Journal of Experimental Marine Biology and Ecology 503 (2018) 86–91

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Ambient nutrient availability drives the outcome of an interaction between a sponge (*Halichondria melanadocia*) and seagrass (*Thalassia testudinum*)

Stephanie K. Archer*, Enie Hensel, Craig A. Layman

Department of Applied Ecology, North Carolina State University, 127 David Clark Labs, Campus Box 7617, Raleigh, NC 27695-7617, USA

ARTICLE INFO

Keywords:
Context-dependent
Ecosystem function
Interspecific interactions
Nutrient loading
Porifera
Productivity
Seagrass

ABSTRACT

Nutrient loading is a common human impact in coastal habitats which is driving significant losses of seagrass habitat worldwide. This is concerning, as seagrass beds provide numerous ecosystem services. Although nutrient loading can result in eutrophication and mass mortality of seagrasses, it more often results in subtle alterations of abiotic conditions which, by themselves, are insufficient to drive widespread seagrass mortality. Here we used a month-long field-based experiment to test the influence of anthropogenic nutrient loading on the outcome of an interaction between an epizootic sponge (*Halichondria melanadocia*) and a seagrass (*Thalassia testudinum*). Using a factorial design we manipulated ambient nutrient concentrations by adding fertilizer to plots with and without a sponge in a relatively pristine seagrass bed on Abaco Island, The Bahamas. We measured seagrass growth, below- and above-ground biomass, and the change in shoot density. We found that low levels of nutrient loading (i.e., insufficient to cause increased algal growth and decreased oxygen levels associated with eutrophication) resulted in a non-significant increase in seagrass growth, biomass, and shoot density when a sponge was absent. When a sponge was present, the same level of fertilization resulted in a significant reduction in the response variables. Our results show that anthropogenic nutrient loading can shift the sponge-seagrass interaction away from commensalism toward an interaction that has negative consequences for the seagrass. Additionally, a shift in the outcome of this context-dependent interaction can alter the impact of nutrient loading on seagrass productivity. Our study provides an example of how simple classifications of interspecific interactions (e.g., commensalism) often mask underlying variability. Characterizing the mechanisms driving the variability will allow us to understand when and how the outcome of an interaction will change. Ultimately, this will allow us to better predict how human activities will indirectly impact various ecosystem functions.

1. Introduction

Seagrass beds are an important, globally distributed, coastal ecosystem (Duffy et al., 2014; Lamb et al., 2017) that are disappearing rapidly as a result of myriad anthropogenic threats (Orth et al., 2006; Waycott et al., 2009). The majority of identified threats, including dredging, damage from boats, and eutrophication, result in direct mortality of seagrass. Much seagrass loss attributed to eutrophication is the result of increases in epiphytic algae and water column productivity, significantly decreasing light availability for seagrasses (Burkholder et al., 2007). Low-level anthropogenic nutrient loading (i.e., insufficient to cause increased algal growth and decreased oxygen levels associated with eutrophication) is a common occurrence in coastal systems. However, we know little about how low-level anthropogenic nutrient loading may impact the function of seagrass ecosystems.

Interspecific species interactions play a central role in maintaining both the structure and function of ecosystems. Understanding the impact of interspecific interactions on ecosystem function is complicated by the fact that outcomes of interactions are often context-dependent; they are influenced by the biotic and/or abiotic conditions in which they occur (Bronstein, 1994; Chamberlain et al., 2014). There is evidence that interactions involving nutrient transfer are particularly sensitive to changes in ambient nutrient availability (Kiers et al., 2010), suggesting that anthropogenic nutrient loading has the potential to disrupt interspecific interactions thereby altering ecosystem function. For example mycorrhizal symbioses, where the plant provides carbohydrates in exchange for nutrients, strongly influence the structure of plant communities (Grime et al., 1987; Hartnett and Wilson, 2002; van der Heijden et al., 1998). These symbioses are context dependent, such that benefits to plants are highest when nutrient availability is low in the soil (Johnson et al., 1997; Neuhauser and Fargione, 2004). Long-term soil fertilization can alter these symbioses and their outcome, influencing plant productivity and the competitive balance between plant

---

* Corresponding author at: Fisheries and Oceans Canada, Pacific Biological Station, 3190 Hammond Bay Road, Nanaimo BC V9T 6N7, Canada.
E-mail address: Stephanie.Archer@dfo-mpo.gc.ca (S.K. Archer).

https://doi.org/10.1016/j.jembe.2018.02.005
Received 19 May 2017; Received in revised form 16 February 2018; Accepted 21 February 2018

species (Johnson, 1993; Johnson et al., 2003; Treseder, 2004). As anthropogenic activities increasingly alter abiotic conditions, human influence on the outcome of species interactions is predicted to increase (Kiers et al., 2010).

Despite their potential importance, we know little about context-dependent interactions in marine systems and even less about how shifts in their outcome may influence ecosystem function. Here, we focus on how a small increase in anthropogenic nutrient loading impacts the commensal interaction between the sponge, *Halichondria melanadocia* (de Laudenfels, 1936) and seagrass, *Thalassia testudinum* (Banks & Sol. ex König, 1805), and in turn influences seagrass productivity. The sponge is an epizootic sponge that utilizes the base of seagrass shoots to grow, ultimately shading the blades. Additionally, the sponge is a source of nitrogen and phosphorus; nutrients presumably available to the seagrasses (Archer et al., 2015). In the oligotrophic waters of The Bahamas, where the interaction was first described, the net outcome of the interaction between the sponge and seagrass is commensal, where the sponge likely benefits from the provision of an attachment point on an otherwise soft-bottom system and the seagrass exhibits no measurable effect of hosting the sponge (Archer et al., 2015). We hypothesized that ambient nutrient concentrations influence the outcome of the sponge-seagrass interaction. If the effect of the sponge on the seagrass is a balance of the cost incurred as a result of shading by the sponge and the benefit of the availability of nutrients released by the sponge, increased ambient nutrient availability should change the balance of the interaction so that sponge presence negatively influences the seagrass. In this manuscript, we present the results of a field experiment where we tested whether anthropogenic nutrient loading alters the outcome of the interaction between the sponge and seagrass and, if so, what the impact is for seagrass productivity.

2. Materials and methods

2.1. Study system

This study was conducted on Great Abaco Island, The Bahamas, from 2 June − 3 July 2014. The location of our experiment was a dense seagrass meadow of *Thalassia testudinum* at Jungle Creek (26° 21′ 53″ N, 77° 01′ 25″W). Jungle Creek is a relatively unimpacted, sheltered, tidal system that is bounded by red mangroves (*Rhizophora mangle*) and contains a mosaic of sand flats, hard bottom, and seagrass communities. *Halichondria melanadocia* is abundant in this area (Archer et al., 2015). This site corresponds to the North Bight of Old Robinson described by Stoner et al. (2011) who found low ambient availability of nitrogen (0.51 μM NH_4^+) and phosphorus (0.11 μM soluble reactive phosphorus).

2.2. Experimental design and setup

We conducted a fully crossed 2 × 2 factorial design with two levels of sponge presence (present or absent) and nutrient enrichment (ambient or fertilized) with 10 plots per treatment combination ($n = 40$). Experimental 20 × 20 cm plots were arranged in five rows of eight. All plots were separated by at least 1 m. The first plot was haphazardly placed within the seagrass bed. From this plot we searched for the presence of a sponge within 1–3 m. If a sponge was found, the next experimental plot was established with the sponge in the center. If no sponge was found, the plot was established 1 m from the previous plot. Ten plots for each level of sponge presence were randomly assigned to receive fertilizer; a total of twenty plots were fertilized. All plots were delineated using PVC stakes placed at the corners of the plot. Although the sponge treatment could not truly be randomly assigned, we were able to ensure that the sponge plots were spread throughout the seagrass bed. When adding fertilizer we followed protocol outlined by Ferdie and Fourqurean (2004) and Stoner et al. (2014). This involved massaging 40 mg (± 0.05 mg) of Plantacote slow-release fertilizer (N:P molar ratio = 19:6, Scotts, Columbus, Ohio, USA) into the first 5 cm of the sediment, once at the beginning of the experiment. The amount of fertilizer was chosen based on the findings of Stoner et al. (2014), to increase nutrient availability without resulting in increased algal growth and decreased oxygen levels associated with eutrophication. Fertilization was considered successful if the % nitrogen (%N) of *T. testudinum* blades was significantly higher in fertilized plots; in oligotrophic systems water column nutrients are an unreliable indicator of nutrient dynamics (Allgeier et al., 2011; Allgeier et al., 2013; Ferdie and Fourqurean, 2004) while seagrass tissue is widely used to assess nutrient availability in similar systems (Atkinson and Smith, 1983; Fourqurean and Zieman, 2002).

2.3. Seagrass sampling

As the plots were delineated the initial density of *T. testudinum* shoots was counted. To assess seagrass growth five shoots in each plot were randomly chosen and marked at the base of the shoot with a surgical needle following a standard blade-hole punching technique to measure seagrass growth (Zieman, 1974); the seagrass shoots were marked with two weeks remaining in the month-long experiment. In plots with sponges the seagrass shoot that the sponge was growing around was intentionally marked to directly test the effect of the sponge on its host seagrass shoot. This was done following the methods described in Archer et al. (2015). At the end of the experiment, we recorded final seagrass density within each plot. We then collected marked seagrass shoots for growth measurements. Then, above and below ground seagrass biomass was collected using a 20 cm diameter core pressed 15–20 cm into the substrate. Each core was sieved in the field to remove excess sediment prior to transport. All samples were frozen then transported to North Carolina State University for processing.

In the laboratory, the cores were thawed and sorted into four components: seagrass blades, sheaths, rhizomes, and roots. All components were rinsed in deionized water to remove sand and other foreign particles. Then seagrass blades were gently scraped to remove epiphytes. When sponges were present, they were separated from seagrass tissue and kept. The shoots collected for growth were also scraped to remove epiphytes, the growth was measured, and then the shoots were added to the biomass collected in the core. All seagrass components and sponges were dried at 65 °C for 48–72 h until a stable weight was reached. The dry weight (g) was recorded and a subset of samples ($n = 21$, 5 per treatment combination with the exception of fertilized non-sponge plots for which $n = 6$) were ground to a fine powder for percent carbon (%C) and nitrogen (%N) determination. For %C and %N determination, 3–6 mg of ground material was weighed into tin capsules and sent to the Analytical Chemistry Lab at the University of Georgia for analysis. In addition, epiphytes were ashed at 500 °C for 3 h and ash-free dry weight was recorded after samples cooled.

2.4. Statistical analyses

The difference in sponge dry weight was compared between fertilized and ambient plots using Welch's two sample *t*-test for unequal variance. If nutrient addition influenced sponge growth we would expect to see a difference in sponge dry weight as a congener of this sponge (*Halichondria panacea*) can grow at a rate of 2% per day (Thomassen and Riisgård, 1995). Seagrass growth ($mm^2\ day^{-1}$), the change in *T. testudinum* shoot density (initial – final shoot density), ephiphyte ash-free dry weight (standardized by blade biomass; $g_{epiphytes}/g_{blade}$), and %C and %N of seagrass blades were each analyzed using a 2-way analysis of variance with nutrient addition (ambient vs fertilized) and sponge presence (sponge vs no sponge) as fixed factors. Although the growth of five seagrass shoots per plot was measured, the average seagrass growth per plot was used as the response variable in our analysis. Type II sums of squares was used for the analysis of %C

Table 1
Mean values of sponge biomass as well as several seagrass response variables. Standard deviations are reported in the parentheses. All sample sizes are $n = 10$ unless otherwise indicated.

	Ambient		Fertilized	
	Sponge	No sponge	Sponge	No sponge
Sponge dry weight (g)	8.1 (5.7)	NA	3.5 (3.4)	NA
Seagrass				
Initial shoot count	8.5 (2.0)	10.1 (3.7)	9.3 (2.9)	9.3 (2.0)
Final shoot count	10.3 (2.6)	12.5 (3.2)	8.1 (2.1)	11.7 (3.1)
Epiphyte biomass $(g_{epiphytes}/g_{blades})$	0.08 (0.04)	0.04 (0.02)	0.09 (0.08)	0.04 (0.02)
Above ground biomass (g)	11.9 (6.2)	8.4 (3.8)	7.2 (4.6)	10.0 (6.6)
Below ground biomass (g)	11.6 (3.2)	10.8 (3.4)	9.1 (2.8)	11.5 (4.1)
% Nitrogen[a]	2.12 (0.05)[b]	1.95 (0.05)[b]	2.21 (0.12)[b]	2.02 (0.21)[c]
% Carbon[a]	37.76 (0.67)[b]	38.16 (1.41)[b]	39.82 (0.85)[b]	38.41 (2.94)[c]

[a] Calculated on tissue from the seagrass blades.
[b] $n = 5$.
[c] $n = 6$.

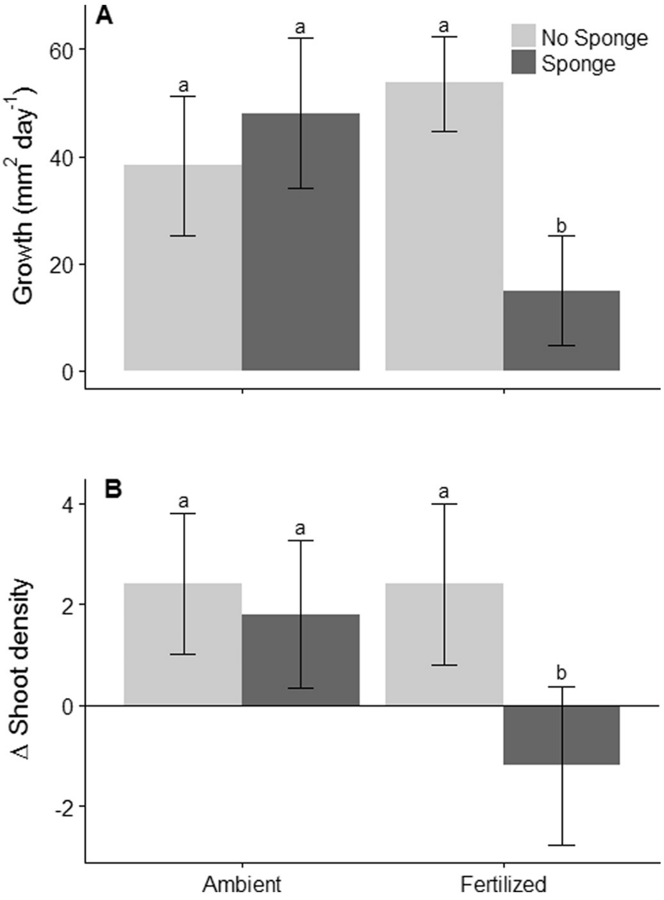

Fig. 1. Mean and 95% confidence intervals of seagrass growth (mm^2 d^{-1}, panel a) and the change in seagrass shoot density (panel b). The letters above the error bars represent statistically similar groups according to Tukey's Honest Significant Difference at $\alpha = 0.05$. In all cases the sample size is n = 10 per treatment combination.

and %N because of unequal sample sizes. Total, above and below ground biomass, as well as their ratio, were each analyzed using analysis of covariance with initial shoot density as the covariate and nutrient addition and sponge presence as fixed factors. When initial shoot density was a significant covariate, the effect of the fixed factors was interpreted using covariate adjusted means. In all cases, residuals were examined to ensure assumptions of the tests were met and post-hoc mean comparisons were conducted using Tukey's Honest Significant Difference. All statistical analyses were completed in SAS version 9.4.

3. Results

There was no difference in sponge dry weight between fertilized and ambient plots ($t_{16} = -1.93$, $p = 0.07$; Table 1). The experimental treatment was successful as fertilization significantly increased the %N of the seagrass blades ($F_{1,17} = 15.69, p = 0.001$, Table 1). The presence of a sponge also increased %N in seagrass blades ($F_{1,17} = 23.25$, $p < 0.001$, Table 1), but the interaction between sponge presence and fertilization was not significant ($F_{1,17} = 0.37$, $p = 0.55$). Fertilization had no effect on the %C of the seagrass blades ($F_{1,17} = 0.85, p = 0.37$, Table 1), neither did the presence of a sponge ($F_{1,17} = 0.34, p = 0.57$, Table 1) nor the interaction ($F_{1,17} = 0.79, p = 0.39$).

In fertilized sponge plots seagrass growth was significantly reduced ($F_{1,36} = 22.12$, $p < 0.001$, Fig. 1a). On average, shoot density also decreased in fertilized sponge plots over the course of the experiment, while all other treatments gained shoots ($F_{1,36} = 5.06$, $p = 0.03$, Fig. 1b). Epiphyte biomass (Table 1) was not affected by the addition of fertilizer ($F_{1,36} = 0.12$, $p = 0.74$), but was significantly higher in the presence of a sponge ($F_{1,36} = 7.47$, $p = 0.01$).

The combination of fertilizer and a sponge significantly affected total seagrass biomass ($F_{1,35} = 7.40$, $p = 0.01$, Fig. 2a), above ground biomass ($F_{1,35} = 7.28$, $p = 0.01$, Table 1), and the ratio of above to below ground biomass ($F_{1,35} = 5.76$, $p = 0.02$, Fig. 2b). Initial shoot density was a significant covariate for all biomass response variables (total biomass: $F_{1,35} = 11.25$, $p = 0.002$; above: $F_{1,35} = 11.12$, $p = 0.002$, below: $F_{1,35} = 4.93$, $p = 0.03$, above:below $F_{1,35} = 8.07$, $p = 0.007$), but the interaction between the covariate and the fixed factors was not significant for any response variable (total biomass: $F_{1,35} = 0.07$, $p = 0.80$; above: $F_{1,35} = 0.32$, $p = 0.58$, below: $F_{1,35} = 1.94$, $p = 0.17$, above: below $F_{1,35} = 1.86$, $p = 0.18$). Below ground biomass was highest in ambient sponge plots and lowest in fertilized sponge plots (Table 1), but this interaction was not significant ($F_{1,35} = 3.75$, $p = 0.06$). Ambient sponge plots had the highest total biomass (24.77 g ± 4.45, adjusted mean ± 95% CI) while fertilized sponge plots had the lowest (16.34 g ± 4.40, Fig. 2a). Similarly, ambient sponge plots had the largest ratio of above to belowground biomass (1.57 ± 0.70, adjusted mean ± 95% CI). This ratio is statistically similar to that observed for the fertilized plots without a sponge (0.88 ± 0.16, adjusted mean ± 95% CI), but significantly larger than both other plot types (Fig. 2b). Ambient sponge plots had both the highest above (12.77 ± 3.00, adjusted mean ± 95% CI) and below ground biomass (12.01 g ± 2.07, adjusted mean ± 95% CI) while fertilized sponge plots had the lowest biomass in both compartments (above: 7.20 g ± 2.99, below: 9.15 g ± 2.05, adjusted mean ± 95% CI) although these differences were not significant.

4. Discussion

Anthropogenic nutrient loading is one of the most pervasive threats to coastal ecosystems (Vitousek et al., 1997). This nearly ubiquitous stressor in coastal systems has the potential to disrupt a wide range species interactions, particularly those involving nutrient transfers (Kiers et al., 2015; Kiers et al., 2010). Here we show that increased anthropogenic nutrient loading can shift the interaction between the sponge *H. melanadocia* and the seagrass *T. testudinum* away from commensalism to either parasitism or amensalism, with significant effects on seagrass productivity.

Results of this experiment corroborate those of Archer et al. (2015) as under ambient, oligotrophic, conditions the presence of the sponge,

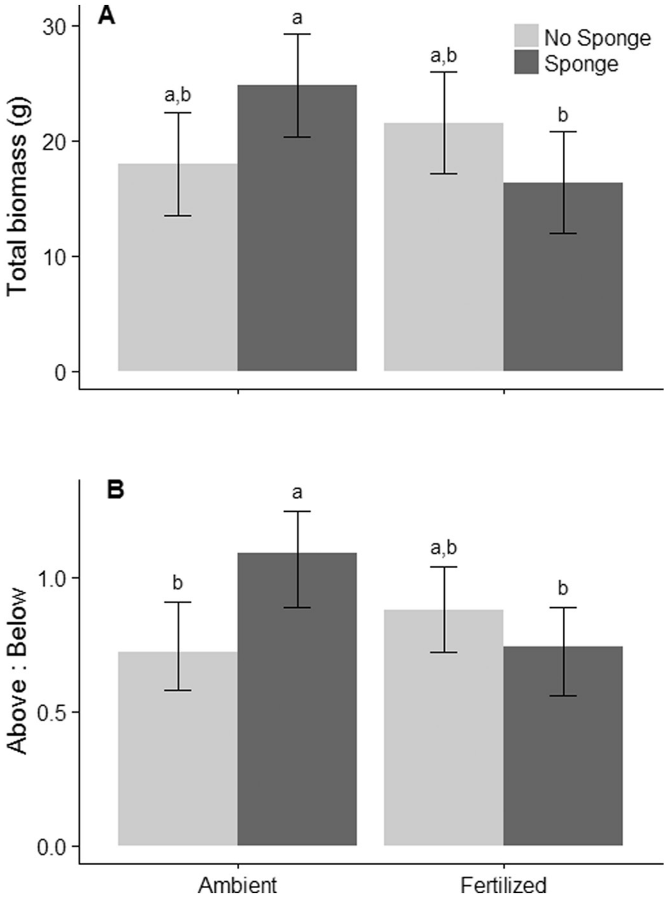

Fig. 2. Covariate adjusted means and 95% confidence intervals for total seagrass biomass (g, panel a) and the ratio of above to below ground biomass (panel b). The letters above the error bars represent statistically similar groups according to Tukey's Honest Significant Difference at $\alpha = 0.05$. In all cases the sample size is $n = 10$ per treatment combination.

H. melanadocia, has no significant impact on *T. testudinum* growth. In non-sponge plots, the addition of fertilizer caused a non-significant increase in seagrass growth (Fig. 1a), which is consistent with reports that seagrass growth is limited by nutrient availability in The Bahamas archipelago (Allgeier et al., 2010; Layman et al., 2013). However, when fertilizer was added to plots containing sponges, seagrass growth was significantly reduced. In three of the fertilized plots with sponges, the seagrass shoot on which the sponge was growing died. Further, despite the fact that only three shoots with sponges died, 7 out of 10 fertilized sponge plots lost shoot density (Fig. 1b). Additionally, fertilized sponge plots had both the lowest above and below ground biomass of any plot type. This indicates that the decrease in total biomass was not only driven by the loss of seagrass shoots but also dieback of the whole plant. Increased shading by epiphytic algae does not seem to explain the decrease in fertilized sponge plots, as epiphyte load was not significantly different between fertilized and ambient sponge plots. Together these lines of evidence suggests that it is not the physical presence of the sponge shading the seagrass as suggested by Wong and Vercaemer (2012). Rather some other, currently unidentified, mechanism is driving seagrass loss in fertilized sponge plots.

The functional equilibrium model of plant growth asserts that the allocation of above and below ground biomass should be determined by relative availability of light and soil nutrients. If light is more limiting, or nutrients replete, the plant should allocate more biomass into above ground tissues (Tilman, 1988). Although this breaks down somewhat for aquatic vegetation that can absorb water column nutrients through their above ground tissues, it still provides a useful framework in which to consider patterns of above and below ground biomass; plants should allocate biomass in such a way to maximize uptake of the most limiting resource (Herbert and Fourqurean, 2009). Duarte and Chiscano (1999) reported an average ratio of above to below ground biomass for *T. testudinum* of 0.89, consistent with the ratio we found in all but the ambient sponge plots. The above-below ground ratio in our ambient plots is consistent with Archer et al. (2015)'s assertion that the sponge shades the seagrass while also providing water column nutrients, both of which would result in increased allocation of above ground biomass (Powell et al., 1989) (Fig. 2b). Interestingly, here we found that sponge presence did increase nitrogen concentration in seagrass tissues, which supports the hypothesis that the sponge acts as a source of nutrients. While this finding is inconsistent with Archer et al. (2015), the scale of sampling may explain the difference. In Archer et al. (2015) seagrass shoots with and without a sponge were collected from the same 1 m^2 where multiple sponges (mean of 11) were present. In this study, sponge and non-sponge plots were separated by at least 1 m, decreasing the likelihood that sponge-derived nutrients could influence seagrass in non-sponge plots. In plots without a sponge we see a small, non-significant, decrease in the ratio of above to below ground biomass in fertilized plots, again consistent with the paradigm that seagrass growth is nutrient limited in this system. The decrease in the ratio of above to below ground biomass for fertilized sponge plots is likely driven by a more rapid decrease in above ground biomass (dead tissue was not included in the biomass estimate) rather than a shift in allocation by the plant since both components of biomass decreased.

We found that the shift in the sponge-seagrass interaction, away from commensalism toward an interaction detrimental for the seagrass, significantly decreased seagrass productivity. The shift in the interaction caused seagrass growth to decrease by an average of 33 mm^2 day^{-1} and caused a reduction in seagrass shoot density, together resulting in lower seagrass biomass. Reduced seagrass productivity may have cascading consequences for secondary productivity. Yeager et al. (2012) found that lower seagrass density was significantly correlated with decreased densities of benthic invertebrates and juvenile White Grunts (*Haemulon plumierii*). Additionally, reduced seagrass productivity may influence carbon storage in the system. Although seagrasses cover $<$ 0.2% of the benthos in the world's oceans, they store a significant amount of carbon (Duarte et al., 2005). Fourqurean et al. (2012) estimated that live seagrass biomass stores between 75.5 and 151 Tg C globally, with significantly more carbon sequestered in the sediments of seagrass beds. Our results suggest that low levels of nutrient loading where the sponge-seagrass interaction occurs will reduce the amount of carbon stored in live seagrass biomass. Future research should be directed at determining if the interaction influences carbon storage in the sediments and how the interaction influences seagrass productivity over long time periods (i.e., years) and larger spatial scales.

We did not collect data that allows us to evaluate the effect of the interaction in the presence of elevated nutrients for the sponge. Although there was no significant difference in dry weight of sponges between the fertilized and ambient plots at the end of the experiment, sponges were larger in ambient plots suggesting increased nutrient availability may directly or indirectly negatively impact the sponge. However, we do not have data on sponge biomass or volume at the start of the experiment. Sponges differ in their response to elevated nutrients. For example, boring sponges (clinoid spp.) are more abundant at sites with elevated nutrients (Holmes, 2000; Ward-Paige et al., 2005) while many other species decline (Easson et al., 2014; Gochfeld et al., 2007). There is some evidence to suggest that phototrophic sponges fair better in eutrophic conditions than heterotrophic sponges (Easson et al., 2015). *Halichondria melanadocia* is a heterotrophic sponge and therefore with more precise measures of the sponge growth it is possible that we would observe a significant negative effect of fertilization on the sponge (Duckworth and Pomponi, 2005).

It is possible that the interaction between the sponge and seagrass will begin to break down under elevated nutrients. For example, a likely benefit that the sponge receives from the seagrass is the seagrass acting as an attachment point for the sessile, filter-feeding sponge, as this species typically grows on mangrove prop roots (Diaz, 2005) and has not been observed growing directly on soft sediments in this system (Archer personal observation). The seagrass shoot loss we observed could result in the loss of this benefit for the sponge and potentially compromise the ability of this sponge to persist in the system. Further work is needed to determine how the sponge is impacted by this interaction under differing nutrient availability regimes.

The results of this study could be viewed within the context of other theoretical frameworks. For example, it is possible that the shading of the seagrass by the sponge is a stressor that the seagrass is able to compensate for under ambient nutrient levels. The elevated nutrient availability created by fertilization in this experiment may also act as a small, typically insignificant, stressor. Combined these multiple stressors (shading and nutrient loading) may drive seagrass loss. Multiple stressors combine to drive the loss of foundation species across a wide range of ecosystems (Crain et al., 2008; Folt et al., 1999; O'Brien et al., 2004; Silliman et al., 2005) including seagrass ecosystems (Orth et al., 2006). It is worth noting that context-dependent species interactions and multiple stressor theories are not mutually exclusive. In fact, multiple stressors can easily be viewed through the lens of context dependency: a stressor's impact on the focal species is dependent on other biotic or abiotic conditions. Ultimately, in this case, the conclusions are the same if you view the sponge as a stressor or as a species interacting with the seagrass: under oligotrophic conditions the sponge does not impact the seagrass, under elevated nutrient levels the presence of a sponge results in seagrass loss even though the nutrient levels themselves do not induce a negative response.

Loss of seagrass beds, and their associated ecosystem functions is an important global conservation issue (Duarte et al., 2005; Fourqurean et al., 2012). Our results show that even low levels of anthropogenic nutrient loading can subtlety shift the effect of an epiphytic sponge on its host seagrass from neutral to negative. Overall, this highlights the idea that seemingly subtle changes in the relative costs and benefits associated with species interactions may correspond to large shifts in ecosystem function.

Author contributions

SKA conceived the ideas and analyzed the data; SKA, EH, and CAL designed methodology; SKA and EH collected the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Acknowledgements

We thank Hannah Levenson and Beth Whitman for their assistance with field work and Katie Lewia and Jill Tucker for their help processing samples in the lab. We also thank the anonymous reviewers whose comments improved this manuscript. This work was supported by donations from Win and Tana Archer, North Carolina State University, and NSF OCE1405198.

Data Accessibility.

Upon acceptance for publication the data will be submitted to Dryad data repository.

References

Allgeier, J.E., Rosemond, A.D., Mehring, A.S., Layman, C.A., 2010. Synergistic nutrient colimitation across a gradient of ecosystem fragmentation in subtropical mangrove-dominated wetlands. Limnol. Oceanogr. 55, 2660–2668.

Allgeier, J.E., Rosemond, A.D., Layman, C.A., 2011. The frequency and magnitude of non-additive responses to multiple nutrient enrichment. J. Appl. Ecol. 48, 96–101.

Allgeier, J.E., Yeager, L.A., Layman, C.A., 2013. Consumers regulate nutrient limitation regimes and primary production in seagrass ecosystems. Ecology 94, 521–529.

Archer, S.K., Stoner, E.W., Layman, C.A., 2015. A complex interaction between a sponge (*Halichondria melanadocia*) and a seagrass (*Thalassia testudinum*) in a subtropical coastal ecosystem. J. Exp. Mar. Biol. Ecol. 465, 33–40.

Atkinson, M., Smith, S., 1983. C: N: P ratios of benthic marine plants. Limnol. Oceanogr. 28.

Bronstein, J.L., 1994. Conditional outcomes in mutualistic interactions. Trends Ecol. Evol. 9, 214–217.

Burkholder, J.M., Tomasko, D.A., Touchette, B.W., 2007. Seagrasses and eutrophication. J. Exp. Mar. Biol. Ecol. 350, 46–72.

Chamberlain, S.A., Bronstein, J.L., Rudgers, J.A., 2014. How context dependent are species interactions? Ecol. Lett. 17, 881–890.

Crain, C.M., Kroeker, K., Halpern, B.S., 2008. Interactive and cumulative effects of multiple human stressors in marine systems. Ecol. Lett. 11, 1304–1315.

Diaz, M.C., 2005. Common sponges from shallow marine habitats from Bocas del Toro region, Panama. Caribb. J. Sci. 41, 465–475.

Duarte, C.M., Chiscano, C.L., 1999. Seagrass biomass and production: a reassessment. Aquat. Bot. 65, 159–174.

Duarte, C.M., Middelburg, J.J., Caraco, N.F., 2005. Major role of marine vegetation on the oceanic carbon cycle. Biogeosciences 2, 1–8.

Duckworth, A.R., Pomponi, S.A., 2005. Relative importance of bacteria, microalgae and yeast for growth of the sponge Halichondria melanadocia (De Laubenfels, 1936): a laboratory study. J. Exp. Mar. Biol. Ecol. 323, 151–159.

Duffy, J.E., Hughes, A.R., Moksnes, P.-O., 2014. Ecology of seagrass communities. In: Bertness, M.D., Bruno, J.F., Silliman, B.R., Stachowicz, J.J. (Eds.), Marine Community Ecology and Conservation. Sinauer Associates, Inc., Sunderland, MA, pp. 271–298.

Easson, C.G., Slattery, M., Baker, D.M., Gochfeld, D.J., 2014. Complex ecological associations: competition and facilitation in a sponge-algal interaction. Mar. Ecol. Prog. Ser. 507, 153–167.

Easson, C.G., Matterson, K.O., Freeman, C.J., Archer, S.K., Thacker, R.W., 2015. Variation in species diversity and functional traits of sponge communities near human populations in Bocas del Toro, Panama. PeerJ 3, e1385.

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. Limnol. Oceanogr. 49, 2082–2094.

Folt, C.L., Chen, C.Y., Moore, M.V., Burnaford, J., 1999. Synergism and antagonism among multiple stressors. Limnol. Oceanogr. 44, 864–877.

Fourqurean, J.W., Zieman, J.C., 2002. Nutrient content of the seagrass *Thalassia testudinum* reveals regional patterns of relative availability of nitrogen and phosphorus in the Florida keys USA. Biogeochemistry 61, 229–245.

Fourqurean, J.W., Duarte, C.M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M.A., Apostolaki, E.T., Kendrick, G.A., Krause-Jensen, D., McGlathery, K.J., 2012. Seagrass ecosystems as a globally significant carbon stock. Nat. Geosci. 5, 505–509.

Gochfeld, D.J., Schloeder, C., Thacker, R.W., 2007. Sponge community structure and disease prevalence on coral reefs in Bocas del Toro, Panama. In: Custódio, M., Lôbo-Hajdu, G., Hajdu, E., Muricy, G. (Eds.), Porifera Research: Biodiversity, Innovation, and Sustainability. Museu Nacional serie livros. Museu Nacional-Univ Federal Do Rio De Janeiro, Rio de Janeiro, Brazil, pp. 335–343.

Grime, J., Mackey, J., Hillier, S., Read, D., 1987. Floristic diversity in a model system using experimental microcosms. Nature 328, 420–422.

Hartnett, D.C., Wilson, G.W., 2002. The role of mycorrhizas in plant community structure and dynamics: lessons from grasslands. In: Smith, S.E., Smith, F.A. (Eds.), Diversity and Integration in Mycorrhizas: Proceedings of the 3rd International Conference on Mycorrhizas (ICOM3) Adelaide, Australia, 8–13 July 2001, 1 ed. Springer, Netherlands, pp. 319–331.

van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., Sanders, I.R., 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396, 69–72.

Herbert, D.A., Fourqurean, J.W., 2009. Phosphorus availability and salinity control productivity and demography of the seagrass Thalassia testudinum in Florida Bay. Estuar. Coasts 32, 188–201.

Holmes, K.E., 2000. Effects of eutrophication on bioeroding sponge communities with the description of new West Indian sponges, Cliona spp. (Porifera: Hadromerida: Clionidae). Invertebr. Biol. 119, 125–138.

Johnson, N.C., 1993. Can fertilization of soil select less mutualistic mycorrhizae? Ecol. Appl. 3, 749–757.

Johnson, N.C., Graham, J.H., Smith, F.A., 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. New Phytol. 135, 575–585.

Johnson, N.C., Rowland, D.L., Corkidi, L., Egerton-Warburton, L.M., Allen, E.B., 2003. Nitrogen enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands. Ecology 84, 1895–1908.

Kiers, T.E., Palmer, T.M., Ives, A.R., Bruno, J.F., Bronstein, J.L., 2010. Mutualisms in a changing world: an evolutionary perspective. Ecol. Lett. 13, 1459–1474.

Kiers, E.T., Ives, A.R., Kawakita, A., 2015. Global Change and Mutualisms. In: Bronstein, J.L. (Ed.), Mutualisms. Oxford University Press, Oxford, UK, pp. 241–267.

Lamb, J.B., van de Water, J.A.J.M., Bourne, D.G., Altier, C., Hein, M.Y., Fiorenza, E.A., Abu, N., Jompa, J., Harvell, C.D., 2017. Seagrass ecosystems reduce exposure to bacterial pathogens of humans, fishes, and invertebrates. Science 355, 731–733.

Layman, C.A., Allgeier, J.E., Yeager, L.A., Stoner, E.W., 2013. Thresholds of ecosystem response to nutrient enrichment from fish aggregations. Ecology 94, 530–536.

Neuhauser, C., Fargione, J.E., 2004. A mutualism–parasitism continuum model and its application to plant–mycorrhizae interactions. Ecol. Model. 177, 337–352.

O'Brien, K., Leichenko, R., Kelkar, U., Venema, H., Aandahl, G., Tompkins, H., Javed, A., Bhadwal, S., Barg, S., Nygaard, L., 2004. Mapping vulnerability to multiple stressors: climate change and globalization in India. Glob. Environ. Chang. 14, 303–313.

Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., Short, F.T., Waycott, M., Williams, S.L., 2006. A global crisis for seagrass ecosystems. Bioscience 56, 987–996.

Powell, G.V.N., Kenworthy, J.W., Fourqurean, J.W., 1989. Experimental evidence for nutrient limitation of seagrass growth in a tropical estuary with restricted circulation. Bull. Mar. Sci. 44, 324–340.

Silliman, B.R., van de Koppel, J., Bertness, M.D., Stanton, L.E., Mendelssohn, I.A., 2005. Drought, snails, and large-scale die-off of southern US salt marshes. Science 310, 1803–1806.

Stoner, E.W., Layman, C.A., Yeager, L.A., Hassett, H.M., 2011. Effects of anthropogenic disturbance on the abundance and size of epibenthic jellyfish Cassiopea spp. Mar. Pollut. Bull. 62, 1109–1114.

Stoner, E.W., Yeager, L.A., Sweatman, J.L., Sebilian, S.S., Layman, C.A., 2014. Modification of a seagrass community by benthic jellyfish blooms and nutrient enrichment. J. Exp. Mar. Biol. Ecol. 461, 185–192.

Thomassen, S., Riisgård, H.U., 1995. Growth and energetics of the sponge Halichondria panicea. Mar. Ecol. Prog. Ser. 128, 239–246.

Tilman, D., 1988. Plant Strategies and the Dynamics and Structure of Plant Communities. Princeton University Press.

Treseder, K.K., 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO2 in field studies. New Phytol. 164, 347–355.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D., 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecol. Appl. 7, 737–750.

Ward-Paige, C.A., Risk, M.J., Sherwood, O.A., Jaap, W.C., 2005. Clionid sponge surveys on the Florida reef tract suggest land-based nutrient inputs. Mar. Pollut. Bull. 51, 570–579.

Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck Jr., K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., Williams, S.L., 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proc. Natl. Acad. Sci. U. S. A. 106, 12377–12381.

Wong, M.C., Vercaemer, B., 2012. Effects of invasive colonial tunicates and a native sponge on the growth, survival, and light attenuation of eelgrass (Zostera marina). Aquat. Invasions 7, 315–326.

Yeager, L., Acevedo, C., Layman, C., 2012. Effects of seascape context on condition, abundance, and secondary production of a coral reef fish, *Haemulon plumierii*. Mar. Ecol. Prog. Ser. 462, 231–240.

Zieman, J.C., 1974. Methods for the study of the growth and production of turtle grass, *Thalassia testudinum* König. Aquaculture 4, 139–143.