

THE EFFECTS OF SUBMARINE GROUNDWATER DISCHARGE ON TROPICAL REEF
BENTHIC COMMUNITY COMPOSITION, STRUCTURE, AND PRIMARY
PRODUCTIVITY

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ABSTRACT

Submarine groundwater discharge (SGD) is recognized as an important source of inorganic nutrients and freshwater to coastal waters worldwide. SGD has been implicated in changing benthic community structure and hypothesized to enhance primary productivity. The goal of this dissertation is to elucidate the role of SGD in structuring benthic macroalgal communities and changing overall reef productivity in a tropical ecosystem (Maunalua Bay, O‘ahu, Hawai‘i) by measuring a suite of variables *in situ* and using a variety of modeling techniques to understand when and where SGD structures benthic communities. I used benthic chamber stable isotope incubations to show that SGD increases carbon uptake of a dominant benthic alga by 82% and water column carbon uptake by 32%. The highest uptake rates occur at intermediate salinities (~21–22), indicating that mixing of nutrient-rich groundwater stimulates the productivity of algae and plankton in specific areas of coral reefs. Using surveys of macroalgae and *in situ* growth experiments, I found that species-specific macroalgal biomass and growth are significantly related to both SGD and long-term integrated indices of wave and wind exposure. I also related multivariate community structure to the temporal and spatial variability in tidally modulated SGD. At SGD seeps I documented low diversity and higher biomass of benthic species that can tolerate the biogeochemistry associated with high concentrations of SGD. Reefs with SGD can be hotspots for algal restoration and for species with large tolerance ranges for temperature, salinity, and nutrients to thrive. The site differences in both the SGD biogeochemistry and community structure underlines the importance of doing these types of studies at the watershed level. Understanding the effects of SGD on coastal communities will help direct and prioritize conservation and management efforts.

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INTRODUCTION

Submarine groundwater discharge in coastal systems

Submarine groundwater discharge (SGD) is defined as “any and all flow of water on continental margins from the seabed to the coastal ocean, regardless of fluid composition or driving force” (Burnett et al. 2003, Moore 2010). Although methods for measuring SGD fluxes have evolved and improved over the last few decades (Burnett et al. 2003), the global importance of SGD remains largely unquantified. However, it is increasingly clear that SGD is an important vector for many different types of solutes (Johannes 1980, Valiela and Costa 1988, Moore 1996, Taniguchi et al. 2002, Zhang and Mandal, 2012). Of particular interest to managers because of concerns over eutrophication, SGD has been recognized as an important source of inorganic nutrients and freshwater to coastal waters worldwide (Kontar and Zektser 1999, Burnett et al. 2003, Slomp and Van Cappellen 2004, Burnett et al. 2006): A recent study showed that the total SGD-derived DIN and DIP fluxes could be approximately 1.4- and 1.6-fold of the river fluxes to the Indo-Pacific and Atlantic Oceans, respectively (Cho et al. 2018).

SGD is an especially important source of both freshwater and solutes in high islands due to high precipitation rates, high shoreline to area ratio, high relief, and immature permeable soil (Kim et al. 2003, Hwang et al. 2005, Moosdorf et al. 2015). The SGD from high volcanic islands often has a high freshwater component due to the high recharge rate; this freshwater vector generally contains the greatest quantity of land-based solutes (Burnett et al., 2003). SGD can include highly variable carbonate chemistry, dissolved organic matter (DOM), dissolved inorganic nutrients, possibly pharmaceuticals and trace metals, which vary by aquifer and watershed land use (Knee et al. 2010, Young et al. 2015, Nelson et al. 2015, Richardson et al. 2017). For instance, studies have mapped and quantified the solutes and microbial communities transported via SGD containing wastewater from leaky sewer systems and on-site sewage disposal systems (OSDS), suggesting

that SGD can be a significant transport mechanisms for bacteria and viruses in some areas (Paytan et al. 2004, Boehm et al. 2004, Whittier & El-Kadi 2009, Yau et al. 2014). Another example of SGD contamination includes the leaching of inorganic nitrogen and pesticides used in agriculture into SGD (Arias-Esteves et al. 2008, Dailer 2010).

In watersheds experiencing high contamination of groundwater by anthropogenic non-point sources of nutrients, SGD is a chronic source of nutrients and freshwater to coastal systems due to its continuous or tidal mode of delivery (Paytan et al. 2006, Nelson et al. 2015, Richardson et al. 2017). Shallow coastal waters with freshwater SGD undergo both nutrient load and salinity changes on small time scales (ie. tidal, hourly) (Johnson et al. 2008). Additionally, in systems experiencing tidally driven SGD, the highest flux rates occur near shore at low tide (Dulaiova et al. 2010, Dimova et al. 2012, Kelly et al. 2013). Tidal pumping of SGD produces strong physical and chemical gradients in the coastline associated with distance from SGD especially at low tide (Johannes 1980, Johnson et al. 2008, Nelson et al. 2015).

Studies have shown that islands surrounded by coral reefs, which experience tidally modulated SGD input, release nutrients via vertical pore water upwelling, tidal pumping, and temperature-driven convection, which can in turn, lead to sustained productivity within coral reef systems (Santos et al. 2010). Tidally dominated SGD inputs can be chronic sources of nutrients to tropical reefs; this contrasts with fluvial nutrient inputs, which vary greatly with precipitation (Amato et al. 2016). Hence, in coastal areas with tidally dominated SGD inputs, SGD may comprise a significant component of reef nutrient inputs producing highly variable biogeochemistry on small temporal scales (ie. hours) and spatial scales (ie. meters).

In areas where the nutrient concentration of coastal groundwater has been substantially increased by residential and agricultural land use activities, nutrient loading to coastal waters via SGD has been associated with macroalgal blooms and shifts in community composition (Naim

1993, McCook 1999, Costa 2008, Lyons et al. 2014). The composition and abundance of the algal assemblage on a reef with SGD depends on which species can thrive in this chemical and physical environment. The multiple stressors associated with SGD (eg. low salinity, high nutrients etc.) leave reef systems vulnerable to permanent ecological transitions to algal-dominated states at sites (LaPointe 1997, Smith et al. 2005, Baker et al. 2008, Fung et al. 2011, Houk et al. 2014). This cyclical set of disturbances associated with SGD can occur at multiple time points throughout the day in a semidiurnal tidal system. In coral reef communities, it is often the interaction of persistent and multiple synergistic disturbances that cause permanent phase shifts (McClanahan et al. 2002).

Algal community structures and productivity in coral reef systems

Algal communities are an essential part of coral reef systems. Coralline algae encourage coral larvae settlement (Vermeij et al. 2008, Ritson-Williams et al. 2016) and reinforce reef structures using their skeletal calcite (McCoy & Kamenos 2015). Turf and fleshy macroalgae are important food sources for herbivores. The balance between coral and algal cover in reef ecosystems is a delicate one. It is well known that continuously elevated nutrient inputs on reef systems along with decreased herbivory are two of the main factors responsible for phase shifts from coral to macroalgal-dominated reefs (McCook 1999, Smith et al. 2001, Littler & Littler 2006, Smith et al. 2010). Experimental manipulation of nutrient and grazing levels show these factors can independently and interactively induce phase shifts from coral to algal dominated reefs in less than 6 months (Smith et al., 2001, Most 2012).

Nutrient increases worldwide have contributed to increased areal cover and biomass of algae (Valiela et al. 1997, Heisler et al. 2008). Nutrient pollution via surface runoff has been studied widely in all aquatic environments (NRC 2000, Smith 2002, Ocean Commission 2004, Ahmad et al. 2016) and has been linked to changes in benthic fauna (Magalhaes & Bailey-Brock

2014), the persistence of deleterious algal blooms (Howarth et al. 2002) and increased macroalgal biomass in coastal ecosystems (Bell 1992, Khan & Ansari 2005). Additionally, the success of certain algal species in reef flats depends on their ability to withstand hydrodynamic forces such as exposure to wind and waves. Water motion directly dictates the algal growth and community composition by imposing physical stress (D'Amours & Sheibling 2007) and varying nutrient delivery depending on the hydrodynamic forces acting on coastal environments (Hurd 2000, Thomas & Cornelisen 2003). The effects of wave and wind action vary with species morphology and structure, creating differential effects on the abundance of different species. This in turn, explains why hydrodynamic forces play a major role in shaping the benthic community composition, diversity, and species richness of marine ecosystems (Costa et al. 2000, Nishira & Terada 2010).

All primary producers have physiologically optimized ranges of tolerance for both inorganic nutrients and salinity (Kirst 1990, Guan et al. 2015, Liu et al. 2016). Although nutrient concentrations have long been known to affect primary productivity (Nixon et al. 1986, Duarte 1995), the effects of salinity on productivity are usually varied and species-specific (Sudhir and Murthy 2004). Experimental factorial studies of salinity pulses and nutrient loading on primary producers show nutrient loading tends to have a long-term effect through complex community interactions (Duarte 1995, Valiela et al. 1997), while salinity pulsing frequency and intensity has an immediate and direct influence on growth and distribution (Boustany et al. 2015).

Chapter objectives

Maunalua Bay, located on the southeastern shore of Oahu, has algal-dominated fringing reefs with localized tidally modulated SGD. These sites both experience two low and two high tides per day that can vary in height to the mixed semi-diurnal tidal regime. Hence, the SGD fluxes

vary on several cyclical scales at different temporal scales. The fringing reefs at two of these sites, Black Point and Wailupe, have well-characterized and chemically distinct SGD (Amato 2015, Nelson et al. 2015, Richardson et al. 2017, Lubarsky et al. 2018). Both sites are dominated by macroalgal species, whose presence and abundance varies across the reef flat, thus providing an interesting study site for the interaction of SGD with macroalgal benthic communities.

Black Point and Wailupe are dominated by invasive macroalgal species and due to the reefs' coastal topography, these sites experience a range of both wave and wind exposure. Chapter 1 explores the relationships between exposure and nutrient load at a local scale (~400 m) with respect to algal biomass, growth, and diversity *in situ*. We hypothesized that (1) species-specific and macroalgal biomass depend on SGD and at least one index of exposure, (2) species-specific growth rates will vary with SGD and herbivory, (3) and that diversity is related to both nutrient load and one or more exposure indices. The overall goal for this chapter is to evaluate the *in situ* associations of SGD gradients with macroalgal distributions and growth in the context of the reef hydrodynamics to better understand their combined effects on macroalgal species.

Chapter 2 measures *in situ* C-uptake of the main primary producers on an algal-dominated reef with SGD influence using benthic chambers. The experimental design was spatially and temporally explicit in order to capture C-uptake rates across a gradient of SGD input. We then coupled this data with benthic cover data to map and estimate both benthic and water column C-uptake on a reef with SGD and a reef without SGD. This work can elucidate the magnitude of the effect of SGD on both benthic and water column algal productivity of reefs. This work can inform managers involved in controlling invasive macroalgal overgrowth.

The major objectives for Chapter 3 are to (1) use a spatiotemporally explicit method to define patterns in the variability of SGD across reef flats which experience complex changes in magnitude and frequency of SGD-related biogeochemistry using salinity as the main tracer for

SGD, (2) assess whether multivariate community structure is related to the SGD spatiotemporal patterns of variation, and (3) elucidate what taxa or functional groups are driving these relationships, if any. Overall this study seeks to explore spatiotemporally explicit patterns in SGD and their effects on macroalgal-dominated benthic community structure on tropical reefs.

CHAPTER 1

MACROALGAL BIOMASS, GROWTH RATES, AND DIVERSITY ARE INFLUENCED BY SUBMARINE GROUNDWATER DISCHARGE AND LOCAL HYDRODYNAMICS IN TROPICAL REEFS

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Florybeth Flores La Valle, Florence I. Thomas, Craig E. Nelson

ABSTRACT: It is critical to evaluate the *in situ* effects of multiple stressors on coastal community dynamics, especially those harboring high diversity such as coral reefs, in order to understand the resilience of these ecosystems, prepare coastal management for future scenarios, and aid in prioritizing restoration efforts. In this *in situ* study, at two sites with gradients of submarine groundwater discharge (SGD), a suite of physical parameters (wave exposure index, wind exposure index, and depth) and an all-encompassing SGD chemical parameter (average N+N daily load) were measured along spatially cohesive and temporally relevant scales and used to model macroalgal growth, biomass, and diversity in Maunalua Bay, Hawai'i. We show that (1) species-specific macroalgal biomass is significantly related to SGD and one of the two exposure indices (ie. wind exposure or wave exposure), (2) SGD and wave exposure play key roles in species-specific growth rates, and (3) SGD supports low diversity and increased biomass of species that can tolerate the biogeochemistry associated with SGD. Our work suggests that SGD and local hydrodynamics predict local variation in macroalgal growth, biomass, and diversity in tropical reefs.

INTRODUCTION

Physicochemical factors are important in shaping the benthic community composition of coastal systems. A major chemical process that alters the community structure and function of coastal systems is nutrient pollution. Nutrient pollution via surface runoff has been studied widely

in all aquatic environments (NRC 2000, Smith 2002, Ocean Commission 2004, Ahmad et al. 2016) and has been linked to changes in benthic fauna (Magalhaes & Bailey-Brock 2014), the persistence of deleterious algal blooms (Howarth et al. 2002) and increased macroalgal biomass in coastal ecosystems (Bell 1992, Khan & Ansari 2005). Continuously elevated nutrient inputs on reef systems along with decreased herbivory have been shown to be the main factors responsible for phase shifts from coral to macroalgal-dominated reefs (McCook 1999, Smith et al. 2001, Littler & Littler 2006, Smith et al. 2010).

Submarine groundwater discharge (SGD), like fluvial runoff, is another potential source of nutrient pollution. Studies have shown that groundwater is a source of nutrients to streams, rivers, and coastal systems (Burnett et al. 2003, Zhang & Mandal 2012). SGD can be a source of land-based nutrients to coral reefs in high island systems (Paytan et al. 2006, Nelson et al. 2015). In areas where the nutrient concentration of coastal groundwater has been substantially increased by land use, nutrient loading to coastal waters via SGD has been associated with macroalgal blooms and shifts in community composition (Naim 1993, McCook 1999, Costa 2008, Lyons et al. 2014).

McClanahan and colleagues (2002) found that the interaction of persistent and multiple synergistic disturbances are often the cause of permanent phase shifts in coral reef communities. SGD in island settings has variable biogeochemical parameters such as salinity, pH, dissolved organic matter (DOM), dissolved inorganic nutrients, as well as other associated chemical factors, which vary by aquifer and land use (Knee et al. 2010, Young et al. 2015, Richardson et al. 2017). In watersheds experiencing high contamination of groundwater by land-based nutrients, SGD is a chronic source of nutrients to coastal systems due to its continuous or tidal mode of delivery (Richardson et al. 2017). Additionally, in systems experiencing semidiurnal tides, SGD and all its associated geochemistry fluxes can be high (Johnson et al. 2008, Holleman 2011). The multiple stressors associated with SGD leave reef systems vulnerable to permanent ecological transitions to

algal-dominated states at sites with large freshwater SGD fluxes with high nutrient concentrations (LaPointe 1997, Smith et al. 2005).

The composition and abundance of the algal assemblage on a reef with SGD depend on which species can thrive in this chemical and physical environment. All marine primary producers have an optimal range of nutrient concentrations and salinity for growth and production. Both of these environmental variables control major physiological functions; salinity drives osmotic and solute regulation (Wiencke & Bishof 2012), while nutrient concentrations drive uptake rates and productivity (Valiela et al. 1997, Thomas & Cornelisen 2003). Shallow coastal waters with freshwater SGD undergo both nutrient load and salinity changes on small time scales (ie. tidal, hourly) (Johnson et al. 2008). Algal communities living in nutrient and salinity variations on this time scale requires fast acclimation to wide ranges of these chemical parameters. These environmental conditions can preferentially spur the growth of some algal species over others. For example, studies have shown that the growth rate of *Gracilaria* sp. is optimal at salinities of 15 to 30 (Israel et al. 1999, Choi et al. 2006). This suggests that there are species better adapted to tolerate coastal systems with high freshwater SGD flux.

Additionally, the success of certain algal species in reef flats depends on their ability to withstand hydrodynamic forces such as exposure to wind and waves. Fringing reef flats are shallow and close to shore therefore making them vulnerable to sedimentation from adjacent land and increased turbidity with wave action (Airoldi 1998, Elfrink & Baldock 2002, Balata et al. 2007). Water motion directly dictates the algal growth and composition by imposing physical stress (D'Amours & Sheibling 2007) and varying nutrient delivery depending on the hydrodynamic forces acting on the area (Hurd 2000, Thomas & Cornelisen 2003). For example, studies have shown that even when nutrient delivery is greatest at a reef crest, macroalgal growth is minimal due to wave action (Hurd 2000, Lilliesköld Sjöo et al. 2011). The physical structure (ie.

morphology, flexibility, size) of benthic species affects their ability to tolerate the mechanical forces of drag and lift and their physiological ability to take up nutrients by creating a thinner or thicker diffusive boundary layer (Koehl 1986, Denny et al. 1989, Carrington 1990, Koch 1993, Hurd 2000). The effects of wave and wind action vary with species morphology and structure, creating differential effects on the abundance of different species. This in turn, explains why wave action and hydrodynamic forces play a major role in shaping the benthic community composition, diversity, and species richness of marine ecosystems (Costa et al. 2000, Nishira & Terada 2010).

Previous work has shown that both eutrophication and wave exposure have significant effects on algal assemblages in the coastal zone (Flores et al. 2015, Pihl et al. 1999) but there are no studies which have looked at these factors together on algae-dominated reef flats with SGD. Maunalua Bay, located on the southeastern shore of Oahu, has 3 algal-dominated fringing reefs, which extend from shore to about 200–400 m offshore, with groundwater input occurring at the shoreline. The SGD at 2 of these sites, Black Point and Wailupe, is lower in salinity (~2–4) and highly enriched in nutrients compared to surrounding coastal waters, thus providing an interesting study site for the interaction of these physical and chemical factors with the macroalgal communities in these systems. Additionally, the fringing reefs of Black Point and Wailupe (Fig. 2) have been the site of a previous study defining biogeochemical zones associated with SGD (Nelson et al. 2015). These zones (ie. spring, transition, diffuse, and ambient zones) are characterized by different degrees of SGD influence, with the spring zone being the site of the groundwater discharge and therefore having the highest SGD influence, to the ambient zone, which is the furthest away from the SGD and has the least amount of SGD influence. The gradient of freshwater and nutrient inputs across these zones also creates interesting study sites for algal growth experiments *in situ*.

Black Point and Wailupe are dominated by invasive macroalgal species and due to the reefs' coastal topography, these sites experience a range of both wave and wind exposure. This study explores the relationships between exposure and nutrient load on a local scale (~400 m) with respect to algal biomass, growth, and diversity *in situ*. We hypothesized that (1) species-specific and macroalgal biomass depend on SGD and at least one index of exposure, (2) species-specific growth rates will vary with SGD and herbivory, (3) and that diversity is related to both nutrient load and one or more exposure indices. Overall our goal is to evaluate the *in situ* effects of SGD in the context of the hydrodynamic state of these reefs to better understand their combined effects on macroalgal population and community dynamics.

METHODS

1. Study site descriptions

The two study sites are areas with known groundwater input along the southern coast of O'ahu, Hawai'i, in Maunalua Bay (21.2743°N, 157.7492°W; Fig. 1 A). Salinity and ²²²Rn surveys of the bay's coastline indicated 3 areas with groundwater signatures and negligible surface water inputs (Richardson et al. 2017). This study focuses on 2 reef flats in the bay: Black Point (21.2586°N, 157.7899°W) and Wailupe (21.2756°N, 157.7624°W) (Fig. 1 B). These two sites are about 4 km away from each other and receive SGD sourced from two different watersheds with different nutrient profiles and sources, possibly due to near-shore geochemical and geological differences (Richardson et al. 2017).

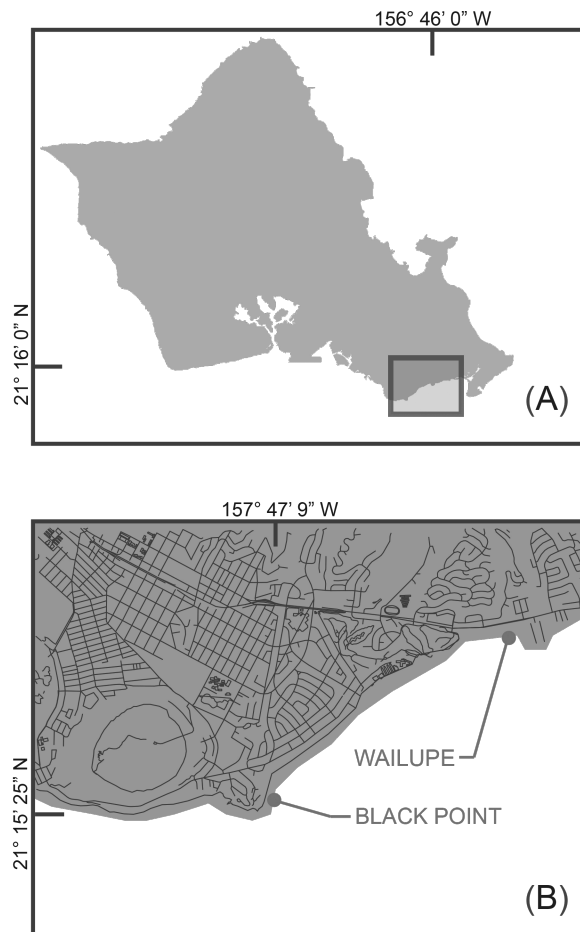


Figure 1. Maps of the sampling locations. (A) Map of O'ahu, Hawai'i, the western half of Maunalua Bay is enclosed in the blue box. (B) Close up of the coastline of western Maunalua Bay. Grey markers indicate two sites with submarine groundwater discharge, Black Point and Wailupe.

The reef flats are algae-dominated and SGD discharge rates range from $128 \text{ m}^3 \text{ d}^{-1} \text{ m}^{-1}$ of coast at Black Point compared to $20 \text{ m}^3 \text{ d}^{-1} \text{ m}^{-1}$ of coast at Wailupe (Holleman 2011). Concentrations of inorganic nutrients at Black Point averaged $190 \mu\text{M NO}_3^- \text{ L}^{-1}$ and $3 \mu\text{M PO}_4^{3-} \text{ L}^{-1}$ resulting in groundwater derived nutrient fluxes of $8902 \text{ mol NO}_3^- \text{ d}^{-1} \text{ km}^{-1}$ shoreline and $238 \text{ mol PO}_4^{3-} \text{ d}^{-1} \text{ km}^{-1}$ shoreline (Holleman 2011). At Wailupe spring, nutrient concentrations averaged $68 \mu\text{M NO}_3^- \text{ L}^{-1}$ and $2 \mu\text{M PO}_4^{3-} \text{ L}^{-1}$, resulting in groundwater derived nutrient fluxes of 1090 mmol d^{-1} and 51 mmol d^{-1} of NO_3^- and PO_4^{3-} , respectively (Holleman 2011, Richardson et al. 2017). Nutrient

concentrations of SGD at both sites are at least two orders of magnitude higher than background levels.

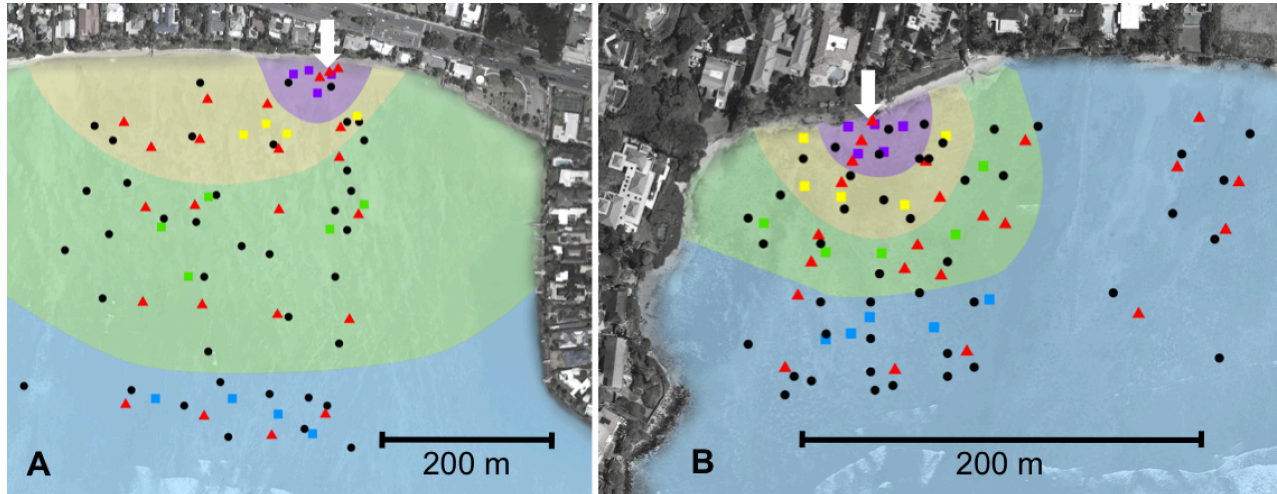


Figure 2. Experimental design and sampling locations at (A) Wailupe and (B) Black Point. White arrows point to localized SGD. Black circles represent sites where species specific biomass, diversity, depth, wave exposure, and wind exposure were measured. Red triangles represent salinity sensor locations. Shading of the reef represents the biogeochemical zones described in Nelson et al. 2015 (purple = spring zone [site of SGD], yellow = transition zone, green = diffuse zone, blue = ambient zone). Color-coded squares represent the sites of the growth experiments, stratified by biogeochemical zone.

2. Calculating nutrient loads

2.1. Water sampling and processing

We established a relationship between salinity and nutrients by compiling a time series of surface and benthic water samples taken synoptically along the SGD gradient at Wailupe and Black Point ($n = 150$ per site) on January 10 and 11 2015, respectively. Water samples were analyzed for salinity using a combination platinum ring electrode thermistor (Metrohm 6.0451.100) on a Metrohm conductivity module with Tiamo software (v2.4). A subset of 48 samples for Black Point and 40 samples for Wailupe were analyzed for inorganic nutrients, covering the largest range of salinities to represent water samples with a range of groundwater fractions. These water samples were filtered through a $0.2\mu\text{m}$ filter (Whatman 6900-2502 PVDF Filtration Medium) and

refrigerated for 3 weeks. The samples were brought to room temperature, mixed, and analyzed on a Seal Analytical Segmented Flow Injection AutoAnalyzer AA3HR for nitrate + nitrite (N + N), silicate (SiO_4^{2-}), total dissolved phosphorus (TDP). These parameters were regressed against salinity and showed strong linear relationships (least squares regressions: $R^2 > 0.964$ and p-values < 0.001). Table 1 shows the linear relationships between the nutrients and salinity by site. The strong relationship between salinity and nutrients allowed us to use salinity as a proxy for the nutrients listed above.

Table 1. Linear relationships for nutrients and salinity at Black Point and Wailupe sites. These relationships were found using linear regressions obtained from salinity vs. nutrients plots. $\text{N+N} = \text{NO}_3^- + \text{NO}_2^-$, total dissolved P (TDP)

Site	Nutrient concentrations ($\mu\text{mol L}^{-1}$)	Linear Relationship with salinity	R^2	p-value
Black Point	N+N	$-5.7 * \text{salinity} + 193.2$	0.995	<0.001
	TDP	$-0.1 * \text{salinity} + 4.1$	0.994	<0.001
	SiO_4^{2-}	$-26.7 * \text{salinity} + 910.7$	0.995	<0.001
Wailupe	N+N	$-2.1 * \text{salinity} + 70.5$	0.990	<0.001
	TDP	$-0.1 * \text{salinity} + 2.1$	0.964	<0.001
	SiO_4^{2-}	$-23.7 * \text{salinity} + 817.6$	0.998	<0.001

2.2. Salinity time series and nutrient load calculations

Autonomous salinity sensors (Odyssey Temperature and Conductivity loggers, 3 to 60 mS cm^{-1}) were deployed in a sparse grid ($n = 23$) across each site (Fig. 2, red triangles). The sensors were deployed at Wailupe for 34 days (17 April – 21 May 2015) and at Black Point for 30 days (29 May – 29 June 2015). The sampling frequency was 1 measurement every 10 minutes. Water samples were taken while each sensor was deployed and were analyzed using a Portasal Salinometer 8410A (accuracy 0.001) and compared to sensor values for QC purposes.

The salinity data was used to calculate TDP, SiO_4^{2-} , and N+N time series across space and time at each location at both sites. The time series data was then used to calculate an average daily

nutrient load at each location. N+N was used as a representative parameter for models and figures but should be treated as a representative variable for this larger suite of SGD-related parameters. Average daily N+N load was then interpolated across space to create a continuous map of daily nutrient loads for both sites (Fig. 3 A–B).

3. Biological parameters

3.1. Determining Algal Biomass

Survey areas for each site were set to encompass areas with high to low SGD-impact based on distinct biogeochemical zones, described in Nelson et al. 2015. The zones are spring (site of SGD), transition, diffuse, and ambient in decreasing order of SGD influence (Fig. 2, shaded areas). The area covered for Wailupe and Black Point respectively was about 0.11 km² (440 m offshore by 250 m alongshore) and 0.020 km² (155 m offshore by 130 m alongshore). The sites were divided into 3 by 3 grids, resulting in 9 cells with the same rectangular dimensions and 4-5 random points were chosen within each cell (ie. stratified random sampling) for benthic algal surveys (Fig. 2, black circles). We used 0.01 m² quadrats to measure species-specific percent algal and coral cover, as well as substrate type. All of the algae within the quadrat were collected, identified, separated by species, dried for 3 days at 60°C and weighed for dry biomass. Seasonal surveys spanned 2014-2016 but only the Fall (ie. September to November) data was used because this season yielded surveys with the highest algal biomass (Fall 2014 and Fall 2015).

3.2. Measuring herbivory and algal growth rates in situ

Algal growth rates and herbivory rates were assessed in caged, open caged, and no cage growth and herbivory assays, using a method similar to Stimson et al. (1996). Unlike Stimson's methodology, our cages were made of plastic-coated galvanized wire (mesh coarseness is 1cm²). A total of 16 to 20 experimental setups made of one cage, one open cage, and one no cage treatment

were deployed at each site (Fig. 2, colored squares), with 4 to 5 replicates in each of the four biogeochemical zones: spring, transition, diffuse, and ambient (Nelson et al. 2015). We placed the experiments in four zones of varying SGD influence in order to capture the SGD gradient. The replicates in each zone by site were deployed simultaneously for periods of 5 days. We used *Gracilaria salicornia* (invasive), *Avrainvillea amadelpha* (invasive), *Acanthophora spicifera* (invasive), and *Halimeda discoidea* (native) at Wailupe because they are the most abundant representatives of the macroalgal species at this site. Likewise, for the herbivory and growth experiments at Black Point, *Bryopsis pennata* (native but invasive at this location), *Avrainvillea amadelpha* (invasive), *Acanthophora spicifera* (invasive), and *Halimeda discoidea* (native) were used. Species- and site-specific three way ANOVAs were run using treatment (ie. cage, open cage, no cage) and zone (ie. spring, transition, diffuse, and ambient) as factors with an interaction between them and only biogeochemical zone was reported as significant in most species (supp. Table 1), therefore herbivory was not significant in either zone, at any site, and for any species. Due to these results and because some algae was detaching from the attachment sites in the cage set-ups during high wave action periods, we switched methodology to one based on Fong et al. 2006, which used mesh bags (5 mm² polyester mesh) to encapsulate the algae and measure growth rates in the absence of herbivory. This methodology allowed us to measure growth in the same manner and it standardized the loss of algal biomass due to wave exposure. All of the sites are less than 1.5 m deep and previous PAR data collected at these sites found that even a 50% decrease by the mesh, would allow for photosynthesis and growth. This method was laid out in the same spatial way as the original method based on biogeochemical zones and replicated 2 times at each site. Roughly 5 g of algae of each algal species were deployed initially. Exact wet weight was measured in the field before deployment and then measured again 5 days after the deployment. This is a common method used especially in Hawaiian algal studies (Vermeij et al. 2009, Reef et al. 2012)

as algae do not grow very large in oligotrophic waters. This length of time allowed for significant changes in biomass of the algae. Growth experiments were done from June to November of 2015.

3.3. Diversity index: Simpson's index

Simpson's diversity index (SDI) in this case is the complement of Simpson's original index (Simpson 1949) and represents the probability that two randomly chosen individuals belong to different species (McCune & Grace 2002). This index was chosen because it includes both a measure of richness and abundance and for its interpretative simplicity. SDI accounts for a small dataset and assumes sampling without replacement. A further strength of this measure is its reduced dependence on sampling effort when compared with species richness (Magurran 2004). Simpson's diversity index is calculated as:

$$D_1 = 1 - \frac{\sum_{i=1}^R n_i(n_i-1)}{N(N-1)}$$

Where R is the number of species (richness), n_i is the percent cover of a particular species and N is the total percent cover of all species. SDI was calculated for all the locations where benthic surveys were done (Fig. 2, black circles).

4. Physical parameter measurements and spatial distribution maps for all parameters

All physical parameters were calculated and measured at the benthic survey locations (Fig. 2, black circles). A wind exposure index (Keddy 1982) was calculated for 26 benthic survey locations at Wailupe and 27 benthic survey sites at Black Point. Wind exposure is given by the following equation:

$$\text{Exp} = \sum_{j=1}^8 (V_j \times P_j \times F_j)$$

where i is i^{th} compass heading (1=N, 2=NE, 3=E, 4=SE, 5=S, 6=SW, 7=W, 8=NW), V is the average monthly maximum wind speed in ms^{-1} , P is percent frequency with which wind occurred from the i^{th} direction, and F is effective local fetch. Effective local fetch was measured using Google Earth Pro (7.1.7.2606 version) as the distance from the point to the closest barrier such as land or reef crest. Average monthly maximum wind speed and percent wind frequency were calculated by taking 2012 data from a buoy (Station OOUH1) located $21^{\circ}18'12''$ N, $157^{\circ}51'52''$ W found on NOAA's national buoy data center (about 9 and 11 km from Black Point and Wailupe respectively).

A wave exposure index was calculated using the same formula as above and substituting monthly average wave height for monthly maximum wind speed. V was substituted with W , the average monthly wave height in meters. Depth was measured at each benthic survey location for each site by measuring the water column height in meters at variable tidal heights and adjusting these values to a mean lower low water (MWWL) tidal height of zero.

Spatial distributions were created using the `interp` function in R (version 1.0.44) on spatially explicit points for each variable. The `interp` function specified a linear interpolation within the boundaries of the data and can be found in the `akima` package (Akima & Gebhardt 2015) supported by R software (Fig. 3 C–H).

5. Generalized Additive Models (GAMs) for biological responses predicted by physical and SGD-related chemical parameters

Generalized Additive Models (GAMs) were used in the `mgcv` package (Wood 2011) in R to explore the relationships between the biological parameters and physical variables including SGD-related nutrient load. GAMs are semi-parametric extensions of Generalized Linear Models (GLMs) that use non-parametric, data-defined smoothers to fit non-linear response curves (Hastie &

Tibshirani 1990, Wood 2006, Zuur et al. 2012). The underlying assumption for GAMs is that the functions are additive and that the components are smooth. The strength of the GAMs is their ability to deal with non-linear and non-monotonic relationships between the response and the set of explanatory variables. We did not want to assume a predetermined relationship between the predictors and the responses. Rather, we wanted the data to determine the nature of the relationship between the response variables and the explanatory variables. The data used for the GAMs included biological responses such as species-specific algal biomass (dry weight, g m^{-2}), total algal biomass (dry weight, g m^{-2}), species-specific average percent growth over 5 days (Table 2), and Simpson's diversity index. The predictors used in the GAMs were an array of SGD-derived chemical (site-specific average N+N load d^{-1}) and local physical parameters (ie. wave exposure index, wind exposure index, and depth). The predictors were checked for collinearity and were not found to be significantly correlated. These models allow us to assess how much variation in the response variable each covariate can explain individually, while accounting for spatial autocorrelation. Species-specific biomass, wind exposure, wave exposure, and depth were all measured at the same locations (Fig. 2, black circles). The values for N+N (proxy for SGD) for the GAMs were calculated from the salinity time series (Fig. 2, red triangles) interpolated to the benthic survey locations (Fig. 2, black circles). Mean growth rates by biogeochemical zones were applied to the benthic survey locations (Fig. 2, shaded areas). This resulted in all response and predictor variables to be on the same spatial grid ($n=82$). All of these datasets were measured within the same grids across the reef flats and therefore are spatially cohesive over longer time scales (ie. seasonally and yearly), which is the temporal scale at which we are measuring the variables used in these models. We measured parameters (SGD as N+N , wave exposure, wind exposure) in a way that would account for their largest cyclical variation. For examples, wave and wind exposure vary with season and therefore were summarized by year. SGD varies with tide and

therefore we measure this through a thirty day period encompassing a spring and neap tide. Our surveys were done in Fall months over two years to capture a time period with most algal biomass. Thus, the datasets used for the GAMs were both spatially cohesive and temporally comprehensive.

It is possible that there would be interactions between wave exposure and SGD but the parameter we chose to represent SGD was an integrated N+N daily loading from SGD using a thirty day high frequency time series. This time series would have implicitly captured any interaction between SGD and exposure. Hence, we did not include an interaction, as the SGD parameter inherently includes this interaction already.

RESULTS

Spatial distributions of biological, chemical, and physical data

Figure 3 A and B show the distribution of average daily nitrate and nitrite (N+N) load over the two reef flats, Black Point and Wailupe, respectively. The nutrient loads differ by site by nearly one order of magnitude (supp. Table 2), which is consistent with past studies (Richardson et al. 2017). Nutrient load decreases with distance from the seep due to mixing and uptake. As mentioned in the methods (section 2.2), N+N will be used for display purposes and as the representative of a larger suite of nutrients which all show strong linear trends with salinity (N+N, TDP, and SiO_4^{2-} ; refer to supp. Table 2) and which we will refer to as “SGD” from now on; average daily N+N load was chosen as a proxy for groundwater influence in the GAMs because it is representative of the time scale relevant to the biological processes we are testing as response variables (ie. growth, biomass, diversity).

Average monthly wave exposure (Fig. 3 C–D) was much higher at Wailupe due to its longer reef flat. This affects the fetch measurement and overall calculation for wave exposure index (equation 2). The distribution of wave exposure was higher close to shore and close to the

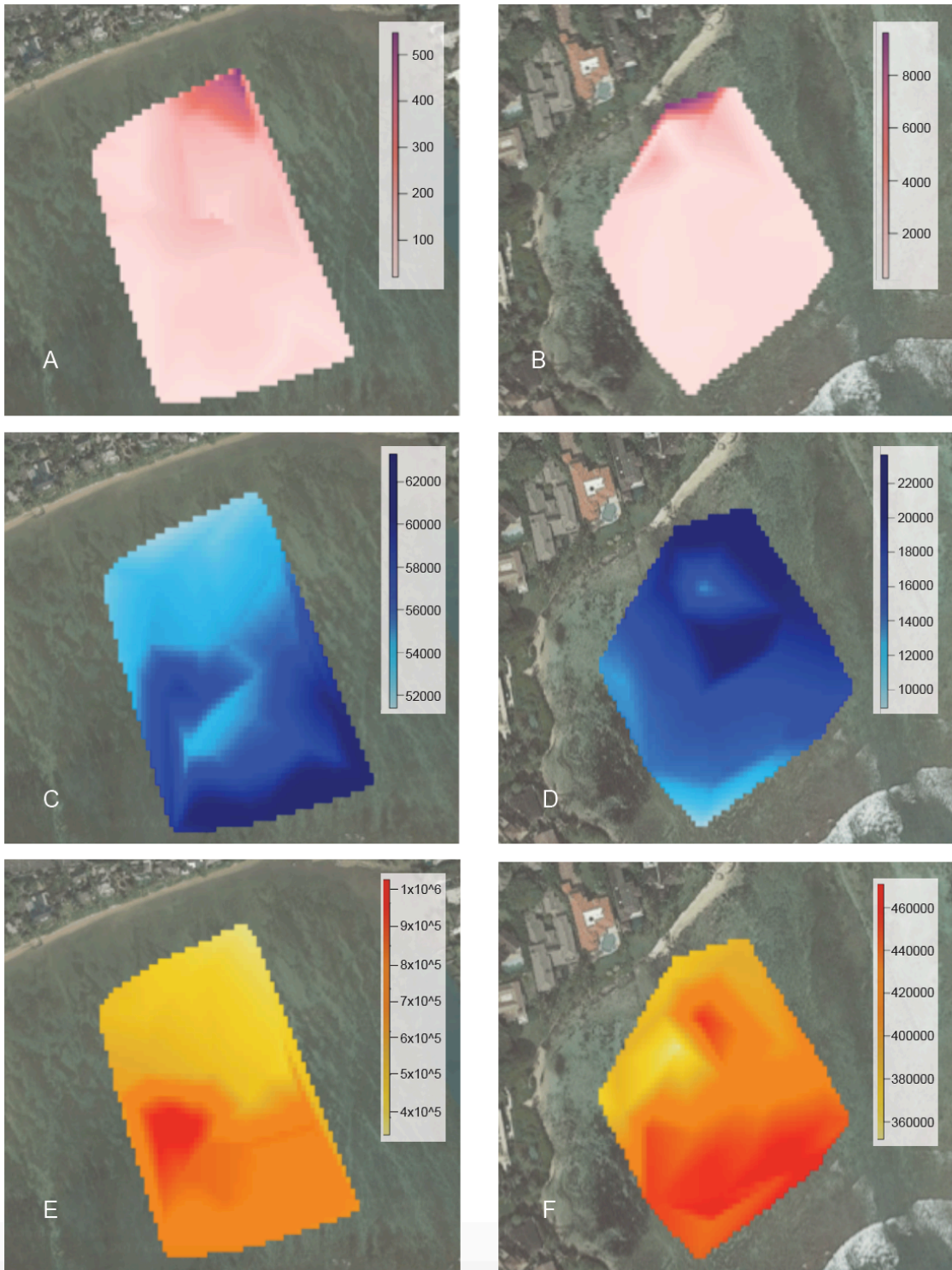
seep at Black Point and was lower away from the seep. The opposite pattern is found at Wailupe. This range difference by site also applies for wind exposure, which uses fetch in its equation as well. Average monthly wind exposure spans 1 order of magnitude difference at the two sites (Table 2). Average monthly wind exposure (Fig. 3 E–F) was generally higher away from the seep and in a patch close to the seep at Black Point. At Wailupe, wind exposure is higher offshore with a peak at a patch on the western mid-reef flat area. Average monthly wave and wind did not show significant correlations at either site and were therefore both used as covariates in the GAMs.

Depth (Fig. 3 G–H) was variable across the reef flat and ranged from about 20 to 105 cm. The major macroalgal species (see Table 2 for list of species) used in the growth experiments and chosen for species-specific biomass analyses in the GAMs are all found both intertidally and subtidally across Hawai‘i (Huisman et al. 2007) and are well adapted to live within this range of depths. Additionally, photosynthetically active radiation (PAR) sensor measurements at different depths in these areas showed that no part of the reef flat is light-limited during daylight hours.

The total biomass (dry weight) maps (Fig. 3 I–J) of total algae show that both reef flats have patches with high algal biomass (~100% cover). The bulk of the biomass at Wailupe is made of *G. salicornia*, which occurs ubiquitously across the reef flat, while at Black Point the dominant macroalga was *B. pennata*, which displays a more patchy distribution.

Black Point is homogeneously low in macroalgal diversity (Fig. 3 K–L), with two small patches with relatively higher diversity: one close to the seep and one offshore. Diversity measures of zero correspond to areas where there is one dominant macroalgae and thus the probability of picking two different algae close to each other is equal to zero. Values closer to one represent areas with high probability of picking different species of algae. The low diversity at Black Point was due to *B. pennata*, which also comprised the majority of the biomass. Areas with “higher” diversity at Black Point were areas with two species. Wailupe generally showed higher diversity with areas

containing up to 5 species per quadrat. Wailupe shows an area with low diversity close to the SGD seep, which is dominated by *G. salicornia*.



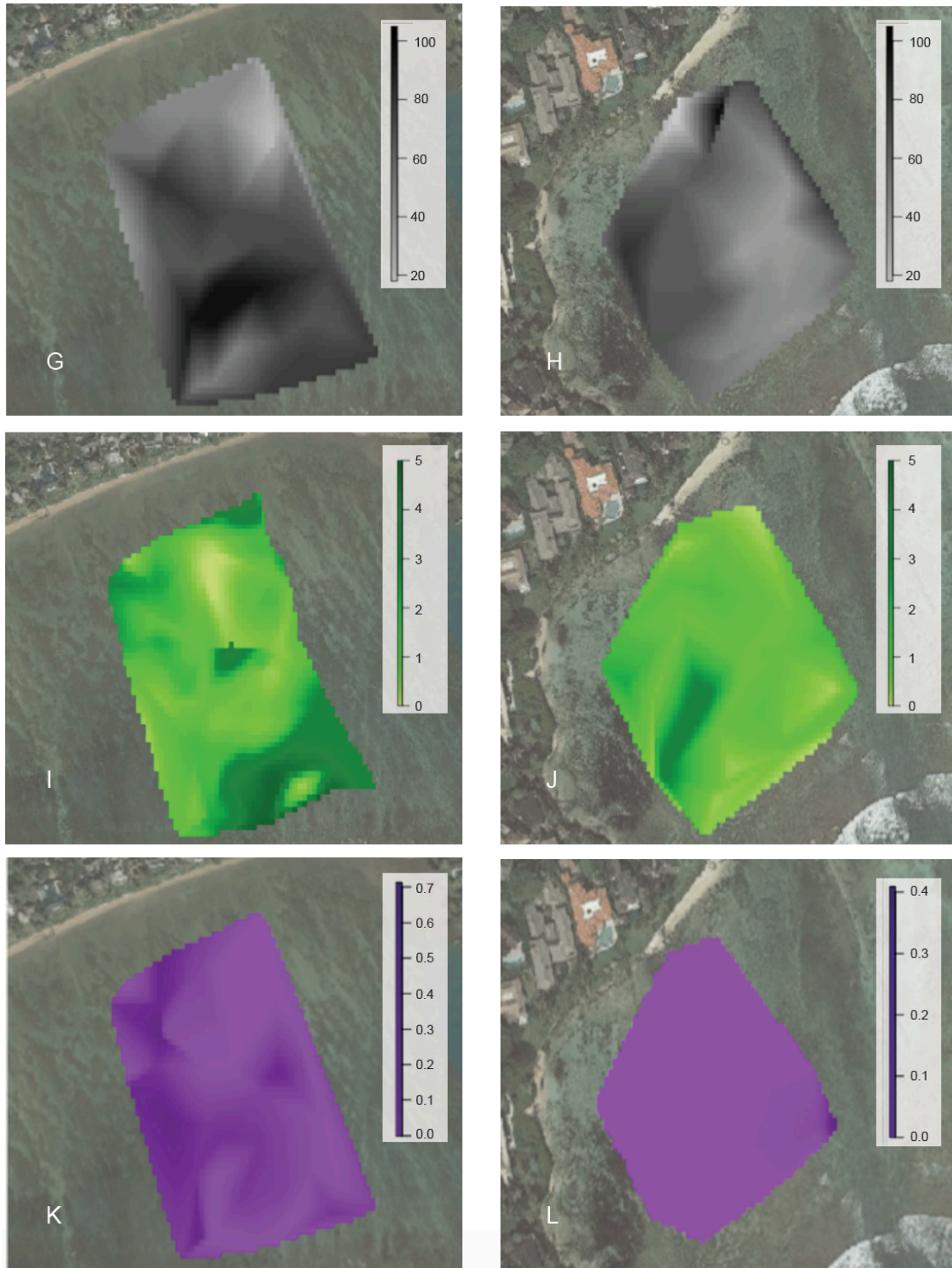


Figure 3. Maps of biological and physicochemical parameters used in the GAMs. Average daily nitrate and nitrate nutrient load (units are $\mu\text{mol L}^{-1} \text{sw}^{-1}$) for Wailupe (A) and Black Point (B). Average monthly wave exposure index (unitless) for Wailupe (C) and Black Point (D). Average monthly wind exposure index (unitless) for Wailupe (E) and Black Point (F). Water column depth (cm) normalized to zero mean lower low water (MWWL) at Wailupe (G) and Black Point (H). Total dry weight (g m^{-2}) Wailupe (I) and Black Point (J). Simpson's diversity index (unitless) for Wailupe (K) and Black Point (L).

Patterns for growth: GAMs and growth experiments

Herbivory did not play a role in macroalgal growth and abundance for the major macroalgal species present at each site. SGD, depth, and wave exposure are important predictors for the GAMs with species-specific growth as a response (Fig. 4). SGD mostly has negative relationships with growth for all species except *B. pennata* at Black Point and *G. salicornia* at Wailupe. These two species also make up the majority of the biomass at these two sites (Fig. 3 K–L). The growth of *B. pennata* is only positive in the ambient zone, farthest away from the seep (Table 2). Despite the negative growth rates in the other biogeochemical zones, during its blooming season, *B. pennata* is found to be the main algae growing abundantly close to the seep and close to shore at Black Point. The results from the growth experiments (Table 2) that shows negative growth in the first 3 zones closest to the seep are most likely due to loss of biomass due to wave action, also suggested by the high standard deviation in each zone. Interestingly, *G. salicornia* showed positive growth in all the biogeochemical zones (Table 2). The zones ranked ambient, spring, transition, diffuse, from smallest to largest average growth rates.

The following pairs of species with significant GAMs have the same directional relationships with all the predictors at the same site: *H. discoidea* at Wailupe and *A. amadelpha* at Wailupe, as well as *H. discoidea* at Black Point and *A. spicifera* at Black Point. This suggests that there are strong site effects, which may be a product of the unique physicochemical environments of each site. On the other hand, the growth of *A. spicifera* shows the same directional relationships for all the predictors at both sites: a negative relationship with SGD and positive relationships with wind exposure and depth.

Wave exposure has mostly negative or non-significant relationships with growth of all the species. Depth mostly has positive relationships with growth for all species except *H. discoidea* at Wailupe, *A. amadelpha* at Wailupe, *B. pennata* at Black Point (Fig. 4). The inconsistency of the

directional relationships of the physical parameters suggests that they are a synthesis of several physical processes that may act on different scales and may affect algal growth across the reef flat in different ways. For example, depth may be a proxy for turbidity at Wailupe since areas close to the seep are both deep and turbid at high tide due to the disturbance of the silty sediment. At Black Point the relationship between depth and turbidity may be different as some deeper areas are covered in sand, while shallower areas are composed of calcified reef both dead and alive.

Table 2. Average growth percentages over 5 days for different species at different sites and zones. The following zones are listed in order closest to and with most SGD fraction to farthest from and with least SGD impact: spring, transition, diffuse, and ambient. ND refers to no data.

Species	Site	Mean % Growth spring zone 5d ⁻¹ ± SD	Mean % Growth transition zone 5d ⁻¹ ± SD	Mean % Growth diffuse zone 5d ⁻¹ ± SD	Mean % Growth ambient zone 5d ⁻¹ ± SD
<i>Acanthophora spicifera</i>	Black Point	10.3 ± 19.2	-8.4 ± 14.6	-0.1 ± 10.3	ND
	Wailupe	-9.6 ± 22.1	-12.1 ± 25.2	6.9 ± 17.3	ND
<i>Avrainvillea amadelpha</i>	Black Point	-6.3 ± 13.6	2.1 ± 7.7	9.0 ± 4.6	ND
	Wailupe	7.8 ± 7.7	6.5 ± 7.3	8.6 ± 8.7	-0.3 ± 1.9
<i>Bryopsis pennata</i>	Black Point	-2.2 ± 34.4	-12.3 ± 39.6	-8.2 ± 37.9	47.2 ± 109.6
<i>Gracilaria salicornia</i>	Wailupe	7.5 ± 7.2	10.1 ± 20.1	14.1 ± 14.0	3.2 ± 8.8
<i>Halimeda discoidea</i>	Black Point	-25.4 ± 14.1	2.2 ± 6.8	17.5 ± 13.3	ND
	Wailupe	-2.6 ± 13.2	-0.3 ± 17.4	12.5 ± 12.7	7.9 ± 2.3

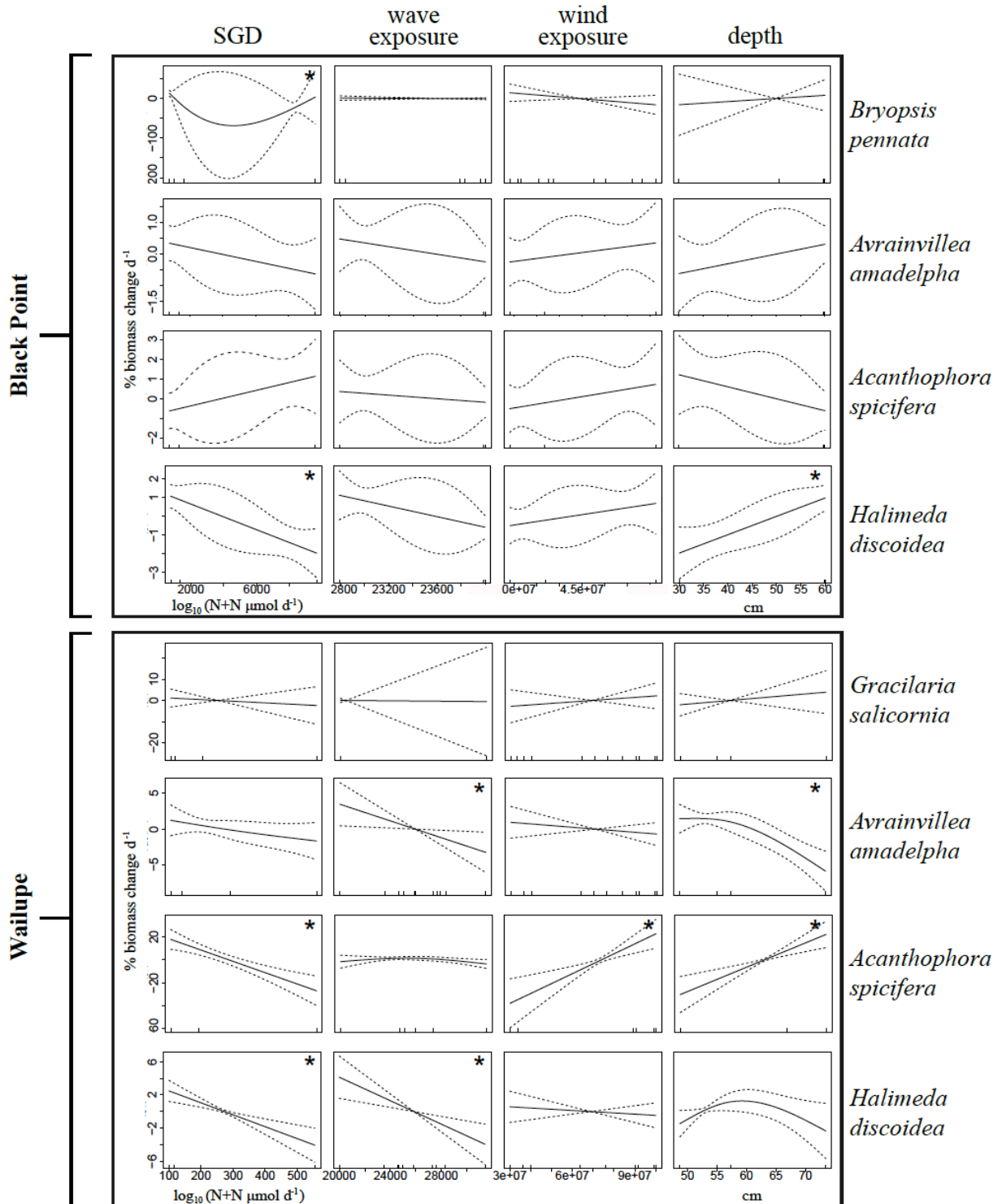


Figure 7. Rug plots of OAVS with growth by species at each site as a response (vertical axis). Asterisk (*) indicates a significant relationship. Predictors are labeled on top of the figure. Wave exposure and wind exposure are unitless indices. Locations of covariate data are plotted as hash marks inside the x axes. Thicker hash marks correspond to multiple data points with corresponding x coordinate.

Patterns for biomass: GAMs

Wave exposure, SGD, and wind exposure are the important predictors for the species-specific biomass models (Fig. 5). Wave exposure generally has positive relationships with species-specific biomass at Wailupe and generally has negative relationships with species-specific biomass at Black Point. Interestingly, *A. spicifera*'s biomass has the opposite relationships with wave exposure at both sites. Two species at Wailupe, *G. salicornia* and *H. discoidea*, showed significant and positive relationships with wave exposure. SGD mostly has a significant positive relationship with biomass for *G. salicornia* at Wailupe and a significant negative relationship with *A. spicifera* biomass at Wailupe. All other relationships with SGD are not significant. The significant relationships between wind exposure and biomass are negative. Depth mostly has negative relationships with biomass for all species except *B. pennata* at Black Point, total biomass at Black Point, and *A. spicifera* at Wailupe. The biomass of *B. pennata* is significantly predicted by wind and wave exposure, two physical parameters, suggesting hydrodynamics are important for the colonization and presence of this algal species.

The following pairs have same directional relationships with all the predictors: *B. pennata* at Black Point and total biomass at Black Point, *G. salicornia* at Wailupe and total biomass at Wailupe. These results are supported by the benthic surveys, which show *B. pennata* and *G. salicornia* are the most abundant algae at Black Point and Wailupe respectively (Fig. 3 I–L), and therefore are strongly related to the total biomass response. The biomass of *A. amadelpha* at Wailupe, *A. spicifera* at Wailupe, and *Jania sp.* at Black Point didn't show significant relationships with any of the physicochemical parameters in the GAMs.

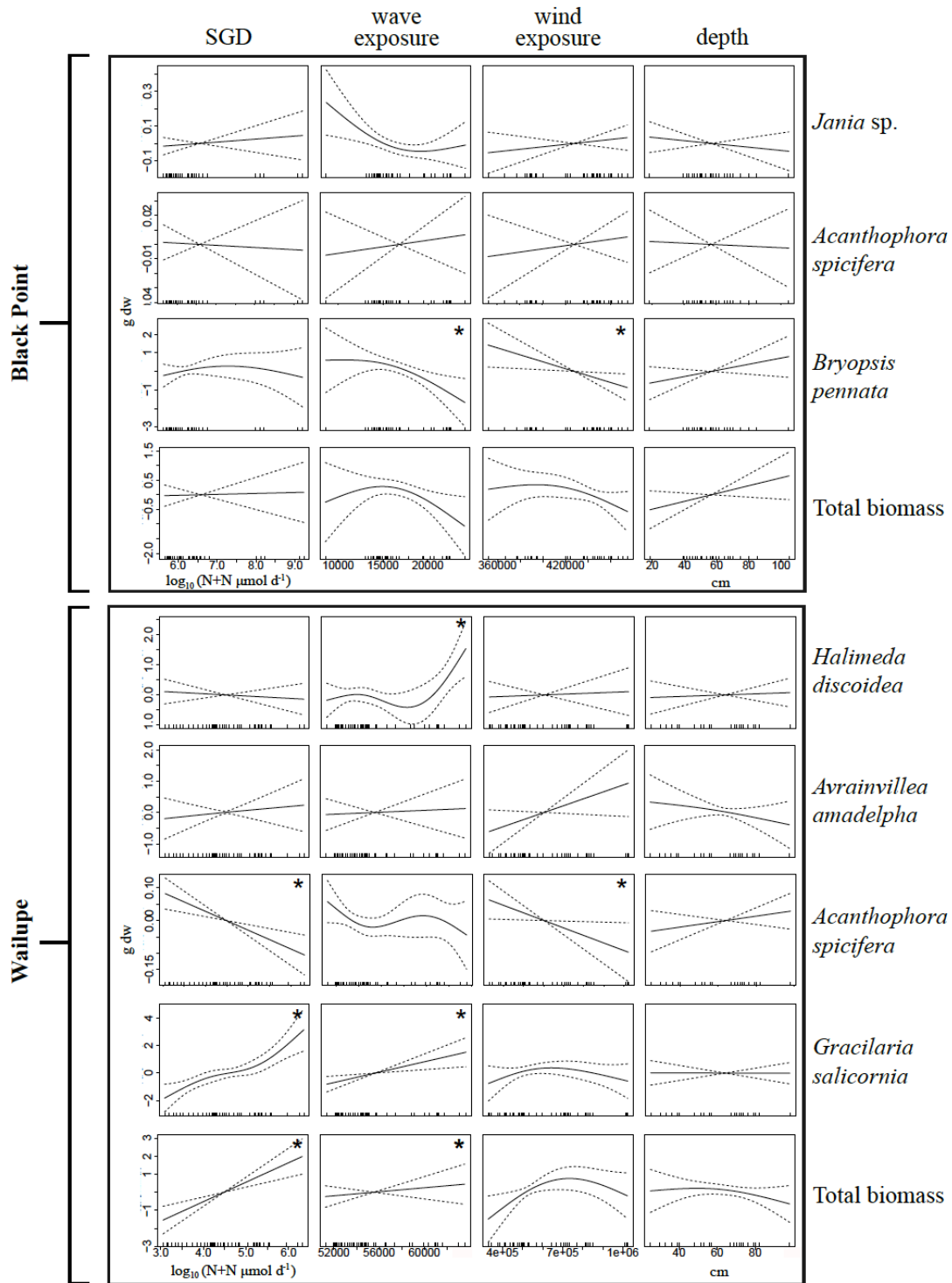


Figure 5. Rug plots of GAMs with biomass by species at each site as a response (vertical axis). Asterisk (*) indicates a significant relationship. Predictors are labeled on top of the figure. Wave exposure and wind exposure are unitless indices. Locations of covariate data are plotted as hash marks inside the x axes. Thicker hash marks correspond to multiple data points with corresponding x coordinate.

Patterns for Simpson's diversity index (SDI): GAMs

SDI was significant at Wailupe mostly with average daily N+N load as well as wind exposure (Fig. 6). SDI was above 0 in areas with N+N loads lower than 250 N+N $\mu\text{mol L}^{-1}$ sw. SDI was increased at higher wind exposure and depths which both occur away from the groundwater seep. The low SDI scores close to the seep show that few species occur in areas with a distinctly different chemical environment brought in by the groundwater. Interestingly, even though diversity of algal assemblage decreased with higher nutrient loads, the total biomass of algae increased with higher nutrient loads (Fig. 5–6).

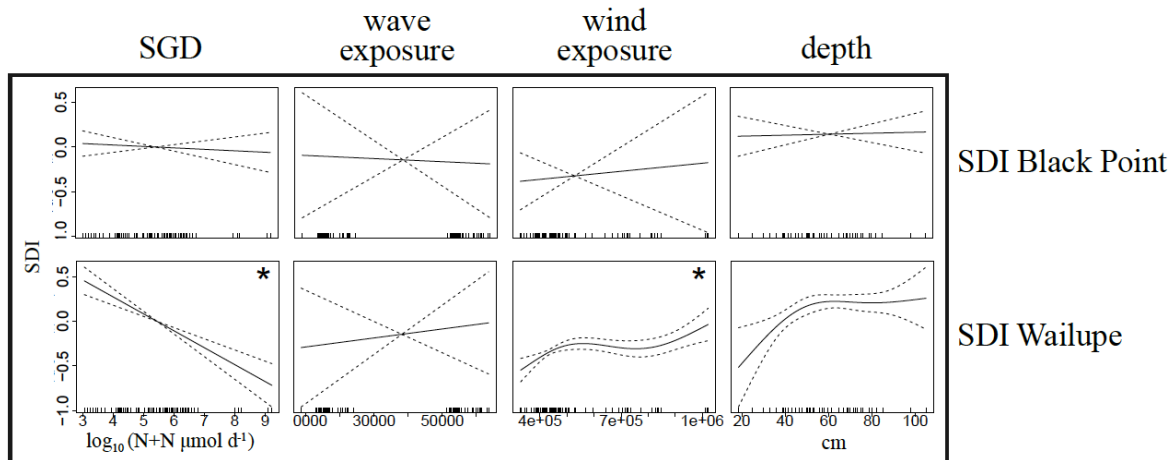


Figure 6. Rug plots of GAMs with Simpson's diversity index (SDI) at each site as a response (vertical axis). Asterisk (*) indicates a significant relationship. Predictors are labeled on top of the figure. Wave exposure and wind exposure are unitless indices. Locations of covariate data are plotted as hash marks inside the x axes. Thicker hash marks correspond to multiple data points with corresponding x coordinate.

DISCUSSION

We calculated and measured a suite of physical, chemical, and biological characteristics on 2 reef flats with SGD input. We ran GAMs for species-specific biomass, total algal biomass, species-specific growth, and Simpson's diversity index against a suite of physicochemical parameters (ie. N+N load, wave exposure, wind exposure, depth). All biological responses for at

least half of the species studied were significant with some combination of the physicochemical parameters.

Factors predicting biomass distributions of macroalgal species

Our first hypothesis stated that species-specific macroalgal biomass depends on SGD and at least one index of exposure. This was true for (1) the biomass *G. salicornia* at Wailupe, which had positive significant relationships with both SGD and wave exposure, as well as for (2) the biomass of *A. spicifera* at Wailupe, which had negative relationships with both SGD and wind exposure.

The rest of the significant species-specific biomass relationships were solely significant with exposure indices. The biomass of *H. discoidea* at Wailupe had a positive significant relationship with wind exposure. The biomass of *B. pennata* had negative significant relationships with both wind and wave exposure. Wave exposure integrates a wide variety of environmental variables and our results are not surprising given that the concept that hydrodynamic conditions influence the distribution of coastal organisms is not new (e.g. Fowler-Walker et al. 2005, Jonsson et al. 2006, Cefalì et al. 2016).

SGD supports low diversity and increased biomass of species that can tolerate low salinities

SGD is positively related to the invasive species *G. salicornia* and the bloom forming species *B. pennata*, indicating that if found or transported to this kind of environment, these species are likely to be successful invaders. SGD has negative effects on growth with all species except *B. pennata* and *G. salicornia*, which also explains the low algal diversity in areas close to the SGD seep.

G. salicornia is found throughout the entire reef flat but is the main species found close to the SGD seep at Wailupe. Its biomass at Wailupe was significantly positive with wave exposure

and its growth was positive in all biogeochemical zones. Its growth was not highest at the spring or transition zone, which suggests that it may not thrive in this environment but it is the most tolerant of it. Smith and colleagues (2004) showed that *Gracilaria* sp. is highly tolerant of varying temperatures and desiccation. Unpublished work in aquaculture with this genus has also shown that intermediate salinities show increased growth of *Gracilaria* sp. (Wally Ito personal communication). From its high biomass across the reef flat, we can infer that *G. salicornia* is a good biological competitor for space. *G. salicornia*'s large range of tolerance for several chemical and physical parameters, as well as its fast-growing mechanism make it a successful invasive species. A study by Lapointe in 1985 showed that a species in the *Gracilaria* genus was able to increase growth after frequent (2 week^{-1}) nutrient pulses. He noted that frequency was more important than nutrient load for growth of this species. This study suggests that *G. salicornia* grows well in coastal systems with high nutrient loads at much higher pulse rates.

B. pennata was the major macroalgal species found at Black Point and both its presence and biomass were not related to any of the chemical or physical parameters. This suggests that there may be other physicochemical or biological factors, such as competition, contributing to the presence and abundance of *B. pennata* across the reef flat. This alga is highly abundant across most of the reef flat but is the dominant alga in the area close to the SGD seep possibly due to lack of competition from other algae. Offshore, although the growth data indicates that the nutrient regime/load is more ideal for its growth, there is increased competition with other algae, which also show positive growth in these biogeochemical areas.

SGD supports increased biomass of species that can tolerate low salinities. If these species are bloom forming or have high growth rates, they can take over reef flats at local spatial scales. This might be able to precipitate a succession of invasive or native algal domination on a reef. It is not surprising that macroalgal biomass is high at these sites when we combine these results with

the lack of herbivory. Wailupe has been macroalgal dominated for at least the last 15 years (McGowan 2004); it would be interesting to pursue work on succession of algae in this system, especially with regards to invasive species.

Diversity and macroalgal community biomass distributions

We hypothesized that diversity is related to both nutrient load and one or more exposure indices. This was supported for Wailupe where Simpson's diversity index was significantly, negatively related to SGD and significantly, positively related to wind exposure.

The effects of exposure on the diversity of macrophytes are often unclear (Kraufvelin et al. 2010, Williams et al. 2013, Norderhaug et al. 2014). Indeed, ecological studies have shown both increasing and decreasing relationships with wave exposure, as well as hump-shaped patterns in the diversity of macroalgae (Bailey 1988, Riis & Hawes 2003, Nishihara et al., 2010, Ricketts et al. 1985, Norderhaug et al. 2014).

At Wailupe, where total macroalgal biomass and diversity were significantly predicted by both exposure and SGD factors, high wind exposure occurs in areas generally away from the groundwater seep. Total biomass was positively related to wave exposure (significant) and SGD (not significant); in contrast, diversity increased with higher wind exposure and decreased with increased SGD. This supports the argument that some macroalgal species, invasive and opportunistic species in this case, can grow and persist in an area with high SGD-derived chemical factors (ie. high nutrient and low salinity daily loads) but that most algae are outcompeted in these environments.

SGD and physical parameters affect growth of algae species in different ways

Finally, we hypothesized that species-specific growth rates differ by biogeochemical zone and have a significant relationship with SGD. Our growth experiment results support that species-specific growth rates do differ by biogeochemical zone (Table 2). For the growth of *A. spicifera* at Wailupe, and *H. discoidea* at both sites, there was a significant negative relationship with SGD (Fig. 4). Growth rates of *G. salicornia* were the only ones that were positive in all zones. The growth rates of the same species also differ by site and biogeochemical zone, this difference could be due to the difference in magnitude of the nutrient concentrations of the SGD at the two sites (Table 1). We did not predict that physical parameters would have significant effects on growth but wave exposure had significant negative relationships with growth of *A. amadelpha* and *H. discoidea* at Wailupe and a positive relationship with growth of *A. spicifera* at Wailupe.

Distributions and growth rates of macroalgal species found away from the SGD seeps

A. spicifera is another invasive species in Hawai'i, common at both Black Point and Wailupe and known to be able to take up nitrate (Leon-Soon, 2017), which may explain its prevalence in an area where the main N source is groundwater-derived nitrate. *A. spicifera*'s biomass decreased with increasing SGD and wind exposure. A study following this species' distribution showed that fragments were broken off by turbulence in the fore reef, transported by currents, and snagged or entangled in the back reef (Kilar & Maclachlan, 1986). *A. spicifera* is also commonly known as “prickly seaweed” in Hawai'i due to the pointy projections from the main axis which allow for it to get easily entangled in things it comes into contact with. This strategy has allowed for it to become one of the most prevalent invasive species in Hawai'i.

H. discoidea grew most and was found in areas away from the groundwater seep with high wave action at Wailupe. This result is in agreement with work by Walters and colleagues (1994,

2002), which suggested that this species could be successful in areas with high wave action and grazing pressure because it is able to clonally propagate via vegetative fragmentation of very small fragments previously cut several times and in several directions. We hypothesize that *H. discoidea* is also not found close to the groundwater seep because it is a calcifying alga and requires certain chemical and physical conditions such as pH of about 8.1 (on total scale at 25°C) (Porzio et al. 2011) and carbonate/bicarbonate availability in order to calcify and grow. The groundwater that discharges at this site has lower pH of about 7.58 ± 0.03 (on total scale at 25°C) (Richardson et al. 2017), which doesn't allow for the dissociation of high concentrations of bicarbonate and carbonate ions. Thus, it is probable that areas most affected by the groundwater do not have ideal carbonate chemistry conditions for calcifying algae.

Caveats and future directions

The limitations of this study include a relatively low sample size for the number of parameters we were fitting in the multivariate models. This is in part the reason we ran univariate models in addition to multivariate models. The analysis presented here looks at the environmental conditions and the correlations with these biological parameters and takes into account the spatial context they occur in. By identifying the key factors associated with increases and declines, as well as presence and absence of algal taxa, the analysis highlights variables that are most clearly related to growth rates, biomass, and diversity as well as the modalities by which they interplay. This opens the way to subsequent analyses of the quantitative linkages among, for example, nutrient toxicity levels and biomass accrual. A seasonal analysis would strengthen and elucidate seasonal trends for this work as we know that both wind and wave exposure are themselves seasonal and have seasonal effects on macrophytes (Wernberg & Vanderklift 2010). Knowledge about the interactive effects of groundwater-derived biogeochemistry and hydrodynamic forces on

macroalgal communities in shallow coastal systems is scant. Therefore, while our experimental results are sound, we acknowledge that several aspects of our interpretation require further support.

It is critical to evaluate the effects of stressors on community dynamics not only independently but also under different combinations to understand how those effects will be played out in more realistic scenarios (Muthukrishnan & Fong 2014). Salinity and nutrient effects on specific algae have been studied, but this is rarely done in conjunction with an exposure index. Intertidal ecologists recognize the importance of all these factors (Kraufvelin 2007, Kraufvelin et al. 2010) and in Hawai‘i and other tropical areas housing coral reefs, we should apply these crosscutting concepts and *in situ* studies on reef systems affected by groundwater. A good start would be to review what we know about invasive algal species in areas with coral reefs and the effects (both interactive and not) of salinity, eutrophication, and hydrodynamics.

Both from observational studies and physiological experiments, we know that macroalgal relationships between tolerance and resistance to stress are variable and species-specific (Hay et al. 2011). This is where the multiple stressor literature is helpful in trying to distill these complex interactions. Coastal systems are especially complex with upwards of 100 two-way interactions (Côté et al. 2016). In Côté and colleagues’ paper about ecosystem stressor interactions, they lay out a guide for identifying generalities about ecosystems, stressors and/or responses that could provide guidance to conservation scientists and managers. Along with physiological studies about stress mechanisms, studies such as this one can accumulate data to ground truth these relationships and interactions *in situ*.

The information we can gather about specific algal species on their biomass and growth in different chemical and hydrodynamic scenarios can help us infer their fate when placed in areas with SGD and can then be expanded to estuaries and many coastal environments experiencing global changes. These systems are also important study sites for tolerance levels and thresholds for

growth in different chemical load with different temporal deliveries scenarios. In the case of groundwater, these are hard to disentangle but mesocosm studies constraining factors have been done and can help elucidate these dynamics and interactions. If we can understand what factors affect biomass and persistence of certain algal assemblages, especially those comprised of invasive and opportunistic algae, we can prioritize restoration efforts. Additionally, understanding how communities of primary producers are functioning and how resilient they may be to predicted climate change scenarios (ie. sea level rise, increasing temperatures, etc.) will help with preparedness for coastal management.

CHAPTER 2

NUTRIENT-RICH SUBMARINE GROUNDWATER DISCHARGE INCREASES ALGAL CARBON UPTAKE IN A TROPICAL REEF ECOSYSTEM

In review with *Limnology and Oceanography Letters*
Florybeth Flores La Valle, Florence I. Thomas, Craig E. Nelson

Abstract: Submarine groundwater discharge (SGD) with high inorganic nutrient content is generally thought to augment primary production in coastal systems. However, there is limited evidence for a direct association between SGD and primary productivity of reefs. To elucidate the response of primary productivity to SGD, we conducted spatially and temporally explicit *in situ* benthic chamber experiments on a reef flat along a gradient of SGD. We found significant quadratic relationships between C-uptake and SGD for both phytoplankton and the benthic macroalga *Gracilaria salicornia*, with uptake maxima at SGD-derived salinities of ~21–22 (24.5–26.6 $\mu\text{mol NO}_3^- \text{L}^{-1}$) suggesting a physiological trade-off between salinity tolerance and nutrient availability for reef primary producers. Spatially explicit modeling of reefs with and without SGD indicate reef-scale *G. salicornia* and phytoplankton C-uptake decreased by 82% and 36% in the absence of SGD, respectively. Thus, nutrient-rich and low salinity SGD has significant effects on algal C-uptake in reef systems.

INTRODUCTION

Submarine groundwater discharge (SGD) is an important source of terrigenous freshwater and nutrients to coastal waters worldwide (Kontar and Zektser 1999, Slomp and Van Cappellen 2004, Burnett et al. 2006). A recent study by Cho and colleagues (2018) showed that the total SGD-derived DIN and DIP fluxes could be approximately 1.4- and 1.6-fold of the river fluxes to the Indo-Pacific and Atlantic Oceans, respectively. SGD is especially important in high islands due

to high precipitation rates, high shoreline to area ratio, high relief, and immature permeable soil (Moosdorf et al. 2015).

Studies have shown that offshore coral islands, which experience tidally modulated SGD input, release nutrients via vertical pore water upwelling, tidal pumping, and temperature-driven convection, which can in turn, lead to sustained productivity within coral reefs (Santos et al. 2010). Tidally dominated SGD inputs can be chronic sources of nutrients to tropical reefs; this contrasts with fluvial nutrient inputs, which vary greatly with precipitation (Amato et al. 2016). Hence, in coastal areas with tidally dominated SGD inputs, SGD may comprise a significant component of reef nutrient inputs but have highly variable biogeochemistry on small temporal scales (ie. hours) and spatial scales (ie. meters).

SGD input to reefs decreases the salinity and increases the nutrient concentration of coastal waters (Burnett et al. 2006, Moore et al. 2010). The primary producers that inhabit these ephemerally estuarine environments must be able to withstand, grow, and maintain productivity while being exposed to rapid fluctuations in both salinity and nutrients. All primary producers have physiologically optimized ranges of tolerance for both inorganic nutrients and salinity (Kirst 1990, Guan et al. 2015, Liu et al. 2016). Although nutrient concentrations have long been known to affect primary productivity (Nixon et al. 1986, Duarte 1995), the effects of salinity on productivity are usually varied and species-specific (Sudhir and Murthy 2004). While increased nutrients in the water column of reefs can increase coral reef photosynthesis (Marubini and Davies 1996, Fabricius 2005), these effects will depend on the structure of the benthic community (Yap et al. 1994, Dizon and Yap 2003). For example, some fleshy macroalgae make use of inorganic nutrients on a reef more efficiently and rapidly than coral (Littler et al. 1991, Dailer et al. 2012), but this may depend on differential tolerance of low salinities in SGD plumes. Experimental factorial studies of salinity pulses and nutrient loading on primary producers show nutrient loading tends to have a long-term

effect through complex community interactions (Duarte 1995, Valiela et al. 1997), while salinity pulsing frequency and intensity has an immediate and direct influence on growth and distribution (Boustany et al. 2015). Most of the studies measuring production rates, especially in macroalgae, are done in laboratory and mesocosm settings. There is a need for *in situ* studies of macroalgal C-uptake on reefs as these are in short supply. Measuring C-uptake rates of primary producers on a reef with SGD characterized by low salinity and high inorganic nutrients could shed light on the relationship between productivity and nutrients delivered via a freshwater medium.

Past *in situ* studies have shown that seagrass growth rates are higher in areas with SGD (Peterson et al. 2012), and phytoplankton communities and productivity vary by biogeochemistry of SGD (Gobler and Boneillo 2003, Troccoli-Ghinaglia et al. 2010). The results from a study by Johnson and Weigner (2013) showed that coastal primary production and respiration respond to surface plumes of SGD over short spatial and temporal scales. Recent work on an intertidal flat showed that phytoplankton was responsible for 30% use of inorganic nutrients from SGD, indicating removal of up to 70% of the nutrients by other primary producers, such as benthic algae (Waska and Kim 2011). In shallow macroalgal-dominated reefs, benthic macroalgae account for the majority of the overall ecosystem productivity (Valiela et al. 1997, Dailer et al. 2012). Hence, it is reasonable to hypothesize that the effect of SGD on the productivity of benthic macroalgae plays an important role in the ecology of reefs and coastal systems with SGD (Lee and Kim 2015).

In this study, we measured *in situ* C-uptake of the main primary producers on an algal-dominated reef with SGD influence using benthic chambers. The experimental design was spatially and temporally explicit in order to capture C-uptake rates across a gradient of SGD input. We then coupled this data with benthic cover data to map and estimate both benthic and water column C-uptake on a reef with SGD and a reef without SGD. This work can elucidate the magnitude of the effect of SGD on both benthic and water column algal productivity of reefs. This has important

management implications, as it is important to discern drivers of algal productivity of coral reefs, which are ecologically and economically important ecosystems.

METHODS

1. Study site description and experimental design

The study site is Maunalua Bay along the southeast coast of O‘ahu, Hawai‘i. Our specific study area, the reef flat adjacent to Wailupe beach park (Fig. 7), has both diffuse and localized SGD (21.2756°N, 157.7624°W) close to the shoreline (Nelson et al. 2015). The reef flat is dominated by macroalgae, with the invasive species, *Gracilaria salicornia*, accounting for an average of 13% of the total benthic cover (areas without algal cover included) and approximately 31% of algal cover. The average SGD discharge rate is 20 m³ d⁻¹ m⁻¹ of coast at Wailupe and average nutrient concentrations at the seep were 68 μM NO₃⁻ and 2 μM PO₄³⁻ resulting in groundwater derived nutrient fluxes of 8902 mol NO₃⁻ d⁻¹ km⁻¹ shoreline and 238 mol PO₄³⁻ d⁻¹ km⁻¹ shoreline (Holleman 2011). SGD is the dominant source of nutrients to the water column and throughout the majority of the year is the only source of terrestrial fresh water (McGowan 2004).

Benthic chambers were constructed out of clear 6 mL polypropylene bags (Lehua Greenhouse tarp, 93% PAR transparency). Each benthic chamber accommodated about 20 L of seawater and encompassed a 0.25 x 0.25 m² area of reef at its base. Benthic chamber deployments were done across the reef flat in an area 0.11 km² (440 m offshore by 250 m alongshore) during peak daylight hours (ie. 9:00–14:00). Experiments were done across space and at different tidal heights in order to capture a range of groundwater input (ie. different salinities) (Fig. 7). Following deployment, benthic chambers were enriched with about 0.3 g of 98 at.% NaH¹³CO₃ to increase total DIC by about 10% (Mateo et al. 2001) and incubated for 1 hour.

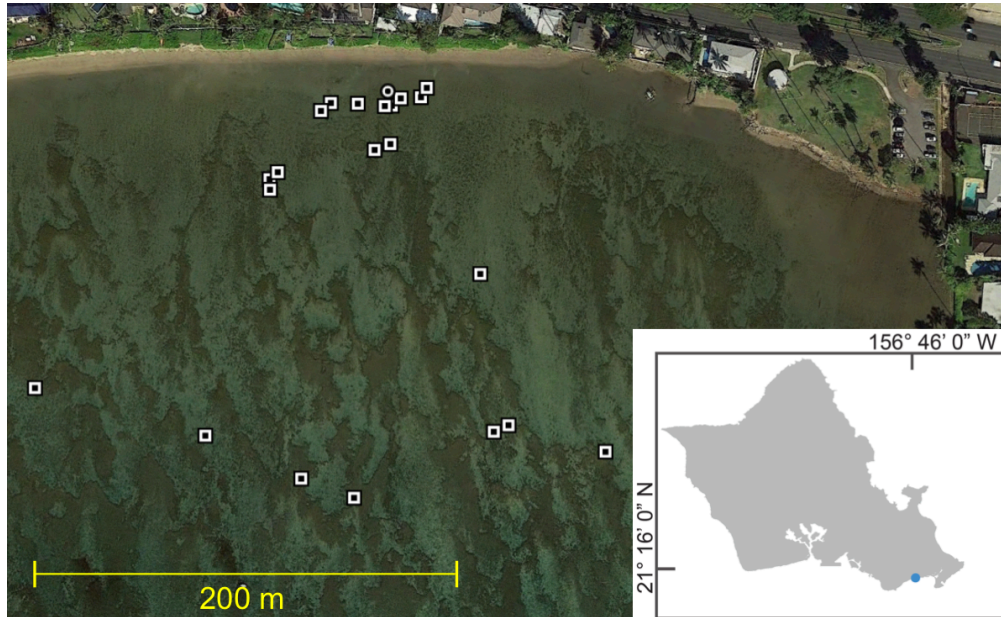


Figure 7. Map of experiment deployments. Exact locations at Wailupe marked by white and black squares; circle shows location of main SGD seep. (Inset) Map of O‘ahu, Hawai‘i, blue dot shows Wailupe’s location.

2. Isotope analyses

Water samples (1 L) were collected before the experiment, right after the $\text{NaH}^{13}\text{CO}_3$ enrichment, and after the experiment from within the benthic chambers and filtered through a 0.7mm glass fiber filter. Water samples were sent to the Biogeochemical Stable Isotope Facility at the University of Hawai‘i at Mānoa for $\delta^{13}\text{C}$ analysis of dissolved inorganic carbon (DIC; Torres et al. 2005). Pre-enrichment and post-experiment filters were analyzed for N chemical species (nitrite, nitrate, ammonium, dissolved organic nitrogen), ^{15}N of nitrate, C, and ^{13}C of dissolved inorganic carbon content using a Carlo Erba 2500 elemental analyzer coupled with a Finnigan Delta S mass spectrometer (internal error was ± 0.05 ‰, and the with total analytical precision ± 0.2 ‰). Ambient samples of all macroalgal species present in the benthic chamber were picked within 0.3 m of the benthic chamber and all of the macroalgae was gathered from within the benthic chamber at the end of the experiment. Macroalgal samples were separated by species, cleaned of epiphytes and

sediment, dried at 60°C over 3 days, and weighed. Dried samples of each ambient and enriched macroalgal species were then analyzed for N, ¹⁵N, C, and ¹³C content.

3. Numerical procedures

Isotopic equations are listed in supplemental Table 2 and were derived from equations in Hayes (2004). Macroalgal uptake rates (P) were calculated using an equation derived from Hama et al. (1983) and used in Mateo et al. (2001). Similar calculations are also specified in Cornelisen & Thomas (2002).

$$P \text{ (mg C d}^{-1} \text{ dw h}^{-1}\text{)} = \frac{C \cdot (a_{is} - a_{ns})}{t \cdot (a_{ic} - a_{ns}) \cdot dw} \quad (1)$$

Where a_{ns} is the atomic % ¹³C in the ambient sample's tissue, a_{is} is the atomic % ¹³C of the enriched sample tissue, taken after a 1 hour incubation, and a_{ic} is the atomic % ¹³C in the dissolved inorganic carbon in the incubation medium after enrichment. C (mg) is the carbon content of the sample, dw refers to dry weight (g) of the sample, and t (h) is time.

Phytoplankton uptake rates were determined using the C-uptake rate equation in Hama et al. (1983).

$$P \text{ (ug C L}^{-1} \text{ h}^{-1}\text{)} = \frac{POC \cdot (a_{is} - a_{ns})}{t \cdot (a_{ic} - a_{ns})} \quad (2)$$

The one deviation from equation 1 is that POC refers to the particulate organic carbon (ug C L⁻¹) in the incubated sample. No correction factor for isotopic discrimination against ¹³C has been applied

to the calculations. C-uptake rates for *G. salicornia*, phytoplankton, and *Acanthophora spicifera* were modeled against salinity and fit with quadratic least squares regressions (Fig. 8).

4. Water chemistry and physical parameter measurements

SGD factors - Due to the approximately conservative mixing of SGD-derived solutes with seawater, nutrients ($\text{NO}_3^- + \text{NO}_2^-$, total dissolved phosphorus, SiO_4^{2-}) were highly correlated with salinity (linear regression $R^2 = 0.99$ for total dissolved phosphorus, $R^2 = 0.96$ $\text{NO}_3^- + \text{NO}_2^-$, $R^2 = 0.998$ for SiO_4^{2-} ; supp Table 3). This high collinearity prevented the separate analysis of individual SGD-derived solutes and only salinity was included as a groundwater tracer in the models; nutrients were assumed to covary with SGD in all analyses.

Salinity and temperature – Two autonomous salinity sensors (Odyssey Temperature and Conductivity loggers, 3 to 60 mS cm^{-1}) and a temperature logger (Onset TidbiT v2 Water Temperature Data Logger) were deployed inside and outside the benthic chambers. The sampling frequency was one measurement per minute for both parameters. Salinity of water samples was measured using the Orion Star Portable meter as well with a conductivity probe. These values were used to groundtruth salinity time series data. Histograms of the mean salinity and temperature values inside the benthic chambers are shown in supp. Fig. 1.

Dissolved inorganic nutrients – Ambient samples for dissolved nutrient analysis, pH, and dissolved inorganic carbon (DIC) (supp. Fig. 1) were taken before incubation started as well as right at the end of the incubation. Water samples for dissolved inorganic nutrient analysis were filtered through a 0.2 μm previously combusted glass fiber filter. The samples were brought to room temperature, mixed, and analyzed on a Seal Analytical Segmented Flow Injection AutoAnalyzer AA3HR for soluble reactive phosphate (PO_4^{3-}) ammonium (NH_4^+), nitrate + nitrite ($\text{N} + \text{N}$; $\text{NO}_2^- + \text{NO}_3^-$), and silicate (SiO_4) at the S-LAB at the University of Hawai‘i at Mānoa.

pH – pH on the total scale (pH_{tot}) was measured using a Thermo Scientific Orion Star A329 portable meter with a pH electrode calibrated against a Tris buffer of known pH from Andrew Dickson’s laboratory at the Scripps Institution of Oceanography. Calculated pH values were corrected for *in situ* temperature recorded by the temperature loggers at each experimental location using CO₂SYS (Lewis & Wallace 1998) using pH, DIC, temperature, and salinity. HSO₄⁻ dissociation constants were taken from Uppstrom 1974 and Dickson 1990, while K₁K₂ dissociation constants were taken from Mehrbach et al 1973 and refit by Dickson & Millero 1987.

DIC – Water samples for DIC analysis (250 ml) were collected from within the benthic chamber using a 1L syringe in 300 mL borosilicate bottles before the experiment, right after the NaH¹³CO₃ spike and at the end of the experiment. Samples were brought back to the lab and fixed with 200 µl HgCl₂ per 250 ml seawater. Samples were analyzed for DIC using the UIC Coulometer and Marianda VINDTA 3D at the S-LAB at the University of Hawai‘i at Mānoa (supp. Fig. 1).

PAR and current – Two autonomous Odyssey Submersible Photosynthetic Active Radiation Logger were deployed; one within and one outside the benthic chamber. The scan rate was one per minute and the detected wavelength was cosine corrected photosynthetic irradiance (400–700nm). A Nortek Vector 3D Acoustic Velocimeter was deployed outside the chamber, set to sample continuously at a sampling rate of 8 Hz, with a nominal velocity range of 0.30 m/s (supp. Fig. 1).

5. Distribution maps for carbon uptake

Percent cover was measured on a 400m by 250m gridded map of Wailupe (n=73; Fig. 10A) using 0.25 x 0.25 m² quadrats and interpolated via linear interpolation within the boundaries of the data (interp function in package akima in R; Akima and Gebhardt 2015). To create C-uptake rate maps of *G. salicornia* for a reef with SGD, the map of percent cover was converted to biomass using an empirically derived regression model ($\text{dw} = 0.0392 \cdot (\% \text{ cover})$, $p < 0.001$, $F = 58.42$, $r^2 = 0.7322$,

supp. Fig. 4). The carbon uptake rate maps on a reef without SGD (Fig. 9B, D) were calculated using a mean value for carbon uptake rates measured in experiments located at least 250m from the SGD seep and with no SGD influence (ie. >34 salinity) (C-uptake rate_{noSGD} = 0.0213 mg C g dw⁻¹ h⁻¹ for *G. salicornia*; 0.0423 mg C g dw⁻¹ h⁻¹ for *A. spicifera*) multiplied with measured dry weight of either *G. salicornia* or *A. spicifera* (dw_{A.spi(i)}, g) (Fig. 9A, C) and mean C-uptake rates on a reef with no-SGD influence for either species (C-uptake rate_{noSGD}, mg C g dw⁻¹ h⁻¹). The model of carbon uptake rates vs. salinity (Fig. 8) was multiplied with biomass (Fig. 10A) to calculate carbon uptake rates of *G. salicornia* across the reef with SGD influence (Fig. 10C) interpolating salinity at a low tide of -0.12 m (Fig. 10B) for each percent cover point. C-uptake at ith benthic survey location (C-uptake_{SGD(i)}, mg C h⁻¹) was calculated by multiplying measured dry weight (dw_{G.sal(i)}, g) of *G. salicornia* by salinity-dependent C-uptake rate (C-uptake rate_{G.sal(i)}, mg C g dw⁻¹ h⁻¹). *A. spicifera* was only found in benthic experiments located away from the groundwater seep, therefore, carbon uptake rates were not calculated or modeled for areas with high SGD input.

6. Relating benthic carbon uptake and water column carbon uptake

The benthic carbon uptake comprised mainly *G. salicornia* as it is the most abundant macroalga on the reef and accounts for the majority of benthic production. The C-uptake values for *G. salicornia* on a reef with or without SGD were given as (mg C h⁻¹ m⁻²) for each benthic location by dividing the respective C-uptake values by the area of the quadrats used for the benthic surveys (0.0625 m²); the values were then square root transformed and C-uptake values for a reef without SGD were subtracted from the C-uptake values for a reef with SGD (Fig. 10D, supp. Fig. 2). Water column productivity on a reef with or without SGD (ug C h⁻¹) was calculated using salinity-dependent C-uptake rates (ug C L⁻¹ h⁻¹) multiplied by water column depth (depth_(i), m) at each benthic survey location (Table 3). For productivity on a reef with no SGD a mean C-uptake rate

(C-uptake rate_{noSGD(i)} = 0.692 mg C h⁻¹ L⁻¹) calculated from experimental data collected about 250m from the SGD seep when there was no SGD influence (ie. >34 salinity) was used (Table 3).

RESULTS

Relationships between productivity and SGD

We modeled C-uptake rate with salinity as a measure of SGD (relationships in supp. Table 2) for *G. salicornia*, phytoplankton, and *A. spicifera*. Quadratic models fit best for *G. salicornia* and phytoplankton (Fig. 8); linear regressions were not found to be significant. *A. spicifera* was only found in 7 experiments and only above salinities of 27 (Fig. 8C), therefore there were not enough samples to determine a significant model between C-uptake rate and SGD. Models peak at approximately 21–22 salinity (24.5–26.6 μmol NO₃⁻ L⁻¹ and 0.54–0.58 μmol PO₃⁺⁴ L⁻¹) for *G. salicornia* and phytoplankton, respectively.

Effects of SGD on benthic and water column productivity

Maps of biomass and C-uptake in the absence of SGD for both species are shown in Figure 9. Even though uptake rates of each species for areas with no SGD was comparable (mean C-uptake rate_{*G. salicornia*} = 0.014 ± 0.082 mg C g dw⁻¹ h⁻¹; mean C-uptake rate_{*A. spicifera*} = 0.0423 ± 0.029 mg C g dw⁻¹ h⁻¹), *G. salicornia*'s biomass is much greater than that of *A. spicifera* and therefore *G. salicornia* is responsible for the majority of the benthic uptake at this site. Even considering the areas with no hard substrate for this alga to grow on, percent cover of *G. salicornia* was 4.6% in 2004 (McGowan 2004) and from our benthic surveys done between 2014–2017 it is more recently approximately 13% of total benthic cover, making it the most abundant algal species at this site.

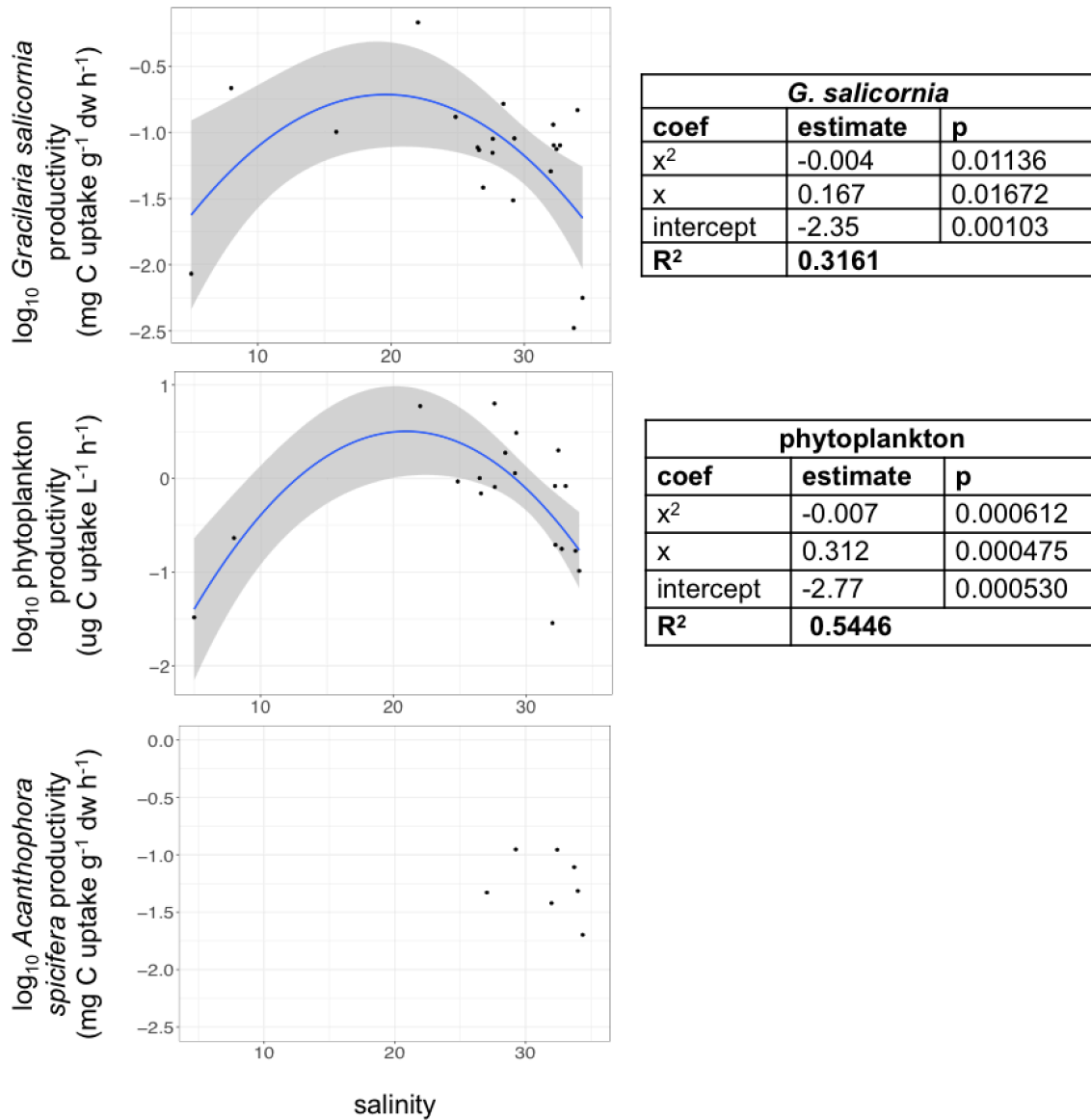


Figure 8. Peak carbon uptake by primary producers at intermediate SGD levels. C-uptake rates modeled against salinity (proxy for SGD factors) on a reef flat with SGD (left) and model statistics (right). Quadratic relationships are significant for *G. salicornia* and phytoplankton vs. salinity.

We calculated C-uptake of *G. salicornia* across the Wailupe reef flat with SGD input (Fig. 10). The uptake map (Fig. 10 C) reflects *G. salicornia*'s biomass and shows hotspots of uptake where there is high biomass and intermediate salinity. The difference between C-uptake on a reef with SGD and a reef without SGD was calculated for *G. salicornia* for each sampling point where we found *G. salicornia* (Fig. 10D, supp. Fig. 2). At low tide, *G. salicornia* is responsible for a

mean additional $0.097 \text{ mg C h}^{-1}$ uptake. The water column contribution and benthic contributions on a reef with SGD are $0.697 \text{ mg C m}^{-2} \text{ h}^{-1}$ and $2.72 \text{ mg C m}^{-2} \text{ h}^{-1}$, respectively. On a reef with no SGD these values decrease by 32% for the water column and 82% for the benthos (Table 3).

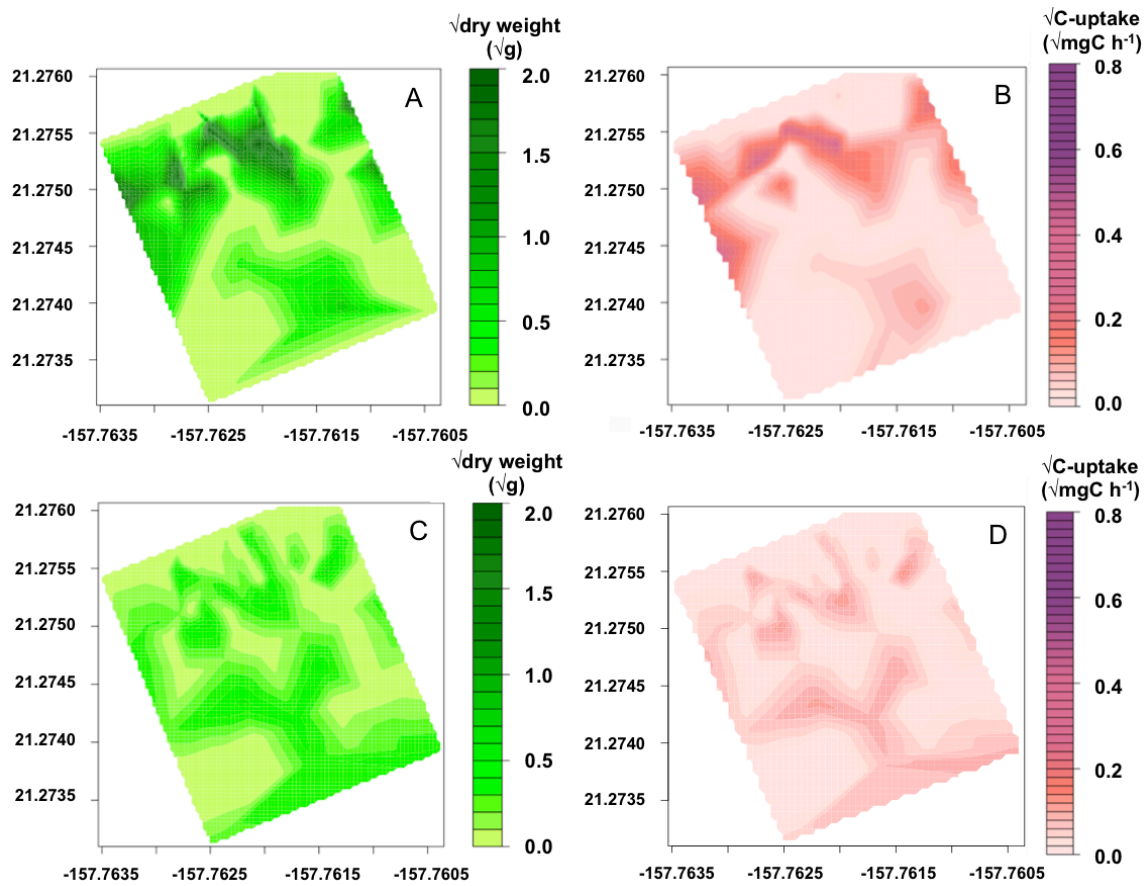


Figure 9. Spatial distributions and modeled C-uptake rates for macroalgal species on a reef without SGD. (A) *G. salicornia* dry weight ($\sqrt{\text{g}}$), (B) *G. salicornia*'s C-uptake on a reef flat without SGD (mg C h^{-1}), (C) *A. spicifera* dry weight ($\sqrt{\text{g}}$), (D) *A. spicifera*'s C-uptake on a reef flat without SGD (mg C h^{-1}).

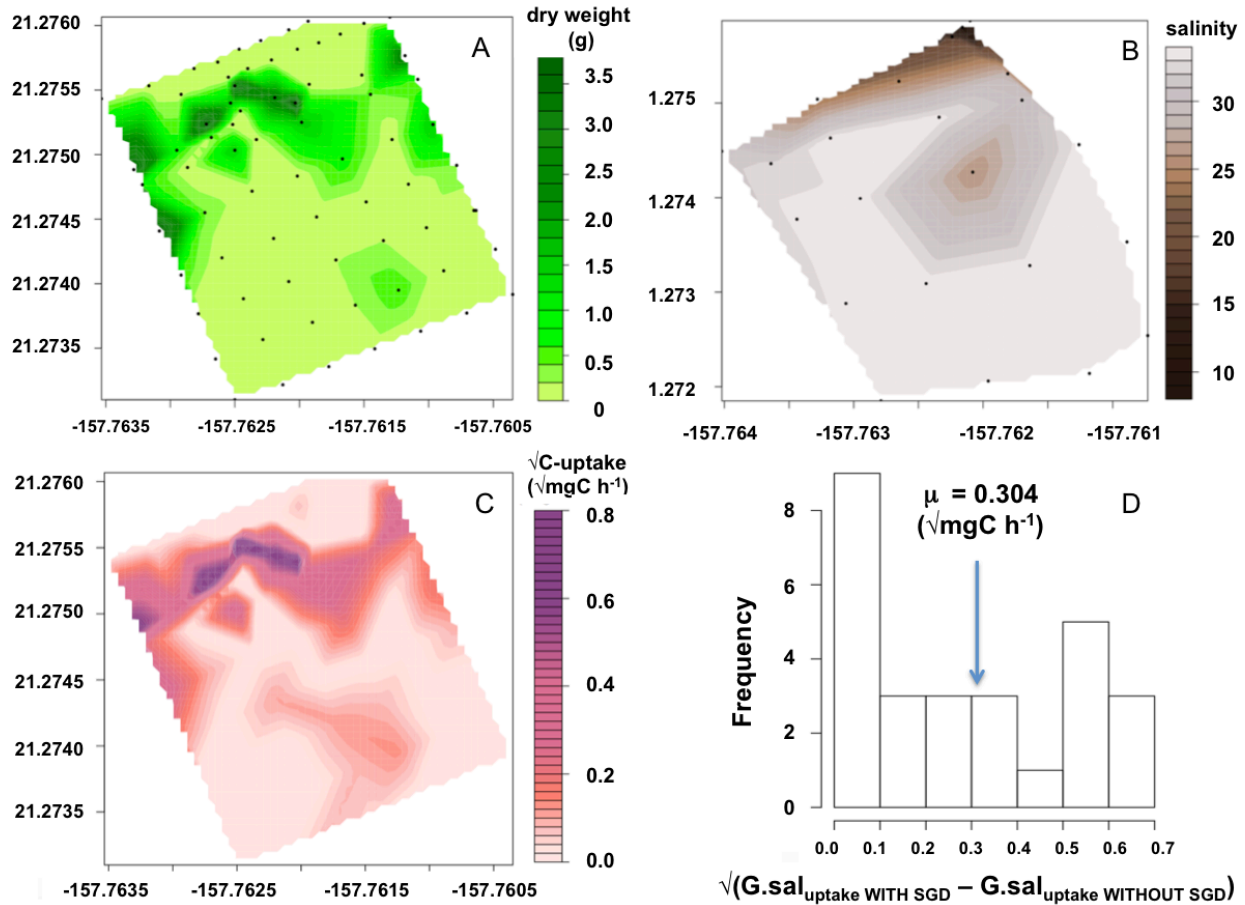


Figure 10. Spatial distribution and modeled C-uptake rates of *G. salicornia* with respect to SGD inputs on a reef flat. Contour plots shaded by (A) *G. salicornia* dry weight (g). Black points on maps refer to actual sampling locations. (B) Salinity at low tide (May 15, 2015), (C) modeled C-uptake on a reef flat with SGD (mg C h^{-1}), (D) Histogram of the square root transformed differences between *G. salicornia* C-uptake on reef with SGD and without SGD. Mean of the square root transformed values is indicated by the blue arrow.

Table 3. C-uptake rates for *G. salicornia* and phytoplankton for reefs with and without SGD. Values in rows 2 and 4 are normalized to benthic area.

	C-uptake on reef with SGD ($\mu \pm \text{se}$)	C-uptake on reef without SGD ($\mu \pm \text{se}$)
<i>G. salicornia</i>	$0.111 \pm 0.031 \text{ mg C g}^{-1} \text{ dw h}^{-1}$	$0.014 \pm 3.274 \text{ mg C g}^{-1} \text{ dw h}^{-1}$
C-uptake rate	$2.72 \pm 0.035 \text{ mg C m}^{-2} \text{ h}^{-1}$	$0.487 \pm 0.005 \text{ mg C m}^{-2} \text{ h}^{-1}$
Phytoplankton	$1.39 \pm 0.423 \text{ ug C l}^{-1} \text{ h}^{-1}$	$0.136 \pm 0.033 \text{ ug C l}^{-1} \text{ h}^{-1}$
C-uptake rate	$0.697 \pm 0.075 \text{ mg C m}^{-2} \text{ h}^{-1}$	$0.449 \pm 0.021 \text{ mg C m}^{-2} \text{ h}^{-1}$

DISCUSSION

Our study shows that SGD is important for both water column and benthic C-uptake in tropical reef systems. Peak C-uptake of both phytoplankton and *G. salicornia* occurred at SGD-derived salinities of ~ 21.5 ($25.5 \mu\text{mol NO}_3^- \text{L}^{-1}$ and $0.54 \mu\text{mol PO}_4^{3-} \text{L}^{-1}$), suggesting a physiological trade-off between salinity tolerance and nutrient availability for reef primary producers. The water column and benthic contributions to C-uptake on a reef with SGD are approximately 36% and 82% higher than a reef without SGD, respectively. This suggests that SGD has a greater impact on benthic macroalgal productivity.

Several studies on species in the *Gracilaria* genus have shown optimal growth rates between 20–30 salinity (Hoyle 1975, Glenn et al. 1999, Choi et al. 2006). One study by Duarte and colleagues (2010) on the native Hawaiian species *Gracilaria coronopifolia* showed quadratic relationships between growth and SGD, with maxima at (27 salinity, 7.51 μmol nitrate, 0.15 μmol phosphate). *G. salicornia*, the main primary producer on this reef is one of the main invasive species in Hawai'i. Our work shows that *G. salicornia* can have high C-uptake rates in areas with SGD (high nutrients and low salinity), which implies that some macroalgae, an invasive species in this case, can thrive in areas with groundwater input and may make it a better competitor in this type of environment.

Phytoplankton uptake rates were boosted 32% in reefs with SGD, emphasizing that the effects of SGD are ecosystem-wide. Sugimoto and colleagues (2017) found uptake rates of phytoplankton to range from 2–50 $\mu\text{g C l}^{-1} \text{h}^{-1}$ in a coastal embayment with SGD. Our values fall on the low end of this range, with 1.39 $\mu\text{g C l}^{-1} \text{h}^{-1}$ on a reef with SGD and decreased by an order of magnitude to 0.136 $\mu\text{g C l}^{-1} \text{h}^{-1}$ on a reef without SGD. These rates are comparable to time series measurements by radioisotope incorporation methods in reef flats of a similar high island (Mo'orea, French Polynesia) away from obvious SGD sources which average $0.423 \pm 0.276 \mu\text{g C}$

$l^{-1} h^{-1}$ (Alldredge and Carlson 2016). C-uptake values of phytoplankton from Paiko lagoon in Maunalua bay (21.2811°N, -157.7296°W) range from 7–15 $mg C m^{-2} d^{-1}$ (K. Peyton, personal communication); our mean C-uptake values are comparable at 10.8 $mg C m^{-2} d^{-1}$ on a reef with no SGD and 17.7 $mg C m^{-2} d^{-1}$ on a reef with SGD.

Several studies have shown that high islands with semidiurnal tides, such as the main Hawaiian islands, have high fluxes of SGD with high nutrient levels (Moosdorf et al. 2015, Dimova et al. 2012). On reefs experiencing semi-diurnal tidal patterns, intermediate salinities can occur up to four times a day, creating a periodicity that could allow for consistently elevated productivity on reefs with SGD. Consistent with previous surveys of spatial distributions (Nelson et al. 2015), we showed that groundwater can have an effect on productivity up to 200 meters offshore (Fig. 7, supp. Fig. 3); in order to create more accurate productivity maps and estimates, we need to keep in mind SGD's spatial "reach" or extent. These coastal systems with SGD can be hotspots for possible phase shifts as well as restoration. These areas should be monitored for changes in community structure and can be targeted by groups engaged in conservation efforts such as local Hawaiian community groups who are currently "replanting" for limu (algae) maunaea (*G. coronopofolia*) as well as other native and endemic species.

Wailupe's benthic community is composed of a variety of macroalgae, zoanthids, and sparse coral. Even considering these species, whose cover is less than 5% individually, *G. salicornia* is most likely responsible for the largest C-uptake on this reef. Our *G. salicornia* uptake values (2.72 $mg C m^{-2} h^{-1}$ on a reef with SGD) account for a small fraction of gross reef productivity, which have been reported to range from 0.33–30 $g C m^{-2} h^{-1}$ (Sorokin 1995, Gattuso et al. 1996, Andrefouet and Payri 2001). The uneven benthic community at Wailupe could explain the lack of productivity at this site. The high abundance of this species could in turn lead to a less

productive coastal system, lacking the variety in functional groups which could boost this system's carbon uptake (Steneck and Dethier 1994, Nyström 2006).

A common interest between management and the SGD research community is the fate of the dissolved inorganic nutrients brought to coastal systems via SGD. *Gracilaria* species have been well studied for aquaculture purposes and are widely known to have high uptake rates for NH_4^+ , NO_3^- , and PO_4^{3-} (Glenn et al. 1999, Yang et al. 2006, Huo et al. 2012). They can remove 13–85% DIN and DIP within 23–45 days (Yang et al. 2006, Huo et al. 2012) in a closed system. Wailupe reef flat has a residence time of approximately 1 day (Wolanski et al. 2009), which suggests that large amounts of DIN and DIP are being exported to the reef crest and other areas offshore.

In this study, we have shown that SGD is an important source of freshwater and nutrients to reef systems. In this macroalgal-dominated reef, the productivity of both the water column and the main macroalgal species are increased at intermediate levels of SGD. Further research on the benthic communities associated with SGD and its biochemistry is warranted. Understanding the effects of SGD on coastal communities will help direct and prioritize conservation and management efforts. Close attention should be paid to the photosynthetic communities present in coastal areas with SGD as these areas may be hotspots for phase shifts and restoration.

CHAPTER 3

TEMPORAL AND SPATIAL PATTERNS IN SUBMARINE GROUNDWATER DISCHARGE CHEMISTRY RELATE TO MACROALGAL COMMUNITY STRUCTURE *IN SITU*

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Abstract: Submarine groundwater discharge (SGD) has been recognized as an important transporter of solutes and freshwater in coastal systems worldwide. In high island systems with a mixed semidiurnal tidal cycle driving the delivery of SGD, the biogeochemistry in coastal systems with SGD is highly variable both temporally and spatially. Past studies have shown that SGD can shape the local species composition, diversity, and richness of biological communities. In this study, we explored empirical orthogonal functions (EOFs) as a dimension-reducing tool used to quantify predictable spatial and temporal dynamics of the delivery of SGD on two reefs. The first two EOFs, one with a 12h period and highest variability at the SGD seep, and the other with a 24h period and highest variability mid-reef, explained an average of 36% and 17% of the total variability at the two sites, respectively. Multivariate community structure was significantly related to the second EOF at one site and both the first and second EOFs at another site. Generally, we found a higher number of taxa away from the SGD, while only 1–2 taxa were more abundant close to the SGD. Among the species that were present away from the SGD were *Acanthophora spicifera*, calcifying macroalgae, *Pterocladia* sp., *Lyngbya* sp., and *Avrainvillea amadelpha*. Zoanths and turf algae were present closer to the SGD source. These results show that benthic communities vary with respect to SGD at the local scale. The site differences in both the SGD chemical composition and its physical properties, as well as coastal community structure underline the importance of studies done *in situ* exploring biological and physicochemical interactions at the watershed level.

INTRODUCTION

Submarine groundwater discharge (SGD) has been recognized as an important source of inorganic nutrients and freshwater in marine systems (Howarth et al. 2002, Burnett et al. 2003, Zhang & Mandal 2012). This is especially true in volcanic high island systems surrounded by oligotrophic waters (Kim et al. 2003, Hwang et al. 2005, Moosdorf et al. 2015). Most of the literature concerning SGD has been focused on characterizing its solutes and chemical composition, with the implication that these distinct water masses can be important vectors for inorganic nutrients, organic matter, metals, microbes, and pharmaceuticals (Moore 2010). However, fewer studies have looked at the impacts of SGD on coastal ecology.

The temporal delivery of SGD-derived solutes is unique; groundwater discharging close to the shoreline in a system that is tidally driven sees high fluxes of SGD at low tide, when there is weak hydrostatic pressure on the seeps, while at high tide there is high hydrostatic pressure on the seeps and groundwater flux to the coastal area is minimal. Additionally, SGD in island systems has a large freshwater component with pH and salinity values lower than surrounding coastal water (Moosdorf et al. 2015, Burnett et al. 2003). This cyclical set of disturbances associated with SGD can occur at multiple time points throughout the day in a semidiurnal tidal system.

In coral reef communities, it is often the interaction of persistent disturbances that cause permanent phase shifts (McClanahan et al. 2002). The many different stressors that are associated with the chemical composition of SGD, especially high nutrient concentrations, and the periodic mode of its delivery may in part cause reef systems to become vulnerable to increased algal biomass (Baker et al. 2008, Fung et al. 2011, Houk et al. 2014). The composition of the algal assemblage that occurs on a degraded reef with SGD depend on which species can thrive in this specific chemical and physical environment. Marine primary producers have an optimal range of nutrient concentrations and salinity for growth and production. Both of these environmental

variables control major physiological functions; salinity drives osmotic and solute regulation (Wiencke & Bishof 2012), while nutrient concentrations drive uptake rates and productivity (Thomas & Cornelisen 2003, Valiela et al. 1997). Shallow coastal waters with SGD undergo concomitant nutrient load and salinity changes on small time scales (i.e. tidal, hourly) (Johnson et al. 2008). Functioning under this highly variable nutrient and salinity environment at this time scale requires fast acclimation or adaptation to wide ranges of chemical parameters. These rapidly changing environmental conditions can preferentially spur the growth of algal species. In areas where the nutrient concentration of coastal groundwater has been substantially increased by changing land use (i.e. agriculture, residential, commercial), nutrient loading via SGD has been associated with macroalgal blooms and shifts in community composition (Naim 1993, McCook 1999, Costa et al. 2008, Lyons et al. 2014).

Current work on SGD and its effects on biological community structure focuses on microbial, phytoplankton, and meiofaunal communities. Most studies have found that microbial (Lee et al. 2017) and phytoplankton communities (Troccoli-Ghinaglia et al. 2010) associated with SGD are distinct from reference sites, species diversity and richness patterns vary by community type and SGD-associated chemistry, and site. The studies focused on meiofaunal community structure associated with SGD have found that areas close to SGD (scale of tens of meters) show increased abundance of specific invertebrates, specifically mollusks (Leitao et al. 2015, Piló et al. 2018) and polychaetes (Encarnação et al. 2015). Studies that have looked at macroalgal and seagrass community structure show decreased abundance and diversity in areas with SGD (Kantún-Manzano et al. 2018, Piló et al. 2018). Specifically, Kantún-Manzano and colleagues found that SGD influenced the spatial pattern of seagrasses by creating monospecific stands of *H. wrightii*, with a spatial arrangement that follows a gradient characterized by increasing biomass and coverage with greater distance from SGD. These studies are showing that SGD can shape the

local species composition, diversity, and richness of biological communities although few studies have explored this in tropical coral reef settings.

Maunalua Bay, located on the southeastern shore of O‘ahu, has algal-dominated fringing reefs with localized tidally-modulated SGD. Sites in the bay experience two low and two high tides per day that can vary in height due to the mixed semi-diurnal tidal regime. Hence, the SGD fluxes vary on several cyclical temporal scales. The fringing reefs at two of these sites, Black Point and Wailupe, have well-characterized and chemically distinct SGD (Amato 2015, Nelson et al. 2015, Richardson et al. 2017, Lubarsky et al. 2018). Both sites are dominated by macroalgae, whose presence and abundance vary across the reef flat, providing an interesting study site for the interaction of SGD with macroalgal benthic communities. Overall this study seeks to explore spatiotemporally explicit patterns in SGD and their effects on macroalgal-dominated benthic community structure on tropical reefs. The specific objectives for this study are to (1) model predictable spatiotemporal variability in SGD across reef flats using high resolution spatial time series of salinity and temperature, (2) assess whether multivariate community structure is related to the SGD spatiotemporal patterns of variation, and (3) elucidate which taxa or functional groups have spatial distributions predictable from modeled SGD dynamics.

METHODS

Site descriptions and SGD characterizations: The study sites are located along the southern shoreline of O‘ahu, Hawai‘i, in Maunalua Bay (Fig 11A; 21.2743°N, 157.7492°W). The two study sites, Black Point (21.2586°N, 157.7899°W) and Wailupe (21.2756°N, 157.7624°W), are on the western side of the bay (Fig. 11B) and are shallow reefs dominated by macroalgae from the shoreline to the reef crest. Black Point and Wailupe SGD flux rates are approximately 280 m³ d⁻¹ m⁻¹ of coast and 181 m³ d⁻¹ m⁻¹ of coast, respectively (Holleman 2011). Nutrient concentrations in

SGD seeps at Black Point averaged $190 \mu\text{M NO}_3^- \text{ L}^{-1}$ and $3 \mu\text{M PO}_4^{3-} \text{ L}^{-1}$ resulting in groundwater derived nutrient fluxes of $55.6 \text{ mol NO}_3^- \text{ d}^{-1} \text{ m}^{-1}$ and $0.915 \text{ mol PO}_4^{3-} \text{ d}^{-1} \text{ m}^{-1}$ (Holleman 2011, Richardson et al. 2017). Wailupe SGD average nutrient concentrations were $68 \mu\text{M NO}_3^- \text{ L}^{-1}$ and $2 \mu\text{M PO}_4^{3-} \text{ L}^{-1}$ resulting in groundwater derived nutrient fluxes of $10.7 \text{ mmol NO}_3^- \text{ d}^{-1} \text{ m}^{-1}$ shoreline and $0.30 \text{ mol PO}_4^{3-} \text{ d}^{-1} \text{ m}^{-1}$ shoreline (Holleman 2011, Richardson et al. 2017). The high nutrient concentrations in the SGD are possibly due to near-shore geochemical and geological changes including high density of on-site sewage disposal systems (OSDS) (Richardson et al. 2017). Salinity and ^{222}Rn surveys of the bay's coastline indicated negligible surface water inputs (Richardson et al. 2017) suggesting that SGD is the dominant source of nutrients and terrestrial freshwater inputs throughout the year, excluding rain.

Salinity time series and spatiotemporal analyses: Previous work has shown strong conservative relationships between salinity and SGD at both Black Point and Wailupe (Nelson et al. 2015, Lubarsky et al. 2018) therefore salinity was used as a proxy for SGD. Twenty-three autonomous salinity sensors (Odyssey Temperature and Conductivity loggers, 3 to 60 mS cm^{-1}) were deployed in a sparse grid across each site (Fig. 2). The sensors were deployed at Black Point for 30 days (29 May—29 June 2016) and for 27 days at Wailupe (4 April—21 May 2016) at a sampling frequency of one reading every 10 minutes. Water samples were taken while each sensor was deployed and were analyzed using a Portasal Salinometer 8410A (accuracy 0.001) and compared to sensor values for quality control. Data from rainy days at the sites were excluded from any analyses.

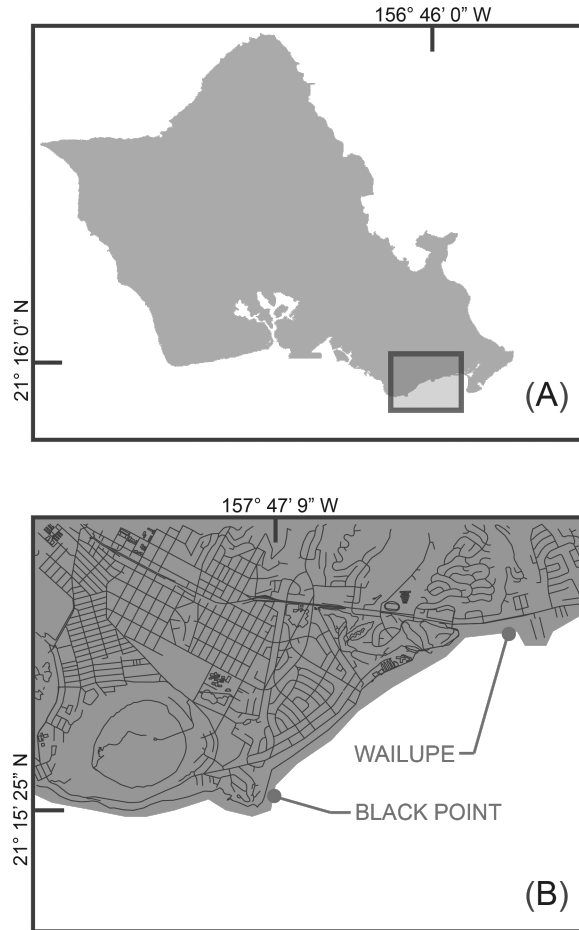


Figure 11. Maps of the site locations. (A) Map of Oahu, Maunalua Bay is enclosed in the grey box. (B) Close up of the coastline of the western half of Maunalua Bay. Grey markers indicate Black Point and Wailupe.

Empirical orthogonal functions (EOFs) were applied on the spatially indexed salinity time series at both sites. EOFs were used to reduce the dimensionality of the spatially explicit time series and to reveal the spatial structure of the time series data. EOFs are the spatiotemporal manifestation of principal components analysis (PCA) (Wikle et al. 2018). The output for EOFs includes a spatial map of loadings and an associated normalized principal-component time series for the salinity dataset obtained using a singular value decomposition of a space-wide matrix. The singular value decomposition was done using the function `svd` in the base library in RStudio (version 1.0.44, R Core Team 2016). Spectral density of the principal component time series for

each EOF using the spectrum function in the stats library in RStudio (R Core Team 2016). The output for the spectral analysis includes a periodogram, from which the most important frequency can be back-calculated (Bai & Silverstein 2018, Wikle et al. 2018).

The salinity sensor locations (Fig. 2) and benthic survey areas (described in the next section) generally overlapped, but some benthic surveys fell outside of the bounds of the salinity sensor locations (Fig. 3). We used Kriging in order to extrapolate the EOF values for the benthic surveys outside the bounds of the EOF maps while accounting for spatial autocorrelation we used the function variogram in the R library spatial (Venables & Ripley 2002) as well as the function krige in the R library gstat (Pebesma 2004, Gräler et al. 2016).

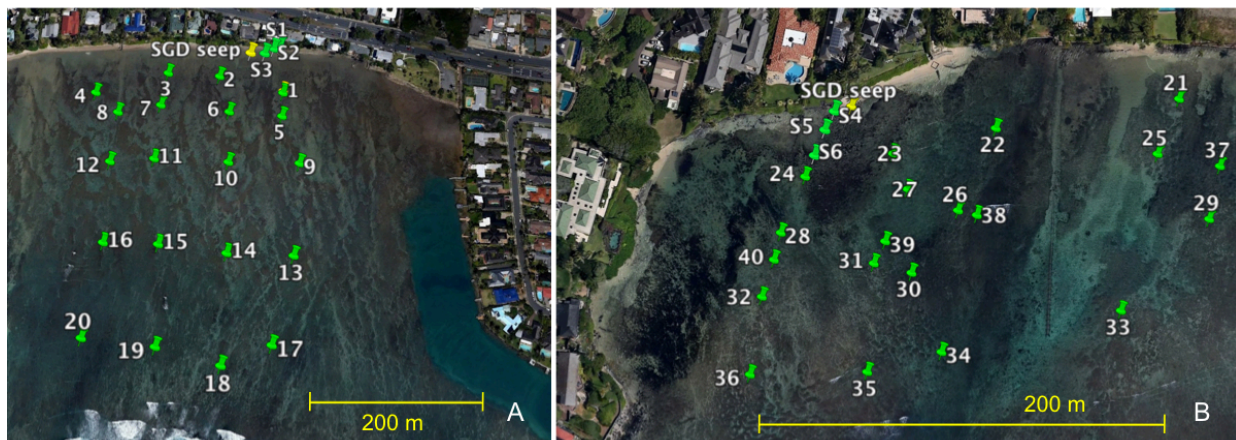


Figure 12. Salinity sensor locations on reef flats (A) Wailupe (B) Black Point. Yellow marker indicates SGD seep in both locations.

Benthic surveys and community structure analyses: The spatial extent of the survey areas for each site was set to encompass a previously characterized gradient of SGD from seep to background oceanic waters (Nelson et al. 2015). The area covered for Black Point was about ~ 85.1 m² (230 m offshore by 370 m alongshore) and 90.0 m² (300 m offshore by 300 m alongshore) for Wailupe (Fig. 3). A grid was superimposed on the site, from groundwater seep to reef crest, and benthic algal surveys were done at the grid intersection points ($n = 97$ for Wailupe and $n = 115$ for

Black Point). The grid includes more points closer to the SGD seep in order to have comparable sampling effort along the gradient of SGD. The survey consisted of 25 cm by 25 cm quadrats, where species-specific percent algal cover, species-specific invertebrate percent cover, and substrate type were measured. All taxa were identified to the lowest taxonomic level. Surveys were completed between May and August 2016. Although salinity time series were not taken synchronously with the benthic surveys, the data was taken during the same season and one year apart and therefore experience similar tidal patterns, mixing, and swell (F. F. La Valle, unpubl.). The most abundant biological communities at these sites are composed of perennials and their presence and absence does not change significantly during the year, but the species-specific biomass can change with season (F. F. La Valle, unpubl.). The tidal ranges that occurred during the period of time the salinity time series were taken were equivalent to the tide ranges that occurred during the benthic surveys. Tide is the main driver for SGD delivery; the thirty-day time series of salinity encompassed spring and neap tides and all the tidal heights variability experiences by this reef flat from May to August 2016. Thus, the SGD flux and variability will be comparable to the time these surveys were taken.

The relative abundance data by taxa ranged from zero to one; it was square root transformed to down-weight ubiquitous taxa and to account for patchiness of reef species. Nonmetric multidimensional scaling (NMDS) was used to visualize community dissimilarity matrices (bray-curtis dissimilarity) using the metaMDS function in the R package vegan (Oksanen et al. 2017) in R.

We ran distance-based linear models (DistLM) on the distance matrix of community data by site using EOF1 and EOF2 as fixed effects (predictors) and substrate type as a random effect was used to explore species composition to quantify variance in benthic community structure explained by EOFs (Zelditch et al. 2012). The function adonis in the R package vegan was used to

create these models (Okansen et al. 2017).

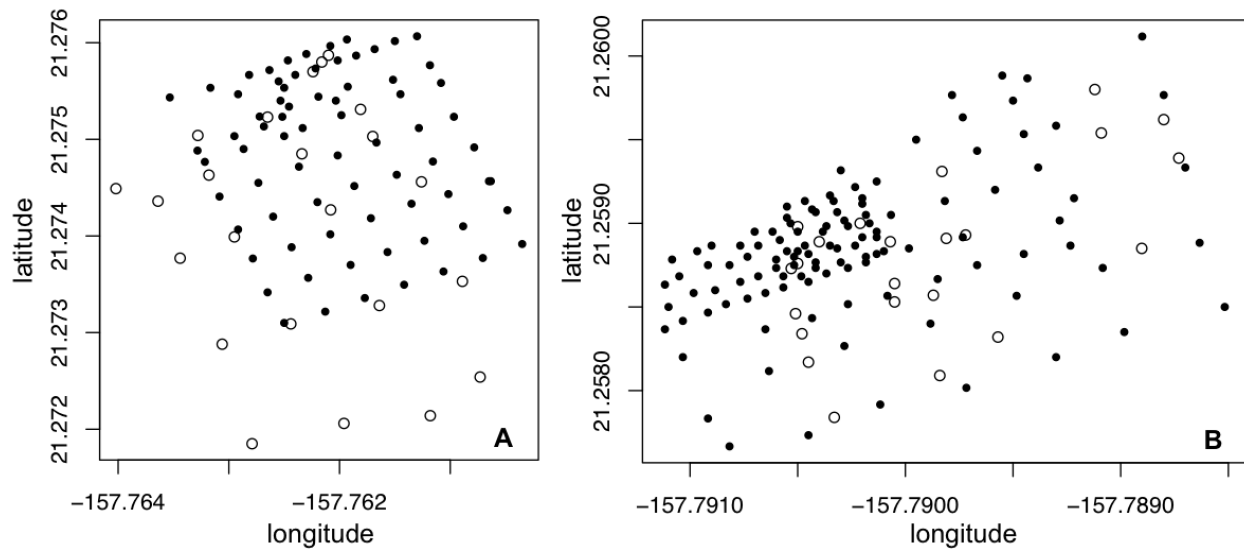


Figure 13. Salinity sensor and benthic survey locations. Open circles represent salinity sensor locations and black filled-in circles represent benthic survey locations at (A) Wailupe and (B) Black Point.

Species-specific relationships with variability in SGD: In order to characterize how presence or absence of each benthic taxon was related to SGD dynamics logistic regression models were run for each site using the glm function in the stats package (R Core Team) with EOF1 and EOF2 as fixed effects (predictors). Only species that appeared in at least three benthic samples were analyzed for univariate relationships to SGD. For Wailupe, the following taxa were selected:

Acanthophora spicifera, *Gracilaria salicornia*, *Hypnea* sp., *Avrainvillea amadelpha*, *Dictyota* spp.,

Lyngbya sp., *Laurencia* sp., crustose coralline algae (CCA), turf, *Spyridia* sp., zoanthids, and a

calcifying macroalgae category, which included *H. discoidea*, *Liagora* sp., *Galaxaura* sp.. At

Black Point, the following taxa were selected: *A. spicifera*, *Bryopsis pennata*, *Pterocaldiella* sp.,

turf, *H. discoidea*, *Liagora* sp., and a calcifying macroalgae group, which included *H. discoidea*,

Liagora sp., and *Jania* sp. to explore community structure. Logistic regression models were run

using the glm function in the stats package (R Core Team) with EOF1 and EOF2 as fixed effects

(predictors) and substrate type as a random effect. Substrate types for Black Point were sand, rock, and reef flat; at Wailupe the substrate types were sand, rock, reef flat, and silt. All logistic regression p-values were controlled for false discovery rate ($\alpha = 0.05$) using the function `p.adjust` with the Benjamini Hochberg method in the R package `stats` (R Core Team, 2016).

RESULTS

Salinity time series and spatiotemporal analyses: EOFs were derived from the spatially explicit salinity time series datasets at both Wailupe and Black Point, resulting in EOF loading maps (Fig. 14 A, C, E, G) and complementary normalized principal components time series (Fig. 14 B, D, F, H). The first and second EOFs at Black Point explained 35.3% and 16.0% of the spatiotemporal variance, respectively. Similarly, at Wailupe EOFs 1 and 2 explained 36.8% and 18.9% of the spatiotemporal variance, respectively. EOF1 shows the main source of variation as a 12.0–12.5 hour cycle, indicating that tidal variation drives the majority of the SGD variability. The EOF loading maps for Wailupe and Black Point (Fig. 14 A, E) show higher loadings close at the SGD seeps at both sites due to the high variability in salinity at the 12 hour frequency at the SGD seeps. SGD seeps and surrounding areas are the most variable with tide in terms of salinity, with low salinity SGD fluxes increasing at low tide and decreasing at high tide at both sites. EOF2 time series show that an average 17.5% of the spatiotemporal variability occurs at a 24-hour cycle. This coincides one full mixed semidiurnal tidal cycle (ie. two high tides and two low tides per day that vary in height, Fig. 14 D, H). Daily variability (EOF2) is highest in the mid-reef zone.

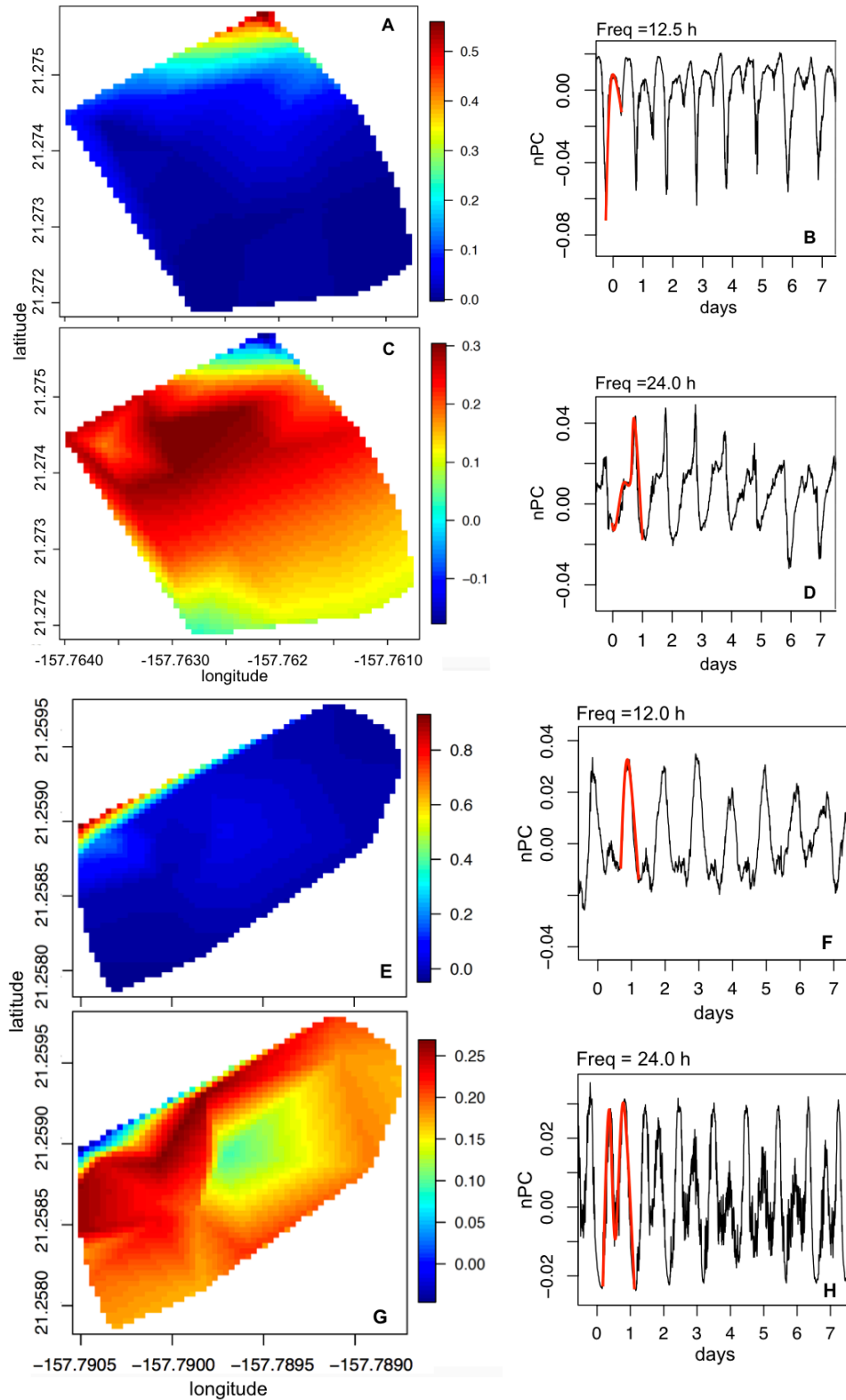


Figure 14. EOF1 and EOF2 loading maps and associated time series showing important patterns of variance in SGD at Wailupe (A–D) and Black Point (E–H). The most important frequencies for the time series are shown on top of the time series and a sample cycle is highlighted in red on the time series plots.

Benthic surveys and community structure analyses: Benthic surveys recorded 30 benthic species (24 macroalgal taxa, 6 invertebrate taxa) at Wailupe; the main species were *A. spicifera* (present in $n = 32$ samples, $\mu = 9.85\%$), *G. salicornia* (present in $n = 29$ samples, $\mu = 13.1\%$), *H. discoidea* (present in $n = 20$ samples, $\mu = 3.86\%$), and *Lyngbya* sp. (present in $n = 18$ samples, $\mu = 3.05\%$). Black Point had generally less macroalgal cover, with 28 benthic species recorded (21 macroalgae taxa, 7 invertebrate taxa). The most abundant species at Black Point were *Pterocladia* sp. (present in $n = 51$ samples, $\mu = 8.54\%$), turf (present in $n = 35$ samples, $\mu = 5.94\%$), and *B. pennata* (present in $n = 13$ samples, $\mu = 2.83\%$).

Multivariate benthic algal and invertebrate community structure was significantly related to EOF1 and EOF2 loadings at Black Point and significantly related to EOF2 loadings at Wailupe (adonis $p < 0.05$), which shows that community structure is in fact related to spatiotemporal structures of variability of SGD (supp. Tables 4–5). NMDS plots of community structure at the two sites are shown in Figure 15 shaded by EOF1 and EOF2. NMDS1 and NMDS2 were both significantly correlated with EOF2 (Fig. 15D, supp. Table 5) at Black Point. NMDS2 was significantly correlated with both EOF1 (Fig. 15A, supp. Table 4) and EOF2 (Fig. 15C, supp. Table 4) at Wailupe.

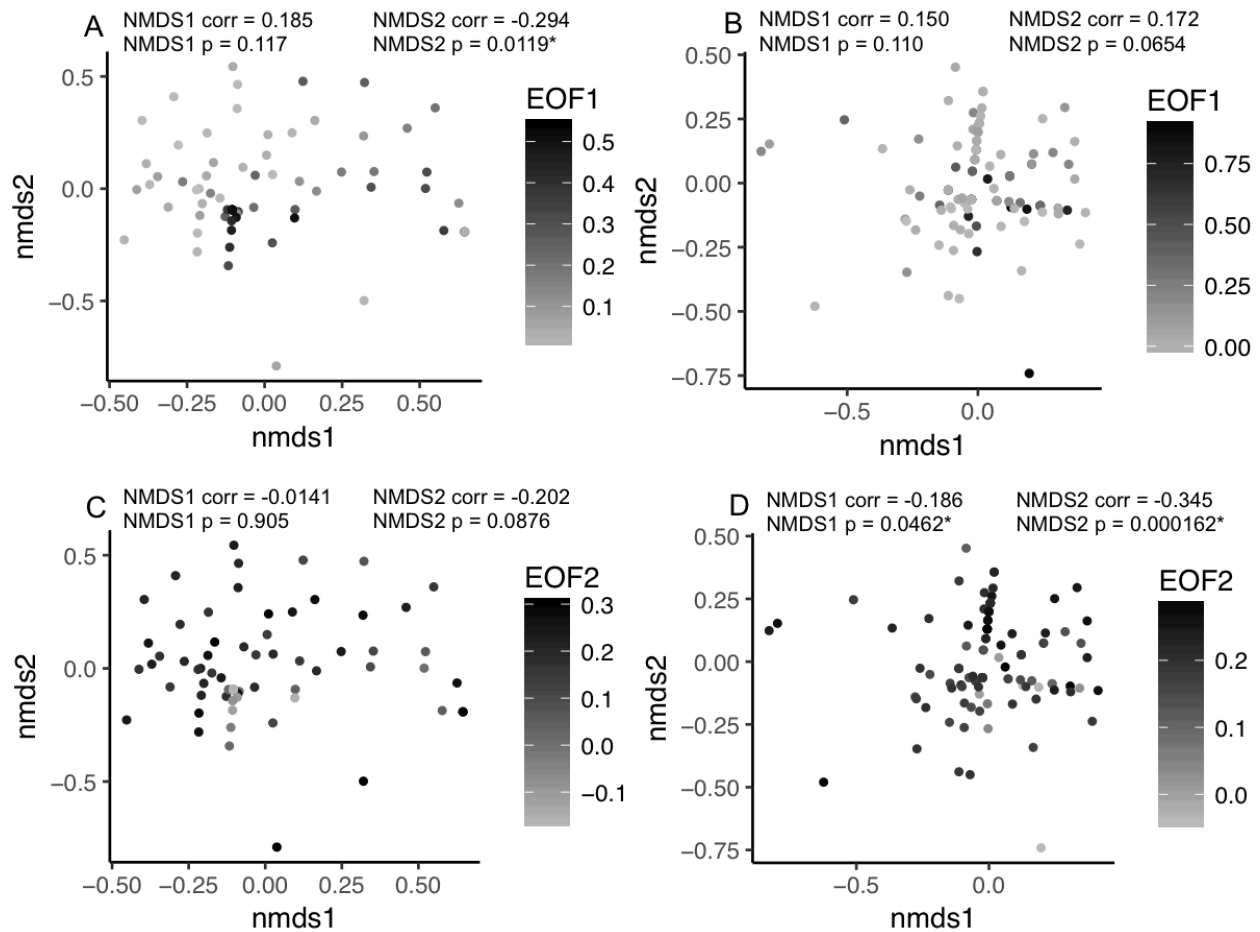


Figure 15. NMDS plots of benthic community structure for Wailupe (A, C) and Black Point (B, D) shaded by EOF1 (A–B) and EOF2 (C–D). Correlation coefficients and associated p values are given for NMDS scores and EOFs shown in each panel. DistLM for Wailupe EOF1: $F= 2.253$, $R^2=0.0256$, $p=0.079$; EOF2: $F= 4.826$, $R^2=0.0556$, $p=0.0039^*$. DistLM for Black Point EOF1: $F= 3.096$, $R^2=0.0252$, $p=0.0113^*$; EOF2: $F= 5.153$, $R^2=0.0419$, $p=0.0001^*$.

Species-specific relationships with variability in SGD: Calcifying algae and *A. spicifera* showed the same inverse relationship with SGD at both sites; they were found mostly away from the seep (Fig. 16, supp. Tables 4–5). Most species with the exception of turf algae and zoanthids at Wailupe were absent (0% cover) near the SGD source, but distances varied by species (Fig. 16A, C; supp. Table 4). Turf and zoanthids at Wailupe were most likely to be present close to the SGD, while *A. amadelpha* and *Lyngbya* sp. were more likely to be absent close to the SGD (Fig. 16A, C; supp. Table 4). At Black Point, *Pterocliadiella* sp. was more likely to be found away from the seep (Fig. 16B, D; supp. Table 5).

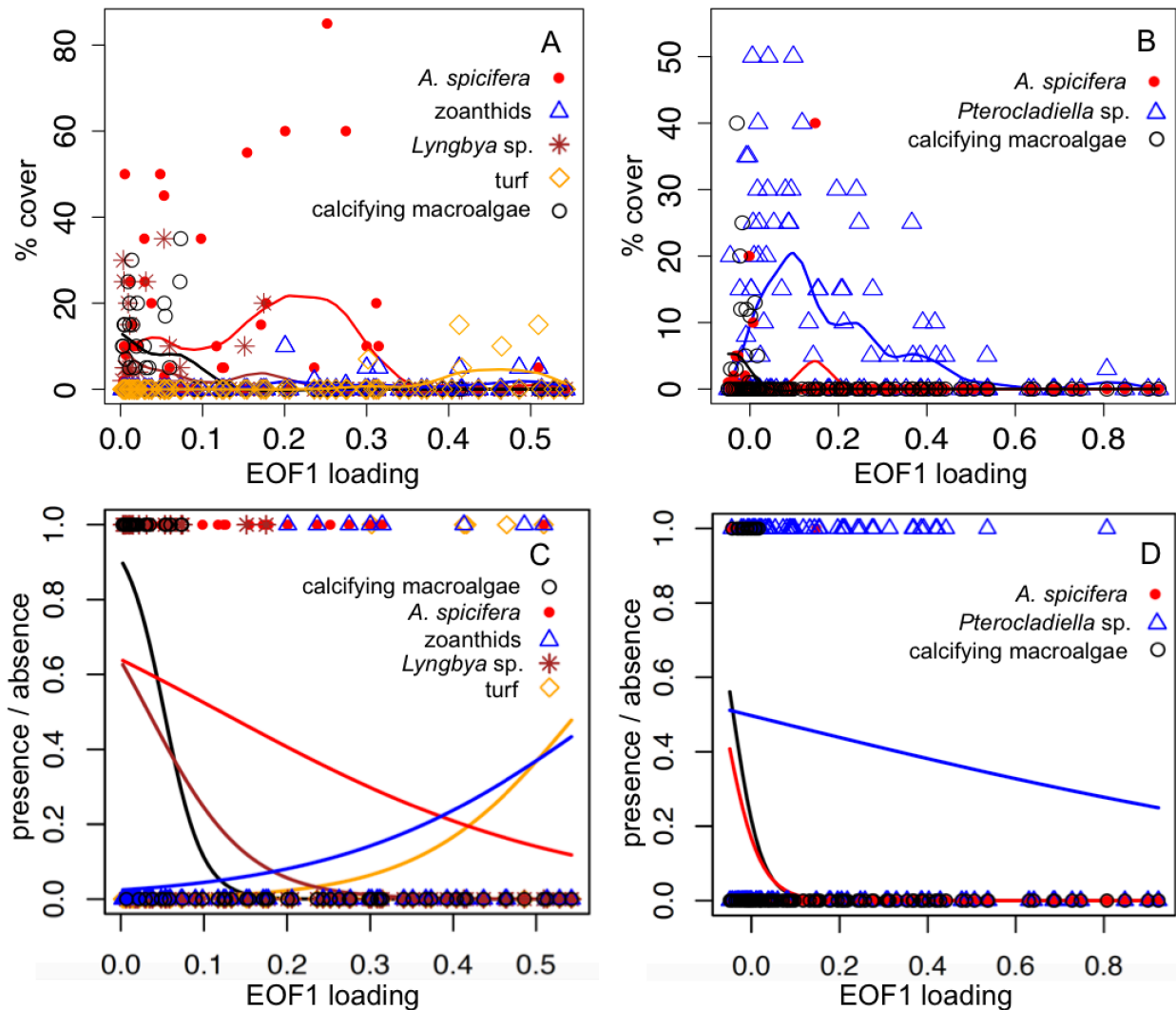


Figure 16. Taxa-specific visualizations of percent cover with EOF1 and logistic regression plots. Only taxa with significant relationships to logistic regression models shown. Percent cover plots for Wailupe (A) and Black Point (B). Lines in A and B are smoothers color-coded by taxa. Logistic regression plots for Wailupe (C) and Black Point (D).

DISCUSSION

Characterizing the spatial and temporal variation in SGD using EOFs

In this study we explored EOFs as a dimension-reducing tool used to account for the spatial and temporal complexity of the delivery of SGD at Black Point and Wailupe. We used EOFs to characterize the spatial and temporal variability in SGD with the first two EOFs explaining more than 50% of the total variability at the two sites. EOF1 shows an SGD gradient mainly driven by

one tidal cycle, low tide to next low tide, in a mixed semi-diurnal tidal regime (Fig. 4A–B, E–F). EOF2 shows a 24-hour cycle (full semidiurnal tidal cycle including 2 low tides and 2 high tides; Fig. 4C–D, G–H). Because the southern coast of O‘ahu has a mixed semidiurnal tidal cycle, the flux of groundwater into coastal zones is highly variable at relatively small time scales. For example, a transition from low to high tide takes approximately 6 hours. In this study, we explored EOFs as a dimension-reducing tool used to account for the spatial and temporal complexity of the delivery of SGD at Black Point and Wailupe. The salinity time series were the input for the EOFs; this statistical technique provided an effective way of summarizing the variability of SGD in space and time. EOF1 described the main source of variance and validated past multi-tracer data showing high variability close to the SGD source (eg. inorganic nutrients, fDOM, radon, etc.) (Holleman 2011, Nelson et al. 2015, Richardson et al. 2017). EOF2 showed spatial patterns of variability we had not captured in past studies and was related to multivariate community structure (DistLM) and univariate species distribution (logistic regressions).

Mapping SGD across space is not a simple endeavor. Reliable tracers for SGD include radon and radium (Burnett et al. 2003), but the equipment to analyze water samples for these elemental tracers is costly. When salinity is an appropriate tracer using autonomous salinity sensors is a relatively easy way to trace SGD over larger areas. Synchronous collection of water samples across space requires several people out in the field at the same time. Salinity sensors can record longer time series and subsequently can capture variability and periodicity with high sampling frequencies. Here we show that we can map multiple aspects of SGD distribution across a reef using a relatively low cost salinity array via EOF modeling.

SGD and benthic community structure

Multivariate community structure was significantly related to EOF1 and EOF2 at Black Point and

EOF2 at Wailupe. Generally, we found higher richness (3-7 taxa) away from the SGD. Close to the SGD we only recorded 1-2 species present. Past research identified similar patterns in seagrass communities (Kantún-Manzano et al. 2018). It is becoming increasingly clear that SGD can shape the local species composition, diversity, and richness of biological communities – the direction of these changes may have to do with turnover time, species initial presence or absence, and mobility (for invertebrates).

Calcifying macroalgae and A. spicifera grow away from the SGD source

At both sites, calcifying macroalgae was not found close to the groundwater seep, possibly due to the groundwater creating an inhospitable environment for the calcification of macroalgae (Fig. 17). Groundwater enters the bay at pH values close to 7.3, which does not allow for the dissociation of bicarbonate ions into carbonate ions. Both bicarbonate and carbonate ions are the building blocks for macroalgal calcification and therefore, without them, macroalgae are not able to calcify. For other reasons (e.g. low salinity, substrate type), calcifying algae may not be able to colonize areas close to the groundwater seep. Future work could focus on the groundwater-based “calcification boundaries” of different calcifying organisms such as crustose coralline algae, branching calcifying algae, and coral. This would involve taking samples to be analyzed for carbonate chemistry at a fine resolution grid in a reef flat with groundwater, mapping the calcifying organisms at a fine scale, and then trying to resolve the parameters where the functional groups occur.

A. spicifera was found at higher abundances distant from the SGD source; this macroalga is the most ubiquitous invader in Hawai‘i’s nearshore and intertidal environments (Russell 1992). It is interesting that *A. spicifera* is only found away from the seep since *A. spicifera* has increased photosynthetic capacity in nutrient enriched experiments (Dailer et al., 2012), although past research has also shown that *A. spicifera*’s distribution is limited by temperature (25–27°C) and

salinity (19–36) (Russell 1992, Pereira et al. 2017). This highlights the multiple chemical and physical characteristics of SGD (ie. low temperature, low salinity, high nutrients) and the differential effects they may have on macroalgal species' distribution.

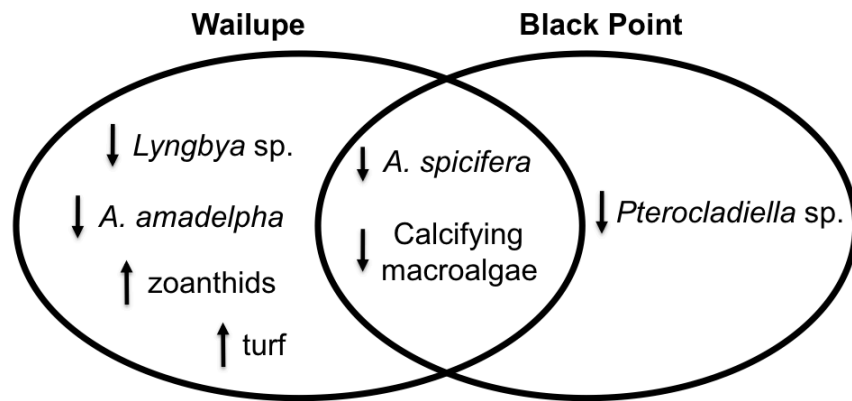


Figure 17. Visualizations of taxa-specific significant relationships with EOF1 (a measure of SGD). Arrows refer to the direction of the relationship. The overlap of the two ellipses shows taxa with the same significant relationships at both sites.

Other taxa that were found away from SGD

At Wailupe we found *Lyngbya* sp. and *A. amadelpha* were most abundant distant from the SGD source (Fig. 17). *Lyngbya* sp. is a cyanobacteria that forms coarse filamentous tufts with toxins, lyngbyatoxin-a and debromoaplysiatoxin, that can cause swimmer's itch on contact (Capper et al. 2005, Huisman et al. 2007). This finding contradicts past work, which has found increased cyanobacterial abundance close to SGD sources (Blanco et al. 2011). It is worth mentioning that this site has high *Lyngbya* sp. cover relative to nearby reef flats. This may mean that although *Lyngbya* sp. does not grow adjacent to the SGD, the SGD is still able to support the cyanobacteria's growth at a certain distance away from the SGD source. Needless to say, its abundance could also be driven by many other physical and biological factors.

A. amadelpha is one of the main invasive macroalgae in Hawai'i (Smith et al. 2002; Cox et

al. 2013, 2017) and a known ecosystem engineer (Littler et al. 2004). *A. amadelpha* is present in high abundance in Paiko Lagoon, on the eastern end of Maunalua Bay. The cover of *A. amadelpha* at Wailupe is much lower than at Paiko Lagoon currently and throughout the past 15 years (McGowan 2004), which could be in part controlled by competition with other invasive species, such as *G. salicornia*.

At Black Point, *Pterocliadiella* sp. was also mainly found away from SGD (Fig. 17). *Pterocliadiella* sp. used to be uncommon in Hawai‘i (Cox et al., 2013), which is why it is surprising it was found in such high abundance at this site. Like, *Lyngbya* sp., it may be that *Pterocliadiella* sp.’s abundance could be supported by SGD in areas where SGD and coastal waters have mixed to create a brackish area. More studies need to be done on this alga and its physiological tolerance for freshwater, high nutrient levels, and lower temperatures.

Taxa found close to the SGD

Zoanths and turf were abundant close to the SGD. Phase shifts from coral to zoanths have been documented in Brazil (Cruz et al. 2015), Palmyra Atoll (Work et al. 2008), as well as Hawai‘i (Walsh & Bowers 1971, Smith et al. 1981, Hunter & Evans 1995, Amato et al. 2016). Amato and colleagues (2016) found that coastal areas in Maui with SGD contaminated by wastewater was barren of corals and almost entirely dominated by colonial zoanths. This study supports this positive relationship between zoanths and SGD, although this relationship is only true in localized areas close to the SGD and shoreline. In the same study, Amato and colleagues (2016) found an inverse relationship between fleshy macroalgae and turf. It may be possible that the SGD supports turf indirectly by creating environments where only a few macroalgae, mainly *G. salicornia* at Wailupe, are able to grow and live.

Future work, limitations and implications

At these two sites, we know SGD is the main source of brackish water. Autonomous salinity sensors are a cost-effective way to gather high-resolution spatial and temporal information across areas with groundwater discharge. This method has the potential to be useful in several types of near-shore or shallow environments and can easily be implemented along with biological surveys to look at spatial relationships between SGD and both individual taxa as well as multivariate community structure. Future work could focus on the groundwater-based “calcification boundaries” of different calcifying organisms such as crustose coralline algae, branching calcifying algae, and coral. This would involve taking samples to be analyzed for carbonate chemistry at a fine resolution grid in a reef flat with groundwater, mapping the calcifying organisms at a fine scale, and then trying to resolve the parameters where the functional groups occur.

The effects of SGD will vary with the chemical composition of SGD and the biological communities present in the areas where SGD enters. The solutes associated with SGD vary with many factors including soil characteristics, flow path and length, land use (eg. agriculture, development, etc.), on-site sewage disposal system density, impervious surface area, recharge and discharge rates, residence time, and more. Hence we cannot generalize for all areas with SGD. Although it may be possible to generalize patterns for functional groups in areas with similar SGD and coastal water characteristics as these sites. It is imperative for coral reef health and management that the effects of SGD on macroalgal communities at these sites are known, particularly given that the macroalgal blooms are often composed of opportunistic invasive species, which can cover and outcompete coral.

CONCLUSIONS AND FUTURE DIRECTIONS

Characteristics of SGD's temporal and spatial delivery

SGD is tidally driven and due to the mixed semidiurnal tidal cycle experienced by Hawaiian coastlines the flux of groundwater into coastal zones is highly variable at relatively small time scales (eg. 6, 12, and 24 hours). This rapid variation in water chemistry in areas with SGD input can create estuarine-like conditions at the localized scale (ie. meters to tens of meters), with increased fluxes of cold, low salinity, and high nutrient SGD at low tide and more typical coastal conditions at high tide. The range in the variability of temperature, salinity, and nutrient concentrations decreases with distance in the direction of flow on the reefs. EOFs provided a simple and effective way of summarizing the variability of SGD in space and time. The first two EOFs characterized over 50% of the total spatiotemporal variability at the two sites.

Hydrological changes in Maunalua Bay and across certain watersheds across the main Hawaiian islands point to possible decreases in the flux of SGD. The decreased freshwater flux along with the change in the solute types and concentrations in SGD can have an impact on the coastal biological communities, such as decreases in freshwater-loving algal species leading to a decrease in biodiversity. Unfortunately baselines of groundwater fluxes and associated chemical compositions prior to the residential development and even prior to agricultural land use are not available. Historical Hawaiian newspapers contain qualitative narratives of the freshwater fluxes at culturally important shorelines (eg. fishponds). These written records may be the closest version of baselines for Hawaiian SGD fluxes. Several researchers and programs at the University of Hawai'i are translating these resources from Hawaiian to English and looking for Hawaiian terms that could be associated with freshwater. This is one key to understanding what SGD fluxes used to be like.

SGD, benthic community composition, and phase shifts

Our findings support the argument that few benthic species, invasive and opportunistic species in this case, can grow and persist in an area with SGD and that most macroalgae (especially calcifying macroalgae) are either outcompeted or unable to withstand these environments. SGD was positively related to the biomass and growth of *G. salicornia* and *B. pennata*, indicating that if found or transported to this kind of environment, these species are likely to be successful invaders. At Wailupe, we found high total biomass but low benthic diversity in areas with high SGD input due to the very few species that can grow and live in areas experiencing the high variability in water quality associated with SGD.

SGD with high concentrations of inorganic nutrients and low salinity can be hotspots for *Gracilaria* spp. growth. Generally, SGD high in inorganic nutrients, discharging in areas with low herbivory and lack of competition from coral and other benthic species can be susceptible to invasive species proliferation. It is becoming increasingly clear that SGD can shape the local species composition, diversity, and richness of biological communities – the direction of these changes may have to do with species-specific turnover time, species initial presence or absence, and mobility (for invertebrates). Phase shifts from coral to zoanthids have been documented in Brazil (Cruz et al. 2015) as well as Hawai'i (Walsh & Bowers 1971, Smith et al 1981, Hunter & Evans 1995, Amato et al. 2016). Amato and colleagues (2016) found that coastal areas in Maui with SGD contaminated by wastewater was barren of corals and almost entirely dominated by colonial zoanthids. This study supports this positive relationship between zoanthids and SGD, although this relationship is only true in localized areas close to the SGD and shoreline.

SGD and productivity on algal-dominated reefs

A common interest between management and the SGD research community is the fate of the dissolved inorganic nutrients brought to coastal systems via SGD. Chapter 2 showed that *G. salicornia* and phytoplankton both had significant hump-shaped relationships between productivity and SGD. Peak C-uptake of both phytoplankton and *G. salicornia* occurred at salinities of ~ 21.5 ($25.5 \mu\text{mol NO}_3^- \text{L}^{-1}$ and $0.54 \mu\text{mol PO}_4^{3-} \text{L}^{-1}$), suggesting a physiological trade-off between salinity tolerance and nutrient availability for reef primary producers. The water column and benthic contributions to C-uptake on a reef with SGD are approximately 36% and 82% higher than a reef without SGD, respectively. This suggests that SGD has a greater impact on benthic macroalgal productivity in coastal systems. Consistent with previous surveys of spatial distributions of factors associated with SGD such as nutrient concentrations, salinity, and dissolved organic matter (Nelson et al. 2015), we showed that groundwater can have an effect on water column and benthic productivity up to 200 meters offshore; in order to create more accurate productivity maps and estimates, we need to keep in mind SGD's spatial extent.

Wailupe's benthic community is composed of a variety of macroalgae, zoanthids, and sparse coral. Even considering these species, whose cover is less than 5% individually, *G. salicornia* is most likely responsible for the largest C-uptake on this reef. Our *G. salicornia* uptake values ($2.72 \text{ mg C m}^{-2} \text{ h}^{-1}$ on a reef with SGD) account for a small fraction of gross reef productivity, which have been reported to range from $0.33\text{--}30 \text{ g C m}^{-2} \text{ h}^{-1}$ (Sorokin 1995, Gattuso et al. 1996, Andrefouet and Payri 2001). The uneven benthic community at this site could explain the lack of productivity at this site. The high abundance of this species could, in turn, lead to a less productive coastal system, lacking the variety in functional groups, which could boost this system's carbon uptake (Steneck and Dethier 1994, Nyström 2006).

Future directions

Both from observational studies and physiological experiments, we know that macroalgal relationships between tolerance and resistance to stress are variable and species-specific (Hay et al. 2011). It is critical to evaluate the effects of stressors on community dynamics not only independently but also under different combinations to understand how those effects will play out in more realistic scenarios (Muthukrishnan & Fong 2014). The multiple stressor literature is helpful in trying to distill these complex interactions. Coastal systems are especially complex with upwards of 100 two-way interactions (Côté et al. 2016). In Côté and colleagues' paper about ecosystem stressor interactions, they lay out a guide for identifying generalities about ecosystems, stressors and/or responses that could provide direction for conservation scientists and managers. Along with physiological studies about stress mechanisms, studies such as the ones presented in this dissertation can accumulate data to ground truth these relationships and interactions *in situ*. If we can understand what factors affect biomass and persistence of certain algal assemblages, especially those comprised of invasive and opportunistic algae, we can prioritize restoration efforts such as identifying areas for native algal replanting and algal removals. Additionally, understanding how communities of primary producers are functioning and how resilient they may be to predicted climate change scenarios (ie. sea level rise, increasing temperatures, ocean acidification, etc.) will help with preparedness for coastal management.

Further research on the benthic communities associated with SGD and its biogeochemistry is warranted. Understanding the effects of the solutes carried by SGD on coastal communities will help direct and prioritize management of land use activities that may contribute to groundwater contamination. This work hints at possible “calcification boundaries” of different calcifying organisms such as crustose coralline algae, branching calcifying algae, and coral in areas with SGD. More work is warranted on calcifying species and their relationship with SGD. Moreover,

close attention should be paid to the photosynthetic communities present in coastal areas with SGD as these areas may be hotspots for phase shifts and restoration. These areas should be monitored for changes in community structure and can be targeted by groups engaged in conservation efforts such as local Hawaiian community groups who are currently “replanting” for limu (algae) maunaea (*G. coronopofolia*) as well as other native and endemic species.

APPENDIX

Supplemental Table 1. Herbivory and growth experiment results by site and species. A three way ANOVA was run with zone, treatment, and an interaction between zone and treatment as factors. Effect size (F), degrees of freedom (df), and p value (p) are presented in the table.

Species	Site	Factor: F (df), p
<i>Acanthophora spicifera</i>	Black Point	Zone: 32.16 (3), 0.0291* Treatment: 2.06 (2), 0.134 Zone*Treatment: 22.57 (5), 0.101
	Wailupe	Zone: 41.66 (3), 0.0193* Treatment: 5.20 (2), 0.381 Zone*Treatment: 4.87 (5), 0.346
<i>Avrainvillea amadelpha</i>	Black Point	Zone: 1.45 (3), 0.562 Treatment: 0.433 (2), 0.894 Zone*Treatment: 0.938 (5), 0.722
	Wailupe	Zone: 30.01 (3), 0.0593 Treatment: 4.05 (2), 0.284 Zone*Treatment: 16.73 (5), 0.221
<i>Bryopsis pennata</i>	Black Point	Zone: 82.09 (3), 0.0057* Treatment: 2.19 (2), 0.831 Zone*Treatment: 6.2 (5), 0.601
<i>Gracilaria salicornia</i>	Wailupe	Zone: 95.77 (3), 0.00164* Treatment: 10.95 (2), 0.420 Zone*Treatment: 14.20 (5), 0.291
<i>Halimeda discoidea</i>	Black Point	Zone: 6.72 (3), 0.678 Treatment: 16.12 (2), 0.221 Zone*Treatment: 4.12 (5), 0.599
	Wailupe	Zone: 26.23 (3), 0.0388* Treatment: 3.08 (2), 0.495 Zone*Treatment: 6.10 (5), 0.319

Supplemental Table 2. Important equations and corresponding descriptors

<p>(1) Ratio of $^{13}\text{C}/^{12}\text{C}$ in the sample</p> $^{13}\text{C}/^{12}\text{C}_{\text{sample}} = (\delta^{13}\text{C}_{\text{sample}} / 1000 + 1) * ^{13}\text{C}/^{12}\text{C}_{\text{STD VPBD}}$ <ul style="list-style-type: none"> • $^{13}\text{C}/^{12}\text{C}_{\text{STD VPBD}}$ = known value for VPBD standard = 0.011237 • solved for $^{13}\text{C}/^{12}\text{C}_{\text{sample}}$ from Hayes (2004) equation (6) • $\delta^{13}\text{C}_{\text{sample}}$ value given by lab and refers to common delta notation
<p>(2) Atomic percent of ^{13}C in the sample</p> $\text{atomic \% } ^{13}\text{C} = (^{13}\text{C}/^{12}\text{C}_{\text{sample}} / (^{13}\text{C}/^{12}\text{C}_{\text{sample}} + 1)) * 100$ <ul style="list-style-type: none"> • Equivalent to Hayes (2004) equation (1): Atomic % $^{13}\text{C} = [^{13}\text{C}_{\text{sample}} / (^{13}\text{C}_{\text{sample}} + ^{12}\text{C}_{\text{sample}})] * 100$ • Atomic percent is the common way to report absolute abundance of isotopes (Hayes, 2004).
<p>(3) C-uptake rate of macroalgae per grams dry weight</p> $\mathbf{P \text{ (mg C g dw}^{-1} \text{ h}^{-1})} = \frac{\mathbf{C \cdot (a_{is} - a_{ns})}}{\mathbf{t \cdot (a_{ic} - a_{ns}) \cdot dw}}$ <ul style="list-style-type: none"> • a_{ns} is the atomic % ^{13}C in the ambient sample's tissue • a_{is} is the atomic % ^{13}C of the enriched sample tissue taken after a 1 hour incubation • a_{ic} is the atomic % ^{13}C in the dissolved inorganic carbon in the incubation medium after enrichment • C (mg) is the carbon content of the sample • dw refers to dry weight (g) of the sample, and t (h) is time.
<p>(4) C-uptake rate of phytoplankton per L filtered</p> $\mathbf{P \text{ (ug C L}^{-1} \text{ h}^{-1})} = \frac{\mathbf{\text{POC} \cdot (a_{is} - a_{ns})}}{\mathbf{t \cdot (a_{ic} - a_{ns})}}$ <ul style="list-style-type: none"> • See Equation 3 specifications. • POC refers to the particulate organic carbon (ug C L⁻¹) in the incubated sample. • No correction factor for isotopic discrimination against ^{13}C has been applied to the calculations.

Supplemental Table 3. Linear relationships for nutrients and salinity at Black Point and Wailupe sites. These relationships were found using linear regressions obtained from salinity vs. nutrients plots. N+N = NO₃⁻ + NO₂⁻, total dissolved P (TDP)

Nutrient concentrations (μmol L ⁻¹)	Linear Relationship with salinity	R ²	p-value
N+N	-2.1*salinity + 70.5	0.990	<0.001
TDP	-0.1*salinity + 2.1	0.964	<0.001
SiO ₄ ²⁻	-23.7*salinity + 817.6	0.998	<0.001

Supplemental Table 4. Model output and R syntax for DistLM, Pearson's correlations, and linear mixed effects models for Wailupe data. † refers for the same model syntax using EOF2 as a predictor. * refers to a *p* value < 0.05.

Model and R syntax	Model output	EOF1	EOF2
Distance-based linear model adonis(distance matrix of community data at Wailupe ~ EOF1 + EOF2 + (1 substrate), distance = "bray", data = benthic community Wailupe)	<i>F stat</i>	2.253	4.826
	<i>R2</i>	0.0256	0.0556
	<i>p</i>	0.0790	0.0039*
Pearson's correlation: NMDS1 †cor.test(Wailupe NMDS1, Wailupe EOF1, use = "complete.obs")	<i>Corr coef</i>	0.185	-0.0141
	<i>p</i>	0.117	0.9048
Pearson's correlation: NMDS2 †cor.test(Wailupe NMDS2, Wailupe EOF1, use = "complete.obs")	<i>Corr coef</i>	-0.294	0.202
	<i>p</i>	0.0119*	0.08763
Logistic Regression: <i>H. discoidea</i> †glm(<i>H. discoidea</i> presence/absence ~ EOF1, family = "binomial", data = benthic community data at Wailupe)	<i>β</i>	-35.2609	10.3336
	<i>SE β</i>	11.6838	3.5579
	<i>p</i>	0.00255*	0.003679*
Logistic Regression: calcifying macroalgae †glm(calcifying macroalgae presence/absence ~ EOF1, family = "binomial", data = benthic community data at Wailupe)	<i>β</i>	-43.4759	12.4112
	<i>SE β</i>	13.8788	3.8624
	<i>p</i>	0.00173*	0.001312*
Logistic Regression: <i>A. spicifera</i> †glm(<i>A. spicifera</i> presence/absence ~ EOF1, family = "binomial", data = benthic community data at Wailupe)	<i>β</i>	-4.7703	5.2874
	<i>SE β</i>	1.6884	2.1544
	<i>p</i>	0.00472*	0.0139*
Logistic Regression: <i>Lyngbya</i> sp. †glm(<i>Lyngbya</i> sp. presence/absence ~ EOF1, family = "binomial", data = benthic community data at Wailupe)	<i>β</i>	-16.8398	8.5354
	<i>SE β</i>	5.5368	3.3307
	<i>p</i>	0.00235*	0.010387*
Logistic Regression: <i>A. amadelpha</i> †glm(<i>A. amadelpha</i> presence/absence ~ EOF1, family = "binomial", data = benthic community data at Wailupe)	<i>β</i>	-13.2609	15.544
	<i>SE β</i>	11.6838	7.067
	<i>p</i>	0.00255*	0.02785*

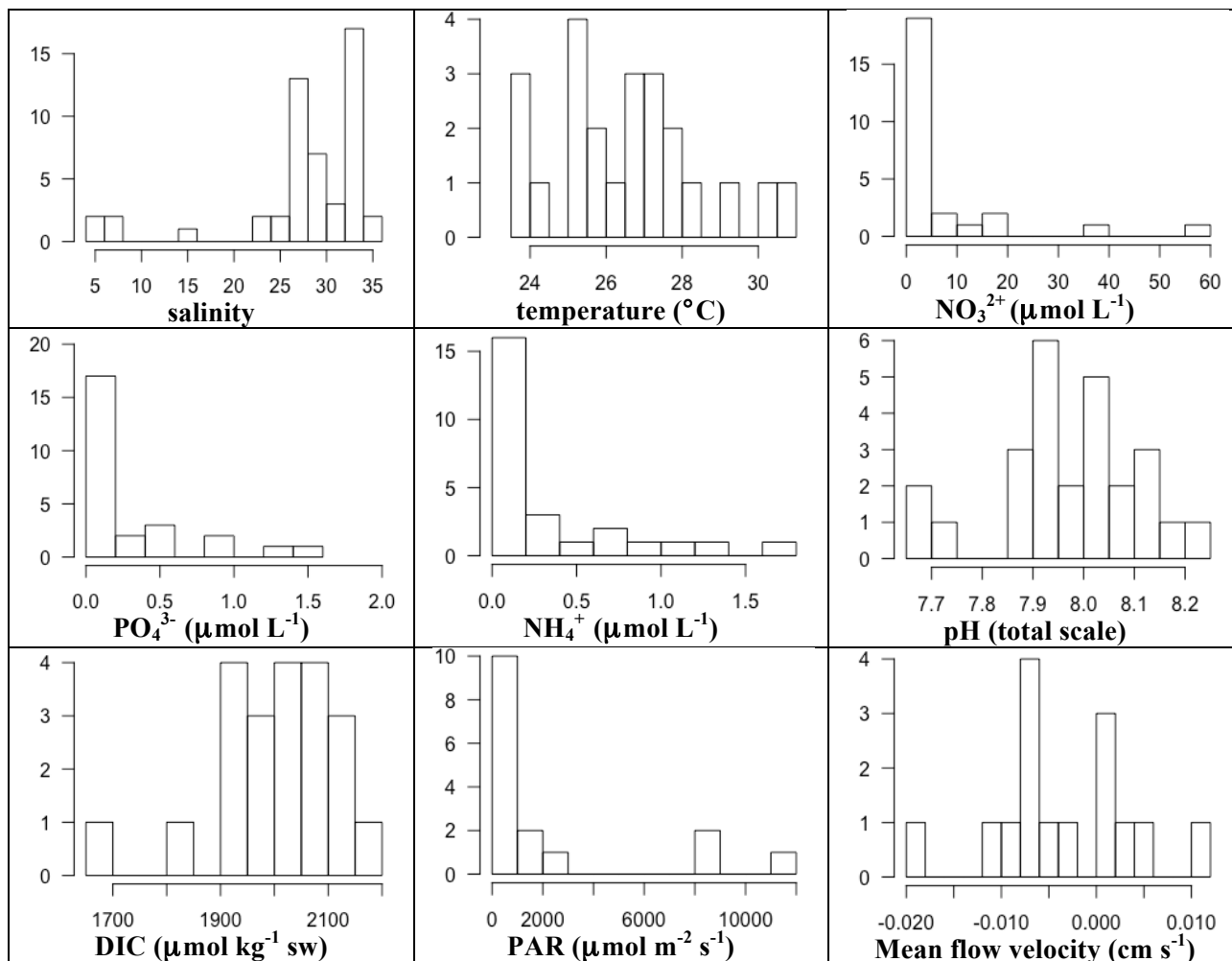
Logistic Regression: turf †glm(turf presence/absence ~ EOF1, family = "binomial", data = benthic community data at Wailupe)	β	10.696	-11.1547
	$SE \beta$	4.262	4.1958
	p	0.012089*	0.007848*
Logistic Regression: zoanthids †glm(zoanthids presence/absence ~ EOF1, family = "binomial", data = benthic community data at Wailupe)	β	6.319	-6.3656
	$SE \beta$	2.485	2.7623
	p	0.011*	0.021196*
Logistic Regression: <i>G. salicornia</i> †glm(<i>G. salicornia</i> presence/absence ~ EOF1, family = "binomial", data = benthic community data at Wailupe)	β	-1.3115	1.6345
	$SE \beta$	1.4797	2.0035
	p	0.375	0.1135
Logistic Regression: <i>Hypnea</i> sp. †glm(<i>Hypnea</i> sp. presence/absence ~ EOF1, family = "binomial", data = benthic community data at Wailupe)	β	-0.3943	1.6345
	$SE \beta$	2.2973	3.1145
	p	0.863717	0.599731
Logistic Regression: <i>Dictyota</i> sp. †glm(<i>Dictyota</i> sp. presence/absence ~ EOF1, family = "binomial", data = benthic community data at Wailupe)	β	-24.4855	5.067
	$SE \beta$	16.9015	4.854
	p	0.147	0.2965
Logistic Regression: <i>Spyridia</i> sp. †glm(<i>Spyridia</i> sp. presence/absence ~ EOF1, family = "binomial", data = benthic community data at Wailupe)	β	-1.8178	5.477
	$SE \beta$	3.9625	6.413
	p	0.646403	0.39308
Logistic Regression: CCA †glm(CCA presence/absence ~ EOF1, family = "binomial", data = benthic community data at Wailupe)	β	-24.5739	3.776
	$SE \beta$	19.0416	4.952
	p	0.1969	0.44582

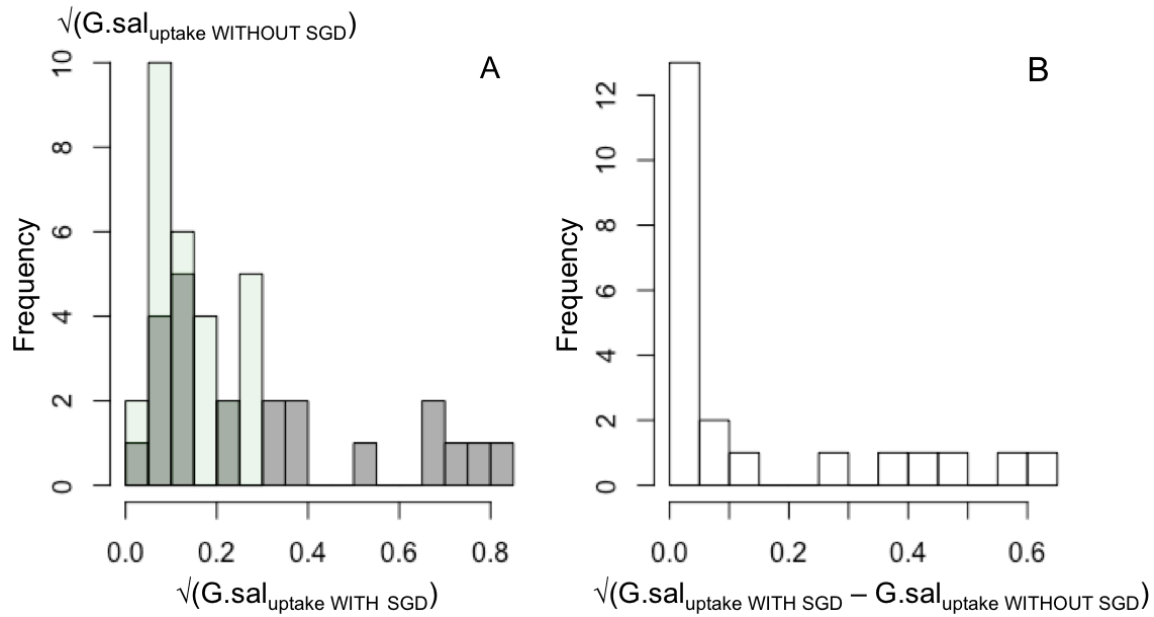
Supplemental Table 5. Model output and R syntax for DistLM, Pearson's correlations, and linear mixed effects models for Black Point data. † refers for the same model syntax for EOF2. * refers to a p value < 0.05.

Model and R syntax	Model output	EOF1	EOF2
Distance based linear model adonis(distance matrix of community data at Black Point ~ EOF1 + EOF2 + (1 substrate), distance = "bray", data=benthic community Black Point)	F stat	3.0959	5.1525
	R^2	0.02518	0.04190
	p	0.0113 *	0.0001*
Pearson's correlation: NMDS1 †cor.test(Black Point NMDS1, Black Point EOF1, use="complete.obs")	$Corr$ coef	0.1496	-0.1863
	p	0.1104	0.0462*
Pearson's correlation: NMDS2 †cor.test(Black Point NMDS2, Black Point EOF1, use="complete.obs")	$Corr$ coef	0.17240	-0.34461
	p	0.06541	0.000162*
Logistic Regression: <i>H. discoidea</i> †glm(<i>H. discoidea</i> presence/absence ~ EOF1, family = "binomial", data = benthic community data at Black Point)	β	-25.405	1.9925
	$SE \beta$	11.277	4.6135
	p	0.0243*	0.665782

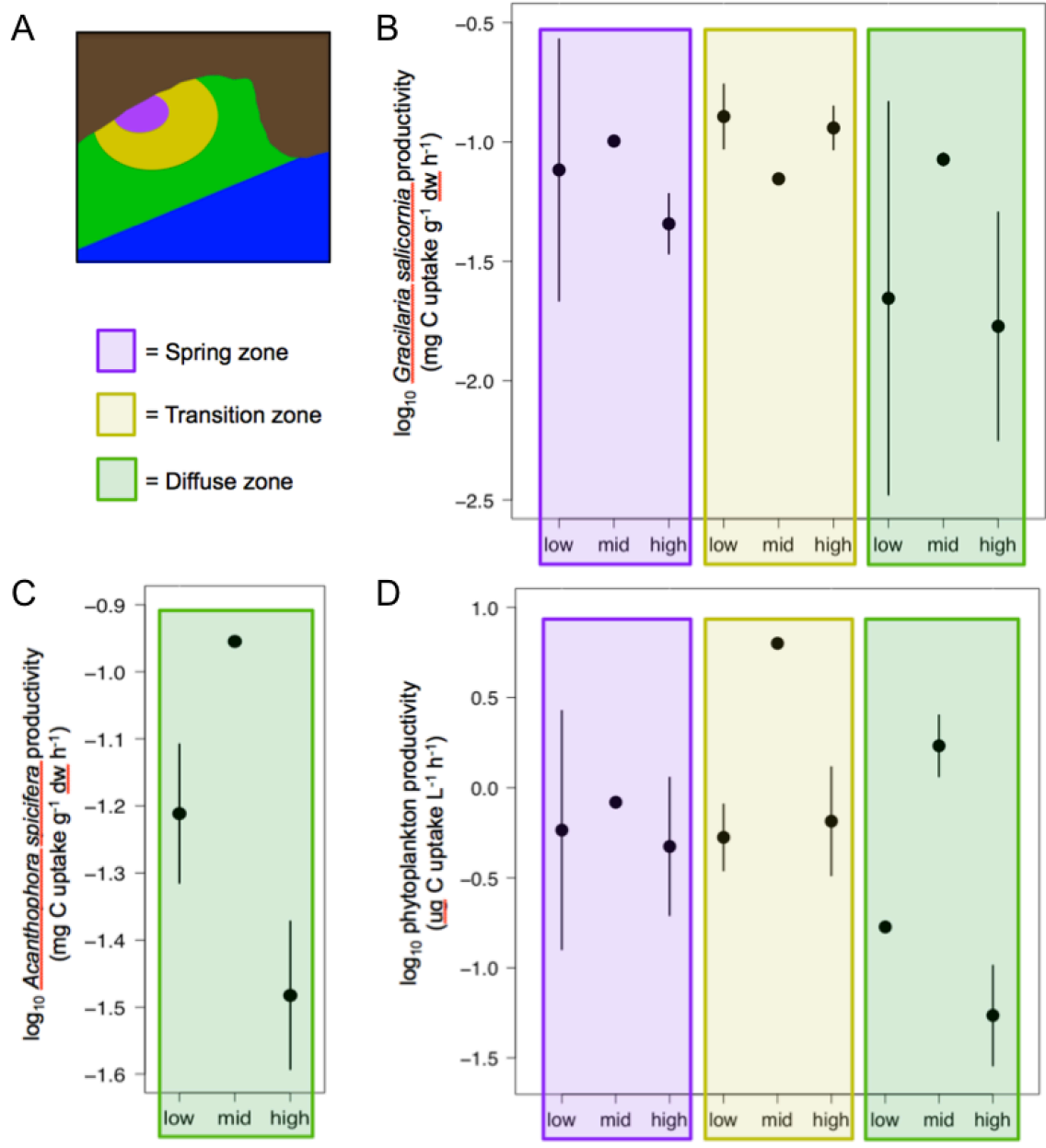
Logistic Regression: calcifying macroalgae †glm(calcifying macroalgae presence/absence ~ EOF1, family = "binomial", data = benthic community data at Black Point)	β	-31.301	2.0380
	$SE \beta$	0.3431	4.2827
	p	0.010341*	0.63417
Logistic Regression: <i>A. spicifera</i> †glm(<i>A. spicifera</i> presence/absence ~ EOF1, family = "binomial", data = benthic community data at Black Point)	β	-25.1905	2.948
	$SE \beta$	11.7020	4.958
	p	0.0313*	0.55208
Logistic Regression: <i>Pterocladia</i> sp. †glm(<i>Pterocladia</i> sp. presence/absence ~ EOF1, family = "binomial", data = benthic community data at Black Point)	β	-1.17977	11.2897
	$SE \beta$	0.82506	3.3746
	p	0.153	0.000821*
Logistic Regression: <i>A. amadelpa</i> †glm(<i>A. amadelpa</i> presence/absence ~ EOF1, family = "binomial", data = benthic community data at Black Point)	β	-17.9706	0.2154
	$SE \beta$	16.1121	8.1066
	p	0.265	0.788
Logistic Regression: turf †glm(turf presence/absence ~ EOF1, family = "binomial", data = benthic community data at Black Point)	β	1.3627	-3.9965
	$SE \beta$	0.817	2.7492
	p	0.0953	0.146
Logistic Regression: anthozoa †glm(anthozoa presence/absence ~ EOF1, family = "binomial", data = benthic community data at Black Point)	β	-2.5205	8.19
	$SE \beta$	3.1489	8.924
	p	0.423	0.3587
Logistic Regression: <i>B. pennata</i> †glm(<i>B. pennata</i> presence/absence ~ EOF1, family = "binomial", data = benthic community data at Black Point)	β	-0.3293	4.8365
	$SE \beta$	1.2748	4.6490
	p	0.796	0.29819
Logistic Regression: <i>Liagora</i> sp. †glm(<i>Liagora</i> sp. presence/absence ~ EOF1, family = "binomial", data = benthic community data at Black Point)	β	-35.716	0.6396
	$SE \beta$	19.981	6.4164
	p	0.0739	0.92060
Logistic Regression: <i>Dictyota</i> sp. †glm(<i>Dictyota</i> sp. presence/absence ~ EOF1, family = "binomial", data = benthic community data at Wailupe)	β	-39.866	1.516
	$SE \beta$	26.771	8.443
	p	0.136	0.8375

Supplemental Figure 1. Histograms of chemical and physical parameters measured for benthic chamber experiments.

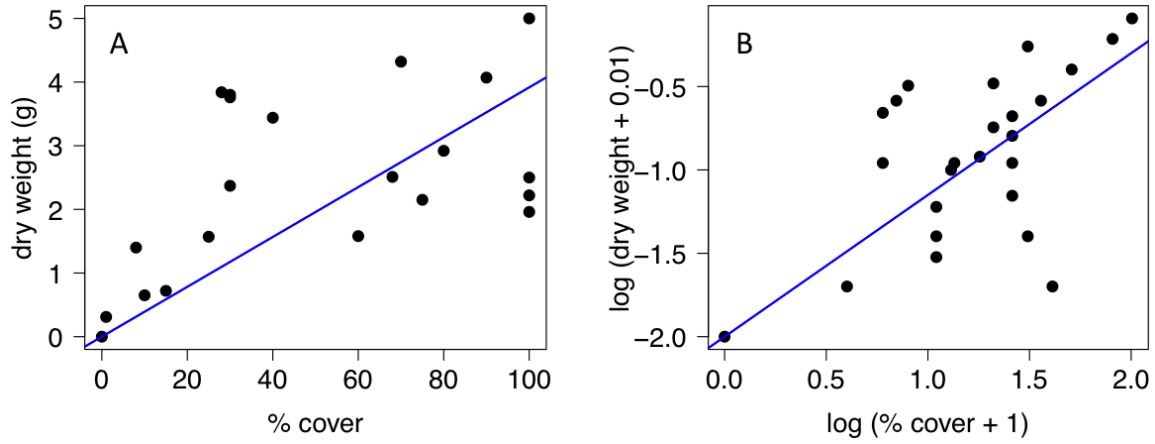




Supplemental Figure 2. (A) Histograms of square root transformed C-uptake by *G. salicornia* on a reef without SGD (light grey bars) and with SGD (mustard bars, overlap between the two histograms is indicated by the dark grey). (B) Histogram of the square root transformed differences between *G. salicornia* C-uptake on reef with SGD and without SGD. $\mu = 0.304 \sqrt{(\text{mg C h}^{-1})} = 0.0923 \text{ mg C h}^{-1}$



Supplemental Figure 3. C-uptake rates for primary producers partitioned by reef zone and tidal cycle. (A) Conceptual maps of biogeochemical zones (modified from Nelson et al. 2015). Legend shows the colors corresponding to biogeochemical zones. C-uptake rates across tide and zone for (B) *G. salicornia*, (C) *A. spicifera* and, (D) phytoplankton.



Supplemental Figure 4. Dry weight vs. % cover regressions. Regression stats for *G. salicornia* (dry weight = $0.0392 \cdot \% \text{ cover}$, $p < 0.001$, $F = 58.4$, $r^2 = 0.732$) and for *A. spicifera* (dry weight = $0.00665 \cdot \% \text{ cover}$, $p < 0.001$, $F = 32.9$, $r^2 = 0.571$).

REFERENCES

- Ahmad ZU, Sakib S, Gang DD (2016) Nonpoint source pollution. *Water Environ Res* 88(10):1594–1619
- Airoidi L (1998) Roles of disturbance, sediment stress, and substratum retention on spatial dominance in algal turf. *ecology* 79(8):2759–2770
- Akima H, Gebhardt A (2015). akima: interpolation of irregularly and regularly spaced data. R package version 0.5–12. <https://CRAN.R-project.org/package=akima>
- Allredge A, Carlson C (2016) MCR LTER: coral reef: water column: nearshore water profiles, CTD, primary production, and chemistry ongoing since 2005. knb-lter-mcr.10.34 doi:10.6073/pasta/ba09a6a0b0bd8741f68a0007ac2cafc4
- Amato, D (2015) Ecophysiological responses of macroalgae to submarine groundwater discharge in Hawai‘i. Ph.D. thesis. University of Hawai‘i at Mānoa
- Amato DW, Bishop JM, Glenn CR, Dulai H, Smith CM (2016) Impact of Submarine Groundwater Discharge on Marine Water Quality and Reef Biota of Maui. *PLOS One* 3 (11):e0165825. doi: 10.1371/journal.pone.0165825. eCollection
- Andrefouet S, Payri C (2001) Scaling-up carbon and carbonate metabolism of coral reefs using *in-situ* data and remote sensing. *Coral Reefs* 19 (3): 259–269.
- Arias-Estevez M, Lopez-Periago E, Martinez-Carballo E et al. (2008) The mobility and degradation of pesticides in soils and the pollution of groundwater resources. *Agric Ecosyst & Environ* 123 (4): 247-260.
- Bai Z, Silverstein JW (2018) Spectral Analysis of Large Dimensional Random Matrices. 2nd Ed. Springer Series in Statistics
- Bailey RC (1988) Correlations between species richness and exposure: freshwater mollusks and macrophytes. *Hydrobiologia* 162: 183–91
- Baker AC, Glynn PW, Riegl B (2008) Climate change and coral reef bleaching: An ecological assessment of long-term impacts, recovery trends and future outlook. *Estuar Coast Shelf Sci* 80: 435–471
- Balata D, Piazzini L, Cinelli F (2007) Increase of sedimentation in a subtidal system: effects on the structure and diversity of macroalgal assemblages. *J Exp Mar Biol Ecol* 351(1–2):73–82
- Bell PRF (1992) Eutrophication and coral reefs: some examples in the Great Barrier Reef lagoon. *Water Res* 26:553–568

- Blanco AC, Watanabe A, Nadaoka K, et al. (2011) Estimation of nearshore groundwater discharge and its potential effects on a fringing coral reef. *Mar Poll Bull* 52(4): 770–785
- Boehm AB, Shellenbarger GG, Paytan A (2004) Groundwater Discharge: A potential association with fecal indicator bacteria in the surf zone. *Environ Sci Technol* 38, 3558-3566
- Boustany RG, Michot TC, Moss RF (2015) Effect of nutrients and salinity pulses on biomass and growth of *Vallisneria americana* in lower St Johns River, FL, USA. *Royal Soc Open Sci* 2 (2):140053 <http://doi.org/10.1098/rsos.140053>
- Burnett WC, Dulaiova H, Stringer C (2006) Submarine groundwater discharge: its measurement and influence on the coastal zone. *Journal of Coastal Research. Proceedings of the 8th International Coastal Symposium (ICS 2004), Vol. I (Winter 2006) Special Issue* 39:35–38
- Burnett WC, Bokuniewicz H, Huettel M, Moore WS, Taniguchi M (2003) Groundwater and pore water inputs to the coastal zone. *Biogeochemistry* 66(1/2):3–33
- Capper A, Tibbetts IR, O’Neil JM, Shaw GR (2005) The fate of *Lyngbya majuscula* toxins in three potential consumers. *J. Chem. Ecol.* 31(7):1595–606
- Carrington E (1990) Drag and dislodgment of an intertidal macroalga: consequences of morphological variation in *Mastocarpus papillatus*. *J Exp Mar Biol Ecol* 139:185–200
- Cefali ME, Cebrian E, Chappuis E, Pinedo S, Terradas M, Mariani S, Ballesteros E (2016) Life on the boundary: Environmental factors as drivers of habitat distribution in the littoral zone. *Estuar Coast Shelf Sci* 172, 81–92
- Cho HM, Kim G, Kwon EY, Moosdorf N, Garcia-Orellana J, Santos IR (2018) Radium tracing nutrient inputs through submarine groundwater discharge in the global ocean. *Sci Rep* 8: 2439
- Choi HG, Kim YS, Kim JH, Lee SJ, Park EJ, Ryu J, Nam KW (2006) Effects of temperature and salinity on the growth of *Gracilaria verrucosa* and *G. chorda*, with the potential for mariculture in Korea. *J Appl Phycol* 18:269–277
- Cornelisen CD, Thomas FIM (2002) Ammonium Uptake by Seagrass Epiphytes: Isolation of the Effects of Water Velocity Using an Isotope Source. *Limnol. Oceanogr.* 47 (4):1223–1229
- Costa JOS, Leao ZMAN, Nimmo M, Attrill MJ (2000) Nutrifcation impacts on coral reefs from northern Bahia, Brazil. *Hydrobiologia* 44:307–315
- Costa JOS, Nimmo M, Attrill MJ (2008) Coastal nutrification in Brazil: a review of the role of nutrient excess on coral reef demise. *J S Am Earth Sci* 25:257–270
- Côte´ IM, Darling ES, Brown CJ (2016) Interactions among ecosystem stressors and their importance in conservation. *Proc R Soc B* 283:2015–2592

- Cox TE, Philippoff J, Baumgartner E, Zabin CJ, Smith CM (2013) Spatial and temporal variation in rocky intertidal communities along the main Hawaiian islands. *Pac Sci* 67(1):23–45
- Cox TE, Spalding H, Foster MS (2017) Spatial and temporal variation of diverse intertidal algal assemblages in Southwest O‘ahu. *Marine Ecol* 38:e12429
- Cruz ICS, Loiola M, Albuquerque T, Reis R, de Anchieta CC, Nunes J, Reimer JD, et al. (2015) Effect of phase shift from corals to zoantharia on reef fish assemblages. *PLOS ONE* 10(1): e0116944. doi:10.1371/journal.pone.0116944
- Dailer ML, Knox RS, Smith JS, Napier M, Smith CM (2010) Using $\delta^{15}\text{N}$ values in algal tissue to map locations and potential sources of anthropogenic nutrient inputs on the island of Maui, Hawai‘i, USA. *Mar Pollut Bull* 60:655–671
- Dailer ML, Smith JE, Smith CM (2012) Responses of bloom forming and non-bloom forming macroalgae to nutrient enrichment in Hawai‘i, USA. *Harmful Algae* 17: 111–125
- D’Amours O, Scheibling RE (2007) Effects of wave exposure on morphology, attachment strength and survival of the invasive green alga *Codium fragile* ssp. *tomentosoides*. *J Exp Mar Biol Ecol* 351:129–142
- Denny M, Brown V, Carrington E, Kraemer G, Miller A (1989) Fracture mechanics and the survival of wave-swept macroalgae. *J Exp Mar Biol Ecol* 127:211–28
- Dickson AG (1990) Standard potential of the reaction: $\text{AgCl(s)} + 1/2 \text{H}_2\text{(g)} = \text{Ag(s)} + \text{HCl(aq)}$, and the standard acidity constant of the ion HSO_4^- in synthetic sea water from 273.15 to 318.15 K. *J Chem Thermodyn.* 22:113–127. doi:10.1016/0021-9614(90)90074-z
- Dickson AG, Millero FJ (1987) A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep Sea Res* 34:1733–1743
- Dimova NT, Swarzenski PW, Dulaiova H, Glenn CR (2012) Utilizing multichannel electrical resistivity methods to examine the dynamics of the fresh water-seawater interface in two Hawaiian groundwater systems. *J Geophys Res Oceans* 117, 12
- Dizon RM, Yap HT (2003) Metabolic changes and compositional shifts in nutrient-enriched tropical reef sediment communities. *Sci Mar* 67 (2): 117–127
- Duarte CM. (1995) Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia* 41:87–112
- Duarte TK, Pongkijvorasin S, Roumasset J, et al. (2010) Optimal management of a Hawaiian Coastal aquifer with nearshore marine ecological interactions. *Water Resour Res* 46: W11545
- Elfrink B, Baldock T (2002) Hydrodynamics and sediment transport in the swash zone: a review and perspectives. *Coast Eng* 45(3–4):149–167

- Encarnaç o J, Leit o F, Range P, Pil  D, Ch charo MA, Ch charo L (2015) Local and temporal variations in near-shore macrobenthic communities associated with submarine groundwater discharges. *Mar Ecol-Evol Persp* 36(4): 926–941
- Fabricius KE (2005) Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. *Marine Pollution Bulletin* 50:125–146
- Flores AAV, Christofoletti RA, Peres ALF, Ciotti AM, Navarrete SA (2015) Interactive effects of grazing and environmental stress on macroalgal biomass in subtropical rocky shores: modulation of bottom-up inputs by wave action. *J Exp Mar Biol Ecol* 463:39–48
- Fong P, Smith TB, Wartian MJ (2006) Epiphytic cyanobacteria maintain shifts to macroalgal dominance on coral reefs following ENSO disturbance. *Ecology* 87(5):1162–1168
- Fowler-Walker MJ, Wernberg T, Connell SD (2005) Differences in kelp morphology between wave sheltered and exposed localities: morphologically plastic or fixed traits? *Mar Biol* 148:755–67
- Fung T, Seymour R, Johnson C (2011) Alternative stable states and phase shifts in coral reefs under anthropogenic stress. *Ecology* 92(4): 967–982
- Gattuso JP, Pichon MR, Delesalle B, Canon C, CopinMontegut G, Pichon M, Frankignoulle M. (1996) Carbon fluxes in coral reefs. I. Lagrangian measurements of community metabolism and resulting air–sea CO₂ disequilibrium. *Mar Ecol Prog Ser* 145:109–121
- Glenn EP, Moore D, Akutagawa M, Himler A, Walsh T, Nelson SG. (1999) Correlation between *Gracilaria parvispora* (Rhodophyta) biomass production and water quality factors on a tropical reef in Hawaii. *Aquaculture* 178:323–331
- Gobler CJ, Boneillo GE (2003) Impacts of anthropogenically influenced groundwater seepage on water chemistry and phytoplankton dynamics within a coastal marine system. *Mar Ecol Prog Ser* 255:101–114
- Gr aler B, Pebesma E, Heuvelink G (2016) Spatio-temporal interpolation using gstat. *R J* 8(1), 204–218
- Guan Y, Hohn S, Merico A (2015) Suitable Environmental Ranges for Potential Coral Reef Habitats in the Tropical Ocean. *PLOS ONE* 10 (6):e0128831. doi:[10.1371/journal.pone.0128831](https://doi.org/10.1371/journal.pone.0128831)
- Hama T, Miyazaki T, Ogawa Y, Iwakuma T, Takahashi M, Otsuki A, Ichimura S. (1983) Measurement of photosynthetic production of a marine phytoplankton population using a stable ¹³C isotope. *Mar Biol* 73:31–36
- Hastie T, Tibshirani RJ (1990) *Generalized additive models*. London, UK: Chapman and Hall

- Hay KB, Poore AGB, Lovelock CE (2011) The effects of nutrient availability on tolerance to herbivory in a brown seaweed. *J Ecol* 99(6):1540–1550
- Hayes JM (2004) *An Introduction to Isotopic Calculations*. Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA
- Heisler J, Glibert PM, Burkholder JM, et al. (2008) Eutrophication and harmful algal blooms: a scientific consensus. *Harmful Algae* 8 (1): 3–13
- Holleman K (2011) Comparison of submarine groundwater discharge, coastal residence times, and rates of primary productivity, Manualua Bay, Oahu and Honokohau Harbor, Big Island, Hawaii, USA. MS thesis, University of Hawai‘i at Mānoa, Honolulu, HI
- Howarth RW, Sharpley A, Walker D (2002). Sources of nutrient pollution to coastal waters in the United. *Estuaries* 25(4b):656–676
- Houk P, Benavente D, Iguel J, Johnson S, Okano R (2014) Coral reef disturbance and recovery dynamics differ across gradients of localized stressors in the Mariana Islands. *PLOS ONE* 9(9): e110068 <https://doi.org/10.1371/journal.pone.0105731>
- Howarth RW, Sharpley A, Walker D (2002) Sources of nutrient pollution to coastal waters in the United States: Implications for achieving coastal water quality goals. *Estuaries* 25(4B): 6556–676
- Hoyle MD (1975) The literature pertinent to the red algal genus *Gracilaria* in Hawaii, Mar Agron Prog, University of Hawaii, Honolulu
- Huisman JM, Abbott IA, Smith CM (2007) *Hawaiian Reef Plants*. Honolulu, Hawaii, University of Hawai‘i Sea Grant College Program.
- Huo YZ, Wu HL, Chai ZY, Xu SN, Han F, Dong L, He PM (2012) Bioremediation efficiency of *Gracilaria verrucosa* for an integrated multi-trophic aquaculture system with *Pseudosciaena crocea* in Xiangshan harbor, China. *Aquaculture* 326:99–105
- Hunter CL, Evans CW (1995) Coral reefs in Kāne‘ohe Bay, Hawai‘i: two centuries of western influence and two decades of data. *Bull Mar Sci* 57(2):501–515
- Hurd CL (2000) Review: water motion, marine macroalgal physiology, and production. *J Phycol* 36:453– 472
- Hwang DW, Kim G, Lee Y-W, Yang H-S (2005) Estimating submarine inputs of groundwater and nutrients to a coastal bay using radium isotopes. *Mar Chem* 96:61–71
- Israel A, Martinez-Goss M, Friedlander M (1999) Effect of salinity and pH on growth and agar yield of *Gracilaria tenuistipitata* in laboratory and outdoor cultivation. *J Appl Phycol* 11:543–549

Ito W. February 16, 2017. Personal communication.

Johnson AG, Glenn CR, Burnett WC, Peterson RN, Lucey PG (2008) Aerial infrared imaging reveals large nutrient-rich groundwater inputs to the ocean. *Geophysical Research Letters* 35:L15606.

Johnson EE, Wiegner TN (2013) Surface water metabolism potential in groundwater-fed coastal waters of Hawaii Island, USA. *Estuaries Coast* 37:712–723

Jonsson PR, Granhag L, Moschella PS, Aberg P, Hawkins SJ, Thompson RC (2006) Interactions between wave action and grazing control the distribution of intertidal macroalgae. *Ecology* 87:1169–78

Kantún-Manzano CA, Herrera-Silveira JA, Arcega-Cabrera F (2018) Influence of coastal submarine groundwater discharges on seagrass communities in a subtropical karstic environment. *Bull Environ Contam Toxicol* 100:176–183

Keddy PA (1982) Quantifying within-lake gradients of wave energy: interrelationships of wave energy, substrate particle size and shoreline plants in Axe Lake, Ontario. *Aquat Bot* 14:41–58

Khan FS, Ansari AA (2005) Eutrophication: an ecological vision. *Bot Rev* 71(4):449–482

Kilar JA, McLachlan J (1986) Ecological studies of the alga, *Acanthophora spicifera* (Vahl) Børg. (Ceramiales: Rhodophyta): Vegetative fragmentation. *J Exp Mar Biol Ecol* 104(1–3):1–21

Kim G, Lee KK, Park KS, Hwang DW, Yang HS (2003) Large submarine groundwater discharge (SGD) from a volcanic island. *Geophys Res Lett* 30: doi:10.1029/2003GL018378

Kirst GO (1990) Salinity tolerance of eukaryotic marine algae. *Annu Rev Plant Biol* 41:21–53

Knee K, Street JH, Grossman EE, Boehm AB, Paytan A (2010) Nutrient inputs to the coastal ocean from submarine groundwater discharge in a groundwater-dominated system: relation to land use (Kona coast, Hawaii, USA). *Limnol Oceanogr* 55(3):1105–1122

Koch EW (1993) The effect of water flow on photosynthetic processes of the alga *Ulva lactuca*. *Hydrobiologia* 260/261:457–62

Koehl MAR (1986) Seaweeds in moving water: form and mechanical function. In: Givnish TJ (ed) on the economy of plant form and function. Cambridge University Press, Cambridge, UK

Kontar EA, Zektser IS (1999) Submarine discharge and its effect on oceanic processes in the coastal zone. *Water Resour* 26: 512

- Kraufvelin P (2007) Responses to nutrient enrichment, wave action and disturbance in rocky shore communities. *Aquat Bot* 87(4):262–274
- Kraufvelin P, Lindholm A, Pedersen MF, Kirkerud LA, Bonsdorff E (2010) Biomass, diversity and production of rocky shore macroalgae at two nutrient enrichment and wave action levels. *Mar Biol* 157:29–47
- Lapointe BE (1985) Strategies for pulsed nutrient supply to *Gracilaria* cultures in the Florida Keys: Interactions between concentration and frequency of nutrient pulses. *J Exp Mar Biol Ecol* 93, 211–222.
- Lapointe BE (1997) Nutrient thresholds for bottom-up control of macroalgal blooms on coral reefs in Jamaica and southeast Florida. *Limnol Oceanogr* 42:5, 1119–1131.
- Lee J, Kim G (2015) Dependence of coastal water pH increases on submarine groundwater discharge off a volcanic island. *Estuar Coast Shelf Sci* 163:15e21
- Lee E, Shin D, Huyn SP, et al. (2017) Periodic change in coastal microbial community structure associated with submarine groundwater discharge and tidal fluctuation. *Limnol Oceanogr* 62(2): 437–451
- Leitao F, Encarnação J, Range P, et al. (2015) Submarine groundwater discharges create unique benthic communities in a coastal sandy marine environment. *Estuar Coast Shelf Sci* 163: 93-98
- Leon-Soon S (2017) Biophysical interactions: influence of water flow on nutrient distribution and nitrate uptake by marine algae. PhD dissertation, University of Hawai‘i at Mānoa, Honolulu, HI
- Lewis ER, Wallace DWR (1998) Program Developed for CO₂ System Calculations, DOE (US Department of Energy), ORNL/CDIAC-105
- Lilliesköld Sjöo GL, Mörk E, Andersson S, Melander I (2011) Differences in top-down and bottom-up regulation of macroalgal communities between a reef crest and back reef habitat in Zanzibar. *Estuar Coast Shelf Sci* 91(4):511–518
- Littler MM, Littler DS, Brooks BL (2004) Extraordinary mound-building forms of *Avrainvillea* (Bryopsidales, Chlorophyta): their experimental taxonomy, comparative functional morphology and ecological strategies. *Atoll Res Bull* 515:1–26
- Littler MM, Littler DS, Brooks BL (2006) Harmful algae on tropical coral reefs: bottom-up eutrophication and top-down herbivory. *Harmful Algae* 5:565–585
- Littler MM, Littler DS, Titlyanov EA (1991) Producers of organic-matter on tropical reefs and their relative dominance. *Mar Biol* 6:3–14

- Liu X, Xiao W, Landry MR, Chiang KP, Wang L, Huang B (2016) Responses of phytoplankton communities to environmental variability in the east China Sea. *Ecosystems* 19: 832–849. doi: 10.1007/s10021-016-9970-5
- Lubarsky KA, Silbiger NJ, Donahue MJ (2018) Effects of submarine groundwater discharge on coral accretion and bioerosion on two shallow reef flats. *Limnol Oceanogr* 63: 1660–1676
- Lyons DA, Arvanitidis C, Blight AJ, Chatzinikolaou E, Guy-Haim T, Kotta J, Orav-Kott H, Queirós AM, Rilov G, Somerfield PJ, Crowe TP (2014) Macroalgal blooms alter community structure and primary productivity in marine ecosystems. *Glob Change Biol* 20:2712–24
- Magalhaes WF, Bailey-Brock JH (2014) Polychaete assemblages associated with the invasive green alga *Avrainvillea amadelpha* and surrounding bare sediment patches in Hawaii. *Mem Mus Vic* 71:161–168
- Magurran AE (2004) *Measuring biological diversity*. 2nd ed. Blackwell Science Ltd., Oxford, UK
- Marubini F, Davies PS (1996) Nitrate increases zooxanthellae population density and reduces skeletogenesis in corals. *Mar Biol* 127:319–328. doi:10.1007/BF00942117.
- Mateo MA, Renom P, Hemminga MA, Peene J (2001) Measurement of seagrass production using the ¹³C stable isotope compared with classical O₂ and ¹⁴C methods. *Mar Ecol Prog Ser* 223:157–165
- McClanahan T, Polunin N, Done T (2002) Ecological states and the resilience of coral reefs. *Conserv Ecol* 6(2):18
- McCook LJ (1999) Macroalgae, nutrients and phase shifts on coral reefs: scientific issues and management consequences for the Great Barrier Reef. *Coral Reefs* 18:357–367
- McCoy SJ, Kamenos NA (2015) Coralline algae (Rhodophyta) in a changing world: integrating ecological physiological, and geochemical responses to global change. *J Phyco* 51 (1): 6-24
- McCune B, Grace JB (2002) *Analysis of ecological communities*. Glenden Beach, Oregon: MjM Software Design.
- McGowan MP (2004) Submarine groundwater discharge: freshwater and nutrient input into Hawaii's coastal zone. Master's thesis. Department of Geology and Geophysics. University of Hawai'i at Mānoa.
- Mehrbach C, Culbertson CH, Hawley JE, Pytkowicz RN (1973) Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnol. Oceanogr.* 18, 897–907

- Moore WS (2010) The effect of submarine groundwater discharge on the ocean. *Annual Rev Marine Sci* 2:59–88
- Moosdorf N, Stieglitz T, Waska H et al. (2015) Submarine groundwater discharge from tropical islands: a review. *Grundwasser* 20 (1):53–67 doi:10.1007/s00767-014-0275-3
- Muthukrishnan R, Fong P (2014) Multiple anthropogenic stressors exert complex, interactive effects on a coral reef community. *Coral Reefs* 33(4):911–921
- Naim O (1993) Seasonal responses of a fringing reef community to eutrophication (Reunion Island, western Indian Ocean). *Mar Ecol Prog Ser* 99:307–315
- National Data Buoy Center, Station OOUH1, NOAA (2017) www.ndbc.noaa.gov/station_history.php?station=oouh1. Accessed May 15, 2017.
- National Research Council (2000) Clean coastal waters: understanding and reducing the effects of nutrient pollution. National Academies Press, Washington D.C.
- Nelson CE, Donahue MJ, Dulaiova H, Goldberg SJ, La Valle FF, Lubarsky K, Miyano J, Richardson C, Silbiger NJ, and Thomas FIM (2015) Fluorescent dissolved organic matter as a multivariate biogeochemical tracer of submarine groundwater discharge in coral reef ecosystems. *Mar Chem* 177(2): 232–243
- Nishihara GN, Terada R (2010) Species richness of marine macrophytes is correlated to a wave exposure gradient. *Phycol Res* 58(4):280–292
- Nixon WS, Oviatt CA, Frithsen J, Sullivan B (1986) Nutrients and the productivity of estuarine and coastal marine ecosystems. *J Limnol Soc South Afr* 12:43–71
doi:10.1080/03779688.1986.9639398
- Norderhaug KM, Christie H, Rinde E, Gundersen H, Bekkby T (2014) Importance of wave and current exposure to fauna communities in *Laminaria hyperborean* kelp forests. *Mar Ecol Prog Ser* 502:295–301
- Nyström M (2006) Redundancy and response diversity of functional groups: implications for the resilience of coral reefs. *Ambio* 35(1): 30–35
- Ocean Commission (2004) An ocean blueprint for the 21st century. Final Report. U.S. Commission on Ocean Policy. Washington D.C.
- Oksanen J, Blanchet FB, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Henry M, Stevens H, Szoecs E, Wagner H (2017) vegan: community ecology package. R package version 2.4–3. <https://CRAN.R-project.org/package=vegan>

- Paytan A, Boehm AB, Shellenbarger GG (2004) Bacterial contamination and submarine groundwater discharge – a possible link. *Environ Chem* 1 (1): 29–30
- Paytan A, Shellenbarger GG, Street JH, Gonneea ME, Davis K, Young MB, Moore WS (2006) Submarine groundwater discharge: an important source of new inorganic nitrogen to coral reef ecosystems. *Limnol Oceanogr* 51(1):343–348
- Pihl L, Svenson A, Moksnes PO, Wennhage H (1999) Distribution of green algal mats throughout shallow soft bottoms of the Swedish Skagerrak archipelago in relation to nutrient sources and wave exposure. *J Sea Res* 41:281–294
- Pebesma EJ (2004) Multivariable geostatistics in S: the gstat package. *Comp Geosci* 30: 683–69
- Peterson BJ, Stabler AD, Wall CC, Gobler CJ (2012) Nitrogen-rich groundwater intrusion affects productivity, but not herbivory, of the tropical seagrass *Thalassia testudinum*. *Aquat Biol* 15:1–9 doi: 10.3354/ab00413
- Piló D, Barbosa AB, Teodósio MA, Encarnação J, Leitão F, Range P, Krug LA, Cruz J, Chícharo L (2018) Are submarine groundwater discharges affecting the structure and physiological status of rocky intertidal communities? *Mar Environ Res* 136: 158–173
- Porzio L, Buia MC, Hall-Spencer JM (2011) Effects of ocean acidification on macroalgal communities. *J Exp Mar Bio Ecol* 400 (1-2): 278-287
- R Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>
- Reef R, Pandolfi JM, Lovelock CE (2012) The effect of nutrient enrichment on the growth, nucleic acid concentrations, and elemental stoichiometry of coral reef macroalgae. *Ecol Evol* 2(8): 1985–1995
- Richardson C, Dulaiova H, Popp B, Ruttenberg K, Fackrell J (2017). Submarine groundwater discharge drives biogeochemistry in two Hawaiian reefs. *Limnol Oceanogr* 10.1002/lno.10654.
- Richardson CM, Dulai H, Whittier RB (2017) Sources and spatial variability of groundwater-delivered nutrients in Maunaloa Bay, Oahu, Hawai‘i. *J Hydrol Reg Stud* 11: 178–193
- Ricketts EF, Calvin J, Hedgpeth JW, Phillips DW (1985) *Between Pacific tides*. Stanford University Press, Palo Alto, CA.
- Riis T, Hawes I (2003) Effect of wave exposure on vegetation abundance, richness and depth distribution of shallow water plants in a New Zealand lake. *Freshw Biol* 48:75–87
- Tirson-Williams R, Arnold SN, Paul VJ (2016) Patterns of larval settlement preferences and post-settlement survival for seven Caribbean corals. *Mar Ecol Prog Ser* 548: 127–138

- Rodgers S, Cox EF (1999) Rate of Spread of Introduced Rhodophytes *Kappaphycus alvarezii*, *Kappaphycus striatum*, and *Gracilaria salicornia* and Their Current Distribution in Kane'ohē Bay, O'ahu Hawai'i. *Pac Sci* 53(3): 232–241
- Russell DJ (1992) The ecological invasion of Hawaiian reefs by two marine red algae, *Acanthophora spicifera* (Vahl) Boerg. and *Hypnea musciformis* (Wulfen) J. Ag., and their association with two native species, *Laurencia nidifica* J. Ag. and *Hypnea cervicornis*. *ICES Mar Sci Symp* 194: 110–125
- Santos IR, Erler D, Tait D et al. (2010) Breathing of a coral cay: Tracing tidally driven seawater recirculation in permeable coral reef sediments. *J Geophys Res* 114: C12010
- Simpson EH (1949) Measurement of diversity. *Nature* 163:688–688
- Slomp CP, Van Cappellen P (2004) Nutrient inputs to the coastal ocean through submarine groundwater discharge: controls and potential impact. *J Hydrol* 295 (1–4): 64–86
- Smith JE, Hunter CL, Smith CM (2001) The effects of herbivory and nutrient enrichment on benthic community structure and development on a Hawaiian Reef. *Coral Reefs* 19:332–342
- Smith SV, Kimmerer WJ, Laws EA, Brock RE, Walsh TW (1981) Kāne'ohē Bay sewage diversion experiment: perspectives on ecosystem responses to nutritional perturbation. *Pac Sci* 35:279–397
- Smith JE, Hunter CL, Smith CM (2002) Distribution and reproductive characteristics of nonindigenous and invasive marine algae in the Hawaiian Islands. *Pac Sci* 56(3): 299–315
- Smith JE, Hunter CL, Smith CM (2010) The effects of top-down and bottom-up factors on benthic coral reef community structure. *Oecologia* 163(2):497–507
- Smith JE, Hunter CL, Conklin EJ, Most R, Sauvage T, Squair C, Smith CM (2004) Ecology of the invasive red alga *Gracilaria salicornia* (Rhodophyta) on O'ahu, Hawai'i. *Pac Sci* 58:325–343.
- Smith JE, Runcie JW, Smith CM (2005) Characterization of a large-scale ephemeral bloom of the green alga *Cladophora sericea* on the coral reefs of West Maui, Hawai'i. *Mar Ecol Prog Ser* 302:77–91
- Sorokin YI (1995) *Ecological Studies Coral Reef Ecology* Vol. 102. Berlin: Springer-Verlag.
- Steneck RS, Dethier MN (1994) A functional group approach to the structure of algal-dominated communities. *Oikos* 69: 476

- Stimson J, Larned S, McDermid K (1996) Seasonal growth of the coral reef macroalga *Dictyosphaeria cavernosa* Borgesen and the effects of nutrient availability, temperature and herbivory on growth rate. *J Exp Mar Biol Ecol* 196:53–77
- Sudhir P, Murhy SDS (2004) Effects of salt stress on basic processes of photosynthesis. *Photosynthetica* 42 (2):481–486.
- Sugimoto R, Kitagawa K, Nishi S, Honda H, Yamada M, Kobayashi S, Shoji J, Ohsawa S, Taniguchi M, Tominaga O (2017) Phytoplankton primary productivity around submarine groundwater discharge in nearshore coasts. *Mar Ecol Prog Ser* 563: 25–33
- Thomas FIM, Cornelisen CD (2003) Ammonium uptake by seagrass communities: effects of oscillatory versus unidirectional flow. *Mar Ecol Prog Ser* 247:51–7
- Torres ME, Mix AC, Rugh WD (2005) Precise $\delta^{13}\text{C}$ analysis of dissolved inorganic carbon in natural waters using automated headspace sampling and continuous-flow mass spectrometry. *Limnol Oceanogr: Methods* 3 (8): 349–360
- Troccoli-Ghinaglia L, Herrera-Silveira JA, Comin FA et al. (2010) Phytoplankton community variations in tropical coastal area affected where submarine groundwater occurs. *Cont Shelf Res* 30 (20):2082–2091
- Uppstrom LR (1974) The boron/chlorinity ratio of deep-sea water from the Pacific Ocean. *Deep Sea Res* 21:161–162. doi:10.1016/0011-7471(74)90074-6
- Valiela I, McClelland J, Hauxwell J, Behr PJ, Hersh D, Foreman K (1997) Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. *Limnol Oceanogr* 42:1105–1118
- Venables WN, Ripley BD (2002) *Modern applied Statistics*. 4th ed. Springer, New York. ISBN 0-387-95457-0
- Vermeij MJA, Dailer ML, Smith CM (2009). Nutrient enrichment promotes survival and dispersal of drifting fragments in an invasive tropical macroalga. *Coral reefs* 28(2), 429–435
- Vermeij MJA, Sandin SA (2008) Density-dependent settlement and mortality structure the earliest life phase of a coral population. *Ecology* 89 (7): 1994–2004
- Walsh GE, Bowers RL (1971) A review of Hawaiian zoanthids with descriptions of three new species. *Zool J Linn Soc* 50(2):161–180. doi: 10.1111/j.1096-3642.1971.tb00757.x
- Walters LJ, Smith CM (1994) Rapid rhizoid production in *Halimeda discoidea* decaisne (Chlorophyta, Caulerpales) fragments: a mechanism for survival after separation from adult thalli. *J Exp Mar Biol Ecol* 175(1):105–120
- Walters LJ, Smith CM, Coyer JA, Huter CL, Beach KS, Vroom PS (2002) Asexual propagation in the coral reef macroalga *Halimeda* (Chlorophyta, Bryopsidales):

- production, dispersal and attachment of small fragments. *J Exp Mar Biol Ecol* 278(1):47–65
- Waska H, Kim G (2011) Submarine groundwater discharge (SGD) as a main nutrient source for benthic and water-column primary production in a large intertidal environment of the Yellow Sea. *J Sea Res* 65:103–113.
- Wernberg T, Vanderklift MA (2010) Contribution of temporal and spatial components to morphological variations in the kelp *Ecklonia* (Laminariales). *J Phycol* 46:153–61
- Whittier RB, El-Kadi AI (2009) Human and environmental risk ranking of onsite sewage disposal systems. Report prepared for State of Hawai'i Safe Drinking Water Branch, Department of Geology and Geophysics, University of Hawai'i at Mānoa. Honolulu, Hawai'i, 142
- Wiencke C, Bischof K (2012) Seaweed biology: Novel insights into ecophysiology, ecology and utilization. Springer-Verlag, Berlin, Germany, 514
- Wikle CK, Zammit-Mangion A, Cressie N (2018) Spatio-Temporal Statistics with R. Chapman and Hall/CRC
- Williams GJ, Smith JE, Conklin EJ, Gove JM, Sala E, Sandin SA (2013) Benthic communities at two remote Pacific coral reefs: effects of reef habitat, depth, and wave energy gradients on spatial patterns. *PeerJ* 1:e81
- Wolanski E, Martinez JA, Richmond RH (2009) Quantifying the impact of watershed urbanization on a coral reef: Maunalua Bay, Hawaii. *Estuar Coast Shelf Sci* 84:259–268
- Wood SN (2006) Generalized additive models. An Introduction with R. Boca Raton, FL: Chapman & Hall/CRC
- Wood SN (2011) Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *J R Stat Soc Series B Stat Methodol* 73(1):3–36
- Yang YF, Fei XG, Song JM, Hu HY, Wang GC, Chung IK (2006) Growth of *Gracilaria lemaneiformis* under different cultivation conditions and its effects on nutrient removal in Chinese coastal waters. *Aquaculture* 254: 248–255
- Yap HT, Montebon ARF, Dizon RM (1994) Energy-flow and seasonality in a tropical coral-reef flat. *Mar Ecol Prog Ser* 103 (1–2): 35
- Yau VM, Schiff KC, Benjamin F, et al. (2014) Effect of submarine groundwater discharge on bacterial indicators and swimmer health at Avalon Beach, CA, USA. *Water Res* 59: 23–36

Young C, Tamborski J, Bokuniewicz H (2015) Embayment scale assessment of submarine groundwater discharge nutrient loading and associated land use. *Estuar Coast Shelf Sci* 158: 20–30

Zelditch M, Swiderski D, Sheets H (2012) *Geometric Morphometrics for Biologists*. 2nd Edition. Academic Press.

Zhang J, Mandal AK (2012) Linkages between submarine groundwater systems and the environment. *Curr Opin Env Sust* 4 (2): 219–26

Zuur AF, Ieno EN, Smith GM (2007) *Analysing ecological data*. Springer Science and Business Media, New York, NY

