig. 1c, Table S1), but unaffected by nutrient enrichment. *Spirorbis* abundance declined during the summer sampling and furthermore, CO_2 enrichment had a significantly greater effect during the winter season; thus the within-subject factor of season, and the interaction between season and CO_2 were significant ($F_{1,24} = 4.52$, $P = 0.044$ and $F_{2,24} = 6.12$, $P = 0.007$, respectively).

Foraminifera abundance was reduced by the presence of the chambers ($F_{2,24} = 12.04$, $P < 0.001$), however there were no differences between the CO_2-enriched and CO_2-ambient chambers (Fig. 1d and Table S1). The within-subject factor of season was significant ($F_{1,24} = 22.41$, $P < 0.001$), as was the interaction between season and carbon treatment ($F_{2,24} = 9.19$, $P < 0.001$), suggesting that seasonal differences were greater within the open plots, as compared to the chambered treatments.

Filamentous algae increased in response to CO_2 enrichment ($F_{2,24} = 5.86$, $P < 0.01$, Fig. 1e), and was unaffected by nutrient enrichment ($F_{1,24} = 0.01$, $P = 0.979$). *Post hoc* analysis revealed that the CO_2-enriched chambers were distinct

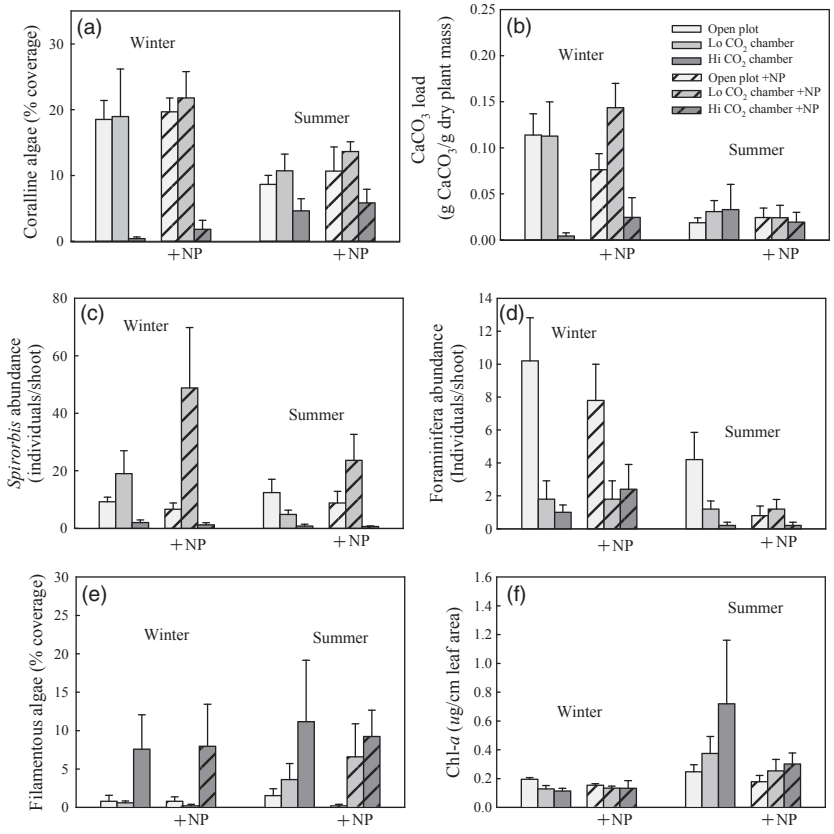

Fig. 1. Shifts in epiphyte community structure under CO_2 and nutrient enrichment. Graphs display epiphyte metrics (means ± SE, $n = 5$) as assessed during a winter (December 2010) and summer (July 2011) sampling.

from both the CO_2-ambient chambers and the control plots. Season had no influence on filamentous algae, and treatment interactions were not significant.

EPIPHYTE CHL-A CHARACTERISTICS

Epiphyte Chl-*a* loads (ug Chl-*a*/cm leaf area) were significantly higher during the summer season ($F_{1,24} = 6.32$, $P = 0.019$, Fig. 1f); however, neither CO_2 nor nutrient enrichment had any effect. Dry epiphyte mass (g/shoot) and leaf-specific epiphyte mass (g/cm leaf area) were unaltered by season, nutrients or CO_2 enrichment (Table S1). Epiphyte autotrophic index (ug Chl-*a*/g epiphyte dry mass) was additionally not affected by CO_2 or nutrient enrichment; however, season did have a significant effect ($F_{1,24} = 26.23$, $P < 0.001$), with higher values during the summer sampling (Table S1).

Discussion

Ocean acidification shifted the community structure of seagrass epiphytes by reducing calcareous taxa and increasing fleshy taxa. Furthermore, seasonal variation revealed that the effects of ocean acidification vary depending upon the sampling period; thus, future studies should make efforts to repeatedly sample across extended time periods. Contrary to our hypotheses, nutrient fertilization had no effect on epiphyte load or community structure, and interactions between fertilization and ocean acidification were not detected. These results suggest that for some locations, broader climate change stressors may take precedence in altering community structure over localized pressures.

The calcareous skeleton of coralline algae renders them sensitive to acidified conditions (Hall-Spencer *et al.* 2008; Jokiel *et al.* 2008; Kuffner *et al.* 2008; Martin *et al.* 2008; Gao & Zheng 2010). We document a decline in CCA coverage from 20% in the CO_2-ambient chambers to nearly 1% within the CO_2-enriched chambers during the winter season. Epiphytic $CaCO_3$ loads followed similar trends. Crustose coralline algae produce carbonate skeletons with a highly soluble mineralogy (high magnesium calcite), which readily dissolves under acidic conditions relative to other forms of $CaCO_3$; thus, future ocean acidification might have drastic consequences for these taxa (Jokiel *et al.* 2008; Kuffner *et al.* 2008). Seasonal variation in both CCA abundance and the effects of ocean acidification might be attributed to multiple factors; however, the limited sampling frequency of this experiment precludes us from drawing strong conclusions from this trend. While CCA abundance and epiphyte $CaCO_3$ load were lower during the summer sampling, we detected no seasonal change in total epiphyte dry mass (g/shoot) or leaf-specific epiphyte mass (g/cm leaf area). Increases in Chl-*a* load and epiphyte autotrophic index were detected during the summer, suggesting a decrease in the contribution of calcified taxa. Across all treatments, these trends suggest temporal shifts in the dominance of one epiphyte group relative to another at our site, with CCA taxa dominating in the winter

and uncalcified taxa dominating in the summer. The overall decline in calcified groups during the summer likely accounts for the significant season × CO_2 interaction.

The abundance of the serpulid worm, *Spirorbis* sp. was reduced with ocean acidification and unaltered by nutrient addition. The mechanisms for these declines are similar to those responsible for declines in CCA abundance, as *Spirorbis* worms secrete protective $CaCO_3$ shells sensitive to low-pH environments (Cigliano *et al.* 2010). Benthic foraminifera were not affected by acidification; however, abundances were reduced within our CO_2-enriched and CO_2-ambient chambers. This unexpected 'caging effect' remains unexplained; thus, we are hesitant to draw strong conclusions in regard to the sensitivity of benthic foraminifera to acidification. We note that this 'caging effect' was not detected for any other epiphyte group.

Ocean acidification increased fleshy filamentous algae. The CO_2-enriched chambers displayed higher abundances as compared to both the CO_2-ambient chambers and open plots. Furthermore, such trends were not dependent upon season or nutrient availability. CO_2-induced increases in uncalcified algal groups (particularly turf taxa) have been previously demonstrated in several studies (Russell *et al.* 2009; Connell & Russell 2010). Uncalcified algal taxa have been considered unresponsive to CO_2 enrichment due to the presence of efficient carbon concentrating mechanisms (CCMs) and carbon saturated photosynthetic rates (Beer & Koch 1996). However, not all algal groups contain and utilize CCMs, as some rely upon the diffusive flux of CO_2 to support photosynthetic rates (Maberly 1990; Maberly, Raven & Johnston 1992; Raven 2003). Thus, these taxa may benefit from acidification and increases in CO_2 (Hepburn *et al.* 2011). Prior work has demonstrated increases in the biomass of temperate turfs under CO_2 enrichment (Russell *et al.* 2009; Connell & Russell 2010). These responses were linked to increases in effective quantum yield, suggesting that CO_2 enrichment may have improved photosynthetic efficiency. A similar mechanism may have operated in our experiment and account for our detected increases in filamentous algae. In addition to being potentially stimulated by CO_2 supply, the dominance of fleshy epiphytes may have resulted from decreased competition with coralline algae (Russell *et al.* 2009; Hepburn *et al.* 2011). While our methodology prevents us from drawing strong conclusions in regard to the role of space competition in our experiment, we submit that acidification may have further favoured filamentous algae by decreasing CCA coverage and increasing the leaf space available for recruitment.

Epiphyte Chl-*a* loads were not affected by either acidification or nutrient enrichment. Furthermore, we failed to detect any treatment effects on dry epiphyte mass or leaf-specific epiphyte mass. Thus, while we document large shifts epiphyte community structure, epiphyte loads remained similar among our treatments. Epiphyte loading has been commonly cited as cause of seagrass decline due to excessive overgrowth and shading (Duarte 1995). The similarity of leaf loading metrics (Chl-*a*, dry epiphyte mass and leaf-specific epiphyte mass) among our treatments suggests that improvements in light transmission to the leaf surface due to declines in CCA may have been offset by increases in filamentous algae. However, note that light transmission through the epiphyte layer may be further modified by the natural orientation and morphology of the epiphyte canopy (Frankovich & Zieman 2005). Thus, light transmission through an epiphyte community comprised of flat, two-dimensional CCA (representative of our control treatments) may be distinct from light transmission through a dense layer of three-dimensional filamentous algae (representative of our acidified treatment). Alterations in light transmission due to shifts in epiphyte morphology may not have been revealed by our Chl-*a* analysis, which removes such structural characteristics.

Nitrogen and phosphorus enrichment had no effect on any of our measured loading metrics, suggesting that epiphyte assemblages were not strongly nutrient limited at this site, and that alternate factors likely exerted more proximate control. Our findings are consistent with the conclusions of prior studies, suggesting that epiphyte responses to nutrient enrichment can be variable and site-specific within this region (Frankovich *et al.* 2009; Armitage, Frankovich & Fourqurean 2011). Additional work has further documented weak relationships between nutrient availability and epiphyte loading across seagrass meadows in Florida Bay and the Florida Keys (Frankovich & Fourqurean 1997; Fourqurean, Muth & Boyer 2010). While the factors controlling epiphyte community structure at any given location are complex, the lack of a response to nutrient enrichment at our site may be attributable to influences from either top-down forces (grazing activity) or other physicochemical parameters (CO_2 availability, salinity and temperature).

Interactions between ocean acidification and nutrient supply were undetected in our experiment, contrasting with other studies aimed at addressing interactions between global climate change and other localized stressors (Russell *et al.* 2009; Falkenberg, Connell & Russell 2013a; Falkenberg, Russell & Connell 2013b). For example, Russell *et al.* 2009 document increased turf recruitment in the presence of CO_2 and nutrient enrichment, and this interaction provided a 34% increase in algal abundance as compared to the sum of individual effects. The lack of significant CO_2 × nutrient interaction, and the disparities between Russell *et al.* 2009 and the present work might result from either methodological distinctions or the site-specific effects of nutrient enrichment (Armitage, Frankovich & Fourqurean 2006). Our experimental design allowed for natural variation in grazing pressure and environmental parameters, both of which might have masked strong responses to nutrient enrichment.

Our findings are in agreement with other studies examining the effects of ocean acidification on the community structure of benthic organisms (Martin *et al.* 2008; Russell *et al.* 2009; Porzio, Buia & Hall-Spencer 2011). As seawater pH continues to decline, current research demonstrates that broad shifts from calcified to fleshy taxa are likely. Coupled to these shifts, are potential alterations in ecosystem diversity and resilience (Kroeker *et al.* 2011). Our results suggest that similar trends may be expected within seagrass meadows,

which harbour a diverse collection of calcified and fleshy organisms. Specific consideration of the epiphyte community entails a number of consequences in the context of ocean acidification, as i) future declines in epiphytic $CaCO_3$ loads may alter rates of biogenic sediment production and ii) increases in fleshy epiphytes may ultimately influence the grazing community and food-web structure in seagrass habitats.

Acknowledgements

We thank Dr. Patrick Rice and the Florida Keys Community College for logistical support. Jennifer Sweatman, Bryan Dewsbury, Nathan Lemoine and Thomas Frankovich provided assistance in the field. This work was supported by the National Science Foundation through the Florida Coastal Everglades Long-Term Ecological Research Program under Grant No. DBI-0620409, and a Graduate School Doctoral Evidence Acquisition Fellowship awarded to J.E.C by Florida International University. This is contribution number 654 from the Southeast Environmental Research Center at FIU. The authors declare no conflict of interest.

References

Andersen, T. & Andersen, F.O. (2006) Effects of CO_2 concentration on growth of filamentous algae and *Littorella uniflora* in a Danish softwater lake. *Aquatic Botany*, **84**, 267–271.

Armitage, A.R. & Fourqurean, J.W. (2009) Stable isotopes reveal complex changes in trophic relationships following nutrient addition in a coastal marine ecosystem. *Estuaries and Coasts*, **32**, 1152–1164.

Armitage, A.R., Frankovich, T.A. & Fourqurean, J.W. (2006) Variable responses within epiphytic and benthic microalgal communities to nutrient enrichment. *Hydrobiologia*, **569**, 423–435.

Armitage, A.R., Frankovich, T.A. & Fourqurean, J.W. (2011) Long-term effects of adding nutrients to an oligotrophic coastal environment. *Ecosystems*, **14**, 430–444.

Armitage, A.R., Frankovich, T.A., Heck, K.L. & Fourqurean, J.W. (2005) Experimental nutrient enrichment causes complex changes in seagrass, microalgae, and macroalgae community structure in Florida Bay. *Estuaries*, **28**, 422–434.

Barron, C., Duarte, C.M., Frankignoulle, M. & Borges, A.V. (2006) Organic carbon metabolism and carbonate dynamics in a Mediterranean seagrass (*Posidonia oceanica*) meadow. *Estuaries and Coasts*, **29**, 417–426.

Beer, S. & Koch, E. (1996) Photosynthesis of marine macroalgae and seagrasses in globally changing CO_2 environments. *Marine Ecology-Progress Series*, **141**, 199–204.

Boesch, D.F. (2002) Challenges and opportunities for science in reducing nutrient over-enrichment of coastal ecosystems. *Estuaries*, **25**, 886–900.

Caldeira, K. & Wickett, M.E. (2003) Anthropogenic carbon and ocean pH. *Nature*, **425**, 365–365.

Caldeira, K. & Wickett, M.E. (2005) Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *Journal of Geophysical Research-Oceans*, **110**, C09S04.

Campbell, J.E. & Fourqurean, J.W. (2011) Novel methodology for *in situ* carbon dioxide enrichment of benthic ecosystems. *Limnology and Oceanography-Methods*, **9**, 97–109.

Campbell, J.E. & Fourqurean, J.W. (2013) Effects of *in situ* CO_2 enrichment on the structural and chemical characteristics of the seagrass *Thalassia testudinum*. *Marine Biology*, **160**, 1465–1475.

Cigliano, M., Gambi, M., Rodolfo-Metalpa, R., Patti, F. & Hall-Spencer, J. (2010) Effects of ocean acidification on invertebrate settlement at volcanic CO_2 vents. *Marine Biology*, **157**, 2489–2502.

Connell, S.D. & Russell, B.D. (2010) The direct effects of increasing CO_2 and temperature on non-calcifying organisms: increasing the potential for phase shifts in kelp forests. *Proceedings of the Royal Society B-Biological Sciences*, **277**, 1409–1415.

Crain, C.M., Kroeker, K. & Halpern, B.S. (2008) Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters*, **11**, 1304–1315.

Dickson, A.G. & Millero, F.J. (1987) A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep-Sea Research*, **34**, 1733–1743.

Duarte, C.M. (1995) Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia*, **41**, 87–112.

Falkenberg, L.J., Connell, S.D. & Russell, B.D. (2013a) Disrupting the effects of synergies between stressors: improved water quality dampens the effects of future CO_2 on a marine habitat. *Journal of Applied Ecology*, **50**, 51–58.

Falkenberg, L.J., Russell, B.D. & Connell, S.D. (2013b) Contrasting resource limitations of marine primary producers: implications for competitive interactions under enriched CO_2 and nutrient regimes. *Oecologia*, **172**, 575–583.

Ferdie, M. & Fourqurean, J.W. (2004) Responses of seagrass communities to fertilization along a gradient of relative availability of nitrogen and phosphorus in a carbonate environment. *Limnology and Oceanography*, **49**, 2082–2094.

Fourqurean, J.W., Muth, M.F. & Boyer, J.N. (2010) Epiphyte loads on seagrasses and microphytobenthos abundance are not reliable indicators of nutrient availability in oligotrophic coastal ecosystems. *Marine Pollution Bulletin*, **60**, 971–983.

Fourqurean, J.W., Zieman, J.C. & Powell, G.V.N. (1992a) Phosphorus limitation of primary production in Florida Bay - Evidence from C-N-P ratios of the dominant seagrass *Thalassia testudinum*. *Limnology and Oceanography*, **37**, 162–171.

Fourqurean, J.W., Zieman, J.C. & Powell, G.V.N. (1992b) Relationships between Porewater Nutrients and Seagrasses in a Subtropical Carbonate Environment. *Marine Biology*, **114**, 57–65.

Fourqurean, J.W., Escorcia, S.P., Anderson, W.T. & Zieman, J.C. (2005) Spatial and seasonal variability in elemental content, $\delta^{13}C$, and $\delta^{15}N$ of *Thalassia testudinum* from South Florida and its implications for ecosystem studies. *Estuaries*, **28**, 447–461.

Frankovich, T.A. & Fourqurean, J.W. (1997) Seagrass epiphyte loads along a nutrient availability gradient, Florida Bay, USA. *Marine Ecology-Progress Series*, **159**, 37–50.

Frankovich, T.A. & Zieman, J.C. (1994) Total epiphyte and epiphytic carbonate production on *Thalassia testudinum* across Florida Bay. *Bulletin of Marine Science*, **54**, 679–695.

Frankovich, T.A. & Zieman, J.C. (2005) Periphyton light transmission relationships in Florida Bay and the Florida Keys, USA. *Aquatic Botany*, **83**, 14–30.

Frankovich, T.A., Armitage, A.R., Wachnicka, A.H., Gaiser, E.E. & Fourqurean, J.W. (2009) Nutrient effects on seagrass epiphyte community structure in Florida Bay. *Journal of Phycology*, **45**, 1010–1020.

Gao, K.S., Ji, Y. & Aruga, Y. (1999) Relationship of CO_2 concentrations to photosynthesis of intertidal macroalgae during emersion. *Hydrobiologia*, **399**, 355–359.

Gao, K.S. & Zheng, Y.Q. (2010) Combined effects of ocean acidification and solar UV radiation on photosynthesis, growth, pigmentation and calcification of the coralline alga *Corallina sessilis* (Rhodophyta). *Global Change Biology*, **16**, 2388–2398.

Gerloff, G.C. & Krombholtz, P.H. (1966) Tissue analysis as a measure of nutrient availability for the growth of angiosperm aquatic plants. *Limnology and Oceanography*, **11**, 529–537.

Hall-Spencer, J.M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., Fine, M., Turner, S.M., Rowley, S.J., Tedesco, D. & Buia, M.C. (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature*, **454**, 96–99.

Halpern, B.S., Walbridge, S., Selkoe, K.A., Kappel, C.V., Micheli, F., D'Agrosa, C. *et al.* (2008) A global map of human impact on marine ecosystems. *Science*, **319**, 948–952.

Hauxwell, J., Cebrian, J., Furlong, C. & Valiela, I. (2001) Macroalgal canopies contribute to eelgrass (*Zostera marina*) decline in temperate estuarine ecosystems. *Ecology*, **82**, 1007–1022.

Hepburn, C.D., Pritchard, D.W., Cornwall, C.E., McLeod, R.J., Beardall, J., Raven, J.A. & Hurd, C.L. (2011) Diversity of carbon use strategies in a kelp forest community: implications for a high CO_2 ocean. *Global Change Biology*, **17**, 2488–2497.

Jokiel, P.L., Rodgers, K.S., Kuffner, I.B., Andersson, A.J., Cox, E.F. & Mackenzie, F.T. (2008) Ocean acidification and calcifying reef organisms: a mesocosm investigation. *Coral Reefs*, **27**, 473–483.

Kitting, C.L., Fry, B. & Morgan, M.D. (1984) Detection of inconspicuous epiphytic algae supporting food webs in seagrass meadows. *Oecologia*, **62**, 145–149.

Kohler, K.E. & Gill, S.M. (2006) Coral Point Count with Excel extensions (CPCe): a Visual Basic program for the determination of coral and substrate coverage using random point count methodology. *Computers & Geosciences*, **32**, 1259–1269.

Kroeker, K.J., Micheli, F., Gambi, M.C. & Martz, T.R. (2011) Divergent ecosystem responses within a benthic marine community to ocean acidification.

Proceedings of the National Academy of Sciences of the United States of America, **108**, 14515–14520.

Kubler, J.E., Johnston, A.M. & Raven, J.A. (1999) The effects of reduced and elevated CO_2 and O_2 on the seaweed *Lomentaria articulata*. *Plant Cell & Environment*, **22**, 1303–1310.

Kuffner, I.B., Andersson, A.J., Jokiel, P.L., Rodgers, K.S. & Mackenzie, F.T. (2008) Decreased abundance of crustose coralline algae due to ocean acidification. *Nature Geoscience*, **1**, 114–117.

Lewis, E. & Wallace, D.W.R. (1998) *Program Developed for CO_2 System Calculations*. ORNL/CDIAC-105. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee.

Lotze, H.K., Lenihan, H.S., Bourque, B.J., Bradbury, R.H., Cooke, R.G., Kay, M.C., Kidwell, S.M., Kirby, M.X., Peterson, C.H. & Jackson, J.B.C. (2006) Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science*, **312**, 1806–1809.

Maberly, S.C. (1990) Exogenous sources of inorganic carbon for photosynthesis by marine macroalgae. *Journal of Phycology*, **26**, 439–449.

Maberly, S.C., Raven, J.A. & Johnston, A.M. (1992) Discrimination between ^{12}C and ^{13}C by marine plants. *Oecologia*, **91**, 481–492.

Martin, S., Rodolfo-Metalpa, R., Ransome, E., Rowley, S., Buia, M.C., Gattuso, J.P. & Hall-Spencer, J. (2008) Effects of naturally acidified seawater on seagrass calcareous epibionts. *Biology Letters*, **4**, 689–692.

McGlathery, K.J. (2001) Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched coastal waters. *Journal of Phycology*, **37**, 453–456.

MCSM (2001) *Monroe County Stormwater Management Master Plan; Volume 1; Section 2.3; Pollution Loads Targets and Analysis*. Monroe County, Key West, Florida.

Mehrbach, C., Culberson, C.H., Hawley, J.E. & Pytkowicz, R.M. (1973) Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnology and Oceanography*, **18**, 897–907.

Morgan, M.D. & Kitting, C.L. (1984) Productivity and utilization of the seagrass Halodule-Wrightii and its attached epiphytes. *Limnology and Oceanography*, **29**, 1066–1076.

Neckles, H.A., Wetzel, R.L. & Orth, R.J. (1993) Relative effects of nutrient enrichment and grazing on epiphyte macrophyte (*Zostera marina*) dynamics. *Oecologia*, **93**, 285–295.

Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L. *et al.* (2006) A global crisis for seagrass ecosystems. *BioScience*, **56**, 987–996.

Porzio, L., Buia, M.C. & Hall-Spencer, J.M. (2011) Effects of ocean acidification on macroalgal communities. *Journal of Experimental Marine Biology and Ecology*, **400**, 278–287.

Raven, J.A. (2003) Inorganic carbon concentrating mechanisms in relation to the biology of algae. *Photosynthesis Research*, **77**, 155–171.

Russell, B.D., Thompson, J.A.I., Falkenberg, L.J. & Connell, S.D. (2009) Synergistic effects of climate change and local stressors: CO_2 and nutrient-driven change in subtidal rocky habitats. *Global Change Biology*, **15**, 2153–2162.

Short, F.T., Burdick, D.M. & Kaldy, J.E. (1995) Mesocosm experiments quantify the effects of eutrophication on eelgrass, *Zostera marina*. *Limnology and Oceanography*, **40**, 740–749.

Strickland, J.D.H. & Parsons, T.R. (1972) *A Practical Handbook of Seawater Analysis*. Fisheries Research Board of Canada, Ottawa.

Tomasko, D.A. & Lapointe, B.E. (1991) Productivity and biomass of *Thalassia testudinum* as related to water column nutrient availability and epiphyte levels - Field observations and experimental studies. *Marine Ecology-Progress Series*, **75**, 9–17.

Valiela, I., McClelland, J., Hauxwell, J., Behr, P.J., Hersh, D. & Foreman, K. (1997) Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography*, **42**, 1105–1118.

Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S. *et al.* (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 12377–12381.

Wernberg, T., Smale, D.A. & Thomsen, M.S. (2012) A decade of climate change experiments on marine organisms: procedures, patterns and problems. *Global Change Biology*, **18**, 1491–1498.

Yates, K.K., Dufore, C., Smiley, N., Jackson, C. & Halley, R.B. (2007) Diurnal variation of oxygen and carbonate system parameters in Tampa Bay and Florida Bay. *Marine Chemistry*, **104**, 110–124.

Received 14 September 2013; accepted 18 February 2014
Handling Editor: Christer Nilsson

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Summary of statistical results of epiphyte community metrics.

Figure S1. Schematic of experimental design.

Figure S2. Time series of pH values.

Figure S3. Photographs of shifts in epiphyte community structure.