



# A complex interaction between a sponge (*Halichondria melanadocia*) and a seagrass (*Thalassia testudinum*) in a subtropical coastal ecosystem



Stephanie K. Archer<sup>a,b,\*</sup>, Elizabeth W. Stoner<sup>b</sup>, Craig A. Layman<sup>a,b</sup>

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

<sup>b</sup> Marine Sciences Program, Department of Biological Sciences, Florida International University, 3000 NE 151st Street, North Miami, FL 33181-3605, USA

## ARTICLE INFO

### Article history:

Received 18 September 2014

Received in revised form 18 December 2014

Accepted 11 January 2015

Available online 24 January 2015

### Keywords:

Context dependence

Foundation species

*Halichondria melanadocia*

Nutrient flux

Species interactions

*Thalassia testudinum*

## ABSTRACT

Foundation species, such as oysters, corals, and seagrasses, form the basis for entire ecosystems and are characterized by positive interactions with community members. However, many species interactions are context dependent, where the outcome or strength of the interaction depends on the biotic or abiotic conditions. Therefore, a mechanistic knowledge of species interactions, especially those involving foundation species, may allow for a more complete understanding of how anthropogenic changes influence nearshore ecosystems. This study describes the interaction between the seagrass *Thalassia testudinum* and the sponge *Halichondria melanadocia*, a species that grows around the base of seagrass shoots. A combination of surveys and experimental manipulations on Abaco Island, The Bahamas, revealed that the interaction between *T. testudinum* and *H. melanadocia* is a commensal relationship with the sponge benefiting from the presence of *T. testudinum* up to medium shoot densities (589–615 shoots per m<sup>2</sup>). The net neutral effect of *H. melanadocia* on *T. testudinum* is likely a balance of the negative effect of the sponge shading the seagrass with the positive effect of nitrogen and phosphorus supplied by the sponge. The mechanisms underlying the interaction between *H. melanadocia* and *T. testudinum* suggest that the interaction is likely context dependent. As such, environmental change, namely eutrophication, has the potential to shift the nature of this interaction from commensal to parasitic. A simple simulation showed that if this relationship becomes parasitic, above ground production in seagrass beds could be reduced. This study highlights the importance of a mechanistic understanding of species interactions involving foundation species when predicting human impact on the environment.

© 2015 Elsevier B.V. All rights reserved.

## 1. Introduction

Foundation species (sensu Dayton, 1972 as refined by Bruno and Bertness, 2001) define entire communities or ecosystems by creating habitat and altering abiotic conditions. As a result of their net positive influence on the organisms which live in and around them, foundation species are typically associated with increased abundance, diversity and distributions of community members (Bracken et al., 2007; Stachowicz, 2001). In addition to their effect on community structure, foundation species are key mediators of ecosystem function (Duffy, 2006; Ellison et al., 2005; McLeod et al., 2011). Seagrasses are a globally distributed group of foundation species (Costanza et al., 1997; Duffy, 2006; Larkum et al., 2006) which influence processes such as nutrient cycling (Hemminga et al., 1991; Marba et al., 2006; Yarbro and Carlson, 2008), sediment stabilization (Folmer et al., 2012), and carbon storage (Fourqurean et al., 2012; McLeod et al., 2011). Despite their

importance, human activities have led to a worldwide decrease in seagrass abundance, potentially affecting their interactions with other species (Orth et al., 2006; Waycott et al., 2009).

Context dependent species interactions, which are common in nature, are defined as interactions where the strength or outcome differs based on the conditions (biotic or abiotic) in which they occur (Bronstein, 1994; Chamberlain et al., 2014). For example, the effect of ulvoid macroalgae on the seagrass *Zostera marina* varies along an estuarine gradient; the effect of macroalgal blooms on the seagrass shifts from neutral at fully marine sites to strongly negative in more riverine portions of the estuary (Hessing-Lewis et al., 2011). In areas impacted by humans, abiotic conditions are often very different from the un-impacted state, which may shift the outcome of some interactions with potential cascading effects on community structure and ecosystem function (Kiers et al., 2010). The importance of species interactions in maintaining both species diversity within seagrass beds and seagrasses themselves is well understood (Heck and Valentine, 2006; Heck et al., 2000; van der Heide et al., 2012). Therefore, a mechanistic understanding of the interactions in seagrass beds can provide insight into how human activities may alter ecosystem structure and function. To this end, the goal of this study was to provide a mechanistic

\* Corresponding author at: Department of Applied Ecology, North Carolina State University, 127 David Clark Labs, Campus Box 7617, Raleigh, NC 27695-7617, USA. Tel.: +1 801 907 1087.

E-mail address: [skraftarcher@gmail.com](mailto:skraftarcher@gmail.com) (S.K. Archer).

description of the interaction between a sponge *Halichondria melanadocia* (de Laudenfels, 1936) and a foundation species, the seagrass *Thalassia testudinum* (Banks & Sol. ex König, 1805).

*T. testudinum* is found in the tropical and sub-tropical western Atlantic and is the dominant seagrass in the shallow waters of the Bahamian archipelago (Wabnitz et al., 2008; Williams, 1990; Zieman, 1982). Though sponges are a common component of seagrass communities, little is known of their role in this system, despite their recognized importance in reef and hard bottom habitats (Bell, 2008; Wulff, 2006). Sponges are filter feeders which host diverse symbiotic microbial communities. As a result, many sponges are known sources of bioavailable forms of nutrients (Corredor et al., 1988; Diaz and Ward, 1997; Maldonado et al., 2012; Southwell et al., 2008) and direct mutualisms involving nutrient transfer between sponges and primary producers, including mangroves (Ellison et al., 1996) and rhodophytes (Davy et al., 2002) have been documented. *H. melanadocia*, typically considered a mangrove sponge (Diaz and Rützler, 2009), is frequently observed in Bahamian seagrass beds (Archer, this study). Unlike many sponges which grow on hard substrates within the seagrass bed, *H. melanadocia* grows surrounding one or more shoots of *T. testudinum* (Fig. 1).

Three potentially co-occurring mechanistic pathways through which *H. melanadocia* and *T. testudinum* may interact were hypothesized. 1) Several species of sponge are sources of bioavailable forms of both nitrogen (N) and phosphorus (P), which are limiting nutrients for seagrass growth in coastal waters of The Bahamas (Allgeier et al., 2013; Maldonado et al., 2012 and references therein). Although nutrient fluxes through *H. melanadocia* have not been published, it is likely that the sponge is a source of N, P or both. Therefore, it was hypothesized that the sponge may help alleviate nutrient limitation in *T. testudinum*

shoots they grow around. 2) The growth of *H. melanadocia* around blades of *T. testudinum* covers a large percentage of photosynthetic tissue of the shoot (12–59%,  $\bar{x} = 37.2$ ,  $sd = 10.8$ , Fig. 1). Consequently, it was hypothesized that by shading shoots, *H. melanadocia* may lead to light limitation in *T. testudinum*. 3) Sponges, as sessile invertebrates, generally require structure for successful settlement and growth. Therefore, it was hypothesized that *T. testudinum* benefits *H. melanadocia* by providing structured habitat. These three hypothesized interaction pathways allowed us to isolate 11 response variables (Table 1) that can be used to describe the nature of the interaction between *T. testudinum* and *H. melanadocia*.

## 2. Materials and methods

### 2.1. Surveys

Surveys were conducted at six sites on Abaco Island, The Bahamas, in May and June 2012 (Fig. 2). At each site, ten, 1 m<sup>2</sup> plots were haphazardly selected. Plots ranged between 0.35 and 1.50 m ( $\bar{x} = 0.77 \pm 0.31$  sd) low tide depth. Within each plot, percent cover and shoot density of *T. testudinum* were estimated and *H. melanadocia* was enumerated. *T. testudinum* shoot density was determined by counting the number of shoots within four 0.15 cm × 0.15 cm quadrats haphazardly placed within the larger sampling plot. The four counts were averaged to get an estimate of shoot density for the entire plot. If more than three *H. melanadocia* were present within the plot, the *T. testudinum* shoots sponges were growing around were collected for morphometric (blade length and width, cm) and nutrient analysis. *T. testudinum* shoots without *H. melanadocia* epibionts were also collected for morphometric and nutrient analysis.

*T. testudinum* shoots collected for morphometric and nutrient analysis were immediately frozen, then transported to Florida International University for analysis. For morphometric analysis, the number of blades per shoot and blade width and length of thawed *T. testudinum* shoots were measured. Measured *T. testudinum* blades were gently scraped to remove epiphytes and then dried at 65 °C for 48–72 h. Dried samples were ground into a fine powder and stored in a desiccator until analysis. Percent carbon (C) and nitrogen (N) of the ground seagrass tissue were determined in duplicate using a Carlo Erba CHN analyzer (Fisons NA1500). Percent phosphorus (P) was determined by dry oxidation acid hydrolysis extraction followed by colorimetric analysis (Fourqurean et al., 1992).

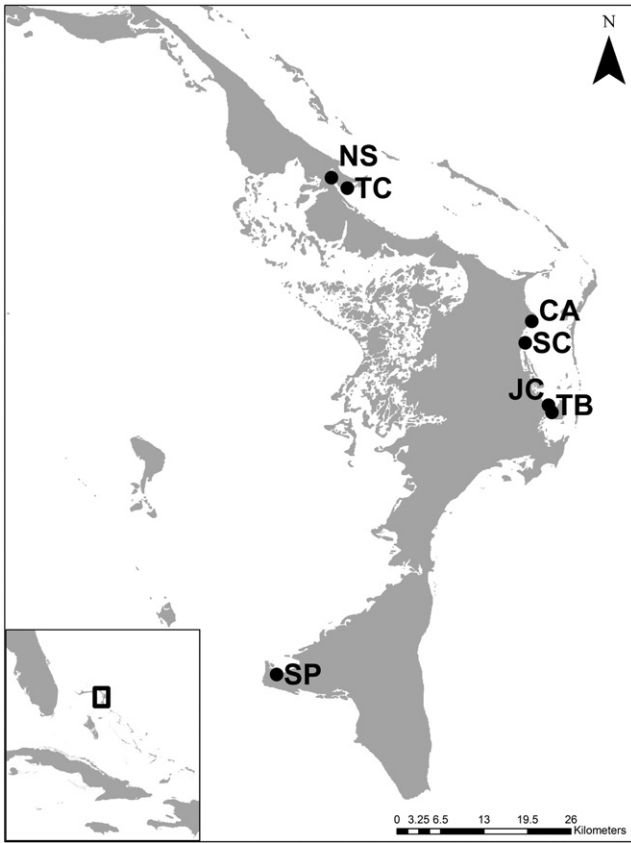


Fig. 1. *Halichondria melanadocia* growing around a *Thalassia testudinum* shoot.

Table 1

Response variables and their expected outcome for each predicted interaction mechanism if the mechanism is acting alone. X indicates that the variable is not predicted to vary directionally in response to the mechanism in question.

	Seagrass benefits from sponge derived nutrients	Sponge leads to light limitation in seagrass	Seagrass provides structure for the sponge
<i>Seagrass nutrient content</i>			
%C	X	–	X
%N	+	X	X
%P	+	X	X
C:N	–	–	X
C:P	–	–	X
<i>Seagrass morphometrics</i>			
Blades per shoot	+	–	X
Blade length	+	–	X
Blade area	+	–	X
<i>Abundance and growth</i>			
Sponge abundance	X	X	+
Seagrass growth	+	–	X
Sponge growth	X	X	+



**Fig. 2.** Location of sites surrounding Abaco, The Bahamas included in this study. Surveys were conducted at the following sites: NS – Nursery Site, TC – Treasure Cay, CA – Camp Abaco, SC – Snake Cay, JC – Jungle Creek, and TB – Turtle Beach. *Thalassia testudinum* growth was determined at SC and JC. Artificial seagrass unit experiments to determine *Halichondria melanadocia* growth were conducted at SP – Sandy Point.

## 2.2. *T. testudinum* growth

*T. testudinum* growth was evaluated at two sites, Snake Cay and Jungle Creek (Fig. 2), between June 22–July 6, 2013 and July 8–22, 2013 respectively using the standard blade hole punching technique (Zieman, 1974). Shoots were marked in groups of three with each group consisting of an unshaded shoot, one with *H. melanadocia* growing around the shoot, and one with a dried, dead sponge cut to cover the same percentage of the shoot as the shoot with *H. melanadocia*. Grouped shoots were located within approximately a  $0.10 \times 0.10$  m area. For *T. testudinum* shoots with *H. melanadocia* growing around the shoot, if the sponge covered the marked area on the seagrass, the sponge tissue was gently pushed up the seagrass shoot to expose the base of the shoot. After the mark was made, the sponge tissue was gently returned to its original location. The dead sponges were dried and cleaned prior to use resulting in only structural components of the sponge remaining using techniques similar to those traditionally used to prepare sponges for commercial sale. Briefly, dead, dry sponges were collected from the beach and then held in mesh bags underwater for 3–7 days prior to placement on the seagrass. The goal of this treatment was to prevent decomposition of the sponge from creating anoxic conditions for the seagrass during the experiment. There were no visible signs of decomposition, although we did not explicitly test for decomposition. Growth was determined for 10 and 15 groups of *T. testudinum* shoots at Snake Cay ( $n = 30$  shoots) and Jungle Creek ( $n = 45$  shoots) respectively (Fig. 2). Fourteen days after marking, *T. testudinum* shoots were collected and the total area of new growth

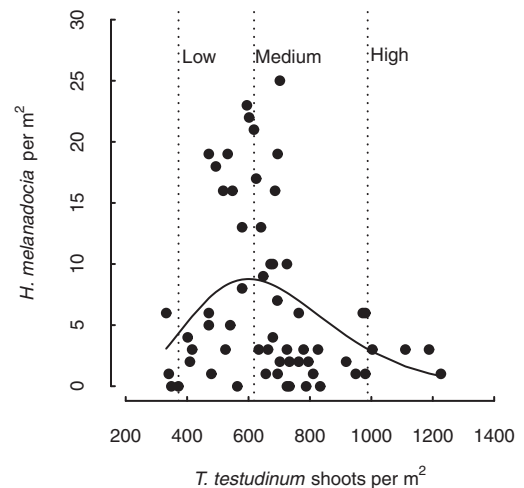
was recorded. The carbon content of the new growth was determined using the same procedure described above.

## 2.3. *H. melanadocia* growth and recruitment

Ten,  $0.5 \times 0.5$  m, artificial seagrass units (ASUs) were constructed in each of three densities, 372, 618, and 988 shoots per  $m^2$  representing the low, medium, and high *T. testudinum* shoot densities observed during the surveys (Fig. 3). ASUs were constructed using pre-soaked 3.75 mesh rug canvas (MCG Textiles®) and green polypropylene ribbon (Splendorette®) cut into  $140 \times 5$  mm strips. Each shoot on the artificial units consisted of two ribbon strips folded in half and attached to the canvas. The length, width, and leaf number of the shoots on the ASUs correspond to the average length, width, and leaf number observed in these surveys. *H. melanadocia* were initially gathered from the Jungle Creek site on May 8, 2014 and transported to the Sandy Point site (Fig. 2). Although surveys were not conducted at Sandy Point, both *T. testudinum* and *H. melanadocia* were present at the site. However, the abundance of *H. melanadocia* was not sufficient to allow for the use of sponges collected at the site. The *H. melanadocia* were never held out of the water for more than 5 s. The volume, to the nearest mL, of each *H. melanadocia* was recorded, then the sponge was placed in the center of an ASU and held loosely in place with plastic zip ties. Subsequently, metal sod staples were used to attach each ASU to the benthos. The ASUs were randomly arranged in five rows, with six units per row. Twenty-one of the initially transplanted *H. melanadocia* did not survive the transplantation. New *H. melanadocia* were gathered from the Jungle Creek site and transplanted onto the ASUs on May 14, 2014. The experiment was monitored every other day for two weeks to ensure the *H. melanadocia* survived the second round of transplantation. After the first two weeks the experiment was monitored weekly, but no additional alterations or additions were made. On July 23, 2014, *H. melanadocia* were removed from the center of each ASU and the volume of each sponge measured to the nearest mL. Any *H. melanadocia* recruits were recorded.

## 2.4. *H. melanadocia* nutrient flux

Four *H. melanadocia* ( $13.8 \text{ mL} \pm 3.6$ , mean  $\pm$  sd) were collected from the Jungle Creek site on July 20, 2013. The sponges were cleaned of all external algae and sediment, and their volume measured to the nearest milliliter. Five high density polyethylene containers were filled with 2 L



**Fig. 3.** The relationship between *Thalassia testudinum* shoot density and *Halichondria melanadocia* abundance. The solid line represents the predicted *H. melanadocia* abundance by the best fitting model:  $H. melanadocia \text{ per } m^2 = (T. testudinum \text{ shoots per } m^2) + \ln(T. testudinum \text{ shoots per } m^2)$ . Dashed lines represent the shoot densities of the three artificial seagrass unit treatments.

of unfiltered seawater and initial water samples were collected prior to placing a sponge into four of the containers, the fifth container served as a control. The containers were placed in a seawater bath to maintain ambient temperatures ( $27.2 \text{ }^\circ\text{C} \pm .7$ , mean  $\pm$  sd). Throughout the incubation, dissolved oxygen concentrations were monitored and the containers were periodically stirred. Water samples were collected every 4 h for 24 h for the determination of ammonium, nitrate/nitrite ( $\text{NO}_x$ ), and soluble reactive phosphorus (SRP). After each sampling event, the volume of water in the containers was brought back up to 2 L with unfiltered seawater.

All water samples were immediately filtered through 0.45  $\mu\text{m}$  Whatman nylon-membrane filter. Samples were analyzed for ammonium immediately using the fluorometric method described by Holmes et al. (1999) as modified by Taylor et al. (2007).  $\text{NO}_x$  and SRP samples were frozen at  $-20 \text{ }^\circ\text{C}$  in 20 mL acid washed (10% HCl) scintillation vials and transported to North Carolina State University (NCSU) for analysis.  $\text{NO}_x$  samples were analyzed by the NCSU Center for Applied Aquatic Ecology Water Quality Laboratory in Raleigh, NC. SRP samples were analyzed using a standard colorimetric technique (Parsons et al., 1984).

### 2.5. Statistical approach

Because abundance datasets are often zero-laden, a negative binomial regression was used to investigate the relationship between sponge abundance and several predictor variables: seagrass shoot density, survey site, and plot depth. Models including all combinations of predictor variables were tested and the best model was selected using model weights calculated using the corrected Akaike's Information Criteria (AICc). The site variable was included to allow for potential drivers of sponge abundance that were not measured in this study. An additional model, a linearized Ricker function, which allows a non-linear relationship between sponge abundance and seagrass shoot densities, was included in the model selection after visual inspection of the data (R Core Team, 2013).

To minimize differences due to sampling location, *T. testudinum* morphometric and nutrient content variables (Table 1) were each analyzed separately by comparing shoots with and without a sponge collected from each plot in a paired t-test. The growth of *T. testudinum*, in  $\text{mg C day}^{-1}$ , was compared between treatments using an ANOVA with treatment and group as fixed factors. When significant, differences among levels of the fixed factors were compared post-hoc using Tukey's Honest Significant Difference (HSD). For analysis, *H. melanadocia* growth was recorded as the relative change in sponge volume per day:  $[(\text{final volume} - \text{initial volume})/(\text{initial volume})^{-1}(\text{number of days on the ASU})^{-1}]$ . Both *H. melanadocia* growth and the number of recruits per ASU were compared between treatments using separate ANOVAs and any significant differences between treatments were compared post-hoc using Tukey's HSD.

When calculating fluxes values below detection limit were replaced with said limit (ammonium:  $0.2 \mu\text{g L}^{-1}$ , SRP:  $0.03 \mu\text{g L}^{-1}$ , and  $\text{NO}_x$ :  $50 \mu\text{g L}^{-1}$ ). As this only affected initial (i.e. those prior to the introduction of the sponge) and control phosphorus values, this served to make our estimates for phosphorus more conservative. Fluxes for each solute of interest were determined by least squares regression of the concentration of the solute against time for each sponge. The coefficient of the time variable from the regression output was then normalized by sponge volume and the volume of the incubation chamber, and divided by 4 to determine hourly flux estimates (water samples were taken at four hour intervals). Fluxes reported are in  $\mu\text{g L}_{\text{sponge}}^{-1} \text{h}^{-1}$ . The mean and standard deviations reported are from the four replicate sponges incubated. For all solutes the time coefficient for the control was not significantly different than zero (see Results below), therefore we concluded that *H. melanadocia* was a significant source (or sink) of each solute if the flux in sponge incubations was significantly different than zero when compared using a t-test.

## 3. Results

### 3.1. Surveys

The best fit model shows that *H. melanadocia* abundance is correlated with *T. testudinum* shoot density in a non-linear fashion (Table 2) with the highest sponge abundances predicted at *T. testudinum* densities between 589 and 615 shoots per  $\text{m}^2$  (Fig. 3). This *T. testudinum* density is near the mean shoot density observed in the surveys ( $\bar{x} \pm \text{sd}$ ,  $676.62 \pm 201.83$ ). Although the linearized Ricker model was clearly the best fit model (model weight = 0.86, Table 2), all models including *T. testudinum* shoot density as a predictor variable (other than the all-inclusive model) performed better than those including only depth, site or a combination of depth and site. There were no differences between the paired samples of seagrass with a sponge and those without for any of the *T. testudinum* morphometric or nutrient content variables (Table 3).

### 3.2. *T. testudinum* growth

*T. testudinum* growth did not differ between sites, but did differ among treatments ( $F_{2,69} = 9.84$ ,  $p < 0.001$ , Fig. 5). The growth of unshaded seagrass shoots did not differ from shoots shaded by live *H. melanadocia* (Tukey adjusted  $p = 0.06$ ), but the growth of unshaded seagrass shoots was significantly higher than shoots shaded by a dead sponge (Tukey adjusted  $p < 0.0001$ ). There was not a significant difference in the growth of *T. testudinum* shoots shaded with live or dead sponge (Tukey adjusted  $p = 0.08$ , Fig. 5).

### 3.3. *H. melanadocia* growth and recruitment

All but three *H. melanadocia* transplanted into the ASUs lost volume over the course of the experiment; the three which grew were on medium density ASUs. Analysis of variance (ANOVA) results show that sponges on medium density ASUs lost less volume than those on low (Tukey adjusted  $p = 0.008$ ) or high (Tukey adjusted  $p = 0.02$ ) density ASUs (Fig. 4a). There was no difference in the sponge volume lost between low and high density ASUs (Tukey adjusted  $p = 0.91$ ).

Although the transplanted *H. melanadocia* did not thrive on the ASUs, recruit sponges did settle onto the seagrass mats. After the removal of one outlier ( $1.5 \times$  the inter-quartile range for the treatment), ANOVA results show that the number of recruits per ASU was significantly different among the treatments (with outlier removed:  $F_{2,26} = 4.12$ ,  $p = 0.03$ , Fig. 4b, including outlier:  $F_{2,27} = 2.72$ ,  $p = 0.08$ ). The number of recruits was highest on medium density ASUs ( $\bar{x} \pm \text{sd}$ ,  $2.5 \pm 2.4$ ) although the difference between medium and high density ASUs was not significant (Tukey adjusted  $p = 0.12$ ) or between high and low density ASUs (Tukey adjusted  $p = 0.81$ ). There was, however, a significant difference in the number of sponge recruits

**Table 2**

AICc and model weights for all potential models predicting *H. melanadocia* abundance. SD represents *T. testudinum* shoot density, Depth is the depth of the sampling plot in m, Site represents the survey site.

Model	AICc	Model weight
SD + ln(SD)	355.08	0.86
SD	360.24	0.06
Depth + ln(Depth)	360.34	0.06
SD + Site + SD $\times$ Site	361.58	0.03
SD + Depth + SD $\times$ Depth	363.16	0.02
Site	363.20	0.01
Depth	363.55	0.01
Site + Depth + Site $\times$ Depth	366.49	0.00
SD + Site + Depth + SD $\times$ Site + SD $\times$ Depth + Site $\times$ Depth + SD $\times$ Depth $\times$ Site	368.54	0.00

**Table 3**

Seagrass nutrient content and morphometric response variables from the surveys conducted in the summer of 2012. Response variables were analyzed for a difference between seagrass shoots both with and without a sponge using a paired t-test with samples collected at the same site in the same plot paired.

Variable	DF	t Value	p-Value
%C	30	0.78	0.44
%N	30	1.04	0.31
%P	31	-0.07	0.94
C:N	30	0.62	0.63
C:P	29	0.01	0.99
Longest blade	32	-0.19	0.85
Blade area	32	0.71	0.48
Blade number	32	1.96	0.06

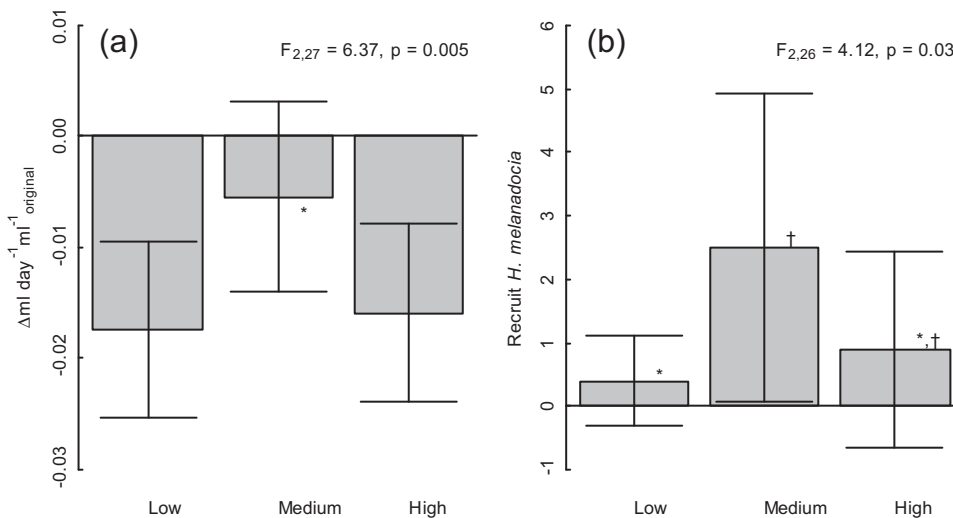
between the medium and low density ASUs (Tukey adjusted  $p = 0.03$ , Fig. 4b).

### 3.4. *H. melanadocia* nutrient flux

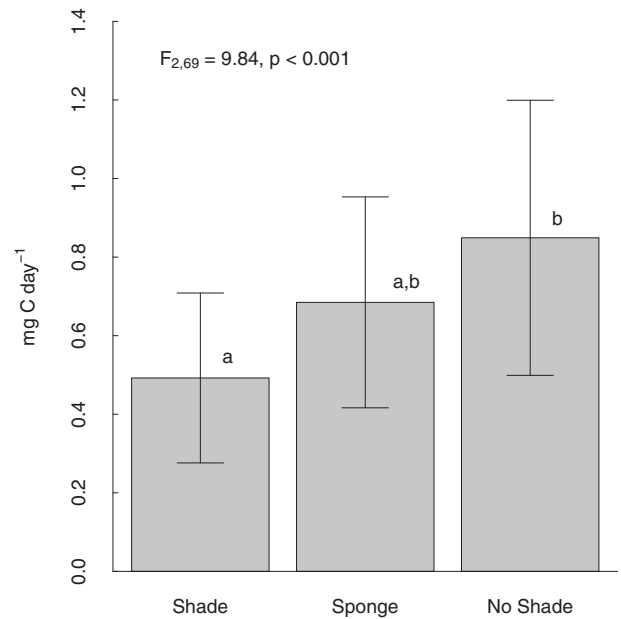
The time coefficient is not significant for the control for any solute (ammonium:  $t_5 = -0.18$ ,  $p = 0.87$ , SRP:  $t_5 = -0.84$ ,  $p = 0.44$ ,  $\text{NO}_x$ :  $t_5 = 0.12$ ,  $p = 0.91$ ). Comparing flux values to zero revealed that *H. melanadocia* is a significant source of both ammonium and SRP ( $t_3 = 5.76$ ,  $p = 0.01$ ,  $\bar{x} \pm \text{sd}$ ,  $325.2 \pm 113.0 \mu\text{g L}_{\text{sponge}}^{-1} \text{h}^{-1}$  and  $t_3 = 3.64$ ,  $p = 0.04$ ,  $21.0 \pm 11.5 \mu\text{g L}_{\text{sponge}}^{-1} \text{h}^{-1}$  respectively). However, *H. melanadocia* is not a significant source or sink of  $\text{NO}_x$  ( $t_3 = -0.82$ ,  $p = 0.47$ ,  $\bar{x} \pm \text{sd}$ ,  $-4.9 \pm 11.8 \mu\text{g L}_{\text{sponge}}^{-1} \text{h}^{-1}$ ).

## 4. Discussion

This study shows that the sponge, *H. melanadocia* benefits from a commensal relationship with *T. testudinum*. However, the interaction appears to be complex and potentially context dependent. *H. melanadocia* abundance is nonlinearly related to *T. testudinum* abundance, with the highest observed sponge abundances occurring in medium seagrass shoot densities. This nonlinearity suggests that multiple mechanisms control sponge abundance in seagrass beds. Complex dynamics also underlie the net neutral effect for seagrass. The data suggest that the neutral effect results from a balance of a negative effect of sponge shading, with a positive effect of sponge nutrient fluxes which help alleviate nutrient limitation for the seagrass.



**Fig. 4.** Results of the artificial seagrass unit (ASU) experiment. Panel (a) represents the change in *Halichondria melanadocia* volume over the course of the experiment standardized by the original volume of the sponge ( $\text{mL day}^{-1} \text{mL}^{-1} \text{original}$ ) for each of the three ASU shoot densities. Panel (b) represents the number of *H. melanadocia* which recruited to the ASUs in each treatment over the course of the experiment. In both panels \* and † represent significantly different groups at the  $\alpha = 0.05$  level after the Tukey's Honest Significant Difference correction for multiple comparisons.



**Fig. 5.** *Thalassia testudinum* growth in  $\text{mg C day}^{-1}$  for shoots shaded with a dead sponge (Shade), a live sponge (Sponge) and with No Shade. Letters represent significantly different groups at the  $\alpha = 0.05$  level after the Tukey's Honest Significant Difference correction for multiple comparisons.

The structural complexity created by *T. testudinum* alters the environment in multiple ways. This environmental alteration is likely sufficient to explain the nonlinear relationship between *H. melanadocia* and *T. testudinum* shoot density. The increase in sponge abundance up to medium densities of seagrass can be explained by two potential mechanisms. First, structure available for *H. melanadocia* settlement and growth increases with increasing *T. testudinum* shoot density. Second, *H. melanadocia* may benefit from higher food availability in denser stands of *T. testudinum*. While food availability was not explicitly measured in this study, previous studies provide support for this hypothesis. Judge et al. (1993) measured in situ food availability for a common benthic suspension feeder, *Mercenaria mercenaria*, at varying heights above the bottom in both vegetated and unvegetated habitats. They found that food is significantly more available in seagrass beds,

specifically at the measurement station closest to the bottom. In their study, the majority of food available was pennate diatoms, with sponges are known to consume (Reiswig, 1971a; Ribes et al., 1999). Additionally, seagrasses are known to leach DOC from their leaves (Ziegler and Benner, 1999), providing another food source for sponges (Maldonado et al., 2012 and references therein).

If the two mechanisms discussed in the previous paragraph were operating alone or in concert, the result would be a linear relationship between sponge abundance and seagrass density. However, the non-linearity of the relationship suggests that another mechanism is driving sponge abundance at high *T. testudinum* shoot densities. There is strong evidence that the structure associated with seagrass beds increases rates of sediment deposition (Gacia et al., 1999; Gacia et al., 2003). While the specific response of *H. melanadocia* to sedimentation is unknown, it is well established that sedimentation can reduce sponge pumping rates (Gerrodette and Flechsig, 1979). Future research is needed to specifically evaluate these potential explanatory mechanisms driving the observed nonlinear relationship between *H. melanadocia* abundance and *T. testudinum* density.

There are many factors not associated with structural complexity which are known to drive sponge abundances at large spatial scales. For example, both sponge species distributions and seagrass density are known to vary with depth (de Voogd and Cleary, 2007; Duarte et al., 2006). However, the depth range covered in this study's surveys was minimal (0.35–1.5 m). Additionally, depth was included as a potential explanatory variable in the models tested, and the highest ranked model including depth had a model weight of only 0.06 (Table 2), indicating that depth was not a strong predictor of sponge abundance. Patterns in sponge abundance can also be driven by predator abundance (Pawlik et al., 2013; Wulff, 2000), prevailing currents and wave patterns (Reiswig, 1971b). While these variables were not specifically measured, they would largely vary at the site level. However, the highest ranking model tested including site as a predictor variable had a model weight of only 0.03.

The results of the ASU experiment support the assertion that seagrass density is a main driver of *H. melanadocia* abundance in this system. Transplanted *H. melanadocia* lost significantly less volume on medium density ASUs than on either the low or high density treatments. In fact, all *H. melanadocia* to increase in volume over the course of this experiment were located on medium density seagrass units. Despite the declining volumes of *H. melanadocia* transplanted onto the ASUs, recruit *H. melanadocia* did settle on the experimental units. If availability of structure was the only mechanism driving settlement and survival of *H. melanadocia*, the highest recruit abundances would be expected on high density ASUs. However, more *H. melanadocia* recruited to medium density ASUs. This result is consistent with the hypothesis that the structure provided by *T. testudinum* shoots benefits *H. melanadocia*, while increased sedimentation resulting from structural complexity of seagrass shoots negatively impacts sponge survival. Again, while the specific response of *H. melanadocia* to sedimentation has not been reported, it is known that sedimentation can negatively affect the survival of recruit sponges (Maldonado et al., 2008). While it is possible that what was classified as recruit *H. melanadocia* simply represent resettled fragments of the transplanted sponges, on average more *H. melanadocia* recruited to medium density ASUs. As a result, sponges on medium density ASUs would have lost less volume and would, therefore, have necessarily either fragmented less or grown significantly since fragmentation.

Seagrasses, in general, have high light requirements and thus are susceptible to light limitation. Epiphyte and epibiont growth on seagrass blades has previously been linked to light limitation (Burkholder et al., 2007). For example, Wong and Vercaemer (2012) found that the presence of an epibiotic sponge, *H. panacea*, led to light limitation in the seagrass *Z. marina*. The morphological measurements and nutrient ratios used as response variables in this study (Table 1) are among the strongest indicators of light limitation in seagrasses

(McMahon et al., 2013). However, the results of the surveys comparing *T. testudinum* shoots with and without *H. melanadocia* showed no evidence of light limitation (Table 3). *T. testudinum* shoots with and without a live sponge grew at similar rates, but when the same percentage of a shoot was shaded with a dead sponge, there was a significant decrease in seagrass growth compared to non-shaded shoots. Consequently, although it appears as though *H. melanadocia* shades enough of the *T. testudinum* shoot to cause a decrease in growth, something is offsetting this effect.

This study shows that *H. melanadocia* is a significant source of bio-available nitrogen ( $\text{NH}_4^+$ ) and phosphorus (SRP). Several studies have documented nutrient transfer between sponges and primary producers growing in close proximity to each other (Davy et al., 2002; Easson et al., 2014; Ellison et al., 1996). Because nitrogen and phosphorus co-limit seagrass growth in the study system, it is likely that the nutrients released by the sponge are taken up by the seagrass. When limiting resources are heterogeneously distributed many clonal plant species translocate resources throughout the clone (Hutchings, 1999; Hutchings and Wijesinghe, 1997; Stuefer, 1998; Stuefer et al., 1994). Seagrass are clonal plants and there is evidence that *T. testudinum* maintains shaded shoots by translocation of resources when shading is restricted to a small number of shoots within the clone (Tomasko and Dawes, 1989). Therefore, it is possible that seagrass shoots with the sponge growing around them transport excess N and P from the sponge to other shoots, while receiving photosynthate from nearby unshaded shoots. If this is occurring, there would be no measurable signature in the growth or nutrient content from either the nutrient supply or the light limitation, as was observed.

Taken together, the data presented suggest that the interaction between *H. melanadocia* and *T. testudinum* is likely a context dependent interaction. Specifically, for *H. melanadocia*, the interaction is likely a balance between the positive effects of increased habitat and food availability with a potential negative effect of increased sedimentation at high seagrass densities. For *T. testudinum*, the interaction ostensibly is the result of a balance between the negative effects of shading by *H. melanadocia* and the positive effect of nutrient supply. In other context dependent interactions where the benefit to at least one of the participants is dependent on nutrient transfer, the relationship will often shift from a positive interaction (e.g. mutualism, commensalism) towards parasitism with increasing ambient nutrient availability. For example, as soil fertility increases, the relationship between plants and their associated mycorrhizae will shift from mutualism to parasitism (Johnson et al., 1997; Neuhauser and Fargione, 2004). If the effect of *H. melanadocia* on *T. testudinum* is a balance between the positive effect of nutrient supply and the negative effect of shading, eutrophication may shift the relationship towards parasitism. It should be noted that seagrass shoots with a live sponge did grow less, although not significantly so, than unshaded shoots. This may be evidence that the interaction, even under the oligotrophic conditions under which we studied it, is bordering on parasitism. Eutrophication is characterized by increased light attenuation and ambient nutrient availability. Increased light attenuation would likely increase the consequences of the shading by *H. melanadocia*, while increased ambient nutrient availability would decrease the benefit of the nutrients supplied by the sponge. Such a shift in the cost–benefit ratio would drive the relationship over the line from commensalism to parasitism.

A simple simulation of the effect of such a shift from commensalism to parasitism suggests that this could impact the rate of above ground productivity by decreasing production by just over 1% per square meter per day. This estimate ignores below ground carbon storage by *T. testudinum*, and does not take into account the direct effect of increased light attenuation and ambient nutrient availability on seagrass productivity. Despite these caveats, the simulation suggests that a parasitic relationship between *H. melanadocia* and *T. testudinum* could contribute to reduced carbon fixation in seagrass beds beyond that predicted by only considering the direct effects of eutrophication

on *T. testudinum* productivity. This simplistic simulation underscores the importance of further characterizing this, and other, species interactions involving foundation species.

Foundation species are critically important for the maintenance of biological diversity because of their positive interactions with community members and tendency to alleviate harsh abiotic conditions. As the scale and magnitude of anthropogenic impacts increase, data allowing us to predict the response of ecosystems to abiotic alteration is paramount. If an interaction is context dependent, human alteration of the environment may result in a shift in the net outcome of previously described interactions (Chamberlain et al., 2014; Kiers et al., 2010). Therefore, mechanistic understandings of species interactions, rather than description of the mean net effects, will lead to a more complete description of the interaction, and its outcomes, in a changing world. Such a description, especially for interactions involving foundation species, may prove valuable for restoration and conservation efforts.

## Acknowledgments

We would like to thank J. Fourqurean for invaluable advice in the experimental design and data interpretation and to A. Armitage for guidance in the design and construction of the ASUs. Many thanks to E. Archer, S. Buhler, C. Burgett, T. Callahan, D. Claridge, C. Dunn, J. Sanchez, and B. Whitman for support and assistance in the field. This study was funded by the EPA STAR Fellowship (EWS), Florida International University's Presidential Fellowship (SKA) and NSF (OCE #0746164 and #1405198). Funding agencies played no role in the experimental design, decision to publish, or collection, analysis, and interpretation of the data presented in this manuscript.

## References

- Allgeier, J.E., Yeager, L.A., Layman, C.A., 2013. Consumers regulate nutrient limitation regimes and primary production in seagrass ecosystems. *Ecology* 94 (2), 521–529.
- Bell, J.J., 2008. The functional roles of marine sponges. *Estuar. Coast. Shelf Sci.* 79 (3), 341–353.
- Bracken, M.E., Bracken, B.E., Rogers-Bennett, L.D., 2007. Species Diversity and Foundation Species: Potential Indicators of Fisheries Yields and Marine Ecosystem Functioning. Bronstein, J.L., 1994. Conditional outcomes in mutualistic interactions. *Trends Ecol. Evol.* 9 (6), 214–217.
- Bruno, J.F., Bertness, M.D., 2001. Habitat Modification and Facilitation in Benthic Marine Communities. In: Bertness, M.D. (Ed.), *Marine Community Ecology*. Sinauer, pp. 201–218.
- Burkholder, J.M., Tomasko, D.A., Touchette, B.W., 2007. Seagrasses and eutrophication. *J. Exp. Mar. Biol. Ecol.* 350 (1), 46–72.
- Chamberlain, S.A., Bronstein, J.L., Rudgers, J.A., 2014. How context dependent are species interactions? *Ecol. Lett.* 17 (7), 881–890.
- Core Team, R., 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Corredor, J.E., Wilkinson, C.R., Vicente, V.P., Morell, J.M., Otero, E., 1988. Nitrate release by Caribbean reef sponges. *Limnol. Oceanogr.* 33 (1), 114–120.
- Costanza, R., d'Arge, R., deGroot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., Oneill, R.V., Paruelo, J., Raskin, R.G., Sutton, P., van den Belt, M., 1997. The value of the world's ecosystem services and natural capital. *Nature* 387 (6630), 253–260.
- Davy, S.K., Trautman, D.A., Borowitzka, M.A., Hinde, R., 2002. Ammonium excretion by a symbiotic sponge supplies the nitrogen requirements of its rhodophyte partner. *J. Exp. Biol.* 205 (22), 3505–3511.
- Dayton, P.K., 1972. Toward an Understanding of Community Resilience and the Potential Effects of Enrichments to the Benthos at McMurdo Sound, Antarctica. *Proceedings of the Colloquium on Conservation Problems in Antarctica*. Allen Press Lawrence, Kansas, USA, pp. 81–96.
- de Voogd, N.J., Cleary, D.F.R., 2007. Relating species traits to environmental variables in Indonesian coral reef sponge assemblages. *Mar. Freshw. Res.* 58 (3), 240–249.
- Diaz, M.C., Rützler, K., 2009. Biodiversity and abundance of sponges in Caribbean mangrove: indicators of environmental quality. *Smithson. Contrib. Mar. Sci.* 38, 151–172.
- Diaz, M.C., Ward, B.B., 1997. Sponge-mediated nitrification in tropical benthic communities. *Mar. Ecol. Prog. Ser.* 156, 97–107.
- Duarte, C.M., Fourqurean, J.W., Krause-Jensen, D., Olesen, B., 2006. Dynamics of Seagrass Stability and Change. *Seagrasses: Biology Ecology and Conservation*. Springer, pp. 271–294.
- Duffy, J.E., 2006. Biodiversity and the functioning of seagrass ecosystems. *Mar. Ecol. Prog. Ser.* 311, 233–250.
- Easson, C.G., Slaterry, 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.
- Ellison, A.M., Farnsworth, E.J., Twilley, R.R., 1996. Facultative mutualism between red mangroves and root-fouling sponges in Belizean mangal. *Ecology* 77 (8), 2431–2444.
- Ellison, A.M., Bank, M.S., Barton, D.C., Colburn, E.A., Elliott, K., Ford, C.R., Foster, D.R., Kloeppe, B.D., Knoepp, J.D., Lovett, G.M., Mohan, J., Orwig, D.A., Rodenhouse, N.L., Sobczak, W.V., Stinson, K.A., Stone, J.K., Swan, C.M., Thompson, J., Von Holle, B., Jackson, R.W., 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Front. Ecol. Environ.* 3 (9), 479–486.
- Folmer, E.O., van der Geest, M., Jansen, E., Olff, H., Anderson, T.M., Piersma, T., van Gils, J.A., 2012. Seagrass–sediment feedback: an exploration using a non-recursive structural equation model. *Ecosystems* 15 (8), 1380–1393.
- Fourqurean, J.W., Zieman, J.C., Powell, G.V., 1992. Phosphorus limitation of primary production in Florida Bay: evidence from C:N:P ratios of the dominant seagrass *Thalassia testudinum*. *Limnol. Oceanogr.* 37 (1), 162–171.
- 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 (7), 505–509.
- Gacia, E., Granata, T.C., Duarte, C.M., 1999. An approach to measurement of particle flux and sediment retention within seagrass (*Posidonia oceanica*) meadows. *Aquat. Bot.* 65 (1–4), 255–268.
- Gacia, E., Duarte, C.M., Marbà, N., Terrados, J., Kennedy, H., Fortes, M.D., Tri, N.H., 2003. Sediment deposition and production in SE-Asia seagrass meadows. *Estuar. Coast. Shelf Sci.* 56 (5–6), 909–919.
- Gerrodette, T., Flechsig, A.O., 1979. Sediment induced reduction in the pumping rate of the tropical sponge *Verongia lacunosa*. *Mar. Biol.* 55 (2), 103–110.
- Heck, K.L., Valentine, J.F., 2006. Plant–herbivore interactions in seagrass meadows. *J. Exp. Mar. Biol. Ecol.* 330 (1), 420–436.
- Heck, K.L., Pennock, J.R., Valentine, J.F., Coen, L.D., Sklenar, S.A., 2000. Effects of nutrient enrichment and small predator density on seagrass ecosystems: an experimental assessment. *Limnol. Oceanogr.* 45 (5), 1041–1057.
- Hemminga, M., Harrison, P., Van Lent, F., 1991. The balance of nutrient losses and gains in seagrass meadows. *Mar. Ecol. Prog. Ser.* 71.
- Hessing-Lewis, M.L., Hacker, S.D., Menge, B.A., Rumrill, S.S., 2011. Context-dependent eelgrass–macroalgae interactions along an estuarine gradient in the Pacific Northwest, USA. *Estuar. Coasts* 34 (6), 1169–1181.
- Holmes, R.M., Aminot, A., Kerouel, R., Hooker, B.A., Peterson, B.J., 1999. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. *Can. J. Fish. Aquat. Sci.* 56 (10), 1801–1808.
- Hutchings, M., 1999. Clonal plants as cooperative systems: benefits in heterogeneous environments. *Plant Species Biol.* 14 (1), 1–10.
- Hutchings, M.J., Wijesinghe, D.K., 1997. Patchy habitats, division of labour and growth dividends in clonal plants. *Trends Ecol. Evol.* 12 (10), 390–394.
- Johnson, N.C., Graham, J.H., Smith, F.A., 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytol.* 135 (4), 575–585.
- Judge, M.L., Coen, L.D., Heck Jr., K.L., 1993. Does *Mercenaria mercenaria* encounter elevated food levels in seagrass beds? Results from technique to collect suspended food resources. *Mar. Ecol. Prog. Ser.* 92, 141–150.
- 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 (12), 1459–1474.
- Larkum, A.W.D., Orth, R.J., Duarte, C.M., 2006. *Seagrasses: Biology, Ecology, and Conservation*. Springer, Dordrecht, The Netherlands.
- Maldonado, M., Giraud, K., Carmona, C., 2008. Effects of sediment on the survival of asexually produced sponge recruits. *Mar. Biol.* 154 (4), 631–641.
- Maldonado, M., Ribes, M., van Duyl, F.C., 2012. Nutrient fluxes through sponges: biology, budgets, and ecological implications. In: Becerro, M.A., Uriz, M.J., Maldonado, M., Turon, J. (Eds.), *Advances in Sponge Science: Physiology, Chemical and Microbial Diversity*. Biotechnology, pp. 113–182.
- Marbà, N., Holmer, M., Gacia, E., Barron, C., 2006. Seagrass beds and coastal biogeochemistry. In: Larkum, A.W.D., Orth, R.J., Duarte, C.M. (Eds.), *Seagrasses: Biology, Ecology and Conservation*. Springer, The Netherlands, pp. 135–157.
- McLeod, E., Chmura, G.L., Bouillon, S., Salm, R., Bjork, M., Duarte, C.M., Lovelock, C.E., Schlesinger, W.H., Silliman, B.R., 2011. A blueprint for blue carbon: toward an improved understanding of the role of vegetated coastal habitats in sequestering CO<sub>2</sub>. *Front. Ecol. Environ.* 9 (10), 552–560.
- McMahon, K., Collier, C., Lavery, P.S., 2013. Identifying robust bioindicators of light stress in seagrasses: a meta-analysis. *Ecol. Indic.* 30, 7–15.
- Neuhauser, C., Fargione, J.E., 2004. A mutualism–parasitism continuum model and its application to plant–mycorrhizae interactions. *Ecol. Model.* 177 (3–4), 337–352.
- 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 (12), 987–996.
- Parsons, T.R., Maita, Y., Lalli, C.M., 1984. *A Manual of Chemical and Biological Methods for Seawater Analysis*. Pergamon Press Inc, Elmsford, NY.
- Pawlik, J.R., Loh, T., McMurray, S.E., Finelli, C.M., 2013. Sponge communities on Caribbean coral reefs are structured by factors that are top-down, not bottom-up. *PLoS ONE* 8 (5), e62573.
- Reiswig, H.M., 1971a. Particle feeding in natural populations of 3 marine Demosponges. *Biol. Bull.* 141 (3), 568–591.
- Reiswig, H.M., 1971b. In-situ pumping activities of tropical Demospongiae. *Mar. Biol.* 9 (1), 38–50.
- Ribes, M., Coma, R., Gili, J.-M., 1999. Natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dendroceratida) throughout an annual cycle. *Mar. Ecol. Prog. Ser.* 176, 179–190.
- Southwell, M.W., Weisz, J.B., Martens, C.S., Lindquist, N., 2008. In situ fluxes of dissolved inorganic nitrogen from the sponge community on Conch Reef, Key Largo, Florida. *Limnol. Oceanogr.* 53 (3), 986–996.

- Stachowicz, J.J., 2001. Mutualism, facilitation, and the structure of ecological communities. *Bioscience* 51 (3), 235–246.
- Stuefer, J.F., 1998. Two types of division of labour in clonal plants: benefits, costs and constraints. *Perspect. Plant Ecol. Evol. Syst.* 1 (1), 47–60.
- Stuefer, J.F., During, H.J., Kroon, H.d., 1994. High benefits of clonal integration in two stoloniferous species, in response to heterogeneous light environments. *J. Ecol.* 82 (3), 511–518.
- Taylor, B.W., Keep, C.F., Hall, R.O., Koch, B.J., Tronstad, L.M., Flecker, A.S., Ulseth, A.J., 2007. Improving the fluorometric ammonium method: matrix effects, background fluorescence, and standard additions. *J. N. Am. Benthol. Soc.* 26 (2), 167–177.
- Tomasko, D.A., Dawes, C.J., 1989. Evidence for physiological integration between shaded and unshaded short shoots of *Thalassia testudinum*. *Mar. Ecol. Prog. Ser.* 54 (3), 299–305.
- van der Heide, T., Govers, L.L., de Fouw, J., Olf, H., van der Geest, M., van Katwijk, M.M., Piersma, T., van de Koppel, J., Silliman, B.R., Smolders, A.J.P., van Gils, J.A., 2012. A three-stage symbiosis forms the foundation of seagrass ecosystems. *Science* 336 (6087), 1432–1434.
- Wabnitz, C.C., Andrefouet, S., Torres-Pulliza, D., Muller-Karger, F.E., Kramer, P.A., 2008. Regional-scale seagrass habitat mapping in the Wider Caribbean region using Landsat sensors: applications to conservation and ecology. *Remote Sens. Environ.* 112 (8), 3455–3467.
- 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 (30), 12377–12381.
- Williams, S.L., 1990. Experimental studies of Caribbean seagrass bed development. *Ecol. Monogr.* 449–469.
- 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 (3), 315–326.
- Wulff, J., 2000. Sponge predators may determine differences in sponge fauna between two sets of mangrove cays. *Belize Barrier Reef. Atoll Res. Bull.* 477, 251–263.
- Wulff, J.L., 2006. Ecological interactions of marine sponges. *Can. J. Zool. Rev. Can. Zool.* 84 (2), 146–166.
- Yarbro, L.A., Carlson, P.R., 2008. Community oxygen and nutrient fluxes in seagrass beds of Florida Bay, USA. *Estuar. Coasts* 31 (5), 877–897.
- Ziegler, S., Benner, R., 1999. Dissolved organic carbon cycling in a subtropical seagrass-dominated lagoon. *Mar. Ecol. Prog. Ser.* 180, 149–160.
- Zieman, J.C., 1974. Methods for the study of the growth and production of turtle grass, *Thalassia testudinum* König. *Aquaculture* 4, 139–143.
- Zieman, J.C., 1982. *The Ecology of the Seagrasses of South Florida: A Community Profile*. U.S. Fish and Wildlife Services, Offices of Biological Services, Washington, DC.