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Los Angeles

Developing tools and approaches for understanding ecological strategies
and promoting resilience across marine algal communities

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

by

Lauren Lee Smith

2023

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ABSTRACT OF THE DISSERTATION

Developing tools and approaches for understanding ecological strategies
and promoting resilience across marine algal communities

by

Lauren Lee Smith

Doctor of Philosophy in Biology

University of California, Los Angeles, 2023

Professor Peggy Marie Fong, Chair

Anthropogenic stressors reshape communities worldwide, motivating research on predictions of future impacts as well as developing tools and approaches for promoting resilience. My research focuses on marine algal communities in two habitats, temperate rocky intertidal zones and tropical fringing reefs, that have undergone widespread shifts due to global and local stressors.

For over four decades, ecologists have improved understanding of how plant communities shift in response to environmental drivers using a trait-based framework. In **Chapter 1**, I develop a quantitative method for determining traits that are critical to measure for understanding the performance of rocky intertidal macroalgae. I measured a wide suite of traits then used ordination and correlation to reduce to six traits that limit collinearities, maximize potential tradeoffs, and create a functionally diverse trait space. I discovered an axis of variation for maximizing resource acquisition that varies between being tall and strong versus maximizing

surface area and being short and weak. This work provides a roadmap for trait selection that I test in **Chapter 2** for macroalgae on a tropical fringing reef. By following this same quantitative approach, I determine a core set of traits important for understanding ecological strategies of tropical algae. I select five traits critical to understand this temperate fringing reef and discover two axes of variation for resource acquisition and resistance to herbivory. By comparing Chapter 1 and 2, we find three traits useful in describing these diverse communities, suggesting there is a set of universally important traits that will facilitate comparisons between algal-dominated communities across multiple scales.

In **Chapter 3**, I test the effects of tropical storms on turf algae, a critical transition community on reefs that can facilitate coral recovery. Understanding the impacts of storms is important as their intensity is projected to increase with climate change. In a field experiment we simulate a storm with physical abrasion, sediment deposition, increased nutrients, and altered herbivore activity as stressors. We found that herbivores can reverse storm-generated transitions on coral reefs.

In summary, my dissertation advances the development of novel approaches for understanding the shifting functions of algal communities and improves our ability to predict the responses of tropical algal communities to climate change.

The dissertation of Lauren Lee Smith is approved.

Kyle C. Cavanaugh

Nathan Jared Boardman Kraft

James O. Lloyd-Smith

Peggy Marie Fong, Committee Chair

University of California, Los Angeles

2023

To Clara and Calder, Emerson, Isaiah, Wade and Attie, Dean, Lena and Rebekah

In you I know hope for the future, for you I work to build a better one.

To Gene, Harry, and Bud

I wish I could share this with you. Miss you.

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LIST OF ACRONYMS

Acronym	Description
ANOVA	Analysis of Variance
BO	Branching order
CaCO ₃	Calcium carbonate
CCA	Crustose corraline algae
CG	Campground, Catalina Island
CO ₂	Carbon dioxide
DW	Dry weight
DW:WW	Dry weight to wet weight ratio
FGM	Functional Group Model
GAMM	General Additive Mixed Model
GLM	General Linear Model
GLMM	General Linear Mixed Model
H	Height
HCl	Hydrochloric acid
H:DW	Height to dry weight ratio
H:WW	Height to wet weight ratio
HF _{ww}	Holdfast wet
HF _{ww} :WW	Holdfast wet weight to total wet weight ratio
HT	Holdfast thickness weight
LH	Little Harbor, Catalina Island
MCR-LTER	Moorea Coral Reef Long Term Ecological Research

N	Nitrogen
TBE	Trait-Based Ecology
T	Toughness
TS	Tensile strength
P	Perimeter (Chapter 1)
P	Phosphorous (Chapter 3)
PC	Principal Components
PCA	Principal Components Analysis
SA	Surface area
SA:DW	Surface area to dry weight ratio
SA:VL	Surface area to volume ratio
SH	Shark Harbor, Catalina Island
SE	Standard Error
SLA	Specific Leaf Area
VL	Volume
W	Width
WEIS	Wrigley Institute for Environment and Sustainability
WW	Wet weight
W2H	West Two Harbors, Catalina Island
%C	Percent calcium carbonate
+/0D	Addition/no addition of a one-time abrasion event
+/0N	Addition/ambient nutrients
+/0S	Addition/ambient sediment deposition

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Chapter 3 is a version of Smith L.L., Soriano A.R.P., Gould K.E., Yee S.K., Rynzar E.R., Fong P. in review *Coral Reefs*. Experimental manipulation of pulse disturbances associated with tropical storms initiate transitions from short to long turf that are reversed by herbivory. This chapter was developed with students, Arianna Rose Soriano, Katie Gould, and Shelby Yee as part of the Marine Biology Quarter. Nathan Hwangbo at UCLA stats consulting helped immensely by giving statistics advice.

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Ryznar ER, **Smith LL**, Hà BA, Grier SR, Fong P. Functional trait variability supports the use of mean trait values and identified tradeoffs for marine macroalgae. *Journal of Ecology*.

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Smith, L.L., Ryznar E.R., and Fong, P. 2019. “High temperatures may facilitate invasion of *Sargassum horneri*”. Poster presentation. Western Society of Naturalists Annual Meeting, Ensenada, MX.

Smith, L.L., Ryznar E.R., and Fong, P. 2018. “Invasive *Sargassum horneri* experiences positive growth at elevated temperatures”. Poster presentation. Western Society of Naturalists Annual Meeting, Tacoma, WA.

CHAPTER 1

What traits are critical to capture ecological processes shaping intertidal macroalgal communities? A roadmap for selecting traits

ABSTRACT

1. Increased impacts from anthropogenic stressors are motivating the search for novel tools and approaches to understand ecosystem shifts and community changes. While terrestrial ecologists made great strides in understanding how plant communities shift in response to environmental drivers using a trait-based framework, the functional ecology of marine macroalgae remains centered on a functional group model from the 1980's that has recently been debunked. Our objective was to determine what traits of rocky intertidal macroalgae are critical to capture the diversity of ecological strategies used to maintain ecological functions.
2. Macroalgal individuals must balance their need to compete for resources, resist damage or loss due to physical disturbance, and avoid herbivory. Leveraging decades of terrestrial research, we identified and measured 16 functional traits on 212 individual algal thalli that contribute to resource acquisition, resistance to herbivory, or resistance to physical disturbance. We used ordination to collapse multidimensional trait space into 2 dimensions (full model), and then used Principal Components Analysis (PCA), loading plots and correlation analysis to select 6 traits for a reduced model.
3. For the full model, 13 traits contributed more than the null expectation of equal contribution to the PCA. Many were strongly and positively correlated, suggesting trait syndromes that captured two ecological strategies for maximizing resource acquisition: macroalgae in the intertidal can be tall and strong or short with high surface area relative

to structure or size. Other traits were negatively correlated revealing tradeoffs; for example, an individual cannot be both tall and have a high surface area to volume ratio. After eliminating traits that were highly correlated or contributed little to creating trait space, we re-ran the analyses using six traits: height, tensile strength, dry weight to wet weight ratio, surface area to volume ratio, and surface area to dry weight ratio. The first two PCs for these six traits explained slightly more variation in traits compared to the original 16.

4. *Synthesis.* We posit that only 6 traits need to be measured to capture trait variation in intertidal macroalgae, streamlining future research. We also furthered understanding of ecological strategies that balance an individual's need to acquire resources and resist removal by physical disturbance or herbivory in marine macroalgae. Finally, our process provides a roadmap for selecting traits that can be used in future studies to rapidly advance exploration of trait-based ecology in other macroalgal-dominated ecosystems. These findings demonstrate the value of a trait-based approach in this system and provide a strong foundation to continue developing trait-based ecology for macroalgae across ecosystems.

INTRODUCTION

There is a critical need for novel tools and approaches for understanding and promoting ecosystem resilience in the Anthropocene as natural systems globally are increasingly challenged by climate change and loss of habitat and biodiversity (Walther et al. 2002; Otto 2018). Over the past few decades, our understanding of community response to environmental drivers in terrestrial plant communities has been accelerated by the development of trait-based ecology (TBE) (e.g. Cornwell and Ackerly 2009; Messier et al. 2010). One novel approach that has

provided insight to ecological strategies is to examine trait tradeoffs, which occur when specializing in one strategy leads to reducing another, and trait syndromes, or suites of traits that frequently co-occur (Macarthur and Wilson 1967; Grime 1977; Law 1979; Rees 1993). For example, three distinct ecological strategies for angiosperms have been identified by syndromes and tradeoffs; plants 1) with a suite of traits that promotes drought tolerance, 2) with a suite that produces shade tolerance, or 3) that trade off fast growth for stress tolerance (Stahl et al. 2013). Understanding ecological strategies advances our ability to predict plant community responses to major challenges, including climate change (Beukhof et al. 2019), invasive species (Chan et al. 2021), and loss of biodiversity (Hillebrand and Matthiessen 2009; Wolf et al. 2021). These rapid advances, driven by a trait-based approach, continue to motivate further expansion of the use of TBE across systems and taxa.

As TBE expands beyond terrestrial plants, researchers in a wide diversity of fields have leveraged the early advances made by plant ecologists to make significant and rapid progress across other taxa. Currently, TBE has furthered our understanding across a wide diversity of community types including phytoplankton (e.g., Litchman and Klausmeier 2008), birds (e.g., Pigot et al. 2016), corals (e.g. Darling et al. 2012), microbes (for review, see Krause et al. 2014) and mammals (e.g. Kohli et al. 2021). For example, in marine fishes TBE has been used to relate traits to environmental conditions and fishing pressure (Beukhof et al. 2019). Another study used traits to understand wind turbine collision risks for over 10,000 species of birds and bats, findings that can inform construction of future wind farms (Thaxter et al. 2017). These examples of the use of TBE propelling rapid advances in our understanding of the relationship between traits and ecosystem functioning in other systems provides strong motivation to continue its expansion to new systems and taxon.

While TBE has proven to be a powerful approach for other producer communities, this approach is just beginning to be developed for marine macroalgae (e.g., Jänes et al. 2017; Cappelatti et al. 2019; Mauffrey et al. 2020; Lloyd et al. 2020; Stelling-Wood et al. 2020; Carvalho et al. 2022; Ryznar et al. 2023). In contrast to terrestrial plant ecology, research on algal communities in benthic marine ecosystems remained mired in the functional group model (FGM) for over 40 years (e.g., Littler and Littler 1980; Steneck and Dethier 1994). The FGM framework relies on the overarching assumption that a few gross morphological traits of macroalgae reflect a shared ecological role, process, or function. This framework has recently been debunked by research demonstrating that the FGM violates this assumption and does not consistently group functionally similar species (Mauffrey et al. 2020; Ryznar et al. 2021). In contrast, early work exploring TBE for macroalgae shows promise that a suite of algal traits can provide insights into habitat provision for associated invertebrate species (Carvalho et al. 2022; Stelling-Wood et al. 2020; Lloyd et al. 2020) and capture ecological functions and strategies (Cappelatti et al. 2019; Fong et al. 2023; Ryznar et al. 2023), motivating our exploration of TBE for understanding key ecological strategies and functions for marine macroalgae.

Selecting relevant functional traits is critical to advance the use of a trait-based framework for marine macroalgae, and it is widely acknowledged that the nature and number of traits that are included in any analysis (de Bello et al. 2021) depends on the question asked or process studied. Functional traits are defined as any metric that is related to performance (Violle et al. 2007), or the ability of an individual to survive, grow and reproduce (de Bello et al. 2021). Thus, to select traits for marine macroalgae, we must identify the environmental drivers constraining individual performance. For marine macroalgae to perform, individuals must balance the need to acquire resources such as sunlight and inorganic nutrients needed to grow

(Carpenter 1990), avoid being consumed (Dayton 1985), and withstand loss due to physical disturbance by abiotic factors such as wave action and desiccation (Dayton 1985; Norton 1991; Kawamata 2001). Here, we seek to understand the different ecological strategies that algal species employ to maximize performance under these constraints. Thus, to determine candidate traits to explore strategies for marine macroalgae, we will leverage the advances in other fields with a strong TBE foundation, such as terrestrial plant and phytoplankton ecology (Funk et al. 2017; Litchman and Klausmeier 2008), where continuous traits are used to represent ecological functions.

Rocky intertidal zones provide an ideal landscape to select algal traits critical to exploring ecological strategies due to the presence of strong vertical gradients in biotic and abiotic environmental drivers that likely create a high diversity landscape of functional traits (Mauffrey et al. 2020). These gradients and drivers can impact the ecological functions of interest: resource acquisition, resistance to physical disturbance, and resistance to herbivory. For example, algae compete to acquire light and nutrients, with the intensity of competition shifting along the gradient from high tide zones, that are fully exposed to light during low tide, to low tidal zones, where algae have access to nutrients in the water. Further, the risk of herbivory changes based on elevation and herbivore species (Thornber et al. 2008). Finally, algae are at increased risk for damage from other abiotic factors such as sun exposure and temperature fluctuations in the higher intertidal (Benedetti-Cecchi and Trussell 2014). Temperate intertidal systems have been the focus for some of the earliest macroalgal TBE (Jänes et al. 2017; Cappelatti et al. 2019; Mauffrey et al. 2020; Lloyd et al. 2020). While there has had some overlap in traits measured, there has been no systematic methodology for selecting traits and no

core set of traits for understanding these three functions. For these reasons, rocky intertidal communities provide an ideal setting for our exploration of trait selection.

Our overarching objective is to explore what traits of rocky intertidal macroalgae are critical to capture the diversity of ecological strategies used to maintain the ecosystem functions of resource acquisition, resistance to physical disturbance, and resistance to herbivory. To meet this objective, we will advance development of TBE for macroalgae by: 1) measuring a wide suite of traits on rocky intertidal algae that we relate to different ecological functions based on existing studies on algae and other producer communities (hereafter defined as the full model), 2) determining what traits are most important in creating a functionally diverse trait space, 3) selecting a subset of these traits based on reducing collinearities and maximizing potential tradeoffs (hereafter defined as the reduced model), and 4) assessing the ability of the reduced model to create a functionally diverse trait space.

METHODS

Overall approach

In the full model we included 16 traits, 5 of which are ratios, based on metrics commonly measured for algae as well as analogs to those measured for other producer groups. We used Principal Components Analysis (PCA) to determine what traits contributed most to creation of trait space for this full model and a correlation approach to explore bivariate relationships between traits. We then constructed a reduced model by: 1) using Pearson's correlation to identify suites of strongly collinear traits, 2) choosing one trait from any suite of positively correlated traits with the same proposed function that is important in creating trait space as representative and eliminating the others, 3) retaining pairs of traits that are negatively correlated as they represent potential tradeoffs, and 4) retaining some traits that are more direct analogs of

traits commonly used for other primary producers. Based on these criteria, we reduced our included traits to six and these were subject to the same analysis as traits in the full model.

Study species and collection methods

We reasoned that capturing both morphological and habitat diversity is important for exploring what traits are critical when building a trait-based framework for macroalgae that assesses the ecosystem functions of resource acquisition, resistance to herbivory and resistance to physical disturbance. Therefore, we designed our sampling protocol to capture the greatest potential diversity of macroalgal traits in the intertidal habitats of Catalina Island, one of the Channel Islands off the coast of Southern California. The Channel Islands are a region with known high algal biodiversity (Murray et al. 1980) and therefore an ideal study site to meet our objective.

To capture a broad range of both interspecific and intraspecific trait variability, we chose seven common species of marine macroalgae with very diverse gross morphologies that occupy habitats ranging from intertidal to shallow subtidal. We collected algae from two leeward and two windward sites ($n = 4$ sites) in two seasons (August, November). We did so because competition in the intertidal change as the tidal amplitudes shift (Benson et al. 1983). In addition, environmental conditions vary across seasons in this location (waves and wind: Cao et al. 2018) and windward and leeward shores are known to be subject to different biotic and environmental drivers such as temperature (Iacchei et al. 2005) and wave action (Kench et al. 2009). Thus, we reasoned that collecting across these sites and seasons should maximize the functional diversity of algal traits.

The seven marine macroalgal species we selected differed across two axes of diversity, taxonomic and functional. Taxonomically, these species span 6 families and include two green

algae (division Chlorophyta; *Ulva spp.* and *Codium fragile*), one red alga (division Rhodophyta; *Laurencia pacifica*), and four brown algae (class Phaeophyceae; *Dictyota flabellata*, *Colpomenia sp.*, *Pelvetiopsis californica*, and *Silvetia compressa*). We will use the genus names hereafter.

These species represent a wide range of complexity and gross morphology. All algal descriptions below are adapted from data collected in Fong et al. (2023) and can be found in their supplemental table.

Ulva and *Dictyota* are the least complex species. *Ulva* is two cells thick, undergoes diffuse growth, and attaches via rhizoidal cells while *Dictyota* is three cells thick, dichotomously branched, and has a discoid holdfast and apical growth (for images of all species, see Fig. 1-1). *Colpomenia*, our next most complex alga, has a globular, prostrate morphology with broad attachment across the base. Next in complexity are *Laurencia* and *Codium*, both of which are upright in the water column. *Laurencia* is irregularly branched with a discoid holdfast and apical growth. *Codium* is coenocytic (single cell, multinucleate), dichotomously branched, has a crustose holdfast, and grows via centripetal invagination. The most complex species are *Silvetia* and *Pelvetiopsis*. Both are dichotomously branched, grow from apical meristems, and have leaf-like blades, branches, and holdfasts with finger-like projections.

Algae that appeared to be healthy and intact were haphazardly collected in each of the 4 sites. All individuals were collected during low tide from the lowest to the highest intertidal zones. Due to differential availability, replication varied across species; *Ulva* - 28, *Dictyota* - 17, *Colpomenia* - 34, *Laurencia* - 44, *Codium* - 26, *S. compressa* - 39, and *Pelvetiopsis* - 24. For algae that do not grow as clear single individuals (some samples of *Pelvetiopsis*, *Silvetia*), we collected samples that, when removed, came off together and remained whole/connected. This is akin to a ‘module’ that is used when collecting grasses (Gubsch et al 2011).

All collections and measurements were performed in August and November 2021, with care taken to collect samples of each species in each season. Algae were kept in outdoor, flow-through tanks at the USC Wrigley Marine Science Center until processed. Measurements were made within 36 hours of collection, except for one individual (*Pelvetiopsis*) that was processed 60 hours post collection; however, we assessed how the traits of this individual compared to conspecifics and found all traits had z scores between -1.32 and 0.98; traits with a z score greater than 3 or less than -3 are considered outliers (Shiffler 1988).

Trait selection and methods

We chose 16 traits that we reasoned had the potential to be functional because they are either analogs to well-vetted traits for terrestrial plants or phytoplankton or traits forming the foundation of the functional group models for marine macroalgae (Littler and Littler 1980; Steneck and Dethier 1994). All 16 traits are continuous variables measured at the individual thallus level. Of these, 11 are direct measurements and 5 are derived traits (ratios). Traits that were measured directly include: wet weight, height, width, perimeter, surface area, volume, dry weight, holdfast wet weight, holdfast thickness, branching order, and tensile strength. Ratios include dry weight to wet weight, surface area to volume, surface area to dry weight, height to dry weight, and holdfast wet weight to total wet weight.

Hypothesized functions of traits in full model

We hypothesized that our 16 traits belong in three functional categories: resource acquisition, resistance to physical disturbance, and resistance to herbivory. Using existing marine and terrestrial literature, we categorized each trait into one or more of these three ecological functions. We have 11 traits that contribute to resource acquisition, six traits that contribute to resistance to physical disturbance, and two traits that contribute to resistance to herbivory (see

Supplement 1, Fig. 1-1). While these traits are not equally distributed between functions, we made the most parsimonious decision and did not differentially weight them as we had no *a priori* reason to do so.

Traits that Maximize Resource Acquisition

Seven of the eight measured traits hypothesized to maximize resource acquisition represent some aspect of thallus size: height, width, surface area, wet weight, dry weight, volume, and perimeter. Size is important as larger algae are better at competing for resources such as light (Carpenter 1990) and nutrients. Height (H) is commonly measured for terrestrial canopy-forming plants, as taller individuals increase light capture and reduce shading (e.g., Maes et al. 2020). Width (W) measures a different aspect of resource capture, as some algae grow wide and not tall (Marks et al. 2018), a trait that may increase access to resources. Surface area (SA) is another aspect of size that relates to resource acquisition; for example, one study shows that net productivity of algae increases with thallus surface area (Stewart and Carpenter 2003), likely because algae take up both nutrients and light over the entire thallus surface. Volume (VL) is another measure of size that can impact growth rate and resource acquisition - both nutrients and light (Kirk 1994). Note that we measured wet weight (WW), dry weight (DW) and perimeter (P) and, to our knowledge, these have not yet been empirically linked to resource acquisition in algae or terrestrial plants, at least as a direct measure. However, WW and DW are commonly used as measures of the size of an algal thallus, and P was easily measured concurrent with SA.

Branching order (BO) is included in resource acquisition, as increased branching can lead to a partitioning of resource acquisition. For example, upper thallus portions can have higher photosynthetic rates than lower portions (Hay 1981) possibly leaving lower branches for nutrient uptake (Colombo-Pallotta et al. 2006).

We include three ratios in resource acquisition as ratios can provide insight into tradeoffs. As surface area to dry weight ratio (SA:DW) increases, we expect increased allocation to resource acquisition (surface area) compared to size (dry weight) (Littler 1980). This is analogous to specific leaf area used for terrestrial plants (e.g., Maes et al. 2020) because resource acquisition occurs across the whole surface of the thallus. Next, we included surface area to volume ratio (SA:VL), an important functional trait for understanding resource acquisition (Castro and Huber 2008) that has been used for phytoplankton (Kruk et al. 2010). As thallus SA:VL increases, the rate of nutrient uptake increases (Odum et al. 1958; Carpenter 1990) and self-shading decreases (Carpenter 1990). The last is height to dry weight ratio (H:DW), a measure of effort put toward growing taller than competitors compared to overall size (Steneck and Dethier 1994).

Traits that Maximize Resistance to Disturbance

We include six traits, of which four are direct measures and two are ratios, that contribute to resistance to disturbance: branching order, tensile strength, holdfast thickness, holdfast wet weight, holdfast wet weight to thallus wet weight ratio, and dry weight to wet weight ratio. The two stressors we focus on for functional traits that increase resistance to disturbance are desiccation and wave action. As the tides recede, algae become exposed to air and can be dried out by sun and wind. Waves in the intertidal can damage and break algae, as well as completely remove them from their attachment point.

BO contributes to resistance to both desiccation and wave action. We reason higher BO increases branch overlap, reducing the area exposed to air and the sun when the water recedes, a mechanism that retains water and keeps individuals from drying out (Norton 1991; Mauffrey et al. 2020). It has also been found that individuals in more wave-exposed areas have increased

branching (Hurd 2000). Thalli with greater tensile strength (TS), a measure of the force it takes to break a thallus, are more resistant to damage from wave action (Carpenter 1990). TS is an analog to stem specific density, a terrestrial plant trait that represents a tradeoff between growth potential and resistance to physical damage (Díaz et al. 2016). Holdfast size, measured as both holdfast thickness (HT) and holdfast wet weight (HFww), is important as the holdfast anchors algae to the substrate. Algae in exposed habitats can increase holdfast size leading to an increase in attachment strength (Kawamata 2001).

Greater holdfast wet weight to thallus wet weight ratios (HFww:WW) indicate increased allocation to resistance to physical disturbance (holdfast wet weight) over resource acquisition (thallus wet weight). Dry weight to wet weight ratios (DW:WW) measure allocation to thallus structure; the higher the ratio, the more the algal thallus is composed of organic and inorganic matter compared to water (Mauffrey et al. 2020). This trait is analogous to plant tissue density, a trait that is often measured for terrestrial plants (Funk et al. 2017). We reason that algae with a higher percentage of dry to wet matter would have more structure that could aid in resisting removal by wave action.

Traits that Maximize Resistance to Herbivory

We hypothesize that two traits, one direct measure and one ratio, contribute to resistance to herbivory. Increased BO maximizes resistance to herbivory as many branching species have apical meristems at the terminal end of each branch. Thus, if a branch is removed by herbivores, the individual is able to continue growing at the remaining terminal ends. Further, high values of branching order may be due to tight aggregates that are less subjected to grazing by herbivores (Hay 1981). We reasoned that high DW:WW, a measure of how much an algal thallus is composed of organic and inorganic matter compared to water, can increase resistance to

herbivory. A previous experiment found a positive correlation between consumption rate and wet weight to dry weight ratio of varying algal species; this suggests consumption is higher in species with more water weight - or lower DW:WW (Angell et al. 2012).

Trait measurement

To measure WW ($\text{g} \pm 0.1$), thalli were placed in a nylon stocking, spun in a salad spinner for 60 seconds, and weighed. To measure DW ($\text{g} \pm 0.01$), thalli, or a subset of the thalli, were wet weighed, rinsed in freshwater, then placed in a drying oven at 60°C until consistent dry weight was reached. H ($\text{cm} \pm 0.1$) was measured from the holdfast or attachment point to the tallest point of each individual. W ($\text{cm} \pm 0.1$) was measured perpendicular to height across the widest point of the thallus when placed to approximate growth position. P (cm) and SA (cm^2) were measured by taking a photograph with a scale bar and using image analysis software (imageJ provided by NIH) to trace the thallus and calculate both measurements (Schindelin et al. 2012).

V ($\text{mL} \pm 0.01$ to 10 depending on thallus size) was measured by displacement of water in an appropriately sized graduated cylinder. Some large individuals were subsampled; subsamples were wet weighed (as above) and displacement of the subsample scaled to the whole thallus. HT ($\text{mm} \pm 0.1$) was measured across the widest portion of the holdfast using calipers. Hww ($\text{g} \pm 0.01$ or 0.1 depending on thallus size) was determined by removing the holdfast and wet weighing (as above).

BO (count) was determined by selecting a terminal branch at random and counting every branching node until the holdfast was reached; we did this five times per individual, where possible, and used the average as a replicate in the analysis (Mauffrey et al. 2020). BO was zero for unbranched species (*Ulva* and *Colopomenia*). TS was measured as the weight needed to

break each individual thallus (Ryznar et al. 2021; 2023). Thalli were wrapped at the basal end and secured to a spring scale with twist ties used to support garden plants as these are designed to minimize damage to plant stems. We pulled the algae from a terminal branch/blade until it broke. The scale was video recorded and reviewed to determine how many grams of force were necessary to break the thallus.

All ratios – DW:WW, SA:VL, SA:DW, H:DW, and HFww:WW – were calculated by division.

Analysis

We used a two-tiered approach, first utilizing ordination and correlation to understand the relationship among all traits included in the full model, then following this same approach with the functional traits included in the reduced model.

Principal Component Analysis (PCA) reduces the dimensionality of data to be visualized to two dimensions. The PCA was conducted in R version 4.0.1 (R Core Team 2020) using the *prcomp* function available in base R individual, which scales all variables to have a mean of zero. We used a PCA to visualize differences among 16 traits for 210 individuals (1 *Pelvetiopsis* and 1 *Silvetia* were excluded in the full model due to missing values). The percent contribution of each trait to the PCA was compared to the null model of equal contribution using the *fviz_contrib* function from the package *factoextra* (Kassambara and Mundt 2020). Percent contribution of each trait was calculated by scaling total contribution for each axis to 100% then dividing this 100% among the traits in proportion to their contribution to each axis following the PCA. The PCA was visualized using the *fviz_pca_biplot* function, also available in *factoextra*. Individual algal thalli were color-coded by species and trait vectors were color-coded by contribution. To further explore the relationships between pairs of traits, we used Pearson's

correlation that calculates correlation strength with R-values. Pearson's correlations were constructed using the *cor* function available in base R.

The same analyses - PCA, trait contributions, and correlation analysis- were used to visualize and quantify the importance of the traits included in the reduced model. These analyses include the two individuals that were excluded from the full model as they were not missing any of the traits included in the reduced model.

RESULTS

Full model

The first two Principal Components (PCs) explain 63.5% of the variation in our full model, with PC1 representing the preponderance at 51% (eigenvalue = 8.31) and PC2 describing 12.5% (eigenvalue = 2.00; Fig. 1-2A). The eigenvalue for the third PC is 1.60 (10%), and the fourth PC is 1.18 (7.4%) with subsequent PC's eigenvalues <1, see supplement. Overall, size-related traits that maximize resource acquisition contribute the most to PC1 while traits for PC2 are more related to resistance to disturbance and herbivory than size.

Ten traits contribute more than the null expectation of equal contribution to PC1, and all contribute positively (Fig. 1-2B). From highest to lowest contribution these rank: H, W, SA, DW, WW, BO, VL, P, TS, and HT. Seven of these ten traits measure aspects of thallus size and are therefore all related to resource acquisition. Of the remaining three, BO is related to all three ecological functions we identify, while TS and HT are traits related to disturbance resistance.

Five traits contribute more than the null expectation of equal contribution to PC2 (Fig. 1-2C). In descending order of importance these rank, DW:WW, HFww, VL, WW, and BO. Three traits contribute positively to PC2, HFww, VL and WW, while DW:WW and BO contribute negatively. VL and WW are both related to resource acquisition. HFww is related to resistance to

disturbance, DW:WW is related to resistance to both disturbance and herbivory, and BO is related to all three functions. VL and WW contribute positively to both PC1 and PC2, while BO contributes positively to PC1 and negatively to PC2.

Species that share traits that are positively associated with PC1 are *Pelvetiopsis*, *Selvetia* and *Codium* (Fig. 1-2A); however, they parse out differentially in trait space created by PC2 due to differences among species in traits associated with PC2. These three species all have the greatest dissimilarity in TH, SA, and TS, meaning they are all relatively large thalli that maximize overall surface area but are also strong (traits that contribute positively to PC1). They are also heavy, with large volumes and are highly branched (traits that contribute to both PC1 and PC2). *Codium* individuals have positive values of PC2 occupying trait space along the vectors for HFww, VL, and WW. HFww contributes positively to PC2. *Selvetia* and *Pelvetiopsis* occupy trait space with negative values of PC2. These species are closer together in trait space and occupy a smaller space near the BO, TS, and DW:WW vectors. DW:WW and BO contribute negatively to PC2.

Ulva, *Dictyota*, *Colpomenia* and *Laurencia* are negatively associated with PC1 and far more similar to one another in both dimensions than the other three species, suggesting they have less inter- and intraspecific variation. Thalli of these four species are overall smaller than those associated with positive values of PC1, with more SA compared to mass or volume. This is a different resource acquisition strategy than the other species utilize - these species maximize surface area at the cost of height. *Laurencia* individuals are tightly clustered near the overall mean trait value (the origin of the PCA). *Ulva* is also tightly clustered, but with overall greater dissimilarity than *Laurencia* in traits associated with negative values of PC2, particularly

SA:DW, H:DW, and SA:VL. *Dictyota* and *Colpomenia* individuals are more closely associated with the SA:DW and H:DW vectors and have the greatest dissimilarity along these vectors.

Overall, there are more positive than negative correlations between traits, and these positive correlations are stronger than the negative correlations (Fig. 1-3). Many of the strongest correlations are between size-related traits and therefore resource acquisition (Fig. 1-2A).

Correlations between pairs of size-related traits range in strength from 51% to 97%. Three of these pairs of traits that measure size have correlations above 0.90: VL and WW (0.97), SA and DW (0.96), as well as DW and P (0.91). Correlations between W and H as well as SA and P rank next (0.89) followed by SA and WW (0.85). The next four highest ranked pairs of traits have at least one of the pair related to size. WW (size) and HFww (resistance to disturbance) as well as H (size) and BO (all 3 functions) are 0.85, while VL (size) to HFww is 0.84 and W (size) to BO is 0.82.

There are fewer negative correlations between traits, and they are weaker than the positive correlations. Further, traits that are ratios had the preponderance of negative correlations. SA:DW is part of all five pairs of traits with the greatest negative correlations, ranging from -0.49 to -0.59. This trait is imputed to be related to resource acquisition, but likely by employing a different strategy than direct measures of size. For example, SA:DW negatively correlates with 2 direct measures of size (H, W). It also negatively correlates with two traits of resistance to disturbance (HT, TS) and one trait that relates to all 3 functions (BO). These might represent tradeoffs, such as intertidal algae cannot be tall and strong while having a high SA:DW ratio.

SA:VL and HFww:WW are two traits that contribute less than the null expectation to both PC1 and PC2 and are not strongly correlated, positively or negatively, with other traits. This

suggests that SA:VL and HFww:WW are orthogonal to the other traits. They also measure different functions from each other, resource acquisition vs resistance to disturbance, respectively. This may mean that once collinearities are reduced, one or both traits could become meaningful in a reduced trait model.

Selecting traits for the reduced model

We retain six functional traits in the reduced model, three for resource acquisition, two that represent resistance to physical disturbance, and one that is multifunctional for resistance to both physical disturbance and herbivory (Fig. 1-4). The three traits that are related to resource acquisition are H, SA:VL, and SA:DW. We retain H to represent the suite of collinear traits that are directly related to size as it contributed the most to PC1. It is also commonly used for terrestrial plants. Thus, we eliminate P, W, SA, WW, VL and DW because each of these correlates strongly with H (range = 0.63 to 0.89, Fig. 1-3).

We retain both SA:VL and SA:DW as traits related to resource acquisition because they do not correlate strongly with any other traits (maximum positive correlation = 0.42, Fig. 1-3) and thus may represent a different mechanism of resource acquisition. While SA:VL does not contribute to either PC in the full model, it is a common trait used in the burgeoning macroalgal literature (e.g., Cappelatti et al. 2019; Mauffrey et al. 2020; Stelling-Wood et al. 2020) that controls fluxes in and out of marine/aquatic organisms that have entire surfaces active in gas and solute exchange. SA:DW is also selected as it contributes to PC2 and it is a close analog for an important trait for terrestrial plants, specific leaf area. We eliminate H:DW as it has a low contribution to both PCs.

We select TS and HFww as traits related to resistance to physical disturbance (Fig. 1-4). TS is correlated with BO (0.75), but has the advantage that it can be measured on every alga.

Thus, we eliminated BO, despite its greater contribution to the PCA, as not all algae have branches (examples: *Ulva* and *Colpomenia*). HFww remains because it contributed the most to PC2. We eliminate HT as it correlates with both H (0.73), and HFww (0.66). We also eliminate HFww:WW because of its low contribution to both PCs.

We retain DW:WW as it is a multifunctional trait related to both resistance to disturbance and resistance to herbivory (Fig. 1-4). DW:WW has the preponderance of the strongest negative correlations. It has also been found to be an important trait in other early studies on macroalgae (Cappelatti et al. 2019; Mauffrey et al. 2020).

Reduced model

The first two PCs in the reduced model using 6 functional traits explains 4.9% more of the variation in the trait data than the full model PCA that included all 16. The first two PCs explain 68.6% of the variation, with PC1 representing the majority at 46.7% (eigenvalue = 2.80) and PC2 at 21.9% (eigenvalue = 1.32; Fig. 1-5A). This represents a greater balance between the first two PCs for the reduced model compared to the full model. The eigenvalues for the remaining PCs are all <1, confirming the better fit to the data for this PCA.

All 6 functional traits in the reduced model contribute significantly to the PCA, with each trait uniquely contributing to either PC1 or PC2, but never both. Three traits contribute more than the null expectation of equal contribution to PC1. In descending order of importance, they rank: H, TS, and SA:DW (Fig 5B). H and TS contribute positively to PC1, while SA:DW contributes negatively. Moreover, while SA:DW was not an important contributor to trait space in the full model, it became important in the reduced model, likely because elimination of multiple measures of size produced more sensitivity to other traits. These three traits alone capture the same two resource acquisition strategies that were captured in the full model:

growing tall to intercept resources (maximizing H) vs. allocation to tissues that effectively capture resources (maximizing SA). TS contributes to resistance to disturbance, suggesting being strong is intrinsically linked to a strategy of growing tall to capture resources.

PC2 also has three traits that contribute more than the expectation of equal contribution; from highest to lowest contributions these rank DW:WW, SA:VL, and HFww (Fig. 1-5C). DW:WW and SA:VL contribute negatively to PC2, although in different directions with respect to PC1. DW:WW resists both disturbance and herbivory while SA:VL is a measure of resource acquisition. HFww contributes positively to PC2 and is a measure of resistance to disturbance.

While algal thalli occupy similar positions within trait space in both the full and reduced models, individuals of four of the seven species occupy relatively more trait space, thus capturing intraspecific variability in these smaller algae that was likely swamped by the multiple measures of size. However, *Laurencia* remains tightly clustered near the origin of the axis demonstrating its relative lack of intraspecific variability. In contrast, *Colpomenia* individuals occupy more trait space along the SA:DW vector, while *Ulva* and *Dictyota* occupy trait space between the SA:DW and SA:VL vectors. Taken together, these patterns suggest these three species are maximizing resource acquisition through allocation to high surface area compared to structure or the physical space they occupy.

The patterns for *Pelvetiopsis*, *Selvetia*, and *Codium* remain largely the same despite eliminating all but one measure for size. This pattern suggests that these three species utilize increased H as a resource acquisition mechanism; this is associated with increased TS to resist wave disturbance. *Codium* is spread widely along the HFww vector that is positively associated with PC2, while *Pelvetiopsis* and *Selvetia* are along the H and TS vectors. The majority of

Pelvetiopsis individuals are in negative PC2 space and found closer to the H and TS vectors than *Selvetia*. *Selvetia* individuals are closer to the DW:WW vector.

Overall, the selected functional traits resulted in weaker positive correlations and a nearly equal balance between positive and negative values (seven and eight, respectively). The highest positive correlation (0.78) is between H, a resource acquisition trait, and TS, a resistance to disturbance trait, suggesting an ecological strategy where maximizing height to acquire resources has a cost of increased drag, thereby requiring allocation to TS to resist this disturbance. The second highest positive correlation (0.54) is between H and HFww, another resistance to disturbance trait. The strongest negative correlation (-0.61) is between H and SA:DW, and this is also the strongest negative correlation in the full model. The next most negative correlation is between TS and SA:DW (-0.54). This suggests a second strategy for resource acquisition in the intertidal, algae that allocate energy to SA and not DW are shorter and therefore do not need to invest in structural strength.

DISCUSSION

We make three novel contributions to the use of trait-based approaches to study marine macroalgae that we believe provide a strong foundation for future research. Our first contribution comes from leveraging approaches and lessons learned from earlier work with other primary producers to select critical traits that map onto specific ecological functions that we believe are vital for performance of all macroalgal species. Second, our work provides a roadmap for determining key traits that can be expanded for use in other macroalgal communities shaped by the need to acquire resources and to resist herbivores and damage by physical disturbance. In this roadmap, we measured a wide suite of 16 traits for a diverse set of algae from different habitats in the rocky intertidal and across seasons and used a quantitative approach to determine their

relative importance in creating trait space. We discovered that the reduced model of six functional traits explained over 68% of trait space for Southern California intertidal macroalgae, a finding that will streamline field and laboratory efforts in future studies. Finally, we determined that the functional trait approach we employed captured two ecological strategies for maximizing resource acquisition: being tall and strong vs. maximizing surface area while being relatively short and weak. We hope these findings advance the use of trait-based approaches for marine macroalgae in the future.

Selecting critical traits that map onto specific ecosystem functions

We determined what functional traits are critical to measure for marine macroalgae in temperate rocky intertidal systems to assess their capacity to support three key ecological functions universal to all macroalgae: the need to acquire resources (Carpenter 1990), limit removal (Dayton 1985; Koehl and Alberte 1988), and avoid being consumed (Angell et al. 2012). We recommend that future work addressing these critical ecosystem functions focus on the six traits from our reduced model because, between them, they explain 68.6% of the trait variation found in a diverse set of species across seasons and sites that likely vary in environmental drivers. Our selection supports their use in other early trait-based approaches. For example, Cappelatti et al. (2019) focused on intertidal species using five of the same, or similar, traits - SA:DW, SA:VL, H, DW:WW, and a holdfast ratio. The ability to confidently measure only six traits compared to the original 16 we chose to assess ecosystem functions for rocky intertidal algae should streamline future development of TBE for marine macroalgae in this habitat.

By leveraging decades of findings in terrestrial plant ecology, we streamlined the process of trait selection by starting with a wide suite of traits and then providing quantitative evidence

that we can reduce this number to far fewer traits with no loss of information. Our approach follows the path laid by terrestrial plant ecologists over the last several decades. Early work in this field began with measuring multiple traits related to bivariate (r/K selected species) and then trivariate (competition-stress-disturbance triangle) tradeoffs (for a recent review see de Bello et al. 2021). These early efforts were followed by a reduction in the number of critical traits using frameworks such as the leaf-height-seed (LHS) schema (Westoby 1998) that allowed comparisons between a greater number of species across the globe (de Bello et al. 2021). Similarly, we started with a larger number of traits relating to our three ecological strategies, then reduced to six without losing explanatory power. Our end result of six critical traits is similar to other recent studies across taxa (trees - Kraft et al. 2008; plants - Albert et al. 2010; birds – Pigot et al. 2016; fishes - Beukhof et al. 2019), providing some confidence that our selection process may be generalizable.

A roadmap for determining critical traits for macroalgal communities

Our work provides a simple roadmap for choosing critical traits in other marine macroalgal systems and/or when exploring different questions, ecosystem functions, and ecological strategies. Our roadmap includes four basic steps: 1) focusing on specific ecosystem functions and/or questions, 2) using existing literature to select a large suite of traits that address these functions/questions, 3) measuring traits of the most diverse set of algae in the most different habitats and seasons that their study will cover, and 4) using multivariate techniques to reduce the number of traits to a subset that captures the full diversity of the study species. While some early trait studies that focused on algae did reduce traits by eliminating those with little explanatory power (Jänes et al. 2017; Stelling-Wood et al. 2020), the majority did not (Cappelatti et al. 2019; Mauffrey et al. 2020; Lloyd et al. 2020; Carvalho et al. 2022). We reason the

approach of starting with more and then reducing to fewer traits reduces the probability of missing critical traits that may not be obvious in a taxon where trait-based approaches are so new. However, by leveraging our trait discovery process, future studies can quickly arrive at the important traits in their study system through formal analysis.

A caveat to our recommended roadmap is that we recognize our initial choice of 16 traits could have been more balanced. We selected a disproportionately large number of traits for resource acquisition compared to the other two ecological functions, resulting in an overemphasis on this function, particularly traits related to size. We recommend future work in understanding algal traits start with a more balanced selection of initial traits, for example including weight to penetrate an algal thallus (Ryznar et al. 2023) or chemical defenses (Fong and Paul 2011) as herbivory resistance traits. Alternatively, traits could be weighed to make them more balanced (de Bello et al. 2021). However, algal trait research does not have the robust literature that terrestrial plant ecologists can rely on when determining what traits or weights are best suited to their study. Therefore, despite this caveat, our process provides a valuable roadmap for future work on trait-based approaches for marine macroalgae.

Using trait-based approaches identified ecological strategies

In both models, we found trait syndromes that elucidate two ecological strategies for resource acquisition employed by intertidal algae. One strategy is characterized by a trait syndrome where thalli of larger sizes, particularly taller individuals, also have some of the strongest thalli. Taller thalli can preemptively intercept light by forming canopies that elevate photosynthetic tissues above competitors (Dayton 1985; Carpenter 1990), much like trees in a forest (Maes et al. 2020). The cost to being tall is that tall individuals experience more drag in current or with wave action (Dayton 1985; Denny 1988), a high-energy condition that is typical

in intertidal systems (Benedetti-Cecchi and Trussell 2014) and requires increased tensile strength (Kitzes and Denny 2005, Carpenter 1990) and to a lesser extent a larger holdfast (Kawamata 2001). This strategy is used by *Pelvetiopsis* and *Silvetia* individuals, who are tall and prioritize TS to resist disturbance, and *Codium* individuals, who are also tall, but prioritize HFww. Díaz et al. (2016) found a similar trend across morphologically diverse plant species, where tall plants also have high stem specific density - a similar resistance to disturbance trait to TS. Identifying trait syndromes that elucidate ecological strategies has proven a powerful tool for terrestrial plant ecologists and our results demonstrate this is also true for marine macroalgae.

The other resource acquisition strategy is characterized by trait tradeoffs. For example, when algae prioritize SA:DW as a resource acquisition strategy, they trade off size, so are smaller individuals. These individuals also lack strength and structure to resist physical disturbance and herbivores, presumably to allocate more energy to support expansion of surfaces. This is comparable to the differences between herbaceous plants that have inexpensive, acquisitive leaves and woody plants that are greater in height (Díaz et al. 2016). Algal species that prioritize increased surface area over dry weight include *Dictyota*, *Ulva*, and *Colpomenia*. A similar tradeoff is found in Neotropical trees, where SLA negatively correlates with laminar toughness and leaf tissue density (Baraloto et al. 2010). This tradeoff between resource acquisition traits and defensive traits is also seen when comparing aquatic, riparian, and terrestrial plants. Aquatic plants tend to have high SLA compared to terrestrial plants that prioritize leaf dry matter content (LDMC), with riparian plants falling in the middle of the spectrum (Freschet et al. 2010). Thus, our study demonstrates that identifying tradeoffs may also prove to be a valuable approach for the future development of TBE for macroalgae.

Conclusions

We hope this work advances the use of trait-based approaches for marine macroalgae in the future by providing a quantitative roadmap for selecting traits in this and other benthic marine systems where macroalgae play an important role. Tropical marine macroalgal habitats would be an ideal system for testing this roadmap, as, to the best of our knowledge, there has been no exploration of trait-based approaches in these systems. Further, the six traits in the reduced model are easy to measure with basic equipment. There is immense value in cost-effective and low-tech methods to study and monitor marine ecosystems as they are accessible to under-resourced local communities. We hope the understanding of ecological strategies employed by marine macroalgae that we advanced during our trait selection process will also prove useful to address other questions in other systems.

Figures

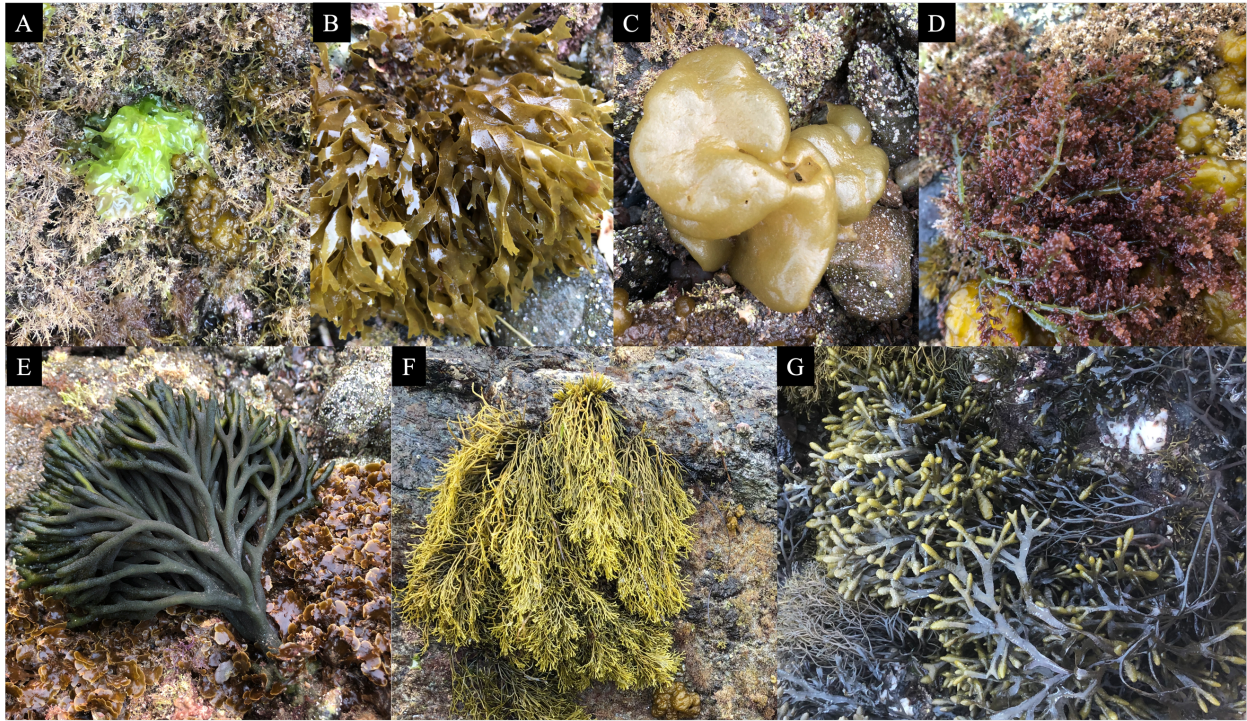


Figure 1-1: Images of each species in the intertidal of Catalina Island, CA. A. *Ulva* spp., B. *Dictyota flabellata*, C. *Colpomenia* sp., D. *Laurencia pacifica*, E. *Codium fragile*, F. *Silvetia compressa*, G. *Pelvetiopsis californica*.

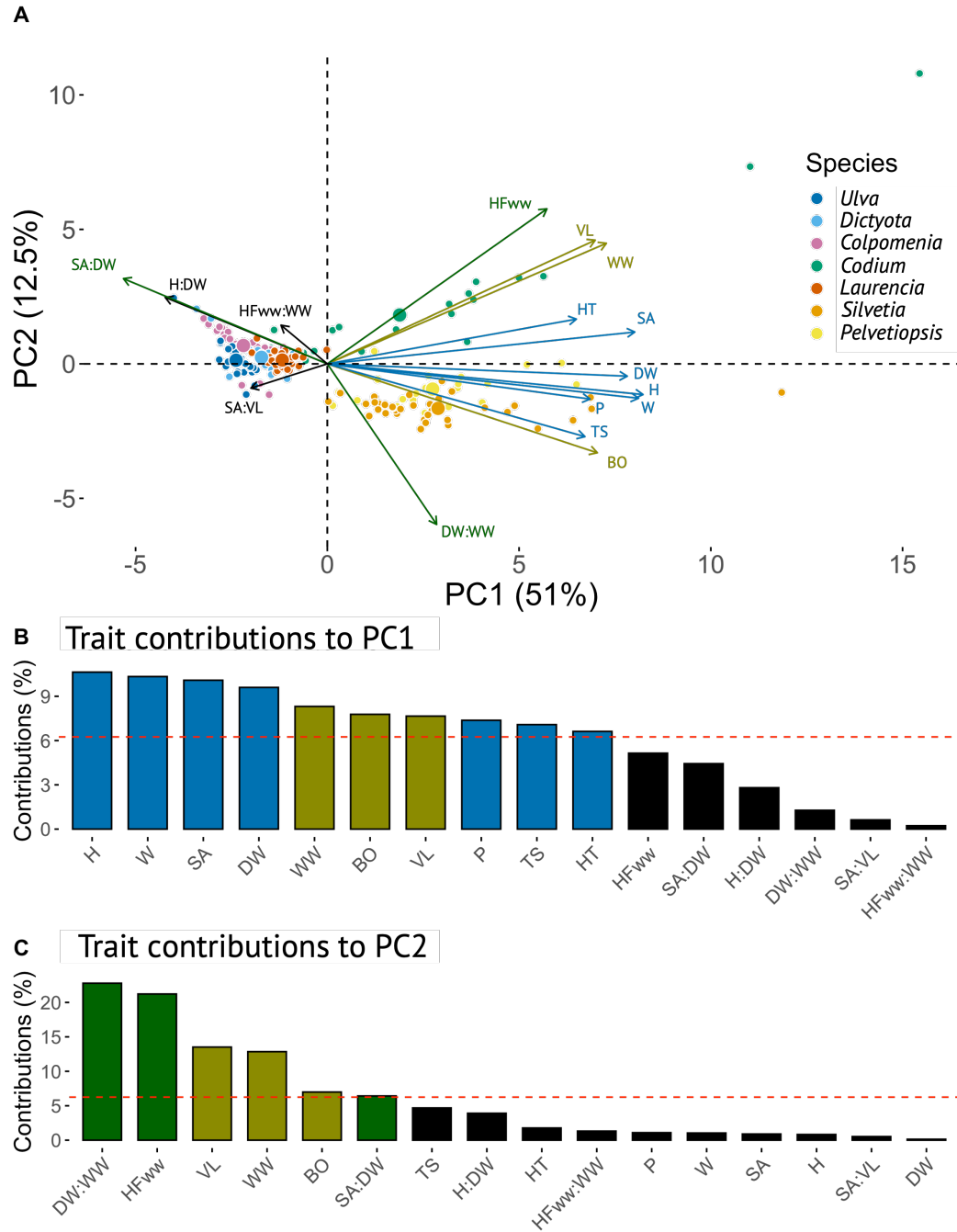


Figure 1-2: A). PCA visualizing trait space using all 16 traits in the full model. Vectors in blue contribute to PC1, vectors in dark green contribute to PC2, vectors in olive green contribute to both PC1 and PC2, and vectors in black do not contribute above the null expectation to either PC. B). The contribution of each trait to PC1. Each small dot represents one individual color-coded by species, while large dots are the centroid for each species. C). The contribution of each trait to PC2. Bars in blue contribute more than the null expectation to PC1, bars in dark green contribute more than the null expectation to PC2, bars in olive green contribute more than expected to both PC1 and PC2, and bars in black do not contribute to PC1 in (B) and PC2 in (C). Abbreviations are: wet weight (WW), height (H), width (W), perimeter (P), surface area (SA), volume (VL), dry weight (DW), holdfast wet weight (HFww), holdfast thickness (HT), branching order (BO), tensile strength (TS), dry weight to wet weight ratio (DW:WW), surface area to volume ratio (SA:VL), surface area to dry weight ratio (SA:DW), height to dry weight ratio (H:DW), and holdfast wet weight to total wet weight ratio (HFww:WW). Note scale change between B and C.

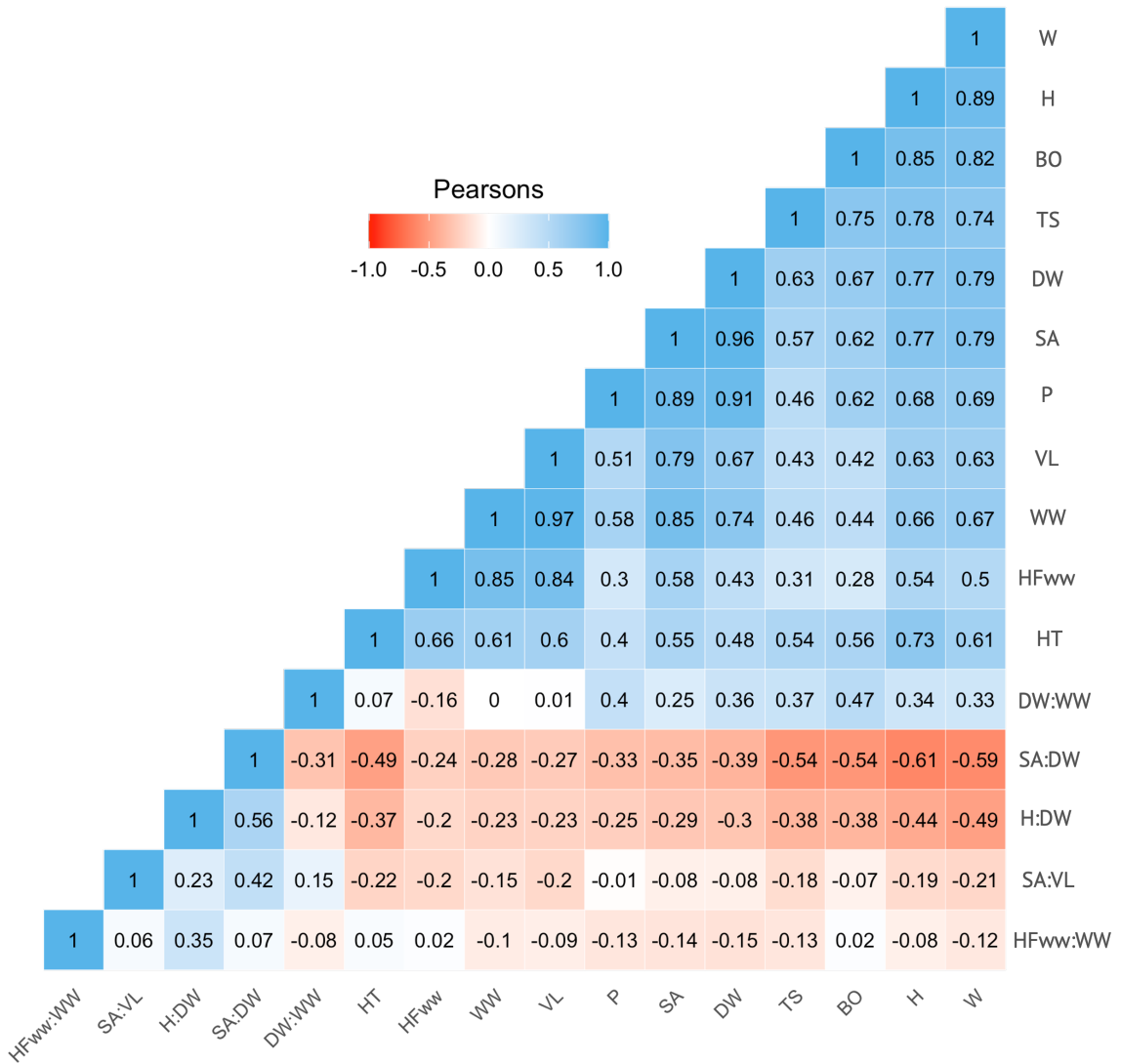


Figure 1-3: A correlation matrix illustrating relationships between traits. Blue squares represent positive correlations while red squares represent negative correlations. Each square contains the pair-wise correlation coefficient (R). Abbreviations are: width (W), height (H), branching order (BO), tensile strength (TS), dry weight (DW), surface area (SA), perimeter (P), volume (VL), wet weight (WW), holdfast wet weight (HFww), holdfast thickness (HT), dry weight to wet weight ratio (DW:WW), surface area to dry weight ratio (SA:DW), height to dry weight ratio (H:DW), surface area to volume ratio (SA:VL) and holdfast wet weight to total wet weight ratio (HFww:WW).

Ecological Function

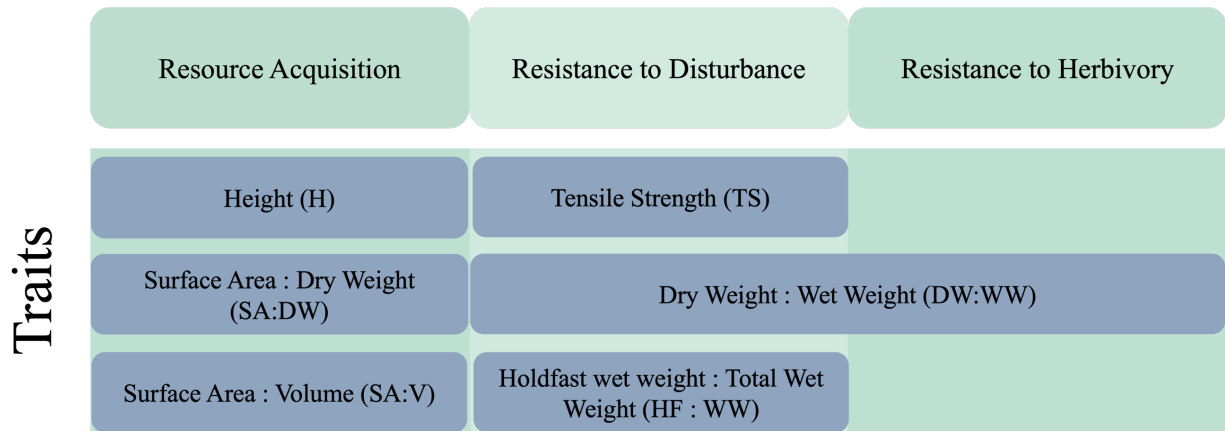


Figure 1-4: Six functional traits selected for the reduced model and their assignments to ecological function(s).

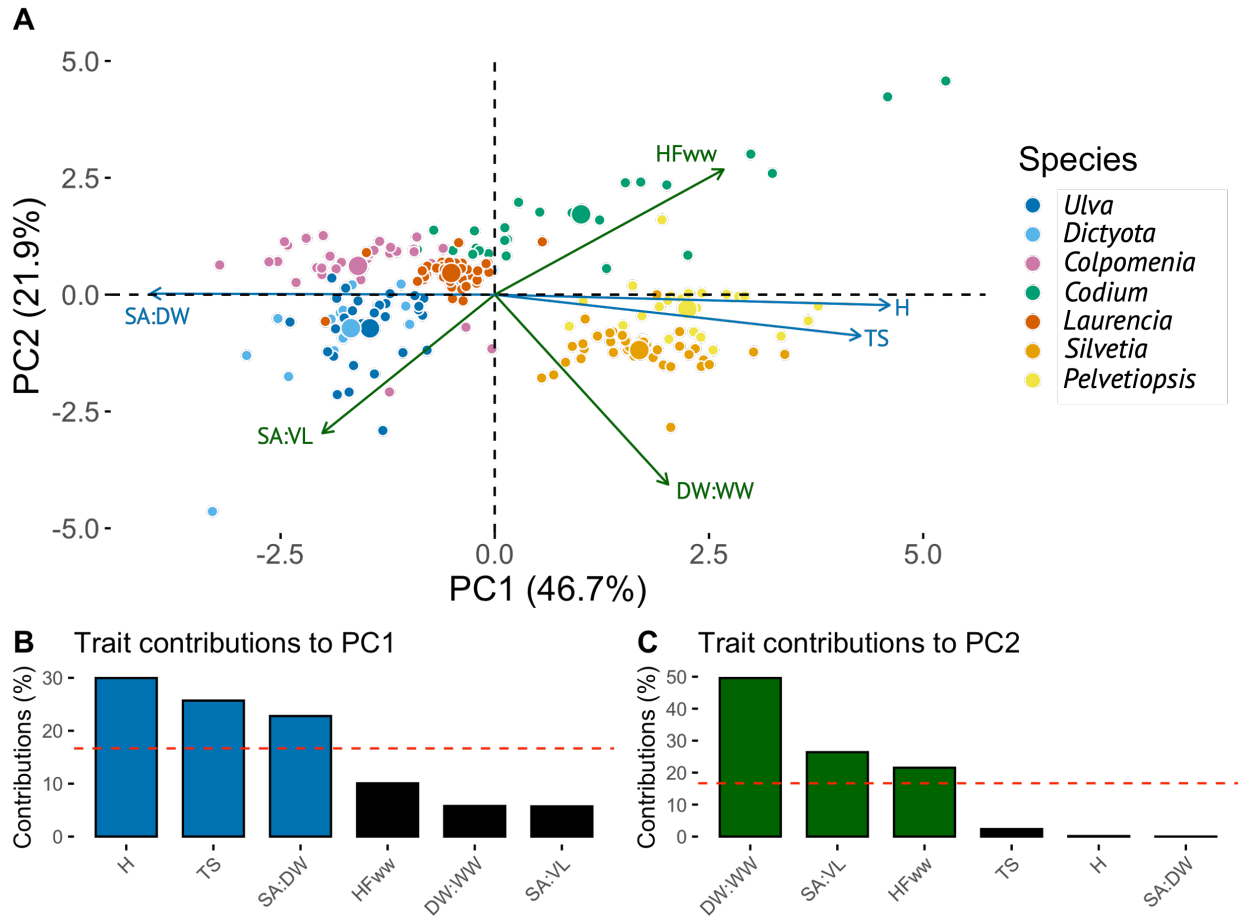


Figure 1-5: A). PCA visualizing trait space using the six functional traits in the reduced model. Each small dot represents one individual color-coded by species and large dots are the centroid for each species. B). The contribution of each trait to PC1. C.) The contribution of each trait to PC2. Vectors and bars in blue contribute more than the null expectation to PC1, vectors and bars in green contribute more than the null expectation to PC2. Abbreviations are: height (H), tensile strength (TS), dry weight to wet weight ratio (DW:WW), surface area to volume ratio (SA:VL), surface area to dry weight ratio (SA:DW), and holdfast wet weight (HFww). Note scale change between B and C.

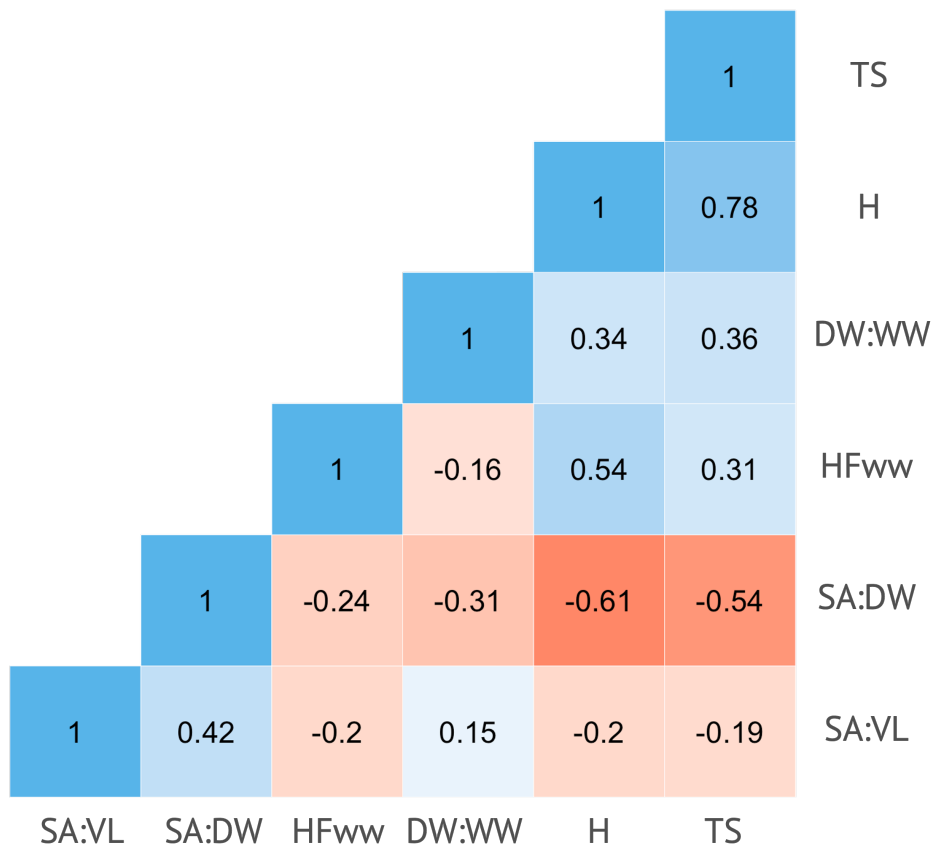


Figure 1-6: A correlation matrix illustrating the positive and negative relationships between traits in the reduced model. Blue shades represent the strength of positive correlations while red squares represent the strength of negative correlations. Each square has the correlation R-values written inside. Abbreviations are: tensile strength (TS), height (H), dry weight to wet weight ratio (DW:WW), holdfast wet weight (HFww), surface area to dry weight ratio (SA:DW), and surface area to volume ratio (SA:VL).

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CHAPTER 2

Testing a roadmap for selecting functional traits: Exploring traits for tropical marine macroalage

ABSTRACT

Trait-based ecology is a powerful tool for exploring ecological strategies in terrestrial plant communities that has motivated expansion of its use across a wide diversity of other taxa. The use of trait-based ecology shows early promise for marine macroalgae, spurring development of a roadmap to guide trait selection that facilitates exploration of new macroalgal communities. Here, we test this roadmap developed for temperate intertidal algae by using it to determine what traits of tropical reef macroalgae are critical to capture the diversity of ecological strategies used to maintain ecological functions in this tropical system compared to the temperate system. This work gains importance because tropical reefs are transitioning from coral to algal domination worldwide, making the need for predictive ecology intense. Following the roadmap, we measured 11 traits that we reasoned contribute to resource acquisition, resistance to herbivory, or resistance to physical disturbance on replicate individuals of 36 species of tropical algae (N=167). We used Principal Component Analysis to collapse these multidimensional data into two dimensions, quantified the contribution of each trait to creating trait space with loading plots, and explored bivariate relationships between traits with correlation analysis. Through these analyses, we selected five core traits for use in a reduced model and reran the same analyses. In the reduced model, we found an axis of variation for resource acquisition strategies between being tall with more structure to reduce removal by wave action versus having high surface area with low structure to capture any light that may penetrate a canopy. We also identified three traits that are critical for understanding ecological strategies across systems with very different environmental contexts, a finding that can inform a nascent marine macroalgal trait database.

These findings provide strong evidence that this roadmap for trait selection can be used in other macroalgal-dominated ecosystems and provide a strong foundation for rapid development of trait-based ecology approaches in tropical reefs and globally.

INTRODUCTION

Over the last several decades, trait-based ecology (TBE) has proven an invaluable approach to explore community and ecosystem functioning in a diversity of terrestrial plant communities (McGill et al. 2006; Westoby and Wright 2006; Díaz et al. 2016). For example, employing trait-based approaches has advanced our ability to understand the impacts of climate change (Willis et al. 2016) and build community resistance to invasive species (Funk et al. 2008). The expansion of TBE across systems and taxa has provided insights as diverse as how to design bird and bat friendly wind farms (Thaxter et al. 2017) and improved our ability to predict the response of mussels to global warming (Archambault et al. 2018). The trait-based approach has driven advances that continue to motivate further expansion of its use.

TBE has advanced our understanding of the ecological strategies organisms use to balance the need to grow, survive, and reproduce across many communities (Baraloto et al. 2010; Gibb et al. 2023). An ecological strategy describes the way an individual, population, or species acquires resources to grow and reproduce, survives disturbance, interacts with neighbors, and ultimately determines its performance (Gibb et al. 2023), an indirect measure of fitness (de Bello et al. 2021). Ecological strategies are evidenced by fundamental ecological or evolutionary tradeoffs among interrelated traits that represent dimensions or axes of variation (Westoby et al. 2002). For example, Baraloto et al. (2010) were able to identify tradeoffs at the stem and leaf level across 16 traits and determine that leaf and stem economics operate independently. While tradeoffs have been observed at the species or population level, the consequences of these

tradeoffs occur at the level of the individual (Agrawal 2020). Consequences occur with tradeoffs, as each tradeoff offers a benefit in one context, while also conveying some disadvantage in that or other contexts (Agrawal 2020). For example, there is a tradeoff between growth potential and mortality risk across stem specific density (Díaz et al. 2016). Understanding ecological strategies informed by trait tradeoffs has advanced our ability to compare and generalize across ecosystems and taxa (Gibb et al. 2023), an increasingly important goal in the Anthropocene as environment contexts shift under climate change.

It is time for a paradigm shift in the way we think about the functional ecology of marine macroalgae, and TBE, though nascent in this taxon, shows early promise as a novel conceptual framework (e.g., Cappelatti et al. 2019; Fong et al. 2023;). Functional group models (FGM) (e.g., Littler and Littler 1980; Steneck and Dethier 1994) have been widely used since their inception in the 1980's (Hanisak et al. 1990; Gaspar et al. 2017; Clausing et al. 2020). These models rely on the largely untested underlying assumption that groups of species with similar gross morphologies perform the same function (for the only tests see Littler 1980; Littler and Littler 1980; Littler and Arnold 1982). However, recent tests of these assumptions found that within group functional diversity often exceeded that between groups (Ryznar et al. 2021; Mauffrey et al. 2021; Fong et al. 2023). In contrast, recent studies using trait-based approaches found that certain traits predicted functions such as habitat support for associated fauna (Carvalho et al. 2022) while other traits responded to changes in environmental drivers. (Ryznar et al. 2023). This early promise for trait-based approaches motivates our search for useful functional traits in macroalgal communities.

Because the use of TBE for marine macroalgae is so new, little effort has focused on developing a systematic method for selecting functional traits. While there is some convergence

in the traits measured in these early studies, there is also considerable divergence (Jänes et al. 2017; Cappelatti et al. 2019; Cappelatti et al. 2019; Lloyd et al. 2020; Stelling-Wood et al. 2020; Carvalho et al. 2022; Fong et al. 2023; Ryznar et al. 2023), limiting comparisons beyond individual studies. Based on data for temperate intertidal algae, in Chapter 1 we proposed a roadmap to select traits that are critical to macroalgal performance - their ability to grow, survive and reproduce. To perform, individuals must balance the need to resist loss due to herbivores (Dayton 1985) and physical removal by disturbance, generally wave action (Denny 1988; Kawamata 2001), with the need to acquire resources such as sunlight and inorganic nutrients for growth (Carpenter 1990). Determining the usefulness of the roadmap for trait selection and examining trait tradeoffs will allow future studies to quickly arrive at the critical traits to measure for their research question.

Here we propose a challenging test of this roadmap by applying it to macroalgae on coral reefs as these ecosystems are subject to very different relative strengths of the environmental drivers that shape trait distributions than ecosystems analyzed in the preponderance of early studies. While early macroalgal trait studies focused on temperate (Jänes et al. 2017; Cappelatti et al. 2019; Mauffrey et al. 2020; Lloyd et al. 2020) or subtropical ecosystems (Stelling-Wood et al. 2020; Carvalho et al. 2022), only one is tropical and includes only two traits (Ryznar et al. 2023). One important difference in drivers between temperate and tropical marine systems is the more intense herbivory pressure typical of coral reef communities, which has led to co-evolution of defense and counter defense adaptations (Ogden and Lobel 1978). Coral reefs are also generally oligotrophic, or nutrient poor, and have higher light penetration than most temperate regions (Fong and Paul 2011). However, sediment deposition is increasing in coral reefs as human development expands (Fabricius 2005) and tropical storms are becoming more intense

under global climate change (IPCC 2019), fueling increases in nutrient supplies via runoff. Finally, while herbivory remains intense in coral reefs, overfishing has eased top-down control (Jackson et al. 2001). These factors have led to macroalgae rapidly expanding in fringing reef systems (Hughes 1994), in many cases creating novel, macroalgal-dominated systems. Knowing what traits are critical for understanding the ecological strategies employed by macroalgae on tropical reefs will expedite future research into these shifting ecosystems.

Our overall objective was to find a core set of traits important for understanding the ecological strategies tropical algae employ to balance the need to acquire resources and resist herbivory and physical disturbance. To do this, we followed the proposed roadmap for trait selection developed for temperate intertidal macroalgae in Chapter 1. This roadmap comprised four steps - 1) measuring and analyzing a wide suite of traits that reflect the need to perform these different ecological functions in the environmental context of the target system (hereafter defined as the full model), 2) determining the relative importance of each trait in creating a functionally diverse trait space, 3) selecting a subset of traits by choosing one of a suite of highly correlated traits as well as retaining pairs of traits that represent tradeoffs (hereafter defined as the reduced model), and 4) quantifying the reduced model's ability to create a functionally diverse trait space.

METHODS

Overall approach

Although we broadly follow the roadmap first proposed in Chapter 1, we selected a different suite of traits that support performance due to the known shifts in the relative importance of different ecological drivers on subtidal tropical reefs compared to temperate intertidal ecosystems. For example, we added toughness and percent calcification as key traits, as

both of these traits function to resist herbivores (Pennings and Paul 1992; Bittick et al. 2016). We removed tensile strength as this trait enables algae to resist physical disturbances, such as wave impacts (Carpenter 1990), and all our algae were from a tropical lagoon, a habitat typically protected from waves. We also eliminated some size-related traits that were highly collinear, although some were included to create ratios (for a full list of traits in both chapters and the differences see Supplement 2, Fig. 2-1 and 2-2).

In the full model we include 11 traits, three of which are ratios, compared to 16 in chapter 1. Broadly, we used Principal Components Analysis (PCA) to determine what traits contributed most to creation of trait space for this full model. We then constructed a reduced model where we chose five traits informed by the full model by: 1) using Pearson's correlation to identify suites of strongly collinear traits, 2) choosing one trait that is important in creating trait space as representative of the suite and eliminating the others, 3) retaining pairs of traits that are strongly negatively correlated as they represent potential tradeoffs, and 4) retaining some traits that are more direct analogs of traits commonly used for primary producers in other ecosystems. The five traits included in the reduced model were subject to the same analysis as the traits in the full model.

Study species and collection methods

As in earlier studies of traits for macroalgae (Ryznar et al. 2023; Fong et al. 2023; Smith Chapter 1), we chose our target species to capture the widest range of algal traits available across as many habitats as possible. Therefore, we selected 36 species based on the Moorea Coral Reef Long Term Ecological Research benthic cover data set (MCR-LTER 2022). These data comprise annual measures, starting in 2005, of benthic cover for six sets of sites, each with three reef types (6 fringing reefs, 6 back reefs, 6 fore reefs) that encircle the island. Our criterion for inclusion

was that each chosen species exceeded 5% cover of the benthos at least once at any point in time or space in the data set through 2021.

We collected all algal individuals from fringing reefs along the north shore of Moorea because this reef type has the highest abundance and diversity of macroalgae (P. Fong personal observation) and therefore enabled us to collect our target species readily. Further, the north shore is easily accessible from the Gump South Pacific Research Station where the laboratory work was conducted. Earlier research demonstrates that interspecific variability exceeds intraspecific variability for at least two algal traits on these fringing reefs (Ryznar et al. 2023; Chapter 1); therefore, we maximized species diversity. We collected five replicates for each of the 30 species that were common, but replication ranged from 1-4 for the six rarer species.

All collections were by hand in water depths of 1-3m and included fringing reef sites from the more open coast as well as within the more protected bays, a method that maximizes intraspecific trait diversity (Ryznar et al. 2023). We collected whole algal thalli that appeared healthy and intact. Care was taken to include holdfasts, where present. Some macroalgae have indeterminate growth, growing both clonally and colonially (Fong and Paul 2011; Fong et al. 2023). Thus, we selected a ‘functional individual’, akin to a ‘module’ that is used when collecting traits for grasses, where individuals can also be difficult to identify (Gubsch et al. 2011); hereafter, modules are called individuals. All collections and measurements were performed in July 2021. In all cases, traits were measured on the same day as individuals were collected.

Trait selection and methods

Using the roadmap developed in Chapter 1, we selected 11 traits we reasoned were functional on tropical reefs based on the literature as well as many that were vetted in Chapter 1

(see methods). All traits are continuous variables, 8 are direct measurements and three derived (ratios). Each trait was measured at the individual level. Directly measured traits include height, width, surface area, wet weight, dry weight, branching order, percent calcification, and toughness. Ratios include surface area to dry weight, height to wet weight, and dry weight to wet weight.

Hypothesized functions of traits

The next step on the roadmap is to relate each trait to one of the three ecological functions of interest using existing literature for terrestrial plants, phytoplankton, or marine macroalgae (Chapter 1). We categorized each trait into one or more of three categories – resource acquisition, resistance to herbivory, and resistance to physical disturbance – such that increasing trait values increased the ability to maximize this particular function. We also reasoned that there would be costs to maximizing a particular function, represented by tradeoffs among these traits.

Traits That Maximize Resource Acquisition

We included eight traits that we reason will maximize resource acquisition of individuals as trait values increase, including six direct measures and two ratios.

Five of the measured traits are related to overall thallus size, a trait we reasoned was an important indicator of resource acquisition as larger marine macroalgae can outcompete neighbors for resources such as light (Carpenter 1990) and nutrients. Traits measured to capture the size of individuals include thallus height, width, surface area, wet weight, and dry weight. Maximizing height (H), in particular, enables preemptive capture of light and reduces self-shading (Carpenter 1990). This is the same function for this trait in terrestrial plants (Bagousse-Pinguet et al. 2017; Asefa et al. 2017; Maes et al. 2020). Width (W) was included as some

species of algae grow wide rather than tall (Marks et al. 2018) and this may increase the area exposed to resources. Net productivity increases with thallus surface area (SA) (Stewart and Carpenter 2003). Wet weight (WW) and dry weight (DW) are common measures of the size of an algal individual and have been linked to resource acquisition in temperate intertidal systems (Chapter 1).

The last directly measured trait for resource acquisition is branching order (BO), a trait that can lead to partitioning of resource uptake, allowing individuals to utilize multiple resources more efficiently. For example, Hay (1981) found that the upper portions of a thallus can have higher photosynthetic rates than lower portions; this could leave lower branches for nutrient acquisition (Colombo-Pallotta et al. 2006).

We included two ratios as contributors to resource acquisition. In marine macroalgae, resource uptake can occur across the entire thallus, therefore a higher surface area to dry weight ratio (SA:DW) represents increased (SA) for resource acquisition compared to size (DW) (Littler 1980). Height to wet weight ratio (H:WW), a similar measure to height to dry weight ratio included in Chapter 1, is a measure of effort put toward growing taller than competitors instead of overall size (Steneck and Dethier 1994). This may be analogous to wood density (as in Funk et al. 2017; Maes et al. 2020), as it estimates structure of a thallus in terms of its size. For example, a thallus that is tall but relatively light devotes more energy to resource acquisition than to physical resistance.

Traits That Maximize Resistance to Herbivory

We included four traits, three direct measures and one ratio, that we reason will maximize resistance to herbivory. The percentage of an algal thallus composed of calcium carbonate (%C) and its toughness (T) are both associated with resistance to herbivory. %C in an algal thallus can

deter herbivores (Pennings and Paul 1992) and propagation of tears in the thallus from the initial bite damage from herbivores (Padilla 1993). T is a measure of the force needed for an herbivore to bite through the surface of an algal thallus. There is experimental evidence that herbivores prefer algae that is less tough when presented with choices (Pennings and Paul 1992; Bittick et al. 2016). Increased BO maximizes resistance to herbivory as many branching species have apical meristems at the terminal end of each branch. Thus, if a branch is removed by herbivores, the individual is able to continue growing at the remaining terminal ends. Dry weight to wet weight ratio (DW:WW) is a measure of the proportion of an algal thallus that is composed of organic and inorganic matter compared to water (Stagnol et al. 2016). Angell et al. 2012 found a positive correlation between the inverse of our trait (wet weight to dry weight ratio) and consumption of algae. This suggests herbivores prefer individuals with lower DW:WW.

Traits That Maximize Resistance to Physical Disturbance

We reasoned that two traits, one direct measure and one ratio, contribute to resistance to physical disturbance.

Calcium carbonate (%C) likely provides mechanical support that protects against wave action (Littler 1976). Further, calcifying algae can slough off outer layers, limiting the growth of epiphytes (Littler 1976). We reasoned that less-heavily epiphytized individuals have less drag and therefore resist physical disturbance. We include DW:WW as high values indicate more structure, which we reason in Chapter 1 could aid in resisting wave action.

Trait measurements

H was measured from attachment position (above the holdfast, if present) to the tallest point of each algal thallus. The W of each thallus was measured at its widest point perpendicular to height. Both H and W were measured in cm (± 0.1). SA was estimated by spreading a thallus

flat, taking a photograph with a ruler in the frame, and digitizing in ImageJ to calculate surface area in cm^2 (Schindelin et al. 2012). Some complex thalli required dissection. To measure WW, each thallus was placed in a nylon bag, spun in a salad spinner for 30 seconds to retain a consistent amount of surface water, then wet weighed in grams (± 0.01). To measure algal DW, a subsample of each thallus was rinsed with fresh water, wet weighed, and placed into a drying oven set to 60°C until constant weight in grams (± 0.01).

BO was determined by randomly selecting a terminal branch or blade and counting the number of nodes from tip to holdfast. When possible, we counted the order of 5 replicate branches/blades per thallus and used the average for each individual in further analysis. To calculate %C, a subsample of each algal thallus was weighed wet and placed in 7.6% hydrochloric acid. Acid was replaced until bubbling ceased, even after replacement, to ensure all CaCO_3 was dissolved (e.g., Pennings and Paul 1992; Clausen et al. 2016; Sura et al. 2021). The subsample was removed from the acid, patted dry, and reweighed. %C was calculated as $[(\text{initial weight} - \text{final weight}) / \text{initial weight}] \times 100$. Algal T was measured as the weight needed to penetrate an algal thallus. We rested the needle of a penetrometer on the middle of the algal thallus or a middle blade and weight was added to the penetrometer until the needle just pierced the surface of the thallus (e.g., Bittick et al. 2016; Bergman et al. 2016; Ryznar et al. 2021; Sura et al. 2021). We chose the middle of each thallus in an effort to standardize estimates across species, though macroalgae can grow from many locations (e.g., apical, basal, diffuse, centripetal invagination). T was recorded in grams (± 0.1 to 0.01).

All ratios – SA:DW, H:WW, and DW:WW – were calculated by division.

Analysis

We followed the two-tiered approach used in Chapter 1, first utilizing ordination and correlation to understand the relationship among our all traits in the full model. We then followed this same approach with our subset of functional traits in the reduced model.

Statistical Methods for the Full Model

We performed a principal component analysis (PCA) to ordinate our 167 individuals based on all 11 traits in multidimensional space (hereafter called trait space). PCA enables visualization of multidimensional data by projecting it into two dimensions. The PCA was conducted in R version 4.0.1 (R core team 2020) using the *prcomp* functional available in base R. To understand how traits shaped trait space, we constructed a loading plot to visualize the contribution of each trait to the PCA. The vectors on the loading plot indicate the contribution to each axis (direction) and strength (vector length) such that weaker predictors have shorter arrows than stronger predictors and arrows in the same direction identify traits that covary. The percent contribution of each trait to each Principal Component (PC) was compared to the null model of equal contribution using the *fviz_contrib* function in the *factoextra* package (Kassambara and Mundt 2020). Percent contribution was calculated by scaling total contribution for each axis to 100% then dividing this 100% among the traits in proportion to their contribution to each axis. The PCA was visualized using the *fviz_pcr_biplot* function, also available in *factoextra*. Vectors were color-coded by contribution. Last, we used Pearson correlation that calculates correlation strength with R-values to quantify the relationship between each pair of traits. Pearson's correlations were constructed using the *cor* function available in base R.

Statistical Methods for the Reduced Model

Based on the analysis of the full model, we reduced our traits to five we argue are important to creating trait space representing our three ecological functions. These functional

traits were selected to reduce collinearities, capture evidence for tradeoffs, and, where possible, to be analogous to well-vetted traits used for vascular plants or phytoplankton. We then repeated the same analysis for these traits as for the full model approach; we constructed a PCA, added a loading plot, determined what traits contribute most to PC1 and PC2, and assessed correlations between traits.

RESULTS

Full Model

Overall, the first two PCs explain 54.4% of the variation in our full model, with PC1 representing 36.6% (eigenvalue = 4.0266) and PC2 describing 17.8% (eigenvalue = 1.9552; Fig. 2-1A). The eigenvalue for the third PC is 1.5000 (13.6%), with subsequent eigenvalues < 1. Over 90% of the variation represented by PC1 is due to five traits: SA, DW, W, WW and H (Fig. 2-1B). All these traits represent different aspects of algal size, a trait often associated with resource acquisition (Steneck and Dethier 1994). Further, these metrics all increase in value with PC1, evidenced by loading plot arrows pointing in the positive direction of PC1.

Almost 85% of the variation explained by PC2 is contributed by only three traits: %C, DW:WW, and T (Fig. 2-1C). DW:WW is associated with both herbivore and disturbance resistance and increases as PC2 values increase. %C and T are both associated with negative values of PC2. We hypothesize that %C contributes to both herbivore and disturbance resistance while T only contributes to resistance to herbivory. Individuals show considerable spread in PC1 space, suggesting that differences in %C, DW:WW, and T occur across individuals of all sizes and all species. The remaining traits, SA:DW, BO, and H:WW, do not contribute more than the null model of equal contribution to either of the first two PCs.

As visualized in the PCA loading plot, all five of the traits that comprise PC1 and represent different measures of thallus size are positively correlated, albeit to different degrees (Fig. 2-2). DW and SA are the most highly correlated ($R = 0.85$), followed by SA to W ($R = 0.79$), SA to H ($R = 0.77$), and WW to DW ($R = 0.77$). The next most highly correlated traits were H to DW ($R = 0.68$), W to DW ($R = 0.68$), W to WW ($R = 0.68$) and SA to WW ($R = 0.65$). In contrast, the three traits that comprise PC2 have weaker overall correlations, the strongest being the negative correlation between DW:WW and %C ($R = -0.52$). Because calcium carbonate is heavy relative to other algal components, we reasoned this correlation should be strongly positive. However, only 15 of the 36 algal species we included calcify, so the majority of %C values are zero. Another, weaker correlation is %C with T ($R = 0.35$). There are also traits that do not correlate strongly with any other trait, for example, BO correlations range from -0.21 to 0.23 and SA:DW from -0.29 to 0.21.

Reduced Model Trait Selection

Given all five traits that contribute to PC1 are collinear and relate to the same function of resource acquisition, we selected H as the single trait representing size in our reduced model (Fig. 2-3). We choose height despite its shorter loading arrow and smaller contribution to the PCA because height is a well-vetted trait for terrestrial plants (e.g., Funk et al. 2017; Kattge et al. 2020), was important in temperate rocky intertidal communities (Chapter 1), and it is extremely easy to measure accurately. For our second PC, %C and T were positively correlated. Given that not all species calcify, we decided to retain T in the reduced model. Additionally, we included DW:WW because it contributed significantly to PC2 and is multifunctional, supporting both resistance to herbivory and physical disturbance. We included SA:DW because it is an analog to traits that have been well-vetted as important for resource acquisition for other primary

producers (e.g., SLA Maes et al. 2020) and does not correlate strongly with any other trait, demonstrating it is orthogonal. Finally, we included H:WW because this trait provides insight into a size vs structure tradeoff and does not strongly correlate with the other selected traits. An additional argument for including these two ratios is that these trait vectors were in different directions than all others and pointed to trait space populated by at least half of our individuals. Our final argument for including these ratios in the reduced model is that it is possible that the importance of these traits was masked by strong collinearities among other traits.

Reduced Model

Overall, the first two PCs of the reduced model with five functional traits explain 56.2% of the variation in our individual algal thalli, with PC1 explaining 29.8% (eigenvalue = 1.4923) and PC2 describing 26.4% (eigenvalue = 1.3201; Fig. 2-4A); eigenvalues for all other PCs were <1. Thus, the reduced model gains a little explanatory power with the reduction in number of traits (54.4% vs 56.2%), produces more balance in the importance of the first two PCs, and reduces the importance of subsequent PCs.

Over 80% of the variation represented by PC1 is contributed by three metrics: SA:DW, H:WW, and H (Fig. 2-4B). Unlike the full model, where PC1 was restricted to simple size metrics, two measures of size as it relates to structure are now included in this PC. In the reduced model, H is associated with negative values of PC1, while the greatest values in H:WW and SA:DW are associated with positive values of PC1. Thus, individuals occur along an axis of variation, the ends of which either maximize overall H as a preemptive resource acquisition strategy or maximize surface area or height compared to mass to allocate relatively more for resource absorption compared to the costs of support or structural material.

Similarly, over 80% of the variation explained by PC2 is contributed by two traits, T and DW:WW (Fig. 2-4C). While both are functional traits for resistance to herbivory, DW:WW is also associated with resistance to physical disturbance. The greatest values in T are associated with negative values of PC2; in contrast, high values of DW:WW are associated with high values of PC2.

Overall, the strength of correlations in the reduced model were weaker than those in our full model (compare Fig. 2-2 and Fig. 2-4). The strongest positive correlation ($R = 0.21$) is between SA:DW and H:WW, as visualized in the PCA (Fig. 2-3A). None of the other positive correlations exceed $R = 0.15$. The strongest negative correlation ($R = -0.29$) is between DW:WW and SA:DW. All other negative correlations $R > -0.25$.

DISCUSSION

Summary

Our results provide three novel findings that we hope will facilitate the use of a trait-based approach to study marine macroalgae in the future. First, we identified five key traits in the reduced model associated with critical ecological functions for marine macroalgae on coral reefs, a macroalgal-dominated system with very different environmental drivers than temperate systems studied previously. This finding provides strong evidence that the roadmap developed in Chapter 1 for temperate systems will be useful across other macroalgal-dominated ecosystems, streamlining field and laboratory efforts in the future. Second, by examining trait tradeoffs we found an axis of variation that provides insights into resource acquisition. This finding furthers our understanding of how environmental drivers shape ecological strategies in this novel macroalgal-dominated habitat where human impacts continue to shift environmental context. Third, we found three traits that are useful in describing tropical macroalgae that were also

important for temperate macroalgae. Identifying traits that are fundamentally important in both systems gives us insight into which traits should be explored as universally relevant across macroalgal-dominated ecosystems. Thus, this finding can be used to begin building a set of universal traits that facilitate comparisons across scales and systems.

Selecting critical traits that map onto specific ecological functions for tropical algae

Our study lays a strong foundation for success in future trait-based research on tropical marine macroalgae by identifying a core set of traits that are critical to measure when exploring questions around three major challenges to algal performance in coral reef ecosystems. By leveraging the roadmap created in Chapter 1, we reduced our initial 11 traits to five that represent resource acquisition, resistance to herbivory and resistance to physical disturbance by removing traits that were highly correlated, had low overall contribution, or were not shared by all species. Through this process, we identified five functional traits that capture over 56% of trait space for common marine macroalgae found in Moorea's fringing coral reefs. Marine macroalgal individuals must balance acquiring resources such as sunlight and inorganic nutrients needed to grow (Carpenter 1990) with the need to resist removal by herbivory (Ogden and Lobel 1978) and physical disturbance (Adjeroud et al. 2002). Being able to confidently measure only the five traits included in the reduced model when exploring these tradeoffs will streamline future studies of the functional ecology of tropical marine macroalgae and could prove useful in monitoring environmental shifts over time within reefs with long term monitoring programs such as the MCR-LTER (2022).

Trait-based approaches identified ecological strategies

Through our search for core traits, we discovered that tropical macroalgae have an axis of variation for acquiring resources that was clearly visualized in the reduced model. Individuals

fall along an axis of variation along PC1 that represents a tradeoff in strategies of resource acquisition of being taller, which requires structure, to being shorter with high surface area, a strategy that does not require as much investment in structure. Individuals at one extreme grow the tallest, often forming canopies that preemptively intercept light above competitors (Dayton 1985; Carpenter 1990), much like canopy trees in a forest (Maes et al. 2020). This results in a tradeoff as tall algae are at higher risk of removal from drag (Dayton 1985; Denny 1988) and therefore require more structure (Chapter 1). At the other extreme of this axis of variation, individuals maximize the amount of photosynthetic area at the cost of overall size. Some studies show greater surface area also increases risk of removal via drag (Kawamata 2001), and perhaps increases palatability to herbivores (Hay 1997), so requires smaller overall height as a tradeoff. Similar tradeoffs occur across herbaceous plants with inexpensive, acquisitive leaves at one end and woody plants that grow taller at the other (Díaz et al. 2016). Identifying trait tradeoffs and relationships between traits that identify ecological strategies is a powerful tool employed by plant ecologists (e.g., Baraloto et al. 2010) and our results provide further evidence this may also be true for marine macroalgae.

We also find variation across PC2 between the two resistance traits, although the evidence that this represents a bivariate tradeoff is not as clear as we find for resource acquisition as they are not directly opposite along this axis. One possible explanation is that one of our resistance traits is multifunctional, or a trait that influences fitness through more than one function (Sack and Buckley 2020). We reasoned that increased DW:WW offers protection from both herbivory and physical disturbance. While multifunctional traits provide an advantage over single function traits in multi-stressor environments (Sack and Buckley 2020), they can also constrain further evolution relative to single function traits (Corn et al. 2021). In our study, as

DW:WW has a relationship with two ecological functions, it could be elevated under multiple scenarios: high wave action and high herbivory, low wave action and high herbivory, or high wave action and low herbivory. Since variation in this trait is driven by two environmental drivers that do not covary, it is likely any tradeoff that does occur is limited by the need to balance performance of two functions simultaneously. Overall, more research is needed to explore traits that provide resistance to herbivory and physical disturbance.

Towards building a database of traits that are universally important for comparing macroalgae globally

Creating a universal database for traits of terrestrial plants has proved invaluable in furthering understanding of these systems (Kattge et al. 2020). Thus, building a similar database for marine macroalgae is an important next step, as advocated by Mauffrey et al. (2020). Based on early work in TBE for macroalgae, there is some support for the existence of a universal set of important traits across systems that can facilitate comparative work. For example, most TBE studies of marine macroalgae include some measure of height (e.g., Jänes et al. 2017; Stelling-Wood et al. 2020), DW:WW, and SA:DW (Capellatii et al. 2019; Mauffrey et al. 2020; Chapter 1). While we acknowledge that trait selection should vary by question and habitat, and we encourage future research to include traits specific for their objectives, our roadmap validates the potential for some that are universally relevant (as in Díaz et al. 2016, Baraloto et al. 2010). Through the roadmap and comparisons with other TBE studies, we uncovered at least three traits that are useful in both coral reefs and temperate intertidal zones. Exploring more macroalgal habitats and traits using this roadmap may validate a core set of traits as universal across all macroalgal-dominated systems.

Figures

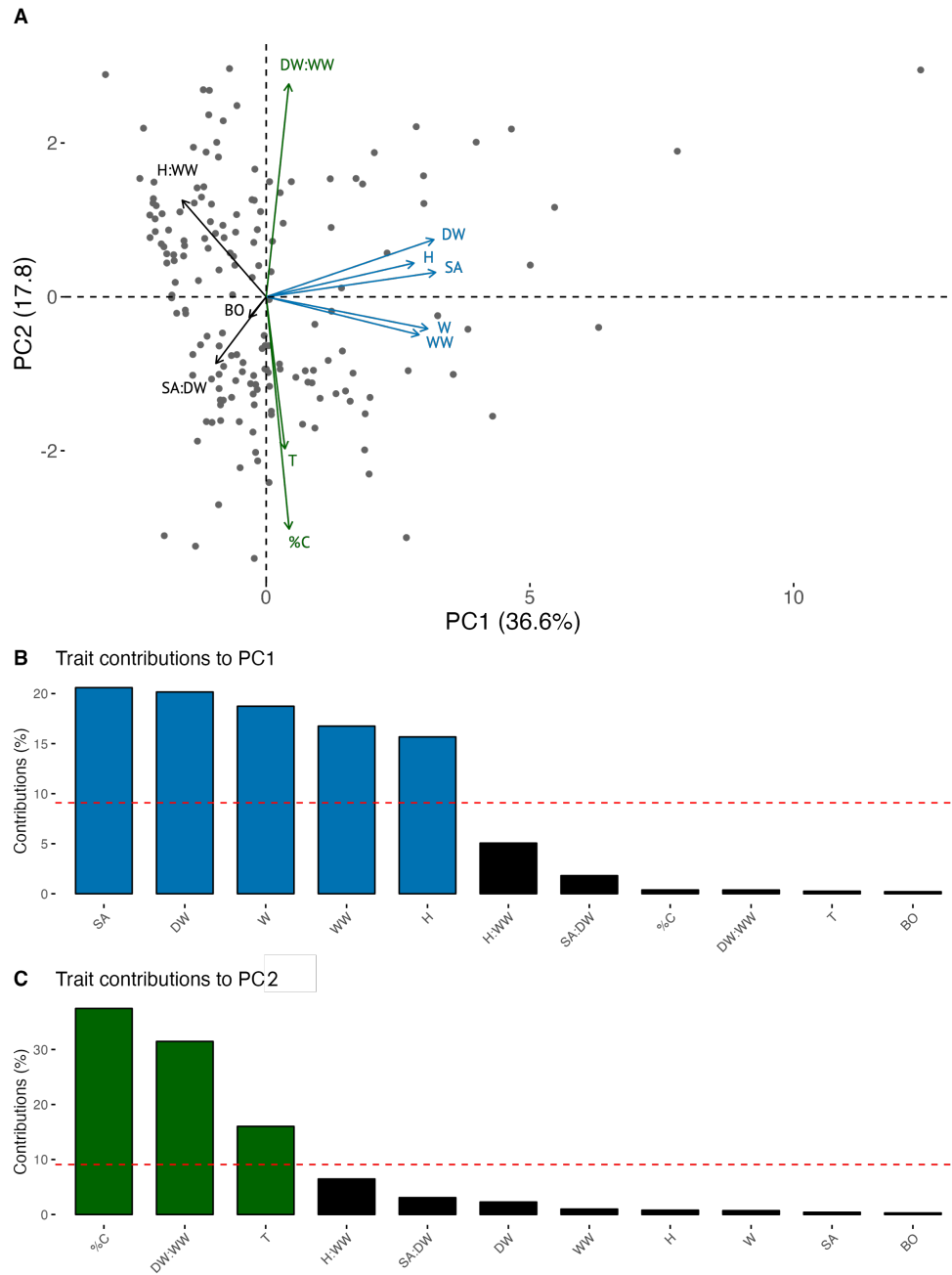


Figure 2-1: A). PCA visualizing trait space created using all 11 traits in the full model. B). The contribution of each trait to PC1. C). The contribution of each trait to PC2. Vectors and bars in blue contribute more than the null expectation to PC1, vectors and bars in dark green contribute more than the null expectation to PC2. Abbreviations are: height (H), width (W), surface area (SA), wet weight (WW), dry weight (DW), branching order (BO), percent calcification (%C), toughness (T), surface area to dry weight ratio (SA:DW), height to wet weight ratio (H:WW) and dry weight to wet weight ratio (SA:DW). Note scale change between B and C.

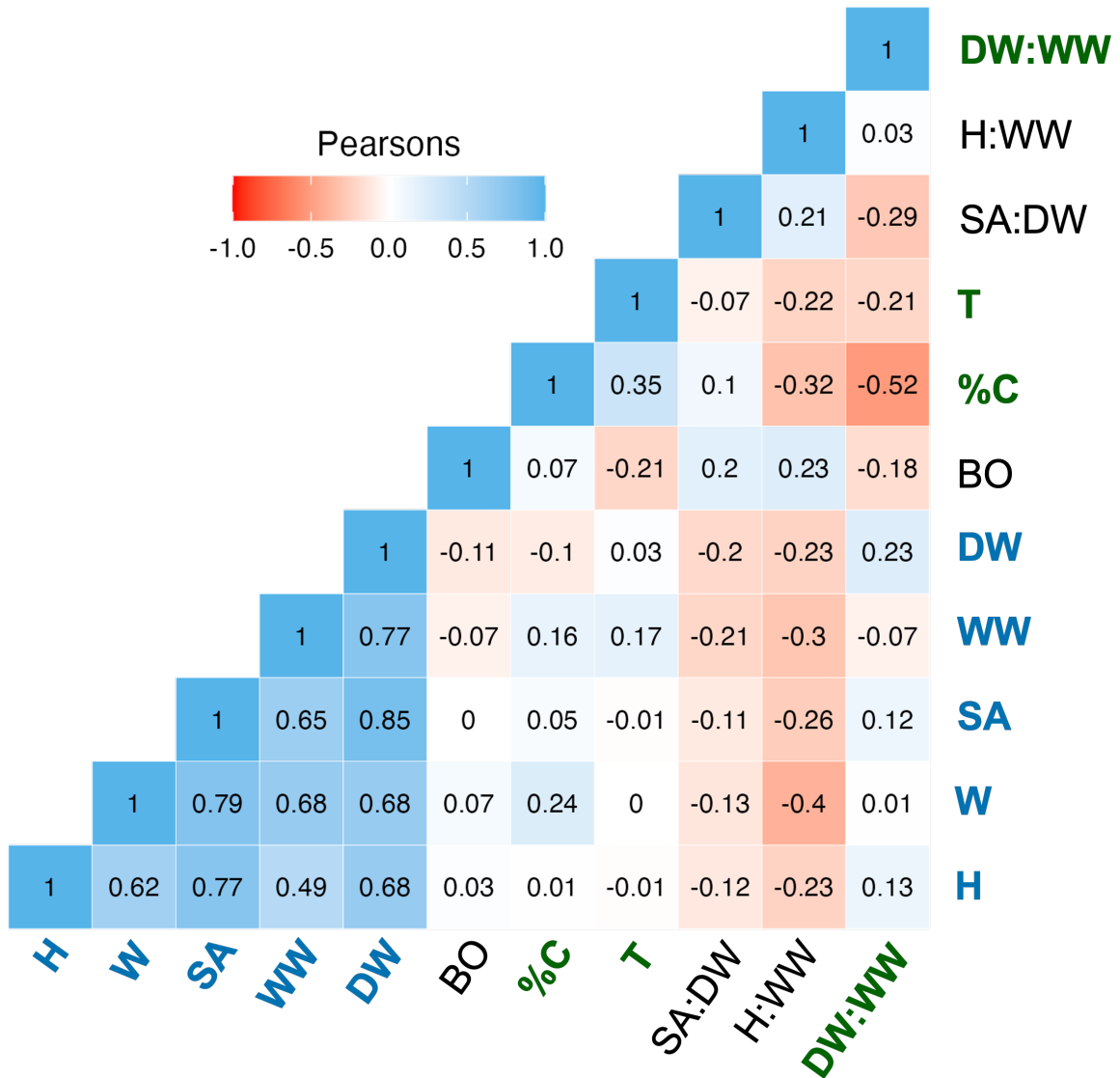


Figure 2-2: A correlation matrix illustrating the relationship between traits. Blue squares represent positive correlations while red squares represent negative correlations. Each square contains the correlation strengths. Trait names are color coded with blue font indicating traits that contribute to PC1, green to PC2, and black not contributing more than the null model to either PC. Abbreviations are: height (H), width (W), surface area (SA), wet weight (WW), dry weight (DW), branching order (BO), percent calcification (%C), toughness (T), surface area to dry weight ratio (SA:DW), height to wet weight ratio (H:WW) and dry weight to wet weight ratio (SA:DW).

Proposed Ecological Functions

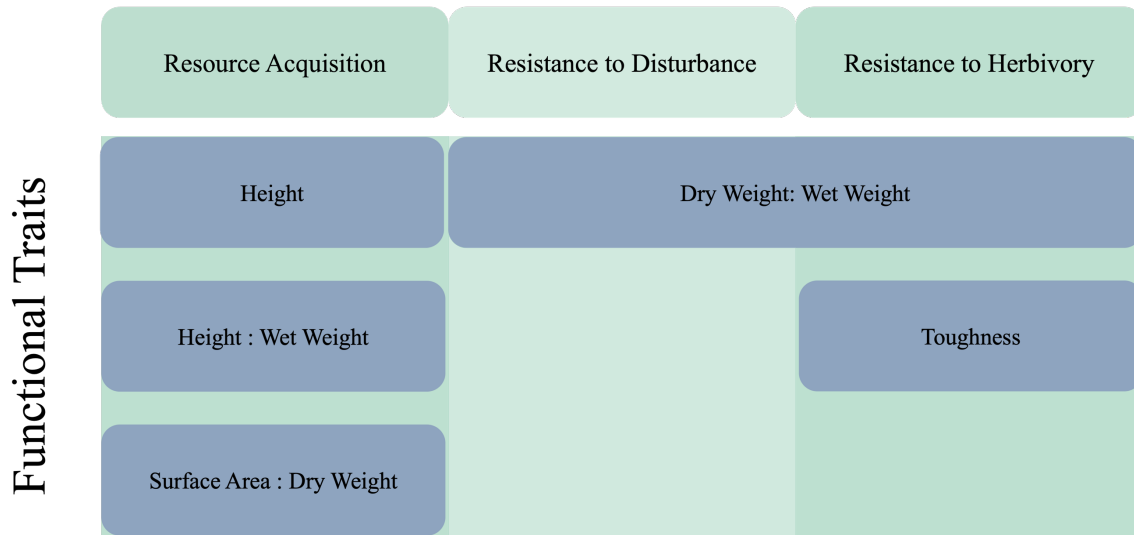


Figure 2-3: Five functional traits selected for the reduced model and their assignments to ecological function(s).

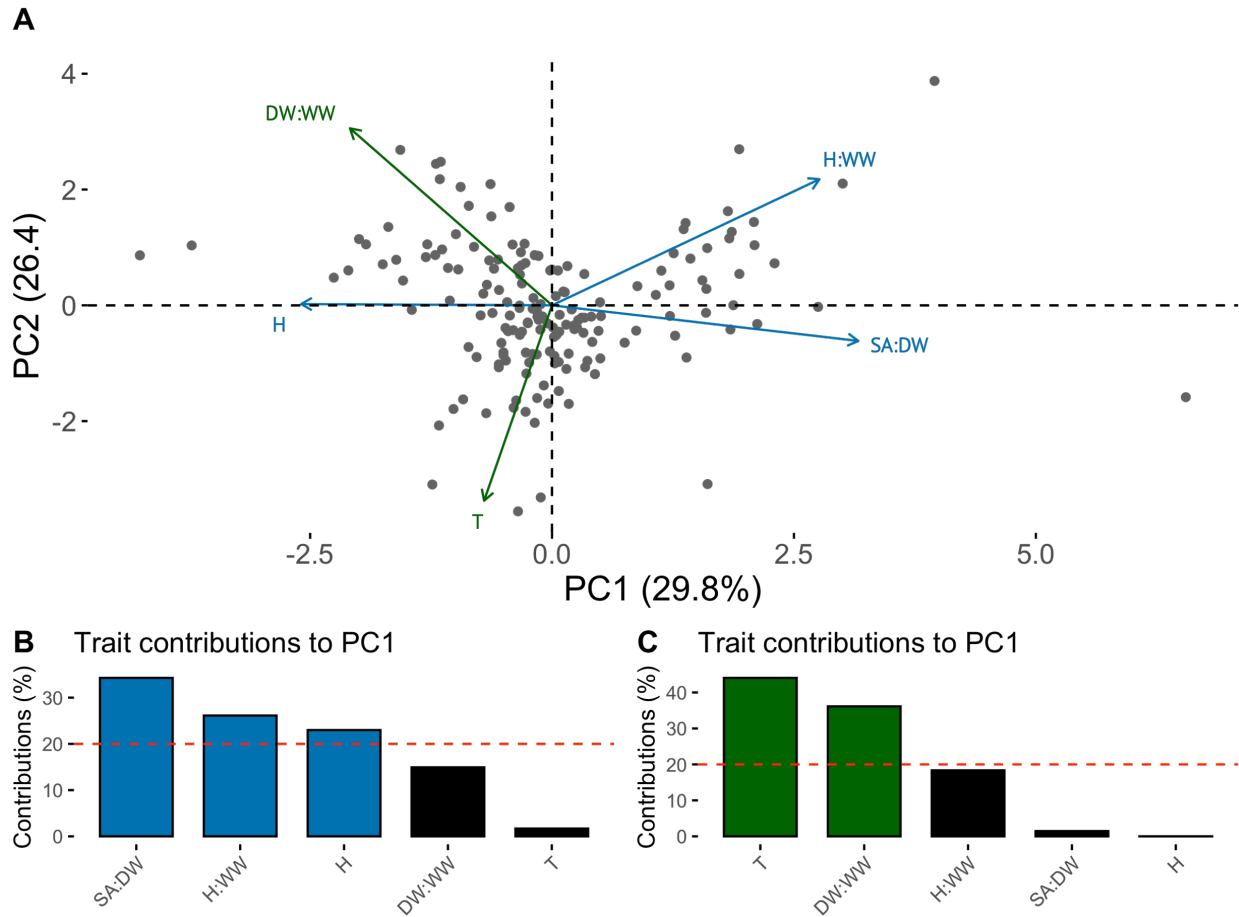


Figure 2-4: A.) PCA visualizing trait space created using the five functional traits in the reduced model. B.) The contribution of each trait to PC1. C.) The contribution of each trait to PC2. Vectors and bars in blue contribute more than the null expectation to PC1, vectors and bars in dark green contribute more than the null expectation to PC2. Abbreviations are: maximum height (H), toughness (T), dry weight to wet weight ratio (DW:WW), surface area to dry weight (SA:DW), and height to wet weight ratio (H:WW). Note scale change between B and C.

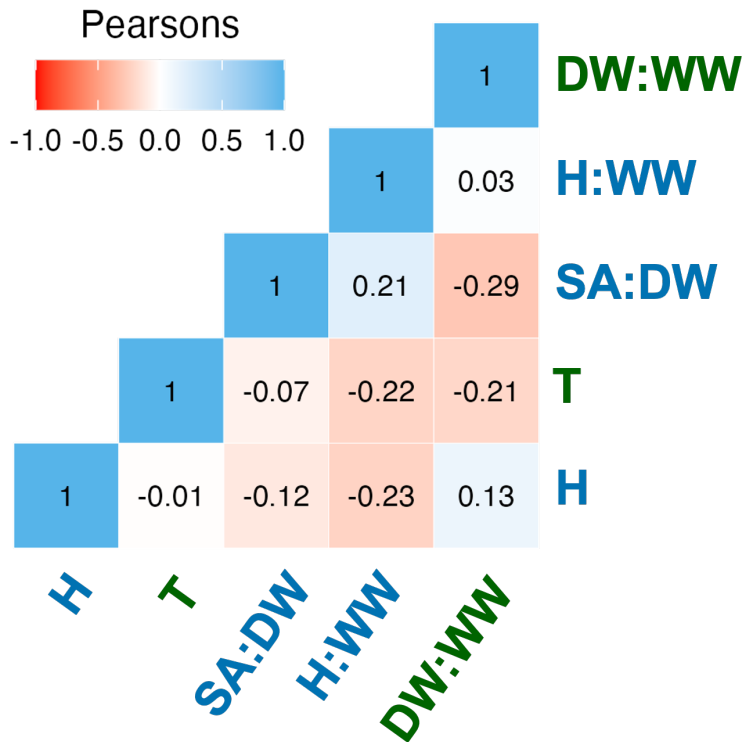


Figure 2-5: A correlation matrix illustrating the relationships between the traits in the reduced model. Blue squares represent positive correlations while red squares represent negative correlations. Each square has the correlation strengths written inside. Abbreviations are: maximum height (H), toughness (T), surface area to dry weight (SA:DW), height to wet weight (H:WW), and dry weight to wet weight (DW:WW).

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CHAPTER 3

Experimental manipulation of pulse disturbances associated with tropical storms initiates transitions from short to long turf that are reversed by herbivory

ABSTRACT

Tropical storms driven by continued climate change are predicted to increase in intensity in the Pacific generating multiple stressors to coral reefs, including to critical transition communities such as algal turf. Stressors include physical abrasion, sediment deposition, increased nutrients, and altered herbivore activity, all of which can co-occur in a pulse disturbance regime. We conducted a three phase field experiment (disturbance, cessation of environmental stressors, and return of herbivores) varying these stressors in a pulse regime on closely-cropped algal turf communities in Moorea, French Polynesia to assess their resilience. We found that experimental simulation of storm-associated stressors initiated a transition to long algal turf, with the major driver being absence of herbivores. Retention of deposited sediment slowed this transition, though this short sediment-laden turf was likely an overall negative for the ecosystem. Removal of cages to allow herbivore access reduced the depth of deposited sediment and turf height, and dramatically reduced turf cover below initial values, suggesting the recovered turf was more palatable than the original. Our findings imply that storm-generated disturbance can initiate community transitions on coral reefs and establish the critical role of herbivorous fishes in reversing them, furthering our understanding of the effects of tropical storms that will only increase in the Anthropocene.

INTRODUCTION

Storms can interact with a myriad of environmental and ecological drivers on coral reefs, including supplies of solubilized nutrients, light penetration, salinity levels, sedimentation (e.g.,

Ban et al. 2014), and the distribution, abundance, and herbivory pressure exerted by coral reef fishes (Kaufman 1983; Walsh 1983; Hayes et al. 2020). It is predicted that the intensity of tropical cyclones in the Pacific Basin will increase with the higher sea surface temperatures associated with increasing global climate change (Walsh and Ryan 2001; Mann et al. 2017; Bacmeister et al. 2018; IPCC 2018; Patricola and Wehner 2018; Bhatia et al. 2019). Due to this intensification of tropical storms, it is also predicted that multiple stressor effects on reef ecosystems and their interactions will intensify (IPCC 2019). Here, we define a stressor as a biotic or abiotic factor that elicits either a positive or negative change in a population (similar to Ban et al. 2014; Fong et al. 2020a), and is often defined as a stressor by the ultimate effect on the ‘desirable’ or natural community. For example, on coral reefs, increased nutrients may positively affect fast-growing or opportunistic algae, which can be considered a negative effect for a coral reef community and therefore a stressor to the reef system. Thus, the predicted increase in storms may alter multiple and interactive abiotic and biotic forces constraining algal growth on tropical reefs, thereby altering the impacts of multiple stressors on reef communities.

Multiple stressors, which can range in both intensity and frequency, have been described as lying on a spectrum from pulse to press regimes (Fong et al. 2020a). Pulse stressor regimes are characterized by shorter duration, lower frequency, and higher intensity episodic disturbance (e.g., Yang et al. 2008; Fong and Fong 2018; Fong et al. 2020a). In contrast, press stressor regimes are comprised of longer duration, higher frequency, and lower intensity chronic disturbance (e.g., Connell 1997; Fong et al. 2020a). Within this framework, tropical storms can produce stress regimes along the pulse vs press spectrum as the effects can vary in magnitude and duration, depending on the stressor. For example, storms can produce punctuated, intense pulses due to physical disturbances (e.g., Lirman and Fong 1997), while changes in other biotic

and abiotic forces may represent pulses of relatively longer duration (e.g., Hayes et al. 2020). Specifically, the potential for tropical storms to produce multiple stressors through increased nutrient supplies, sediment deposition, abrasion, and modification of top-down controls (Clausing and Fong 2016; Fong et al. 2020b; Hayes et al. 2020) has motivated our research to understand the effects of storm disturbance and the potential for recovery of coral reef communities.

Tropical storms produce pulses of precipitation (ICPP 2019), increasing terrestrial runoff and elevating nutrient supplies to coastal waters (Carlo et al. 2007; Clausing and Fong 2016; Fong et al. 2020b). For example, a storm in French Polynesia increased rainfall, elevating concentrations of inorganic nitrogen (N) and phosphorous (P) by 1-2 orders of magnitude over baseline in Pao Pao Bay (also known as Cook's) for 9 days (Fong et al. 2020b). Other studies in Pao Pao Bay also document that, as rainfall increases, relative N and P levels increase (Clausing and Fong 2016). Although previous studies have examined how macroalgae responds to press (Fong et al. 2020a) and pulse nutrient enrichment (e.g., Fong et al. 2003; Dailer et al. 2012; Fong and Fong 2018; Sura et al. 2022), the responses of other benthic primary producers to pulses of nutrient enrichment supplied by tropical storms remain unexplored.

Tropical storms in coastal ecosystems can mobilize benthic sediments, causing pulse disturbances of different regimes. One extremely short-term, high-intensity disturbance associated with high wave action during storms is abrasion by suspended sediment, loose rubble, and macroalgal thalli that can cause damage to and removal of other epilithic organisms (Sousa 1984; Kendrick 1991; Cheroske et al. 2000). Another disturbance occurs when increased water motion mobilizes benthic sediments and re-deposits them on hard substrates (Ban et al. 2014). Sediment suspension can be of longer duration than abrasion, in one case lasting 6 days

following an extreme rainfall event in Moorea (Fong et al. 2020b) and increased deposition has been documented on the Great Barrier Reef following tropical cyclones (Cheal et al. 2010). Increased sediment loads associated with deposition can have complex effects with some finding sediment accumulation reduces herbivory thereby facilitating algal growth (Bellwood and Fulton 2008; Goatley and Bellwood 2012) while others found algae traps sediment (Latrille et al. 2019) causing anoxia that inhibits algal growth (Clausing et al. 2014). While several studies document the effects of these pulse disturbances on algal growth, few (e.g., Airoidi 1998; Airoidi 2000a; Airoidi 2000b) have explicitly quantified the ability of algal communities to recover following storm-induced physical abrasion and increased sedimentation.

Tropical storms may suppress top-down control by herbivores. Surveys following a storm documented changes in herbivorous fish communities, such as reductions in foraging group size, that lasted for 22 days (Kaufman 1983), albeit in this study the remaining herbivorous fishes continued to forage. Another study documented a complete habitat shift of reef fishes from shallow to deeper waters that also lasted for several weeks after a severe tropical storm (Walsh 1983). Given the important role of herbivory in limiting proliferation of algal communities (Goatley and Bellwood 2013; Clausing et al. 2014; Fong et al. 2018), reductions in herbivory during and after intense storms may be a critical factor mediating algal response to storms. While studies document the suppression of top-down control by herbivory following a tropical storm (e.g., Hayes et al. 2020), they also document the return of coral reef fishes (Walsh 1983), suggesting reinstatement of top-down control after a time lag. More research is needed on how algae respond to the absence of herbivores during storms and the reintroduction of herbivory following the cessation of storm-induced pulse stressors.

Considerable recent attention has been concentrated on the effects of multiple stressors

on algal turf, as turf represents a critical transition community on coral reefs (e.g., Fong et al. 2018; 2020a; Schmitt et al. 2019; for a review see Tebbett and Bellwood 2019). Algal turf is comprised mostly of filamentous algae (Fong and Paul 2011; Littler and Littler 2013), is an early colonizer that rapidly covers newly-opened hard substrate (Birrell et al. 2005), and has become a dominant benthic space-holder following coral loss (Holbrook et al. 2016). In a reef community with intense herbivory, algal turf is generally < 1cm in height (Connell et al. 2014) and is often much shorter (e.g., Fong et al. 2018). Closely-cropped turf is highly productive and provides trophic support (Fong and Paul 2011; Harris 2015), while longer turf can reduce coral settlement (Birrell et al. 2005) and lower reef productivity (Tebbett and Bellwood 2020). Strong herbivory, low nutrient availability, and low sediment loads can maintain short, closely-cropped algal turf, at least over the short-term (Fong et al. 2018). In contrast, press environmental stressors, such as decreased herbivory, increased nutrients, and increased sediment, can shift short turf to a longer, sediment-laden state (Fong et al. 2018), at least in part by deterring herbivory (Goatly and Bellwood 2012; Clausing et al. 2014). The boundaries between what is considered healthy short and degraded long turf, however, vary across environmental contexts such as water flow and sediment type (e.g., Gaynus 2019; Tebbett and Bellwood 2019). Although previous studies have evaluated the resilience of algal turf under multiple stressors (e.g., Muthukrishnan and Fong 2014; Fong et al. 2018; Sura et al. 2019; Gaynus 2019; Fong et al. 2020a), less is known about the response of algal turf to changes in pulse stressors associated with episodic disturbance by tropical storms. Even less is known about the ability of longer turf to recover to a shorter state after pulse disturbances cease.

Our objective is to determine how a closely-cropped algal turf community responds to the experimental application and then cessation of multiple pulse stressors that we reason may result

during and after a tropical storm. These stressors include short-term addition of nutrients, simulated abrasion, sediment deposition, and loss of herbivores. The resilience of the turf community will be assessed by its ability to return to its original short state once these simulated storm disturbances are relieved.

METHODS

Overall approach

To simulate an episodic storm event and evaluate the effects on and recovery of an algal turf community on a fringing reef, we designed a three phase field experiment. Phase 1 (days 0 to 8) modeled a storm with pulse stressors. This was a 3-factor fully-crossed field experiment in which we simulated all possible combinations of a one-time abrasion event (+/0D) and 8 days of addition of nutrients (+/0N) and deposition of benthic sediments (+/0S). There were 10 replicates for each of the 8 experimental treatments. During phase 1, herbivores were excluded from all experimental units. In phase 2 (days 8 to 22), we ceased nutrient and sediment treatments and quantified the response of algal turf to their cessation. In this phase we continued to limit herbivory as evidence suggests changes in herbivore behavior can last well after initial storm disturbance (Kaufman 1983; Walsh 1983; Goatley et al. 2016). In phase 3 (days 22 to 24), we evaluated turf response to the return of herbivores. It should be noted that this 22-day absence of herbivores likely represents the extreme end of a spectrum of herbivore response to tropical storms and is therefore a very conservative test of whether return of herbivores can control algal turf transitions. During all three phases, we measured sediment depth, algal turf height, and percent cover of algal turf.

Site Characterization and Plot Selection

We conducted our experiment on a fringing reef (17°28'56.82"S, 149°48'49.01"W) off

the north shore of the island of Moorea, French Polynesia, a site in the Long-Term Ecological Research (LTER) Network. All work was conducted from April to May 2019. Some Moorean coral reefs have demonstrated high resilience following past disturbances, including tropical storms, bleaching events, and crown of thorns sea star (*Acanthaster planci*) outbreaks (Adjeroud et al. 2018; Edmunds et al. 2019).

Our experiment was conducted on a fringing reef that was ~ 100m from the north shore of Moorea and in relatively shallow water (1.8 - 4.9m). We reasoned this type of reef may be especially vulnerable to severe episodic disturbances (Highsmith et al. 1980; Kobluk 1992) and changes in herbivore activity (Kaufman 1983; Walsh 1983). We established plots on high topography hard substrate comprised largely of dead skeletons of coral in the genus *Porites* (hereafter bommies) that were killed during an outbreak of the voracious coral predator, *Acanthaster plancii* (Kayal et al. 2012). Bommies on fringing reefs along the north shore of Moorea are dominated by algal turf that is typically grazed to <1cm by herbivorous fishes (Gaynus 2019; Fong et al. 2020a).

Experimental plots were placed on bommies with tops that had adequate flat areas not comprised of damselfish territories. All plots were enclosed by cylindrical fences 14cm in diameter and 3cm in height that were nailed into the hard substrate surrounding each plot. Previous studies on Moorean fringing reefs demonstrated that turf was unlikely to grow taller than 3cm during the experimental duration (e.g., Clausing et al. 2014; Fong et al. 2020a). We created herbivore exclusion cages from fences by attaching removable lids to allow access for measurements during the experiment (similar to cages used for turf on Moorean reefs in Clausing et al. 2014; Sura et al. 2019; Fong et al. 2020). Caging material was metal hardware cloth with 1cm x 1cm openings to exclude macro-herbivores. Studies of cages of a variety of sizes made

from this material found no difference in flow among partial cages, full cages, or completely open plots (Grier unpublished data; Bittick unpublished data), and the reduction of irradiance by the caging material was <10% (Clausing unpublished data). Further, cages were used on all plots, so any cage effects that might have occurred were equal across treatments. Treatments were assigned randomly to each plot.

Experimental design and set up

Experimental Phase One: Storm Disturbance. To initiate phase 1, we applied disturbances as nutrient addition, abrasion, and sediment deposition, all in the absence of herbivory. To simulate the influx of added nutrients during the storm, 20g of Osmocote slow-release fertilizer (N:P:K 19:6:12) were placed in nylon bags and attached to the tops of cages to create enriched nutrient (+N) plots. This level exceeds the 10g used in Fong and Fong (2014), which was sufficient to produce significant localized nutrient effects. We doubled this value as we were simulating a storm where nutrient influxes can increase dramatically (Fong et al. 2020b). Unenriched nutrient (0N) plots received no fertilizer. Plots were placed at least 1m apart to limit cross-contamination of nutrients (Fong and Fong 2014).

We considered our abrasion treatment to be a physical disturbance as it modeled physical removal of algal turf by abrasion during a storm. To simulate abrasion, we scrubbed half of our plots with a stiff-bristled brush for 30 seconds to create the +D treatment. Undisturbed (0D) plots were not scrubbed.

Added sediment plots modeled pulsed additions that may occur due to resuspension and deposition of benthic sediment during a storm event (Goatley et al. 2016). For +S plots, benthic sediment was collected adjacent to the experimental units. An earlier study documented that these marine sediments comprised 92% sand, 8% silt and 0% clay with 2% organic matter

(Gaynus 2019). Sediment was placed in Ziploc bags with one corner cut out. These were used as piping bags to apply the sediment to a uniform depth of 2mm, as measured by a calibrated, millimeter-marked measuring device (Fong et al. 2018). We chose to add sediment to a specific depth rather than mass. Mass is more difficult to apply replicably in the field as pre-weighed ‘doses’ can be partially removed by current during application. Further, a review by Tebbett and Bellwood (2019) includes sediment depth as one of three ways that sediments are typically characterized. Previous experiments at a different site along the north shore of Moorea found that 2mm additions of sediment had negative effects on algal turf in the absence of herbivory (Clausing et al. 2014). This 2mm depth was maintained every 2 days through phase 1, while ambient sediment (0S) plots were left with ambient sediment depths.

All plots remained caged during phase 1.

Experimental Phase Two: Recovery. On day 8, we removed the Osmocote bags and ceased adding sediment. All plots remained caged during phase 2.

Experimental Phase Three: Restoration of Herbivory. On day 22, cages were completely removed from all plots, opening them to herbivores. This 22-day simulation of herbivory reduction should be considered an extreme end of a spectrum of reduction that can occur due to storms. Nails were left to mark the plot.

Measuring response variables

Sediment depth was measured at ten predetermined points within a 10cm x 10cm quadrat using a calibrated measuring device marked in 1mm increments. Depth was estimated to the nearest mm. Sediment was added to 2mm depth on day 0 of the experiment and maintained at this depth every 2 days through phase 1 (last sediment addition on day 6). To evaluate sediment retention between additions in phase 1, we measured sediment depth prior to replenishing it to

2mm on day 4. We measured retention again on day 8, but did not replenish it. During phase 2, no sediment was added, but sediment depth was measured every 4 days starting on day 10 to monitor any accumulation or dissipation of sediments across treatments. Sediment depth was measured again at the end of the experiment in Phase 3 on day 24.

Initial algal turf height and percent cover were measured on all experimental plots immediately following the abrasion treatment (if applicable) and then remeasured every 2 to 4 days throughout all three phases of the experiment. Turf height was measured at ten predetermined points within the same quadrat as we measured sediment depth using the same calibrated measuring device. Height was estimated to the nearest millimeter. If there was no algal turf at the point of measurement, the height was recorded as 0 mm.

Algal turf cover was measured initially (day 0, post-abrasion) and throughout all three phases of the experiment. Turf cover was measured using the point-intercept method. A 10cm x 10cm quadrat with 25 intercepts was used to assess the presence or absence of turf at each point. Points without turf were either abraded down to bare substrate, CCA, or (rarely) invertebrates. Percent cover was calculated by dividing the number of points where turf was present by the total number of points on the quadrat.

Statistical Analysis

We used generalized additive mixed models (GAMMs) to analyze all three response variables (sediment depth, turf height, turf cover) across the 3 phases because the patterns appear to be non-linear time series. To use GAMMs, we collapsed our 3 factors into a single factor with 8 levels, each of which is one of the 8 experimental treatment combinations. For sediment depth and turf height we used a gamma distribution with a "log" link function and for percent cover a binomial distribution with a "logit" link function. Because we were interested in exploring how

each treatment changed over time (day), we fit the GAMM with treatment plus 'day' as a continuous smoothed factor and with interactions between day and each treatment as a smoothed factor. We included plot with a random effect spline.

To explore the effects of our three fully-crossed factors (disturbance, nutrients, sediment) and their interactions within each experimental phase, we followed significant GAMMs with post hoc analyses. These were conducted for each experimental phase separately for sediment depth, algal turf height, and percent cover of algal turf. We used general linear mixed models (GLMMs) for phases one and two because the data did not meet assumptions of parametric statistics (normality tested with Shapiro, homogeneity of variances with Levine's). For sediment depth and turf height we used a gamma distribution with a "log" link function and for percent cover a binomial distribution. Fixed predictors included physical disturbance (categorical), nutrients (categorical), sediments (categorical), as well as interactions between these factors. For phases 1 and 2, plot number and day were included as random effects. Phase 1 had two sampling points for sediment depth (day 4 and 8) and three sampling points for algal turf height and percent cover (day 0, 4, and 8). Phase 2 had four sampling points for each measure (day 10, 14, 18, and 22). Phase 3 was analyzed with a general linear model with the same distributions, link functions, and predictors as data did not meet assumptions of parametric statistics (tested as above) except percent cover which had a quasibinomial distribution. However, we did not include day as a random effect as phase 3 had one sampling point for each measure (day 24).

All data were analyzed in R version 4.2.2. The generalized additive mixed models were conducted using GAM in the mgcv package (v1.8-41; Wood 2011) in R. The general linear mixed models were conducted using GLMER in the lme4 package (1.1-32; Bates et al. 2015) in R. The general linear models were conducted using GLM in the stats package in base R.

RESULTS

Sediment Depth

Initial sediment depth measurements taken on day 4 (2 days after sediment application to 2mm depth) averaged 1.4 ± 0.09 (SE) mm in added sediment treatments and 0.14 ± 0.03 (SE) mm in ambient sediment conditions. The efficacy of our sediment treatment was evidenced by the maintenance of a consistent difference in average sediment depth between added and ambient sediment treatments throughout all three experimental phases (Fig. 3-1a,b, compare dotted vs solid lines), despite cessation of our additions after day 6. Even after cages were removed and sediment depth decreased, there were visual differences maintained between ambient and added sediment treatments.

When all three phases were analyzed together, we found an effect of day on sediment depth (Table 3-1A, Fig. 3-1). The overall temporal pattern was for sediment to remain relatively constant over time until the last sampling point, where it dropped after cages were removed. However, this pattern was not consistent across all 8 treatments, resulting in a two-way interaction between day with the treatment with no disturbance, added nutrients, and ambient sediment (0D, +N, 0S, solid yellow line with triangle symbols). In this treatment, sediments accumulated naturally over time then decreased in the last 3 sampling periods rather than only after day 22. While the interaction is significant, the difference in the pattern appears small and likely driven by low variances in this treatment. There was also a random effect of plot.

When each phase was analyzed separately, sediment treatment had a significant main effect on sediment depth during all 3 experimental phases (Table 3-2), demonstrating that sediment treatments were retained even after additions ceased (Fig. 3-1). After restoration of herbivory (phase 3, day 22-24), there was rapid sediment loss in all treatments; despite this loss,

there was still a main effect of sediment treatment.

Algal Turf Height

Initial algal turf height on day 0 averaged 0.08 ± 0.05 (SE) mm in abrasion treatments (+D) and 0.27 ± 0.05 (SE) mm in undisturbed plots (0D) (Fig. 3-2a,b). When all 3 phases were analyzed together, there was a strong effect of day (Table 3-1B), with the overall pattern of increases in turf height across all 8 treatments until cages were removed in day 22 and turf height decreased precipitously.

When each phase was analyzed separately, sediment addition had a significant main effect on turf height during the first 2 experimental phases (Fig 3-2, Table 3-2). By the end of the disturbance phase 1 (day 8), added sediment resulted in an overall ~50% decrease in turf height compared to ambient sediment treatments regardless of the level of disturbance or nutrients. During recovery (phase 2, day 8-22), after sediment and nutrient additions ceased, growth of algal turf continued to be impeded by the sediment added in phase 1. However, turf in all plots continued to increase in height (Figure 3-2), albeit the variability within treatments increased. For example, during phase 1, SE across all treatments ranged from ± 0.01 to 0.34 mm while SE ranged from ± 0.19 to 2.42 mm in phase 2. After restoration of herbivory (phase 3, day 22-24), there was a rapid reduction in algal turf height (Fig. 3-2) that was not affected by any of the prior experimental treatments (Table 3-3). During this period, algal turf heights in all treatments were grazed down to averages ranging from 0.48 ± 0.13 mm to 0.92 ± 0.38 mm, similar to their initial values.

Percent Cover of Algal Turf

Initial abrasion treatments (day 0) were not even across all experimental units. Rather, one treatment (+D,+N,0S) was reduced to a much lower average cover ($5.70\% \pm 5.53$ SE) than

the three other disturbed treatments, where averages ranged from $26.8\% \pm 3.21$ SE to $44.8\% \pm 4.65$ SE (Fig. 3-3). As for the other response variables, when data for all 3 phases were analyzed together, there was an effect of day (Table 3-1C), but with 2 interactions demonstrating this pattern was not universal. The first interaction was between day and the treatment with disturbance only (+D, 0N, 0S – solid maroon line with circle symbols). This treatment recovered much faster to a high % cover, but also declined more precipitously than the other treatments. The second interaction was between day and the disturbed, added nutrient, ambient treatment (+D,+N,0S -the solid blue line with triangle symbols). This treatment was the heavily damaged one that did not increase cover as much as the other treatments in phase 1 nor decrease as much in phase 2.

During the disturbance (phase 1, day 0-8) there were main effects of both disturbance and sediment on algal turf cover as well as a marginally significant two-way interaction between them (Table 3-4). Cover increased in disturbed treatments but remained high or decreased in undisturbed treatments (Fig. 3-3a,b) while sediment addition had an overall negative effect. The marginal interaction was generated because there was much less cover in the disturbed, added nutrients and ambient sediment treatment (+D, +N, 0S – solid blue line with triangles) the treatment with much lower initial cover due to abrasion when the experiment started and never recovered.

During the recovery phase (2, day 8-22), there were no main effects or interactions. Rather, treatments plateaued, experiencing little to no overall change in percent cover (Fig. 3-3). During the restoration of herbivory phase (3, day 22-24), the cage removal drove a rapid reduction in percent cover across all treatments (Fig. 3-3). There was a main effect of sediment during this phase (Table 3-4), where declines were less with added sediment.

DISCUSSION

Overall, we found that our closely-cropped algal turf community was strongly impacted by pulse disturbances associated with tropical storms, but was also capable of rapid recovery facilitated by restoration of herbivory. Disturbances that simulated storms released algal turf from growth constraints on height and cover, with the most important being removal of herbivores. Sediment deposition posed the greatest limitation on transitions from short to long turf, and this limitation lasted well beyond the period of addition. Cover of algal turf that was reduced by abrasion had the capacity to regain cover rapidly. Re-covering of hard substrate by turf was strongly curtailed by enduring effects of sediment deposition and perhaps initial level of abrasive damage. In contrast, restoration of herbivory rapidly and effectively counteracted stressor effects by removing sediment, reducing turf height, and opening benthic space. However, restored herbivory reduced turf cover below initial pre-disturbance levels, suggesting that the turf that recovered may have been more palatable than the initial community. These results imply that, while algal turf communities may begin to transition to longer, less desirable states during storms, restoration of strong top-down control will reverse these transitions.

Pulse disturbance from tropical storms can disrupt typically strong constraints on algal turf growth and expansion

Our study demonstrated that transitions from short to long algal turf may be initiated by the experimental application of the multiple and pulsed stressors associated with major tropical storms. We found algal turf has the capacity to grow taller and recover newly-opened space rapidly during and for at least two weeks after our experimental simulation of storm disturbance. These results imply that our experimental treatments released algal turf from the typically strong constraints that limit growth of these communities and keep algal turf in a short, heathy, and

likely sparse state on coral reefs (for reviews see Fong and Paul 2011; Tebbett and Bellwood 2019). Although nutrient limitation has been shown to be a critical constraint in several studies (Clausing et al. 2014; Fong et al. 2018, 2020a), it is not the driving force in our study. Rather, growth and expansion of algal turf was likely due to release from top-down control by herbivores, a well-established and strong force controlling primary producers on coral reefs (e.g., Goatley and Bellwood 2013; Clausing et al. 2014; Fong et al. 2018).

We found that pulsed sediment deposition strongly constrains the development of long turf/high cover states. While sediment deposition restricted the transition from short sparse to long dense turf, it is unlikely that turf maintained in a short state by sediment smothering is a net positive for the reef. This finding supports those that also found sediment deposition can effectively smother turf and slow growth in the absence of herbivory (Clausing et al. 2014; Tebbett and Bellwood 2020), but contrasts others that show sediment may provide a benefit in the form of a nutrient source (Kendrick 1991; Russ and McCook 1999). While short turf is usually considered a hallmark of a healthy reef state (Tebbett and Bellwood 2019), it is unlikely that sediment-smothered turf, even in a short state, provides the same ecosystem functions as closely-cropped sediment-free turf (Tebbett and Bellwood 2020). Thus, shorter turf associated with sediment deposition may have significant costs rather than benefits to coral reef environments as past studies have shown increased sedimentation to inhibit coral recovery (Gil et al. 2016). Overall, our finding implies that increased sedimentation induced by tropical storms may limit transitions from short to long turf, but this may be at a net cost to the ecosystem.

Our results also suggest that algal turf may be strongly constrained by available space as initial abrasion in treatments that reduced cover of turf by over 50% returned to pre-disturbance cover within four days of the initial disturbance. Other algal turf communities have also been

shown to rapidly return to a state of high percent cover after disturbance (Airoldi 1998; Airoldi 2000a; Airoldi 2000b). This response is congruent with other literature that establishes the overall opportunistic life history of algal turf, including rapid regeneration (Littler et al. 2006) and the ability to colonize newly-opened substrate (Vroom et al. 2006). Our study demonstrates that algal turf will rapidly colonize open space when pressure from herbivory is reduced, suggesting competition within algal turf communities for available benthic space on coral reefs is fierce.

We found indirect evidence implying there may be a threshold of damage below which algal turf is constrained in its ability to recover open space. The treatment that sustained the most abrasive removal of turf, down to an average of about ~6% cover, did not regain cover as rapidly and plateaued at a lower level than treatments with more moderate initial damage. One study in the Mediterranean found recovery of algal turf from full removal took 4 months while less damaged algal communities recovered more rapidly (Airoldi 1998). Thus, our results demonstrate that algal turf communities on coral reefs can be extremely resilient to physical disturbance, but the community's ability to re-cover hard substrates may be constrained by the intensity of physical abrasion.

The critical role of restored herbivory

Our results demonstrate that closely-cropped algal turf communities on fringing reefs are resilient to pulse stressors associated with storm disturbance, as long as herbivorous fishes return to these systems once disturbance ceases. The resilient nature of algal turf to storm-associated stressors is consistent with a long-term study that demonstrates coral reef communities can recover faster after short-term shifts induced by pulse stressors than by chronic, press stressors (Connell 1997). Our results also align with previous studies that demonstrated the strong positive

influence of herbivores on maintaining coral reef resilience (Hughes et al. 2007; Cheal et al. 2010). However, a study on the Great Barrier Reef found grazing by herbivorous fishes remained suppressed by ~90% with a concurrent increase in algal turf height for one year after repeated storm disturbances (Goatley et al. 2016). It is likely that the disturbances from sequential tropical storms that lasted over a year in that study should be considered a press rather than a pulse disturbance, with very different outcomes. Our rapid and complete reversal of the trajectory toward a transition between turf states, even on a fringing reef in close proximity to human impacts, suggests that these reefs may be resilient to the predicted increase in storm intensity associated with climate change.

In our study, herbivory rapidly reverses trajectories toward long algal turf. Allowing herbivores access to plots rapidly and efficiently returned the algal turf to its original short state, regardless of the longer-term retention of deposited sediment. Previous studies suggest that even very small increases in sediment loads could reduce herbivore feeding rates and limit reef resilience (Goatley and Bellwood 2012). In contrast, we observed fishes feeding on the algal turf communities immediately after removal of cages (Fig. 3-4), suggesting that pulse sediment loads <2mm do not deter herbivore foraging in Moorea. Past experiments that document little change in algal turf height in the presence of herbivory regardless of sedimentation in Moorea (levels 0, 2, and 4mm) also support strong top-down control of algal turf by herbivores regardless of sediment deposition (Clausing et al. 2014). Our results suggest that herbivores can reduce short term increases in algal turf height that may be facilitated by storms, thereby fostering the resilience of reef ecosystems following tropical storms.

It is likely that restored herbivory also functioned to clear deposited sediments. Removal of cages that prevented herbivores from entering plots effectively removed deposited sediments

that had remained relatively stable for the 2-week period after our experimental additions ceased. While sediments were not completely cleared from the addition treatments in 2 days, the loss was rapid and dramatic with no indication it would not continue. It is possible that cage removal itself reduced the benthic boundary layer and resulted in sediment loss. However, another possible mechanism is bioturbation by the bristle tooth surgeonfish, *Ctenochaetus striatus*, that sweeps sediment-laden algal turf and ingests the trapped organic matter (Purcell and Bellwood 1993; Krone et al. 2011; Goatley and Bellwood 2010), though some have found a limit to their ability to remove sediment (Tebbett et al. 2017, 2020). Sura et al. (2021) identified *Ctenochaetus striatus* as one of the most common detritivorous fishes in Moorea. Reduction of deposited sediment by herbivorous/detritivorous fishes was also documented in Moorea as fishes bioturbated the sediment as they forage (Clausing et al. 2014). Taken together, these studies imply that some herbivorous/detritivorous fishes may play an additional, indirect role in restoring short algal turf on coral reefs following disturbance through removal of deposited sediment.

In our experiment, restored herbivory opened space within turf communities. Herbivores over compensated for the experimentally-induced hiatus from herbivory that we used to simulate fish behavior during and after storms (Kaufman 1983; Walsh 1983). Final turf cover was driven lower than initial levels in undisturbed plots and was greatly reduced in disturbed plots, albeit not to initially disturbed levels. One possible explanation was that the lack of herbivory facilitated an increase in dominance by rapidly-growing and highly palatable species of turf algae (Thacker et al. 2001). Our research suggests a storm-induced increase in palatability of algal turf communities may open up space for other organisms to settle. Whether it increases coral recruitment and enhances coral recovery requires more attention.

Tables

Table 3-1. Results of GAMM with all factors collapsed to one factor with 8 levels for A) sediment depth, B) turf height, and C) percent cover of turf. We used treatment as a fixed effect plus day and interactions between day and each treatment as two continuous smoothed factors, and plot as a random effect spline. Significant values are bolded.

Term(s)	A) Sediment depth			
	edf	Ref.df	F	<i>p</i> -value
Day	1.6882	1.882	4.638	0.0133
Plot	0.447	1	1.756	0.0480
Day x 0D,0N,0S	16115	1.839	2.005	0.1512
Day x 0D,0N,+S	1	1	0.634	0.4264
Day x 0D,+N,0S	1.8167	0.916	8.18	0.0012
Day x 0D,+N,+S	1	1	2.437	0.1191
Day x +D,0N,0S	0.7244	0.916	6.341	0.0163
Day x +D,0N,+S	1	1	1.449	0.2292
Day x +D,+N,0S	1.0001	1	0.961	0.3274
Day x +D,+N,+S	1	1	0.968	0.3256

Term(s)	B) Algal turf height			
	edf	Ref.df	F	<i>p</i> -value
Day	1.989	1.999	100.703	<2E-16
Plot	2.90E-06	1	0	0.3258
Day x 0D,0N,0S	1	1	2.713	0.1001
Day x 0D,0N,+S	1.196	1.352	0.511	0.4169
Day x 0D,+N,0S	1.06E-05	2.12E-05	0.426	0.9976
Day x 0D,+N,+S	1	1	2.819	0.0937
Day x +D,0N,0S	1	1	0.03	0.8627
Day x +D,0N,+S	1	1	1.106	0.2934
Day x +D,+N,0S	1.801	1.96	0.873	0.0598
Day x +D,+N,+S	1	1	0.429	0.5129

Term(s)	C) Percent Cover			
	edf	Ref.df	F	<i>p</i> -value
Day	1.91	1.978	8.595	0.0202
Plot	5.21E-06	1	0	0.5026
Day x 0D,0N,0S	1.197	1.3234	0.102	0.8001
Day x 0D,0N,+S	1.562	1.7967	2.947	0.3022
Day x 0D,+N,0S	6.26E-05	0.0001	0	0.99414
Day x 0D,+N,+S	1.618	1.8434	2.126	0.40249
Day x +D,0N,0S	1.875	1.983	11.301	0.0061
Day x +D,0N,+S	1	1	2.062	0.15103
Day x +D,+N,0S	1.835	1.9694	11.438	0.0073
Day x +D,+N,+S	1	1.0001	2.888	0.0893

Table 3-2. Results of general linear mixed-effects models on sediment depth for phases a) one and b) two of the experiment, with physical disturbance (D), nutrients (N), and sedimentation (S) as fixed factors and day and plot as random factors. Results of c) general linear model on sediment depth of phase three with the 3 fixed factors as phase 3 was only one day. Significant values are bolded.

Factor	a) Phase 1			b) Phase 2			c) Phase 3		
	Sediment depth			Sediment depth			Sediment depth		
	Chisq	Df	<i>p</i> -value	Chisq	Df	<i>p</i> -value	Chisq	Df	<i>p</i> -value
D	0.0605	1	0.8057	0.0321	1	0.8579	0	1	0.7777
N	1.2805	1	0.2578	0.2190	1	0.6398	0	1	0.5308
S	309.5590	1	< 2x10⁻¹⁶	120.3805	1	< 2x10⁻¹⁶	36157	1	<2E-16
D x N	0.2888	1	0.5910	1.3547	1	0.2445	1	1	0.4728
D x S	0.3598	1	0.5486	0.7881	1	0.3747	0	1	0.7887
N x S	0.0199	1	0.8879	0.0230	1	0.8796	0	1	0.5197
D x N x S	1.5568	1	0.2121	0.0018	1	0.9657	-415	1	1

Table 3-3. Results of general linear mixed-effects models of algal turf height for phases a) one and b) two of the experiment, with physical disturbance (D), nutrients (N), and sedimentation (S) as fixed factors and day and plot as random factors. Results of c) general linear model on sediment dept of phase three with the 3 fixed factors as phase 3 was only one day. Significant values are bolded.

Factor	a. Phase 1			b. Phase 2			c. Phase 3		
	Algal turf height			Algal turf height			Algal turf height		
	Chisq	Df	<i>p</i> -value	Chisq	Df	<i>p</i> -value	Chisq	Df	<i>p</i> -value
D	2.1756	1	0.1402	0.0212	1	0.8841	0.0821	1	0.7745
N	0.5378	1	0.4633	0.0482	1	0.8263	0.0767	1	0.7819
S	13.8892	1	0.0002	5.7851	1	0.0162	0.0548	1	0.815
D x N	0.0011	1	0.9138	0.0241	1	0.8767	2.3476	1	0.1255
D x S	0.8309	1	0.3620	1.4291	1	0.2319	1.3278	1	0.2492
N x S	0.4107	1	0.5216	0.1140	1	0.7356	1.0620	1	0.3028
D x N x S	0.2309	1	0.6309	0.2565	1	0.6125	0.0292	1	0.8643

Table 3-4. Results of general linear mixed-effects models of algal turf cover for phases a) one and b) two of the experiment, with physical disturbance (D), nutrients (N), and sedimentation (S) as fixed factors and day and plot as random factors. Results of c) linear model on sediment dept of phase three with the 3 fixed factors as phase 3 was only one day. Significant values are bolded.

Factor	a. Phase 1			b. Phase 2			c. Phase 3		
	Percent cover			Percent cover			Percent cover		
	Chisq	Df	<i>p</i> -value	Chisq	Df	<i>p</i> -value	Chisq	Df	<i>p</i> -value
D	11.6516	1	0.0006	0.0163	1	0.9072	0.9791	1	0.3224
N	1.1289	1	0.2880	2.1656	1	0.1411	0.0893	1	0.765
S	5.4035	1	0.0201	0.0020	1	0.9644	5.1386	1	0.0234
D x N	2.1991	1	0.1381	0.9562	1	0.3281	0.4054	1	0.5243
D x S	3.6758	1	0.0552*	0.0000	1	0.9952	0.9549	1	0.3285
N x S	0.7006	1	0.4026	0.0010	1	0.9930	0.1185	1	0.7306
D x N x S	0.5666	1	0.4516	0.0000	1	0.9998	0.433	1	0.5105

Figures

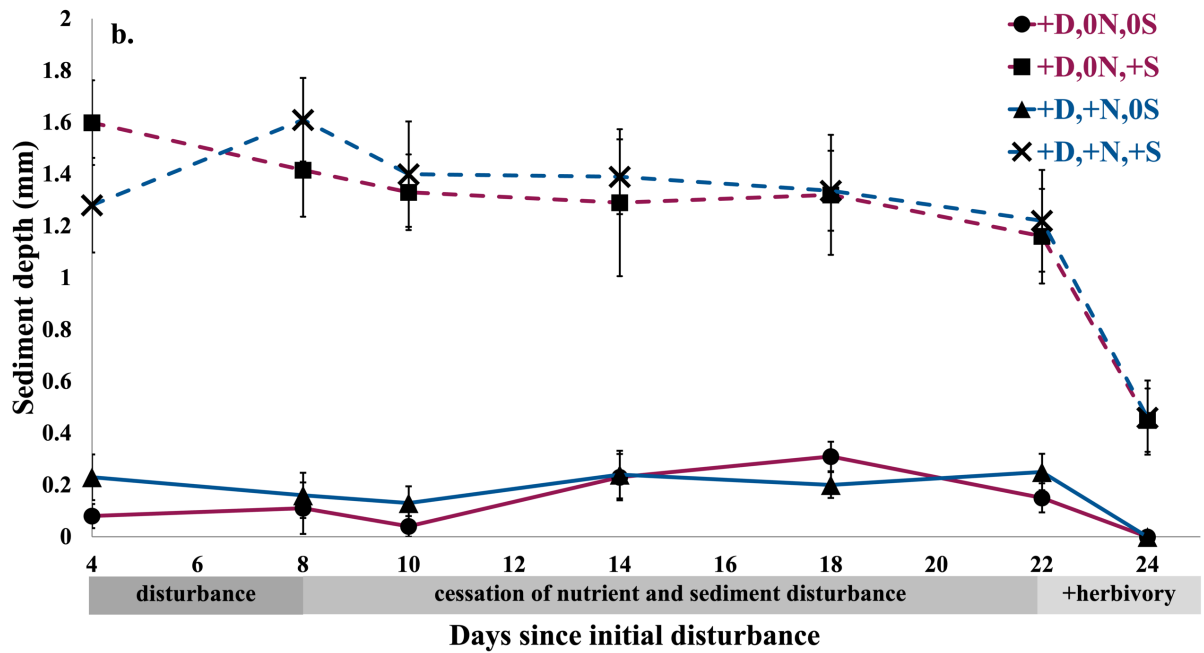
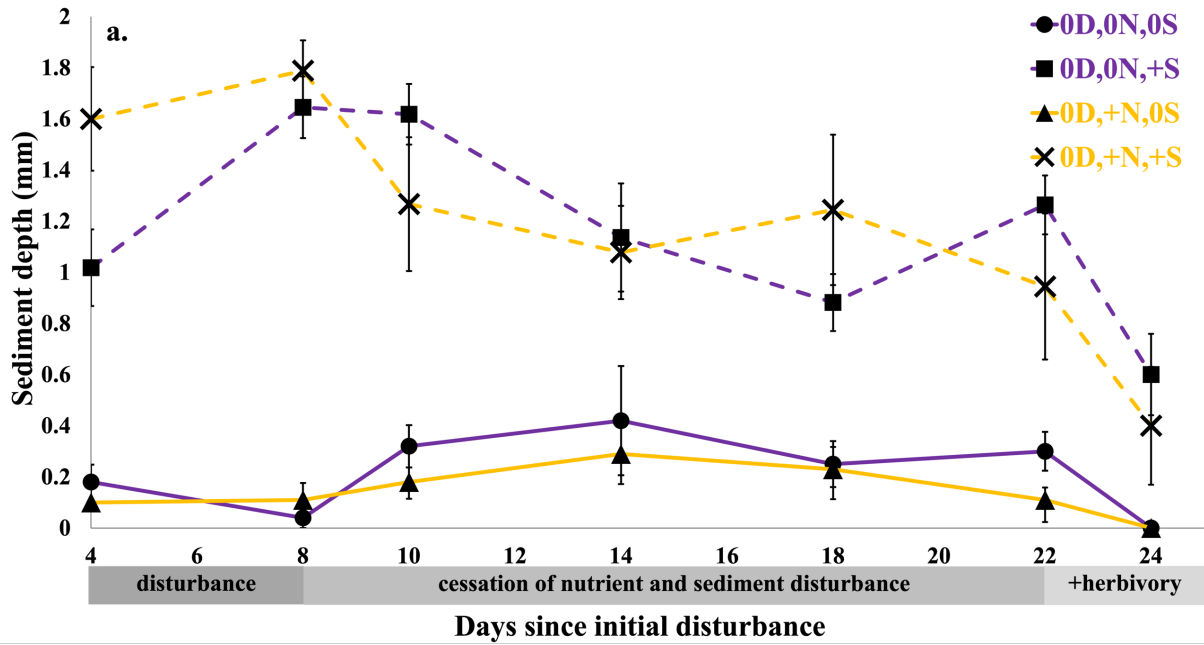


Figure 3-1: Sediment depth (mm) of treatments over time. Graphs are separated by undisturbed treatments (purple and yellow, Fig. 3-1A) and disturbed treatments (blue and maroon, Fig. 3-1B). Sediment depth measurements began on day 4 of the experiment. Dashed lines indicate added sediment treatments. Error bars are \pm SEM

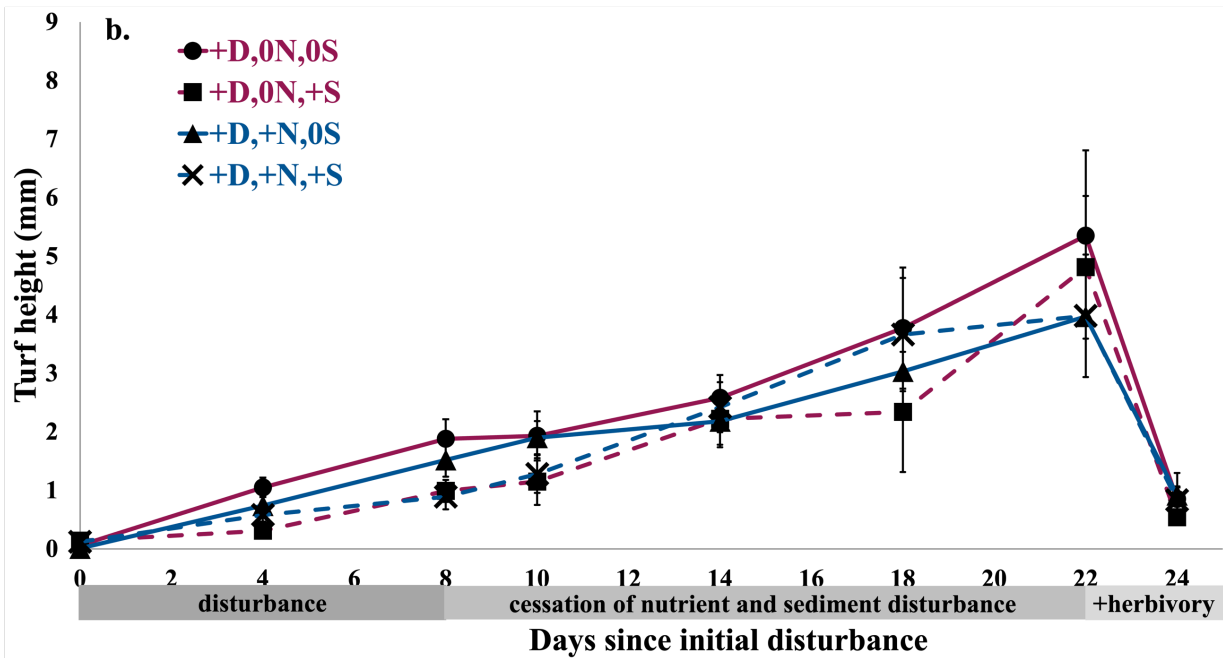
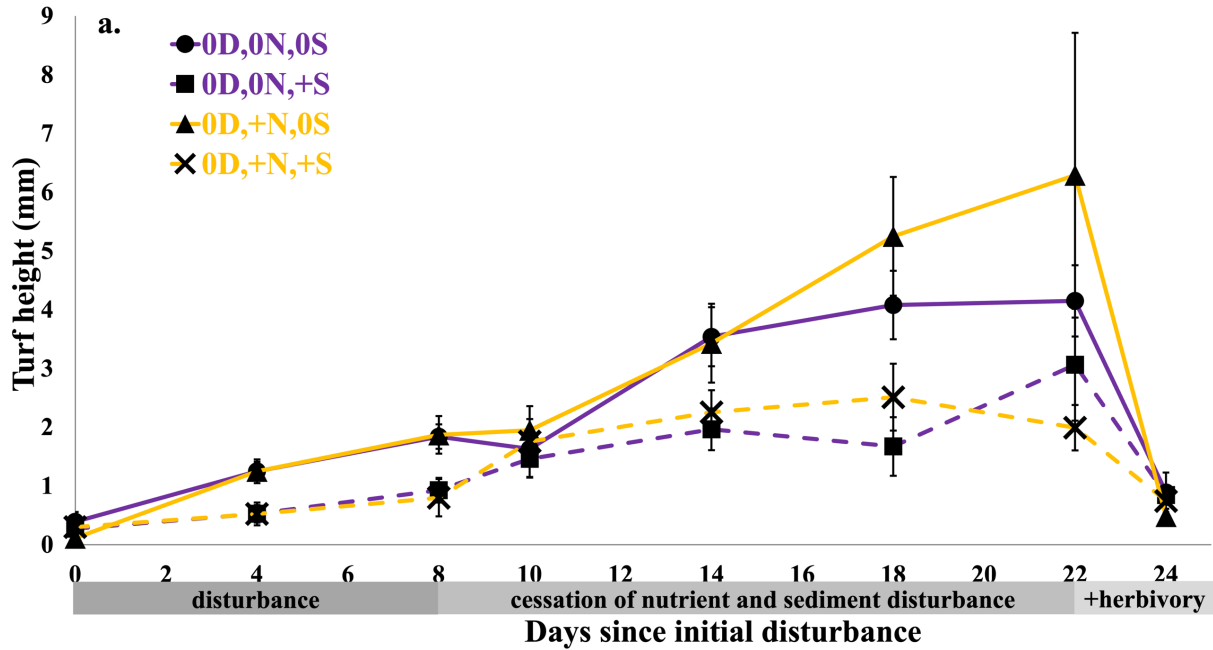


Figure 3-2: Time series of mean algal turf height (mm) per treatment. Days 1-8 simulated episodic disturbance, during which time nutrient and sedimentation treatments were maintained. Days 8-22 simulated recovery period, which added nutrients were removed and added sediment allowed to dissipate, respectively. Days 22-24 were all open to herbivory. Error bars are \pm SEM

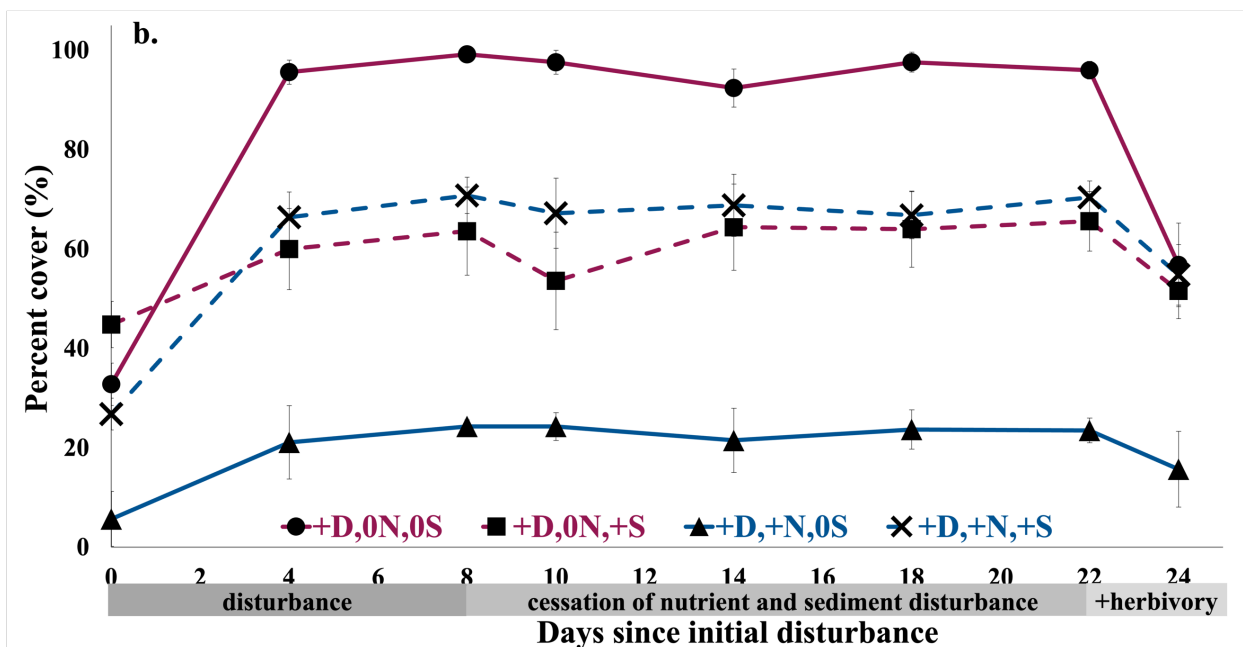
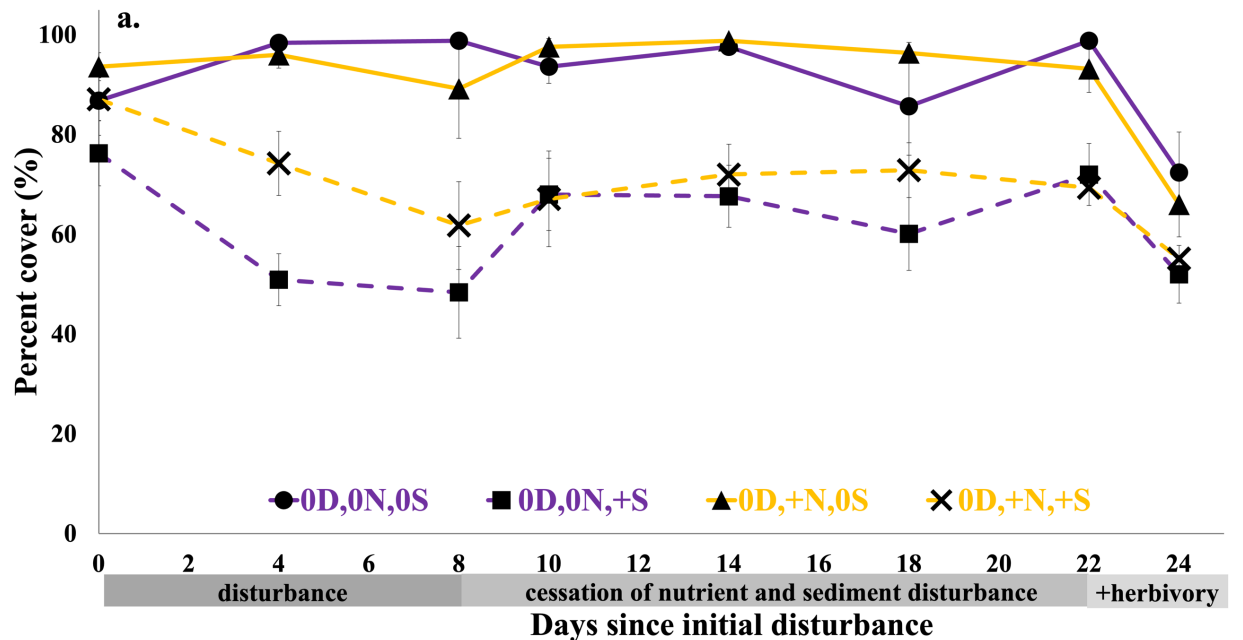


Figure 3-3: Time series of percent cover per treatment. Days 1-8 simulated episodic disturbance, during which time nutrient and sedimentation treatments were maintained. Days 8-22 simulated recovery period, which added nutrients were removed and added sediment allowed to dissipate, respectively. Days 22-24 were all open to herbivory. Error bars are \pm SEM

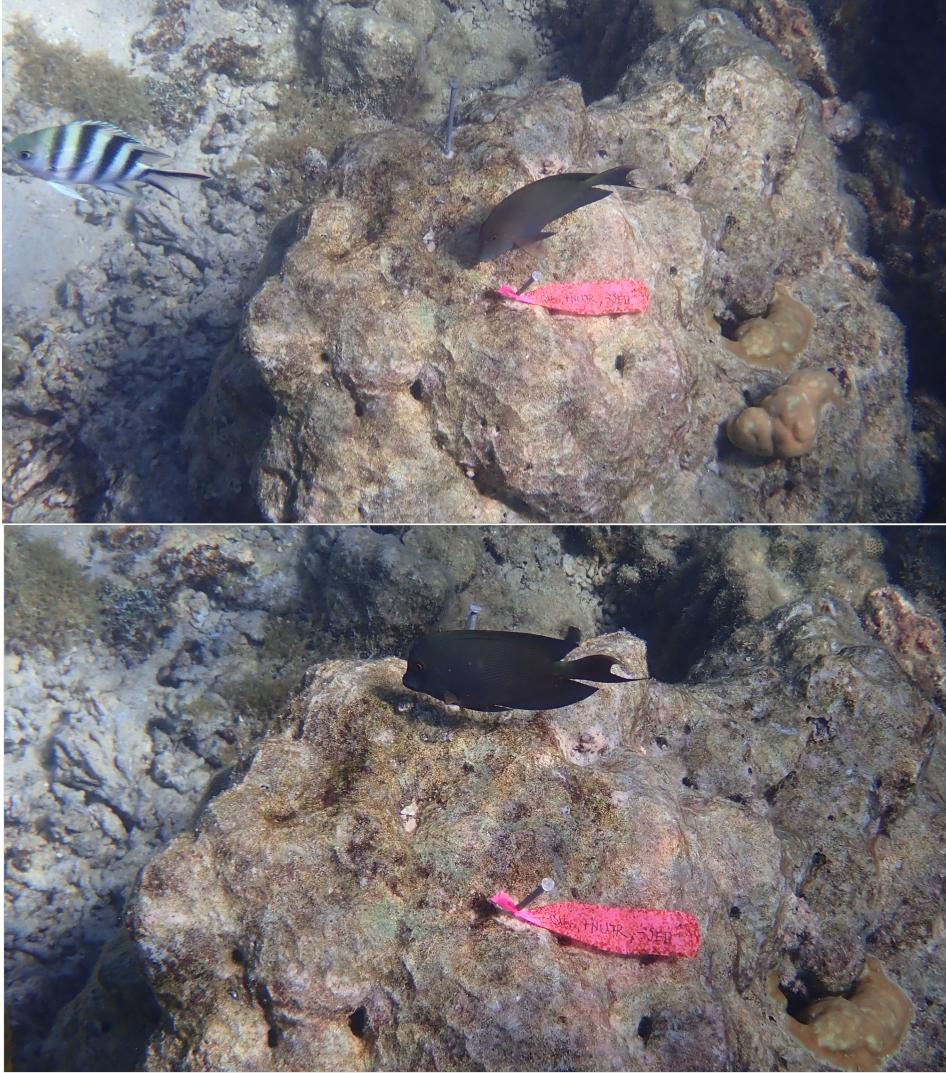


Figure 3-4: An Acanthuridae pictured grazing on algal turf immediately following the removal of cages on day 22 of the experiment. Photo by Lauren Smith.

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APPENDIX 1 – CHAPTER 1 SUPPLEMENT

Supplementary results

Table S1-1: This table shows the principal components for the full model along with their eigenvalue and percent explanation.

Principal Component	Eigenvalue	Percent Explanation
PC1	8.161	51%
PC2	2.004	12.5%
PC3	1.600	10%
PC4	1.183	7.4%
PC5	0.761	4.8%
PC6	0.548	3.4%
PC7	0.508	3.2%
PC8	0.350	2.2%
PC9	0.279	1.7%
PC10	0.232	1.4%
PC11	0.126	0.8%
PC12	0.109	0.7%
PC13	0.074	0.5%
PC14	0.040	0.2%
PC15	0.017	0.1%
PC16	0.007	0%

Proposed Ecological Function

	Resource Acquisition	Resistance to Disturbance	Resistance to Herbivory
Traits Measured	Wet Weight (WW)	Tensile Strength (TS)	
	Height (H)	Holdfast wet weight	
	Width (W)	Holdfast thickness	
	Surface Area (SA)	Holdfast wet weight : Total Wet Weight	
	Dry Weight (DW)	Dry Weight : Wet Weight (DW:WW)	
	Volume (V)		
	Perimeter (P)		
	Height : Dry Weight (H:DW)		
	Surface Area : Volume (SA:V)		
	Surface Area : Dry Weight (SA:DW)		
	Branching Order (BO)		

Figure S1-1: Typology of the traits included in the full model for algae collected in Chapter 1. Traits are placed in the ecological function where the maximum trait value indicates an investment in that function.

Supplementary Figures

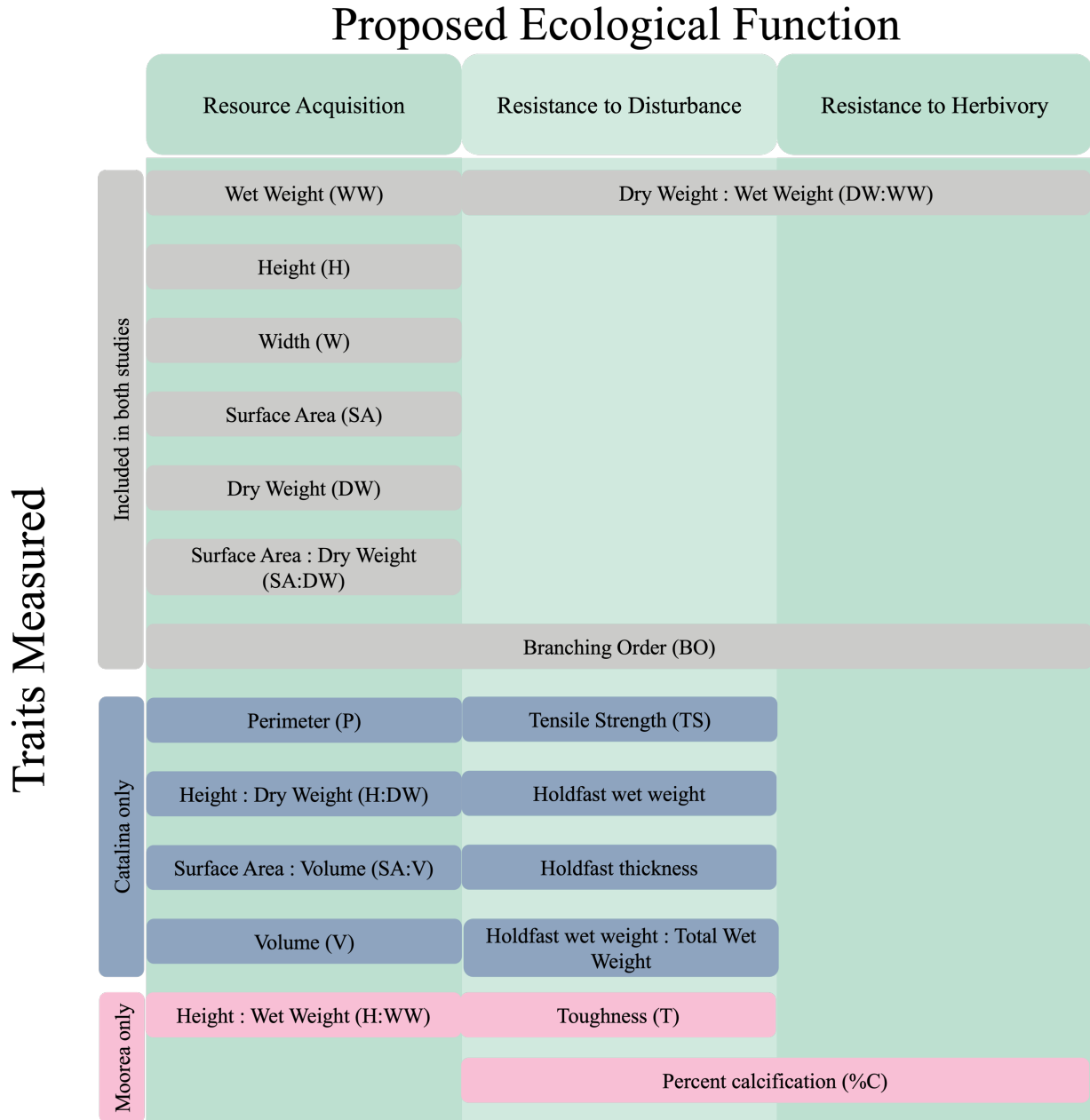


Figure S2-1: Typology of the traits included in the full model for algae collected in Catalina, a temperate rocky intertidal, and Moorea, a tropical fringing reef. Traits in gray were measured in both studies, traits in blue were measured in the Catalina study, and traits in pink were measured in the Moorea study. Traits are placed in the ecological function where the maximum trait value indicates an investment in that function.

Proposed Ecological Function

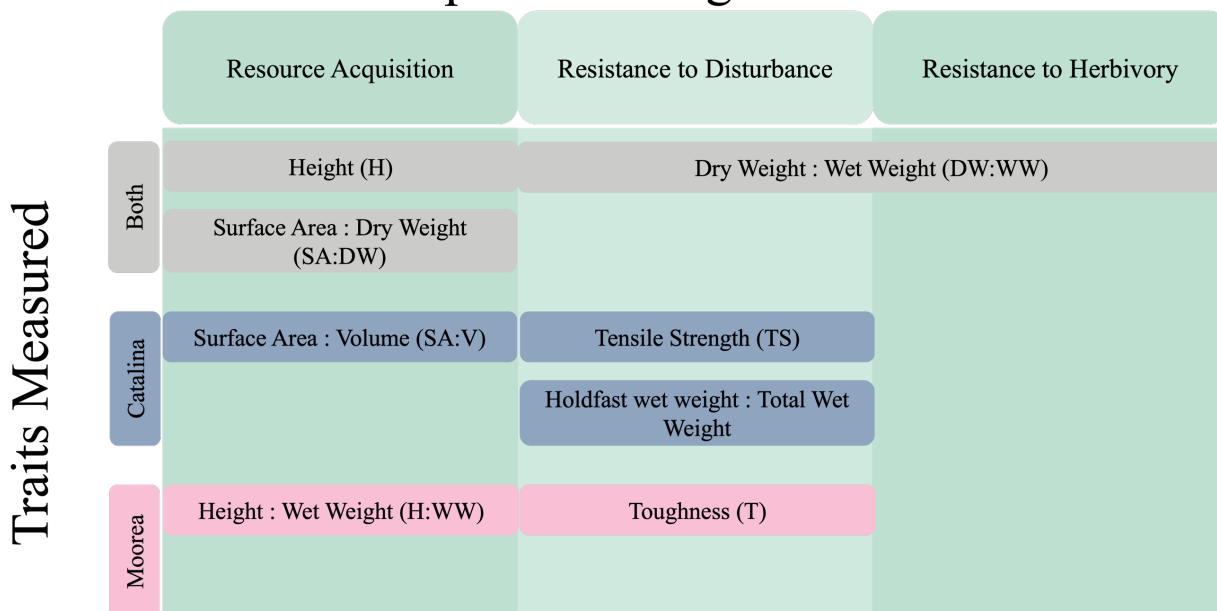


Figure S2-2: Typology of the traits included in the reduced model for algae collected in Catalina, a temperate rocky intertidal, and Moorea, a tropical fringing reef. Traits in gray were measured in both studies, traits in blue were measured in the Catalina study, and traits in pink were measured in the Moorea study. Traits are placed in the ecological function where the maximum trait value indicates an investment in that function.