

***RECRUITMENT AND SYMBIONT INTERACTIONS IN ANTHROPOGENICALLY ALTERED HABITATS: THE
PORIFERA OF MAUNALUA BAY, O'AHU***

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BY

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DEDICATION

I dedicate this thesis to my beautiful wife, Alyssa M. Austin.

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Abstract

Marine ecosystems around the world are affected by anthropogenically-induced disturbances, impacting the competition for benthic space by sessile invertebrates. To investigate the recruitment of Porifera in Maunalua Bay, O'ahu, Hawai'i, Porifera abundances were compared to two earlier studies, Coles *et al.* (2002) and Longenecker *et al.* (2011), to understand changes through time. Cyanobacterial symbiont interactions were tested by determining chlorophyll concentration in the sponge holobiont. There has been a significant increase in Porifera since 2011 and Porifera are now more prevalent in areas where the invasive alga, *Gracilaria salicornia*, is dominant compared to nearby native sea grass beds. Recruitment of Porifera to the invasive algal mats was not explained by resource partitioning facilitated by cyanobacterial symbionts. Over time and through successional regimes, endemic, native, and non-native Porifera have become established in invasive algal mats and the new niche space that they provide.

Table of contents

Acknowledgments	4
Abstract	5
List of Figures	7
List of Tables	8
Introduction	9-11
Materials and Methods	11-14
Results	14-19
Discussion	19-21
Reference	21-28

List of Figures

Figure 1: Map of the study locations.	12
Figure 2: Number of observations of Porifera organisms regardless of species at each individual site in Maunalua Bay, O'ahu.	15
Figure 3: Number of species of Porifera observed at each site in Maunalua Bay, O'ahu.	16
Figure 4: Number of observations of Porifera at all sites across all studies.	17
Figure 5: Number of species of Porifera observed at all sites across all studies.	18
Figure 6: Range of chlorophyll-a concentrations observed.	19

List of Tables

Table 1: Identified sponge organisms observed in Maunalua Bay, O'ahu.

14-15

Introduction

Coastal reefs are a critical component of natural marine ecosystems and world economies (Wilkinson 1996; Brander *et al.* 2012). Coral reefs provide a range of ecosystem services, generating an estimated \$9.6 billion in annual revenue globally, which is a compelling reason to maintain and conserve these ecosystems (Cesar *et al.* 2003, Barbier 2017). Understanding the dynamic interactions of species community assembly is a necessary component to guide coastal reef conservation.

Marine benthic substrates are under fierce competition for space and resources by sessile and non-sessile marine invertebrates (Birkeland 1997). This competition leads to the exclusion of one organism over another that competes for some of the same resources, which in turn leads to the organism defining its niche in the environment (e.g., Elton 1927; Hardin 1960; MacArthur and Levins 1964). Invasive species, often aided by anthropogenic factors, can radically alter the community composition of an ecosystem. A prime example of such an alteration is the result of the invasive *Gracilaria salicornia* which has produced dense algal mats in Maunalua Bay and largely replaced open native seagrass beds (Smith *et al.* 2002; Smith *et al.* 2004).

Invasive ecological engineers

Ecological engineers are organisms that either directly or indirectly regulate resource availability, facilitated by the organism's capacity to cause physical state changes to the biotic and abiotic resources in the ecosystem, and are, in some cases, invasive organisms (Jones *et al.* 1994; Jones *et al.* 1997; Crooks 2002; Cuddington and Hastings 2004). Ecological engineers are of great interest in many degraded coastal ecosystems and marine restoration efforts (Bouma *et al.* 2009 & 2010). Much of the focus in marine conservation of tropical coastal ecological engineers has been on reef-building coral species. Because corals can be so fragile and are keystone species in the reef environment, research has predominantly focused on their response to anthropogenic stressors, while the responses of other reef invertebrates are less well known (Bell *et al.* 2013). Two common non-coral ecological engineers in Hawai'i are the non-native invasive algae, *Gracilaria salicornia* and *Avrainvillea amadelpha* (Smith *et al.* 2004; Magalhaes and Bailey-Brock 2014; Foster *et al.* 2019). Studies and management efforts in Hawai'i have largely focused on the negative impacts of these species and their removal (Smith *et al.* 2002; Coles *et al.* 2002; Smith *et al.* 2004; Longenecker *et al.*, 2011), but have largely overlooked some of the broader range of interactions of algal ecological engineers in altered environments.

A species invasion is an inherently dynamic non-equilibrium process. The effects of invasive species can dramatically change over time; however, most studies of invasive species neglect long-term temporal effects (Strayer *et al.* 2006). Generally, species diversity decreases after an invasion; however, recovery of species diversity is sometimes observed after the initial phases of an invasion (e.g., Morrison 2002; Brockerhoff *et al.* 2003). In contrast to typical assumptions, introduced species can also promote native species success (e.g., Lane 1993; Magalhaes and Bailey-Brock 2014). In some cases, invasive plants can increase both native and exotic species richness compared to native-only habitats (Tecco *et al.* 2006; Sax *et al.* 2007; Magalhaes and Bailey-Brock 2014; Ramus *et al.* 2017).

While some removal of invasive algae might enhance species diversity (i.e., intermediate disturbance theory, *sensu* Ramus *et al.* 2017), large-scale removal of invasive species can have unexpected effects and even negatively impact native species diversity (Ballari *et al.* 2016; Lurgi *et al.* 2018). This can also set the stage for the establishment of additional invasive species (Stachowicz *et al.* 1999; Hulme *et al.* 2006). In sum, there is a great deal of uncertainty about the effects of invasive algae on invertebrate diversity, especially considering temporal effects, and conservation efforts may not fully realize their intended goals without more information about altered environments.

The role of Porifera and microorganism interactions

Sponges contribute to many processes within the reef ecosystem by providing shelter for smaller organisms and food for larger organisms. Sponges also have the potential to both negatively and positively affect the stability of other organisms and contribute to nutrient cycling through the removal of particulate organic matter (Yahel *et al.* 2003; De Goeij *et al.* 2008; González-Rivero *et al.* 2011; Wulff 2012; De Goeij *et al.* 2013; Pawlik and McMurray 2020). However, the role of sponge species diversity and their effects on altered habitats has been dramatically understudied relative to many other marine organisms in the Indo-Pacific (e.g., Keyse *et al.* 2014).

The sponge-microorganism complex known as the sponge holobiont has emerged as a key model for understanding host-microbe interactions (Moreno-Pino *et al.* 2020). Bacteria comprise nearly 50% of the sponge biomass (Weigel and Erwin 2016; Yang 2019) leading to three types of interactions (i.e., mutualism, commensalism, and parasitism) in the coevolution of sponge-microbe associations (Taylor *et al.* 2007; Thacker and Freeman 2012). Sponges frequently harbor symbiotic microorganisms that may benefit their hosts through nutrient supplementation (Wilkinson 1992; Vacelet *et al.* 1995), skeleton stabilization (Rützler 1990), processing of metabolic waste (Beer and Ilan 1998), and the production of secondary metabolites (Bewley *et al.* 1996; Schmidt *et al.* 2000). Sponge-associated cyanobacteria include approximately six genera (Steindler *et al.* 2005; Taylor *et al.* 2007). The most prevalent sponge-associated photo-symbionts are the filamentous cyanobacterium *Oscillatoria spongelia* and the single-celled cyanobacterium *Synechococcus spongiarum* (Erwin and Thacker 2008a, Gao *et al.* 2014). Although the sponge holobiont model has been studied globally, relatively little is known regarding Hawaiian sponge microbiome interactions. Studies that have examined this interaction either focused on fungal symbionts or were limited to two invasive species *Mycale armata* and *Suberites zeteki* (Gao *et al.* 2008; Wang *et al.* 2008; Zhu *et al.* 2008; Wang *et al.* 2009).

Symbiotic mutualisms have evolved to be host-specific and critical to sponge survival. Outplant studies in the Caribbean show that some sponges die without energy from light/cyanobacteria (Thacker 2005; Erwin and Thacker 2008b), suggesting that cyanobacterial-associated resource allocation plays an important role in sponges. It is hypothesized that energy and nutrients from cyanobacteria in unshaded habitats enable sponge resources to be reallocated from a larger emphasis on energy and nutrition production to defense from predation relative to shaded sponges with an absence of cyanobacteria (Erwin and Thacker 2008b). Sponges that specialize in these alternative strategies, with and without cyanobacterial symbionts and the associated resource portioning, can potentially coexist in shaded and unshaded areas of coastal

ecosystems. The presence of symbiotic cyanobacteria hypothetically enhances niche diversity in these mats with a positive effect on species diversity.

Understanding dynamic changes in Maunalua Bay

Maunalua Bay is a highly disrupted ecosystem on the island of O‘ahu that has experienced a seagrass-to-algal phase shift with *Gracilaria salicornia* and *Avrainvillea amadelpha* now dominating most of the bay. This phase shift is due to several complex anthropogenic sources such as the introduction of invasive *Gracilaria salicornia*, changes in submarine groundwater discharge, and increased sedimentation due to runoff from urbanization and agriculture (Smith *et al.* 2002; Wolanski *et al.* 2009). *Avrainvillea amadelpha* was first reported on the south shore of O‘ahu in 1981 and in Maunalua Bay in 1985 (Brostoff 1989) and has subsequently become a dominant invasive alga in Maunalua Bay (Longenecker *et al.* 2011). *Gracilaria salicornia* was first introduced to the South Shore of O‘ahu, Waikīkī, in 1971 and was not surveyed or recorded in Maunalua Bay until the study by Smith *et al.* (2004). It is likely that *Gracilaria salicornia* was present in Maunalua bay prior to the Smith *et al.* (2004) study given that *Gracilaria salicornia* is easily transported through fragmentation and Maunalua Bay’s proximity to the introduction point. Furthermore, *Gracilaria salicornia* can withstand a broad intensity of light environments while also taking advantage of a wide range of nutrients that are made available through submarine groundwater discharge (Beach *et al.* 1997, Larned 1998, Smith *et al.* 2002). However, historically, sponges have only been observed in low abundance or not at all in Maunalua Bay (Coles *et al.* 2002; Longenecker *et al.* 2011).

Recovery after a species invasion is a dynamic process, yet many investigations do not include a temporal aspect—the ultimate effects upon other species in the ecosystem, when a relatively stable phase is ultimately reestablished, are unknown. Symbiotic microbes may act as keystone species, interacting with invasive algae to increase habitat diversity and sponge species diversity. Studies at other locations found that the massive algal mats that cover the benthic substrate created niches for alternative organisms to recruit and thrive (Bell *et al.* 2013; Ainsworth & Mumby 2015). In comparison to open sandy seagrass beds, the invasive algal mats in Maunalua Bay provide a dense complex mesh structure that sponges can utilize as anchors as well as protection from predation and light (Dunlap and Pawlik 1996). Understanding sponge recruitment and community assembly is critical to making predictions about long-term anthropogenic effects and changes in tropical reefs. This study aimed to 1) understand Porifera species diversity over time in areas with invasive *Gracilaria salicornia* and *Avrainvillea amadelpha* mats in Maunalua Bay and 2) evaluate whether symbiotic sponge cyanobacteria are at higher concentrations in unshaded regions of invasive *G. salicornia* mats. The first goal was made possible by comparisons to two previous studies, Coles *et al.* (2002) and Longenecker *et al.* (2011).

Materials and Methods

Observational study

Sponges were surveyed and collected from 10/24/2018 to 02/23/2020 at five locations (Figure 1) in Maunalua Bay, O‘ahu. The first site was Channel Marker 22 (21°16’49.37” N 157°43’05.86” W) near the public boat ramp in Hawaii Kai. The second site was a native Hawaiian sea grass bed, *Halophila hawaiiiana*, maintained by the community organization Malama Maunalua

(21°16'58.87" N 157°43'31.35" W). The third location was Wailupe Beach Park (21°16'27.25" N 157°43'38.26" W). The fourth site was near the Longenecker *et al.* (2011) study site off of Paiko Drive (21°16'52.07" N 157°43'52.97" W). The fifth collection site was at Paiko Beach where little to no invasive algal removal has taken place (21°17'02.63" N 157°43'12.10" W). The Paiko Beach site is relatively more susceptible to sedimentation than the other sites in the study (Wolanski *et al.* 2009).

Two 30 m belt transects were set up horizontal and perpendicular to the shoreline and two 0.25-meter photo-quadrats were randomly placed along the transects to record images and temperature. Random placements were made by using previously randomly generated integer values from 0-30 corresponding to the meter position along the transect. New values were generated for each transect. All the algal material, when present, within each 0.25-meter quadrat was collected and brought back to the lab and placed into saltwater aquaria prior to sponge removal. The organisms were then collected from the saltwater aquaria and separated into morphospecies for further taxonomic identification. Sponges were only observed when algae were present. There was no delineation between algal types in quadrats as several species of algae including *Gracilaria salicornia* and *Avrainvillea amadelpha* were often found together when observed. A total of 61 sponge specimens were collected. Sponges were identified to the lowest taxonomic level using gross morphological characters with further identifications through spicule and molecular analysis.

It should be noted that Coles *et al.* (2002) study collected material in a haphazard fashion with the intent on observing all biota present within the study area. They sampled from three locations in the Maunalua Bay area from one reference substrate, fouling. Organisms collected from the fouling substrate were collected in a semi-quantitative way in two parts. The first part was a visual search and collection of whole macro-organisms by hand where when present complete macroalgae plants were collected and transported back to the lab for later inspection and removal of cryptic species. The second part consisted of 3 scrapings of 0.10 m², collected organisms and substratum from both parts ranged from 4-8 liters in total volume from each location. Both *G. salicornia* and *A. amadelpha* were observed in their study but there was no specification as to whether there was an association between the algae and sponge species they observed.

Likewise, Longenecker *et al.* (2011) removed *Avrainvillea amadelpha* from more than nine hectares in three treatment regimes: 3-, 6-, and 9-months post-removal. They randomly sampled eight points from four reference habitats, mudweed (*Avrainvillea amadelpha*), native algae (*Halimeda discoidea*, *Spyridia filamentosa*, *Gracilaria coronopifolia*), seagrass (*Halophila hawaiiiana* and *H. decipiens*) and unvegetated sand; as well as the three post-removal treatments. At each habitat and treatment point, samples were collected by inserting a 10.16-cm-diameter core up to 10 cm into the substrate. Like the Coles *et al.* (2002) study their intent was to quantify all invertebrate organisms. However, they did not observe either *G. salicornia* or sponges. In the present study the Paiko Drive and native seagrass (*Halophila hawaiiiana*) locations were purposefully selected for their similarity to the Longenecker *et al.* (2011) conditions.

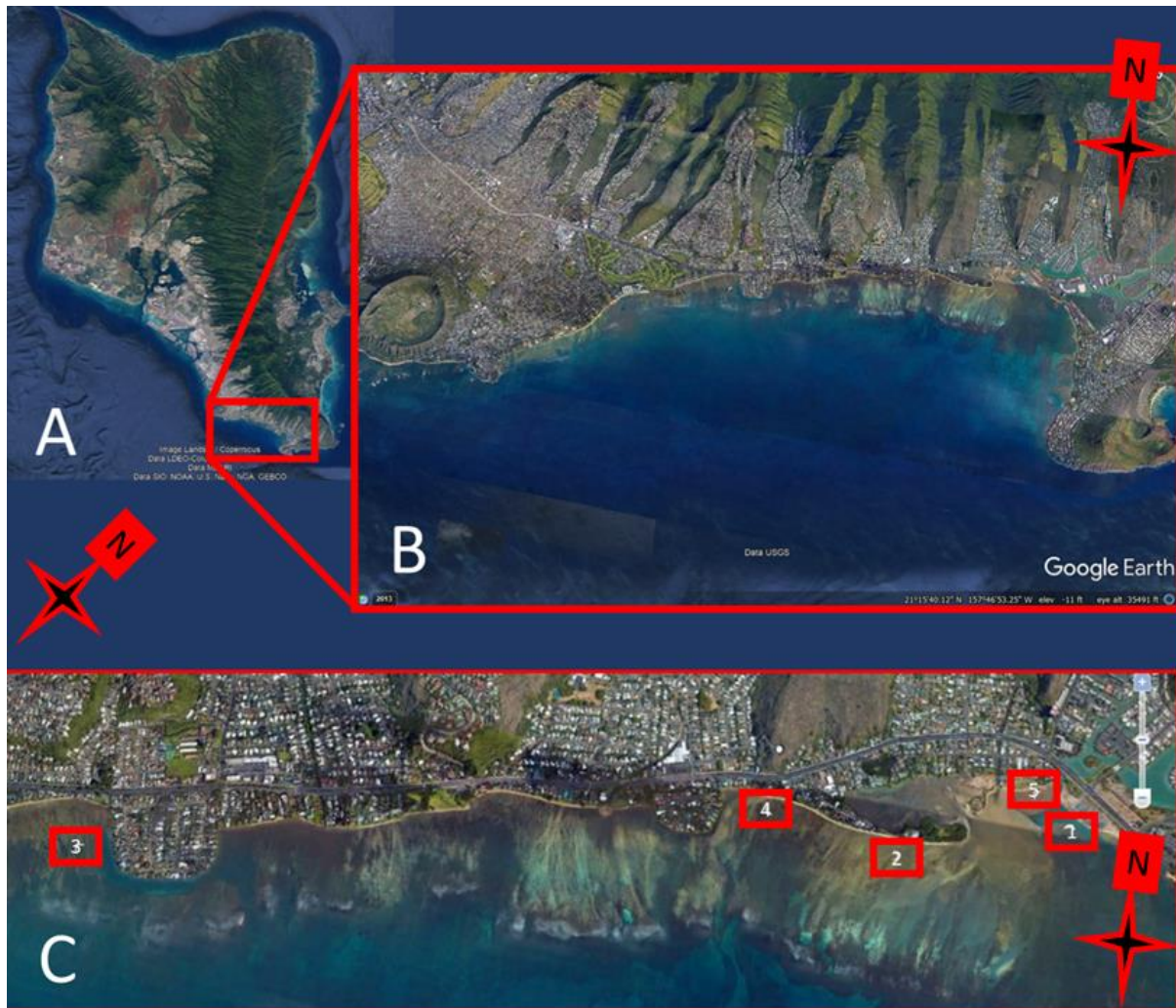


Figure 1: Map of the study locations. A) Google earth image of O'ahu; Maunalua Bay is highlighted. B) Expanded Image of Maunalua Bay. C) Zoomed in view of Maunalua Bay with collection sites outlined in red. 1) Channel Marker 22, 2) native seagrass site, 3) Wailupe Beach Park, 4) Longenecker (2011) site location (Paiko Drive), 5) Paiko Beach.

Chlorophyll-a quantification

A total of 43 sponges were collected between October 10, 2019 to October 31, 2019 from two discrete substrates, one in direct sunlight (light) and the other in indirect sunlight (dark). Light substrates were the surface of a concrete pylon (Channel Marker 19) while dark substrates were under and within the invasive *Gracilaria salicornia* mats surrounding the marker. These *Gracilaria salicornia* mats were homogenous for *G. salicornia* while the *G. salicornia* mats in the observational study were heterogenous with several algal species observed. All visible sponges and algal mat material at each site were collected by snorkel and brought back to the lab where the specimens were divided into two pieces. The first piece was placed into a 1.5 ml microcentrifuge tubes containing 70% ethanol for genetic analysis. The second piece was cut into approximately 25 mm³ pieces, which were then wet weighed, and placed into 10 ml of 90% acetone. The samples were then wrapped in foil in order to inhibit photodegradation and stored

at 4° C overnight for further Chlorophyll-a analysis. Aliquots of the 90% acetone extracts were centrifuged in 1.5 ml Eppendorf tubes; 1.4 ml of supernatant was then placed into quartz cuvettes and absorbances were quantified at 750 nm, 664 nm, 647nm, and 630 nm. This was repeated three times for each specimen. Chlorophyll-a concentrations were estimated for all samples using the equations of Parsons *et al.* (1984); to account for variation in concentration due to differences in mass all samples were standardized by using the extracted sponge wet weight.

Molecular methods

DNA was extracted from 104 sponge samples using a Qiagen DNeasy® Blood & Tissue Kit following the manufacturer's protocol. Initial identification of the samples was confirmed with COI and 28S markers. Cyanobacteria were identified using CYA359F and CYA781F (Nübel *et al.* 1997) forward primer and CYA23S1R (Primer 340; Itean *et al.* 2000) reverse primer.

Fragments of mitochondrial Cytochrome Oxidase subunit I (COI) were PCR amplified using primers:

LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3',

HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'

(Folmer *et al.* 1994) and the following temperature protocol: 95° C for 1 min, 30 cycles of 95° C for 20 s, 40° C for 40 s, and 72° C for 5 min. 28S rDNA primers were also used as they are commonly employed for taxonomic identification throughout Porifera (e.g., Erpenbeck *et al.* 2012; Gazave *et al.* 2013). Fragments of nuclear 28S rDNA were PCR amplified using primers:

RD3A: 5'-GACCCGTCTTGAAACACGA-3',

RD5B2: 5'-ACACACTCCTTAGCGGA-3'

(McCormack *et al.* 2002; Erpenbeck *et al.* 2012) and the following temperature protocol: 95° C for 1 min, 30 cycles of 95° C for 20 s, 50° C for 40 s, and 68° C for 5 min with a final extension step at 68° C for 1 min. All reactions were composed of 13 µl ddH₂O, 25 µl Taq® 2X Master Mix buffer from New England Biolabs Inc., 1.0 µl forward primer, 1.0 µl reverse primer, and 10 µl of DNA sample. PCR products were cleaned with USB® ExoSAP-IT® PCR Product Cleanup (Affymetrix) following manufacturer guidelines, and sequenced in both directions from each primer by Sanger sequencing at the Advanced Studies in Genomics, Proteomics, and Bioinformatics Core Facility, University of Hawai'i at Mānoa on an Applied Biosystems 3730XL sequencer. DNA sequences were manually checked and aligned using Geneious prime 2019.1.3 (<http://www.geneious.com>).

A portion of DNA from *Aplysilla sp. aff. rosea* samples was sequenced using the Illumina MiSeq system with Nextera DNA Flex kit library preparation. Sequencing was performed with v3 chemistry and 600 cycles (15 Gb) paired end fragmented enzymatically with a 136 bp adapters/indexes. Reads were assembled and aligned using Geneious prime 2019.1.3 (<http://www.geneious.com>). This resulted in 22,143,472 reads. *Synechococcus spongiarum* 16S

ribosomal RNA sequence (GenBank Accession EF656438.1, Erwin and Thacker 2007) was used to search for similar sequences in the sample data.

Spicule analysis

From each of the 61 sponge samples collected in the observational study, approximately 1 mm³ of material was placed on a slide then treated with bleach for 10 minutes to remove fleshy material. Spicules were then observed using a compound microscope at 400x. Images of spicules were captured using an AmScope MU1000 (10 Mega Pixel) digital microscope camera. Specimens that were not identified by DNA barcodes were compared to specimens with DNA barcodes for taxonomic placement.

Data analysis

Species diversity for the observational data was calculated using the Shannon diversity index (H) and the Simpson index (F). The Kruskal-Wallis test was then used to test for any statistically significant difference in species diversity between all sites through time and location. Post-hoc comparisons were performed using the Mann–Whitney–Wilcoxon rank sum test to determine which sites were distinct from each other. Differences in Chlorophyll-*a* concentration were also analyzed with Mann–Whitney–Wilcoxon rank sum test.

Results

The 61 sponges collected represented seven orders, eight families, nine genera, and seven species (Table 1), plus six specimens not taxonomically identified. The six unidentified specimens were placed into two Operational Taxonomic Units (OTU's) based on their gross morphological characters and spicules, for data analysis. Of the seven organisms identified to the species level, one was determined to be endemic, two were native, three were non-native and three were not known. Sponges that were identified as either endemic, native, non-native, or not known were determined by their type locality according to the World Porifera Database (<http://www.marinespecies.org/porifera/index.php>). The World Porifera Database, a sub-registry of the World Registry of Marine Species (WoRMS), is considered the authority regarding sponge species taxonomy and locality in the sponge biologist community (Van Soest et al. 2012, Costello et al. 2013). Note that, *Sigmadocia caerulea* (Coles et al. 2002) is not currently accepted as a species name; however, it is accepted as *Haliclona caerulea* and was recorded as such in this study.

Table 1: Identified sponge organisms observed in Maunalua Bay, O'ahu. The following abbreviations, E=endemic, N=ative, NN=Non-native, NK=not known are used.

Class	Order	Family	Genera	Specific (if determined)	E, N, NN, NK
Demospongiae	Dendroceratida	Darwinellidae	Aplysilla	rosea	NK
Demospongiae	Dictyoceratida	Dysideidae	Dysidea	sp.	NK
Demospongiae	Suberitida	Halichondriidae	Halichondria	bowerbanki	NN
Demospongiae	Suberitida	Suberitidae	Pseudosuberites	sp.	NK
Demospongiae	Suberitida	Suberitidae	Suberites	diversicolor	N
Demospongiae	Haplosclerida	Chalinidae	Haliclona	amboinensis	NN
Demospongiae	Poecilosclerida	Coelosphaeridae	Lissodendoryx	hawaiiiana	E
Demospongiae	Verongiida	Pseudoceratinidae	Pseudoceratina	purpurea	N
Demospongiae	Tetractinellida	Ancorinidae	Stelletta	clavosa	NN

Comparisons among sites in the present study

The number of sponges and the number of sponge species varied significantly among sites (observed abundance of individual organisms, Kruskal-Wallis chi-squared = 10.56, df = 4, p-value = 0.032, Figure 2; observed number of species present, Kruskal-Wallis chi-squared = 10.29, df = 4, p-value = 0.036, Figure 3). Post hoc analysis of individual pairwise comparisons showed that the native seagrass site was statistically significantly different from Paiko Beach for both the number of sponges observed and the number of species (observed abundance of individual organisms, Mann-Whitney-Wilcoxon rank sum test $W = 16$, p-value = 0.02; observed number of species present, Mann-Whitney-Wilcoxon rank sum test $W = 16$, p-value = 0.02) while all other sites compared to the native seagrass site were not statistically significant (Mann-Whitney-Wilcoxon rank sum test, Paiko Drive, $W = 2$, p-value = 0.07; Wailupe, $W = 0$, p-value = 0.06; Marker 22, $W = 4$, p-value = 0.07). Also, all other sites, when compared to each other in a pairwise fashion, resulted in no statistically significant difference.

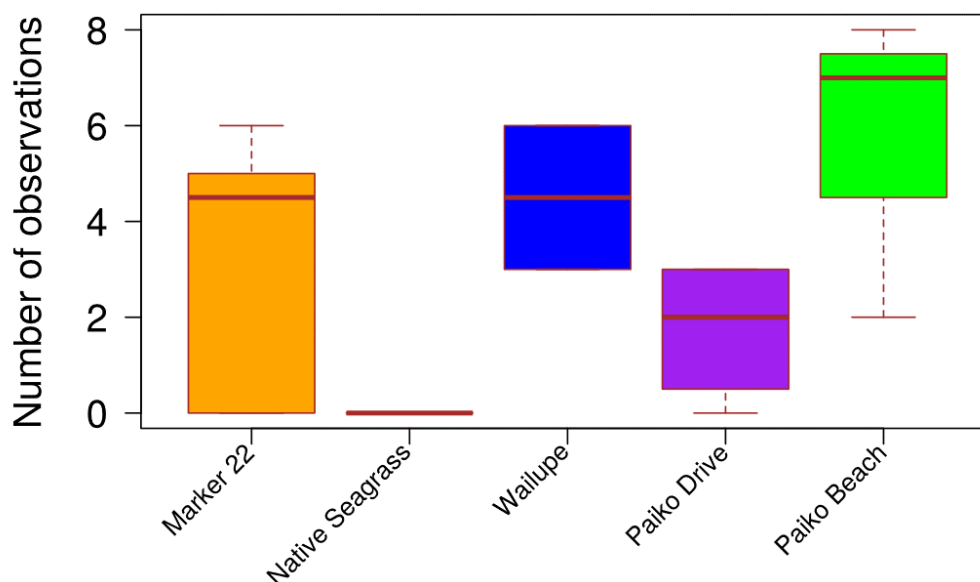


Figure 2 Number of observations of Porifera organisms regardless of species at each individual site in Maunalua Bay, O'ahu. Here and for all other graphs a boxplot representation is used. The

central thick bars indicate median values, the upper and lower boundaries of the box indicate the interquartile range (75% and 25% percentiles respectively) and dashed lines indicate the maximum and minimum values if they fall outside of the interquartile range. The difference in number of sponges counted across all sites was statistically significant (Kruskal-Wallis chi-squared = 10.56, df = 4, p-value = 0.032). The native seagrass site was statistically significantly different from Paiko Beach (Mann-Whitney-Wilcoxon rank sum test $W = 16$, p-value = 0.02).

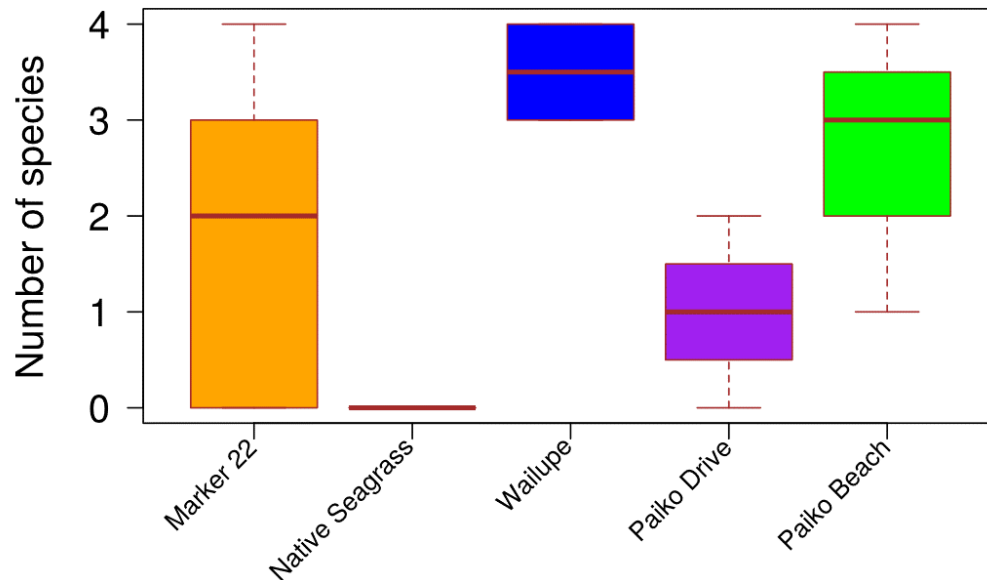


Figure 3: Number of species of Porifera observed at each individual site in Maunalua Bay, O'ahu. There was a statistically significant difference in the number of species of Porifera observed across all sites (Kruskal-Wallis chi-squared = 10.29, df = 4, p-value = 0.036). The native seagrass site was statistically significantly different from Paiko Beach (Mann-Whitney-Wilcoxon rank sum test $W = 16$, p-value = 0.02).

Comparison to earlier surveys

When the number of sponge observations and the number of species were compared from all three studies (Coles *et al.* 2002, Longenecker *et al.* 2011, present study), there was a statistically significant difference in the number of observed Porifera (observed abundance of individual organisms, Kruskal-Wallis chi-squared = 34.49, df = 8, p-value = 3.31×10^{-5} , Figure 4; observed number of species present Kruskal-Wallis chi-squared = 34.10, df = 8, p-value = 3.89×10^{-5} , Figure 5). Further comparisons of both the observed number of Porifera and the observed species, showed that the results of Longenecker *et al.* (2011) were significantly different from this study (observed abundance of individual organisms, Mann-Whitney-Wilcoxon rank sum test $W = 396$, p-value = 5.46×10^{-6} ; observed number of species present, Mann-Whitney-Wilcoxon rank sum test $W = 396$, p-value = 5.29×10^{-6}) and the Coles *et al.* (2002) study (observed abundance of individual organisms, Mann-Whitney-Wilcoxon rank sum test $W = 24$, p-value = 6.7×10^{-3} ; observed number of species present, Mann-Whitney-Wilcoxon rank

sum test $W = 24$, $p\text{-value} = 6.7 \times 10^{-3}$). Note that nonparametric tests were used which do not make any assumptions about the underlying distribution from which the data are sampled from. This allows for statistical testing in cases where the data appear to follow very different distributions such as the zero observations of Longenecker *et al.* (2011) and the present study.

However, this meta-analysis can be misleading as there was a difference in the areas sampled. Coles *et al.* (2002) sampled an area of 0.9 m^2 and Longenecker *et al.* (2011) sampled an area of 0.454 m^2 while the present study sampled 5 m^2 . Re-scaling the total of 61 observations to the sampled areas of the two earlier studies gives an expected number of observations (if abundance were constant over time) of 12.2 per square meter. A Poisson distribution was then used to calculate the probability of the number of observations over the area sampled in the previous studies (based on current abundances). There was a significant difference between the present study and both the Longenecker *et al.* (2011) and Coles *et al.* (2002) studies with P-values of $P = 0.0422$ and $P = 0.000204$ respectively.

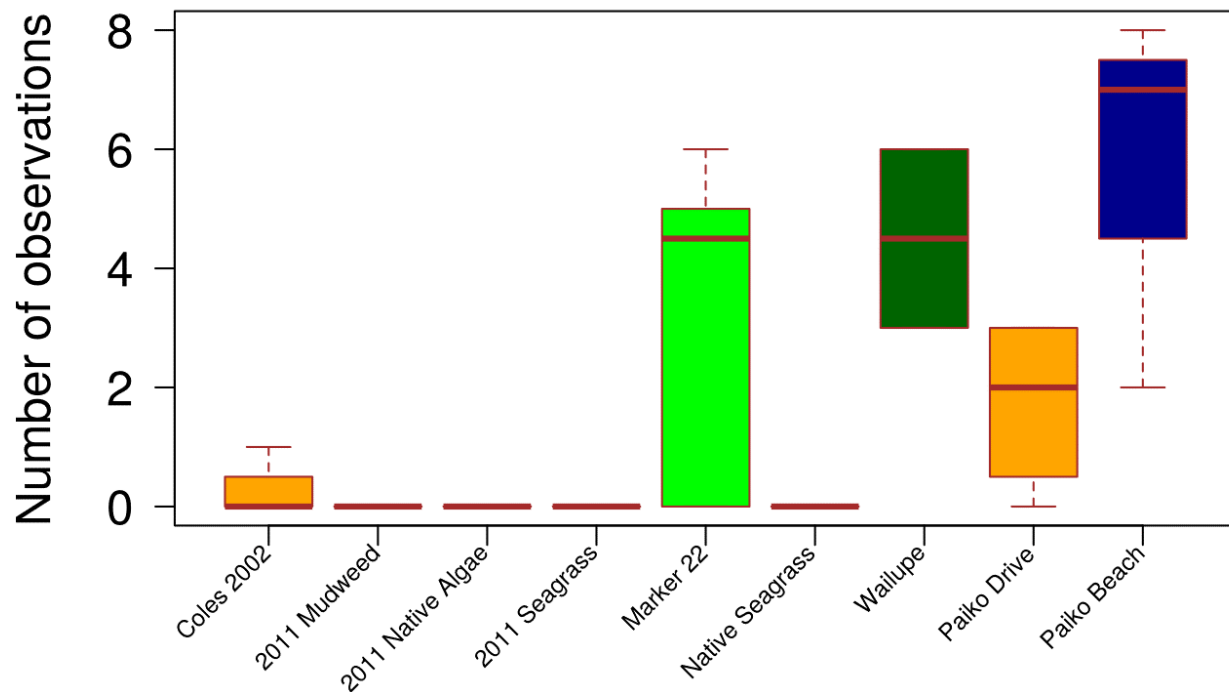


Figure 4: Number of observations of Porifera at all sites across all studies. There was a statistically significant difference in the number of observed Porifera across all three studies (Kruskal-Wallis chi-squared = 34.49, $df = 8$, $p\text{-value} = 3.31 \times 10^{-5}$). There was a statistically significant difference between the Longenecker *et al.* (2011) study and both the current study and the Coles *et al.* (2002) study (current study, Mann-Whitney-Wilcoxon rank sum test $W = 396$, $p\text{-value} = 5.46 \times 10^{-6}$; Coles *et al.* (2002) study, Mann-Whitney-Wilcoxon rank sum test $W = 24$, $p\text{-value} = 6.7 \times 10^{-3}$). 2011 refers to data obtained from the Longenecker *et al.* (2011).

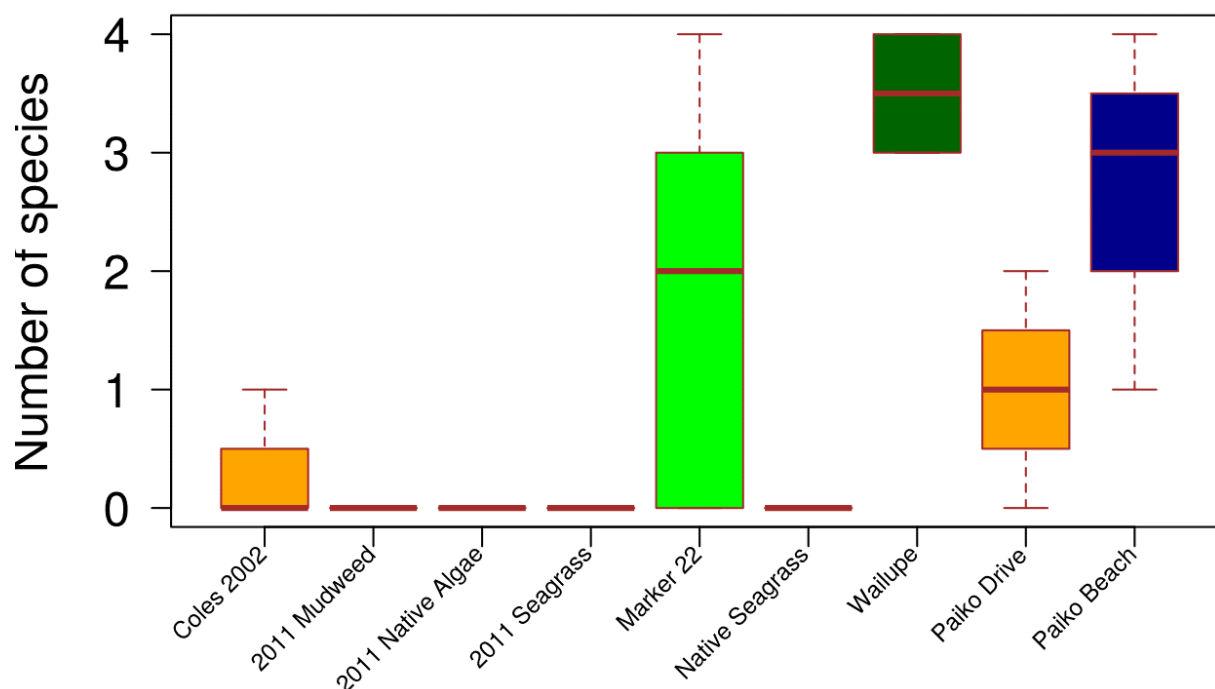


Figure 5: Number of species of Porifera observed at all sites across all studies. There was a statistically significant difference in the number of species of Porifera observed across all three studies (Kruskal-Wallis chi-squared = 34.10, df = 8, p-value = 3.89×10^{-5}). There was a statistically significant difference between the Longenecker *et al.* (2011) study and both the current study and the Coles *et al.* (2002) study (current study, Mann-Whitney-Wilcoxon rank sum test $W = 396$, p-value = 5.29×10^{-6} ; Coles *et al.* (2002) study, Mann-Whitney-Wilcoxon rank sum test $W = 24$, p-value = 6.7×10^{-3}). 2011 refers to data obtained from Longenecker *et al.* (2011).

There was no statistically significant difference across all sites and studies for either the Shannon's diversity and Simpson's diversity indices (Kruskal-Wallis chi-squared = 8, df = 8, p-value = 0.41; Kruskal-Wallis chi-squared = 8, df = 8, p-value = 0.43). Likewise, the inverse relationship between the Shannon's diversity and Simpson's diversity indices was not significantly different (Kruskal-Wallis chi-squared = 2.39, df = 1, p-value = 0.12).

Cyanobacteria results

There was no statistically significant difference in Chlorophyll-a concentration between the light and dark sponge extracts ($W = 284$, p-value = 0.1747; Figure 6). Ninety-one reads from the genomic sequencing were identified as being similar to the *Synechococcus spongiarum* barcode used to search the dataset. However, when these sequences were searched on GenBank they matched a cyanobacterium that appears to be an undescribed *Synechococcus* sp. (accession EU919110.1) that differs in sequence identity from *Synechococcus spongiarum* by 5.16% of the base pairs in an alignment generated by BLAST. This cyanobacterium is associated with sponges-*Mycale armata* in Hawai'i (GenBank EU919110.1), *Haliclona simulans* (GenBank FJ999601.1) and *Gelliodes carnosus* (Li *et al.* 2011) in the South China Sea,

Hymeniacidon heliophila in the Atlantic (Weigel and Erwin 2016), and *Ircinia* spp. in the Mediterranean (Erwin *et al.* 2012).

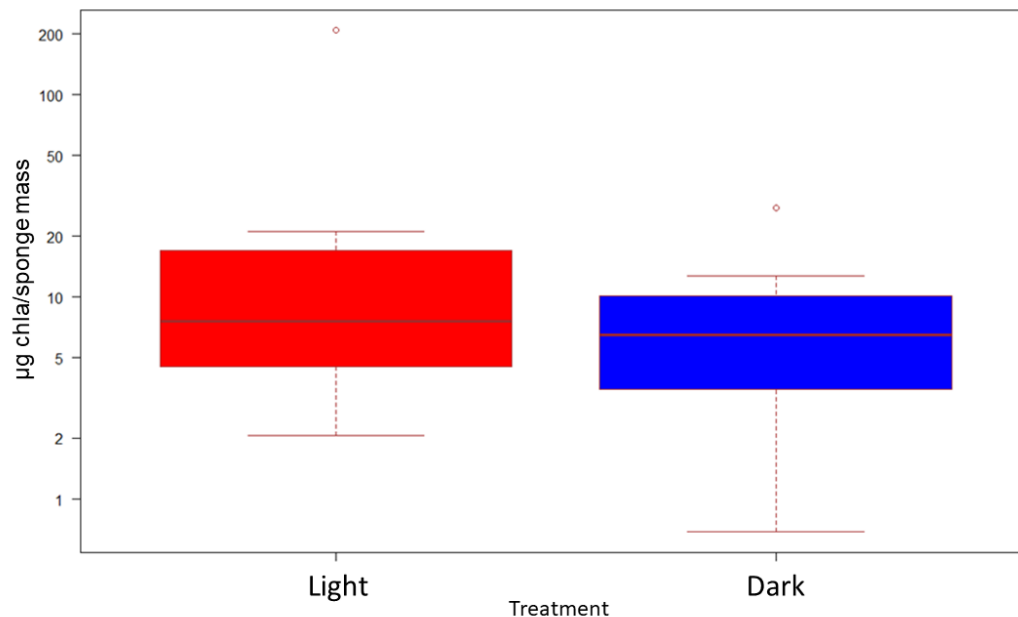


Figure 6: Range of Chlorophyll-a concentrations observed. Statistical outliers are indicated in the boxplot by small dots. N=24 for the light treatment and N=19 for the dark treatment.

Discussion

Maintaining biodiversity in native ecosystems is an important aspect of conservation, and careful consideration regarding disturbances to the ecosystem should be a part of any restoration plan. It has been hypothesized that the greater the biodiversity of an ecosystem the less likely potential invasive species will successfully invade, the premise being that if the niche space is already occupied there is less opportunity for invasive species to become established (Stachowicz *et al.* 1999; Hulme *et al.* 2006; Ballari *et al.* 2016; Lurgi *et al.* 2018). Disturbances such as sedimentation or invasive algae removal increase substrate availability for primary native and invasive successors to settle, influencing the likelihood for native species to recruit to the site. Little to no work has been done to understand successional patterns over extended periods of time in Maunalua Bay, O'ahu. This study has shown that there has been a significant increase in Porifera species within the last nine years suggesting that they might be tertiary colonizers, utilizing the novel niche space provided by *Gracilaria salicornia* and *Avrainvillea amadelpha*. Although invasive algal mats have been present in Maunalua Bay for nearly four decades, it is still unclear as to why the recent abundance of Porifera has dramatically increased. This may be the result of complex interactions with additional anthropogenic stressors over time and/or lags in species establishment and abundance.

Comparisons of sponge species across all three studies (Coles *et al.* 2002, Longenecker *et al.* 2011, and the current study) may be misleading. The present study covered a broader geographic range of Maunalua Bay, while the previous studies focused on more specific regions. If there are significant long-term temporal effects in community assembly following a disturbance (*sensu* Strayer *et al.* 2006), much of the variation among the sites may be due to a lag in successional patterns. It is possible that the Porifera observed by Coles *et al.* (2002) were just beginning to become established. While the absence of Porifera in the Longenecker *et al.* (2011) study is surprising, it suggests a potentially dynamic change in the successional regime, i.e. transient reductions in species abundance in the intermediate stage.

Nearly half (3 of 7) of the sponges observed in 2019 were, when they could be identified to the species level, endemic or native to Hawai'i. It is not clear whether these species were present prior to the shift from seagrass to invasive algae that the bay has experienced; however, given that there were no Porifera observed in the native seagrass bed sites in 2019, it is unlikely that they were present prior to this shift. Given the relatively low percentage of native seagrass area surveyed in detail, one should be cautious in making such assumptions even though haphazard observations of native seagrass beds suggested no Porifera were present. The presence of sponges on the concrete pylons of the channel markers suggests that sponges can potentially be more widespread in the bay where there is hard substrate (i.e., Paiko Beach). The significant difference in the abundance of sponges in terms of both number of observations and number of species between sites and the high number at Paiko Beach, despite high rates of sedimentation, suggest hard benthic substrates are conducive to Porifera colonization regardless of the presence of invasive algae. Future studies should include a wider variety of substrates when accessible.

There was no significant statistical difference among sites in terms of measures of species diversity (Shannon diversity and the Simpson index). This may suggest that no one substrate type is best suited to support the present sponge community of Maunalua Bay. Further comparisons are necessary to understand if there is a difference in species that inhabit the different substrates; however, this will also need to be accompanied by a long-term time series to capture any changes in a possible successional regime.

The links between species diversity and species richness and productivity are notoriously difficult to establish (Adler *et al.* 2011; Hillebrand *et al.* 2018). However, there is evidence that, apparently paradoxically, increasing nutrients can result in lower biodiversity (Isbell *et al.* 2013). One hypothesis is that invasive algae have sequestered resources, lowering nutrient availability, and thus increasing Porifera species diversity. Beta diversity between sites is positively correlated with stability in reef systems (Mellin *et al.* 2014). Another potentially useful follow-up question would be to compare diversity from other sites around Hawai'i with and without invasive algae.

Although there was no statistical difference in the abundance of cyanobacteria in this study, as evidenced by similar Chlorophyll-a concentrations in light and shaded areas, it is possible that the algal mats did not provide a sufficient level of darkness to inhibit cyanobacterial growth in its sponge host. Recent studies suggest that approximately 25% of all cyanobacteria have the ability to harvest weaker far-red light in low light conditions by incorporating Chlorophyll-f into their photosystem I complexes (Gisriel *et al.* 2020). Although this study suggests that sponge recruitment in invasive algal mats is most likely not driven by resource partitioning of

cyanobacteria, there is evidence that sponges may still be using cyanobacteria as an optional resource. Whole genome sequence data revealed the presence of *Synechococcus* sp. in the sponge *Aplysilla rosea*, which was only found in heavily shaded sections of *G. salicornia* mats out of direct sunlight, suggesting that it may be using the alternative Chlorophyll-f. Further studies should investigate how these mats may provide protection from disturbance or predation of sponges by other species.

While this study has shown an apparently increasing presence of Porifera species within the greater Maunalua Bay area, and that this abundance is dynamic, it underscores the need for further time series investigations within and outside of Maunalua Bay. Importantly, comparisons on the order of decades are needed to further understand the overall trajectory of species change.

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