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Human manipulations of bottom-up and top-down controls cause degradation of  
primary and facilitation of secondary foundation species

A dissertation submitted in partial satisfaction  
of the requirements for the degree  
Doctor of Philosophy in Biology

by

Sarah Joy Bittick

2017

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2017

## ABSTRACT OF THE DISSERTATION

Human manipulations of bottom-up and top-down controls cause degradation of primary and facilitation of secondary foundation species

by

Sarah Joy Bittick

Doctor of Philosophy in Biology

University of California, Los Angeles, 2017

Professor Peggy Marie Fong, Chair

As human-induced global change impacts nearly every ecosystem worldwide, there is increasing concern for the degradation of foundation species. These species play key roles in ecosystem functioning by facilitating other species and supporting community structure through amelioration of harsh conditions and/or provision of habitat structure. Human alteration of forces controlling foundation species' abundance and dominance, such as nutrient limitation and herbivory, can result in not only the decline of foundation species, but also a shift in spatial dominance to other, often less desirable species. Replacement species are often fast growing and ephemeral, but there are cases in which "secondary" foundation species establish and expand. Secondary foundation species are

dependent on the original (primary) foundation species to establish and persist. I investigated the influence human manipulation of nutrients has on dominance of a primary (a temperate seagrass) and secondary (a coral reef macroalga) foundation species, and the possible consequences to trophic structure in two of the most threatened marine systems worldwide.

In chapter 1, I demonstrated that anthropogenic increases in nutrients can indirectly, through stimulating algal blooms, drive declines in a primary foundation species, the temperate seagrass, *Zostera marina*. It is widely recognized that these bloom-forming algal species tend to grow quickly in response to nutrients, and can negatively affect seagrasses by inhibiting light and changing flow and sediment conditions. However, the abundance of macroalgae that can be tolerated by seagrass beds and the possible impacts to epiphytes, which are an important trophic resource, are unclear. To address this knowledge gap, I conducted two caging experiments in a *Z. marina* bed in Bodega Harbor, California where I maintained six densities of *Ulva* and *Gracilariopsis*, two common bloom-forming algal species worldwide. Both species caused declines in seagrass health by decreasing shoot density and epiphyte abundance. Further, reduced epiphyte load suggests that not only the habitat structure afforded by *Z. marina* is degraded by macroalgal enrichment, but that there will be trophic consequences through a decrease in resources. Changes to bottom-up control cause significant impacts on this foundation species.

In chapters 2-3, I demonstrated that anthropogenic increases in nutrients may facilitate dominance by *Turbinaria ornata*, a secondary foundation species of brown algae that has been expanding both in range and habitat usage, likely at the

expense of the primary foundation coral species. Coral reefs are typically overgrown by the same sorts of ephemeral, fast growing macroalgae commonly found in seagrass beds. In contrast, I established that *T. ornata* is a persistent foundation species as it facilitated epiphytes, other macroalgae, and herbivorous fish in a field study on fringing reefs in Mo'orea, French Polynesia. However, as *T. ornata* is dependent on the hard substrate created by corals to establish persistent aggregations, it is a secondary, not a primary foundation species, and shifts to this community may ultimately result in reef degradation. To understand mechanisms driving the expansion of *T. ornata*, I examined the role of nutrients and herbivory in facilitating *T. ornata* through a combination of field and mesocosm experiments. My results showed a unique interaction whereby enhanced physical defences with nutrient enrichment release *T. ornata* from herbivore control, which may allow expansion of *T. ornata* to habitats where it is usually controlled by high herbivory. Thus, on coral reefs in the South Pacific, I found a secondary macroalgal species was likely being indirectly facilitated by human impacts. In this case nutrient enrichment by humans caused an expansion of a secondary foundation species indirectly, through weakening of control by herbivory.

Overall, my results demonstrate that human alterations of nutrient supplies can cause both degradation of primary and facilitation of secondary foundation species. In some cases, the facilitation of a secondary foundation species may result in direct replacement of primary foundation species, which may prove to be difficult to reverse, as these species are, by definition, more persistent once established.

The dissertation of Sarah Joy Bittick is approved.

Thomas Welch Gillespie

James O Lloyd-Smith

Peggy Marie Fong, Committee Chair

University of California, Los Angeles

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## EPIGRAPH

*"When one tugs at a single thing in nature, he [she] finds it attached to the rest of the world."*

-John Muir

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Ecology and Evolutionary Biology (EEB) and MS from the Southern California Coastal Water Research Project. All authors designed the study. SJB conducted field work with assistance from PF and wrote the first manuscript draft, MS and PF edited multiple drafts.

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Clausing RJ, **Bittick SJ**, Fong CR, & Fong P. (2016). Sediments differentially alter drivers of two dominant macroalgal species biomass accumulation on a fringing coral reef. *Coral Reefs*, 35: 1297-1309.

Bergman J\*, Dang BN\*, Tabatabaee MM\*, McGowan MM\*, **Bittick SJ**, Fong CR, & Fong P. (2016). Nutrients induce and herbivores maintain thallus toughness, a structural anti-herbivory defense in *Turbinaria ornata*. *Marine Ecology Progress Series*, 559: 35-43.

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Clausing RJ, Annunziata C\*, Baker G\*, Lee C\*, **Bittick SJ**, & Fong P. (2014). Effects of sediment depth on algal turf height are mediated by interactions with fish herbivory on a fringing reef. *Marine Ecology Progress Series*, 517: 121-129.

**Bittick SJ** & Chung GWK. (2011). The use of narrative: Gender differences and implications for motivation and learning in a math game. (CRESST Report 804). University of California, National Center for Research on Evaluation, Standards, and Student Testing.

**Bittick SJ**, Bilotti ND, Peterson HA, & Stewart HL. (2010). *Turbinaria ornata* as an herbivory refuge for associate algae. *Marine Biology*, 157: 317-323.

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### **Selected Presentations**

**Bittick SJ**, Clausing RJ, Fong CR, & Fong P. Bolstered physical defenses under nutrient enriched conditions may facilitate a macroalgal species in the South Pacific. International Coral Reef Symposium. Honolulu, Hawaii, June 19-24, 2016, poster presentation.

**Bittick SJ**, Sutula M, & Fong P. A tale of two algal blooms: Differential negative effects of *Ulva* and *Gracilariopsis* on seagrass. Coastal and Estuarine Research Federation (CERF) Biennial Conference. Portland, Oregon, November 8-12, 2015, paper presentation.

**Bittick SJ**, Scoma S\*, Clausing RJ, Fong CR, & Fong P. A foundational marine macroalga supports epiphytes that facilitate foraging by herbivorous fish in the South Pacific. Western Society of Naturalists Annual Meeting. Tacoma, Washington, November 13-16, 2014, paper presentation.

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**Bittick SJ**, Clausing RJ, Fong CR & Fong P. Interaction of top-down and bottom-up processes facilitate the rapid range expansion of *Turbinaria ornata* in the South Pacific. Ecology Society of America (ESA) Annual Conference. Minneapolis, Minnesota, August 4-9, 2013, paper presentation.

## CHAPTER 1:

### **A tale of two algal blooms: predictable negative effects of two common macroalgae on seagrass health**

#### **Abstract**

Recent evidence suggests macroalgal blooms may play a role in the worldwide decline in seagrass, but the shape of the relationship between seagrass health and dominant bloom-forming macroalgae is poorly characterized. The response may occur as a sudden threshold or in a smooth predictable pattern. We investigated the impact of varying abundances of two cosmopolitan bloom-forming macroalgal genera on the health of the eelgrass *Zostera marina*. We conducted two caging experiments in a shallow *Z. marina* bed (~1 m depth) in Bodega Harbor, California, USA where we maintained six densities within the range of natural abundances of macroalgae, *Ulva* (0-4.0 kg m<sup>-2</sup>) and *Gracilariopsis* (0-2.0 kg m<sup>-2</sup>), as well as uncaged controls over a 10-week period. Shoot density, blade growth, and epiphyte load were measured every two weeks and algal treatments reset. We tested for threshold transitions using sigmoidal and broken-stick analyses for each data set, which are models commonly used to identify thresholds for ecological management. However, we did not find support for a threshold relationship between algal abundance and measures of seagrass bed health. Instead, final measurements of shoot density and epiphyte load were best modeled as linear or

slightly non-linear declines with increasing *Ulva* abundance. A negative linear relationship also existed between shoot density and *Gracilariopsis* abundance and a trend towards linear negative effects on epiphyte load. Growth of existing blades was not related to macroalgal abundance in either experiment. Thus, at algal abundances that are commonly observed, we found smooth and predictable negative impacts to *Z. marina* by decline in shoot density and potential impacts to food webs by loss of epiphytes, rather than sudden threshold shifts or “ecological surprises”. The predictable relationship between seagrass health and macroalgal load can be used to alert managers to systems that require immediate action.

**Keywords:** *Zostera marina*, Eelgrass, Seagrass decline, Epiphyte load, Macroalgal blooms, density-dependence

## **Introduction**

Foundation species are being lost or degraded in terrestrial and aquatic systems globally; this is of special concern as loss of these habitat-forming species results in ecosystem loss (e.g. kelp forests, Steneck and Erlandson 2002; temperate forests, Ellison et al. 2005; seagrass beds, Orth et al. 2006; grasslands, Curtin and Western 2008; sagebrush steppe, Prevéy et al. 2009; corals, Hughes et al. 2010). Seagrasses are important foundation species in shallow waters worldwide, harboring a diverse assemblage of organisms, including invertebrates and juvenile fishes, and carrying out essential processes such as nutrient cycling, organic carbon production, and filtering water from the land as it enters bays and estuaries (for reviews see Beck et al. 2001, Orth et al. 2006). Drastic declines have been documented globally, including the coasts of Canada, Mexico, the eastern

United States, several Caribbean Islands, Central and South America, Europe, Korea, and Australia (Duarte 2002, Baden et al. 2003, reviewed in Orth et al. 2006, Waycott et al. 2009, Cardoso et al. 2010). The loss of this important foundation species has resulted in dramatic losses of associated species. For example, in the 1930s, eelgrass (*Zostera marina*) wasting disease on both sides of the North Atlantic resulted in the collapse of scallop fisheries, a decline in waterfowl abundance, and the extinction of a marine gastropod (Rasmussen 1977, Carlton et al. 1991). Many pressures may contribute to seagrass decline such as eutrophication, sedimentation, and increased temperature often by allowing algal abundance to increase and compete with seagrass (reviewed in Orth et al. 2006). However, while the negative impacts of nutrient-driven phytoplankton blooms and epiphyte loads to seagrasses have been well-documented, more recent evidence suggests blooms of ephemeral macroalgae may also play a role in seagrass loss (reviewed in Orth et al. 2006). Regardless of the cause of loss, cascading impacts are widespread, including to large endangered herbivores such as green sea turtles, dugongs, and manatees (Preen et al. 1995, Orth et al. 2006). It is essential we understand the ecological forces driving losses of these globally important foundation species to conserve habitat and prevent further cascading effects.

Shifts in dominance by primary producers under a gradient of environmental conditions have been documented across systems (e.g. coral reefs, Hughes 1994; reviewed in Scheffer and Carpenter 2003; forests, Odion et al. 2010). Species experience degradation along environmental gradients such as climate warming, nutrient input, or changes to herbivory pressure. These shifts can vary from smooth and gradual to sudden, catastrophic declines (see Scheffer and Carpenter 2003).

Much recent attention has focused on the latter as they may be common responses to anthropogenic stressors, yet are extremely difficult to predict (see examples in Scheffer and Carpenter 2003, seagrass beds in Viaroli et al. 2008, coral reefs in Hughes et al. 2010), presenting formidable management challenges (Suding and Hobbs 2009). One reason the pattern of responses to environmental change varies is because functional forms of primary producers often respond to abiotic conditions differentially and competitive outcomes can shift based on resource availability. For example, lower soil water content can increase competition between herbaceous and woody plants in transition zones between grassland and deciduous forest (Davis et al. 1998), while increased CO<sub>2</sub> (Mckee and Rooth 2008) and winter temperature (Saintilan et al. 2014) enable mangrove expansion into salt marshes. In aquatic systems, a suite of environmental characteristics—water movement, sedimentation, nutrient availability, and CO<sub>2</sub> availability—can influence dominance by phytoplankton versus benthic primary producers (reviewed by Sand-Jensen and Borum 1991). Following these changes to abiotic conditions, the original dominant species may be replaced and this is of particular concern when the replacement species do not provide the same community and ecological support. This is predicted for seagrasses and macroalgae, as macroalgae may not provide the same habitat services for juvenile fishes and invertebrates (Deegan et al. 2002, see Heck et al. 2003 for a review). Research to understand the shape of the relationship between environmental gradients and competing primary producers is important, especially in cases where a foundation species is replaced by a “less desirable” species. Knowing when impacts are predictable and reversible will enable managers to identify, a priori, when systems are at risk.



Many studies examining seagrass decline have identified watershed development and associated nutrient-rich runoff as a driver of loss (Valiela et al. 1997, Cardoso et al. 2004, Orth et al. 2006, Burkholder et al. 2007). A review of 47 studies reported 15 in which eutrophication contributed at least a 1 km<sup>2</sup> area of seagrass loss with five associated with loss > 100 km<sup>2</sup> (Orth et al. 2006). Increased nutrient inputs resulting in blooms of phytoplankton or small epiphytes have been shown to reduce light penetration to seagrass blades (e.g., Borum 1985, Short et al. 1995, Moore et al. 1996, Mcglathery 2001, Drake et al. 2003) as well as to reduce gas and nutrient exchange (Sand-Jensen 1977); it has been well-established that both of these processes increase seagrass mortality. However, ephemeral macroalgae also may increase in response to nutrient input, and a few studies have found that the resultant blooms increased competition for light and ultimately replaced seagrasses (Valiela et al. 1997, Hauxwell et al. 2001, Mcglathery 2001, Borum et al. 2004). Such macroalgal blooms have been shown to cause declines in seagrass in the genus *Zostera* on both sides of the Atlantic Ocean by decreasing recruitment and growth rates through light inhibition and creation of toxic biogeochemical conditions (Sand-Jensen and Borum 1991, Valiela et al. 1997, Hauxwell et al. 2001, Mcglathery 2001, Nielsen et al. 2002, Cardoso et al. 2004, Pulido and Borum 2010; see Appendix S1.1 for a detailed review). Furthermore, studies in upwelling-influenced estuaries on the west coast of the United States found negative impacts of macroalgae to *Z. marina* to be variable and highly context dependent (Hessing-Lewis et al. 2011, 2015). Seagrass beds have often been hypothesized to transition abruptly to micro- or macro- algae at threshold levels but there have been few empirical tests of a threshold relationship (see

model in van der Heide et al. 2007, reviewed in Viaroli et al. 2008). While links have been made between macroalgal blooms and seagrass decline, these studies have not evaluated seagrass responses along a gradient of macroalgal stress to identify the shape of the response. Whether macroalgal loads cause a smooth, predictable degradation of seagrass or whether catastrophic loss occurs above particular loads is key knowledge needed to inform management strategies.

Bloom-forming macroalgae that may negatively affect seagrass exist in a variety of functional forms such as filamentous or calcareous epiphytes or sheet-like green and branching red macroalgae. While each of these forms can respond to nutrients with increased growth, branching reds are less responsive to nutrients than sheet-like green algae (Rosenberg and Ramus 1982, Pedersen and Borum 1996). Epiphytes on seagrasses, especially filamentous forms, have accelerated growth rates in response to nutrients during certain seasons (Borum 1985, Frankovich and Fourqurean 1997, reviewed by Hughes et al. 2004), and negatively impact seagrasses through light attenuation (Short et al. 1995, Brush and Nixon 2002). Red algae can grow in lower light conditions than greens due to accessory pigments (Häder and Figueroa 1997, Fong and Paul 2011) and many types of macroalgae can be found deeper than seagrass (Nielsen et al. 2002). Nutrient-stimulated blooms of free-floating macroalgae may raft onto seagrass and cause an increase in competition with epiphytes for light and nutrients (see Cardoso et al. 2004) with a subsequent decline in epiphyte loading on *Z. marina*. While this may alleviate some of the negative impacts of epiphytes to *Z. marina*, there may be cascading impacts as epiphytes support ecosystem functions such as trophic support to invertebrates (Hughes et al. 2004, Rohr et al. 2009, Angelini and

Silliman 2014) and light attenuation by the macroalga is an issue for seagrass as well (Mcglathery 2001). However, it is unknown whether different functional forms of bloom-forming macroalgae will impact seagrass communities differentially. Therefore, this study evaluates impacts to *Z. marina* and its epiphytes of two common bloom-forming macroalgal species of different functional forms.

Quantifying the shape and predictability of the relationship between stressors and responses of foundation species is of key importance to inform ecosystem management. For example, it is difficult to predict when negative impacts are imminent when the relationship is strongly non-linear and catastrophic decline occurs past a critical level (Scheffer and Carpenter 2003), resulting in a history of “ecological surprises” or unpredicted degradative shifts in coral reef (McCook and McCook 1999), savannah (Ludwig et al. 1997), and lake systems (Carpenter et al. 1999, reviewed in Scheffer et al. 2001). Two common methods typically used to attempt to identify thresholds points of decline for an ecosystem or species in response to an environmental stressor include fitting sigmoid models and performing piecewise (broken stick) regression (e.g., Toms and Lesperance 2003, Samhuri et al. 2010). In contrast, in other systems that respond in a predictable, linear or gently curved way to changes in abiotic factors such as nutrient levels in estuaries (e.g., Nedwell et al. 1999) and urbanization in streams (Morley and Karr 2002), negative responses are more easily predicted and can provide early warning signs that management action is needed. However, the shape of the response of seagrass health (shoot density, growth, and epiphyte load) to macroalgal blooms has not been determined, even in prior manipulative experiments as they lack sufficient treatment levels (see Appendix S1.1 for a review). Therefore, the key

questions asked in this study were: Will there be similar negative responses to increased abundances of two macroalgal species of different functional forms measured by (1) *Zostera marina* shoot density and growth and (2) trophic support via epiphytes? If so, can we (3) identify whether the response to increased abundance of each macroalgal species is a sudden threshold transition or a smooth and predictable?

## **Methods**

### ***Macroalgal species***

Dominant bloom-forming macroalgae are usually either sheet-like or filamentous green (Valiela et al. 1997, Mcglathery 2001) or coarsely branching red algae (Hauxwell et al. 2001, 2003, Huntington and Boyer 2008). Both of these growth forms respond to nutrient addition with rapid increases in growth (Fong et al. 1993, McGlathery 1995, Kamer et al. 2001). Blooms of green algae can produce floating mats that raft over seagrass, blanketing the beds with various abundances and depths (Mcglathery 2001). In contrast, branching red algae tend to form masses that intercalate within the bases of seagrass shoots (Huntington and Boyer 2008). Previous studies showed separately that red or green algal additions can have negative impacts on seagrasses (see Appendix S1.1: Table S1.1), but did not compare species or test multiple levels of algal addition (but see Hauxwell et al. 2001, Huntington and Boyer 2008, Rasmussen et al. 2012 for 3 treatments). Our study compared impacts of 2 functional forms of algae and included enough treatment levels to determine the shape of the seagrass community response.

## **Experimental design**

Two field experiments assessed changes over time in seagrass health, measured as shoot density, blade growth rate, and epiphyte load, with additions of two functional forms of macroalgae. A seagrass bed near the mouth of Bodega Harbor, California, USA (38°18'41.81"N, 123° 3'37.63"W) with a range in tidal height of -0.24 to +2.00 m relative to mean lower low water was the site for both experiments. Prior to the experiments, all existing macroalgae were removed from 44 1 m<sup>2</sup> plots. To retain (or exclude) algae, 5-sided cages with dimensions of 1 m<sup>3</sup> constructed from a PVC frame and hardware mesh with 2.5 cm x 2.5 cm openings were placed on all plots; the 1m height allowed algae to float up and down with the tides if they did so naturally, but maintained experimental treatments (Green et al. 2014).

For the first experiment, six treatments of *Ulva spp.* were added to seagrass plots with densities of 0, 1.0, 1.5, 2.0, 3.0, and 4.0 kg m<sup>-2</sup> wet weight (n=4 for algal treatments; n=5 for no addition plots used in both experiments (see below)). Marked but uncaged control (UCC) plots (n=4) evaluated artifacts due to cages alone. There were no differences due to cages for any response variable but epiphyte load, which was reduced by cages (see Appendix S1.2). Treatments were based upon Olyarnik and Stachowicz (2012) finding negative impacts at 4.0 kg m<sup>-2</sup> of *Ulva* during one year of their study; we added a gradient of algal abundance below this value to characterize the overall shape of the relationship.

The second caging experiment evaluated the impact of the branching red alga, putatively *Gracilariopsis spp.* There were six treatments of macroalgae—0,

0.75, 1.0 1.5, 1.75, and 2.0 kg m<sup>-2</sup> wet weight (n=3). Additions of *Gracilariopsis* were determined from Huntington and Boyer (2008) who found negative effects at 1.7 kg m<sup>-2</sup> but not 0.325 kg m<sup>-2</sup>. Both the 0 kg m<sup>-2</sup> and UCC plots were used for both experiments.

Treatments were initiated by collecting the appropriate alga, weighing out the randomly assigned densities for each experimental unit with a hanging fish scale, and placing the algae within experimental plots. To prevent trapping fish within cages, a PVC pipe was moved back and forth throughout the plot prior to securing cages. The same procedure was replicated on UCC plots as well. This likely disturbed the epiphyte community, so we began measuring epiphytes in week 2. We used a shovel to sever rhizomes to a depth of ~30 cm around each plot to prevent movement of nutrients and photosynthate from outside the experimental area. Every two weeks (see below) we collected all algae from within each plot, measured its biomass, and added or subtracted macroalgae to re-establish initial treatment levels. The amount of macroalgae present in each plot after each two-week period estimated the persistence of macroalgae over time and treatment (Appendix S1.3). Overall, *Ulva* biomass remained constant at the treatment levels except between the last two weeks, while *Gracilariopsis* biomass was reduced between each interval.

### ***Field and Laboratory Methods***

The experiments ran for ten weeks from early July-mid September 2012; previous work demonstrated algal effects on intertidal mudflat communities within this timeframe (Green et al. 2014). We sampled all plots within both experiments

initially and five times over the 10-week duration approximately every 14-days at the spring low tides. Sampling occurred in a 0.25 m x 0.25 m (0.0625 m<sup>2</sup>) quadrat placed in a different predetermined location within each plot for each sampling event. We counted the number of seagrass shoots (see Hauxwell et al. 2001 for method) and normalized density to shoots per m<sup>2</sup>. We collected three shoots from each plot to quantify epiphyte load. Shoots were separated into individual blades and both sides were scraped with a microscope slide to remove epiphytes (method adapted from Short et al. 1995, Kendrick and Lavery 2001). Epiphytes from each blade were composited for each shoot and transferred to separate pre-weighed aluminum foil, dried at 60° C to a constant weight, and dry weighed. Epiphyte load per shoot was calculated as the average of the 3 shoots per plot. Epiphyte load per m<sup>2</sup> was calculated as the average epiphyte dry weight (g) on the three collected shoots multiplied by the total number of shoots m<sup>-2</sup> (epiphyte load = epiphyte biomass (g)/shoot \* #shoots m<sup>-2</sup>).

Two weeks prior to the end of the experiment at least four shoots per plot were marked to measure seagrass growth. Two holes were punched through the shoots within the sheath using a needle (method adapted from Duarte and Kirkman 2001). The first hole was punched approximately 5 cm from the sediment and the second directly above it to make them distinguishable from other damage or grazing scars. After two weeks, shoots were collected and growth measured as the distance from the initial mark on the outer sheath (which does not elongate) to the hole on each interior blade. The tissue between the hole in the sheath and in each blade is comprised of new tissue as seagrass grows from a basal meristem (see Short et al. 1995, Kendrick and Lavery 2001). Lengths of new blades with no holes

were also measured. The total length of new tissue from each blade was summed for a given shoot and averaged for all shoots from a plot for average total blade elongation (cm shoot<sup>-1</sup>) (see Duarte and Kirkman 2001).

### ***Threshold Analysis and Model Fitting***

We tested for a sudden threshold shift in response variables (shoot density, growth, epiphyte load per shoot, and epiphyte load per m<sup>2</sup> from the final week 10 measurement) in response to macroalgal abundance with two common approaches: (1) testing the fit of a sigmoid function and (2) conducting piecewise regression (Toms and Lesperance 2003, Samhuri et al. 2010). Figure 1.1 (a) shows the function:

$$R = \frac{C_1}{1 + \frac{C_2^{-t}}{S}} \quad (\text{Equation 1})$$

where  $R$  = the ecosystem response variable,  $S$  = the stressor on the system,  $C_1$  is the y-axis starting value, and  $t$  is varied to determine the steepness in the relationship between the ecosystem response and pressure at point  $C_2$ . As the value of  $t$  declines, the shape of the negative relationship between the pressure and ecosystem response switches from being a very abrupt threshold transition (e.g.  $t=50$ ) to a very smooth relationship (e.g.  $t=1$ ). We used the non-linear regression, `nls`, routine (R Core Team 2015) and `bbmle` package (Bolker 2008) in R to estimate values for parameters  $C_1$ ,  $C_2$ , and  $t$  for each of our seagrass response variables using maximum likelihood estimation (as in Samhuri et al. 2010). In cases where there was not support for a sharp threshold transition (e.g.  $t$  closer to or less than 1), the smooth sigmoid model was compared by Akaike Information Criterion, using the correction for small sample sizes (AICc), to two other predictable stress-



response models based on their ecological relevance to the possible effect of macroalgae on seagrass and their epiphytes: (1) steady negative decline (linear) and (2) seagrass decline occurs more quickly at low values of the stressor (exponential decay). If AICcs were similar ( $\Delta AICc < 3-4$ ; see criteria in Burnham et al. 2011), we report multiple models. Appendix S1.4 lists all models and comparisons; non-linear  $R^2$  values were estimated by squaring the correlation between predicted and actual response values.

As a second test for threshold behavior, which could accommodate a broader range of functional relationships, we conducted piecewise regression through the iterative search method in R (see method in Crawley 2007, R Core Team 2015). In this case, two linear regressions:

$$\begin{aligned} R &= b_1 + m_1 * S \text{ when } S \leq S_b, \text{ and} \\ R &= b_2 + m_2 * S \text{ when } S \geq S_b \end{aligned} \quad \text{(Equation 2)}$$

were conducted to describe the data before and after a break-point,  $S_b$  (Figure 1.1 b). The breakpoint that yields a model with the lowest residual mean standard error (MSE) was selected. We show any significant piecewise models, critically consider the nature of the transition at the breakpoint, and present the  $\Delta AICc$  compared to the models described above (see similar analysis in Sutula et al. 2014).

All analyses were conducted independently for the two (*Ulva* and *Gracilariaopsis*) experiments. Temporal responses of shoot density and epiphyte load to macroalgal abundance are presented in Appendix S1.5 and S1.6. UCC plots were not included in analyses, as they do not represent an experimental treatment but compared to 0 kg m<sup>-2</sup> plots to assess cage effects in Appendix S1.2.

## Results

### *Ulva* experiment

The data did not support the existence of a steep transition or threshold point as *Z. marina* shoot density declined with increasing *Ulva* abundance (Figure 1.2 a). The maximum likelihood estimate (MLE) of  $t$  for the sigmoid function was 1.55, resulting in a smooth curve (Fig 1.2 a, green) similar in shape to the exponential decay model (Fig 1.2 a, blue). The piecewise model (Figure 1.2 b), was significant ( $p < 0.001$ , adjusted  $R^2 = 0.5554$ ) with a breakpoint at  $S_b = 2$  but the model was not preferred by AICc comparison. The exponential, sigmoid, linear (Fig 1.2 a, red), and piecewise models had  $\Delta\text{AICc}$ s of 0, 2.4, 3.1, 7.2 respectively. Thus, based upon the criteria outlined in the methods, the exponential model was selected (Table 1.1; see Appendix S1.4 for full model results). Final shoot density in treatments  $> 2.0 \text{ kg m}^{-2}$  was at least 60% less than in the  $0 \text{ kg m}^{-2}$  treatment and compared to initial values ( $\bar{x} = 110 \pm 4.9 \text{ SEM shoots m}^{-2}$ ).

Epiphyte load was negatively impacted by *Ulva* abundance when considered at the per  $\text{m}^2$  scale, but did not exhibit a threshold pattern. The sigmoid curve was smooth with a MLE for  $t$  less than 1 (Fig 1.2 c, green). The exponential decay (Fig 1.2 c, blue) model is slightly preferred over the linear (Fig 1.2 c, red) and sigmoid (Fig 1.2 c, green) models by  $\Delta\text{AICc}$  (exponential=0, linear=2.4, and sigmoid=2.4). In addition, the adjusted  $R^2$  value was higher for the exponential model than linear (0.33 vs. 0.22). ML parameter estimates and adjusted  $R^2$  values for each of these models are included in Table 1.1. The piecewise model was significant, at the

breakpoint  $S_b=1$  ( $p=0.04$ , adjusted  $R^2=0.25$ , Figure 1.2 d) but was not preferred by AICc ( $\Delta AICc=5.6$  compared to the other 3 models). Compared to initial levels ( $\bar{x} = 12.2 \pm 1.2$  SEM  $g\ m^{-2}$ ) average epiphyte load ( $g\ m^{-2}$ ) decreased at least 3-fold in all treatments except for the  $0\ kg\ m^{-2}$  added (Figure 1.2 c).

There was no relationship between *Ulva* abundance and growth of *Z. marina* blades (Figure 1.3 a). Total blade elongation (cm) per shoot was highly variable with a range from 2 to 171  $cm\ shoot^{-1}$  ( $\bar{x} = 45.3 \pm 9.1$  SEM  $cm\ shoot^{-1}$ ). While there were no differences by treatment, blade elongation never exceeded 100  $cm\ shoot^{-1}$  in treatments  $>1.5\ kg\ m^{-2}$ . There was also no relationship between *Ulva* abundance and epiphyte load on individual shoots ( $g\ shoot^{-1}$ ) (Figure 1.3 b). Mean epiphyte load per shoot was initially  $0.11 \pm 0.01\ g$  and none of the treatments recovered to these levels. Non-significant results for each analysis as described in the methods are included in Appendix S1.4.

### ***Gracilariopsis* experiment**

There was a significant negative linear or slightly non-linear (exponential decay) relationship between *Gracilariopsis* abundance and final shoot density (Figure 1.4 a). The sigmoid curve was smooth with MLE of  $t=2.8$ , but this parameter was not significant ( $p=0.0796$ ). The piecewise model was significant (Figure 1.4b,  $p=0.03$ , adjusted  $R^2=0.38$ ) with the breakpoint  $S_b=1$  but was least preferred by  $\Delta AICc$ .  $\Delta AICc$  values were 0 for exponential decay, 1.1 for linear, 1.4 for sigmoid, and 7.9 for piecewise. Therefore, the exponential is slightly preferred but MLE and adjusted  $R^2$  for the top three models are presented in Table 1.2. Overall, for treatments  $>1.5\ kg\ m^{-2}$ , average number of shoots per  $m^2$  was lower than initial

values ( $\bar{x} = 90.4 \pm 9.0$  SEM shoots  $m^{-2}$ ) while the three lower treatments (0, 0.75, and 1.0 kg  $m^{-2}$ ) all experienced increases.

There were no clear relationships between *Gracilariopsis* abundance and final measurements of blade elongation (cm shoot $^{-1}$ ), epiphyte load per shoot, or epiphyte load per  $m^2$ . Growth measured as blade elongation (cm) per shoot over the final two weeks was highly variable (range from 6.7 to 149.6 cm shoot $^{-1}$ ), with no apparent trends with algal abundance (Figure 1.5 a). There were also no strong relationships between algal abundance and the two measures of epiphyte load (Figure 1.5 b, c), although there was a trend towards a negative linear ( $p=0.14$ ) relationship when epiphyte load (g) was considered at the per  $m^2$  scale. As in the *Ulva* experiment, none of the treatments recovered to the initial epiphyte load values of  $\bar{x} = 0.17 \pm 0.02$  SEM g shoot $^{-1}$  and  $\bar{x} = 13.9 \pm 2.0$  SEM g  $m^{-2}$ .

## **Discussion**

We documented a simple, predictable relationship rather than a sudden threshold shift between the biotic stress gradient produced by macroalgal blooms and the responses of a critical seagrass foundation species. Identifying the shape of the relationship between stressors and foundation species is especially important as foundation species are becoming increasingly subject to a suite of pressures (e.g. forested systems in Ellison et al. 2005, seagrass beds in Orth et al. 2006, coral reefs in Hughes et al. 2010). In contrast to the predictable linear or slightly non-linear relationship we observed, many foundation species have been found to exhibit strongly non-linear responses to stressors that are difficult to predict and

therefore make management options uncertain. Numerous examples of strongly non-linear shifts have been documented in terrestrial, aquatic, and marine systems worldwide and many argue this pattern may be the “new normal” in systems subject to human disturbance (see examples in Scheffer and Carpenter 2003, Folke et al. 2004). These highly non-linear shifts have also been predicted for seagrass beds (e.g. Viaroli et al. 2008). However, we found a predictable relationship that is more similar to responses of alpine plants and salt marsh/mangrove systems responded to warming temperatures. For example, Lesica and McCune (2004) found 4 of 7 alpine plants declined linearly in relation to increased temperatures over a decade. As winter temperatures increase in temperate latitudes, there is a concurrent predictable shift from dominance by salt marsh plants to invasion by mangroves (Saintilan et al. 2014). As in these examples, we found that health of *Z. marina* declined smoothly with the environmental stress of increased abundance of two functionally different macroalgal species. Thus, the relationship between the community of interest (seagrass and epiphytes) and the environmental stressor (macroalgae) was predictable and gradual rather than being a tipping point with a resultant “ecological surprise” (*sensu* King 1995, Lindenmayer et al. 2010); this is essential information for managers because they can identify early warning signs of decline.

We found that epiphytes on seagrass, which provide a key ecosystem function of trophic support, declined in the same simple predictable manner with the biotic stress of added macroalgae as the seagrass itself. By definition, a foundation species creates an entire community through its structural and functional characteristics (*sensu* Dayton 1972, reviewed by Ellison et al. 2005) and

degradation of the foundation species inevitably causes losses to species diversity and ecosystem functioning. As in our study, others found that degradation or replacement of foundation species caused cascading effects, including losses of higher trophic levels as their habitat, food source, or both disappeared (tropical rain forests, Turner 1996; kelp forests, Graham 2004; grasslands, Krauss et al. 2010; coral reefs, Kayal et al. 2012). In seagrass systems, some organisms feed directly on seagrass or benthic macroalgae, but many rely on epiphytes (Hughes et al. 2004). Many epifaunal invertebrates, for example, depend on epiphytes as a food source (Thayer et al. 1978) and their possible decline in abundance because of reduced epiphyte load may have cascading effects to juvenile fish that prey on epifauna (Marsh 1973). However, there are complex interactions between seagrass, macroalgae, epiphytes, and invertebrate abundance, which may depend on the taxa in question. Whalen et al. (2013), for example, found that epiphytes declined in the presence of macroalgae due to a positive benefit of macroalgal abundance to herbivorous caprellid amphipods and isopods. In other systems (tropical rain forests, kelp forests, grasslands, and coral reefs; see previous citations), there are clear and often linear declines in abundance and/or diversity of associated species in response to the loss of a foundation species. Most studies, however, evaluate the response of secondary or tertiary consumers, while we measure the direct losses to a primary producer in response to a biotic pressure. Negative effects to epiphytes in our system were strongest for *Ulva*, possibly due to greater light attenuation from the sheet-like morphology compared to the more open branching pattern of *Gracilariopsis*. *Ulva* also had strong negative effects on trophic support in intertidal mudflats, where abundance and richness of infaunal invertebrates declined at

abundances of  $<1.0 \text{ kg wet wt m}^{-2}$  (Green et al. 2014). To understand the full consequences of stressors to a foundation community, we must consider not only negative impacts to the foundation species itself, but to the associated species it supports. Thus, it is important to extend our approach in future work to assess the relationship between epiphyte loss and invertebrate and fish abundance to enable managers to establish targets for macroalgal endpoints that limit negative community impacts.

### ***Implications for management***

We hypothesize that, while biotic and abiotic context likely affects the negative relationship between macroalgae and seagrass communities, the changes will be to the rate of decline (slope) and background shoot density in the absence of macroalgae (intercept) rather than the overall predictable pattern. To test this hypothesis, our relatively simple experimental approach should be utilized in other locations; however, we found predictable negative effects to *Zostera marina* and its epiphytes at abundances of *Ulva* and *Gracilariopsis* that are found to occur naturally in seagrass beds around the world (see studies with similar species from East Coast USA, Hauxwell et al. 2001; Australia, Cummins et al. 2004; Portugal, Cardoso et al. 2004; Japan, Sugimoto et al. 2007; West Coast USA, Huntington and Boyer 2008, Olyarnik and Stachowicz 2012; Denmark, Rasmussen et al. 2012). As to abiotic context, our study was conducted near the mouth of Bodega Harbor in California. This is an expansive eelgrass bed under high flow and flushing conditions (Olyarnik and Stachowicz 2012); under this best-case scenario, we still identified negative effects of macroalgal loads. Unless nutrient input into these systems declines it is

likely that such blooms will continue to occur, propagating further seagrass declines with concurrent trophic disruptions. However, our study shows that the pattern of this degradation is predictable, not an ecological surprise or sudden transition. The discovery of a smooth and predictable  $x, y$  (stressor-response) relationship is critical information for resource managers because, rather than managing for unpredictable and catastrophic crashes, managers can monitor increases in macroalgal biomass as an indicator of future declines in seagrass health and initiate management action before negative effects become severe. In addition, it is likely that managers will be able to implement interventions in already degraded systems with predictable positive outcomes.

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## Tables

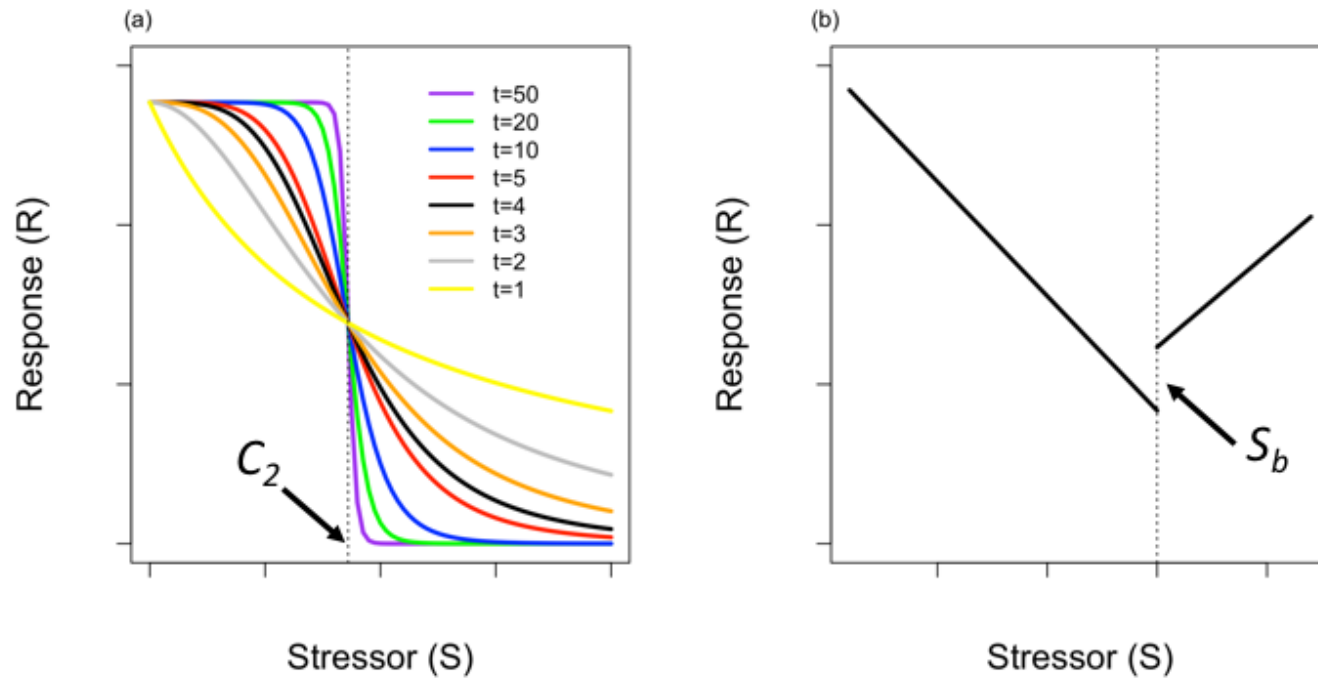
**Table 1.1.** Best fit models by AICc comparison and corresponding parameter values for each seagrass bed response to the stressor (S) of *Ulva* abundance. Only responses with significant models are presented here. Parameters marked in bold are significant at the 0.01 level. Adjusted R<sup>2</sup> values for non-linear models are estimated in R using the square of the correlation between actual and predicted shoot density values.

Response (R)	Form	Parameters	Adjusted R <sup>2</sup>
Shoot density n=25	Exponential decay $R = a * e^{bS}$	<b>a=138.74, b=-0.39</b>	0.5939
Epiphyte load (g/m <sup>2</sup> ) n=23	Exponential decay $R = a * e^{bS}$	<b>a=7.51, b=-0.62</b>	0.3330
	Linear $R = b + mS$	<b>b=6.2, m=-1.55</b>	0.2238
	Sigmoid $R = \frac{C_1}{1 + \frac{C_2}{S^{-t}}}$	<b>C<sub>1</sub>=7.75, C<sub>2</sub>=0.68, t=0.83</b>	0.3476

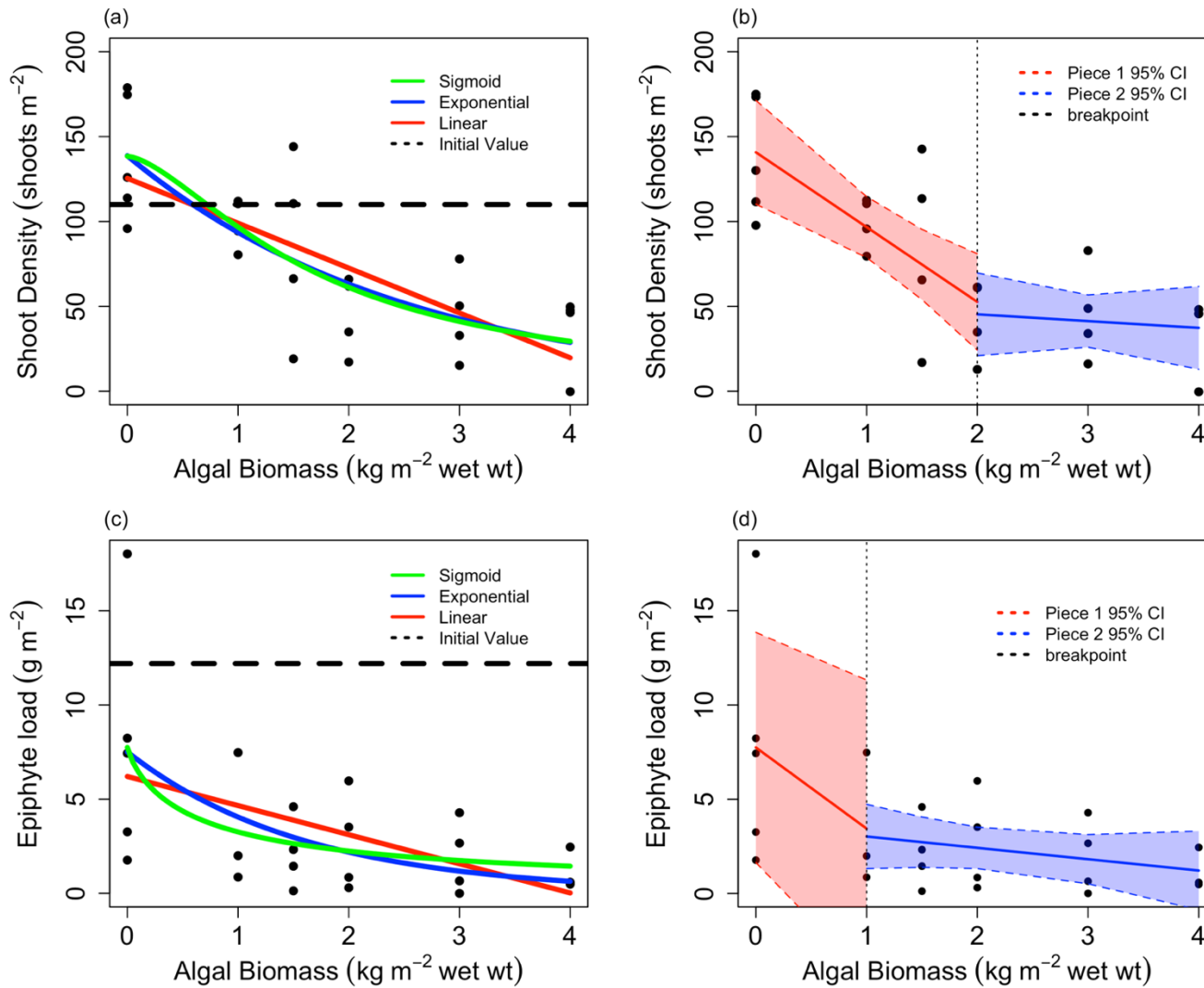
**Table 1.2.** Best fit models by AICc comparison and corresponding parameter values for seagrass bed response to the stressor (*S*) of *Gracilariopsis* abundance. Only responses with significant models are presented here. Parameters marked in bold are significant at the 0.01 level. Adjusted R<sup>2</sup> values for non-linear models are estimated in R using the square of the correlation between actual and predicted shoot density values.

Response (R)	Form	Parameters	Adjusted R <sup>2</sup>
Shoot density n=19	Exponential decay $R = a * e^{bS}$	<b>a=147.94, b=-0.37</b>	0.3924
	Linear $R = b + mS$	<b>b=148.51, m=-42.97</b>	0.3912
	Sigmoid $R = \frac{C_1}{1 + \frac{C_2}{S^{-t}}}$	<b>C<sub>1</sub>=141.5, C<sub>2</sub>=1.68,</b> $t=2.82$	0.4741

## Figures

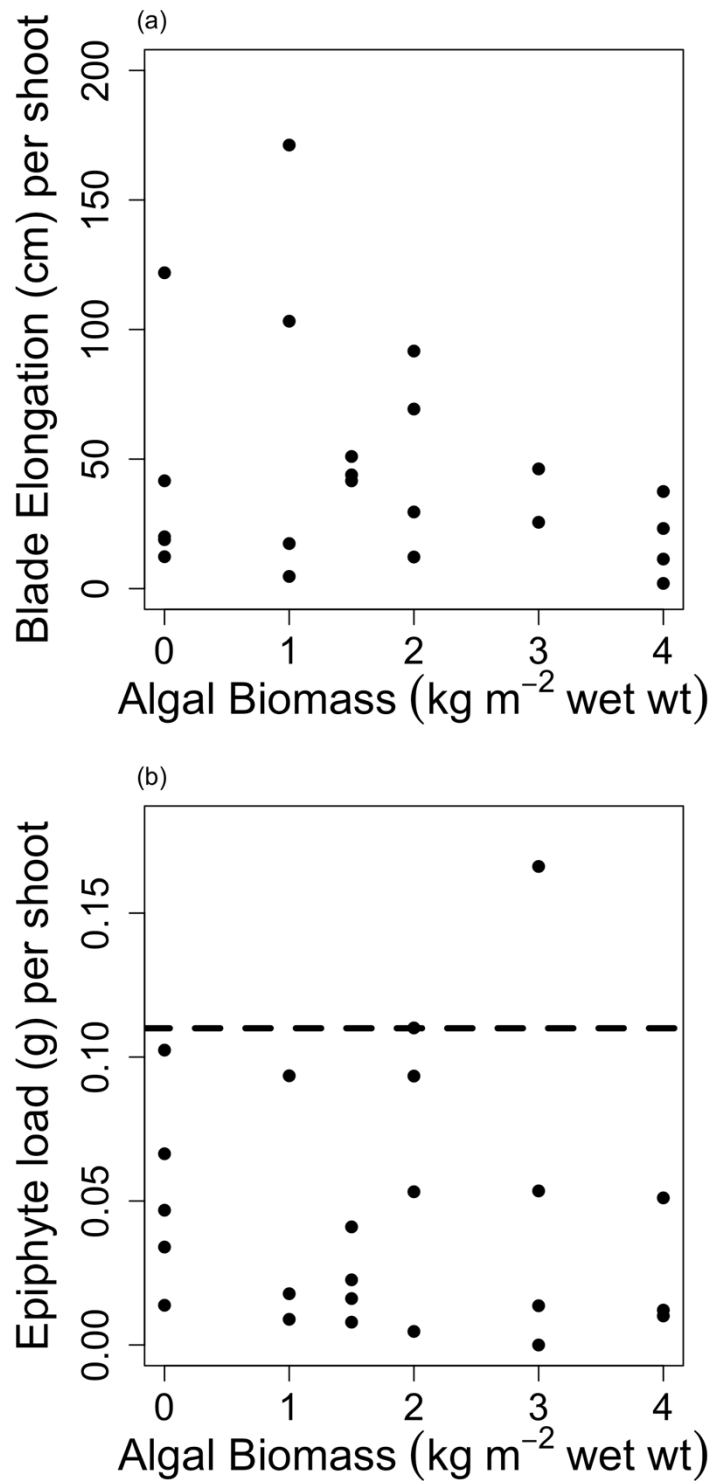


**Figure 1.1.** Examples of possible ecosystems response (R) to a stressor (S) following a threshold pattern either through (a) a sigmoid function (Eq. 1) or (b) piecewise regression (Eq. 2) model with breakpoint at  $S_b$ . The different colors of the lines in the sigmoid (a) example represent a drastic threshold response (purple,  $t=50$ ) at point  $C_2$  (dotted line) to smooth, predictable relationship (yellow,  $t=1$ ).



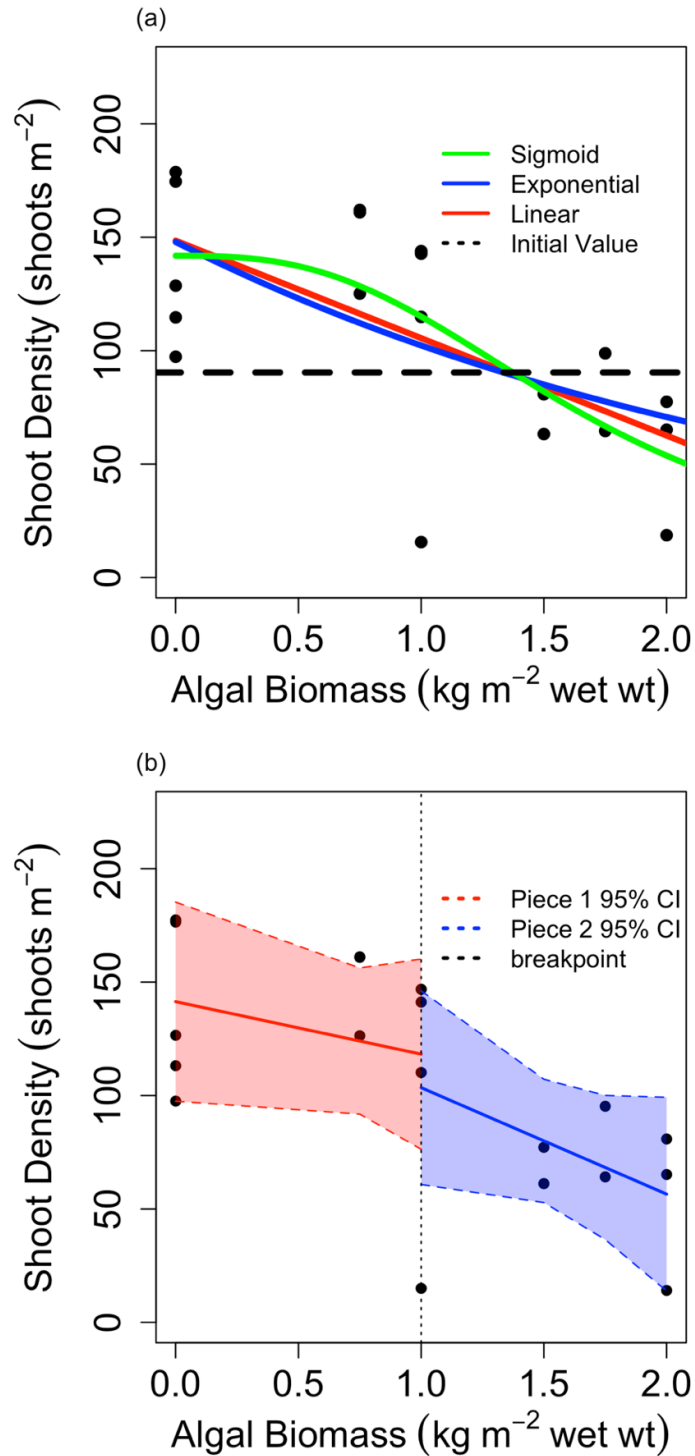
**Figure 1.2.** *Zostera marina* shoot density (n=25) and epiphyte load (g) per m<sup>2</sup> (n=23) responses to *Ulva* abundance (kg m<sup>-2</sup>). Linear, exponential and sigmoid models were fit to *Z. marina* shoot density (a) and epiphyte

load (c) ( $\text{g m}^{-2}$ ). Black dotted horizontal lines indicate initial values ( $n=25$ ). Piecewise regression with 95% confidence intervals for each linear piece (shaded areas) are also plotted for (b) shoot density with breakpoint  $S_b=2$  ( $p<0.001$ ,  $R^2=0.56$ ) and (d) epiphyte load ( $\text{g m}^{-2}$ ) with breakpoint  $S_b=1$  ( $p=0.04$ ,  $R^2=0.04$ ).



**Figure 1.3.** Scatter plots of responses with no significant relationship to *Ulva* abundance, (a) shoot growth over last two-week period (n=22) and (b)

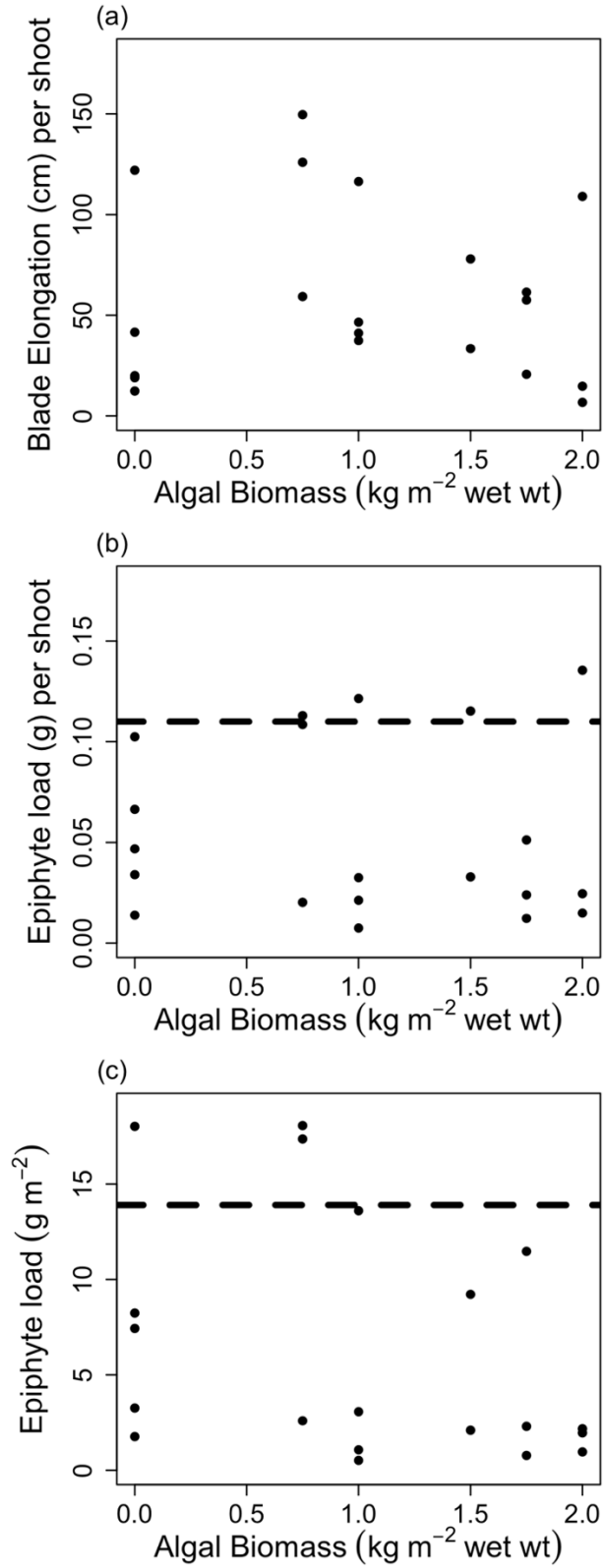
epiphyte load (g) per shoot (n=23). Black dotted horizontal lines indicate initial values (n=25). Note that there is no initial value for growth because this is a measurement over a two-week period.



**Figure 1.4.** Response of shoot density to *Gracilariopsis* abundance (kg m<sup>-2</sup>) comparing (a) linear, exponential and sigmoid models (n=19) and (b)



piecewise regression for shoot density with 95% confidence intervals at  
breakpoint  $S_b=1$  ( $p=0.03$ ,  $R^2=0.25$ ).



**Figure 1.5.** Scatter plots of responses with no significant relationship to *Gracilariopsis* abundance, (a) shoot growth over last two-week period

(n=19), (b) epiphyte load (g) per shoot (n=20), and (c) epiphyte load per meter (n=19). Black dotted horizontal lines indicate initial values (n=20). Note that there is no initial value for growth because this is a measurement over a two-week period.

## References

- Angelini, C., and B. R. Silliman. 2014. Secondary foundation species as drivers of trophic and functional diversity: Evidence from a tree-epiphyte system. *Ecology* 95:185–196.
- Baden, S., M. Gullström, B. Lundén, L. Pihl, and R. Rosenberg. 2003. Vanishing seagrass (*Zostera marina*, L.) in Swedish coastal waters. *Ambio* 32:374–377.
- Beck, M. W., K. L. Heck, K. W. Able, D. L. Childers, D. B. Eggleston, B. M. Gillanders, B. Halpern, C. G. Hays, K. Hoshino, T. J. Minello, R. J. Orth, P. F. Sheridan, and M. P. Weinstein. 2001. The Identification, Conservation, and Management of Estuarine and Marine Nurseries for Fish and Invertebrates. *BioScience* 51:633.
- Bolker, B. 2008. *bbmle: Tools for general maximum likelihood estimation*. R Development Core Team.
- Borum, J. 1985. Development of epiphytic communities on eelgrass (*Zostera marina*) along a nutrient gradient in a Danish estuary. *Marine Biology*.
- Borum, J., C. M. Duarte, D. Krause-Jensen, and T. M. Greve. 2004. European seagrasses : an introduction to monitoring and management. Page Management.
- Brush, M., and S. Nixon. 2002. Direct measurements of light attenuation by epiphytes on eelgrass *Zostera marina*. *Marine Ecology Progress Series* 238:73–79.
- Burkholder, J., D. Tomasko, and B. Touchette. 2007. Seagrasses and eutrophication. *Journal of Experimental Marine Biology and Ecology* 350:46–72.
- Cardoso, P. ., M. . Pardal, A. . Lillebø, S. . Ferreira, D. Raffaelli, and J. . Marques. 2004. Dynamic changes in seagrass assemblages under eutrophication and implications for recovery. *Journal of Experimental Marine Biology and Ecology* 302:233–248.
- Cardoso, P. G., S. Leston, T. F. Grilo, M. D. Bordalo, D. Crespo, D. Raffaelli, and M. A. Pardal. 2010. Implications of nutrient decline in the seagrass ecosystem success. *Marine Pollution Bulletin* 60:601–608.
- Carlton, J. T., G. J. Vermeij, D. R. Lindberg, D. A. Carlton, and E. C. Dudley. 1991. The first historical extinction of a marine invertebrate in an ocean basin: the demise of the eelgrass limpet *Lottia alveus*. *Biological Bulletin* 180:72–80.
- Carpenter, S. R., D. Ludwig, and W. A. Brock. 1999. Management of eutrophication for lakes subject to potentially irreversible change.

Ecological Applications 9:751–771.

- Crawley, M. J. 2007. Regression. Pages 387–448 *The R Book*. John Wiley & Sons, Ltd, Chichester, UK.
- Cummins, S. P., D. E. Roberts, and K. D. Zimmerman. 2004. Effects of the green macroalga *Enteromorpha intestinalis* on macrobenthic and seagrass assemblages in a shallow coastal estuary. *Marine Ecology Progress Series* 266:77–87.
- Curtin, C., and D. Western. 2008. Grasslands, people, and conservation: Over-the-horizon learning exchanges between African and American pastoralists. *Conservation Biology* 22:870–877.
- Davis, M. A., K. J. Wrage, and P. B. Reich. 1998. Competition between tree seedlings and herbaceous vegetation: support for a theory of resource supply and demand. *Journal of Ecology* 86:652–661.
- Dayton, P. K. 1972. Toward an understanding of community resilience and the potential effects of enrichments to the benthos at McMurdo Sound, Antarctica. Page Proceedings of the colloquium on conservation problems in Antarctica.
- Deegan, L. A., A. Wright, S. G. Ayvazian, J. T. Finn, H. Golden, R. R. Merson, and J. Harrison. 2002. Nitrogen loading alters seagrass ecosystem structure and support of higher trophic levels. *Aquatic Conservation: Marine and Freshwater Ecosystems* 12:193–212.
- Drake, L. A., F. C. Dobbs, and R. C. Zimmerman. 2003. Effects of epiphyte load on optical properties and photosynthetic potential of the seagrasses *Thalassia testudinum* Banks ex König and *Zostera marina* L. *Limnology and Oceanography* 48:456–463.
- Duarte, C. M. 2002. The future of seagrass meadows. *Environmental Conservation* 29:192–206.
- Duarte, C. M., and H. Kirkman. 2001. Methods for the measurement of seagrass abundance and depth distribution. *Global Seagrass Research Methods*:1–15.
- Ellison, A. M., M. S. Bank, B. D. Clinton, E. A. Colburn, K. Elliott, C. R. Ford, D. R. Foster, B. D. Kloeppel, J. D. Knoepp, G. M. Lovett, J. Mohan, D. A. Orwig, N. L. Rodenhouse, W. V Sobczak, K. A. Stinson, J. K. Stone, C. M. Swan, J. Thompson, B. Von Holle, and J. R. Webster. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment* 3:479–486.
- Folke, C., S. Carpenter, B. Walker, M. Scheffer, T. Elmqvist, L. Gunderson, and C. S. Holling. 2004. Regime Shifts, Resilience, in *Ecosystem Management*. *Annual Review of Ecology, Evolution, and Systematics*

35:557–581.

- Fong, P., and V. J. Paul. 2011. Coral Reef Algae. Page (Z. Dubinsky and N. Stambler, Eds.) *Coral Reefs: An Ecosystem in Transition*. Springer Netherlands, Dordrecht.
- Fong, P., J. B. Zedler, and R. M. Donohue. 1993. Nitrogen vs . phosphorus limitation of algal biomass in shallow coastal lagoons. *Limnology and Oceanography* 38:906–923.
- Frankovich, T. A., and J. W. Fourqurean. 1997. Seagrass epiphyte loads along a nutrient availability gradient, Florida Bay, USA. *Marine Ecology Progress Series* 159:37–50.
- Graham, H. M. 2004. Effects of local deforestation on the diversity and structure of Southern California giant kelp forest food webs. *Ecosystems* 7:341–357.
- Green, L., M. Sutula, and P. Fong. 2014. How much is too much? Identifying benchmarks of adverse effects of macroalgae on the macrofauna in intertidal flats. *Ecological Applications* 24:300–14.
- Häder, D., and F. Figueroa. 1997. Photoecophysiology of Marine Macroalgae. *Photochemistry and Photobiology* 66:1–14.
- Hauxwell, J., J. Cebrian, C. Furlong, and I. Valiela. 2001. Macroalgal Canopies Contribute to Eelgrass ( *Zostera marina* ) Decline in Temperate Estuarine Ecosystems. *Ecology* 82:1007–1022.
- Hauxwell, J., J. Cebrián, and I. Valiela. 2003. Eelgrass *Zostera marina* loss in temperate estuaries: relationship to land-derived nitrogen loads and effect of light limitation imposed by algae. *Marine Ecology Progress Series* 247:59–73.
- Heck, K. L., G. Hays, and R. J. Orth. 2003. Critical evaluation of the nursery role hypothesis for seagrass meadows. *Marine Ecology Progress Series* 253:123–136.
- van der Heide, T., E. H. van Nes, G. W. Geerling, A. J. P. Smolders, T. J. Bouma, and M. M. van Katwijk. 2007. Positive Feedbacks in Seagrass Ecosystems: Implications for Success in Conservation and Restoration. *Ecosystems* 10:1311–1322.
- Hessing-Lewis, M. L., S. D. Hacker, B. A. Menge, S. O. McConville, and J. Henderson. 2015. Are large macroalgal blooms necessarily bad? Nutrient impacts on seagrass in upwelling-influenced estuaries. *Ecological Applications* 25:1330–1347.
- Hessing-Lewis, M. L., S. D. Hacker, B. A. Menge, and S. S. Rumrill. 2011. Context-Dependent Eelgrass-Macroalgae Interactions Along an Estuarine Gradient in the Pacific Northwest, USA. *Estuaries and Coasts* 34:1169–

1181.

- Hughes, A. R., K. J. Bando, L. F. Rodriguez, and S. L. Williams. 2004. Relative effects of grazers and nutrients on seagrasses: A meta-analysis approach. *Marine Ecology Progress Series* 282:87–99.
- Hughes, T. 1994. Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. *Science* 265:1547–1551.
- Hughes, T. P., N. A. J. Graham, J. B. C. Jackson, P. J. Mumby, and R. S. Steneck. 2010. Rising to the challenge of sustaining coral reef resilience. *Trends in ecology & evolution* 25:633–42.
- Huntington, B., and K. Boyer. 2008. Effects of red macroalgal (*Gracilariopsis* sp.) abundance on eelgrass *Zostera marina* in Tomales Bay, California, USA. *Marine Ecology Progress Series* 367:133–142.
- Kamer, K., K. a. Boyle, and P. Fong. 2001. Macroalgal Bloom Dynamics in a Highly Eutrophic Southern California Estuary. *Estuaries and coasts* 24:623.
- Kayal, M., J. Vercelloni, T. Lison de Loma, P. Bosserelle, Y. Chancerelle, S. Geoffroy, C. Stievenart, F. Michonneau, L. Penin, S. Planes, and M. Adjerdou. 2012. Predator Crown-of-Thorns Starfish (*Acanthaster planci*) Outbreak, Mass Mortality of Corals, and Cascading Effects on Reef Fish and Benthic Communities. *PLoS ONE* 7:e47363.
- Kendrick, G. A., and P. S. Lavery. 2001. Assessing biomass, assemblage structure and productivity of algal epiphytes on seagrasses. *Global seagrass research methods*:199–222.
- King, A. 1995. Avoiding Ecological Surprise: Lessons From Long-Standing Communities. *Academy of Management Review* 20:961–985.
- Krauss, J., R. Bommarco, M. Guardiola, R. K. Heikkinen, A. Helm, M. Kuussaari, R. Lindborg, E. Ockinger, M. Partel, J. Pino, J. Poyry, K. M. Raatikainen, A. Sang, C. Stefanescu, T. Teder, M. Zobel, and I. Steffan-Dewenter. 2010. Habitat fragmentation causes immediate and time-delayed biodiversity loss at different trophic levels. *Ecology Letters* 13:597–605.
- Lesica, P., and B. McCune. 2004. Decline of arctic-alpine plants at the southern margin of their range following a decade of climatic warming. *Journal of Vegetation Science* 15:679–690.
- Lindenmayer, D. B., G. E. Likens, C. J. Krebs, and R. J. Hobbs. 2010. Improved probability of detection of ecological “surprises”. *Proceedings of the National Academy of Sciences of the United States of America* 107:21957–21962.
- Ludwig, D., B. Walker, and C. Holling. 1997. Sustainability, stability, and

resilience. Conservation ecology.

- Marsh, G. A. 1973. The *Zostera* Epifaunal Community in the York River , Virginia. Chesapeake Science 14:87–96.
- Mccook, L. J., and L. J. Mccook. 1999. Macroalgae, nutrients and phase shifts on coral reefs: scientific issues and management consequences for the Great Barrier Reef. Coral Reefs 367:357–367.
- McGlathery, K. 1995. Nutrient and grazing influences on a subtropical seagrass community. Marine Ecology Progress Series 122:239–252.
- Mcglathery, K. J. 2001. Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched coastal waters. Journal of Phycology 37:453–456.
- Mckee, K. L., and J. E. Rooth. 2008. Where temperate meets tropical: Multifactorial effects of elevated CO<sub>2</sub>, nitrogen enrichment, and competition on a mangrove-salt marsh community. Global Change Biology 14:971–984.
- Moore, K., H. Neckles, and R. Orth. 1996. *Zostera marina* (eelgrass) growth and survival along a gradient of nutrients and turbidity in the lower Chesapeake Bay. Marine Ecology Progress Series 142:247–259.
- Morley, S. A., and J. R. Karr. 2002. Assessing and Restoring the Health of Urban Streams in the Puget Sound Basin. Conservation Biology 16:1498–1509.
- Nedwell, D., T. Jickells, M. Trimmer, and R. Sander. 1999. Nutrients in estuaries. Advances in Ecological Research 29:43–92.
- Nielsen, S. L., K. Sand-Jensen, J. Borum, and O. Geertz-Hansen. 2002. Phytoplankton, nutrients, and transparency in Danish coastal waters. Estuaries 25:930–937.
- Odion, D. C., M. A. Moritz, and D. A. Dellasala. 2010. Alternative community states maintained by fire in the Klamath Mountains, USA. Journal of Ecology 98:96–105.
- Olyarnik, S., and J. Stachowicz. 2012. Multi-year study of the effects of *Ulva* sp. blooms on eelgrass *Zostera marina*. Marine Ecology Progress Series 468:107–117.
- Orth, R. J., T. J. B. Carruthers, W. C. Dennison, C. M. Duarte, J. W. Fourqurean, K. L. Heck Jr, A. R. Hughes, G. A. Kendrick, W. J. Kenworthy, S. Olyarnik, and others. 2006. A global crisis for seagrass ecosystems. Bioscience 56:987–996.
- Pedersen, M., and J. Borum. 1996. Nutrient control of algal growth in estuarine waters. Nutrient limitation and the importance of nitrogen



- requirements and nitrogen storage among phytoplankton and species of macroalgae. *Marine Ecology Progress Series* 142:261–272.
- Preen, A. R., W. J. Lee Long, and R. G. Coles. 1995. Flood and cyclone related loss, and partial recovery, of more than 1000 km<sup>2</sup> of seagrass in Hervey Bay, Queensland, Australia. *Aquatic Botany* 52:3–17.
- Prevéy, J. S., M. J. Germino, N. J. Huntly, and R. S. Inouye. 2009. Exotic plants increase and native plants decrease with loss of foundation species in sagebrush steppe. *Plant Ecology* 207:39–51.
- Pulido, C., and J. Borum. 2010. Eelgrass (*Zostera marina*) tolerance to anoxia. *Journal of Experimental Marine Biology and Ecology* 385:8–13.
- R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rasmussen, E. 1977. The wasting disease of eelgrass (*Zostera marina*) and its effects on environmental factors and fauna. Pages 1–51 in C. McRoy and C. Helfferich, editors. *Seagrass Ecosystems*. Marcel Dekker, New York.
- Rasmussen, J. R., B. Olesen, and D. Krause-Jensen. 2012. Effects of filamentous macroalgae mats on growth and survival of eelgrass, *Zostera marina*, seedlings. *Aquatic Botany* 99:41–48.
- Rohr, J. R., C. G. Mahan, and K. C. Kim. 2009. Response of arthropod biodiversity to foundation species declines: The case of the eastern hemlock. *Forest Ecology and Management* 258:1503–1510.
- Rosenberg, G., and J. Ramus. 1982. Ecological Growth Strategies in the Seaweeds *Gracilaria foliifera* (Rhodophyceae) and *Ulva* sp. (Chlorophyceae): Photosynthesis and Antenna Composition. *Marine ecology progress series*. Oldendorf 8:233–241.
- Saintilan, N., N. C. Wilson, K. Rogers, A. Rajkaran, and K. W. Krauss. 2014. Mangrove expansion and salt marsh decline at mangrove poleward limits. *Global change biology* 20:147–57.
- Samhuri, J. F., P. S. Levin, and C. H. Ainsworth. 2010. Identifying thresholds for ecosystem-based management. *PLoS ONE* 5:e8907.
- Sand-Jensen, K. 1977. Effect of epiphytes on eelgrass photosynthesis. *Aquatic Botany* 3:55–63.
- Sand-Jensen, K., and J. Borum. 1991. Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. *Aquatic Botany* 41:137–175.
- Scheffer, M., S. Carpenter, J. a Foley, C. Folke, and B. Walker. 2001. Catastrophic shifts in ecosystems. *Nature* 413:591–6.

- Scheffer, M., and S. R. Carpenter. 2003. Catastrophic regime shifts in ecosystems: linking theory to observation. *Trends in Ecology & Evolution* 18:648–656.
- Short, F. T., D. M. Burdick, and J. E. I. Kaldy. 1995. Mesocosm experiments quantify the effects of eutrophication on eelgrass, *Zostera marina*. *Limnology and Oceanography* 40:740–749.
- Steneck, R., and J. M. Erlandson. 2002. Kelp Forest Ecosystems : Biodiversity , Stability , Resilience and Future. *Environmental Conservation* 29:439–459.
- Suding, K. N., and R. J. Hobbs. 2009. Threshold models in restoration and conservation: a developing framework. *Trends in Ecology & Evolution* 24:271–279.
- Sugimoto, K., K. Hiraoka, S. Ohta, Y. Niimura, T. Terawaki, and M. Okada. 2007. Effects of ulvoid (*Ulva* spp.) accumulation on the structure and function of eelgrass (*Zostera marina* L.) bed. *Marine pollution bulletin* 54:1582–5.
- Sutula, M., L. Green, G. Cicchetti, N. Detenbeck, and P. Fong. 2014. Thresholds of Adverse Effects of Macroalgal Abundance and Sediment Organic Matter on Benthic Habitat Quality in Estuarine Intertidal Flats. *Estuaries and Coasts* 37:1532–1548.
- Thayer, G., P. Parker, M. LaCroix, and B. Fry. 1978. The Stable Carbon Isotope Ratio of Some Components of an Eelgrass, *Zostera marina*, Bed. *Oecologia* 34:1–12.
- Toms, J. D., and M. L. Lesperance. 2003. Piecewise Regression: A tool for identifying ecological thresholds. *Ecology* 84:2034–2041.
- Turner, I. M. 2008. Species loss in fragments of tropical rain forest : a review of the evidence. *Journal of Applied Ecology* 33:200–209.
- Valiela, I., J. McClelland, J. Hauxwell, P. J. Behr, D. Hersh, and K. Foreman. 1997. Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography* 42:1105–1118.
- Viaroli, P., M. Bartoli, G. Giordani, M. Naldi, S. Orfanidis, and J. M. Zaldivar. 2008. Community shifts, alternative stable states, biogeochemical controls and feedbacks in eutrophic coastal lagoons: a brief overview. *Aquatic Conservation: Marine and Freshwater Ecosystems* 18:S105–S117.
- Waycott, M., C. M. Duarte, T. J. B. Carruthers, R. J. Orth, W. C. Dennison, S. Olyarnik, A. Calladine, J. W. Fourqurean, K. L. Heck, A. R. Hughes, G. A. Kendrick, W. J. Kenworthy, F. T. Short, and S. L. Williams. 2009. Accelerating loss of seagrasses across the globe threatens coastal

ecosystems. *Proceedings of the National Academy of Sciences of the United States of America* 106:12377–81.

Whalen, M. A., J. E. Duffy, and J. B. Grace. 2013. Temporal shifts in top-down vs. bottom-up control of epiphytic algae in a seagrass ecosystem. *Ecology* 94:510–520.

## **SUPPORTING INFORMATION**

**Appendix S1.1:** Summary of macroalgal impacts on seagrasses in previous studies

Dominant bloom forming macroalgae are usually either sheet-like or filamentous green algae (Chlorophyta) such as *Ulva* and *Cladophora* (Valiela et al. 1997; McGlathery 2001) or coarsely branching red algae (Rhodophyta) from the family Gracilariaceae (Hauxwell et al. 2001; Huntington and Boyer 2008). See Table S1.1 for some comparable studies adding macroalgae to seagrass.

## **Tables.**

**Table S1.1.** Previous work investigating impacts of macroalgae on seagrasses. This list is not exhaustive but represents some of the work done previously that is relevant to our study. The table summarizes the: (a) year and first authors of the study, (b) location the study, (c) seagrass species examined, (d) algal species added, (e) number of algal addition treatments (controls, ambient or mimics not included), (f) algal abundance at which negative effects were observed, (g) seagrass measures for which negative effects occurred, and (h) a comparison of whether the values indicated as having a negative impact were lower, higher, or approximately equal to ours.

(a) Study	(b) Location	(c) Seagrass species	(d) Algal species	(e) # of algal addition treatments	(f) Negative abundance	(g) Seagrass measures	(h) Comparison
Cummins et al. 2004	Tuggerah Bay, New South Wales, Australia	Multi-species	<i>Ulva intestinalis</i> (formerly <i>Enteromorpha</i> )	1	4.5 kg m <sup>-2</sup>	-Macrofauna assemblage -seagrass biomass	>
Hauxwell et al. 2001	Waquoit Bay, Massachusetts, USA	<i>Zostera marina</i>	Mix of <i>Cladophora</i> and <i>Gracilaria</i>	2-3 depending on site	9-12 cm	-shoot density -recruitment -growth	>
Hessing-Lewis et al. 2014	Newport, Oregon, USA	<i>Zostera marina</i>	<i>Ulva sp.</i>	1 addition for mesocosm*	6000-8000 mL**	-shoot density -shoot biomass - morphology	>
Holmer and Nielsen 2007	Fyn, Denmark	<i>Zostera marina</i>	<i>Chaetomorpha</i>	2***	by 5cm	-sulfide content -Growth rate -Below ground biomass	≥
Huntington and Boyer 2008	Tomales Bay, California, USA	<i>Zostera marina</i>	<i>Gracilariopsis sp.</i>	3	1.7 kg m <sup>-2</sup>	-shoot density -growth	≈
Irlandi et al. 2004	Biscayne Bay, Florida, USA	<i>Thalassia testudinum</i>	Drift algae, unspecified	1	500 g / 0.25 m <sup>2</sup> or 2.0 kg m <sup>-2</sup>	-above ground biomass	≥
Olyarnik and Stachowicz 2012	Bodega Bay, California, USA	<i>Zostera marina</i>	<i>Ulva sp.</i>	1 (also an ambient)	>4.0 kg m <sup>-2</sup>	Shoot biomass	>
Rasmussen et al. 2012	Aarhus Bay, Denmark	<i>Zostera marina</i>	<i>Chaetomorpha</i>	3***	9 cm	Seedling growth and mortality	>
Sugimoto 2007	Seto Inland Sea, Japan	<i>Zostera marina</i>	<i>Ulva sp.</i>	2***	13-25 cm	-seagrass survival -number of seedlings	>

-growth  
-carbon content

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- \*This study had an *in situ* and mesocosm experiment but only found negative results in the mesocosm
- \*\*The researchers list this value as converting to 0.4-0.5 kg dry weight/0.25 m<sup>2</sup> or 1.6-2.0 kg m<sup>-2</sup> dry weight. Which is a much higher abundance than our 1.5 kg m<sup>-2</sup> wet weight.
- \*\*\*These studies only conducted mesocosm/aquaria experiments

## References

- Cummins, S. P., Roberts, D. E., & Zimmerman, K. D. (2004). Effects of the green macroalga *Enteromorpha intestinalis* on macrobenthic and seagrass assemblages in a shallow coastal estuary. *Marine Ecology Progress Series*, 266, 77-87.
- Hauxwell, J., Cebrián, J., Furlong, C., & Valiela, I. (2001). Macroalgal canopies contribute to eelgrass (*Zostera marina*) decline in temperate estuarine ecosystems. *Ecology*, 82(4), 1007-1022.
- Hauxwell, J., Cebrián, J., & Valiela, I. (2003). Eelgrass *Zostera marina* loss in temperate estuaries: relationship to land-derived nitrogen loads and effect of light limitation imposed by algae. *Marine Ecology Progress Series*, 247(1), 59-73.
- Hessing-Lewis, M. L., Hacker, S. D., Menge, B. A., McConville, S. O., & Henderson, J. (2015). Are large macroalgal blooms necessarily bad? nutrient impacts on seagrass in upwelling-influenced estuaries. *Ecological Applications*, 25(5), 1330-1347.
- Holmer, M., & Nielsen, R. M. (2007). Effects of filamentous algal mats on sulfide invasion in eelgrass (*Zostera marina*). *Journal of Experimental Marine Biology and Ecology*, 353(2), 245-252.
- Huntington, B. E., & Boyer, K. E. (2008). Effects of red macroalgal (*Gracilariopsis* sp.) abundance on eelgrass *Zostera marina* in Tomales Bay, California, USA. *Marine Ecology Progress Series*, 367, 133-142.
- Irlandi, E. A., Orlando, B. A., & Biber, P. D. (2004). Drift algae-epiphyte-seagrass interactions in a subtropical *Thalassia testudinum* meadow. *Marine Ecology Progress Series*, 279(9), 81-91.
- McGlathery, K. J. (2001). Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched coastal waters. *Journal of Phycology*, 37(4), 453-456.
- Olyarnik, S. V., & Stachowicz, J. J. (2012). Multi-year study of the effects of *Ulva* sp. blooms on eelgrass *Zostera marina*. *Marine Ecology Progress Series*, 468, 107.
- Rasmussen, J. R., Olesen, B., & Krause-Jensen, D. (2012). Effects of filamentous macroalgae mats on growth and survival of eelgrass, *Zostera marina*, seedlings. *Aquatic botany*, 99, 41-48.
- Sugimoto, K., Hiraoka, K., Ohta, S., Niimura, Y., Terawaki, T., & Okada, M. (2007). Effects of ulvoid (*Ulva* spp.) accumulation on the structure and



function of eelgrass (*Zostera marina* L.) bed. *Marine pollution bulletin*, 54(10), 1582-1585.

Valiela, I., McClelland, J., Hauxwell, J., Behr, P.J., Hersh, D. and K. Foreman. 1997. Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography* 42 (5): 1105-1118.

## Appendix S1.2: Analysis of caging effects

**Methods.** Uncaged control (UCC) plots were set up randomly along with the experimental treatments to assess the impact adding a cage may have to seagrass shoot density, growth, or epiphyte load. Final (week 10) means were compared by Welch two-sample t-test to the 0 kg m<sup>-2</sup> treatment as UCC plots generally had low or no algae rafting in. Since UCC plots and 0 kg m<sup>-2</sup> plots were used for both experiments, these results do not differ between the *Ulva* and *Gracilariopsis* experiments. A PVC rod was run through each plot to avoid trapping juvenile fish in cages, and the same was done to UCC plots for consistency.

### Results.

*Shoot density*—For seagrass shoot density, week 10 densities were not significantly different for the UCC plots than for the 0 kg m<sup>-2</sup> plots (Welch two-sample  $t(5.1) = -0.7$ ,  $p = 0.53$ ). Final shoot densities for UCC plots ( $n=4$ ,  $\bar{x} = 116 \pm 27.2$  SEM shoots m<sup>-2</sup>) and the 0 kg m<sup>-2</sup> ( $n=5$ ,  $\bar{x} = 138 \pm 16.5$  SEM shoots m<sup>-2</sup>) treatment were higher than initial values ( $n=29$ ,  $\bar{x} = 104 \pm 6.1$  SEM shoots m<sup>-2</sup>).

*Growth*— Variances for blade elongation over the last time period (week 8 to 10) were high in 0 kg m<sup>-2</sup> ( $n=5$ ,  $\bar{x} = 42.9 \pm 20.3$  SEM cm shoot<sup>-1</sup>) and UCC plots ( $n=4$ ,  $\bar{x} = 17.2 \pm 8.3$  SEM cm shoot<sup>-1</sup>), but means were not significantly different (Welch two-sample  $t(5.2) = -1.2$ ,  $p = 0.29$ ).

*Epiphyte load*—For UCC plots ( $n=3$ ,  $\bar{x} = 18.0 \pm 2.1$  SEM  $\text{g m}^{-2}$ ), final epiphyte load (g) per  $\text{m}^2$  was significantly higher than 0  $\text{kg m}^{-2}$  plots ( $n=5$ ,  $\bar{x} = 7.7 \pm 2.8$  SEM  $\text{g m}^{-2}$ ), by a Welch two-sample t-test ( $t(6) = 2.9$ ,  $p=0.027$ ).

Similarly, final epiphyte load at the per shoot level was significantly higher in UCC plots ( $n=3$ ,  $\bar{x} = 0.14 \pm 0.01$  SEM  $\text{g shoot}^{-1}$ ) than 0  $\text{kg m}^{-2}$  ( $n=5$ ,  $\bar{x} = 0.05 \pm 0.02$  SEM  $\text{g shoot}^{-1}$ ), by a Welch two-sample t-test ( $t(5.3) = 4.2$ ,  $p < 0.01$ ).

**Discussion.** Our results showed that cages did not have any effects on seagrass shoot density or growth but did negatively impact epiphyte load. Despite cage effects, all caged treatments were impacted in the same way and there was still an overall negative impact of macroalgae to epiphyte load. Epiphytes may be more susceptible to caging effects than *Zostera marina*. This is consistent with other studies that found epiphytes were sensitive to light attenuation (Cardoso et al. 2004). However, another explanation could be a trophic cascade where herbivorous invertebrates received a refuge from consumers, such as fish. Thus, epiphytes would be subjected to higher herbivory pressure inside caging where invertebrates are provided a refuge from consumption. These sorts of trophic cascades were observed in Hughes et al. (2013) where a quaternary trophic cascade existed in seagrass beds where presence of otters resulted in decreased crab abundance, increased invertebrate abundance, and decreased epiphyte loading on *Z. marina*. The true reason for caging effects on epiphyte loading needs to be investigated further but regardless, our main result showed a

negative impact of macroalgae, at least for *Ulva*. The impact of top-down (herbivores) and bottom-up (nutrients) processes on epiphytes is important to consider not only because epiphytes can cause negative effects to *Z. marina* by shading, but they are also an important food source in seagrass community trophic structure.

## References

- Cardoso, P. G., Pardal, M. A., Lillebø, A. I., Ferreira, S. M., Raffaelli, D., & Marques, J. C. (2004). Dynamic changes in seagrass assemblages under eutrophication and implications for recovery. *Journal of Experimental Marine Biology and Ecology*, 302(2), 233-248.
- Hughes, B. B., Eby, R., Van Dyke, E., Tinker, M. T., Marks, C. I., Johnson, K. S., & Wasson, K. (2013). Recovery of a top predator mediates negative eutrophic effects on seagrass. *Proceedings of the National Academy of Sciences*, 110(38), 15313-15318.

### **Appendix S1.3:** Macroalgal abundance and persistence over time.

**Methods.** Every two weeks we collected all algae from within each plot, measured its biomass, and added or subtracted biomass to re-establish initial treatment levels. Macroalgal biomass after 2 weeks was analyzed by a two-factor (treatment X time) ANOVA after meeting assumptions over four time points (week 2, 4, 6, 8). Week 0 is not included in the analysis because the initials started at our randomly assigned treatment value. We also did not consider week 10 because algal treatments were not reset at the end of the experiment. An ANOVA is used for this analysis as opposed to a repeated measures ANOVA as we reset algal values during each sampling period and so measurements are independent.

**Results.** Overall, biomass of *Ulva* remained relatively close to the initial treatment levels across all treatments during the 2-week interval between sampling (Figure S1.1), with the exception of week 8. There was a significant difference between the treatments, with each treatment level approximately reflecting the macroalgal dose that was applied 2 weeks prior (Table S1.2). There was also a significant difference in mean algal biomass due to time (week, Table S1.2), with no interaction. This was associated with the decrease in biomass between week 6 and 8, when there was consistently some loss or senescence in all treatments. This may reflect natural seasonal decreases in growth and increases in decomposition at the onset of Fall.

Overall, *Gracilariopsis* declined in abundance from initial treatment levels across all experimental units and all time intervals (Figure S1.2). *Gracilariopsis* abundance after 2 weeks significantly depended on the initial treatment level, but was always much lower than the respective treatment after two weeks (Table S1.3). For example, biomass of *Gracilariopsis* declined from initial levels of 0.75 to a maximum average of 0.50 kg/m<sup>2</sup> in one treatment while treatments that were initially 2.00 kg/m<sup>2</sup> never weighed more than an average of 1.25 kg/m<sup>2</sup> after 2 weeks. Moreover, this decline increased over time, with final macroalgal weights quite low (<0.50 kg m<sup>2</sup>) across all treatments by week 8 (Table S1.3). There was never any *Gracilariopsis* biomass found in uncaged controls or 0 kg/m<sup>2</sup>, demonstrating that, unlike *Ulva*, *Gracilariopsis* did not grow significantly *in situ* at this site. By contrast, there was some *Ulva* biomass in UCC and 0 kg m<sup>-2</sup> plots after two weeks (see Figure S1.1). Additionally, Figure S2 shows dry weights of algae collected from 0 kg m<sup>-2</sup>, *Ulva*, and *Gracilariopsis* plots starting in week 2. These values are an order of 10 lower than wet weights measured in the field (e.g. 4.0 kg m<sup>-2</sup> wet weight is approximately 0.4 kg m<sup>-2</sup> dry weight), although values are highly variable.

**Discussion.** *Ulva* treatments were more persistent throughout time during this experiment than *Gracilariopsis* and this factor may contribute to the clearer negative effect for *Z. marina* and its epiphytes in the *Ulva* experiment in main results and Appendix 1.4 and 1.5. Whether the lower persistence of *Gracilariopsis* during this experiment is a natural occurrence or an artifact of

the experimental design (i.e. caging less successful) is unknown and should be investigated.

**Tables.**

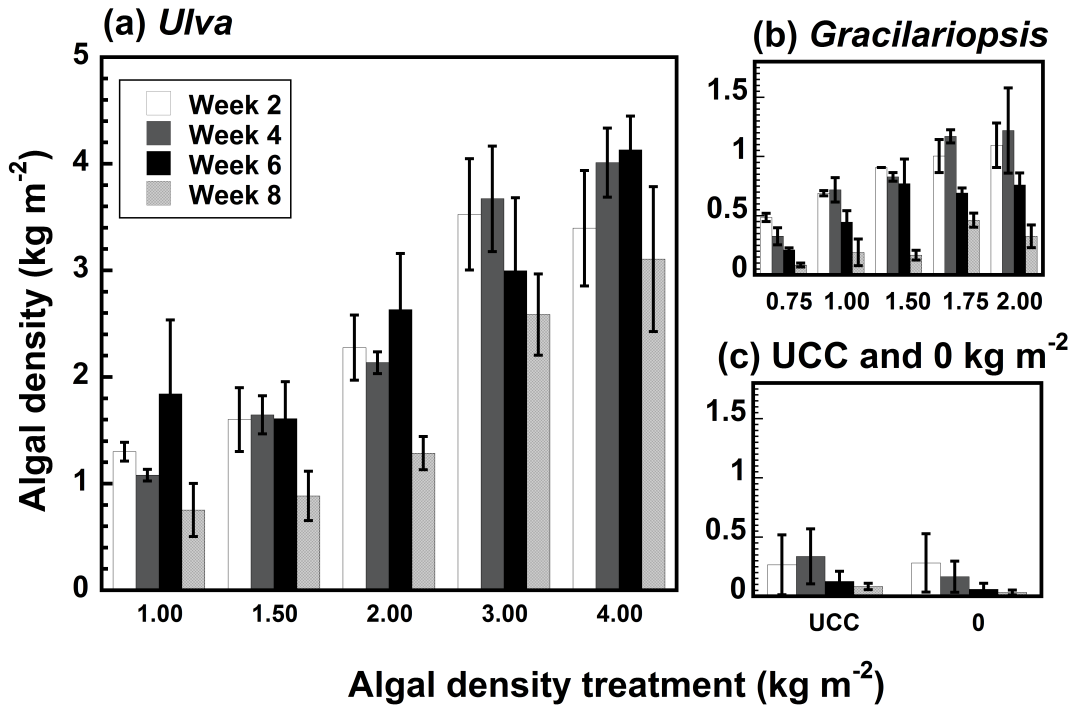
**Table S1.2:** Two factor ANOVA on *Ulva* wet weight by treatment and week

Factor	DF	F ratio	Prob>F
Treatment	6	69.8295	<0.0001*
Week	1	8.8057	0.0038*
Treatment*week	6	0.5602	0.7610

**Table S1.3:** Two factor ANOVA on *Gracilariopsis* abundance by treatment and week.

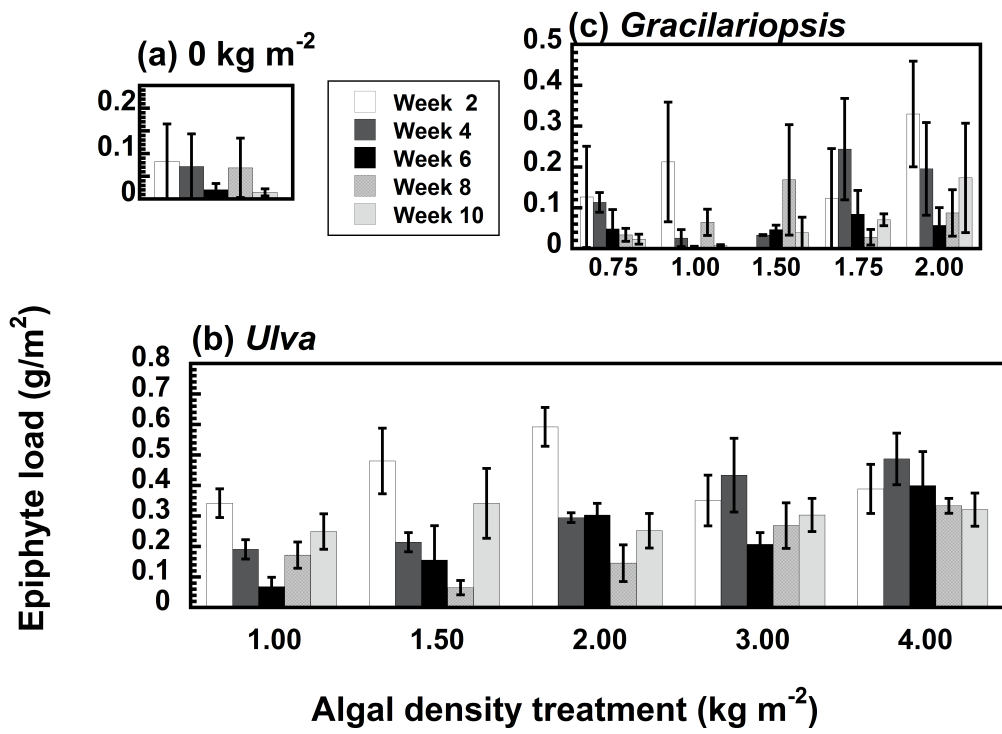
Factor	DF	F ratio	Prob>F
Treatment	4	13.4231	<0.0001*
Week	1	57.5926	<0.0001*
Week*treatment	4	0.8409	0.5059

**Figures.**



**Figure S1.1:** Persistence of (a) *Ulva* and (b) *Gracilariopsis* and (c) regrowth of *Ulva* in UCC and 0 kg m<sup>-2</sup> plots by week. Note: there was no or an immeasurable amount of *Gracilariopsis* in UCC and 0 kg m<sup>-2</sup> plots.





**Figure S1.2:** Dry weight of algae for (a) 0 kg m<sup>-2</sup>, (b) *Ulva* experiment, and (c) *Gracilariopsis* experiment

**Appendix S1.4:** Least squares model fitting and piecewise regression between macroalgal abundance and all seagrass responses. This includes data sets with no significant relationships.

**Table S1.4.** Comparison of linear, exponential, and quadratic least squares regression models and piecewise regression for each measurement. Selected models are in bold.

Response (R)	Algal species (S)	Form	Equation	Adjusted R <sup>2</sup>	P-value	ΔAICc		
Shoot density	<i>Ulva</i>	Linear	$R = 125.45 - 26.44S$	0.5207	<0.0001	3.1		
		<b>Exponential decay</b>	<b><math>R = 138.74e^{-0.39S}</math></b>	<b>0.5939</b>	<b>&lt;0.0001</b>	<b>0.0</b>		
		Sigmoid	$R=138.5/[1+(1.72/S)^{-1.55}]$	0.6009	0.001	2.4		
		Piecewise	$R= 137.28 - 36.06S$ when $S \leq 2$ , $R= 68 - 8S$ when $S \geq 2$	0.5554	<0.001	7.2		
	<i>Gracilariopsis</i>	<b>Linear</b>	<b><math>R = 148.51 - 42.97S</math></b>	<b>0.3912</b>	<b>0.0025</b>	<b>0.0</b>		
		<b>Exponential decay</b>	<b><math>R = 147.94e^{-0.37S}</math></b>	<b>0.3914</b>	<b>&lt;0.01</b>	<b>1.1</b>		
		Sigmoid	$R=141.8/[1+(1.68/S)^{-2.82}]$	0.4741	0.0796	1.6		
		Piecewise	$R= 137.6 + 15.65S$ when $S \leq 1$ , $R= 140.2 - 44.4S$ when $S \geq 1$	0.3843	0.0268	7.9		
		Growth	<i>Ulva</i>	Linear	$R = 59.04 - 7.86S$	0.0205	0.2441	0.0
				Exponential decay	$R = 57.55e^{-0.15S}$	0.0556	0.3723	0.3
Sigmoid	$R=54.5/[1+(3.46/S)^{-4.54}]$			0.1075	0.5655	2.0		
Piecewise	$R= 42.9 -12.5S$ when $S \leq 1$ , $R= 70.6 -12.5S$ when $S \geq 1$			0.0253	0.3446	4.0		

Epiphyte load (g/shoot)	<i>Gracilariopsis</i>	Linear	$R = 62.27 - 3.56S$	-0.0517	0.8009	0.0
		Exponential decay	$R = 61.62e^{-0.049S}$	0.0030	0.8370	0.0
		Sigmoid	$R=65.8/[1+(2.19/S)^{-5.05}]$	0.0465	0.7305	2.3
		Piecewise	$R= 42.9 + 91.6S$ when $S \leq 1$ , $R= 89.3 - 23.4S$ when $S \geq 1$	0.1112	0.2271	3
	<i>Ulva</i>	Linear	$R = 0.0491 - 0.0022S$	-0.0428	0.7585	0.0
		Exponential decay	$R = 0.049e^{-0.046S}$	0.0044	0.7719	0.0
		Sigmoid	NF*	--	--	--
		Piecewise	$R= 0.054 - 0.019S$ when $S \leq 2$ , $R= 0.16 - 0.034S$ when $S \geq 2$	-0.0404	0.5489	6.9
<i>Gracilariopsis</i>	Linear	$R = 0.0585 - 0.0035S$	-0.0517	0.8012	0.0	
	Exponential decay	$R = 0.058e^{-0.061S}$	0.0585	0.8116	0.0	
	Sigmoid	$R=0.06/[1+(3.49/S)^{-2.73}]$	0.0879	0.8850	3.1	
	Piecewise	$R= 0.053 + 0.003S$ when $S \leq 0.75$ , $R= 0.045 + 0.003S$ when $S \geq 0.75$	-0.1098	0.7734	5.5	

Epiphyte load (g/m <sup>2</sup> )	<i>Ulva</i>	Linear	$R = 6.20 - 1.55S$	0.2238	0.0131	2.4
		<b>Exponential decay</b>	<b><math>R = 7.51e^{-0.62S}</math></b>	<b>0.3330</b>	<b>0.0156</b>	<b>0.0</b>
		Sigmoid	$R=7.75/[1+(0.68/S)^{-0.83}]$	0.3476	0.481	2.4
		Piecewise	$R= 7.2 - 0.45S$ when $S \leq 1$ , $R= 2.6 - 0.45S$ when $S \geq 1$	0.2475	0.0386	5.6
	<i>Gracilariopsis</i>	Linear	$R = 9.41 - 3.43S$	0.0669	0.1417	0.0
		Exponential decay	$R = 9.01e^{-0.38S}$	0.0997	0.2248	0.4
		Sigmoid	$R=8.73/[1+(1.59/S)^{-3.57}]$	0.1497	0.4625	2.4
		Piecewise	$R=7.7 + 6.6S$ when $S \leq 1$ , $R=18.6 - 8.3S$ when $S \geq 1$	0.0994	0.2454	6.6

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**Appendix S1.5:** Repeated measured analyses of shoot density over time.

**Methods.** Response of shoot density (shoots  $\text{m}^{-2}$ ) to macroalgal abundance was analyzed by repeated measures ANOVA over the biweekly time points. Shoot density was analyzed over all six-time points (weeks 0, 2, 4, 6, 8, and 10). Growth was not analyzed with repeated measures due to low samples size in early weeks. Mauchley sphericity tests were used to validate use of repeated measures and Greenhouse-Geisser corrections are included if sphericity was violated.

**Results.** Both time and *Ulva* treatment had a significant impact on seagrass shoot density (Table S1.5). For treatments with  $\leq 1.5 \text{ kg m}^{-2}$  of added *Ulva*, overall shoot densities either increased or remained the same from weeks 0-10, except for a temporary decrease across all treatments in week 2 (Figure S1.3). By the end of the experiment, shoot density in these treatments exceeded  $100 \text{ shoots m}^{-2}$ , approximating initial conditions. In the  $2.0 \text{ kg m}^{-2}$  treatment, there was increased shoot density from week 2-8, but then a sharp decline at week 10. In the two highest macroalgal density treatments (additions of  $3.0$  and  $4.0 \text{ kg m}^{-2}$  *Ulva*), while there was some variability over time, shoot density declined sharply by the final sampling date ending with  $\sim 50 \text{ shoots m}^{-2}$ , which is about half of initial densities.

Treatment and time also had a significant effect on shoot density, with no interaction, in the *Gracilariopsis* experiment (Table S1.6, Figure S1.3). Although there was high variability with time, and week 8 showed high shoot density values for all treatments, there was an overall negative effect of *Gracilariopsis* on shoot

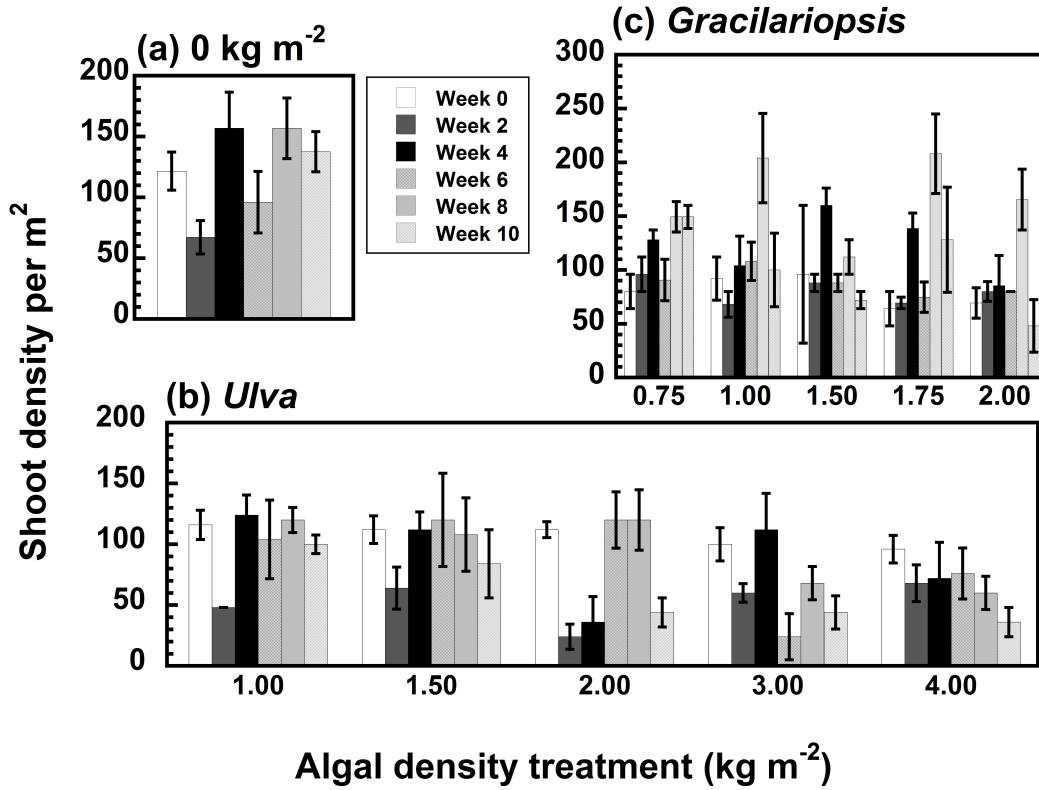
density for treatments  $\geq 1.5 \text{ kg m}^{-2}$ . Although final (week 10) shoot densities for these treatments are not different from initials, they were lower than treatments  $< 1.5 \text{ kg m}^{-2}$ , which increased in some cases.

**Discussion.** Both the *Ulva* and *Gracilariopsis* experiments indicated a negative impact of macroalgal abundance on *Z. marina* shoot density. In both cases, these effects appeared to occur  $\sim 1.5 \text{ kg m}^{-2}$  indicating that *Z. marina* shoot density can be negatively impacted by *Ulva* and *Gracilariopsis* at levels that naturally occur in California estuaries (see Huntington and Boyer 2008; Olyarnik and Stachowicz 2012). However, temporal patterns were also significant and consistent across treatments (no interaction). The dramatic and temporary reduction in shoot density in week 2 occurred across all treatments. These results suggest it is important to quantify duration of negative effects on seagrasses to determine if they are part of natural fluctuations or a result of macroalgal blooms.

## References

- Huntington, B. E., & Boyer, K. E. (2008). Effects of red macroalgal(*Gracilariopsis* sp.) abundance on eelgrass *Zostera marina* in Tomales Bay, California, USA. *Marine Ecology Progress Series*, 367, 133-142.
- Olyarnik, S. V., & Stachowicz, J. J. (2012). Multi-year study of the effects of *Ulva* sp. blooms on eelgrass *Zostera marina*. *Marine Ecology Progress Series*, 468, 107.

**Figures and Tables.**



**Figure S1.3.** Shoot density over time for (a) 0 kg m<sup>-2</sup>, (b) *Ulva* spp. and (c) *Gracilariopsis* spp.



**Table S1.5:** Shoot density over time with respect to varying *Ulva* biomass.

Interactions calculated by Wilks Lambda (Repeated Measures ANOVA). Mauchley sphericity test validates use of RMANOVA ( $\chi^2 = 15.0$ ,  $df = 14$ ,  $Prob > \chi^2 = 0.3756$ ).

Source	<i>d.f.</i>	<i>F</i>	<i>P</i>
Between Subjects (treatment)	6	5.99	0.0020
Within Subjects (Time)	5	10.53	<0.001
Time*Treatment	25	1.41	0.1403

**Table S1.6:** Shoot density over time with respect to varying *Gracilariopsis*

biomass. Interactions calculated by Wilks Lambda (Repeated Measures ANOVA).

Mauchley sphericity test validates use of RMANOVA ( $\chi^2 = 11.2$ ,  $df = 14$ ,  $Prob > \chi^2 = 0.6666$ ).

Source	<i>d.f.</i>	<i>F</i>	<i>P</i>
Between Subjects (treatment)	5	3.33	0.0373
Within Subjects (Time)	5	14.07	0.0118
Time*Treatment	30	2.022	0.7080

**Appendix S1.6:** Repeated measures analysis of epiphyte load ( $\text{g m}^{-2}$ ) over time.

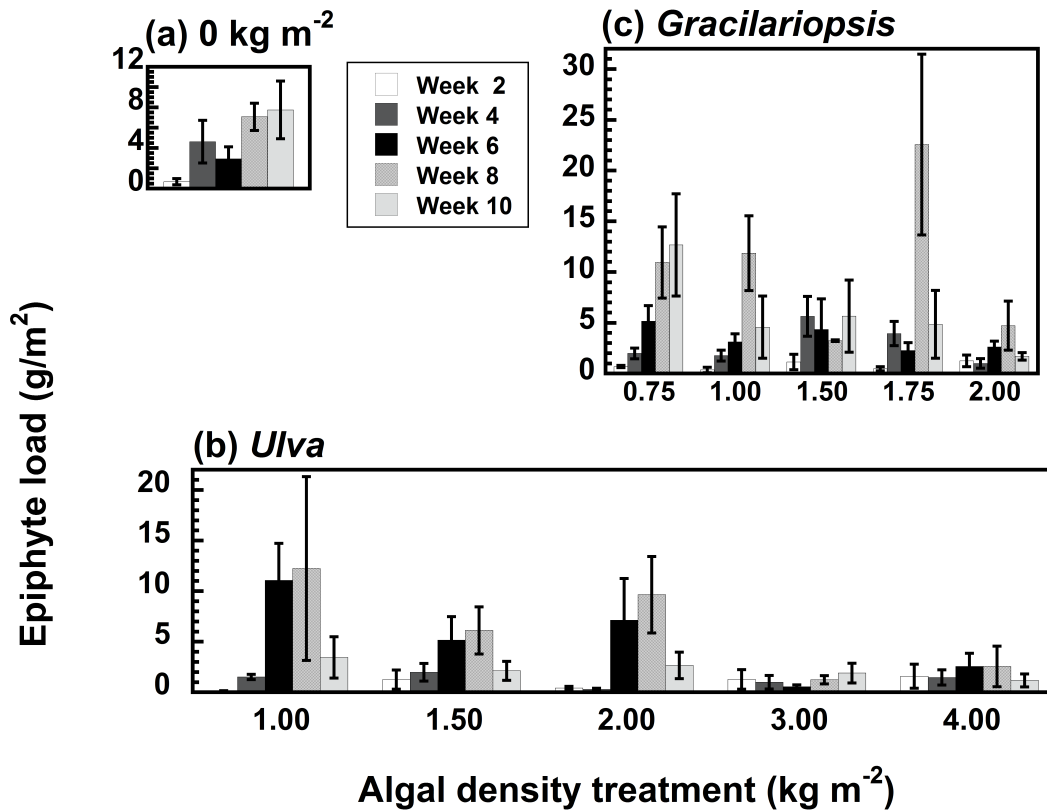
**Methods.** Response of epiphyte load ( $\text{g m}^{-2}$ ) to macroalgal abundance was analyzed by repeated measures ANOVA over five time points (weeks 2, 4, 6, 8, 10). Epiphyte load analysis started with week 2, as the difference between week 0 and 2 would be an artifact of disturbing plots prior to the initial deployment of cages. Mauchley sphericity tests were used to validate use of repeated measures and Greenhouse-Geisser corrections are included if sphericity was violated.

**Results.** Data for both experiments violated assumptions of sphericity and Greenhouse-Geisser corrections are included in Tables S1.7 and S1.8. Using the correction, only treatment is significant for *Ulva* (time is marginal at  $p=0.07$ ) and only time was significant in the *Gracilariopsis* experiment. In the *Ulva* experiment, low epiphyte load values in week 2 are followed by recovery nearly to initial levels ( $\bar{x} = 12.2 \pm 1.2 \text{ SEM g m}^{-2}$ ) in plots with no algae ( $0 \text{ kg m}^{-2}$ ). There was recovery in treatments of  $1.0\text{-}2.0 \text{ kg m}^{-2}$  over weeks 4-8 followed by a decline in week 10. Finally, treatments  $3.0\text{-}4.0 \text{ kg m}^{-2}$  experienced little to no epiphyte recovery in any week (Figure S1.4). Although treatment was not significant for the *Gracilariopsis* experiment, final epiphyte loads for treatments  $\geq 1.0 \text{ kg m}^{-2}$  were lower than those for 0 and  $0.75 \text{ kg m}^{-2}$  (Figure S1.4). There was very high temporal variability and within week variance for the *Gracilariopsis* experiment.

**Discussion.** Epiphytes may be more sensitive to *Ulva* abundance than to *Gracilariopsis* as treatment was significant for *Ulva* and not *Gracilariopsis*. There

may also be negative effects due to *Gracilariopsis* but due to high variability, this was not significant with the Greenhouse-Geisser correction. We suggest more work examining the impact of macroalgal abundance on epiphytes in seagrass communities, but if *Gracilariopsis* has less of an impact on epiphytes at naturally occurring levels of algae, there may also be less drastic impacts to seagrass bed food chains in estuaries with more frequent *Gracilariopsis* blooms than *Ulva* blooms. One explanation for greater effect of *Ulva* than *Gracilariopsis* may be the open branching thallus form of *Gracilariopsis* compared to the flattened sheets of *Ulva*; open branches may allow more water and gas exchange and block less light to the epiphytes.

Figures and Tables.



**Figure S1.4.** Epiphyte load (g m<sup>-2</sup>) on *Z. marina* shoots over time for (a) 0 kg m<sup>-2</sup>, (b) *Ulva* spp., (c) *Gracilariopsis* spp. Initial values ( $\bar{x} = 12.2 \pm 1.2$  SEM g m<sup>-2</sup>) were disturbed by running a rod through plots upon experimental set up to avoid trapping of fish in cages and excluded from RMANOVA.

**Table S1.7:** Epiphyte load ( $\text{g m}^{-2}$ ) over time with respect to varying *Ulva* biomass. Interactions calculated by Wilks Lambda and Greenhouse-Geisser (G-G) correction due to violation of sphericity by Mauchly test ( $\chi^2 = 26.5$ ,  $\text{df} = 9$ ,  $\text{Prob} > \chi^2 = 0.0017$ ).

Source	<i>d.f.</i>	<i>F</i>	<i>P</i>
Between Subjects (treatment)	5	3.17	0.0433
Within Subjects (Time)	4	4.84	0.0196
G-G correction (Time)	1.87	2.93	0.0750
Time*Treatment	20	1.39	0.1943
G-G correction (Time*Treatment)	9.36	0.822	0.6057

**Table S1.8:** Epiphyte load ( $\text{g m}^{-2}$ ) over time with respect to varying *Gracilariopsis* biomass. Interactions calculated by Wilks Lambda and Greenhouse-Geisser (G-G) correction due to violation of sphericity by Mauchly test ( $\chi^2 = 29.4$ ,  $\text{df} = 9$ ,  $P < 0.001$ ).

Source	<i>d.f.</i>	<i>F</i>	<i>P</i>
Between Subjects (treatment)	5	1.54	0.2440
Within Subjects (Time)	4	17.27	<0.001

G-G correction (Time)	2.34	10.13	<0.001
Time*Treatment	20	1.17	0.3335
G-G correction (Time*Treatment)	11.72	1.78	0.0994

## CHAPTER 2:

### **Bolstered physical defences under nutrient enriched conditions may facilitate a secondary foundational algal species in the South Pacific<sup>1</sup>**

#### **Abstract**

Humans have a long history of changing species' ranges and habitat distributions, making studies of the ecological processes that may facilitate these changes of key importance, particularly in cases where a primary foundation species is replaced by another, less desirable species. We investigated the impact of nutrients and herbivory on *Turbinaria ornata*, a secondary foundational macroalga that depends on and likely competes with coral, the primary foundational community. *T. ornata* is also rapidly expanding in range and habitat across the South Pacific. We conducted: 1) a mesocosm experiment assessing relative nutrient limitation, 2) a field experiment comparing importance of nutrients (+/-) and herbivory (+/-) to biomass accumulation, and 3) an herbivory assay and toughness test comparing enriched and ambient thalli to assess changes to anti-herbivory defences. We found no evidence of growth being nutrient limited in *T. ornata*; rather than stimulating growth, nutrient addition deterred herbivores. However, when physical toughness was removed, enriched algae were preferred, with consumption rates 25-fold those of unenriched algae. Additionally, enriched thalli

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<sup>1</sup>The chapter here is a version of the article published in *Journal of Ecology*. S.J. Bittick, R.J. Clausing, C.R. Fong, & P. Fong (2016). Bolstered physical defences under nutrient enriched conditions may facilitate a secondary foundational algal species in the South Pacific. *Journal of Ecology*, 104: 646–653.

were tougher than ambient thalli, suggesting physical defences were bolstered by nutrient enrichment. *Synthesis.* We found a unique interaction where nutrients inhibit herbivory and facilitate *Turbinaria ornata* biomass accumulation. While concern is often placed on degradation of foundation species via anthropogenic change, instead here we show that anthropogenic change can facilitate secondary foundation species. This facilitation may allow a secondary foundation species to better compete with primary foundation species.

**Key-words:** anti-herbivory defences; aquatic plant ecology; coral reefs; secondary foundation species; human impacts; top-down and bottom-up control; *Turbinaria ornata*

## **Introduction**

Human alterations of major ecological processes have resulted in worldwide changes in species' geographic ranges as well as their distribution among habitats (e.g., Ellison et al. 2005, temperate and tropical forests; Orth et al. 2006, seagrass beds; Rohr et al. 2009, hemlock groves; Veldman and Putz 2011, Amazon basin grasslands and forests; Saintilan et al. 2014, mangroves and saltmarsh plants). While human impacts usually result in overall loss of foundation species (Ellison et al. 2005, Orth et al. 2006), in some cases humans may facilitate a switch from one dominant foundation species to a secondary, often less desirable, foundation species (Rohr et al. 2009, Veldman and Putz 2011, Saintilan et al. 2014). While all foundation species, by definition, support community structure, species composition, and ecosystem functioning (see Ellison et al. 2005 for a review), secondary foundation species are dependent on primary foundation species for



space or habitat stabilization such as moss epiphytes to their oak hosts (Angelini and Silliman 2014) or mussels in a cordgrass habitat (Altieri et al. 2007). As humans impact the physical and abiotic environment, there can be consequences to interactions between primary and secondary foundation species that will have cascading effects to the communities they support (see Angelini et al. 2011). Because shifts between foundational species have large impacts on their dependent communities' structure and functioning, studies of the ecological processes that may facilitate these changes are of key importance.

Anthropogenic changes to major ecological controlling forces, such as nutrient limitation and its interaction with herbivory, are known to impact the composition and abundance of primary producers in many ecosystems (e.g., grasslands in Tilman and Downing 1994; terrestrial and aquatic systems in Hillebrand et al. 2007). Overall, increased nutrients have been found to stimulate herbivory on coral reefs (Boyer et al. 2004, Chan et al. 2012) in salt marshes (He and Silliman 2015), and on rocky reefs (Gruner et al. 2008). Further, theory predicts enrichment will translate directly to increased primary productivity and more diverse food webs (e.g., Oksanen et al. 1981; with concurrent high consumption in Worm et al. 2002; but see Rosenzweig 1971 for a discussion of the paradox of enrichment). As for many primary producers, coral reef macroalgae are thought to be controlled by interactions between top down and bottom up forces. Although there is high spatial and temporal variability in algal response to nutrient addition, many tropical macroalgae experience opportunistic growth when exposed to inputs of nitrogen and phosphorous (e.g., Fong et al. 2003), as these nutrients tend to be limiting in tropical systems (for a review, see Fong and Paul 2011).

Macroalgae is also strongly controlled by high herbivory on coral reefs, though overfishing can result in a decrease in herbivory pressure (Hughes et al. 2010, Fong and Paul 2011). Further, a meta-analysis found that nutrients only increased algal abundance in the absence of herbivores (Burkepile and Hay 2006). Subsequent studies follow similar trends but vary across time, space and species (e.g., Smith et al. 2010). Both empirical studies and theoretical predictions suggest that interactions between nutrients and herbivory should increase both primary productivity and consumption by herbivores. However, what is unknown is whether these predictions hold true for secondary foundation species when humans manipulate top-down and bottom up controls.

Macroalgae have increased on reefs over the last 40 years (e.g., Bellwood et al. 2004) and can: (1) compete directly with coral for space and light, as well as (2) inhibit recruitment of juvenile corals (see Fong and Paul 2011 for a review; also Box and Mumby 2007). Typically, these macroalgae are opportunistic species that respond quickly to nutrients and are highly palatable (Littler and Littler 1980). In contrast, *Turbinaria ornata* is a secondary foundational brown macroalga (see methods for rationale) that has been increasing in range and habitat usage within its range in the South Pacific over the last 35 years. Prior to 1980, *T. ornata* was confined to the Austral and Society islands but by 1990 appeared on the northern and southern Tuamotu islands (Martinez et al. 2007). While it is still unclear what processes are driving this expansion (Stiger and Payri 2005), possible explanations include changes in top-down and bottom-up processes and their interactions. Only a handful of studies have examined the effect of nutrients and herbivory on *T. ornata*, and results from these studies have been mixed. For example, one study on

the Great Barrier Reef showed that inshore *T. ornata* did not respond to nutrients by increasing biomass but did store nutrients in their tissues (Schaffelke 1999). A study from Tahiti found *T. ornata* had greater concentrations of phenols, the aromatic molecules responsible for chemical defence, in an area with high nutrient input (Stiger et al. 2004). This contrasts with *Fucus distichus*, a closely related brown alga, that had no or a negative relationship between nutrient availability and phenol content (Yates and Peckol 1993, Koivikko et al. 2005); rather, herbivory induced chemical defences in this alga (Koivikko et al. 2005). Finally, Chan et al. (2012) found a congener, *T. turbinata*, became more palatable in response to nutrient input in the Caribbean. Thus, a range of interactions between nutrients and herbivory have been found in closely related algae. It is important to further our understanding of these complex interactions as human-induced alterations of these controlling top-down and bottom-up processes will only intensify in the future.

We evaluated the role of anthropogenic alterations of nutrients and herbivory in promoting the persistence of *T. ornata* on coral reefs of the South Pacific. Specifically, we asked: (1) Does *T. ornata* grow in response to nutrients? (bottom-up control), (2) Does herbivory control *T. ornata* biomass? (top-down control), and (3) Is there any interaction between nutrients and herbivory that may lead to the persistence of *T. ornata*?

## **Materials and Methods**

*Study Species*—*T. ornata* forms dense aggregations ( $\bar{x} = 267.2 \pm 17.1$  SEM thalli per  $m^2$ ,  $n = 80$ ) on hard reef substrates formerly dominated by corals and has been observed to facilitate other species by providing habitat structure, refuge, or

both to other macroalgae (Bittick et al. 2010), juvenile fish (Harvey et al., in prep) and invertebrates (S. Briley, unpublished data). By supporting a high density of epiphytes, *T. ornata* also provides trophic support to herbivores (Bittick et al., Chapter 3). Because *T. ornata* facilitates a diverse community but depends on corals to form the hard substrate for attachment, it can be considered a secondary foundation species.

*T. ornata* belongs to the thick-leathery functional form group that is predicted to be slower growing and less responsive to nutrients compared to other types of more opportunistic macroalgae (Littler and Littler 1980). Previous studies showed that *T. ornata* has high morphological plasticity where flow increases tensile (breaking) strength (Stewart 2006a). Reproductive *T. ornata* thalli detach seasonally through natural senescence and as a result of high wave energy. This results in formation of rafts that may facilitate dispersal, though the direct effect on dispersal has not been studied (Stewart 2006b). *T. ornata* also contains chemical (Deslandes et al. 1997, Stiger et al. 2004) and mechanical (physical toughness; Payri et al. 2004) defences to deter herbivores, though in Australia *T. ornata* was found to be highly palatable (Mantyka and Bellwood 2007).

*Study sites*— To evaluate whether our results showed the same overall pattern across space and time, all experiments were repeated for two different patch reef sites; however, logistical constraints prohibited running the experiments simultaneously. Our two sites were: Gump Reef (17°29'17.25"S, 149°49'32.26"W) situated at the mouth of Cook's Bay and Sailing School (17°28'59.81"S, 149°50'45.70"W) located at the mouth of Opunohu Bay. We expected higher ambient nutrients at Gump Reef due to a larger human population, more

agricultural development, and greater mean riverine input than Cook's Bay (Letourneur et al. 2013). This expectation was evaluated by analysing tissue nitrogen and phosphorous for *T. ornata* thalli collected from the two sites because *T. ornata* store nutrients in its tissues (Schaffelke 1999). Tissue nutrient concentrations (% nitrogen and phosphorous) confirmed lower nutrient levels at Sailing school (see Appendix S2.1). Additionally, a follow-up study determined herbivore abundance to be much lower at Gump than Sailing School (Bergman et al. 2016).

*Experimental approaches*—To determine whether *T. ornata* growth was nutrient limited, we conducted a two-factor bioassay varying the supply of nitrogen (+/- N) and phosphorous (+/- P) in a fully crossed factorial design. Forty small *T. ornata* (5–10cm tall) thalli were collected from two sites, Gump Reef on April 25, 2012 and Sailing School Reef on May 6, 2012. All (-) treatments used ambient seawater from each site. For enriched (+) treatments, nitrate (NaNO<sub>3</sub>) and phosphate (NaH<sub>2</sub>PO<sub>4</sub>) were added to ambient seawater from each site to achieve concentrations 20µM N and 2µM P above ambient as in Fong et al. (2003). Each thallus was spun in a salad spinner for 1 min, wet weighed ( $\bar{x} = 3.45\text{g} \pm 0.12 \text{ SEM}$ ) and randomly assigned to one of the four treatments with 10 replicates. Eight hundred mL of the appropriate treatment seawater (-N-P, +N-P, -N+P, +N+P) and a *T. ornata* thallus were put into each of 40 1000mL plastic cups. The cups were set haphazardly in a flow through water table for temperature regulation. After five days, thalli were reweighed, and growth was calculated as per cent change from initial biomass. Data from both sites met the assumptions of normality and homogeneity of variance and were analysed by a two-factor ANOVA using the

statistical software JMP version 11 (JMP® 2015).

An *in situ* two-factor, fully crossed experiment varying nutrient addition (+/- N) and access to herbivores (+/- H) was repeated at Gump (April 25-30, 2012) and Sailing School (May 9-14, 2012) reefs to determine whether *T. ornata* biomass was controlled by bottom up and/or top down processes or their interaction. Herbivore access was limited by exclusion cages (6-sided;  $10 \times 10 \times 15 \text{cm}^3$  L×W×H) constructed from hardware cloth with 1 cm openings. Light restriction by caging material was <10% and there was no measured restriction to flow in similar cages used at the same site (Clausing et al. 2014). Thalli open to herbivores were attached to square (10×10cm) bases of the same hardware cloth. Forty *T. ornata* thalli were collected from Gump Reef and Sailing School reefs and wet weighed ( $\bar{x} = 6.26\text{g} \pm 0.25 \text{ SEM}$ ). Individual thalli were assigned to four treatments: ambient nutrients and uncaged (-Nutr+Herb), ambient nutrients and caged (-Nutr-Herb), nutrient enriched and uncaged (+Nutr+Herb) and nutrient enriched and caged (+Nutr-Herb). Nutrient enrichment was achieved by placing 20 g of slow-release fertilizer (Osmocote 19N:6P:12K) in a mesh bag attached either to a cage bottom or open platform. Ten replicates of each treatment were deployed at both sites by randomly attaching the cage or open platform to hard substrate. After 6 days all experimental units were collected, and the final wet weight and height of each thallus were recorded. Data from both sites were non-normal and heteroscedastic, and these issues were not resolved by common transformations. We used a two-factor univariate variation of permutational multivariate analysis of variance (PERMANOVA) in PRIMER-e v6 (Clarke and Gorley 2006). This technique has been found to be robust against violations of normality and heterogeneity of variance

(McArdle and Anderson 2001, Anderson and Walsh 2013). Similarity matrices were constructed using Euclidean distances, which is appropriate for biomass change data where values can be zero or negative (Norkko et al. 2013). The model was run on untransformed data for 999 permutations to obtain P-values.

A one-factor experiment was conducted to determine whether nutrient-enriched thalli deter herbivores due to increases in chemical defence. Dried nutrient-enriched and ambient thalli from the Gump Reef *in situ* experiment were ground into as fine a powder as possible to remove the influence of any physical defence. The ground material was added to trays of hot agar solution and poured over window screen mesh as thinly as possible. This method was adapted from a technique used by Hay et al. (1994). No algae were added to a third tray resulting in a control treatment of only agar. Ten experimental units (~5cm × 7cm) were cut from each of the three trays and set out in a blocked pattern on Gump Reef on May 23, 2012. Photos were taken of each plate before and after 3 days in the field. Initial and final surface areas were estimated using ImageJ software (U.S. National Institutes of Health 2016). Herbivory was calculated as per cent change in area. Data met the assumptions of parametric statistics and were analysed in JMP using a one-factor blocked ANOVA.

To evaluate whether physical defences were impacted by nutrients, an enrichment experiment was conducted, and then a penetrometer used to determine the relative toughness of nutrient-enriched and ambient *T. ornata* thalli. Twenty *T. ornata* thalli were collected from Gump Reef on April 20, 2013, assigned to nutrient enriched (as described for field experiment) or ambient treatments, and placed back on Gump Reef. Thalli were collected 6 days later and a blade selected from the

most apical (newest algal growth) ring was placed on a platform under an insect-dissecting pin with a small plastic cup attached. Incremental weight was then added to the cup until the pin just pierced the *T. ornata* blade. This method was adapted from Duffy and Hay (Duffy and Hay 1991). The weight was recorded as the mass required to penetrate the thallus, and differences between means for ambient and enriched thalli were determined by a t-test in JMP.

## Results

Although patterns of growth varied between Sailing School and Gump Reef, there were no significant increases in growth in response to either nitrogen or phosphorous addition. For both sites, growth ranged from <1–6% over the 5-day experiment (Figure 2.1).

At Sailing School there was a significant interaction between nutrients and herbivory on change in algal biomass (PERMANOVA, NutrXHerb interaction  $p = 0.027$ ), where herbivores only consumed algae under ambient nutrient conditions (Table 2.1; Figure 2.2 a). The pattern was the same at Gump Reef (Figure 2.2 b) but the interaction was not significant ( $p=0.107$ ). While both sites had greatest herbivory rates when nutrients were ambient and herbivores allowed access, net loss in this treatment (-N+H) was nearly threefold greater at Gump compared to Sailing School. When nutrients were added, consumption by herbivores was greatly reduced, resulting in only small losses in biomass at Gump Reef, and increased biomass at Sailing School, presumably due to growth exceeding herbivory in this treatment. When caged, algae at Sailing School grew an average of 10–15% while those at Gump Reef either did not change or showed small losses. Similarity in the



magnitude of the caging by nutrient interaction between the two sites (net difference of 25–30% change in biomass between –N+H treatments and all others) indicated that the difference between sites was driven by higher growth at Sailing School than Gump as opposed to different herbivory rates.

In contrast to the field manipulation, when physical defences were removed, herbivores preferentially grazed on agar containing nutrient enriched thalli (Figure 2.3 a). Only the agar plates containing thalli enriched by fertiliser showed significant loss in surface area by herbivory (blocked ANOVA,  $p$ -value = 0.0021). The agar containing algal thalli grown under ambient nutrients and those with no *T. ornata* thalli were not significantly different from each other, and their mean per cent change was near zero.

There was a significant effect of nutrient enrichment on the toughness of *T. ornata* thalli (t-test,  $p$ -value = 0.0002; Figure 2.3 b). There was nearly a 30% increase in the weight needed to pierce the thalli of nutrient enriched compared to ambient *T. ornata* thalli.

## **Discussion**

We found a unique interaction whereby enhanced physical defences with nutrient enrichment released *T. ornata* from herbivore control, which may allow expansion of *T. ornata* to habitats where it is usually controlled by high herbivory. This is a novel finding because, in this case, reduction in herbivory is the indirect result of nutrient enrichment, not the direct result of overfishing, as is commonly found in other reef systems (e.g., Hughes et al. 2010). The finding that nutrients decrease herbivory contrasts to previous empirical work and theoretical predictions

that enrichment will, if anything, stimulate herbivory and trophic complexity (see Oksanen et al. 1981, Hillebrand et al. 2007; marine examples in Gruner et al. 2008, He and Silliman 2015). Additionally, most other studies examining interactions of nutrients and herbivory on coral reef algae have found herbivory to be more important, and that nutrients are only important in the absence of herbivores (see Burkepile and Hay 2006 for a meta-analysis).

Our results suggest that human impacts that alleviate nutrient limitation, and thereby reduce herbivory, may facilitate *T. ornata*'s expansion of its range and habitat usage. This expands upon previous evidence that biotic and abiotic interactions have strong effects on communities supported by foundation species (Ellison et al. 2005) by suggesting strong interactions are also important for a secondary foundation species that may be replacing a primary foundation species. Human alterations of abiotic factors have been found to change the outcome of interspecific interactions, specifically competition, across ecosystem types (Briggs et al. 2005, Veldman and Putz 2011, Saintilan et al. 2014). Mangroves, for example, have expanded into salt marshes in areas where climate change has resulted in warmer winter temperatures (Saintilan et al. 2014). Competitive outcomes between mangroves and salt marsh graminoids are mediated by environmental conditions, and mangroves are predicted to be competitively dominant when winter temperatures are higher and droughts more frequent (Osland et al. 2013, Saintilan et al. 2014). Similarly, the reduction of the abiotic controlling force of fire on grassland prairies allowed shrubs to recruit, become competitively dominant, and replace previously dominant C4 grasses (Briggs et al. 2005). Once large stands established, more frequent fire regimes did not

necessarily reverse the shift because the large and persistent shrubs also protected fire-sensitive recruits and species. Changes to nutrient availability have also resulted in shifts in species dominance in each of these ecosystems (for a review see Smith et al. 1999). Although the drivers occur at different scales, human-induced change of abiotic forces (winter temperature, fire frequency and nutrient levels) has caused each of these foundation species to be released from biotic controls (competition and herbivory) that previously restricted their distribution. This caused large community and ecosystem shifts for salt marsh, grassland and forest systems, and we predict this is true for reef systems where *T. ornata* is expanding and potentially competing with coral as well. Our study adds to a growing body of literature that suggests human alterations of key abiotic forces, such as nutrient limitation, can cause shifts in foundation species by changing the strength and even direction of interspecific interactions (e.g., Briggs et al. 2005, Veldman and Putz 2011, Osland et al. 2013, Saintilan et al. 2014).

The bolstering of *T. ornata*'s physical defences in response to increased nutrients is a unique finding, as previous work on the effects of nutrients on defences have either considered chemical defences or cases where physical defences decline. Some studies have found enhanced phenols may serve as chemical defences in *T. ornata* (Stiger et al. 2004) and temperate brown macroalgae (Yates and Peckol 1993, Koivikko et al. 2005) in response to nutrients, but increased phenols in brown algae may not always result in decreased herbivory pressure (Targett and Arnold 1998). A physical anti-herbivore response to nutrients appears to be novel except in calcifying forms of algae. Calcium carbonate ( $\text{CaCO}_3$ ) mineralization has been shown to decrease in response to elevated levels of

phosphates in several species of calcifying algae presumably making them more susceptible to herbivory (e.g., *Halimeda* in Demes et al. 2009). However, the direction of the nutrient effect is opposite; nutrients stimulated defences in *T. ornata*, but reduced them in calcifying algae. In terrestrial systems, resource allocation and carbon nutrient balance theories predict that nutrient-rich environments will yield plants with decreased physical defence, such as sclerophylly or leaf hardening, although a meta-analysis of 50 studies did not find conclusive results (Endara and Coley 2011). Previous studies of physical toughness as a defence in non-calcifying macroalgae have usually focused on herbivory by amphipods and found differences based on algal species (e.g., Duffy and Hay 1991) and tissue type (e.g., Taylor et al. 2002), but have not been linked to nutrient regimes. Rather, nutrients have been found to increase the palatability of *T. turbinata*, a Caribbean congener (Chan et al. 2012). However it is unknown whether this has any relation to a change in defences, though when physical defences were removed, we found enriched *T. ornata* became more palatable as well. It is likely that increased toughness has additional population-level effects other than deterrence of herbivores, such as increased breaking strength that may reduce dispersal via thalli detachment and rafting. Clearly more research is needed to explore the full implications of this finding. However, our results demonstrated *T. ornata*'s distribution and abundance may be controlled, at least in part, by a unique interaction between top-down and bottom-up processes that facilitate *T. ornata* populations by increasing physical defences in contrast to the potential decreases in calcifying algae and in terrestrial systems.

*Turbinaria ornata* does not have an opportunistic life-history strategy like

other algae that typically dominate in response to nutrient enrichment in marine and estuarine ecosystems in general (for a review see Fong 2008) and coral reefs in particular (e.g., Folke et al. 2004). Rather, our results support *T. ornata*'s designation as a species with a "persistor" life-history strategy (Littler and Littler 1980), as it did not respond directly to nutrient input by increasing growth. In comparison, some species of opportunistic macroalgae can increase growth by 20% or more in just 3 days when subjected to nutrient enrichment (Fong et al. 2003). Overall, *T. ornata* grows relatively slowly, like many other foundation species (e.g., Ellison et al. 2005). In contrast to our results, where nutrients facilitated a secondary foundational species, in systems dominated by slower-growing foundation species, nutrient enrichment often causes replacement by rapidly growing opportunists. In seagrass beds, for example, nutrient runoff causes increased micro- and macroalgal growth and subsequent seagrass loss (Orth et al. 2006). Similarly, higher nutrients on coral reefs can cause phytoplankton blooms or corals can be overgrown by opportunistic macroalgae (Hughes et al. 2010, Fong and Paul 2011). However, there are several terrestrial examples where slow growing, long lived foundation species such as hardwood forest trees (Rohr et al. 2009), grassland shrubs (Briggs et al. 2005) and mangroves (Saintilan et al. 2014) are the replacement species. Our study adds a novel aquatic example to these terrestrial cases and replacement by a persistent secondary foundation species may be of greater concern than by more temporary, ephemeral algal species.

Although the global decline of foundation species is well documented (Ellison et al. 2005), much work is still needed to understand the potentially complex drivers of change. In many systems, a suite of human impacts has changed the

range and habitat boundaries of foundation species by releasing them from limiting ecological forces (Briggs et al. 2005, Rohr et al. 2009, Veldman and Putz 2011, Saintilan et al. 2014). In some cases, expansions into new geographic regions and local habitats have occurred at the expense of an original foundation species. While it is unknown whether *T. ornata* directly replaces other foundation species in the system (such as corals), as with mangroves and salt marsh plants, recruits of both occupy the same substrate. Further, as a secondary foundation species dependent on the hard substrate afforded by calcium carbonate deposition by corals, we expect a shift from a coral to *T. ornata* dominated reef to result in an overall decline in reef resilience. Further, a recent study suggested that *T. ornata* recruits may negatively affect coral recruits (Brandl et al. 2013). As human impacts are becoming more pervasive, it is essential not only to understand the numerous systems in which foundation species are degraded but also to evaluate drivers in cases where secondary foundation species are expanding at the expense of the original foundation community.

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## Tables

**Table 2.1:** Results from the permutational analysis (PERMANOVA) of differences in *T. ornata* biomass change for the *in situ* two-factor (Nutrients and Herbivory) experiments conducted at Gump and Sailing School reefs.

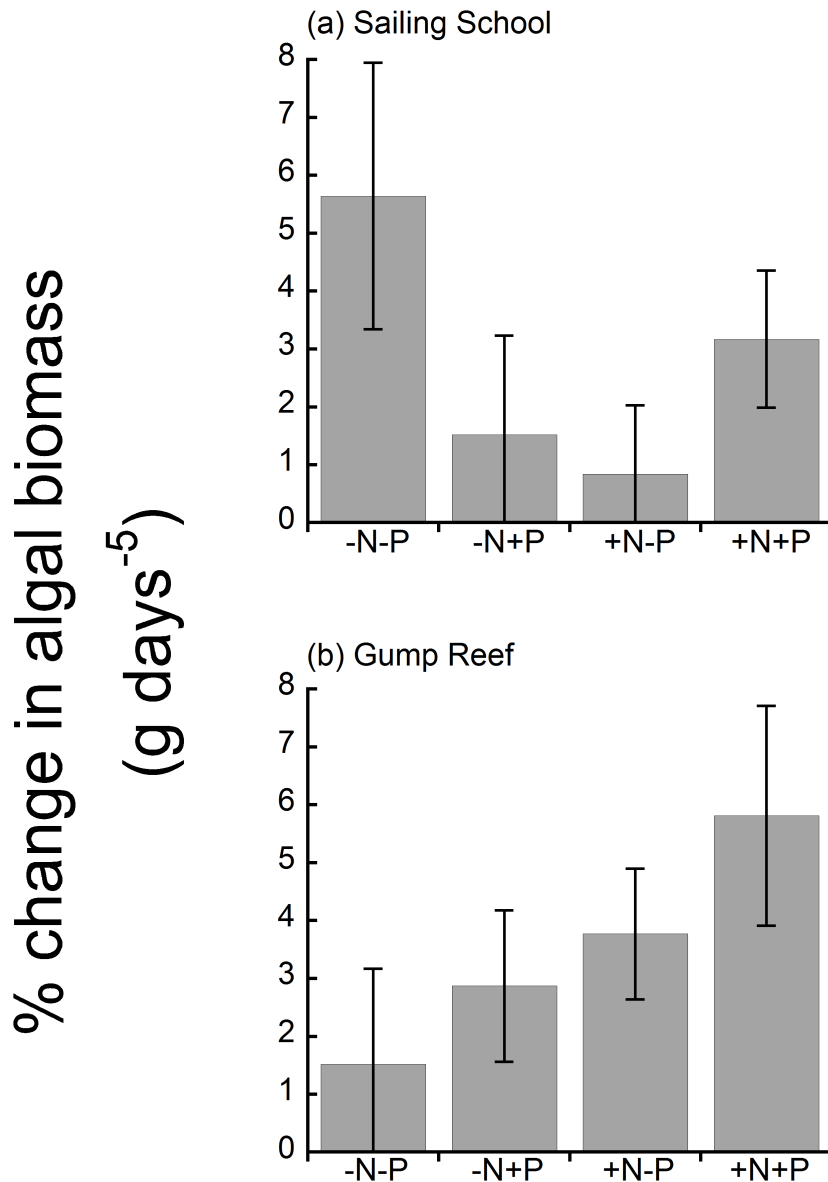
PERMANOVA	df	SS	MS	Pseudo-F	<i>P</i> (perm)
Sailing School					
Nutrients (Nutr)	1	529.62	529.62	1.9731	0.179
Herbivory (Herb)	1	1752.4	1752.4	6.5285	<b>0.018</b>
Nutr × Herb	1	1508.4	1508.4	5.6193	<b>0.027</b>
Res	33	8858	268.42		
Total	36	12947			
Gump					
Nutrients (Nutr)	1	1016.3	1016.3	2.2475	0.165
Herbivory (Herb)	1	1878.8	1878.8	4.1547	0.053
Nutr × Herb	1	1331.5	1331.5	2.9444	0.107
Res	35	15827	452.21		
Total	38	19842			

Bold numbers represent significant P-values (<0.05). This table is duplicated from the online version of this article in *Journal of Ecology*:

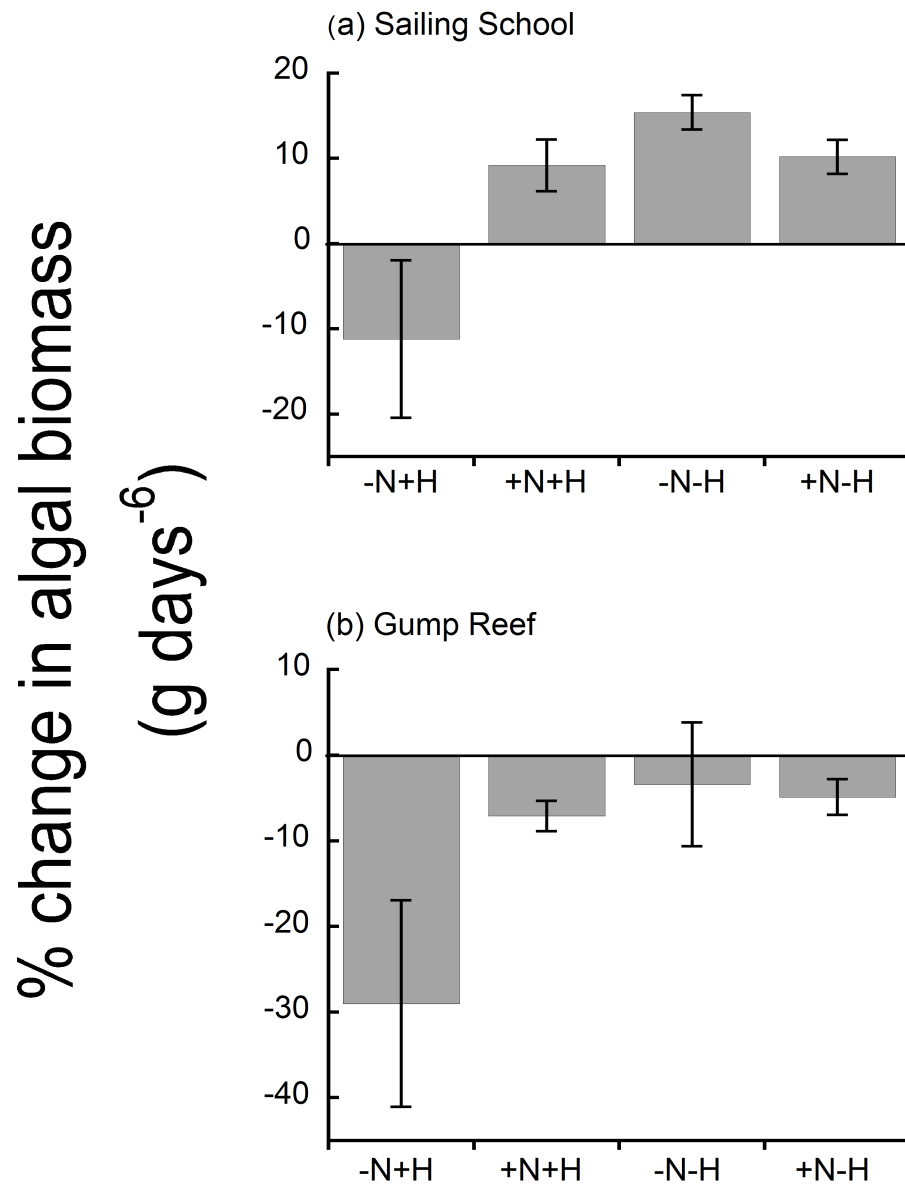
<http://onlinelibrary.wiley.com/doi/10.1111/1365-2745.12539/full>



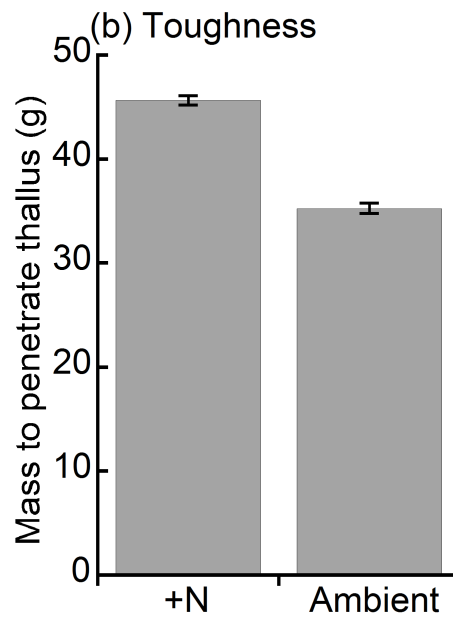
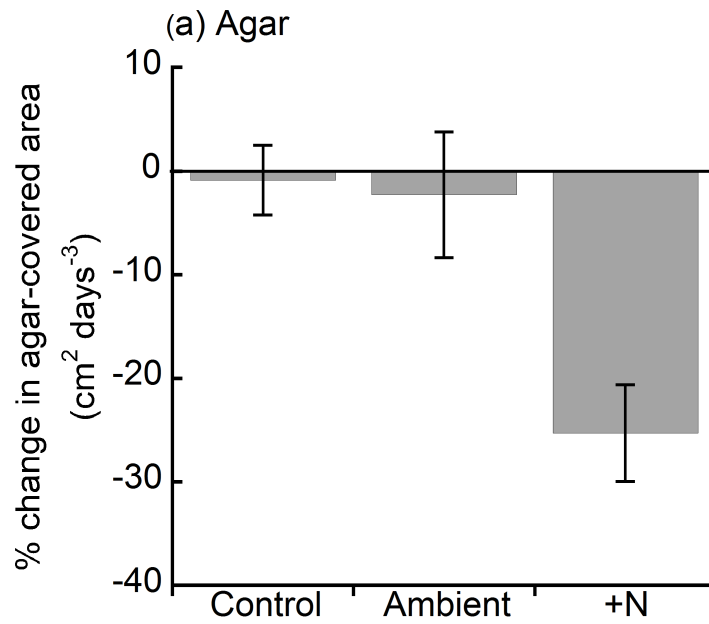
## Figures



**Figure 2.1:** Results of a two-factor nutrient addition, +/- nitrogen (N) and +/- phosphorous (P), mesocosm experiment at Sailing School reef (a) and Gump reef (b). Bars show mean per cent change (+/- SEM) of algal biomass.



**Figure 2.2:** Results of a two-factor *in situ* experiment manipulating nutrients (+/- N) and herbivory (+/- H) repeated at two sites—Sailing School (a) and Gump (b). Bars are mean per cent change (+/- SEM) of algal biomass.



**Figure 2.3:** (a) Palatability study of ground nutrient enriched (+N) and ambient *T. ornata* placed in agar shows that enriched thalli are consumed

preferentially (ANOVA,  $p=0.0021$ ) and, thus, there is not a chemical deterrent associated with increased nutrient load. (b) Confirmation that *T. ornata* thalli are protected from herbivory by nutrients through a strengthening of physical defences, or thalli toughness (T-test,  $p=0.0002$ ).

## References

- Altieri, A. H., B. R. Silliman, and M. D. Bertness. 2007. Hierarchical organization via a facilitation cascade in intertidal cordgrass bed communities. *The American Naturalist* 169:195–206.
- Anderson, M., and D. Walsh. 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: what null hypothesis are you testing? *Ecological Monographs* 83:557–74.
- Angelini, C., A. H. Altieri, B. R. Silliman, and M. D. Bertness. 2011. Interactions among Foundation Species and Their Consequences for Community Organization, Biodiversity, and Conservation. *BioScience* 61:782–789.
- Angelini, C., and B. R. Silliman. 2014. Secondary foundation species as drivers of trophic and functional diversity: Evidence from a tree-epiphyte system. *Ecology* 95:185–196.
- Bellwood, D., T. Hughes, C. Folke, and M. Nyström. 2004. Confronting the coral reef crisis. *Nature* 429:827–33.
- Bergman, J., B. Dang, M. Tabatabaee, M. McGowan, C. Fong, S. Bittick, and P. Fong. 2016. Nutrients induce and herbivores maintain thallus toughness, a structural anti-herbivory defense in *Turbinaria ornata*. *Marine Ecology Progress Series* 559:35–43.
- Bittick, S. J., N. D. Bilotti, H. A. Peterson, and H. L. Stewart. 2010. *Turbinaria ornata* as an herbivory refuge for associate algae. *Marine Biology* 157:317–323.
- Box, S., and P. Mumby. 2007. Effect of macroalgal competition on growth and survival of juvenile Caribbean corals. *Marine Ecology Progress Series* 342:139–149.
- Boyer, K. E., P. Fong, A. R. Armitage, and R. A. Cohen. 2004. Elevated nutrient content of tropical macroalgae increases rates of herbivory in coral, seagrass, and mangrove habitats. *Coral Reefs* 23:530–538.
- Brandl, S. J., A. S. Hoey, and D. R. Bellwood. 2013. Micro-topography mediates interactions between corals, algae, and herbivorous fishes on coral reefs. *Coral Reefs* 33:421–430.
- Briggs, J. M., A. K. Knapp, J. M. Blair, J. L. Heisler, G. a. Hoch, M. S. Lett, and J. K. McCARRON. 2005. An Ecosystem in Transition: Causes and Consequences of the Conversion of Mesic Grassland to Shrubland. *BioScience* 55:243.
- Burkepile, D. E., and M. E. Hay. 2006. Herbivore vs . Nutrient Control of Marine Primary Producers : Context-Dependent Effects. *Ecology* 87:3128–3139.
- Chan, A., K. Lubarsky, K. Judy, and P. Fong. 2012. Nutrient addition increases consumption rates of tropical algae with different initial palatabilities. *Marine Ecology Progress Series* 465:25–31.
- Clarke, K., and R. Gorley. 2006. *PRIMER v6: User Manual PRIMER-E*. Plymouth, UK.
- Clausing, R., C. Annunziata, G. Baker, C. Lee, S. Bittick, and P. Fong. 2014. Effects

- of sediment depth on algal turf height are mediated by interactions with fish herbivory on a fringing reef. *Marine Ecology Progress Series* 517:121–129.
- Demes, K. W., S. S. Bell, and C. J. Dawes. 2009. The effects of phosphate on the biomineralization of the green alga, *Halimeda incrassata* (Ellis) Lam. *Journal of Experimental Marine Biology and Ecology* 374:123–127.
- Deslandes, E., V. Stiger, and C. Payri. 1997. Chemical defenses (as antifeedant) of two invasive brown seaweeds on a Polynesian barrier reef. *Phycologia* 36:25.
- Duffy, J., and M. Hay. 1991. Food and shelter as determinants of food choice by an herbivorous marine amphipod. *Ecology* 72:1286–1298.
- Ellison, A. M., M. S. Bank, B. D. Clinton, E. A. Colburn, K. Elliott, C. R. Ford, D. R. Foster, B. D. Kloeppel, J. D. Knoepp, G. M. Lovett, J. Mohan, D. A. Orwig, N. L. Rodenhouse, W. V. Sobczak, K. A. Stinson, J. K. Stone, C. M. Swan, J. Thompson, B. Von Holle, and J. R. Webster. 2005. Loss of foundation species : consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment* 3:479–486.
- Endara, M. J., and P. D. Coley. 2011. The resource availability hypothesis revisited: A meta-analysis. *Functional Ecology* 25:389–398.
- Folke, C., S. Carpenter, B. Walker, M. Scheffer, T. Elmqvist, L. Gunderson, and C. S. Holling. 2004. Regime Shifts , Resilience , in *Ecosystem Management. Annual Review of Ecology, Evolution, and Systematics* 35:557–581.
- Fong, P. 2008. Macroalgal-dominated ecosystems. Page *in* E. J. Carpenter and D. G. Capone, editors. *Nitrogen in the Marine Environment*. Springer, New York.
- Fong, P., K. Boyer, K. Kamer, and K. Boyle. 2003. Influence of initial tissue nutrient status of tropical marine algae on response to nitrogen and phosphorus additions. *Marine Ecology Progress Series* 262:111–123.
- Fong, P., and V. J. Paul. 2011. Coral Reef Algae. Page (Z. Dubinsky and N. Stambler, Eds.) *Coral Reefs: An Ecosystem in Transition*. Springer Netherlands, Dordrecht.
- Gruner, D. S., J. E. Smith, E. W. Seabloom, S. A. Sandin, J. T. Ngai, H. Hillebrand, W. S. Harpole, J. J. Elser, E. E. Cleland, M. E. S. Bracken, E. T. Borer, and B. M. Bolker. 2008. A cross-system synthesis of consumer and nutrient resource control on producer biomass. *Ecology Letters* 11:740–755.
- Hay, M. E., Q. E. Kappel, and W. Fenical. 1994. Synergisms in plant defenses against herbivores---interactions of chemistry, calcification, and plant-quality. *Ecology* 75:1714–1726.
- He, Q., and B. R. Silliman. 2015. Biogeographic consequences of nutrient enrichment for plant-herbivore interactions in coastal wetlands. *Ecology Letters* 18:462–471.
- Hillebrand, H., D. S. Gruner, E. T. Borer, M. E. S. Bracken, E. E. Cleland, J. J. Elser, W. S. Harpole, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Consumer versus resource control of producer diversity depends on ecosystem type and producer community structure. *Proceedings of the National Academy of Sciences of the United States of America* 104:10904–9.

- Hughes, T. P., N. A. J. Graham, J. B. C. Jackson, P. J. Mumby, and R. S. Steneck. 2010. Rising to the challenge of sustaining coral reef resilience. *Trends in ecology & evolution* 25:633–42.
- JMP. 2015. . SAS Institute Inc., Cary, NC.
- Koivikko, R., J. Loponen, T. Honkanen, and V. Jormalainen. 2005. Contents of soluble, cell-wall-bound and exuded phlorotannins in the brown alga *Fucus vesiculosus*, with implications on their ecological functions. *Journal of Chemical Ecology* 31:195–212.
- Letourneur, Y., T. Lison de Loma, P. Richard, M. L. Harmelin-Vivien, P. Cresson, D. Banaru, M. F. Fontaine, T. Gref, and S. Planes. 2013. Identifying carbon sources and trophic position of coral reef fishes using diet and stable isotope ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) analyses in two contrasted bays in Moorea, French Polynesia. *Coral Reefs* 32:1091–1102.
- Littler, M., and D. Littler. 1980. The evolution of thallus form and survival strategies in benthic marine macroalgae: field and laboratory tests of a functional form model. *American Naturalist* 116:25–44.
- Mantyka, C. S., and D. R. Bellwood. 2007. Direct evaluation of macroalgal removal by herbivorous coral reef fishes. *Coral Reefs* 26:435–442.
- Martinez, E., K. Maamaatuaiahutapu, C. Payri, and A. Ganachaud. 2007. *Turbinaria ornata* invasion in the Tuamotu Archipelago, French Polynesia: ocean drift connectivity. *Coral Reefs* 26:79–86.
- McArdle, B. H., and M. J. Anderson. 2001. Fitting multivariate models to community data: A comment on distance-based redundancy analysis. *Ecology* 82:290–297.
- Norkko, A., A. Villnäs, J. Norkko, S. Valanko, and C. Pilditch. 2013. Size matters: implications of the loss of large individuals for ecosystem function. *Scientific reports* 3:2646.
- Oksanen, L., S. D. Fretwell, J. Arruda, and P. Niemela. 1981. Exploitation Ecosystems in Gradients of Primary Productivity. *The American Naturalist* 118:240–261.
- Orth, R. J., T. J. B. Carruthers, W. C. Dennison, C. M. Duarte, J. W. Fourqurean, K. L. Heck Jr, A. R. Hughes, G. A. Kendrick, W. J. Kenworthy, S. Olyarnik, and others. 2006. A global crisis for seagrass ecosystems. *Bioscience* 56:987–996.
- Osland, M. J., N. Enwright, R. H. Day, and T. W. Doyle. 2013. Winter climate change and coastal wetland foundation species: salt marshes vs. mangrove forests in the southeastern United States. *Global change biology* 19:1482–94.
- Payri, C. E., A. D. R. N'Yeurt, and J. Orempüller. 2004. *Algae of French Polynesia*. Au Vent des Iles Editions, Tahiti.
- Rohr, J. R., C. G. Mahan, and K. C. Kim. 2009. Response of arthropod biodiversity to foundation species declines: The case of the eastern hemlock. *Forest Ecology and Management* 258:1503–1510.
- Rosenzweig, M. L. 1971. Paradox of enrichment: destabilization of exploitation ecosystems in ecological time. *Science* 171:385–387.

- Saintilan, N., N. C. Wilson, K. Rogers, A. Rajkaran, and K. W. Krauss. 2014. Mangrove expansion and salt marsh decline at mangrove poleward limits. *Global change biology* 20:147–57.
- Schaffelke, B. 1999. Short-term nutrient pulses as tools to assess response of coral reef macroalgae to enhanced nutrient availability. *Marine Ecology Progress Series* 182:305–310.
- Smith, J. E., C. L. Hunter, and C. M. Smith. 2010. The effects of top-down versus bottom-up control on benthic coral reef community structure. *Oecologia* 163:497–507.
- Smith, V. H., G. D. Tilman, and J. C. Nekola. 1998. Eutrophication: Impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution* 100:179–196.
- Stewart, H. L. 2006a. Ontogenetic Changes in Buoyancy, Breaking Strength, Extensibility, and Reproductive Investment in a Drifting Macroalga *Turbinaria Ornata* (Phaeophyta). *Journal of Phycology* 42:43–50.
- Stewart, H. L. 2006b. Morphological variation and phenotypic plasticity of buoyancy in the macroalga *Turbinaria ornata* across a barrier reef. *Marine Biology* 149:721–730.
- Stiger, V., E. Deslandes, and C. E. Payri. 2004. Phenolic contents of two brown algae, *Turbinaria ornata* and *Sargassum mangarevense* on Tahiti (French Polynesia): interspecific, ontogenic and spatio-temporal variations. *Botanica Marina* 47:402–409.
- Stiger, V., and C. Payri. 2005. Natural settlement dynamics of a young population of and phenological comparisons with older populations. *Aquatic Botany* 81:225–243.
- Targett, N. M., and T. M. Arnold. 1998. Predicting the effects of brown algal phlorotannins on marine herbivores in tropical and temperate oceans. *Journal of Phycology* 34:195–205.
- Taylor, R., E. Sotka, and M. Hay. 2002. Tissue-specific induction of herbivore resistance: seaweed response to amphipod grazing. *Oecologia* 132:68–76.
- Tilman, D., and J. A. Downing. 1994. Biodiversity and stability in grasslands. *Nature* 367:363–365.
- U.S. National Institutes of Health. 2016. ImageJ. Bethesda, Maryland, USA.
- Veldman, J. W., and F. E. Putz. 2011. Grass-dominated vegetation, not species-diverse natural savanna, replaces degraded tropical forests on the southern edge of the Amazon Basin. *Biological Conservation* 144:1419–1429.
- Worm, B., H. K. Lotze, H. Hillebrand, and U. Sommer. 2002. Consumer versus resource control of species diversity and ecosystem functioning. *Nature* 417:848–851.
- Yates, J., and P. Peckol. 1993. Effects of Nutrient Availability and Herbivory on Polyphenolics in the Seaweed *Fucus Versiculosus*. *Ecology* 74:1757–1766.



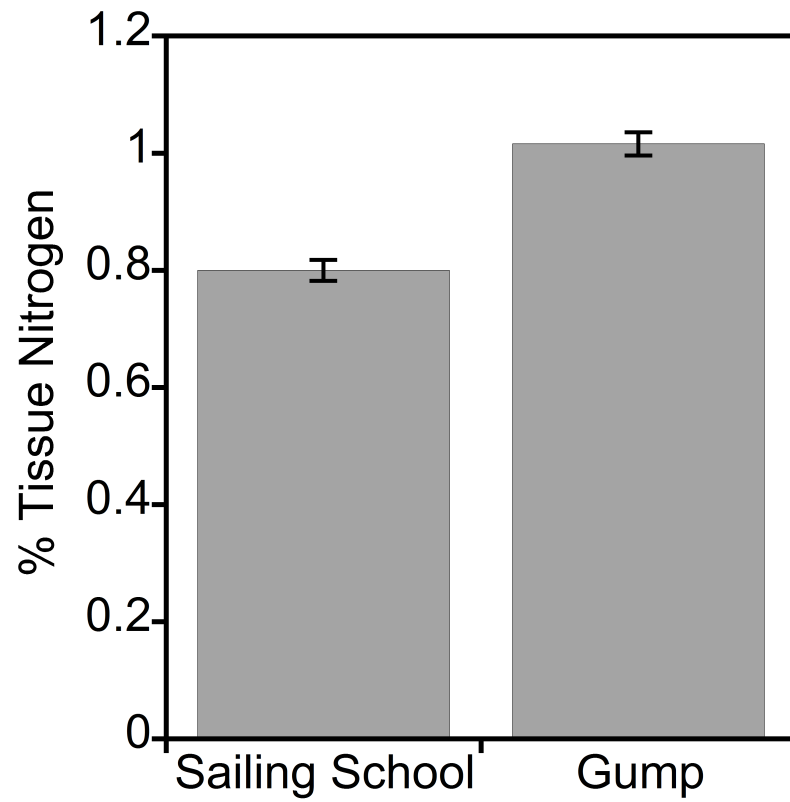
## SUPPORTING INFORMATION

### **Appendix S2.1: Tissue nutrient data from *T. ornata* collected at Gump Reef and Sailing School Reef.**

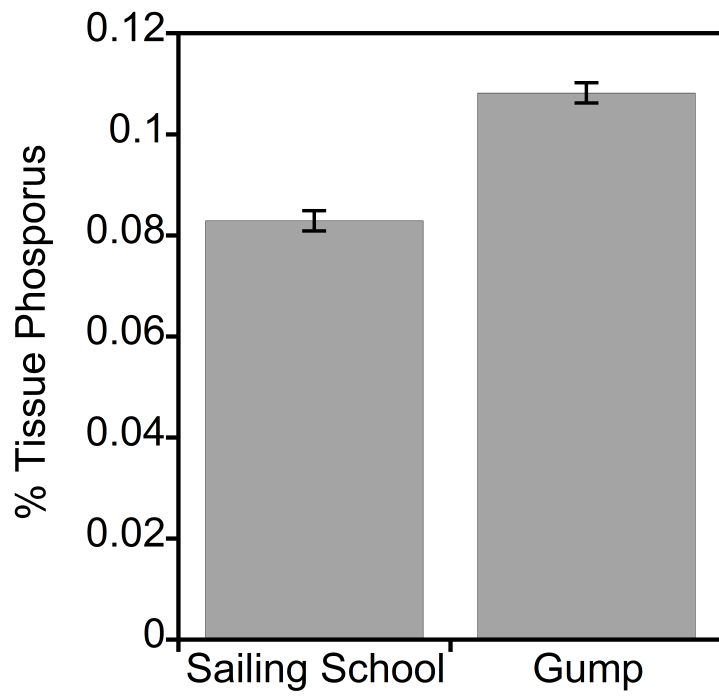
**Methods.** *T. ornata* thalli were collected from our two sites, Gump and Sailing School reefs, to evaluate nitrogen and phosphorous content. 10 samples of algae from each site were rinsed in freshwater, dried at <60°C, ground with a mortar and pestle, and sent to the University of California Davis Isotope Facility.

**Results.** Tissue nutrients, nitrogen and phosphorous, by percentage of dry weight were significantly higher for *T. ornata* collected from Gump than Sailing School Reef (see Figures S2.1 and S2.2 below).

## Supplemental Figures



**Figure S2.1:** Graph of tissue % Nitrogen at each site. % Nitrogen is significantly higher at Gump than Sailing School by T-test (p-values <.0001).



**Figure S2.2:** Graph of tissue % Phosphorous at each site. % Phosphorous is significantly higher at Gump than Sailing School by T-test (p-values <.0001).

## CHAPTER 3:

### **A rapidly expanding alga acts as a secondary foundational species providing novel ecosystem functions in the South Pacific**

#### **Abstract**

Foundation species facilitate associated communities and provide key ecosystem functions, yet many are at risk of replacement due to anthropogenic change. Community function of these secondary species is largely unknown as current research focuses on degradation of primary foundation species. On South Pacific coral reefs, the macroalga *Turbinaria ornata* has expanded its range and habitat and may be a novel alternative foundation species to coral. We predicted increasing *T. ornata* density would facilitate growth of associated algae, resulting in a positive but novel trophic cascade to herbivorous fish. An experiment manipulating *T. ornata* densities showed a unimodal relationship between *T. ornata* and growth of understory algae, with optimal growth occurring at the most frequent natural density. Epiphyte cover also increased with density until the same optimum, but remained high with higher *T. ornata* densities. Foraging by herbivorous fishes increased linearly with *T. ornata* density. An herbivore exclusion experiment confirmed *T. ornata* facilitated epiphytes, but resource use of epiphytes by herbivores, though significant, was not affected by *T. ornata* density. Therefore, *T. ornata* functions as a secondary foundation species because it provides novel habitat to understory and epiphytic macroalgae and trophic

support to consumers, though likely this novel function is at the expense of corals.

**Key-words:** facilitation cascade; foraging behavior; secondary foundation species

## **Introduction**

Foundation species facilitate associated species and support ecosystem functions through amelioration of harsh conditions, increased trophic support, and/or provision of habitat (*sensu* Dayton 1972, Stachowicz 2001, Ellison *et al.* 2005). Foundation species often form habitat by providing physical structure for associated organisms to grow on directly or in close proximity. For example, some epiphytes grow directly on foundation species and are important for trophic support across systems (e.g. as on seagrasses, Hughes *et al.* 2004; freshwater macrophytes, Jaschinski *et al.* 2011; oak trees, Angelini & Silliman 2014). In addition, foundation species in diverse systems can provide canopy that ameliorates harsh conditions (e.g. nutrient limitation, photoinhibition, high wind or wave energy) for plants and macroalgae in the understory (for example terrestrial forests in Gentry & Dodson 1987, Ellison *et al.* 2005; kelp forests in Graham 2004).

Most studies have focused on the role of primary, or original, foundation species (reviewed in Angelini *et al.* 2011), but recent research has begun to recognize the importance of secondary foundation species – defined as those species with facilitation functions that rely on primary foundation for existence (Altieri *et al.* 2007, Thomsen *et al.* 2010, Dijkstra *et al.* 2012,

Bishop et al. 2012, Angelini and Silliman 2014, Angelini et al. 2015). In all these examples, the secondary foundation co-exists with the primary. What has received less attention is the role of secondary foundation species that both rely upon and replace primary species.

It is likely that secondary foundation species do not support the same associated organisms or ecosystem functions as the primary foundation species they replace. Studying the functioning of secondary foundation species is increasingly important as they can have large positive effects on community diversity and species abundance, though this has only been documented for co-occurring species (Altieri et al. 2007, Thomsen et al. 2010, Angelini et al. 2011, Angelini and Silliman 2014). However, shifts to secondary foundation species may come at a cost—undesired shifts due to anthropogenic impacts can promote proliferation of the secondary species over the primary. For example, warmer winter temperatures resulted in mangrove encroachment into salt marsh habitat (Osland et al. 2013), fire suppression caused historically oak dominated forests to shift to shade tolerant trees such as maples (Nowacki and Abrams 2008), and increasing human population density and fire frequency turned shrubland into grassland (Talluto and Suding 2008). In each case, shifts in terrestrial foundation species due to anthropogenic influence resulted in drastic changes to community structure and trophic support.

In marine systems, foundation species tend to be structure-forming invertebrates (e.g. mussels, Suchanek 1992; corals, Hughes et al. 2010) and marine macrophytes (e.g. kelp, Graham 2004; seagrasses, Orth et al. 2006;

rocky shore macroalgae, Korpinen *et al.* 2010). In pristine tropical systems with hard substrates corals are usually the dominant foundation species, while in nutrient-rich temperate waters fleshy macroalgae often fill this role. Coral reef macroalgae tend to be smaller, more cryptic, and more ephemeral than temperate macroalgae (reviewed by Fong & Paul 2011); however, there has been a recent increase in fleshy macroalgae on coral reefs (e.g. *Turbinaria* in Payri 1984, *Sargassum* in Hughes *et al.* 2007) attributed to decreased herbivory for *Sargassum* (Hughes *et al.* 2007) combined with increased nutrient input for *Turbinaria* (Bittick *et al.* 2016). Whether these macroalgae function as secondary foundational species in coral systems has not been evaluated. Though it is widely acknowledged that algal-domination cannot sustain net reef growth because loss of coral results in lower calcification (Gattuso *et al.* 1997), some coral reef macroalgae have been found to have positive impacts on biomass of fish (turf algae, Tootell & Steele 2016), abundance and diversity of invertebrates (Roff *et al.* 2013), and macroalgal richness (Bittick *et al.* 2010). As fleshy macroalgae have increased on many coral reefs, it is important to determine whether they can function as replacement foundation species and what functions, if any, they may provide.

Our overall objective was to evaluate if *Turbinaria ornata*, a marine macroalga that is expanding its range and habitat use in the South Pacific (Payri 1984), functions as a secondary foundation species when it replaces corals after disturbance to tropical reefs. Negative impacts of *T. ornata* on coral have been documented, including inhibiting coral recruits (Brandl *et al.*

2013) and outcompeting coral in high flow conditions (Brown and Carpenter 2014). Further, *T. ornata* benefits from anthropogenic change as increased nutrients caused a decrease in herbivory (Bittick et al. 2016). However, aggregations of *T. ornata* benefit understory macroalgae (Bittick et al. 2010) by providing a refuge from herbivores thereby increasing species richness and it may protect invertebrates and juvenile fish (*personal obs*). We ask: (1) Does *T. ornata* facilitate epiphytic and understory macroalgae? and (2) Does this facilitation cascade up to herbivorous fish through increased resources?

## Methods

*Study site and survey* — The study site was a fringing patch reef at the mouth of Opunohu Bay in Mo'orea, French Polynesia (17°28'59.81"S, 149°50'45.70"W). *Turbinaria ornata* requires hard substrate to settle and often grows in patches, or aggregations, of varying density (see Appendix S3.1, Figure S3.1 in Online Only Supporting Information). To characterize the aggregations, we constructed a density-frequency distribution from counts of thalli in 0.0625 m<sup>2</sup> areas. We randomly placed five 30 m transects, selected six random points along each, and counted the number of thalli in the nearest aggregation (N=30). Surveys were conducted in May 2012.

*Density manipulation experiment*— To measure the effect of *T. ornata* density on growth of epiphytic and understory algae and the consequences to herbivore foraging, we thinned existing aggregations of *T. ornata* (randomly selected, but initially with  $\geq 30$  thalli/0.0625m<sup>2</sup>) to create plots of 8



densities: 0, 3, 7, 10, 15, 20, 25, and 30 thalli/0.0625 m<sup>2</sup> (n=3). We avoided damselfish territories (family Pomacentridae), although a territory subsequently encroached on a plot of 15 thalli/0.0625 m<sup>2</sup> (reducing n to 2 for this treatment). Treatments were maintained for 18 days in May 2012, during which we conducted a growth bioassay within the experimental plots using a locally abundant macroalga, *Padina boryana*. Two grams (standardized wet weight) of *P. boryana* were placed in fine mesh cages and attached within the understory of each plot (see Fong et al. 2006 for method). Algae were collected after 48 hours, wet weighed, and growth was calculated as % change from initial wet weight. At the end of the experiment, three *T. ornata* thalli (5-12 cm tall) were collected randomly (except for plots where n=3) from each density plot. Photos were taken (see Appendix S3.1, Figure S3.2) and epiphyte percent cover quantified using the point intercept method in ImageJ (U.S. National Institutes of Health).

To determine the relationship between *T. ornata* density and herbivorous fish, we observed and recorded foraging behavior within density plots. Acanthuridae and Scaridae were the two most common families, though occasional Siganidae were noted (see Appendix S3.2). These families eat filamentous and fleshy macroalgae, particularly acanthurids (Francini-Filho et al. 2010), which dominated the site. Each plot was observed by the same individual on snorkel three times over the 18 days for 10 minutes (total 30 min/plot). The observer remained at least 5 meters away from the plot and recorded when fish: 1) came within 0.25 meters of the plot and 2) took a bite from the canopy, stipe, or understory of the algal aggregation.

*Epiphyte herbivory experiment*—To determine the influence of *T. ornata* density and herbivory on epiphyte load, we conducted an *in situ* 2-factor experiment manipulating *T. ornata* density (as above) and access to herbivores (+/- H). The experiment was fully crossed with three replicates of each treatment (n = 48). Herbivore access was limited by exclusion cages (5-sided; 25×25×30cm<sup>3</sup> L×W×H) constructed from hardware cloth with 1 cm openings. Light restriction by caging material was <10% with no measureable restriction to water flow in similar cages used at the same site (Clausing et al. 2014). Thalli were collected from each plot and photos were taken of 10 randomly selected thalli for analysis of initial percent cover by epiphytes. After 16 days during May-June 2014, cages were removed and three thalli were collected from each plot, photographed, and analysed in ImageJ for final percent cover by epiphytes. Initial epiphyte load was 61.6 +/- 5.6 % SEM.

*Statistical analysis*—All analyses were conducted in R (R Core Team 2015). For all response variables, linear and/or non-linear least squares models were fit to the data and compared by Akaike Information Criterion (AICc). We tested whether the relationships between *T. ornata* density and both epiphytes and understory macroalgae were best explained as either: (1) linear, (2) logistic (i.e. positive effects saturate at a certain density), (3) exponential (i.e. positive effects increase fastest at lower densities with no saturation) or (4) quadratic (i.e. positive effects decline after an optimal density) equations. The model with the lowest AICc value ( $\Delta AIC=0$ ) and highest AICc weight or, if AICs were similar ( $\Delta AIC<3-4$ ), the equation with

the lowest number of parameters was chosen by rule of parsimony (Burnham et al. 2011) and presented for each data set. Full model comparisons and fit are provided in Appendix S3.3. Further, we expected foraging behavior of herbivorous fish (as bites over a 10-minute observation period) would also follow one of these patterns in response to availability of resources. The epiphyte herbivory experiment was analysed using analysis of covariance (ANCOVA) with caging as the explanatory variable and density as a covariate.

## Results

*Survey— Turbinaria ornata* density was normally distributed (Shapiro-Wilk W Test,  $W=0.98$ ,  $P < W=0.80$ ) ranging from 0-40 thalli per  $0.0625 \text{ m}^2$ . Average density was  $19.8 \pm 1.9 \text{ SEM thalli}/0.0625 \text{ m}^2$  and 83% of the aggregations were 30 thalli or less (Figure 3.1 a).

*Density manipulation experiment—* There was an increase in epiphyte cover on *T. ornata* thalli with density until an optimum of 15 thalli/ $0.0625 \text{ m}^2$  area (Figure 3.1 b). Treatments with 3 thalli had ~40% cover by epiphytes, which increased to ~65% cover in the 15 thalli treatments and remained at this level at higher densities; thus, cover saturated in a logistic fit (Fig 3.1 b; Appendix S3.1). Similarly, macroalgae used as a bioassay for understory growth increased in biomass with *T. ornata* density up to 15 thalli/ $0.0625 \text{ m}^2$  (max = 30% growth per 48 hrs; Figure 3.1 c). After this optimum, growth declined precipitously to nearly zero in treatments with 30 thalli; this was best fit with a quadratic equation (Figure 3.1 c; Appendix S3.3)

Approximately 80 percent of all fish observed approaching and foraging in the density plots were acanthurids (see Appendix S3.2 for distribution). Foraging behaviour measured as bites per 10 minutes was modelled as a linear increase (Figure 3.1 d; Appendix S3.3). The relationship between bites per 10 minutes and *T. ornata* density was positive, with no evidence of a decline. Of the 408 observed bites, 51% were taken from the canopy, 8% along the algal stipe, and 40% in the understory at the margins of the aggregation.

*Epiphyte herbivory experiment*— *T. ornata* density facilitated and herbivores reduced abundance of epiphytes. Exclusion of herbivores and increasing *T. ornata* density both resulted in higher epiphyte cover relative to low density with presence of herbivores. As in the density manipulation experiment in 2012, the 2014 experiment showed a positive effect of *T. ornata* density on epiphytes; however, this relationship was linear instead of logistic (Figure 3.2; Appendix S3.3). We found a significant effect of caging ( $F=16.92$ ,  $P = 0.0002$ ) on percent epiphyte coverage, which was further explained by the covariate *T. ornata* density ( $F=36.43$ ,  $P<0.0001$ ). However, the accumulation of epiphytes with density (slope) is not significantly different between herbivore treatments (t-test,  $p=0.16$ ). The range in percent cover by epiphytes in 2012 and 2014 were also comparable across years (28.2–72.6 and 27.2–76.3, respectively).

## **Discussion**

Our results demonstrated *Turbinaria ornata* functions as a novel

secondary foundation species when it replaces coral on reefs of the South Pacific. We suggest this represents a facilitation cascade (e.g. Thomsen et al. 2010) where *T. ornata* attaches to hard substrate formed by dead corals after a disturbance and then becomes a secondary foundation species supporting a suite of new ecosystem functions. One line of evidence for its role as a foundation species is that, up to an optimum, increasing density of *T. ornata* also increases the abundance of associated primary producer groups such as epiphytes and understory macroalgae that are not associated with coral dominated reefs (Fong and Paul 2011). Other positive effects that have been documented to increase with density of a macroalgal foundation species include more efficient nutrient cycling (Human et al. 2015) and reduced photoinhibition (Franklin et al. 1996). In addition, the decline in growth of holdfast macroalgae in our experiment at high *T. ornata* densities, but not epiphytes, may be attributed to density-dependent increases in intensity of competition for light or nutrients. This relationship has also been found in terrestrial forests where understory species can survive in reduced light up to a critical threshold (Anderson et al. 1969) and are positively impacted by tree thinning (Canham et al. 1990, Lieffers et al. 1999), but canopy-occupying species such as epiphytes benefit from larger trees and denser canopies (Woods et al. 2015). Similarly, epiphytes in the “canopy” of *T. ornata* aggregations may not experience the same reduction in light or nutrients as understory macroalgae. Whatever the mechanism involved, our study demonstrated that *T. ornata* acts as a secondary foundation species because, once it replaces coral after a disturbance, it facilitates an associated

community of primary producers.

A second line of evidence that *T. ornata* is a secondary foundation species is its facilitation of reef consumers through enhanced food resources. Increased densities of *T. ornata* aggregations caused a facilitation cascade in which herbivory by fish was stimulated as epiphyte load and macroalgal understory increased. This is consistent with examples in terrestrial and aquatic systems in which trophic support and/or consumer abundance and diversity is negatively impacted by the loss of a foundation species (Hughes et al. 2004, Rohr et al. 2011, Angelini and Silliman 2014); however, in our study a secondary foundation species supplied this trophic support. In other systems, primary producers such as macroalgae and understory plants also increase trophic support and consumer species diversity (e.g. kelp forests, Graham 2004; temperate forests, Gilliam 2007; marshes, Angelini et al. 2015). While the majority of grazing occurred on epiphytes on the surface of the thalli within aggregations, understory macroalgae at the aggregation's edges provided additional resources to grazers. Taken together these findings suggest *T. ornata* aggregations provide more food to herbivorous fish via increased supplies of epiphytes and understory macroalgae, demonstrating *T. ornata's* role as a foundation species through enhanced trophic support.

The effects of *T. ornata* were strongly density-dependent, a phenomenon that has rarely been evaluated in studies examining foundational communities. Rather, most studies assess impacts to associated species in the presence and absence of a focal foundation species (e.g.

Graham 2004, Angelini et al. 2015). However, there are terrestrial studies that showed decreased tree canopy cover, which may be a proxy for density, reduced richness and abundance of associated species (e.g. Caners et al. 2010, Cach-Pérez et al. 2013), suggesting density effects may be important across systems. Further, we found that density effects varied across associated functional groups, with epiphytes responding linearly or logistically and understory macroalgae responding unimodally to *T. ornata* density. One possible explanation for the macroalgal response is nutrient or light limitation, which may have parallels in terrestrial systems. For example, in forests, canopy cover can have a unimodal effect on understory plant growth and diversity; in this case, nutrient input from the canopy has a positive effect while growth and diversity are negatively affected by canopy closure, creating a hump-shaped response to canopy cover (reviewed in Gilliam 2007). Thus, facilitation in the case of *T. ornata*, as in terrestrial forests, is highly density-dependent, and the density of *T. ornata* that arises after corals are removed by a disturbance can have a profound effect on reef community structure.

In summary, our results demonstrated that *T. ornata* acts as a foundation species where aggregations facilitate both primary producers and consumers on tropical reefs. Further, we suggest this represents a facilitation cascade (Thomsen et al. 2010) where corals form the hard substrate to which *T. ornata* attaches, and *T. ornata* is a secondary foundation species that provides habitat for epiphytes and increased trophic support for herbivorous fish. Much work is still needed to understand the complexity of

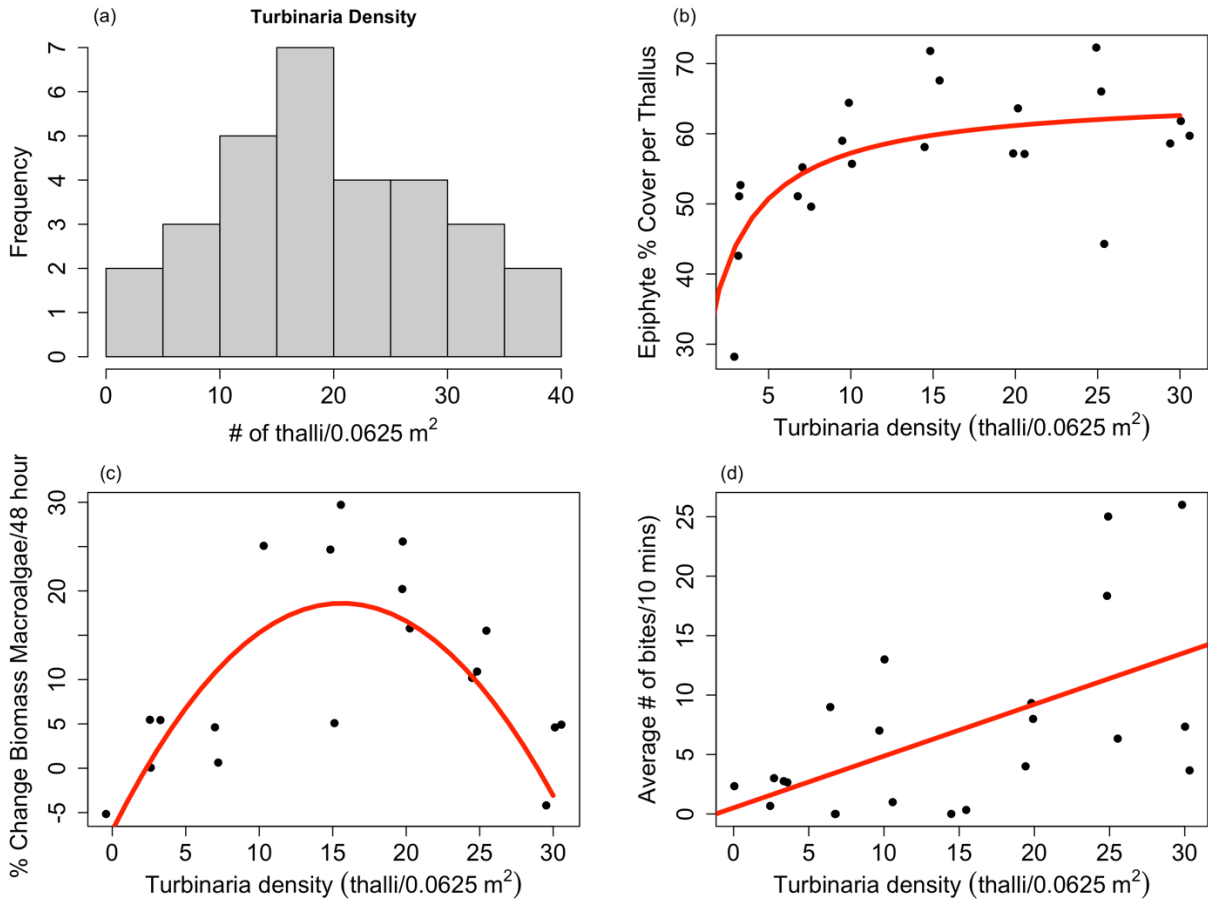
secondary foundation species in all systems, especially those in which one foundation species is replaced by another and trophic impacts are density dependent. Overall, support of a co-occurring secondary foundation species by a primary foundation species results in increased positive impacts to species diversity, abundance, and trophic interactions (as in Altieri et al. 2007, Angelini et al. 2011, 2015, Angelini & Silliman 2014). However, in cases where one foundation species is replaced by another that is independent, it is often the result of human impacts and there are “undesirable” changes to ecosystem functioning and community structure (see Ellison et al. 2005, Osland et al. 2013). Our study shows that secondary foundation species like *T. ornata*, that replace the primary foundation species they depend upon, provide a novel suite of ecosystem functions. However, these functions are likely not sustainable if *T. ornata* domination persists at the expense of the primary foundational coral community.

### **Acknowledgments**

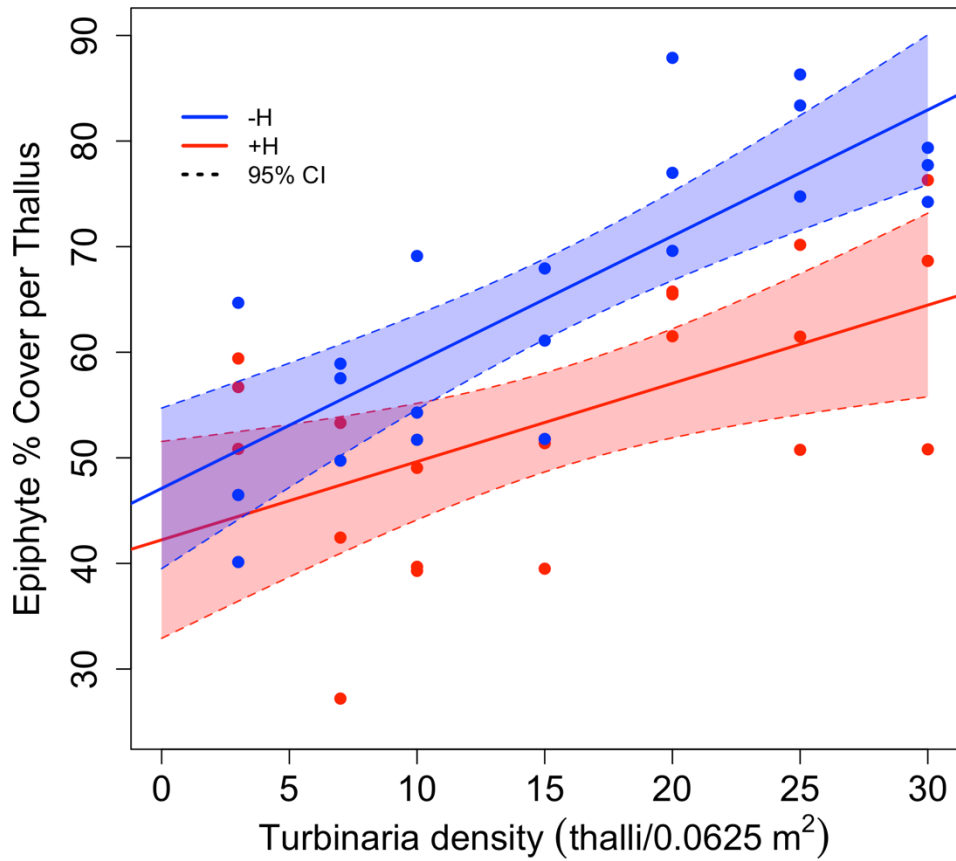
Thank you to the undergraduate students from UCLA’s field courses 2012-2016, and ImageJ processing by Von Phan and Meera Solanki. A special thanks for 2014 field assistance from Briana Fodor and funding from Aquarium of the Pacific. Funding in the field for SJB and PF was provided by UCLA’s OID and the EEB Department; RJC was funded by these sources and the NSF GRFP; and CRF was funded by a Sigma Xi Grant-in-Aid of Research (GIAR). Funding while writing was provided to SJB by the Eugene Cota-Robles Fellowship Program and NSF GRFP.



## Figures



**Figure 3.1.** (a) Results of survey of density of *T. ornata* aggregations on a fringing reef in Mo'orea, French Polynesia (b) relationship between *T. ornata* density and percent epiphyte cover modelled as a logistic fit ( $y = \frac{65.66x}{1.47+x}$ ,  $R^2=0.45$ ,  $p<0.001$ ) (c) Growth of understory macroalgae in response to *T. ornata* canopy ( $y = -7.01 + 3.28x - 0.10x^2$ ,  $R^2=0.62$ ,  $p<0.001$ ) (d) The number of bites by all fish had a positive linear relationship with *T. ornata* density ( $y = 1.49 + 0.42x$ ,  $R^2=0.30$ , and  $p<0.01$ ).



**Figure 3.2.** Relationship between *T. ornata* density and percent epiphyte cover with herbivores present (red,  $y = 42.228555 + 0.7414138 \cdot x$ ,  $r^2=0.32$ ,  $p < 0.01$ ) or absent (blue,  $y = 47.105735 + 1.1951281 \cdot x$ ,  $r^2=0.65$ ,  $p < 0.0001$ ) and the respective 95% confidence intervals between dotted lines.

## References

- Altieri, A. H., B. R. Silliman, and M. D. Bertness. 2007. Hierarchical organization via a facilitation cascade in intertidal cordgrass bed communities. *The American Naturalist* 169:195–206.
- Anderson, R. C., O. L. Louck, and A. M. Swain. 1969. Herbaceous Response to Canopy Cover, Light Intensity, and Throughfall Precipitation in Coniferous Forests. *Ecology* 50:255–263.
- Angelini, C., A. H. Altieri, B. R. Silliman, and M. D. Bertness. 2011. Interactions among Foundation Species and Their Consequences for Community Organization, Biodiversity, and Conservation. *BioScience* 61:782–789.
- Angelini, C., T. van der Heide, J. N. Griffin, J. P. Morton, M. Derksen-Hooijberg, L. P. M. Lamers, A. J. P. Smolders, and B. R. Silliman. 2015. Foundation species' overlap enhances biodiversity and multifunctionality from the patch to landscape scale in southeastern United States salt marshes. *The Royal Society Proceedings B* 282:20150421-.
- Angelini, C., and B. R. Silliman. 2014. Secondary foundation species as drivers of trophic and functional diversity: Evidence from a tree-epiphyte system. *Ecology* 95:185–196.
- Bishop, M. J., J. E. Byers, B. J. Marcek, and P. E. Gribben. 2012. Density-dependent facilitation cascades determine epifaunal community structure in temperate Australian mangroves. *Ecology* 93:1388–1401.
- Bittick, S. J., N. D. Bilotti, H. A. Peterson, and H. L. Stewart. 2010. *Turbinaria ornata* as an herbivory refuge for associate algae. *Marine Biology* 157:317–323.
- Bittick, S. J., R. J. Clausing, C. R. Fong, and P. Fong. 2016. Bolstered physical defences under nutrient-enriched conditions may facilitate a secondary foundational algal species in the South Pacific. *Journal of Ecology* 104:646–653.
- Brandl, S. J., A. S. Hoey, and D. R. Bellwood. 2013. Micro-topography mediates interactions between corals, algae, and herbivorous fishes on coral reefs. *Coral Reefs* 33:421–430.
- Brown, A. L., and R. C. Carpenter. 2014. Water flow influences the mechanisms and outcomes of interactions between massive *Porites* and coral reef algae. *Marine Biology* 162:459–468.
- Burnham, K. P., D. R. Anderson, and K. P. Huyvaert. 2011. AIC model selection and multimodel inference in behavioral ecology: some

background, observations, and comparisons. *Behavioral Ecology and Sociobiology* 65:23–35.

- Cach-Pérez, M. J., J. L. Andrade, N. Chilpa-Galván, M. Tamayo-Chim, R. Orellana, and C. Reyes-García. 2013. Climatic and structural factors influencing epiphytic bromeliad community assemblage along a gradient of water-limited environments in the Yucatan. *Tropical Conservation Science* 6:283–302.
- Caners, R. T., S. E. Macdonald, and R. J. Belland. 2010. Responses of boreal epiphytic bryophytes to different levels of partial canopy harvest. *Botany* 88:315–328.
- Canham, C. D., J. S. Denslow, W. J. Platt, J. R. Runkle, T. A. Spies, and P. S. White. 1990. Light regimes beneath closed canopies and tree-fall gaps in temperate and tropical forests. *Canadian Journal of Forest Research* 20:620–631.
- Clausing, R., C. Annunziata, G. Baker, C. Lee, S. Bittick, and P. Fong. 2014. Effects of sediment depth on algal turf height are mediated by interactions with fish herbivory on a fringing reef. *Marine Ecology Progress Series* 517:121–129.
- Dayton, P. K. 1972. Toward an understanding of community resilience and the potential effects of enrichments to the benthos at McMurdo Sound, Antarctica. Page Proceedings of the colloquium on conservation problems in Antarctica.
- Dijkstra, J. A., J. Boudreau, and M. Dionne. 2012. Species-specific mediation of temperature and community interactions by multiple foundation species. *Oikos* 121:646–654.
- Ellison, A. M., M. S. Bank, B. D. Clinton, E. A. Colburn, K. Elliott, C. R. Ford, D. R. Foster, B. D. Kloeppel, J. D. Knoepp, G. M. Lovett, J. Mohan, D. A. Orwig, N. L. Rodenhouse, W. V Sobczak, K. A. Stinson, J. K. Stone, C. M. Swan, J. Thompson, B. Von Holle, and J. R. Webster. 2005. Loss of foundation species : consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment* 3:479–486.
- Fong, P., and V. J. Paul. 2011. Coral Reef Algae. Page (Z. Dubinsky and N. Stambler, Eds.) *Coral Reefs: An Ecosystem in Transition*. Springer Netherlands, Dordrecht.
- Fong, P., T. B. Smith, and M. J. Wartian. 2006. Epiphytic cyanobacteria maintain shifts to macroalgal dominance on coral reefs following ENSO disturbance. *Ecology* 87:1162–1168.

- Francini-Filho, R. B., C. M. Ferreira, E. O. C. Coni, R. L. de Moura, and L. Kaufman. 2010. Foraging activity of roving herbivorous reef fish (Acanthuridae and Scaridae) in eastern Brazil: influence of resource availability and interference competition. *Journal of the Marine Biological Association of the United Kingdom* 90:481–492.
- Franklin, L. A., G. G. R. Seaton, C. E. Lovelock, and A. W. D. Larkum. 1996. Photoinhibition of photosynthesis on a coral reef. *Plant Cell and Environment* 19:825–836.
- Gattuso, J. P., C. E. Payri, and M. Pichon. 1997. Production, calcification, and air-sea CO<sub>2</sub> fluxes of a macroalgal-dominated coral reef community (Moorea, French Polynesia). *Journal of Phycology* 33:729–738.
- Gentry, A. H., and C. Dodson. 1987. Contribution of nontrees to species richness of a tropical rain forest. *Biotropica* 19:149–156.
- Gilliam, F. S. 2007. The Ecological Significance of the Herbaceous Layer in Temperate Forest Ecosystems. *BioScience* 57:845–858.
- Graham, H. M. 2004. Effects of local deforestation on the diversity and structure of Southern California giant kelp forest food webs. *Ecosystems* 7:341–357.
- Hughes, A. R., K. J. Bando, L. F. Rodriguez, and S. L. Williams. 2004. Relative effects of grazers and nutrients on seagrasses: A meta-analysis approach. *Marine Ecology Progress Series* 282:87–99.
- Hughes, T. P., N. A. J. Graham, J. B. C. Jackson, P. J. Mumby, and R. S. Steneck. 2010. Rising to the challenge of sustaining coral reef resilience. *Trends in ecology & evolution* 25:633–42.
- Hughes, T. P., M. J. Rodrigues, D. R. Bellwood, D. Ceccarelli, O. Hoegh-Guldberg, L. McCook, N. Moltschaniwskyj, M. S. Pratchett, R. S. Steneck, and B. Willis. 2007. Phase Shifts, Herbivory, and the Resilience of Coral Reefs to Climate Change. *Current Biology* 17:360–365.
- Human, L. R. D., G. C. Snow, J. B. Adams, G. C. Bate, and S. C. Yang. 2015. The role of submerged macrophytes and macroalgae in nutrient cycling: A budget approach. *Estuarine, Coastal and Shelf Science* 154:169–178.
- Jaschinski, S., D. C. Brepohl, and U. Sommer. 2011. The trophic importance of epiphytic algae in a freshwater macrophyte system (*Potamogeton perfoliatus* L.): Stable isotope and fatty acid analyses. *Aquatic Sciences* 73:91–101.
- Korpinen, S., V. Jormalainen, and E. Pettay. 2010. Nutrient availability modifies species abundance and community structure of *Fucus*-

- associated littoral benthic fauna. *Marine Environmental Research* 70:283–292.
- Lieffers, V. J., C. Messier, K. J. Stadt, F. Gendron, and P. G. Comeau. 1999. Predicting and managing light in the understory of boreal forests. *Canadian Journal of Forest Research* 29:796–811.
- Nowacki, G. J., and M. D. Abrams. 2008. The demise of fire and "Mesophication" of forests in the Eastern United States. *BioScience* 58:123–138.
- Orth, R. J., T. J. B. Carruthers, W. C. Dennison, C. M. Duarte, J. W. Fourqurean, K. L. Heck Jr, A. R. Hughes, G. A. Kendrick, W. J. Kenworthy, S. Olyarnik, and others. 2006. A global crisis for seagrass ecosystems. *Bioscience* 56:987–996.
- Osland, M. J., N. Enwright, R. H. Day, and T. W. Doyle. 2013. Winter climate change and coastal wetland foundation species: salt marshes vs. mangrove forests in the southeastern United States. *Global change biology* 19:1482–94.
- Payri, C. E. 1984. The effect of environment on the biology and morphology of *Turbinaria ornata* (Phaeophyta) from the Tiahura Reef (Moorea Island, French Polynesia). *Botanica Marina* 27:327–333.
- R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Roff, G., C. C. C. Wabnitz, A. R. Harborne, and P. J. Mumby. 2013. Macroalgal associations of motile epifaunal invertebrate communities on coral reefs. *Marine Ecology* 34:409–419.
- Rohr, N. E., C. S. Thornber, and E. Jones. 2011. Epiphyte and herbivore interactions impact recruitment in a marine subtidal system. *Aquatic Ecology* 45:213–219.
- Stachowicz, J. J. 2001. the Structure of Ecological Communities. *BioScience* 51:235–246.
- Suchanek, T. H. 1992. Extreme biodiversity in the marine environment: Mussel bed communities of *Mytilus californianus*. *Northwest Environmental Journal* 8:150–152.
- Talluto, M. V., and K. N. Suding. 2008. Historical change in coastal sage scrub in southern California, USA in relation to fire frequency and air pollution. *Landscape Ecology* 23:803–815.
- Thomsen, M. S., T. Wernberg, A. Altieri, F. Tuya, D. Gulbransen, K. J.

McGlathery, M. Holmer, and B. R. Silliman. 2010. Habitat cascades: The conceptual context and global relevance of facilitation cascades via habitat formation and modification. *Integrative and Comparative Biology* 50:158–175.

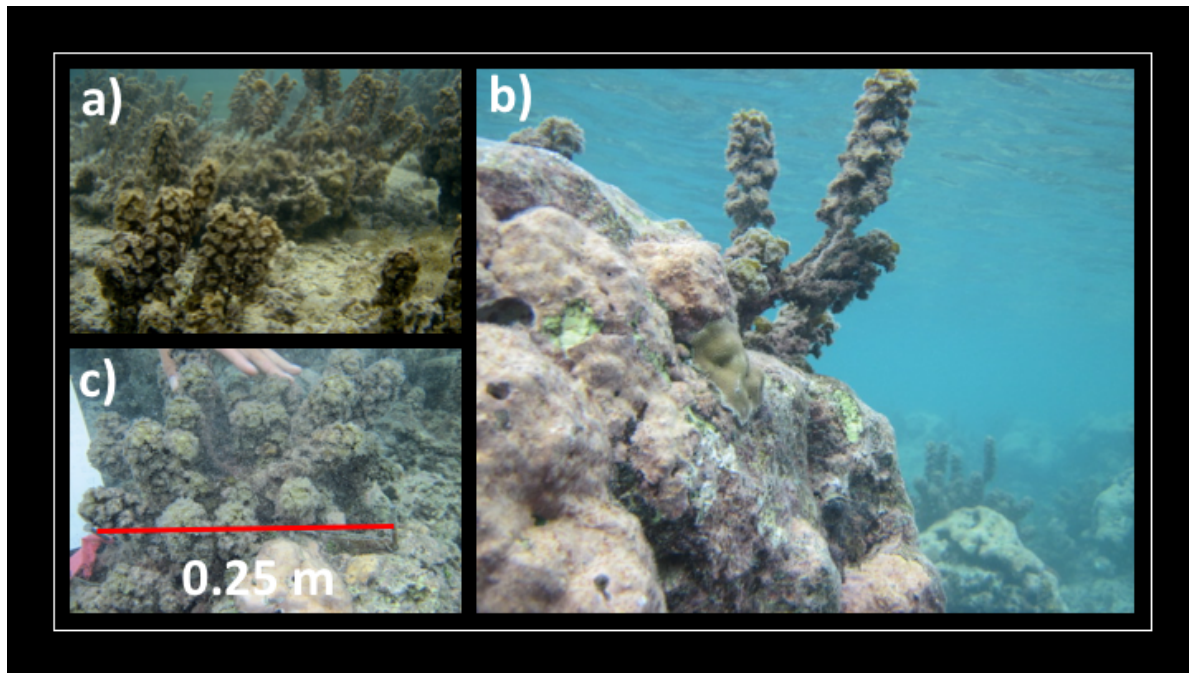
Tootell, J. S., and M. A. Steele. 2016. Distribution, behavior, and condition of herbivorous fishes on coral reefs track algal resources. *Oecologia* 181:13–24.

U.S. National Institutes of Health. 2016. ImageJ. Bethesda, Maryland, USA.

Woods, C. L., C. L. Cardelús, and S. J. Dewalt. 2015. Microhabitat associations of vascular epiphytes in a wet tropical forest canopy. *Journal of Ecology* 103:421–430.

## SUPPORTING INFORMATION

### Appendix S3.1: Sample images of *Turbinaria ornata*.



**Figure S3.1.** Examples of *Turbinaria ornata* aggregations on the reef. (a) Shows a reef flat in the lagoon with many aggregations of *T. ornata*, (b) A small aggregation (thalli = 3) growing on a nearly dead coral colony, (c) Close up of an experiment plot showing a dense (thalli = 25) aggregation of *T. ornata*.

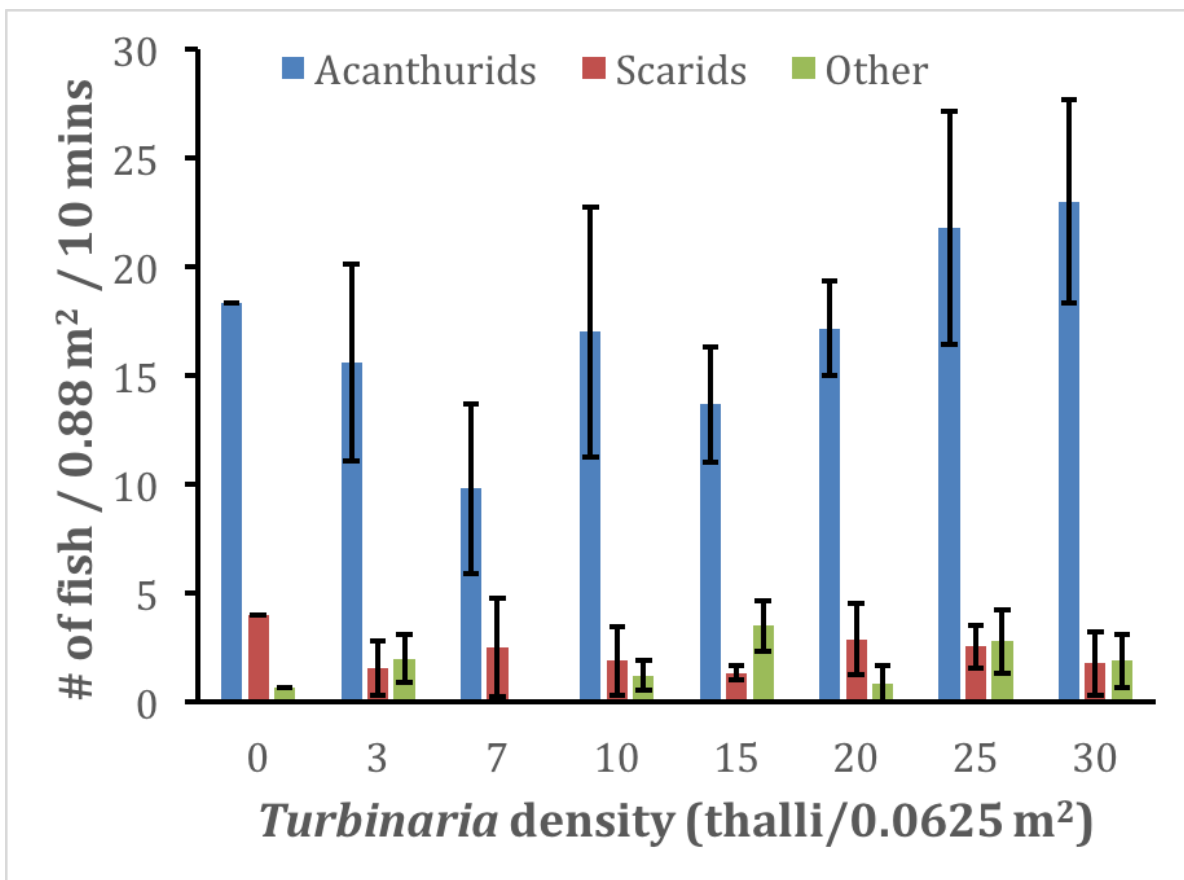




**Figure S3.2.** Example image of a *Turbinaria ornata* thallus with red and green algal epiphytes growing on its blades.

**Appendix S3.2:** Fish abundances by *Turbinaria ornata* density at our site.

**Methods.** Fish of the families Acanthuridae, Scaridae, and Siganidae were counted during the 10-minute plot observations if they came within 0.25 meters of the plot in any direction. Each density plot was observed 3 times for 10 mins, and the average +/- standard error is plotted below.



**Figure S3.3:** Average abundances of fishes by family and *T. ornata* density

**Appendix S3.3:** Least squares model fitting of the relationship between macroalgal abundance and its epiphytes

**Table S3.1.** Comparison of linear, logistic, exponential, and quadratic least squares models. Selected models are in bold.

Dependent variable (y)	Form	Equation	R <sup>2</sup>	P-value	AICc	dAICc	AICc weight
Epiphyte % cover (Figure 3.1 b)	Linear	$y = 48.64 + 0.53x$	0.2529	0.0171	163.92	6.67	0.0265
	<b>Logistic/Michaelis-Menten</b>	<b><math>y = (65.66x)/(1.47 + x)</math></b>	<b>0.4483</b>	<b>&lt;0.001</b>	<b>157.25</b>		<b>0.7440</b>
	Exponential	$y = 49.5e^{0.01x}$	0.2376	0.1350	164.37	7.12	0.0212
	Quadratic	$y = 37.49 + 2.60x - 0.06x^2$	0.4600	0.0029	159.80	2.55	0.2083
% Change macroalgal biomass (Figure 3.1 c)	Linear	$y = 7.41 + 0.18x$	0.0292	0.4839	149.65	14.74	0.0006
	Logistic	$y = 14.23 / (1 + e^{-8.99*(x-7.17)})$	0.3182	0.0481	146.20	11.29	0.0035
	Exponential	$y = 11.87 - 1.6 \times 10^{-169} * (e^{10.7x})$	0.1301	0.0906	150.83	15.92	0.0003
	<b>Quadratic</b>	<b><math>y = -7.01 + 3.28x - 0.10x^2</math></b>	<b>0.6236</b>	<b>0.0004</b>	<b>134.91</b>		<b>0.9955</b>
# bites by fish (Figure 3.1 d)	<b>Linear</b>	<b><math>y = 1.49 + 0.42x</math></b>	<b>0.3273</b>	<b>0.0054</b>	<b>149.42</b>	<b>0.13</b>	<b>0.3024</b>
	Logistic	$y = (662970.84x)/(1442196.9 + x)$	0.3259	<0.01	149.47	0.18	0.2950
	Exponential	$y = 2.38e^{0.06x}$	0.3314	<0.01	149.29		0.3230
	Quadratic	$y = -0.14 + 0.41x + 0.01(x-14.48)^2$	0.3380	0.0199	152.09	2.8	0.0796

## CONCLUSION

As anthropogenic changes to major ecological controlling forces, such as nutrient limitation and its interaction with herbivory, continue to increase worldwide it is essential that we understand the community and trophic consequences. I demonstrated how nutrient input can cause drastic changes to trophic interactions, in one case by degrading a primary foundation species and by facilitation of a secondary foundation species in the other. Human manipulations of nutrient regimes are known to impact the composition and abundance of primary producers in many ecosystems (e.g., grasslands in Tilman and Downing 1994; terrestrial and aquatic systems in Hillebrand et al. 2007). Following these changes to abiotic conditions, the original dominant species may be replaced and this is of concern when the replacement species do not provide the same community and ecological support. In a temperate seagrass bed, nutrient input and consequential blooms of macroalgae resulted in degradation of the primary seagrass foundation species. Such shifts from seagrasses to a “less desirable” macroalgal species, which do not provide the same habitat and resource services for juvenile fishes and invertebrates (Deegan et al. 2002, see Heck et al. 2003 for a review), result in drastic declines of many of these species (Mcglathery 2001, Orth et al. 2006, Waycott et al. 2009).

An increase in nutrient availability also stimulated biomass accumulation of a macroalga in the South Pacific, but through the indirect interaction of discouraging herbivores by increased algal toughness. Further, my work in chapter 3 showed that *Turbinaria ornata* provides ecosystem functions that are novel compared to those of corals. However, *T. ornata*'s interaction with the primary coral foundation species and long term effects on the reef are unclear. The overall impact to

ecosystem function by a secondary foundation species such as *T. ornata* can be negative (e.g., mangroves replacing salt marsh plants in Osland et al. 2013, Saintilan et al. 2014), positive (e.g., tree-epiphyte systems in Angelini and Silliman 2014, Angelini et al. 2015), or redundant (e.g., invasive species of seagrass replacing *Zostera marina* in Knight et al. 2015, Althea and Duffy 2016) as compared to the primary foundation species. Studies have suggested that reefs in Moorea are able to recover after disturbance (Adam et al. 2011, Kayal et al. 2012). We need to determine whether the habitat and resources provided by *T. ornata* play a role in mechanisms of reef recovery or if the alga's increase on the reef represents a stable shift to a macroalgal dominated reef (see review of phase-shifts in Hughes et al. 2010).

*Zostera marina* responded to the stressor of increased macroalgal loading, a proxy for nutrient enrichment in a smooth and predictable way (Chapter 1). *T. ornata* may respond in a similar, but positive, way to nutrient enrichment (Figure C.1 a). Alternatively, *T. ornata* may become dominant at a threshold level of nutrient input (Figure C.1 b) or exist as an alternative stable state with coral (Figure C.1 c). Alternative stable states occur when two different community-types (for example *T. ornata* and coral) can occur under the same environmental conditions, a situation known as hysteresis (between the two arrows in Figure C.1 c). In this example, only *T. ornata* may be able to persist at high nutrient levels. While there are a wide range of nutrient levels in which corals could potential coexist, it would take not only a decrease in nutrients, but a large disturbance such as physical removal by storm to push the system back to coral dominance (e.g. from  $F_2$  to  $F_1$ ).

In a world of global change, it is of utmost importance to understand how degraded systems such as coral reefs and seagrass beds will respond to a local stressor that has drastic implications for trophic relationships and biodiversity. The work presented in my dissertation provides evidence for nutrient enrichment as a key local factor responsible for community shifts. Nutrients also interact synergistically with global pressures such as warming to negatively impact foundation species in terrestrial and marine systems (Falkenberg et al. 2012, Alatalo et al. 2014, Tomas et al. 2015). Management of local stressors can reduce the negative impacts of these global stressors and improve the resilience of foundation species and their associated communities (Falkenberg et al. 2013, Strain et al. 2015). Therefore, below I propose three areas of future research to understand the consequences of, and where necessary, successfully mitigate the replacement of foundation species.

## **Future Directions**

### **1) A focus on local stressors and their interaction with global stressors**

Studies focused on the impact of local stressors, such as increased nutrient input and overfishing, to foundation species and corresponding ecosystem functioning are an important direction to inform ecosystem management. These stressors can have a profound impact on the prevalence of foundation species; in this work, negatively for seagrass and positive for *T. ornata*. Further, local stressors interact with global stressors to cause acceleration of degradation and shift competitive outcomes to favor “less desirable” species (Falkenberg et al. 2013, Russell and Connell 2014). For example, there is a synergistic benefit of increased

CO<sub>2</sub> and nutrient enrichment to turfing algal mats as they replace kelp canopies on rocky reefs in Australia (Falkenberg et al. 2013). Similar benefits have been documented for epiphytic algae in seagrass beds (Martínez-Crego et al. 2014) and macroalgae on coral reefs. Additive effects models have predicted that focus on a reduction in local stressors can reduce the impact of global stressors overall (Brown et al. 2014). This is good news for conservation efforts as we have concrete examples of success at reducing local pressures (e.g. water quality/nutrient examples and Marine Protected Areas/protection of key consumers). One reason for these successes is that it is possible to mitigate at these local levels where regional entities may have some jurisdiction.

## **2) Investigation of the resilience of primary foundation species and possibility of functional redundancy or complementarity by secondary foundation species**

However, the likelihood of success and overall need for management effort should first be evaluated through consideration of ecosystem resilience and the overall impacts of replacement species. Once local pressures that are responsible in part for ecosystem shifts are identified, we must know whether the degraded system is able to recover once the shift has occurred. In both terrestrial and aquatic systems, herbivores can compensate for a shift to weedy species (Ghedini et al. 2015). On coral reefs for example, an initial disturbance that removes coral may first result in increase of macroalgae on the reef and a subsequent increase in herbivorous fish. The herbivores reduce macroalgal cover which allows corals to recover (e.g., recovery of coral after crown-of-thorns seastar outbreak and storms

in Adam et al. 2011, Kayal et al. 2012). Therefore, there are built in mechanisms and feedbacks by which systems recover after disturbance but these feedbacks are precarious especially with increasing human influence.

Basic ecological research is still necessary in many systems to assess the complexities of ecological functions provided by primary foundation species and replacement species before resources are used on restoration. For example, the seagrass study presented in Chapter 1 is the first to identify the predictable nature of the relationship between macroalgal loading and loss of seagrass shoot density and epiphytes. This information can now be used in monitoring programs to prevent the shift from seagrass to macroalgal domination in these systems. In the case of *T. ornata* on coral reefs in the South Pacific, we are still at the point of determining the overall impact of the secondary species to the coral reef community. In the case of added (positive) or redundant ecosystem functioning by replacement species, there is not a need to expend effort to restore the system to dominance by the primary foundation species.

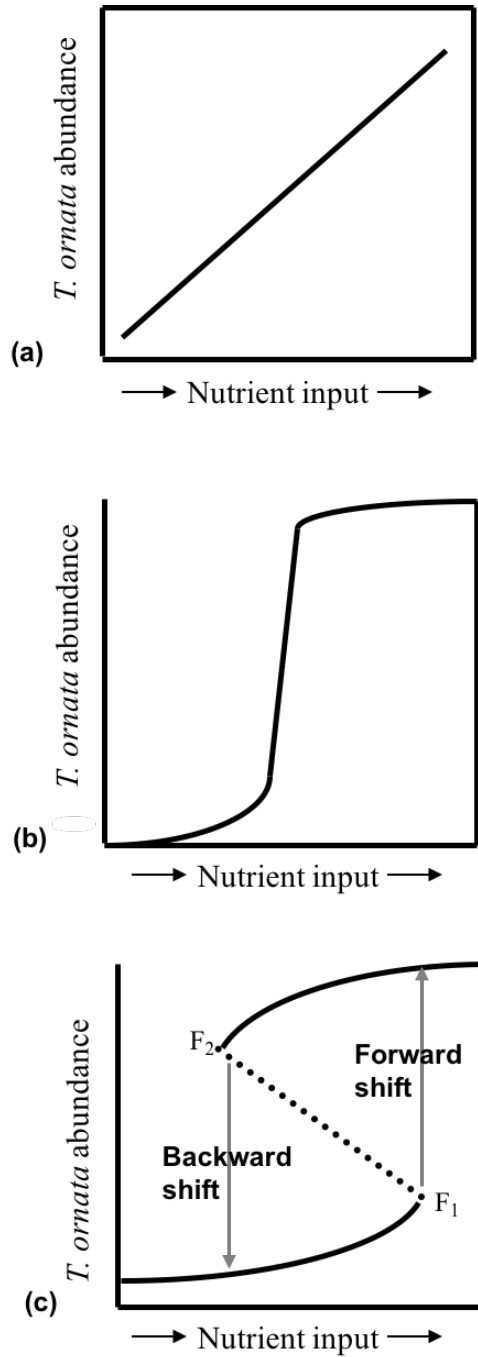
### **3) An integrated approach to understanding the complex relationships between multiple interacting species as they respond to anthropogenic influence**

The complexities of multiple interacting species, such as primary and secondary foundation species, all responding to anthropogenic influence makes ecosystem based management a difficult task. However, an approach that integrates spatial modeling and basic empirical data as evidence for development of such programs is essential. For example, fairly simple experimental approaches,



such as the nutrient enrichment and herbivore exclusion experiments conducted here, can identify how multiple species respond to multiple anthropogenic pressures. We can also use existing literature to consider the factors that determine natural range expansion and retraction (Svenning et al. 2014) and the coexistence of foundation species (examples in Bulleri et al. 2016). Spatial models can then be utilized to examine potential range shifts of multiple species move through a landscape (see Hodgson et al. 2016 for an example model) under historical, degraded, and predicted management intervention conditions. As multiple human manipulations of forces controlling species abundance and range boundaries continue to increase, we will need to use every tool possible to ensure any opportunity for effective management is not missed.

## Figures



**Figure C.1.** *T. ornata* vs coral dominance on reefs under three predictions of phase shifts: (a) predictable linear, (b) simple threshold, and (b) alternative stable states response with regard to the stressor of increasing nutrients.

## References

- Adam, T. C., R. J. Schmitt, S. J. Holbrook, A. J. Brooks, and P. J. Edmunds. 2011. Herbivory, Connectivity, and Ecosystem Resilience: Response of a Coral Reef to a Large-Scale Perturbation. *PLoS ONE* 6:e23717.
- Alatalo, J., A. Jägerbrand, and U. Molau. 2014. Seven years of experimental warming and nutrient addition causes decline of bryophytes and lichens in alpine meadow and heath communities. *PeerJ*:1–22.
- Althea, F. P. M., and J. E. Duffy. 2016. Foundation species identity and trophic complexity affect experimental seagrass communities. *Marine Ecology Progress Series* 556:105–121.
- Angelini, C., T. van der Heide, J. N. Griffin, J. P. Morton, M. Derksen-Hooijberg, L. P. M. Lamers, A. J. P. Smolders, and B. R. Silliman. 2015. Foundation species' overlap enhances biodiversity and multifunctionality from the patch to landscape scale in southeastern United States salt marshes. *The Royal Society Proceedings B* 282:20150421-.
- Angelini, C., and B. R. Silliman. 2014. Secondary foundation species as drivers of trophic and functional diversity: Evidence from a tree-epiphyte system. *Ecology* 95:185–196.
- Brown, C. J., M. I. Saunders, H. P. Possingham, and A. J. Richardson. 2014. Interactions between global and local stressors of ecosystems determine management effectiveness in cumulative impact mapping. *Diversity and Distributions* 20:538–546.
- Bulleri, F., J. F. Bruno, B. R. Silliman, and J. J. Stachowicz. 2016. Facilitation and the niche: Implications for coexistence, range shifts and ecosystem functioning. *Functional Ecology* 30:70–78.
- Deegan, L. A., A. Wright, S. G. Ayvazian, J. T. Finn, H. Golden, R. R. Merson, and J. Harrison. 2002. Nitrogen loading alters seagrass ecosystem structure and support of higher trophic levels. *Aquatic Conservation: Marine and Freshwater Ecosystems* 12:193–212.
- Falkenberg, L. J., S. D. Connell, and B. D. Russell. 2013. Disrupting the effects of synergies between stressors: Improved water quality dampens the effects of future CO<sub>2</sub> on a marine habitat. *Journal of Applied Ecology* 50:51–58.
- Falkenberg, L. J., B. D. Russell, S. D. Connell, C. Folke, and B. Walker. 2012. Stability of Strong Species Interactions Resist the Synergistic Effects of Local and Global Pollution in Kelp Forests. *PLoS ONE* 7:e33841.
- Ghedini, G., B. D. Russell, and S. D. Connell. 2015. Trophic compensation reinforces resistance: Herbivory absorbs the increasing effects of multiple

- disturbances. *Ecology Letters* 18:182–187.
- Heck, K. L., G. Hays, and R. J. Orth. 2003. Critical evaluation of the nursery role hypothesis for seagrass meadows. *Marine Ecology Progress Series* 253:123–136.
- Hillebrand, H., D. S. Gruner, E. T. Borer, M. E. S. Bracken, E. E. Cleland, J. J. Elser, W. S. Harpole, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Consumer versus resource control of producer diversity depends on ecosystem type and producer community structure. *Proceedings of the National Academy of Sciences of the United States of America* 104:10904–9.
- Hodgson, J. A., D. W. Wallis, R. Krishna, and S. J. Cornell. 2016, December. How to manipulate landscapes to improve the potential for range expansion. *Methods in Ecology and Evolution* 7:1558–1566.
- Hughes, T. P., N. A. J. Graham, J. B. C. Jackson, P. J. Mumby, and R. S. Steneck. 2010. Rising to the challenge of sustaining coral reef resilience. *Trends in ecology & evolution* 25:633–42.
- Kayal, M., J. Vercelloni, T. Lison de Loma, P. Bosserelle, Y. Chancerelle, S. Geoffroy, C. Stievenart, F. Michonneau, L. Penin, S. Planes, and M. Adjeroud. 2012. Predator Crown-of-Thorns Starfish (*Acanthaster planci*) Outbreak, Mass Mortality of Corals, and Cascading Effects on Reef Fish and Benthic Communities. *PLoS ONE* 7:e47363.
- Knight, N. S., C. Prentice, M. Tseng, and M. I. O'Connor. 2015. A comparison of epifaunal invertebrate communities in native eelgrass *Zostera marina* and non-native *Zostera japonica* at Tsawwassen, BC. *Marine Biology Research* 11:564–571.
- Martínez-Crego, B., I. Olivé, and R. Santos. 2014. CO<sub>2</sub> and nutrient-driven changes across multiple levels of organization in *Zostera noltii* ecosystems. *Biogeosciences* 11:7237–7249.
- Mcglathery, K. J. 2001. Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched coastal waters. *Journal of Phycology* 37:453–456.
- Orth, R. J., T. J. B. Carruthers, W. C. Dennison, C. M. Duarte, J. W. Fourqurean, K. L. Heck Jr, A. R. Hughes, G. A. Kendrick, W. J. Kenworthy, S. Olyarnik, and others. 2006. A global crisis for seagrass ecosystems. *Bioscience* 56:987–996.
- Osland, M. J., N. Enwright, R. H. Day, and T. W. Doyle. 2013. Winter climate change and coastal wetland foundation species: salt marshes vs. mangrove forests in the southeastern United States. *Global change biology* 19:1482–94.
- Russell, B. D., and S. D. Connell. 2014. Ecosystem Resilience and Resistance to Climate Change. Pages 133–139 *Global Environmental Change*. Springer

Netherlands, Dordrecht.

- Saintilan, N., N. C. Wilson, K. Rogers, A. Rajkaran, and K. W. Krauss. 2014. Mangrove expansion and salt marsh decline at mangrove poleward limits. *Global change biology* 20:147–57.
- Strain, E. M. A., J. van Belzen, J. van Dalen, T. J. Bouma, and L. Airoidi. 2015. Management of Local Stressors Can Improve the Resilience of Marine Canopy Algae to Global Stressors. *PLOS ONE* 10:e0120837.
- Svenning, J. C., D. Gravel, R. D. Holt, F. M. Schurr, W. Thuiller, T. Münkemüller, K. H. Schiffers, S. Dullinger, T. C. Edwards, T. Hickler, S. I. Higgins, J. E. M. S. Nabel, J. Pagel, and S. Normand. 2014. The influence of interspecific interactions on species range expansion rates. *Ecography* 37:1198–1209.
- Tilman, D., and J. A. Downing. 1994. Biodiversity and stability in grasslands. *Nature* 367:363–365.
- Tomas, F., B. Martínez-Crego, G. Hernán, and R. Santos. 2015. Responses of seagrass to anthropogenic and natural disturbances do not equally translate to its consumers. *Global Change Biology* 21:4021–4030.
- Waycott, M., C. M. Duarte, T. J. B. Carruthers, R. J. Orth, W. C. Dennison, S. Olyarnik, A. Calladine, J. W. Fourqurean, K. L. Heck, A. R. Hughes, G. A. Kendrick, W. J. Kenworthy, F. T. Short, and S. L. Williams. 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences of the United States of America* 106:12377–81.