ECOLOGY OF MESOPHOTIC MACROALGAE AND *HALIMEDA KANALOANA* MEADOWS IN THE MAIN HAWAIIAN ISLANDS

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DEDICATION

This dissertation is dedicated to the infamous First Lady of Limu, Dr. Isabella Aiona Abbott. She was my inspiration for coming to Hawai‘i, and part of what made this place special to me. She helped me appreciate the intricacies of algal cross-sectioning, discover tela arachnoidea, and understand the value of good company

(and red wine, of course).

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ABSTRACT

This dissertation focused on the distribution and abundance of mesophotic macroalgal assemblages (MMA) in mesophotic coral ecosystems (MCE) in the Main Hawaiian Islands (MHI), with an emphasis on the natural history and recovery response of *Halimeda kanaloana* meadows.

Submersibles, a remotely operated vehicle, and technical diving were used to survey MMA at 59 sites from 40 to 212 m depths around Oʻahu, Maui, Lānaʻi, Kahoʻolawe and Molokaʻi. Seventy-six species of frondose macroalgae were described. Thirty species were new records for Hawaiʻi or new species, with 45% of the flora only found at mesophotic depths. Eleven dominant algal assemblages were encountered, covering several hundred meters to kilometers squared. Mesophotic algal meadows of an invasive species (*Avrainvillea amadelpha*) and a proposed new genus (resembling *Udotea*) were discovered off Oʻahu. MMA in the MHI are abundant, diverse, and spatially heterogeneous. The biology of mesophotic macroalgae will have substantial implications for tropical food web ecology, biodiversity, and biogeography. MCE in Hawaiʻi offer decades of research potential given their areal extent, ecological importance, and reservoir of genetic diversity.

H. kanaloana meadows cover large portions of the sand-dominated environment in the MHI, yet little is known about their ecology or contribution to carbonaceous sediments. To help close this gap, the growth, densities, lifespan, herbivory, quantity of calcium carbonate ($CaCO_3$), and reproduction of *Halimeda* meadows were surveyed at multiple locations over a four year period around Maui from 10 to 85 m depths. *Halimeda* were

generally long-lived perennials, with rapid growth and high densities (up to 314 plants per m^2), producing up to 1883 g $CaCO_3$ m^{-2} y^{-1}. Deep psammophytic algal meadows appear to be an integral and highly productive part of the tropical ecosystem in Hawai‘i.

Both natural and anthropogenic disturbance were observed in *H. kanaloana* meadows, stimulating a manipulative experiment on the recovery of *Halimeda* to anchor scar damage at 23 m depth. Recovery in a near-by anchor scar at 27 m depth was also monitored. Recovery in the experiment and anchor scar occurred in 1½ to 2 years. The use of moorings would be beneficial to the health and stability of deep *Halimeda* meadows.

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CHAPTER I:

GENERAL INTRODUCTION AND BACKGROUND

"The mesophotic coral ecosystem (MCE) is characterized by the presence of light-dependent corals and associated communities that are typically found at depths ranging from 30 to 40 m and extending to over 150 m in tropical and subtropical regions. Coral, sponges, and macroalgae are the dominant communities in the mesophotic zone providing structural habitat and contributing to overall biodiversity." (Hinderstein 2010).

This definition of MCEs was first described at a scientific workshop on 12–15 July 2008, in Jupiter, Florida. The workshop was hosted by the U.S. National Oceanic and Atmospheric Administration (NOAA) Center for Sponsored Coastal Ocean Research (CSCOR), Office of Ocean Exploration and Research/NOAA's Undersea Research Program (OER/NURP), and U.S. Geological Survey (USGS) to identify critical research and resource management needs for MCEs. The outcome was a special issue of *Coral Reefs* dedicated to review and research papers that reflect the current scientific understanding of these ecosystems and the underlying mechanisms that regulate them, as well as potential resource management implications. I was an invited participant in the workshop, and was a contributing co-author towards papers on the distribution (Rooney et al. 2010), community ecology (Kahng et al. 2010), and diversity (Reaka et al. in prep) of macroalgae and corals in MCEs. The following sections on MCEs were prepared for these publications, and together provide a comprehensive literature review of this system in terms of macroalgal assemblages. Topics in this introduction include the abiotic and

biotic factors affecting mesophotic macroalgal abundance, vertical zonation, comparisons between the Indo-Pacific and Caribbean floras, and invasive species in MCE's. This information provides the background for Chapter 2 on the distribution and diversity of mesophotic macroalgae in Hawai'i. The remaining introduction focuses on information pertaining to *Halimeda kanaloana* meadow ecology and carbonate sand production, which relates to Chapters 3 and 4. Chapter 5 includes a summary of the overall conclusions, significance, and suggested future research.

Irradiance

The low light environment of the mesophotic requires photosynthetic organisms, such as algae and zooxanthellate corals, to be highly efficient at light capture and photosynthesis. Surprisingly, mesophotic algae are often as productive as shallow water algae despite the low light environment of the mesophotic (Jensen et al. 1985, Littler et al. 1986). Certain groups of algae are well adapted to growth in the mesophotic, and are conspicuous members of the deep-water algal assemblage. Green algae in the Bryopsidales (e.g. *Halimeda*, *Codium*, *Caulerpa*, *Udotea*, and *Avrainvillea*) are often particularly abundant, and may form continuous beds or meadows (Littler et al. 1985, Litter et al. 1986, Blair & Norris 1988, Drew and Abel 1988, Norris & Olsen 1991, Friele et al. 1995, Aponte & Ballantine 2001, Drew 2001, Leichter et al. 2008).

The success of green algae in deeper tropical waters may be attributable to an optimum spectral composition enriched in blue and green wavelengths for green algal photosynthesis at depth (Kirk 1994). Some green algae possess unique pigments, such as the carotenoid siphonaxanthin, that enhance the absorption of light (Yokohama et al.

1977, Yokohama 1981). Physical changes in plant construction and morphology can enhance photon capture. Species such as *Codium* are optically thick, and have pigment concentrations that appear almost black (Kirk 1994). All of the deep water green algae described from San Salvador Is. had siphonous or giant-celled morphologies that increased total surface area, and increased light capture for photosynthesis (Norris & Olsen 1991). For instance, a deep water endemic species (*Cladophora vandenhoekii*) has cell sizes much larger than those of other shallower species (Norris & Olsen 1991). Deep water *Halimeda copiosa* (to 152 m depths) increases surface area by increasing the diameter of the surface utricles by 15% as compared to shallower plants (Blair & Norris 1988). A thinner lamina (and subsequently lower specific carbon content) also ensures higher specific growth rates than thick plants when light is limiting (Markager & Sand-Jensen 1992). The thin plants of Dictyotalean deep-water species were found to have a higher photosynthetic capacity than species with thicker forms, such as calcified, coarsely branched or leathery (Peckol and Ramus 1988). Thin plants may also increase the alga's nitrogen-uptake ability (Peckol and Ramus 1988).

Metadata for lower depth limit vs K_d PAR for algae

The extinction coefficient for downwelling irradiance (K_d) in the PAR frequency band is a useful relative measure for comparing light attenuation at different locations. If irradiance is the sole limiting factor at the lower depth limit for specific algae across a number of locations, then a linear relationship could exist between K_d and the lower depth limits of algal distribution. To examine this relationship, data on the lower depth limits and K_d of algal species were collected from published mesophotic algal surveys

(Agegian & Abbott 1985, Hillis-Colinvaux 1985, Littler et al. 1985 (K_d calculated from Table 1), Blair and Norris 1988, Liddell and Ohlhorst 1988, Liddell et al. 1988, Aponte & Ballantine 2001 (K_d calculated for this region from Reed 1985). Algae were categorized by species or functional group (Steneck and Dethier 1994) because of discontinuities among species data at most locations. A functional grouping was necessary to compare data from multiple locations. Although this approach may mask species-specific variability, the functional grouping incorporates both productivity and herbivore-induced disturbance potentials of the environment, and often produces similar patterns in algal biomass, diversity and dominance when examined at a functional group level (Steneck and Dethier 1994). Those functional form groups containing at least four data points were selected for analysis, including the articulated corallines (or jointed calcified Chlorophytes: *Halimeda copiosa*, *H. gracilis*, *H. cryptica*, *H. distorta*), crustose corallines and related crusts (Rhodophyte *Peyssonnelia* spp. and nongeniculate coralline algae), and a corticated foliose alga (Phaeophyceae *Lobophora variegata*). Linear regression analyses were performed using Minitab® 15.1.1.0. There was a significant relationship between the lower depth limits and K_d for articulated corallines and encrusting red algae (Figure 1), suggesting that light may be a major limiting factor for the lower depth limit for these groups. However, there was no significant difference (Figure 1) in the corticated foliose group (*L. variegata*), indicating that other factors besides irradiance are important in determining the vertical extension of this alga. This analysis can be improved with more samples (n=4 in this study). The heavier calcification found in the articulated and encrusting algae is complemented by both *Halimeda* spp. and *L. variegata* also containing anti-herbivory compounds (Norris &

Fenical 1982, Gerwick et al. 1985, Paul and van Alstyne 1992, Meyer et al. 1994). Despite this chemical defense, the abundance of *L. variegata* has been dramatically affected by urchins and can experience a high turn-over (Ruyter van Steveninck and Breeman 1987). *L. variegata* may also exhibit seasonal fluctuations in abundance (Peckol and Searles 1984, Nugues and Bak 2008) that may cause variation in the lower depth limit observed. Additional mesophotic algal surveys are needed in other locations with differing water clarity to test these models more rigorously and to develop models for other functional form groups.

Temperature, nutrients, and hydrodynamics

The worldwide distribution patterns of macroalgae are mainly determined by global temperature gradients (Lüning 1990) . On a smaller scale at a particular site, the thermocline may influence the lower depth limit of tropical algae sensitive to colder water. For instance, chlorophytes containing siphonaxanthin are mainly of tropical origin, and may require, as a group, higher temperatures for growth than the rhodophytes and phaeophyceae (Kirk 1994). Predominantly tropical floras also have a low tolerance to the long exposure of low winter temperatures. Temperature fluctuations of 10° C may account for the strong seasonality in the deep water flora in Florida (Cheney and Dyer 1974). This colder water, however, may also encourage more temperate algal species to thrive in tropical waters, and may increase the diversity of algae found in the mesophotic. Graham et al. (2007) predicted the occurrence of deep-water kelp forests in the Galapagos Islands by pinpointing areas of upwelling in tropical regions. Temperate species, such as *Desmarestia ligulata* and *Kallymenia* sp., have also been found in deep

water in the Northwestern Hawaiian Islands (NWHI) (Abbott and Huisman 2003, McDermid and Abbott 2006).

Despite the negative effects of colder temperatures on some tropical algae, the synergistic effect of temperature, nutrients, and hydrodynamics as cold, nutrient-rich upwelled water may positively influence the abundance and distribution of tropical mesophotic algae. In the Great Barrier Reef, tidally-driven jet upwelling provides nutrients for deep water *Halimeda* meadows, and may have provided the physical conditions conducive to *Halimeda* growth for much of the Holocene period (Wolanski et al. 1988, Drew and Abel 1988, Drew 2001). Cooler nutrient-rich bottom water, presumably of continental-slope origin, from the Carolina outer continental shelf increased ambient nitrogen three-fold, and provided deep water macroalgae with a critical source of nutrients during the summer (Peckol and Ramus 1988). In Florida, decreased bottom temperatures and increased nutrients indicated short-term upwelling events that influenced the high rates of benthic productivity observed in deep water algae from 40 to 70 m depths (Leichter et al. 2008). However, temperature, nutrients, and hydrodynamics may not always explain patterns of high algal abundance. Parrish and Boland (2004) found no difference in yearly temperature records between areas of high-cover (> 70%) and low-cover (<30 %) algal biotopes in the NWHI.

Geomorphology

Calcified, mesophotic algae may have a profound impact on the geomorphology of the deep reef. The vertical carbonate sediment accretion of deep water *Halimeda* is as fast, and probably faster, than those of nearby coral reefs (Marshall & Davies 1988). Large

algal bioherms resulting from the accretion of algal-derived carbonate sediments are found throughout the tropics, from the Southwest Caribbean Sea (Hine et al. 1988) to the Great Barrier Reef (Drew 2001) and India (Purnachandra Rao et. al 1994). Deep water rhodolith beds are also important producers of calcareous sediments and critical in paleoenvironmental interpretations (Littler et al. 1991). Psammophytic (sand-dwelling) algae, such as dense *Halimeda* meadows, can also change their surrounding physical environment by acting as a baffle and entraining finer-grained sediments (Friele et al. 1995).

Physical disturbances

Physical disturbances, such as sediment burial or scour by shifting sands, may negatively affect algal abundance and/or their lower depth limits. Leichter et al. (2008) found an overriding effect of substratum availability and sand scour on the lower depth limit of deep-water algae in Florida. Algae were absent from areas of extensive sand with migrating sand waves and ripples, and the lower depth limit of macroalgae coincided with extensive sand deposits from 70 to 80 m depths. The sites with the highest overall cover of sand also had the lowest macroalgal cover. Aponte & Ballantine (2001) also recognized sedimentation as having a substantial influence on algal cover with increasing depth through the movement of sand down the fore reef.

Competition for space

Although competition for space (and therefore optimum light capture) likely affects the abundance of mesophotic algae, experimental studies testing competition have not yet

been conducted. Heavy ephiphyte loads on perennial algae, such as dense growths of *Lobophora variegata* covering *Halimeda* (Drew and Abel 1988), would most likely have a negative impact on the host species through increased drag in currents and shading. *Halimeda* may also be outcompeting corals for space by producing more carbonate sediments and creating a habitat unsuitable for coral growth (Drew and Abel 1988). Invertebrates may also be competing for space with mesophotic algae. Gilmartin (1960) observed burrowing invertebrates in the sand that may have prevented deep water macroalgae from occurring in soft sediments.

Herbivory and Faunal Interactions

The high abundance of some deep-water algae may be influenced by low herbivory in the mesophotic environment. Low herbivore activity may be due to a low abundance of herbivores (Brokovich et al. 2010) and/or low grazing pressure due to the anti-herbivory characteristics of the algae. Larkum et al. (1967) noted very few echinoderms below 30 m depth, although numerous gastropods were found in macroalgal samples that could have a significant effect on algal abundance. The *Diadema* die-off in 1983 in Curaçao had a minor impact on the percent cover of deep water (40 m) *Lobophora*, most likely from a low density of *Diadema* at that depth (Nugues & Bak 2008). Gilmartin (1960) noted that herbivorous fish did not appear to influence the composition of abundance of deep water algae to 65 m. However, Leichter et al. (2008) found algal recruitment on settlement plates was low in close proximity to the coral reef, and observed herbivory halos at 50 - 60 m depths around wrecks. The calcium carbonate in calcified algae (e.g. *Halimeda* and *Udotea*) may deter herbivory (Schupp and Paul 1994). Many of the Bryopsidales are also

chemically defended with anti-herbivory compounds (Meyer et al. 1994, Paul and Van Alstyne 1992). Phaeophyceae belonging to the Dictyotales, such as *Lobophora* and *Stypopodium*, are also common constituents of the deep-water flora (Van den Hoek et al. 1978, Peckol and Ramus 1992), and have highly toxic anti-herbivory compounds for both fish and urchins (Norris & Fenical 1982, Gerwick et al. 1985). Studies are specifically needed with deep water algae to determine grazer susceptibility given possible intraspecific variations in secondary metabolites (Peckol and Ramus 1992).

Vertical zonation of macroalgae in the Indo-Pacific

The vertical zonation of mesophotic algae in the Indo-Pacific has not been as well studied spatially as the Caribbean. The most thorough Indo-Pacific mesophotic algae studies have focused primarily on sites around Hawaii and Johnston Atoll. Doty et al. (1974) did not find any distinctive deep water flora, zonation, or dominance by one phylum from dredges between 10 to 165 m in Hawai‘i, although patterns of zonation may have been difficult to discern from dredged samples over a wide depth range. In contrast, Agegian and Abbott (1985) described three slightly different algal zones at Penguin Bank (southwestern tip of Moloka‘i) and Johnston Atoll based on submersible surveys. At Penguin Bank, the shallow zone (45-70 m) contained a diverse assemblage of algae (i.e. *Lobophora variegata*, *Dictyota friabilis*, *Halimeda* sp., coralline algal maerl/rhodoliths, *Mesophyllum mesomorphum*, and *Peyssonnelia rubra*), the mid-depth zone (90-110 m) was characterized by *Codium mamillosum* and crustose coralline algae, while the deep zone (110-182) was composed primarily of crustose coralline algae. Macroalgae at Johnston Atoll were less diverse but found at greater depths. The shallow water zone (45-

120 m) contained siphonous green algae (i.e. *Halimeda* spp. and *Caulerpa* spp.) with a patchy distribution, the mid-depth zone (120-180 m) consisted of a low abundance of *Halimeda gracilis* and crustose coralline algae, and the deep zone (180-250 m) contained crustose coralline algae covering 40-60% of the substratum. The occurrence of crustose coralline algae in the deepest zone in Hawai‘i was similar to Littler et al. (1986) at San Salvador, Bahamas.

Caribbean vs. Indo-Pacific mesophotic flora

Mesophotic algal surveys in the Caribbean and Indo-Pacific have described several new species and records, and the floras share some of the same abundant, conspicuous species. This includes species such as the Chlorophytes *Halimeda copiosa* (Hawai‘i, Agegian & Abbott 1985; Enewetak, Hillis-Colinvaux 1985; Bahamas, Blair & Norris 1988; Jamaica, Liddell et al. 1988), *Halimeda discoidea* (Hawaii, Doty et al. 1974; Bahamas, Blair & Norris 1988; Jamaica, Liddell et al. 1988; Florida, Leichter et al. 2008), and the Phaeophyceae *Lobophora variegata* (Hawai‘i, Agegian & Abbott 1985; Florida, Leichter et al. 2008; Curaçao, Nugues and Bak 2008;), and *Dictyopteris plagiogramma* (Hawai‘i, Agegian & Abbott 1985; Bahamas, Blair & Norris 1988; Florida, Leichter et al. 2008). The mesophotic Rhodophytes are diverse, but appear less similar between regions. This may partly be due to sampling bias, as it is often difficult to adequately sample less conspicuous species such as turf algae. Unfortunately, nongeniculate coralline algae and rhodoliths are usually not identified to species given the difficulty of identifying these species *in situ*. Floristic studies on these groups are desperately needed to properly interpret biogeographical patterns in the mesophotic zone.

Invasive species in MCEs

Biological invasions by introduced species have a profound effect on species diversity (Levine 2000, Stachowicz and Byrnes 2006, Worm et al. 2006) and fundamentally shift the ecology of a region by modifying ecosystem processes, community composition, and food-web dynamics (Vitousek 1990, Semmens et al. 2004). Consistent with Williams and Smith (2007), "introduced" is defined as a species introduced beyond its native range by human activities and successfully established. The "invasibility" of a system is the susceptibility of a native community to the establishment of an introduced species. The term "invasive" refers to a condition whereby a species becomes excessively abundant, usually causing ecological or economic harm (Boudouresque and Verlaque 2002, Williams and Smith 2007). Despite the high number of introduced species in marine environments (Cole 1999), few actually become invasive. Williamson and Fitter's (1996) tens rule states that on average, one in 10 imported species appear in the wild and become introduced, one in 10 of the introduced become established, and one in 10 of the established become invasive. This simplistic rule has been applied to invasive species in the marine environment, and appears to fit well (Boudouresque and Verlaque 2002). Frequently cited vectors for marine species introductions include aquarium introductions (Semmens et al. 2004), intentional or accidental transport in shipments of fish products, deliberate release to enhance commercial fisheries (Randall 1987), ballast water (Carlton and Geller 1993), hull-fouling, aquaculture, opening of navigable canals (Occhinipinti-Ambrogi 2007), fishing gear, shellfish farming, and researchers (Williams and Smith 2007). Even the political climate can influence introductions; Cole (1999) found two

periods of relatively high introduction rates in Pearl Harbor corresponding to wartime periods. Once introduced, invasion success may be influenced by intrinsic growth rates, trophic status, type of reproduction, dispersal strategies (Ceccherelli et al. 2002, Inderjit et al. 2006), propagule supply (Levine 2000), and persistence, as well as attributes of the recipient community, such as degraded habitat, productivity, and species interactions (Semmens et al. 2004). Although many of the vectors listed primarily affect nearshore marine environments, transport of invasive species from shallow to deeper waters is a likely scenario given the effective dispersal strategies and generalist nature of many invasive species.

The invasibility of MCEs and the resulting effect on biodiversity is challenging to predict given the lack of experimental studies in this environment. However, current invasion biology theory suggests that isolated, island-based MCEs would be sensitive to invasion given that most extinctions resulting from species invasions have been on islands as opposed to mainlands (Sax and Gaines 2008). The biodiversity of a given MCE may also affect its invasibility, although the relationship between diversity and invasibility is often-contested (Stachowicz and Byrnes 2006). Experimental and observational studies often produce conflicting results regarding the effects of native species diversity on community susceptibility to invasion. Although many studies predict that diverse communities are less susceptible to invasion (Stachowicz et al. 1999, Kennedy et al. 2002, Worm et al. 2006), others suggest the most diverse are the most invaded (Levine 2000, Riccardi 2001). The degree to which biodiversity confers resistance to invasion is likely linked to levels of resource availability (Levine 2000, Shea and Chesson 2002**,** Stachowicz and Byrnes 2006). Tilman's Rule R* (where R is resource

availability) predicts that when the resident's R* is greater than the invader's R*, invasion is favored (Tilman 1982). This can happen when 1) the invader has a higher resource acquisition rate than the resident or 2) when the invader has lower maintenance requirements than the resident (Shea and Chesson 2002). The limiting resource for invasive photosynthetic organisms in MCEs may be irradiance. Although some invasive macroalgae like *Caulerpa racemosa* can photoacclimate (Raniello et al. 2006) and occur to 70 m depths (Klein and Verlaque 2008), their abundance is generally low at deeper depths, with the highest abundance occurring from 0 to 30 m depths. The structural complexity of the native community may also influence the invasibility. Ceccherelli et al. (2002) found that an increased number of species in macroalgal assemblages reduced invasion by *Caulerpa* species, but the structural complexity (i.e. encrusting, turf, and erect species) in the assemblage was more important than the number of species. MCEs are typically in remote, offshore locations, and are often considered isolated from human-related stressors (such as introduced species) and the subsequent degradation of shallower reefs (Bak et al. 2005, but see Menza et al. 2007). However, numerous introduced invasive species have been described in MCEs around the world. In Hawai‘i, Kahng and Grigg (2005) observed the invasive octocoral *Carijoa riisei* overgrowing large beds of the scleractinian plate corals *Leptoseris* sp. and *Pavona* sp., creating an underwater "prairie" of white polyps at 60-70 m depths. *C. riisi* also causes large-scale mortality of Hawaiian black corals (*Antipathes dichotoma* and *A. grandis*) from 75-100 m depths (Grigg 2003). The success of the *C. riisei* invasion may be attributable to its smothering, vegetative growth and Hawai‘i's depauperate octocoral fauna (Kahng and Grigg 2005). Although successful invasions of tropical marine fish are considered rare,

this may be due to a lack of attention to marine as compared to freshwater environments (Semmens et al. 2004). In the Western North Atlantic, the invasive Indo-Pacific lionfish *Pterois volitans/miles* complex is now found from 30 to 100 m depths (Whitfield et al. 2007). The potential impacts of lionfish to native communities are direct predation, competition, and overcrowding, with a high number of lionfish increasing the potential for cascading impacts throughout the food chain (Whitfield et al. 2007). In the Bahamas, the presence of lionfish was correlated with a significant decrease in several guilds of native coral reef fish, causing a phase transition to algal-dominated (50% benthic cover) communities and decrease in the percent cover of corals and sponges (Lesser and Slattery 2011). Between 1955 and 1961, the Hawaiian Islands Division of Aquatic Resources attempted to introduce 11 species of marine fishes (Randall 1987). Of these, I've frequently observed the bluestripped snapper (*Lutjanus kasmira*) in MCEs in Hawai'i, although their impact on MCEs remain unknown.

MCEs have been neglected from the majority of long-term studies documenting shifts to algal abundance in degraded coral reefs. Species such as the brown alga *Lobophora variegata* have increased on many degraded reefs (Mumby et al. 2005), and have been found to have a negative effect on corals in deep reefs (Nugues and Bak 2008). In the Mediterranean Sea, the introduced green algae *Caulerpa taxifolia* and *Caulerpa racemosa* are among the most well-known invasive species, and can be found at similar depths as MCEs (Belsher and Meinesz 1995, Klein and Verlaque 2008). *C. taxifolia* and *C. racemosa* have a tremendous impact on invaded environments, reaching a high abundance and potentially threatening biodiversity (Boudouresque et al. 1995, Ceccherelli and Cinelli 1997).

Studies on the composition and distribution of species in MCEs are few compared to shallower coral reefs. Thus, the decline of existing MCE populations from impacts of invasive species could easily be overlooked from a lack of previous data or insufficient taxonomic resolution (Occhinipinti-Ambrogi 2007). Removing large populations of established invasive species from deep water environments is highly unlikely given the difficulty of accessing MCE, and the biocontrol of marine invasives is not advised (Secord 2003). Feasible alternatives for restricting the spread of invasive species in MCE includes more investment in invasion prevention tools (such as informed management strategies), continued monitoring and descriptions of existing populations, and early detection and eradication when invasions are small (Secord 2003). Technical diving and the use of rebreathers might allow enough time at deeper depths for limited removals. Rigorous, manipulative experiments over broad temporal and spatial scales are needed to elucidate the impacts of invasive species on MCE biodiversity, as well as comparable studies between the invaded habitat and the invasive species native range.

Halimeda ecology

Keystone species are regarded as extremely important for conservation purposes, and have been (conceptually) sustaining communities since first described by Paine (1966, 1969). The original keystone species concept applied to predators that increased diversity (Paine 1969), whose impact on their community was large and disproportionate relative to their abundance (Power et al. 1996). Application of the keystone label to species other than predators led to the development of other terms, each referring to the specific mode

of action or behavior (Piraino et al. 2002). The keystone species concept has been applied to ecological dominants as "key species" (sensu Odum 1971) due to their bio-architectural complexity (e.g. plants, corals, and other less-known reef-building organisms) hosting a variety of interstitial spaces with the bio-construction (Huston 1994). Similarly, "ecosystem engineers" (Jones et al. 1994, 1997), or "habitat modifiers" (Mills et al. 1983), were shown to have keystone effects because they modify the chemical and/or physical features of their environment (Piraino et al. 2002).

Regardless of the term used, *Halimeda kanaloana* (Ellis) Lamouroux is a key species in Hawaiian benthic reef communities, providing an expansive habitat for numerous macrofauna through the creation of structural complexity over otherwise featureless expanses of sand. *Halimeda* meadows occur from 10 to 90 m depths with often 100% cover over soft sediments (Figure 2) in the Maui Nui complex (Verbruggen et al. 2006). Yet, there have been few studies examining the role of *Halimeda* meadows in coastal benthic reef communities in Hawaii, and little is known about their biology in this area. Basic information for the population dynamics of this coral reef ecosystem keystone species is needed for resource managers to design ecologically effective marine protected areas, and to understand the habitat requirements of organisms found to associate with these meadows. *Halimeda* meadows host a likely unique assemblage of fish and invertebrates, may be a nursery for juveniles and larvae, a settling substrate for invertebrates and epiphytic algae, are a food source for herbivorous fish and turtles, and likely produce more sand in the Maui Nui region than corals.

Halimeda morphology and taxonomy

Halimeda kanaloana is a sand-dwelling calcified green alga (Chlorophyta, Bryopsidales) with a coenocytic, unicellular, multiaxial body that may attain 25 cm in stature. Often described as “corn flakes” or “money plant”, *Halimeda* is composed of calcified segments joined by uncalcified flexible nodes. *Halimeda* forms dense, upright algal populations, or meadows, that may exist as a long continuous zone or as a series of discontinuous patches over soft sediments (Friele et al. 1995). Originally, *H. kanaloana* was described as *H. incrassata*. Molecular and morphological studies by Verbruggen et. al (2006) split *Halimeda incrassata* into 2 pseudo-cryptic species plus the original species, thus describing *Halimeda incrassata* in Hawai‘i as a new species (*Halimeda kanaloana*). At the start of this research, *H. kanaloana* was only described from the Maui Nui complex. Little was known about the distribution of this species in Hawai‘i, or the factors affecting its distribution. Baseline data were needed on its current distribution for proper management of this essential species.

Studies on deep-water *Halimeda* meadows in Hawai‘i would provide useful information on *Halimeda* standing crop and lower depth limits that could be used to produce sediment generation and accretion rates to be compared with productivity estimates from other *Halimeda* meadows in the Atlantic and Pacific (Table 1). *Halimeda* growth (Table 1) can vary greatly depending on the biotic and abiotic factors at a particular site, necessitating *in situ* data on growth and densities for a particular species at specific depths. There are no studies that have examined these aspects of *H. kanaloana* meadows at multiple depths greater than 20 m (Table 1), even though the quantification of their numerical importance is essential to understanding reef biogenesis, deep-sea

sedimentary processes, and the geological record of the tropics (Jensen et al. 1985). Despite the low irradiances found in deep-water (< 1% surface irradiance), carbon incorporation rates from other deep-water (76 m) *Halimeda* species suggest mesophotic populations of *Halimeda* to be important producers of carbonate sediments (Jensen et al. 1985).

Ecological role of *Halimeda*

Our knowledge of the specific ecological role(s) of macroalgal meadows and beds in Hawai‘i is of importance to resource managers interested in the population dynamics of keystone species and related organisms in coral reef ecosystems. Studies on *Halimeda* meadows in other regions of the tropics provide some clues to the possible ecological roles of mesophotic assemblages meadows in Hawai‘i. In Florida, numerous species of epiphytic algae (Beach et al. 2003, Vroom et al. 2003), and invertebrates (Naim 1988; Llobet et al. 1992; Coma et al. 1992; Kerr and Paul 1995; Sotka et al. 1999) use *Halimeda* as a settling substrate, or food source (Kampfer and Ott 1995). Epiphytic communities on *Halimeda* in the Seychelles were composed of 84 species of algae and main taxons of benthic animals, with a strong variation in communities with depth, emphasizing the need to examine epiphytic communities along a depth gradient (Ivin et al. 2000). *Halimeda* meadows in New Caledonia hosted up to 86 spp. of fish, with some unique fish, such as emperors (Lethrinidae), associated specifically with the meadows (Rossier and Kulbicki 2000). The abundance of numerous reef fish (*Stegastes leucostictus* (Pomacentridae), *S. partitus* (Pomacentridae), and *Halichoeres bivittatus* (Labridae)) were positively correlated with the abundance of *Halimeda incrassata* in

Tague Bay, St. Croix, over spatial scales ranging from 1 to 200 m^2 (Chattaro 2004). As a food source, *Halimeda* species form a significant part of the diet of parrotfishes (Scaridae) (Lobel and Ogden 1981; Overholtzer and Motta 1999, 2000; Munoz and Motta 2000), and have been found to serve as a spawning site for Labridae and Scaridae (Colin and Bell 1991). Because herbivores potentially increase *Halimeda* abundance through the clonal propagation of "bitten" fragments (Walters and Smith 1994, Walters et al. 2002), observations on grazing are important for understanding *Halimeda* dispersal and growth.

Organization of Dissertation

This dissertation is a comprehensive survey of mesophotic macroalgal assemblages in deep water tropical region, and reveals a naturally-occurring, macroalgal-dominated system with astounding diversity and spatial complexity. *Halimeda kanaloana* meadows were one of the most abundant mesophotic assemblages we encountered, and occurred as a continuous meadow into shallower waters (to 10 m) offshore of west Maui. This access allowed me to use these meadows as a model system to delve into more specific questions concerning the basic ecology of an alga, and how it varies across a broad depth gradient into the mesophotic. While diving in the *Halimeda* meadows, we noticed both natural (sting ray feeding pits) and anthropogenic (anchor scars) disturbance in this soft sediment environment, similar to the anchor scars described from seagrass meadows. This inspired a manipulative experiment that mimicked different levels of disturbance while we concurrently monitored recovery from an anchor scar at a similar depth. Aside from the overall introduction and synthesis, there are three research chapters within this dissertation (Chapters 2, 3, and 4). Chapter 2 describes the composition, distribution,

and abundance of mesophotic macroalgae at 59 sites around Hawai‘i using submersible diving, remotely operated vehicle (ROV) operations, and technical diving. Chapter 3 focuses on aspects of *H. kanaloana* field biology, such as the temporal and spatial variability in growth, densities, and age, and develops an estimate of calcium carbonate production. This chapter will eventually be split into two research papers, with one concentrating on the field biology of *H. kanaloana*, and the other on *H. kanaloana* carbonate production. Chapter 4 describes the recovery of *H. kanaloana* to a manipulative experiment mimicking anchor scar damage, and an actual anchor scar at 30 m depth. Additional studies conducted on mesophotic macroalgal physiology, *Halimeda kanaloana* ecophysiology, and mesophotic coral (*Leptoseris* spp. and *Montipora capitata*) physiology were not included as part of this dissertation, but will be published elsewhere as separate papers.

The grammatical person used in each research chapter is usually “we” instead of “I”. Although I am the main person responsible for conducting this research, it was a collaborative effort. Each moment of my data collection was shared with a dive buddy, a person in a submersible, or a room full of observers watching remotely operated vehicle video. The resulting publications will have several co-authors. Given that each chapter will be published as a separate paper, I felt it was most appropriate to acknowledge these contributions by using the first person plural throughout this dissertation.

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TABLES

Table 1. Growth of *H. incrassata* with increasing depth.

Segments/plant/day	Depth (m)	Location	Reference
3.3	0.5	Tahiti	Payri 1988
1.73	1-2.5	Antigua	Multer 1988
0.16 *	5-8	Australia	Drew 1983
0.6	11	New Caledonia	Garrigue 1991
0.2	18	NW Mediterranean	Ballesteros 1991
1	20	US Virgin Islands	Williams 1988

* *H. copiosa* and *H. opuntia*

FIGURES

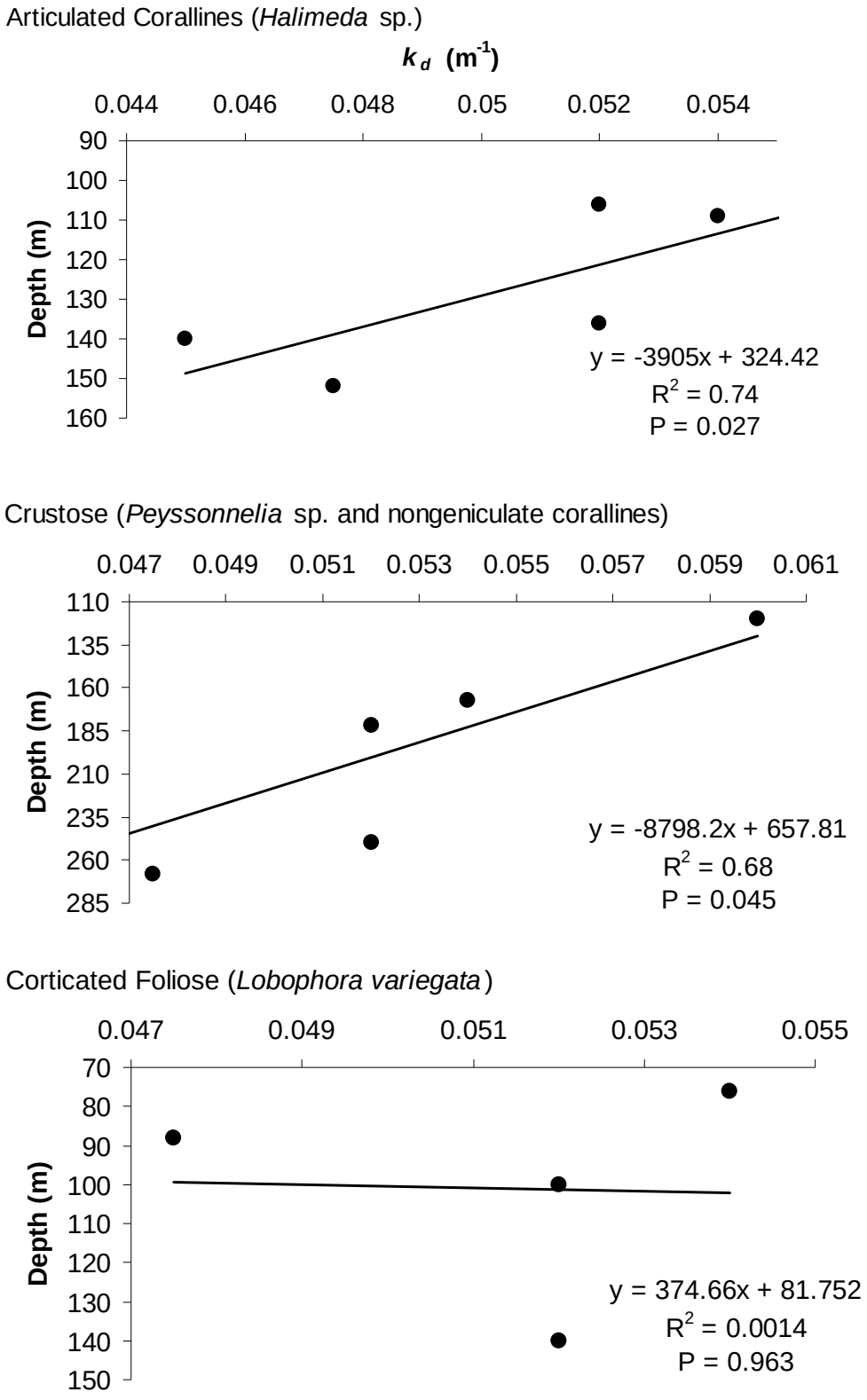

Figure 1. Lower depth limits for mesophotic algae in relation to K_d (PAR).

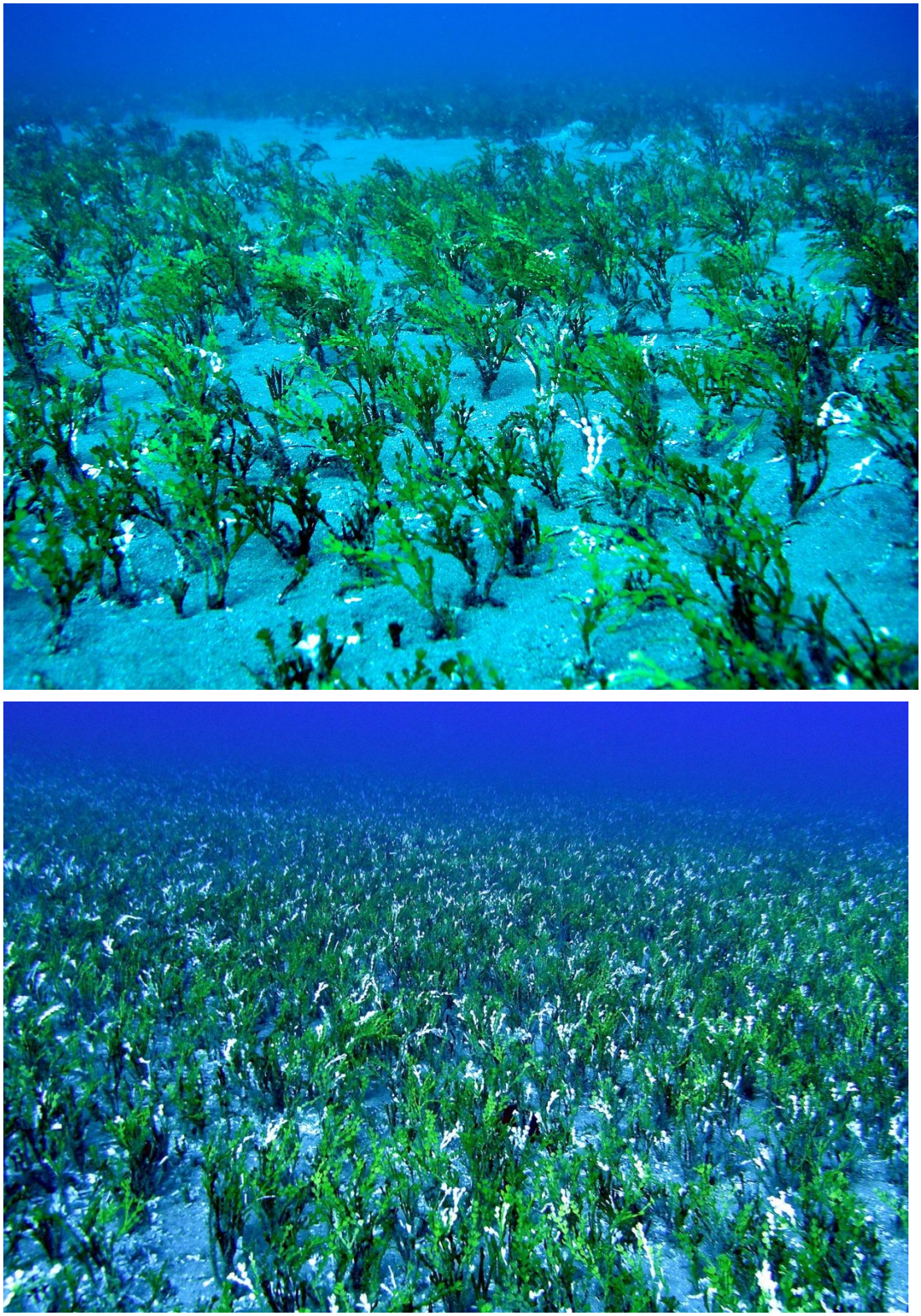

Figure 2. *Halimeda kanaloana* meadows at Kahekili Beach Park, west Maui, at 10 m (top) and 30 m (bottom) depths.

CHAPTER 2

MESOPHOTIC MACROALGAL ASSEMBLAGES IN THE MAIN HAWAIIAN ISLANDS

ABSTRACT

Submersible dives, remotely operated vehicle (ROV) operations, and technical diving were used to survey and collect representative samples from mesophotic macroalgal assemblages at 59 sites from 40 to 212 m depths around the Hawaiian islands of O‘ahu, Maui, Lāna‘i, Kaho‘olawe and Moloka‘i. Seventy-six species of frondose macroalgae were described, including 32 Chlorophyta, 32 Rhodophyta, and 12 Phaeophyceae. The mesophotic zone contained many new or distinct algae as compared to shallower water, and contained genera with tropical, subtropical, and cold-temperate biogeographic affinities. A total of 30 species were new records for the Hawaiian flora or new species to science, with 45% of the flora only found at depths ≥ 40 m. The most distinctive changes in diversity and abundance occurred at 80-90 m and 120-130 m depths. These depths corresponded to ~3% and 0.5% of surface irradiance, respectively, and included depths where large changes in seasonal thermoclines were observed. The water column at most sites was characterized by high clarity and deep light penetration, with a low average attenuation coefficient (-0.042 m^{-1} ± 0.001 SE), although sedimentation from nearshore terrigenous sources appeared to reduce macroalgal abundance at a few sites. Twenty-five species of macroalgae were common constituents of the mesophotic assemblage (at least 10% cover). Of these, 11 were commonly a dominant mesophotic assemblage with a distinct flora, with up to 75 - 100% cover ranging from several hundred meters to kilometers square. *Halimeda kanaloana* meadows were the most abundant assemblage in

the Maui Nui complex at 40 to 90 m depths. The discovery of expansive mesophotic populations of the invasive alga *Avrainvillea amadelpha* off O‘ahu highlights the need for understanding the dynamics of mesophotic community composition if we are to understand the origin and extent of invasive species. Mesophotic macroalgal assemblages in Hawai‘i are abundant, diverse, and spatially heterogeneous. The biology of these key species in mesophotic ecosystems will ultimately have substantial implications for tropical food web ecology, biodiversity, and biogeography.

INTRODUCTION

The mesophotic coral ecosystem (MCE) is characterized by the presence of light-dependent corals and other communities that are typically found at depths ranging from 30 to 40 m and extending to over 150 m in tropical and subtropical regions (Hinderstein 2010). Macroalgae, sponges, and coral form the dominant communities, provide structural habitat, and contribute to overall biodiversity. Submersible surveys of MCE’s in Hawai‘i reveal a high coverage of macroalgae (Kahng and Kelley 2007, Rooney et al. 2010), but provide little information regarding the species composition and spatial heterogeneity of mesophotic macroalgal assemblages. Previous deep water floristic studies in Hawai‘i were restricted to one location (Agegian and Abbott 1985), or collected by dredging (Doty et al. 1974). Each study presented contrasting results, with Agegian and Abbott (1985) finding distinct deep water species, while Doty et al. (1974) concluded that there was no distinctive deep water flora, zonation, or dominance by one phylum of macroalgae. Knowledge of the species composition and depth distribution of present-day mesophotic macroalgae assemblages has far-reaching implications for understanding ecosystem-wide energy flow via the coupling of primary and secondary

productivity, algal biodiversity, carbonate sand production, biogeography, the occurrence of invasive alien algae, and ecological interactions with macrofauna.

One of the most serious threats to the coastal marine environment in the Main Hawaiian Islands (MHI) is the overgrowth of natural reefs by invasive macroalgae, which contribute either directly or indirectly to phase shifts from coral to algal dominance (Smith et al. 2001, 2002; Stimson et al. 2001). Invasive and non-indigenous macroalgae have been found around all of the MHI in shallow water (< 20 m), with the largest blooms occurring in Maui (Smith et al. 2002), costing Maui County an estimated 20 million dollars a year in lost revenue (Cesar and van Beukering 2004). Local fishermen in Maui have reported large mats of the invasive macroalga *Cladophora sericea* to at least 60 –70 m depths during bloom cycles.

The distribution and impacts of invasive macroalgae on MCEs are unknown. Determining the species composition of mesophotic macroalgae is necessary for determining the distribution of native, non-indigenous, and invasive species, and can be used as a basis for monitoring any changes in community composition over time. The mesophotic flora may also include new taxa, and provide a window to an evolutionarily interesting niche that has probably experienced little of the change found in shallower waters.

Some macroalgae form relatively dense, upright algal populations, or meadows, that may exist as a long continuous zone or as a series of discontinuous patches in soft sediments (Friele et al. 1995). We expand this definition of meadows to include dense, low-lying algal populations over soft or hard substrate. These meadows are often

monospecific and may be composed of calcareous algae, important producers of carbonate sediment on coral reefs and in adjacent lagoons (Milliman 1993). This significant production of unconsolidated carbonate sediment is often quantitatively more important than the carbonate produced by the reef framework itself (Milliman 1974), with *Halimeda* being the most important contributor to the geomorphology of modern and ancient reef structures (Hillis-Colinvaux 1980). The availability of gently sloping substrate offshore of O‘ahu and the Maui Nui island complex (Moloka‘i, Maui, Lāna‘i, and Kaho‘olawe) is ideal for the formation of algal meadows in the mesophotic zone. *Halimeda kanaloana* meadows have been observed to 90 m depths off Maui (Verbruggen et al. 2006, Kahng and Kelley 2007, Rooney et al. 2010), and other algal assemblages are likely present.

Mesophotic coral ecosystems typically occur in relatively remote offshore locations, and are often considered buffered from human-related stressors, such as eutrophication and over-fishing, which cause the degradation found on shallower reefs (Bak et al. 2005, but see Menza et al. 2007). This supposed isolation from anthropogenic inputs simplifies our ability to predict the factors that may regulate the abundance and distribution of mesophotic algae. However, increasing depth and the interplay of biotic and abiotic factors likely influence any algal abundance at site-specific depths and locations, and fine scale factors are also certain to be important. Some of the factors influencing mesophotic algal abundance and distribution include: availability of appropriate substrate, resistance to herbivory, efficient nutrient uptake, upwelling of cold nutrient-rich water, resistance to physical disturbances (such as sand scour), low

respiration rates, and changes in irradiance quantity and quality (Kirk 1994, Leichter et al. 2008).

The aim of this study was to describe the species composition and distribution of mesophotic macroalgae and dominant macroalgal assemblages from O‘ahu to Maui. However, macroalgal abundance and species composition can vary greatly at scales ranging from centimeters to meters, thus making our ability to accurately identify the species composition of assemblages on the scale of kilometers problematic. Moreover, most mesophotic studies are limited to a few sites given the difficulty and cost of accessing mesophotic depths. Hawai‘i is uniquely poised for mesophotic research given the large areal coverage in the mesophotic zone (see Kahng et al. 2010, Rooney et al. 2010) and the technical support provided by the Hawai‘i Undersea Research Laboratory (HURL), with two manned submersibles, a remotely operated vehicle (ROV), and technical diving capability. These research tools enabled us to describe the depth distribution and spatial extent of mesophotic macroalgae at 59 sites across five islands in the MHI. Specifically, our objectives were to: 1) identify the species composition and dominant assemblages of mesophotic macroalgae over a broad spatial scale; 2) quantify the relative abundance of each species; 3) determine if mesophotic macroalgal communities are taxonomically distinct from shallow water communities, or if they are composed of shallow water species with a few distinct deep-water species; 4) examine whether mesophotic habitats have been invaded by non-indigenous and invasive species; and 5) quantify the depth-specific variability in photosynthetically active radiation (PAR) and temperature at mesophotic depths, and their possible impact on algal diversity.

MATERIALS AND METHODS

Study sites

Fifty-nine sites from Oʻahu to Maui were surveyed from September 2004 to March 2011 (Figure 1; Tables 1, 2). The islands of Molokaʻi, Maui, Lānaʻi, Kahoʻolawe, and the submerged platform called Penguin Bank are known collectively as the Maui Nui complex. Maui Nui was connected as a single landmass that reached its maximum areal extent ~1.2 million years ago (Ma), when it was larger than the current island of Hawaiʻi (Price and Elliott-Fisk 2004). Subsidence, changes in sea level, and geomorphological processes split the landmass into their current islands, with the islands of Maui and Lānaʻi last connected about 10,000 years ago (Grigg et al. 2002). Sand and sediment plains less than 100 m in depth form limestone bridges that connect the Maui Nui islands underwater (Grigg et al. 2002), and provide optimum habitat for the formation of psammophytic algal meadows. In constrast, Oʻahu is surrounded by submerged shorelines that form insular shelves bordered by steep fossil carbonate slopes (Jones 1993, Fletcher and Sherman 1995). Oceanographic conditions during summer/fall months are characterized by consistent northwest trade winds, low rainfall, and larger waves on south-facing shores, while winter/spring months experience heavy rain with increased nearshore turbidity and large waves on north-facing shores.

Sites were selected based upon availability of gently sloping substrate from 40 to 200 m depths, and included sandy (soft) and carbonate or basalt (hard) substrata. Highly exposed areas from north- and east-facing shores were generally not surveyed, given the difficulty and danger of accessing these locations during winter months when HURL

submersible and ROV operations were possible. Archived 1998 to 2003 videos that showed algae from previous HURL ROV and submersible dives were reviewed to pinpoint areas of high algal abundance or potentially unique species that could be used as dive targets.

Collections and identification

Macroalgae were collected with HURL submersibles *Pisces IV* and *Pisces V* from 40 to 212 m depths (Table 1) and by technical divers using open circuit SCUBA or closed circuit rebreathers from 40 to 60 m depths (Table 2). Algae serendipitously caught in the skids of the ROV *RCV-150* were collected, with their specific collection depth given as the range of depths surveyed. Given the safety hazards of the submersible operating in shallower (< 60 m) depths close to shore, submersible collections tended to occur in deeper (> 60 m) depths, especially around O‘ahu. Thus, technical divers were used for additional shallow collections around O‘ahu to resolve spatial distributional patterns.

Any macroalga visible with the naked eye was targeted for collection. The submersibles were equipped with a Schilling Orion manipulator, a collection box with 18 distinct bins, and a general purpose collections basket. Algae collected by the submersibles were photographed *in situ* and given a unique collection number, with the GPS location and depth noted for each specimen. The GPS location was determined using a tracking system which consisted of acoustic transponders and hydrophones on both the ship and the submersible. Submersible dives typically lasted ~ 6-8 hours, and occurred during daylight hours; additional illumination was provided by external 250 W or 500 W tungsten lights, or 400 W HMIs (Hydrargyrum Medium-arc Iodide lights),

mounted on the submersibles. Technical divers had ~10 – 20 minutes of bottom time depending on the depth, resulting in a haphazard collection of the most abundant algae in a ~25 by 1 m area. Time and logistics prohibited random sample collection.

Collected macroalgae were saved in triplicate as herbarium presses for voucher specimens, in silica gel for molecular analyses, and in a 4% buffered formaldehyde solution in seawater, when possible. Each herbarium press was examined with a dissecting scope for reproductive structures, herbivorous grazing marks, or other distinguishing features. Preserved slides of cross-sections and squash mounts were made for microscopic analyses according to Tsuda and Abbott (1985). Algae were tentatively identified using the systematic keys and taxonomic information provided in Abbott (1999), Abbott and Huisman (2003), Abbott and Huisman (2004) and Huisman et al. (2007), and sent to the appropriate experts for verification based upon morphology and molecular analyses. The reported depth ranges for each alga was noted from Agegian and Abbott (1985), Abbott (1999), and Abbott and Huisman (2003). Depth ranges in the literature recorded as intertidal or subtidal were reported as 1 and 5 to 10 m, respectively.

New species descriptions are not included in this account. Voucher specimens are to be deposited at the Herbarium Pacificum, B. P. Bishop Museum, Honolulu, Hawai‘i. All other macrofauna (invertebrates and fish) collected with the macroalgae were preserved in either formalin or 95% ethanol, and also deposited in the appropriate collections, B.P. Bishop Museum. Descriptions of associated invertebrates and fish will be described elsewhere by R. Langston (University of Hawai‘i, Windward Community College) and K. Longenecker (Bishop Museum).

Video surveys

Video surveys were conducted with the submersibles and the ROV along track lines pre-determined for a site based upon substrate type and the desired survey depths. Upon reaching the bottom, the underwater vehicles followed the track line, recording the benthos on video with the lasers in view for scale. Submersibles used an Insite Pacific MINI-ZEUS HDTV camera starting in 2009 and a Remote Ocean Systems (ROS) color analog video camera prior to 2009 equipped with four lasers calibrated according to Tusting and Davis (1993). The ROV used an ROS color analog video camera with a pan/tilt mount, and was fitted with two fixed lasers 15 centimeters apart and equipped with six 250-W lights. The relative abundance of macroalgae was quantified in each video using CyberLink PowerDVD software. When algae were observed in the video, a record was made noting the highest taxonomic level possible, depth, substrate type, a visual estimate of abundance, and other pertinent ecological information (such as meadows or associated organisms). Abundance was based on a scale of 1 to 5 in a ~ 1 m^2 area following a general DACOR assessment and based upon the orientation of the lasers, where 1 = ≤ 9% cover; 2 = 10 – 25% cover; 3 = 26 - 50% cover; 4 = 52 - 75% cover, 5 = 76 - 100% cover. To verify the identification of an alga, representative screen captures and video segments were taken of each taxon, and compared with algal collections by the submersible. The end result was a record of all algae observed on the dive, with a relative estimate of their abundance. However, the abundance estimates only refer to the range of abundance for a particular entity in the video, not the frequency of occurrence.

Video quality between the submersible and ROV dives varied dramatically with differences in lighting, the time of surveying, and occasional technical difficulties, thus

limiting our ability to make more definitive measurements of abundance across all sites. Further, submersible video surveys during the day were predominantly monochromatic (blue), making it difficult to identify algae unless the submersible focused on a particular area with full lighting. However, battery power over the course of the dive was sometimes limited, and thus full lighting (which is energetically costly for the batteries) was not always possible. The algae in videos from ROV surveys during the night were more visible due to better lighting in the darkness, but the surveys were limited by the speed of the ship, which determined how quickly the tethered ROV moved over the bottom. Small filamentous red algae and crustose coralline algae remain undersampled. The lower algal depth limits of smaller species or depth ranges of rare algae were also difficult to determine given their sparse abundance; these depth ranges should be considered an estimate. Rooney et al. (2010) and Kahng and Kelley (2007) recently provide a broader discussion of the cover of mesophotic macroalgae (as a functional group) and macrofauna for similar sites in Maui Nui.

Irradiance and temperature

Underwater irradiance was measured at sites 6, 40, 45, and 47 on 8 June 2006, 4 August 2008, 5 August 2008, and 14 July 2010, respectively, by lowering a calibrated spherical (4π) quantum sensor (Underwater LI-193SA, LI-COR, Lincoln, NE, USA) through the water via a profiling rig; data were stored with a LI-COR LI-1400 datalogger. The sensor was attached to a 1 m long arm mounted on a polyvinyl chloride housing to reduce instrument shading. The housing was integrated with a calibrated pressure transducer for depth (m) and a temperature sensor. PAR (μmol photons m^{-2} s^{-1}) was recorded every 0.2 to 1 m in the water column to a depth of 52 – 89 m on calm, clear days. Profiles were

done on the sunny side of the vessel to reduce any shadows. The vertical attenuation coefficient (K_o) from the downward portion of each profile was calculated according to the relationship in Beer's Law:

$$I_z = I_o \times e^{-kz}$$

where z represents depth, I_z represents the intensity of irradiance at depth z, and I_o represents the intensity of irradiance just beneath the surface (Kirk 1994). The percent of subsurface irradiance (% SI) at any one depth was extrapolated from the average K_o from the 4 sites. The average irradiance ± Standard Error (SE) was calculated by averaging irradiances at specific depths from each site based upon the K_o determined for each site.

Seawater temperature was continuously recorded on all submersible dives and ROV deployments (Table 1) with an Applied Microsystems or a Falmouth Scientific Micro CTD profiler, respectively. Temperature data were averaged into 1 m bins, and examined for maximum and minimum temperatures and possible thermoclines on each deployment.

RESULTS

Collections and surveys

A total of 160 km of track lines were surveyed with submersibles and the ROV at 48 sites lines and 225 hours of submersible and ROV video were recorded (Table 1). Only 10 of the 48 sites were at depths greater than 150 m because of the difficulty of accessing both deeper and shallower depths over the course of one dive. Thus, we generally focused on depths less than 150 m, where algae were more apparent and abundant. Eleven other sites

were used for collections by technical divers (Table 2), and were useful in supplementing distributional pattern data. For instance, divers found small patches of *H. kanaloana* growing at sites 16, 18, and 21 off south O‘ahu, while the submersibles and ROV did not record any *H. kanaloana* in its surveys around that island.

Macroalgae were an abundant and diverse component of the mesophotic coral ecosystem. We collected 1073 specimens, and spent ~500 hours identifying species based on morphology and ultrastructure. Seventy-six species of large macroalgae were described, including 32 Chlorophyta (green algae), 32 Rhodophyta (red algae), and 12 Phaeophyceae (brown algae) (Table 3). This does not include small epiphytes or turf, which often formed a thick covering over the base of *H. kanaloana* and on other hard surfaces such as dead coral. Rhodoliths and nongeniculate algae were each treated as single species, but are also likely to be composed of several species (R. Riosmena-Rodriguez, pers. comm.)

Twenty-five species of macroalgae were common constituents of the mesophotic assemblage, with at least 10% cover (Table 3). Of these 25 species, nine were meadows with 75 - 100% cover in areas ranging from several hundred meters to at least a kilometer squared. *Cladophora* sp. nov. 1 was also found in high abundance, but was classified as a bloom instead of a bed or meadow because it was loosely attached to other algae or substrate, or was found floating along the bottom.

The mesophotic zone contained many new or distinct algae as compared to shallower water. When combining reported depth ranges for each species from this study and the literature (Table 3), the following species were found with lower depth limits

shallower than 40 m or ≥ 40 m depths, respectively: green algae with 17 and 15 species, brown algae with 11 and 1 species, and red algae with 14 and 18 species. Thus, 55% of the 76 species were also found at depths less than 40 m depth, and 45% were only found at depths ≥ 40 m (Table 3). Twelve species were new to science, three were new records, and 15 were either new species or new records for the Hawaiian flora (Table 3). In summary, a total of 30 species were new records or new species to science, or ~40% of the mesophotic flora described here. These 30 species were considered unique to deep water because they had not been previously collected or observed at shallower depths (less than 40 m) in Hawai‘i. Most of these species, such as the Rhodymeniales, *Halimenia* spp., *Dasya* spp, and *Kallymenia* spp. were large in stature (from ~10 to 75 cm in length) and visually apparent, suggesting they would have been collected historically if they occurred as part of the shallow water flora.

The total number of macroalgal species as a function of depth generally peaked between 60 and 110 m depths, with the highest number of total species found between 80 and 90 m depths (Figure 2). The brown algae were less diverse, with little change in the number of species between 40 and 120 m depths. Both red and green algae peaked in species diversity between 60 and 110 m, which influenced the peak in the total number of species. Large red algal blades, such as species of *Halymenia* and *Kallymenia* (Table 3), were largely responsible for the peak in red algal diversity. Overall, all algal groups experienced a sharp decline in the total number of species below 120 m, with a steady gradual decline over the depths from 130 to 210 m (Figure 2).

The most abundant mesophotic macroalgal assemblages, and coral reefs with associated algae, were summarized by depth with their distributions by island (Table 4).

We commonly encountered 11 different assemblages with distinct floras, including 5 species that also occurred as either subdominant or dominant assemblages (Table 4; Figures 3, 4, 5). Dominant was defined as the most abundant species forming the majority of the assemblage. Subdominant species co-occurred with dominant species but had less biomass. Four assemblages were found ubiquitously around all the islands; rhodolith beds and Ulvales with large red blades (species of *Kallymenia* and *Halymenia*) were found around all islands except the soft substrate-dominated Kahoʻolawe. Dense and large *Avrainvillea amadelpha* and *Udotea* sp. meadows were only found off west and south Oʻahu. Mesophotic coral reefs (*Leptoseris* spp. and branching *Montipora capitata*), dense and expansive *H. kanaloana* meadows, *Cladophora* spp. blooms, and *Microdictyon umbilicatum* were distinct to the Maui Nui complex.

Major assemblages

Avrainvillea amadelpha and *Udotea* sp. were the most commonly encountered bed-forming macroalgae at mesophotic depths around west and south Oʻahu, although no *Udotea* was found east of site 15 (Table 4; Figures 3 a, b). Both species were absent from locations surveyed in the Maui Nui complex. Archived HURL video from 12 Nov 2000 (ROV *RCV-77*) documented *Udotea* in an area around sites 11 and 12 from at 71 to 94 m depths, but we were not able to discern any *A. amadelpha* in the area from this video in 2000. Each species formed monospecific beds that were patchy in distribution, occasionally growing in close proximity, but not intermixed. *Udotea* sp. occurred only in soft sediments and possessed a small, shallow fibrous holdfast. *A. amadelpha* had a more elongate and narrow blade as compared to intertidal populations, with a deep, penetrating holdfast that sequestered fine sediments. Divers observed *A. amadelpha* overgrowing

patches of coral (*Porites* spp.) and other macroalgae (e.g. *Codium* spp.) on hard substrate, and transforming areas of hard substrate into mounds of *A. amadelpha* with soft sediments (Figure 3a). Small fish used the blades of *A. amadelpha* and *Udotea* sp. as habitat (to be reported elsewhere).

Meadows of *Halimeda kanaloana* (Figure 3c) were the most abundant assemblage in the Maui Nui complex from 40 to 90 m depths. Dense meadows were found at 30 of 37 sites surveyed in Maui Nui. There were no luxuriant *H. kanaloana* meadows found at sites around O‘ahu, although small patches (5 to 20 individuals) of heavily epiphytized *H. kanaloana* were found by divers off south O‘ahu (sites 16, 18, and 21). Dense H. *kanaloana* meadows were found predominantly in soft sediments, with *C. mexicana*, *Codium* spp., and sponges intermixed in low abundance. Ulvales, various filamentous reds, *Sporochnus dotyi*, and *C. filicoides* formed localized patches of epiphytes covering patches of *H. kanaloana* meadows.

Halimeda distorta (Table 4, Figure 3d) was found on hard substrata, either as small mounds up to 0.25 m in height or as larger (~10 – 100 m^2) beds. *H. distorta* stands were often observed cascading down from the edges of *Leptoseris* reefs. Small individuals were also observed scattered from 40 to 138 m depths in low abundance and mixed with other macroalgal assemblages. Large individuals of *Halimeda discoidea* were occasionally observed mixed with *H. distorta*, and could be distinguished by their lighter, lime green coloration and larger, circular-shaped segments. Areas in the Maui Nui complex with a mix of hard and soft substrate contained intermixed patches of both *H. distorta* and *H. kanaloana*. Encrusting invertebrates, including sponges, tunicates, and bryozoans, and Rhodymeniales, were found entangled in the larger *H. distorta* mounds.

Leptoseris spp. reefs contained a mix of large macroalgae, with *Codium mamillosum* balls and *Distromium flabellatum* (described below) blades being the most apparent species inside the reef (Figure 3e). Ulvales and *H. distorta* were more common around the fringes of the reefs. *Peyssonnelia inamoena* and nongeniculate coralline algae were found on collected coral, but were difficult to distinguish in the videos or *in situ*. Rooney et al. 2010 gives a description of *Leptoseris* reefs at the sites surveyed.

Distromium flabellatum (Figure 3f in sand, Figure 4a in *Montipora*) was the most commonly encountered brown alga, with populations found on all of the islands surveyed and in a range of habitats, such as with *Leptoseris* spp. and branching *Montipora capitata* coral reefs, in sand, and on carbonate substrate. The kidney-shaped blades often formed sparse beds in the sand from 60 to 160 m depths, or grew epiphytically over other algae and coral. Although *Lobophora* closely resembles *Distromium*, no *Lobophora* was observed or collected in any submersible or technical dive. Other brown algae were present, though less abundant. Small beds of *Spatoglossum* were observed around Penguin Bank. A large, foliose species of *Padina* resembling *Padina moffittiana* Abbott & Huisman was observed in archived ROV video (*RCV-043*, 18 Sept 1999), forming dense ~ 5 m wide patches in the sand at locations around Penguin Bank from 140 to 160 m depths. This species of *Padina* was not observed during ROV and submersible surveys, limiting attempts to verify its identity. Delicate, elongate blades of *Dictyopteris plagiogamma* were commonly encountered in collections, but were not numerically abundant. Three species of *Dictyota* were present, but were often cryptic and low in abundance.

Unique reefs dominated by *Montipora capitata* with a thin, branching morphology were discovered during surveys in Maui Nui. These reefs formed expansive, low-relief (up to ~15 cm in height) reefs around west Maui (described in Rooney et al. 2010). A host of macroalgal species were found covering up to 100% of these reefs, with *Montipora* branches barely discernible, poking through the algal canopy (Table 4, Figure 4). These algal assemblages were patchy, often alternating between species on the scale of several hundred meters squared, or absent from the reef. Some species, such as *Microdictyon umbilicatum* (Figure 4c) and *Caulerpa filicoides* (Figure 4d), formed dense assemblages in areas beyond the *Montipora* reef. A bright green mat-forming alga that morphologically resembled *Boodlea* sp. was also observed with the ROV to be in high abundance in the *Montipora* reef, but no collections were able to be made to verify its identity.

Rhodolith beds were found in areas with soft sediments off all islands except Kahoʻolawe (Table 4). Beds were most abundant off Oʻahu at site 3 from 46 to 103 depths, and off west Maui at sites 36, 37, and 38 in shallower water (39 to 42 m depths). However, rhodoliths did not co-occur with *H. kanaloana* meadows despite the preference shown by rhodoliths for similar substrates. Large blades of Ulvales were sometimes present as epiphytes at depths greater than 50 m (Figure 5a). Submersible pilots commented that rhodolith beds were observed at many locations throughout the Main Hawaiian Islands in previous surveys, and nicknamed the beds “puppy chow” (M. Cremer, personal communication).

Nongeniculate coralline algae (Table 4) were present in every assemblage that contained a hard surface, but were often difficult to document unless a large area of

carbonate or basalt was in view, or samples were collected with other algae or corals. For instance, coralline algae were found on the underside of *Leptoseris* spp. plates, epiphytically on *H. kanaloana* and *H. distorta* segments, and on dead black coral (*Antipathes* spp.), or formed abundant assemblages on basalt rocks or carbonate. Nongeniculate coralline algae were most abundant at ~80 m depth at Site 53 on large basalt boulders (Figure 5b).

An assemblage of large green blades of *Ulva* sp. and *Umbraulva* spp. (collectively called Ulvales, Figure 5c) were commonly found off all islands except Kahoʻolawe from 65 to 125 m depths. Ulvales were also observed from 178 to 204 m depths at site 33, but it was unclear whether these specimens were attached or drift, and thus were not considered in the depth range. Large red blades of *Kallymenia* spp. and *Halymenia* spp. were often intermixed with Ulvales, but present in lower abundance.

Collections and molecular analyses were required to identify green and red bladed species. From these analyses, generally, *Umbraulva* spp. were olive in color and found towards lower depths, while *Ulva* sp. was bright green and found throughout the depth range. *Ulva* sp. blades were up to 78 cm in length, and either perforate or nonperforate. Representatives of the Ulvales were found growing epiphytically (i.e., on rhodoliths or *H. kanaloana*), attached to hard substrate, with up to 75% cover in localized patches (~1 - 5 m^2), or as small single blades attached to small pieces of shell in soft sediment areas.

Species of *Cladophora* (Figure 5d) were found from 57 to 211 m depths at sites off Lānaʻi and Maui (Table 4), either attached or drifting through the water column. We identified seven different species, including three species new to science. *Cladophora* sp.

nov. 1 was found in high abundance in localized patches, and was also the deepest species found at 212 m on a rocky outcrop at site 33 in September 2004 (Figure 5d). This specimen was attached and was bright green in coloration, with a similar morphology to the bloom-forming species found in shallow water (*Cladophora sericea*). However, no large bloom of *C. sericea* was reported in shallow water off west Maui during 2004. We returned to this site in Dec 2004, but only found a few small individual clumps of *Cladophora* sp. nov. 1.

Although most foliose red algae were not numerically abundant, they were frequently collected at most sites because they were visually apparent from a distance. Foliose red algae were the most diverse morphologically and in size, ranging from large, leathery blades of *Grateloupia* sp. 1 (42 cm in length), to a perforate, sheer *Kallymenia* sp. 3 (55 cm in length), to microscopic filamentous red algae (not described here). Over half of the red macroalgal species identified were new records for Hawai‘i or new to science. New species or records not identified to the species level were distinct enough (morphologically, reproductively, and based on ultrastructure) to be designated as separate species. Rhodymeniales 1, Rhodymeniales 3, and *Amansia glomerata* were found on every island at most sites, often entangled in other assemblages.

Irradiance and temperature

Water-column irradiance profiles at sites 6, 40, 45 and 47 (Figure 6) yielded vertical attenuation coefficients, K_o (m^{-1}), of -0.045 ($r^2 = 0.99$), -0.042 ($r^2 = 0.97$), -0.041 ($r^2 = 0.99$), and -0.041 ($r^2 = 0.98$), respectively. Across all sites, the mean ± SE of K_o was -0.042 m^{-1} ± 0.001. The % subsurface irradiance (% SI) was calculated based on the mean

K_o, leading to 10% SI at 55 m, 1% SI at 109 m, 0.1% SI at 165 m, and 0.01 % SI at 210 m (Table 5). The mean ± SE irradiance from the profiles at each of the sites was calculated and is shown for every 10 m of depth (Table 5). Representative temperature profiles at 6 sites from northern O‘ahu to southwest Maui are shown in Figure 7. The vertical positions of thermoclines varied with site, but were generally found at ~40 m depth and were between 80 - 120 m depths (Figure 7). Temperatures ranged from 25.1 to 26.7˚ C at the surface to 18.5˚ C at 160 m depth.

The water column at most sites was characterized by high clarity and deep light penetration, as shown by the low average light attenuation coefficient (0.042 m^{-1} ± 0.001 SE). However, high turbidity which limited visibility was observed during surveys at sites 33, 51, 56, and 57. Fine sediments that were easily disturbed by currents or physical agitation by the submersible were observed on the ocean bottom. No algae were observed at site 56 despite the availability of appropriate substrate. This site was directly offshore of a river basin bordered with steep valleys. The upper depth limits of *H. kanaloana* meadows at sites with high turbidity around Kaho‘olawe were deeper (i.e. 56 m instead of 40 m depth) than at other near-by sites off Maui.

DISCUSSION

The mesophotic ecosystem in Hawai‘i is an expansive area that hosts a variety of stable apparent climax communities. Coral diversity was limited, but macroalgal assemblages were diverse and abundant, forming complex distributional patterns from O‘ahu to Maui in the range from 40 to 212 m depths. The availability of gently sloping substrate affording ample surface area for the formation of expansive mesophotic algal meadows,

beds, and coral reefs is also ideal for anthropogenic uses, such as the deployment of industrial communication or energy-based cables, aquaculture, and sand extraction. Adequate consideration to mesophotic corals and macroalgae must be given in planning such development of the mesophotic benthos, with the distribution and abundance of the affected organisms carefully reviewed. Hawai‘i’s mesophotic flora contained many unique native species when compared to shallower water. As investigations into this realm continue, we anticipate additional discoveries of new species and a sharp increase in the rate of endemism for Hawai‘i’s marine flora.

Description of major assemblages

Hawai‘i’s mesophotic region contained a high abundance of green calcified (*H. kanaloana*, *H. distorta*, *Udotea* sp.) and uncalcified (*A. amadelpha*) algae. Given the high frequency of occurrence and abundance, their areal coverage is likely greater than that of coral (*Leptoseris* spp. and *Montipora capitata*) in Hawai‘i’s mesophotic ecosystem. Green algae in the Bryopsidales (e.g. *Halimeda*, *Codium*, *Caulerpa*, *Udotea*, and *Avrainvillea*) are often particularly abundant in mesophotic regions (Littler et al. 1985, Litter et al. 1986, Blair & Norris 1988, Drew and Abel 1988, Norris & Olsen 1991, Friele et al. 1995, Aponte & Ballantine 2001, Drew 2001, Leichter et al. 2008). The success of green algae in tropical waters may arise in part from the optimal irradiance field in deeper water for green algae; the irradiance field at these depths is rich in the blue and green wavelengths (Kirk 1994). Some green algae also possess unique pigments, such as the carotenoid siphonaxanthin, that enhance the absorbance of blue-green spectral regions (Yokohama et al. 1977, Yokohama 1981). Physical changes in plant construction and morphology also optimize light capture (Vroom and Smith 2001). For example, species

such as *Codium* are optically opaque to the human eye, and have pigment concentrations that appear almost black in color (Kirk 1994). Also, all of the deep water green algae described from San Salvador Island had siphonous morphologies that increased total surface area, and increased light capture for photosynthesis (Norris & Olsen 1991). For instance, deep water *Halimeda copiosa* (to 152 m depths) increases surface area by increasing the diameter of the surface utricles by 15% as compared to shallower plants of the same species (Blair & Norris 1988).

The success of mesophotic algae in soft sediments may also be influenced by their ability to efficiently utilize nutrients from localized upwelling and/or from the sediment. Elevated nutrient levels in sandy sediments (*e.g.*, Falter and Sansone, 2000; Jahnke et al., 2000) suggest that knowledge of the sediment nutrient dynamics may be critical in understanding the distribution and productivity of psammophytic macroalgae (Williams 1984). Dense *H. kanaloana* meadows were found predominantly in the Au‘au Channel – an area with high frequency internal bores and strong alongshore currents (Storlazzi and Jaffe 2008) that could introduce nutrients from deeper water. Long-term temperature data and nutrient analyses of the water column at mesophotic depths, coupled with physiological studies on *Halimeda* similar to Smith et al. (2004), would be necessary to determine if the upwelling of nutrients is influencing the occurrence of this species. In the Great Barrier Reef, tidally-driven jet upwelling provides nutrients for deep water *Halimeda* meadows, and may have provided the physical conditions conducive to *Halimeda* growth for much of the Holocene period (Wolanski et al. 1988, Drew and Abel 1988, Drew 2001). In Florida, decreased bottom temperatures and increased nutrients indicated that short-term upwelling events influenced the high rates of benthic

productivity observed in deep water algae from 40 to 70 m depths (Leichter et al. 2008). Tropical upwelling was also likely responsible for large *Microdictyon* meadows found at 30 m depth at Pearl and Hermes Atoll in the Northwestern Hawaiian Islands (Vroom and Braun 2010). Cooler nutrient-rich bottom water, presumably of continental-slope origin, from the Carolina outer continental shelf increased ambient nitrogen three-fold, and provided deep water macroalgae with a critical source of nutrients during the summer (Peckol and Ramus 1988). However, temperature, nutrients, and hydrodynamics may not always explain patterns of high algal abundance. Parrish and Boland (2004) found no difference in yearly temperature records between similar areas of high-cover (>70%) and low-cover (<30%) algal biotopes from deep terraces and banks in the Northwestern Hawaiian Islands.

The *Udotea* sp. discovered at sites off south O‘ahu has never been described from Hawai‘i, yet was one of the most abundant mesophotic assemblages in this area, in the regions with soft sediment. Based on the position of the ribosomal DNA sequence from the Hawaiian material within a phylogeny of the Udoteaceae, and on a morphological study, Peyton (2009) proposed *Udotea* sp. as a new genus and species (currently described as Unnamed green alga Peyton *et* Ballantine sp. nov. and Unnamed gen. nov. Ballantine *et* Peyton). The closed canopy of the *Udotea* sp. appeared to form habitat for small fish and invertebrates, as well as a hunting ground for larger apex predators, such as Ulua jack (*Carangoides ferdau*), that would feed on the small fish hovering above the canopy (Peyton 2009). The ecosystem services provided by this newly-discovered assemblage merit additional study, and highlight the novelty and importance of the mesophotic flora to the coral reef ecosystem.

Although *Distromium* was only recently described in Hawai‘i (Abbott and Huisman 2003), this phaeophyte was the most abundant and ubiquitous brown alga in our surveys. Phaeophyte blades from the Dictyotales, such as *Distromium*, *Lobophora*, or *Stypopodium*, are common constituents of mesophotic floras world-wide (Van Den Hoek et al. 1978, Peckol and Ramus 1992). For instance, *Lobophora variegata* or *Distromium flabellatum* have been described from the mesophotic in Hawai‘i (Agegian and Abbott 1985, Kahng and Kelley 2007, this study), Florida (Hanisak and Blair 1988, Leichter et al. 2008), Curaçao (Nugues and Bak 2008), and Japan (Kajimura 1987). *Distromium* resembles *Lobophora* in habit (blade-like, recumbent, somewhat flabellate), and can only be distinguished in cross-section (*Distromium* has 2 cell layers, *Lobophora* has 4-8). Specimens identified as *Lobophora* by Agegian and Abbott (1985) and Kahng and Kelley (2007) from Hawai‘i may have been *Distromium* as well; we did not find any *Lobophora* in our collections at similar sites. Unfortunately, the disposition of Agegian's collections remains unknown.

The success of thin, algal blades from the Dictyotales in the mesophotic region may be due to physiological, morphological, and chemical (anti-herbivory) adaptions. A thinner blade (and subsequently lower specific carbon content) ensures higher specific growth rates than thick plants when light is limiting (Markager & Sand-Jensen 1992). The thin plants of Dictyotalean deep water species were found to have a higher photosynthetic capacity than species with thicker forms, such as calcified, coarsely branched or leathery (Peckol and Ramus 1988). The thin plants may also increase the alga’s nitrogen-uptake ability (Peckol and Ramus 1988) by favorable shifts in surface-area: volume ratio.

Dictyotales, such as *Lobophora* and *Stypopodium*, also have highly toxic, anti-herbivory compounds that repel both fish and urchins (Norris & Fenical 1982, Gerwick et al. 1985). Despite this chemical defense, the abundance of *L. variegata* has been dramatically affected by urchins and can experience a high turn-over (Ruyter van Steveninck and Breeman 1987). Physiological measurements of these species along a depth gradient *in situ* in the mesophotic region could offer insight into the reasons for their success at such low light levels.

Rhodolith beds, or maërl, form important habitat for invertebrates and other macroalgae, and are significant producers of calcium carbonate (see reviews by Foster (2001) and Kahng et al. 2010). Rhodolith beds rank as one of the "Big Four" benthic communities, along with kelp forests, seagrass meadows, and non-geniculate coralline reefs (Foster 2001), and are ubiquitous to Hawai'i's mesophotic region and several other locations. Mesophotic rhodolith beds have been reported in the Bahamas from 67 to 91 m depths (Littler et al. 1991), Bermuda at 50 m depths (Reid & Macintyre 1988), the Caribbean from 30 to 60 m depths (Focke and Gebelein 1978), Puerto Rico from 45 to 60 m (Ballantine et al. 2008, Rivero-Calle et al. 2008), and Brazil from 40-100 m depths (Amando-Filho 2012). Fossilized rhodolith beds are found directly onshore from site 3 at Kaena Point, suggesting that rhodolith beds off North O'ahu have been in existence since the Pleistocene (Sherman 1992). Studies on the biology and physiology of rhodoliths in Hawai'i from shallow to mesophotic depths would be helpful in determining their role ecologically in this region, and the potential impact of ocean acidification. Calcified organisms, such as mesophotic rhodolith beds (Amando-Filho et al. 2012) and crustose

coralline algae (Diaz-Pulido et al. 2012), may experience a profound restructuring in response to ocean acidification in the coming decades.

Crustose coralline algae (CCA) are often one the deepest algae reported from mesophotic studies. The record for the deepest occurring macrophyte is 268 m for a crustose coralline algae off San Salvador Island (Littler et al. 1985). CCA were previously reported to 182 m depth in Hawai‘i (Agegian and Abbott 1982), while our lower depth limit for this group was 170 m. It is likely that CCA occur at depths below what was observed in this study given the difficulty of detecting CCA *in situ* and the lower abundances found in deeper water (> 170 m depths).

Potential nuisance species

Macroalgae are important components of coral reef communities, but phase shifts to algal dominance in degraded, nearshore coral reef systems are highly detrimental to coral reef health (Hughes 1994, Pandolfi et al. 2005, Jackson et al. 2008). Algal phase shifts may be the result of an algal bloom, which are generally perceived as an increase in algal biomass from an unbalance between growth and loss processes (Carstensen et al. 2007). In the case of harmful algal blooms (HABs), the trophic consequences are generally seen as unfavorable to the ecosystem and are often linked to both top-down processes (increases in nutrient supply, e.g. LaPointe 2007), and/or bottom-up processes (decreases in herbivory, e.g. Hughes et al. 1999).

Mesophotic ecosystems typically occur in remote, offshore locations, and are often isolated from anthropogenic stressors (such as the overfishing of herbivores and nutrient pollution) that cause algal proliferation and the subsequent degradation of

shallower reefs (Bak et al. 2005). Despite this isolation from anthropogenic stressors, and the inherently low light levels found in the mesophotic zone, there is often a surprisingly high abundance and/or diversity of macroalgae (Cheney & Dyer 1974, Agegian & Abbott 1985, Littler et al. 1985, Blair and Norris 1987, Searles & Schneider 1987, Hanisak & Blair 1988, Peckol and Ramus 1992, Friele et al.1995, Aponte & Ballantine 2001, Leichter et al. 2008, Littler and Littler 2012). This is also consistent with what is observed at remote tropical systems, such as the Northwestern Hawaiian Islands (NWHI), which have a naturally high abundance of algae and intact ecological processes (Parrish and Boland 2004, Vroom et al. 2006, Vroom and Braun 2010). Many macroalgal genera generally considered opportunistic and capable of forming blooms, such as *Ulva*, *Cladophora*, and *Caulerpa*, were found in high abundance in the mesophotic region in Hawai‘i, including the deepest alga observed (*Cladophora* sp. nov. 1 at 212 m). Our findings of an abundant, algal-dominated mesophotic zone in the MHI further supports that algal dominance is not necessarily a simple measure of reef health or degradation, and can reflect a natural state of variation (Vroom et al. 2006, Leichter et al. 2008). However, physiological studies and repeated monitoring of existing algal populations would be beneficial in evaluating whether areas with high algal biomass are ephemeral or episodic in nature and have a deleterious effect on other mesophotic communities.

Our discovery of expansive mesophotic populations of the invasive alga *Avrainvillea amadelpha* off O‘ahu stresses the need for understanding the dynamics of mesophotic macroalgal community composition if we are to understand the origin and extent of invasive species. Although *Avrainvillea amadelpha* is a common Indo-Pacific species, it was first reported in Hawai‘i in 1981 in 13 m of water off Kahe Point, O‘ahu

(Brostoff 1989). Presently, the distribution of this monospecific genus in Hawai‘i extends along most of O‘ahu’s southern and western shores (Nanakuli to Hanauma Bay in <1 to 90 m depth) and Smith et al. (2002) report a one-time collection of *A. amadelpha* from Kaua‘i. *A. amadelpha* spread from its first collection site at Kahe Point to Maunalua Bay (50 km distance) within about six years (Brostoff 1989), and has displaced native seagrass populations in the intertidal (Peyton 2009). The mechanism(s) of its rapid propagation is not known. Recent field surveys of some of the original collection sites cited in Brostoff (1989) revealed a 20-year persistence of *A. amadelpha* along the southern and western shores of O‘ahu, growing on both sand and rock (Peyton 2009). We now know that mesophotic populations exist offshore of the reported shallow collections; the species’ high abundance in the mesophotic introduces the possibility that it may have originated in deep water and then moved into shallower water. Within its natural range, Gepp and Gepp (1911) report *A. amadelpha* from 90 m depth in Mauritius (Indian Ocean), and Littler and Littler (1992) collected a West Indian species of *Avrainvillea* (*A. levis*) from 125 m depth on a Bahamian seamount. *Avrainvillea* has not yet been found in the Maui Nui complex, and its competitive impact on *Halimeda kanaolana* meadows in this region is unknown. *Avrainvillea*’s propensity to grow on either hard or soft substrate makes it particularly invasive, and increases the likelihood of its transport between the islands by potentially contaminating construction materials for underwater development, such as pipelines for an energy network.

New species

Explorations of MCEs often yield new records and descriptions of new species. We found 45% of the mesophotic flora exclusively in deep water (>40 m depth), suggesting

that Hawai‘i’s deep water flora is somewhat unique as compared to the shallow flora. The number of new species or records will likely increase as nongeniculate coralline algae, rhodoliths, and small, epiphytic algae are identified to the species level. Our results are most consistent with Agegian and Abbott (1985), who conducted two submersible dives on Penguin Bank (offshore Moloka‘i) yielding 40 to 50 macroalgal species, many of which were new to science or new records for Hawai‘i. In contrast, Doty et al. (1974) stated that Hawai‘i has no distinctive deep water seaweed community based on 54 dredge hauls off Kaua‘i, O‘ahu, Moloka‘i, and Maui. Methodological differences between dredge hauls versus *in situ* submersible collections, or spatial differences in the communities at the locations surveyed (discussed below), may account for these different interpretations. Mesophotic algal assemblages often contain a combination of shallow algae and some deep water specialists adapted to low-light (Searles & Schneider 1987, Hanisak & Blair 1988, Kajimura et al. 1987). Kajimura (1987) found 243 species from dredging in the Oki Islands, Sea of Japan, with only 4.2% of species growing below 50 m, although attenuation of irradiance is greater (estimated at 0.07 m^{-1} based on Secchi depth of ~20; Walker 1982) in this general region than in Hawai‘i. Hanisak and Blair (1988) found 42 new records (20% of the taxa identified) of deep water algae on the East Florida continental shelf. The high rate of new species and records in Hawai‘i’s mesophotic flora, which drives the differences between the shallow and deep floras, may be due to the isolation of the Hawaiian Islands and subsequent evolution of endemic species.

Distributional patterns

Hawai‘i’s mesophotic flora is composed of a diverse range of species forming complex distributional patterns varying spatially among islands and among depth ranges at a specific site. We found at least 11 dominant macroalgal and coral assemblages, suggesting that large spatial studies with numerous sites are necessary to adequately describe the variation in species composition. This spatial complexity may be illustrated by the large differences in dominant species found between surveys of the mesophotic flora in Hawai‘i. Surprisingly, Doty et al. (1974) described the deep water flora in Hawai‘i to be impoverished and lacking large green algae, with siphonous genera represented by few species with relatively small specimens in limited quantities. Thus, Doty et al. (1974) did not sample the expansive assemblages of *H. kanaloana*, *Udotea* sp., *Avrainvillea amadelpha*, *Caulerpa filicoides*, or *Microdictyon umbilicatum* reported in this study. Conversely, we did not observe the large stands of *Sargassum hawaiiensis* reported by Doty et al. (1974) from dredging off O‘ahu. These differences may also reflect temporal changes in the species composition of the communities over the past ~30 years, or seasonal differences in *Sargassum* abundance. However, in the case of *H. kanaloana* meadows, archived HURL video data from the 1980’s shows *H. kanaloana* meadows dominating areas of the Maui Nui complex.

Macroalgal abundance and species composition varies seasonally depending upon fluctuating abiotic factors (e.g., temperature and light) and the number of perennials versus annuals in the community. The majority of our sampling occurred during winter and spring months (November to April) due to the availability of the submersibles and ROV, suggesting that the flora described may be lacking a seasonal component. Most

studies examining abundance over summer and winter months find a strong seasonal signal, with maximum abundance in the summer (Cheney and Dyer 1974, Hanisak and Blair 1988, Peckol & Ramus 1988, Sanson et al. 2002). Hanisak and Blair (1988) described taxonomic diversity in deep water algae from the East Florida continental shelf as maximal during late spring and summer and minimal during fall and winter. Peckol and Ramus (1988) attributed the seasonality in abundance on the Carolina outer shelf to changes in the cover of *Dictyopteris* and *Dictyota*, while Sanson et al. 2002 described an ephemeral spring-summer flora characterized by changes in red algal composition (e.g. *Dudresnaya* and *Ganonema*) in the Canary Islands. Changes in seasonal abundance may also be due to inconspicuous stages in an algal life cycle or changes in morphology with season. Cheney and Dyer (1997) found species possessing foliaceous habits and weak holdfast systems in summer only, while species with crustaceous, creeping, or prostrate morphologies or extensive holdfasts were dominant in both summer and winter. However, some mesophotic algal communities may be stable seasonally as well as annually; Nugues and Bak (2008) found no significant differences in the percent cover of *Lobophora variegata* over a 30 year period at 40 m, despite large fluctuations at 20-30 m depths.

The vertical zonation of the 11 dominant macroalgal and coral assemblages in the present study overlapped, although they were often island or region-specific and were marked by the termination of most assemblages at two distinct isobaths. For example, psammophytic algal meadows and beds and *Montipora* reefs ended at 80 to 90 m depths, while *Leptoseris* reefs, species within the Ulvales, rhodophyte blades, rhodoliths, and *H. distorta* beds ended at 120 to 130 m depths. However, species of *Cladophora*,

nongeniculate coralline algae, and *Distromium flabellatum* were found throughout the mesophotic zone, with overlapping depth ranges extending into deeper water. Kahng and Kelley (2007) described the vertical zonation of megabenthic taxa in the Au‘au Channel, and found shallower depth limits with less diversity in algal assemblages; *Halimeda* and foliose macroalgae ended at 80 m, with the lower limit of macroalgae occurring at 100 m and relatively few coral and algal taxa dominating each zone. The zonation patterns for macroalgal divisions in clear, oceanic waters from other studies are usually consistent with Dring (1981), with rhodophytes growing deeper than chlorophytes and ochrophytes (Gilmartin 1960, Larkum et al. 1967, Agegian and Abbott 1985, Littler et al. 1985, Littler et al. 1986, Hanisak and Blair 1988). Littler et al. (1986) recognized four algal zone assemblages between 81 to 268 m depths on San Salvador, Bahamas, with each zone dominated by a particular alga: the *Lobophora* zone (81 to 90 m), the *Halimeda* sp. zone (90 to 130 m), the *Peyssonnelia* zone (130 to189 m), and the crustose coralline algae zone (189 – 268 m). However, off Lee Stocking Island in the Bahamas, Aponte and Ballantine (2001), found a slightly different zonation pattern, with the green endolithic alga *Ostreobium* as the deepest occurring alga. The dominant species in each zone were *Lobophora* and *Halimeda* (45 – 60 m), a Corallinales/*Peyssonnelia* group (60 – 120 m, with *Ostreobium* from 90 – 120 m), and *Ostreobium* (150 – 200 m). The high level of spatial variability and diversity of dominant macroalgal assemblages we observed illustrates the complexity of factors that likely affect the species composition and abundance of mesophotic macroalgae in Hawai‘i. Classifying zones may be useful for descriptive purposes, but their biological relevance is limited in diverse, spatially complex ecosystems (Foster and Schiel 1992, Spalding et al. 2003).

Abiotic and biotic factors

Mesophotic macroalgae are generally buffered from wave damage, given their distance below the water's surface (Leichter et al. 2008), but their occurrence may be affected by other physical factors such as nutrient availability, light, temperature, substrate availability and inclination, and sedimentation or sand scour from currents. The most distinctive changes in the diversity and abundance of mesophotic macroalgal assemblages from this study occurred at 80-90 m and 120-130 m depths. This corresponded to ~3% and 0.5% SI, respectively, and included the depths where large thermoclines were observed, depending on the site. The %SI values at the lower depth limits for macroalgae range from 0.05 to 0.1% for multicellular algae (Luning and Dring 1979, Lobban and Harrison 1994), or more specifically 0.5% SI for leathery algae, 0.1% for foliose and delicate algae, and 0.01% for encrusting algae (Markager and Sand-Jensen 1992). While temperature may be influencing the lower depth limits of macroalgae at both the 80-90 and 120-130 m depths, light appears to be less important at 80-90 m, and more influential at 120-130 m depths. The impact of sedimentation appears minimal, except at sites closer to shore with a nearshore terrigenous input, such as sediment run-off from the denuded island of Kahoʻolawe or from nearby river basins at site 57. Sand scour from shifting sands, as described by Leichter et al. (2008), did not appear to impact the lower depth limits of the algae observed.

Herbivory does not appear to be an influential factor structuring mesophotic macroalgae in Hawaiʻi, given the high abundance of luxuriant algal assemblages and the lack of feeding scars on collected macroalgae. Steep decreases in grazing pressure have been found for herbivorous fish in the mesophotic region in the Red Sea due to decreases

in fish biomass with increasing depth (Brokovich et al. 2010). However, manipulative experiments with grazer exclusions and the proper controls along a depth gradient would be most effective at evaluating the importance of herbivory in Hawai‘i’s mesophotic region. A natural, and unfortunate, experiment illustrating the unknown importance of herbivory is the introduction of the invasive, predatory lionfish into the mesophotic region in the Bahamas (Lesser and Slattery 2011). The presence of lionfish was correlated with a significant decrease in several guilds of native coral reef fish, causing a phase transition to algal-dominated (50% benthic cover) communities and decrease in the percent cover of corals and sponges (Lesser and Slattery 2011).

Biogeography

The origin of fishes and mollusks in Hawaiian waters are generally described as having Indo-western Pacific relationships, but the biogeography of macroalgae is more complex, with Caribbean, Indian, and even western Pacific origins (Abbott 1999). This has been illustrated in descriptions of mesophotic macroalgae from the Northwestern Hawaiian Islands (NWHI), which contain a mixture of tropical to cold-temperate species (McDermid and Abbott 2006). The mesophotic flora from the Main Hawaiian Islands shared many similarities with the NWHI, such as the occurrence of *C. campanulatum*, *C. desultorium*, *C. hawaiiense*, *Distromium flabellatum*, and *Sporochnus dotyi*, suggesting some continuity across the Hawaiian Archipelago. Although we did not find the cold-temperate species *Desmarestia ligulata* reported from the NWHI, we found genera such as *Kallymenia* and *Sporochnus* that typically have cold-temperate water biogeographic affinities (Abbott 1999). Several of the red algal specimens from the order Rhodymeniales, and the genera *Kallymenia*, *Halymenia*, and *Grateloupia* were of such

considerable size that they were reminiscent of the central California intertidal zone instead of the tropical waters of Hawai‘i. Detailed floristic studies combining morphological and molecular analyses are needed before more detailed biogeographical interpretations can be developed. The geographic isolation of the Hawaiian Islands makes the identification of these species of key importance to our understanding of biogeography in the Pacific. For instance, Hanisak and Blair (1988) found the mesophotic flora to be relatively continuous over a large portion of the tropical and subtropical western North Atlantic, suggesting that biogeographic boundaries, such as Cape Canaveral, may only be appropriate for shallow water populations.

Conclusions

The mesophotic environment in Hawai‘i and other tropical locales provide a unique combination of clear oceanic water and suitable substrate, allowing light penetration to depths with cooler water and, in some cases, higher nutrients. In essence, this creates a productive euphotic zone similar to temperate regions underneath tropical, nutrient-poor water (Santelices 2007). The synthetic oceanographic and ecophysiological modeling used by Graham et al. (2007) to pinpoint mesophotic tropical kelp refugia illustrates the potential to discover other productive areas containing mesophotic macroalgae and corals. The findings from this study suggest that these mesophotic reefs in Hawai‘i are expansive, abundant, and diverse, but spatially variable in terms of distribution. The development of spatial modeling using specific ecophysiological parameters for key macroalgal assemblages would aid in discovering additional areas of productivity.

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TABLES

Table 1. Submersible (*Pisces IV* and *Pisces V*) and ROV *RCV-150* survey locations from 2004 to 2011. See Figure 1 for site locations.

Site	Dive no.	Location	Date	Transect Length (m)	Duration (hr:min)	Depth Range (m)	Major Features and Assemblages
2	*PIV-183*	N. O‘ahu	16-Nov-06	4470	4:37	52 - 103	Sand and carbonate with isolated algae
3	*RCV-371*	N. O‘ahu	16-Nov-06	3240	3:00	36 - 102	*Sporolithon* spp. with Ulvales, carbonate boulders
5	*RCV-370*	W. O‘ahu	15-Nov-06	1810	1:21	37 - 140	*H. distorta*, *Avrainvillea*, *Montipora*^{P} on carbonate ledge
7	*RCV-369*	S.W. O‘ahu	15-Nov-06	2330	1:24	39 - 172	*Udotea* and *Avrainvillea*
8	*PIV-188*	S. O‘ahu	27-Nov-06	3710	7:31	49 - 221	*Udotea* and *Avrainvillea*, carbonate ledge
9	*RCV-303*	S. O‘ahu	13-Dec-04	4320	3:55	28 - 67	*Udotea* and *Avrainvillea*
10	*PIV-182*	S. O‘ahu	15-Nov-06	5820	7:57	45 - 104	*Udotea*, carbonate ledge with *Montipora*^{P} and *H. distorta*
11	*PV-562*	S. O‘ahu	3-Sep-04	3520	6:50	54 - 322	*Udotea* and *Avrainvillea*
12	*PV-606*	S. O‘ahu	13-Dec-04	2300	7:40	51 - 179	*Udotea* and *Avrainvillea*
14	*RCV-376*	S. O‘ahu	27-Nov-06	1260	1:05	32 - 106	Sand with sparse algae
20	*RCV-377*	S.E. O‘ahu	27-Nov-06	1200	0:50	42 - 58	Sand with sparse algae
23	*PV-564*	Penguin Bank	5-Sep-04	2810	6:18	50 - 371	Nongen. coralline algae, Ulvales, *H. distorta*
24	*PIV-184*	Penguin Bank	17-Nov-06	4820	7:15	77 - 132	Sand and carbonate with isolated algae
25	*RCV-304*	Penguin Bank	14-Dec-04	4430	4:03	63 - 120	Mixed fleshy macroalgae
26	*PIV-189*	Penguin Bank	28-Nov-06	4490	7:45	57 - 83	Carbonate with *Montipora*^{P} reefs
27	*PV-563*	Penguin Bank	4-Sep-04	3460	7:34	80 - 250	*H. kanaloana*, *Spatoglossum*, Ulvales, and *Cladophora* spp.
28	*PIV-133*	Penguin Bank	17-Dec-04	3790	7:05	65 - 164	*Distromium* and *H. kanaloana*
29	*RCV-378*	S. Moloka‘i	28-Nov-06	5710	3:50	30 - 64	*H. kanaloana*, *H. distorta*, *Montipora*^{B} reefs
30	*RCV-372*	N. Lāna‘i	17-Nov-06	5020	2:29	33 - 106	*H. kanaloana*, *H. distorta*, *Leptoseris* and black coral reefs
31	*PIV-185*	W. Lāna‘i	18-Nov-06	4100	6:12	25 - 96	*H. kanaloana*, shallow *Porites* reef
32	*PIV-186*	N.E. Lāna‘i	19-Nov-06	3010	7:30	48 - 104	*H. kanaloana*, carbonate reef with black coral
33	*PV-565*	W. Maui	6-Sep-04	3150	7:38	56 - 211	*Cladophora* spp., *H. kanaloana*, *H. distorta*, *C. filicoides*
34	*PIV-131*	W. Maui	15-Dec-04	1770	6:07	77 - 203	*Leptoseris* reefs with *Codium mamillosum* and *H. distorta*
35	*RCV-434*	W. Maui	4-Apr-09	784	0:13	33 - 55	*Montipora*^{B} reefs, *H. kanaloana*
36	*RCV-432*	W. Maui	3-Apr-09	3760	2:00	32 - 52	*Montipora*^{B} reefs, *H. kanaloana*
37	*RCV-435*	W. Maui	4-Apr-09	5710	2:48	42 - 65	*Montipora*^{B} reefs, *H. kanaloana*
38	*RCV-431*	W. Maui	2-Apr-09	1990	1:22	32 - 51	*Montipora*^{B} reefs, *H. kanaloana*
39	*RCV-383*	S. Maui	30-Nov-06	2070	1:00	53 - 72	*H. kanaloana*, *Montipora*^{B} reefs with *Distromium*
40	*RCV-283*	E. Lāna‘i	8-Sep-04	3600	2:30	48 - 99	*H. kanaloana*
41	*PV-757*	W. Maui	2-Mar-11	3933	7:14	65 - 100	*Microdicyton*, *Leptoseris* reefs, Ulvales, *H. kanaloana*

42	*PIV-233*	W. Maui	19-Jan-10	2629	7:09	64 - 116	*Leptoseris* reefs, Ulvales, *H. kanaloana*
43	*RCV-433*	W. Maui	3-Apr-09	1330	0:55	48 - 57	*Montipora*^{B} reefs with *Distromium, H. kanaloana*
44	*RCV-375*	W. Maui	19-Nov-06	2170	1:43	56 - 63	*H. kanaloana, Leptoseris* and *Montipora*^{B} reefs
45	*PV-755*	W. Maui	28-Feb-11	3280	7:16	42 - 117	*Leptoseris* reefs, Ulvales, *H. kanaloana*
46	*PV-736*	W. Maui	5-Apr-09	2944	7:32	84 - 113	*Leptoseris* reefs with mixed algae, *H. kanaloana*
47	*PV-734*	W. Maui	3-Apr-09	2815	7:39	63 - 121	*Leptoseris* reefs with mixed algae
48	*PIV-190*	W. Maui	29-Nov-06	5840	8:32	60 - 141	*H. distorta* and *H. kanaloana* with *Leptoseris* reefs
49	*PIV-232*	W. Maui	18-Jan-10	1145	7:36	95 - 151	*Leptoseris* reefs, Ulvales, *H. kanaloana*
50	*RCV-373*	W. Maui	18-Nov-06	5780	3:50	54 - 137	*H. kanaloana, H. distorta, Leptoseris* and black coral reefs
51	*RCV-379*	S.W. Maui	29-Nov-06	3710	2:12	54 - 96	*H. kanaloana, H. distorta, Leptoseris* and *Montipora*^{B} reefs
52	*RCV-380*	S.W. Maui	29-Nov-06	1470	1:02	76 - 82	*H. kanaloana, H. distorta*
53	*PIV-191*	S.W. Maui	30-Nov-06	5930	6:31	54 - 83	*H. kanaloana,* basalt with nongen. coralline algae
54	*RCV-381*	S. Maui	30-Nov-06	2600	2:10	34 - 89	*H. kanaloana* with epiphytes
55	*RCV-382*	S. Maui	30-Nov-06	1180	1:40	63 - 78	*H. kanaloana* with epiphytes
56	*PIV-192*	S. Maui	1-Dec-06	2400	2:52	77 - 113	mud, no algae
57	*RCV-305*	N. Kahoʻolawe	16-Dec-04	4810	4:00	47 - 96	*H. kanaloana*
58	*PV-566*	W. Kahoʻolawe	7-Sep-04	3760	7:33	55 - 135	*H. kanaloana* with epiphytes, Ulvales, *Dasya* spp.
59	*PIV-132*	W. Kahoʻolawe	16-Dec-04	4120	7:48	49 - 129	*H. kanaloana* with *Spyridia filamentosa* epiphytes
			Total	160.3 km	225:03		

Montipora^{P}, plating morphology on carbonate substrate; *Montipora*^{B}, branching morphology on soft substrate

Table 2. Location information for technical diving collections around O‘ahu. See Fig. 1 for site number locations. A range of depths is given if collections were made from multiple depths.

Site no.	Description	Dates	Depth (m)
1	Mokuleia	16-Nov-09	46
4	Makua	29-Sep-05	40
6	Kalaeloa (Koolina)	3-May-05, 12-May-05, 13-May-05, 15-Dec-05	40
13	Barber's Point	22-Jan-04	63
15	Ewa Beach	24-Apr-09	40 - 45
16	Sea Tiger wreck	20-Oct-05	57
17	Ala Wai Ledge	23-Jun-08, 25-Jun-08, 27-Jun-08	45 - 60
18	Offshore Ala Wai Harbor	6-Jul-05	50
19	Waikiki (Diamond Head)	21-Jul-05	40 - 49
21	Maunalua Bay	19-May-07	50
22	Portlock	4-Apr-05, 2-Aug-05, 2-Dec-05	41 - 43

Table 3. Species collected by technical divers, submersibles, and the ROV from 40 to 212 m depths. Depth range is the depths over which a species was collected and/or observed from video (if discernible at the species level) from all sites. Previous depth range is the depth range previously reported in the literature from Hawai‘i (Abbott 1999, Abbott and Huisman 1994). “na” is not applicable because this species is a new record or species. Abundance is the maximum visual estimate of abundance per site where 1 = ≤ 9% cover; 2 = 10 – 25% cover; 3 = 26 - 50% cover; 4 = 52 - 75% cover, 5 = 76 - 100% cover. A range of abundance is given if maximum values are different between sites.

	Depth range (m)		
Species by phylum	This study	Previous	Abundance
CHLOROPHYTA			
Anadyomene wrightii Harvey ex J. E. Gray	85	100	1
Avrainvillea amadelpha (Montagne) A. Gepp & E. S. Gepp	40 - 90	1 - 13	1 - 5‡
Boodlea montagnei (Harvey ex J. E. Gray) Egerod 1952	40	1	1
+*Caulerpa filicoides* Yamada	55 - 93	na	1 - 5‡
+*Caulerpa mexicana* Sonder ex Kützing	40 - 129	na	1 - 4‡
+*Cladophora albida* (Nees) Kutzing	90	na	1
+*Cladophora* cf. *sakai* I. A. Abbott	90 - 96	na	1
Cladophora dotyana W. J. Gilbert	80 - 170	1 - 60	1
**Cladophora* sp. nov. 1	86 - 212	na	5
**Cladophora* sp. nov. 2	57 - 70	na	1
**Cladophora* sp. nov. 3	71 - 94	na	1
Cladophora vagabunda (Linnaeus) Hoek	40 - 129	1 - 5	1
Codium campanulatum P. C. Silva & M. E. Chacana	69 - 100	56 - 185	1
Codium desultorium P. C. Silva & M. E. Chacana	40 - 122	27 - 77	1
Codium hawaiiense P. C. Silva & M. E. Chacana	46 - 122	1 - 55	1
Codium mamillosum Harvey	55 - 117	1 - 200	1 - 2
Codium reediae P.C.Silva	40 - 109	5 - 10	1
Codium subtubulosum Okamura	45 - 80	26 - 92	1
Halimeda discoidea Decaisne	40 - 86	1 - 10	1
Halimeda distorta (Yamada) Hillis-Colinvaux	40 - 138	55 - 106	1 - 5‡
Halimeda kanaloana Vroom	40 - 95	1 - 90	2 - 5‡
Microdictyon umbilicatum (Velley) Zanardini	75 - 94	1 - 59	5‡
Microdictyon japonicum Setchell	83 - 109	1 - 59	1
Palmophyllum crassum (Naccari) Rabenhorst	94	2 - 12	1
**Struvea* sp. nov.	100 - 122	na	1
Siphonocladus tropicus (P. L. Crouan & H. M. Crouan) J. Agardh	40 - 55	1 - 2	1
**Udotea* sp. nov.	40 - 90	na	5‡
**Ulva* sp. nov.	93	na	1 - 4
**Umbraulva* sp. nov. 1	85	na	1 - 4
**Umbraulva* sp. nov. 2	125	na	1 - 4
Valonia trabeculata Egerod	80	1	1
Dictyosphaeria cavernosa (Forsskål) Børgesen	40	1 - 59	1
PHAEOPHYCEAE			
Dictyopteris australis (Sonder) Askenasy	83 - 122	1 - 50	2
Dictyopteris plagiogramma (Montagne) Vickers	40 - 134	1 - 80	2
Dictyopteris repens (Okamura) Børgesen	40 - 85	1 - 50	1
Dictyota stolonifera E. Y. Dawson	93	1 - 60	1
Dictyota sandvicensis Sonder	87 - 100	1 - 7	1
Dictyota ceylanica Kützing	55 - 129	1 - 75	1
Distromium flabellatum Womersley	57 - 160	20 - 100	1 - 5‡

Padina boryana Thivy	40	5 - 10	1
Padina melemele I. A. Abbott & Magruder	55	10 - 54	1
*+*Padina* sp.	109 - 129	na	2
Spatoglossum macrodontum J. Agardh	83 - 208	1 - 62	4
Sporochnus dotyi Brostoff	50	7 - 40	2
RHODOPHYTA			
Acanthophora pacifica (Setchell) Kraft	85	1 - 5	1
Amansia glomerata C. Agardh	40 - 113	1 - 30	1 - 3
Cryptonemia umbraticola E. Y. Dawson	43 - 76	4 - 10	1
*+*Dasya* sp. 1	40 - 134	na	1
*+*Dasya* sp. 2	129	na	1
Dichotomaria marginata (J. Ellis & Solander) Lamarck	45	1 - 10	1
Gibsmithia Hawai'iensis Doty	40	10 - 25	1
Gracilaria parvispora I. A. Abbott	40 - 45	1 - 10	1
*+*Grateloupia* sp. 1	94 - 107	na	1
**Grateloupia* sp. nov 1	76 - 80	na	1
*+*Halymenia* sp. 1	92	na	1
*+*Halymenia* sp. 2	85	na	1
*+*Halymenia* sp. 3	40 - 49	na	1
**Halymenia* sp. nov. 1	71 - 113	na	1
**Halymenia* sp. nov. 2	104	na	1
**Halymenia* sp. nov. 3	97	na	1
*Hypnea spinella (*C.Agardh) Kützing	40 - 45	1 - 10	1
*+*Kallymenia* sp. 1	115 - 118	na	1
*+*Kallymenia* sp. 2	76	na	1
*+*Kallymenia* sp. 3	76 - 94	na	1
Martensia fragilis Harvey	55 - 102	1 - 47	1
Nongeniculate coralline algae	40 - 170	1 - 182	1 - 5
Peyssonnelia inamoena Pilger	63 - 115	1 - 30	1 - 2
Portieria hornemannii (Lyngbye) P. C. Silva	75	1 - 33	1
*+Rhodymeniales 1 (twisted)	76 - 104	na	2
*+Rhodymeniales 2 (strap-like)	41 - 122	na	1
*+Rhodymeniales 3 (large cells)	54 - 144	na	2
*+Rhodymeniales 4	87 - 94	na	1
*+Rhodymeniales 5	104 - 108	na	1
Scinaia furcata Zablackis	40	1 - 10	1
Sporolithon spp. (rhodoliths)	40 - 129	1 - 171	1 - 5‡
Spyridia filamentosa (Wulfen) Harvey	40 - 125	1 - 10	1 - 2

*New species verified with molecular analyses
+New record for Hawai'i
*+ New species and/or new record, awaiting verification with molecular analyses
‡Meadow or bed-forming

Table 4. Most abundant macroalgal and coral assemblages, with subdominant taxa observed during surveys. Depth range includes the depths where the dominant assemblage is most abundant. Assemblages on Penguin Bank are included with Molokaʻi. "All islands surveyed" include Oʻahu, Molokaʻi, Lānaʻi, Maui, and Kahoʻolawe . Algae with "*" also occur as a monospecific assemblage outside of the dominant assemblage.

Depth range (m)	Dominant assemblage	Subdominant taxa	Substrate	Distribution by island
40 - 80	*Avrainvillea amadelpha* beds	small epiphytes	soft sediments	Oʻahu
40 - 80	*Montipora capitata* reef (branching)	*Distromium flabellatum** *Caulerpa filicoides** *Microdictyon umbilicatum** *Cladophora* spp.* Rhodymeniales	soft sediments	Maui, Lānaʻi
40 - 80	*Udotea* sp. beds	small epiphytes	soft sediments	Oʻahu
40 - 95	*Halimeda kanaloana* meadows	*Caulerpa mexicana** *Codium* spp. *Sporochnus dotyi* (epiphyte) red algal epiphytes	soft sediments	All islands surveyed (small patches on Oʻahu)
40 - 120	*Sporolithon* spp. beds (rhodoliths)	Ulvales Rhodymeniales *H. distorta* *Codium* spp.	soft sediments	Oʻahu, Molokaʻi, Lānaʻi, Maui
40 - 130	*Halimeda distorta* beds	Rhodymeniales *Codium* spp.	carbonate	All islands surveyed
40 - 170	Nongeniculate coralline algae	*Peyssonnelia inamoena* Rhodymeniales	carbonate basalt	All islands surveyed
40 - 212	*Cladophora* spp. blooms	none	rocky outcrops small shell fragments	Maui, Lānaʻi
50 - 130	*Leptoseris* spp. reef	*Codium mamillosum* *Peyssonnelia inamoena* *Distromium flabellatum* *Halimeda distorta* Ulvales	soft sediments carbonate	Molokaʻi, Lānaʻi, Maui
60 - 160	*Distromium flabellatum* beds	*Caulerpa mexicana*	soft sediments	All islands surveyed
65 - 125	Ulvales and large red blades	Rhodymeniales *H. distorta*	carbonate	Oʻahu, Molokaʻi, Lānaʻi, Maui

Table 5. Calculated mean percent of subsurface irradiance and measured mean irradiance (PAR) at depth for a water column with a vertical attenuation coefficient of 0.042 m^{-1}. Irradiance and SE based on mean light profiles from 4 sites in Figure 6.

Depth (m)	Irradiance ($\mu E\ m^{-2}\ s^{-1}$)	SE	Percent Subsurface Irradiance
0.01	1066.80	17.30	99.96
10	698.38	10.30	65.70
20	457.15	6.33	43.17
30	299.34	4.07	28.37
40	196.06	2.75	18.64
50	128.46	1.93	12.25
60	84.19	1.38	8.05
70	55.19	1.00	5.29
80	36.19	0.73	3.47
90	23.74	0.53	2.28
100	15.58	0.38	1.50
110	10.22	0.27	0.99
120	6.71	0.19	0.65
130	4.41	0.14	0.43
140	2.89	0.10	0.28
150	1.90	0.07	0.18
160	1.25	0.05	0.12
170	0.82	0.03	0.08
180	0.54	0.02	0.05
190	0.36	0.02	0.03
200	0.23	0.01	0.02
210	0.15	0.01	0.01
220	0.10	0.01	0.01

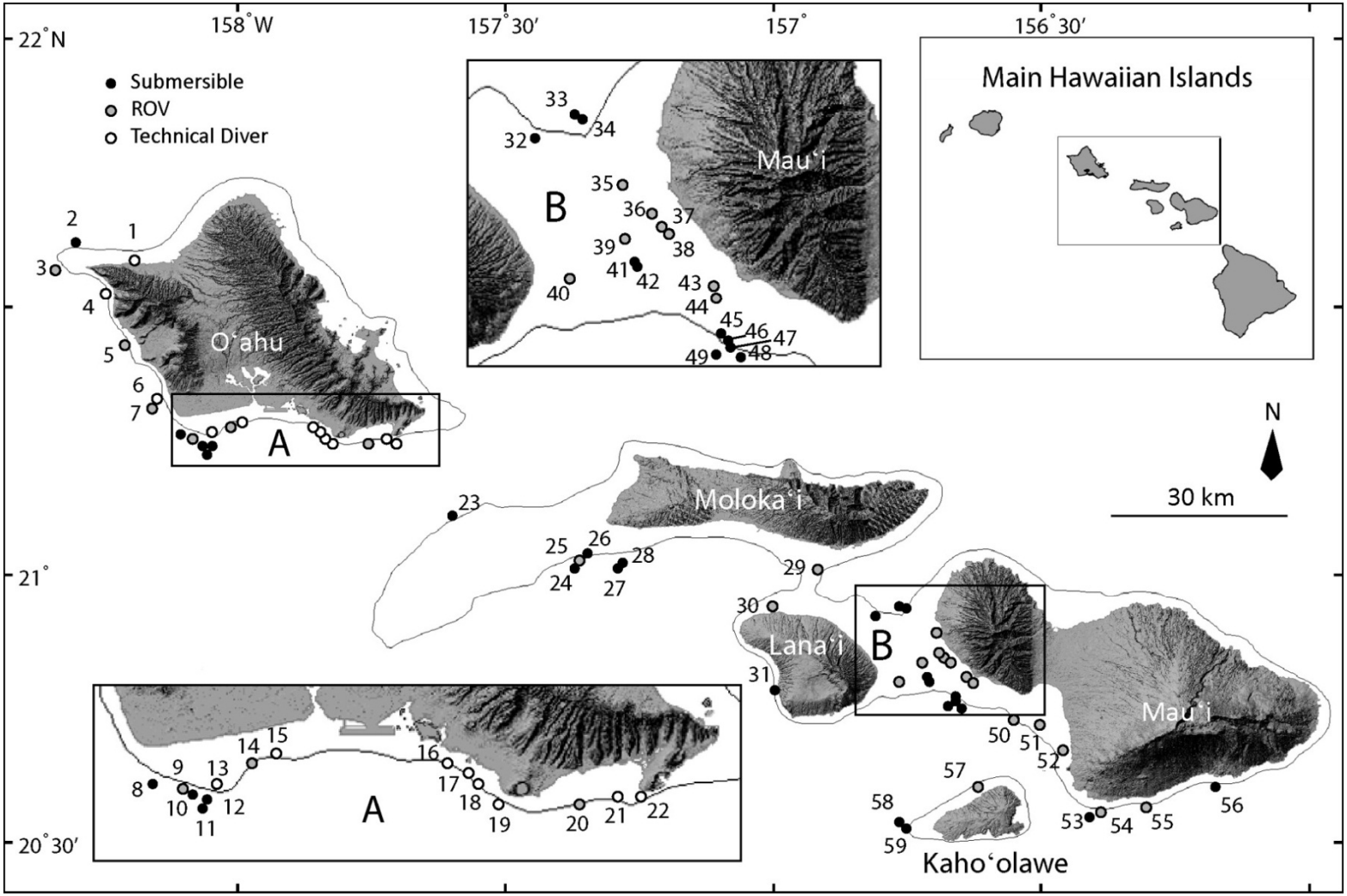

Figure 1. Sites surveyed by technical divers, HURL submersibles (*Pisces IV* and *Pisces V*), or the HURL ROV *RCV-150*. Grey contour lines are at 100 m depth. See Tables 1 and 2 for site information.

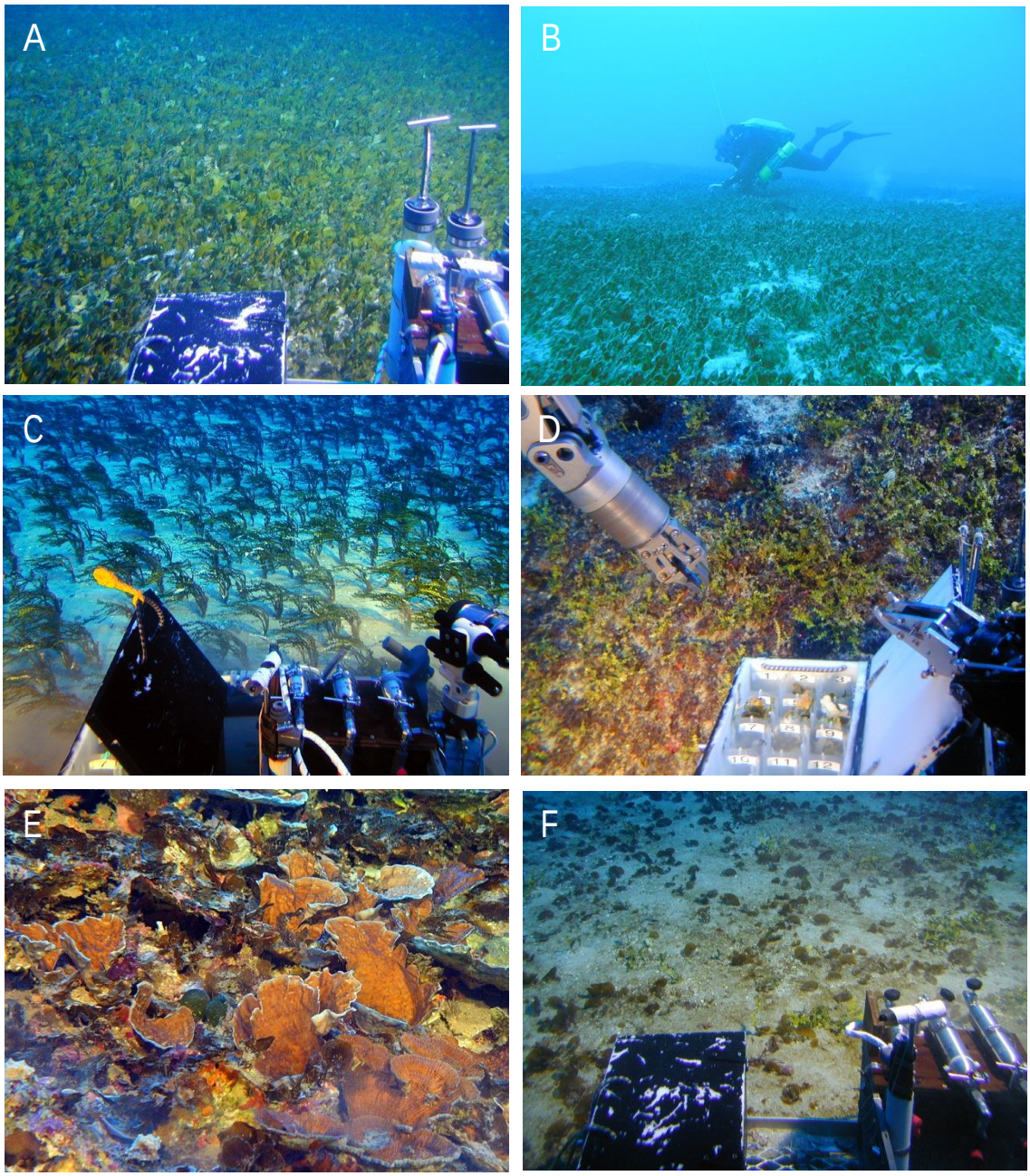

Figure 2. Representative images of mesophotic macroalgal assemblages. A) *Udotea* sp. bed at 67 m. B) *Avrainvillea amadelpha* bed at 50 m. Note the mounding of sediments behind the diver. Photo credit: Heather Spalding. C) *Halimeda kanaloana* meadow at 85 m. D) *Halimeda distorta* bed at 75 m. E) *Leptoseris* spp. coral reef with *Codium mamillosum* and *Distromium flabellatum* at 95 m. F) *Distromium flabellatum* in sand at 110 m. Photo credit for images A, C, D, E, and F: Hawai‘i Undersea Research Laboratory.

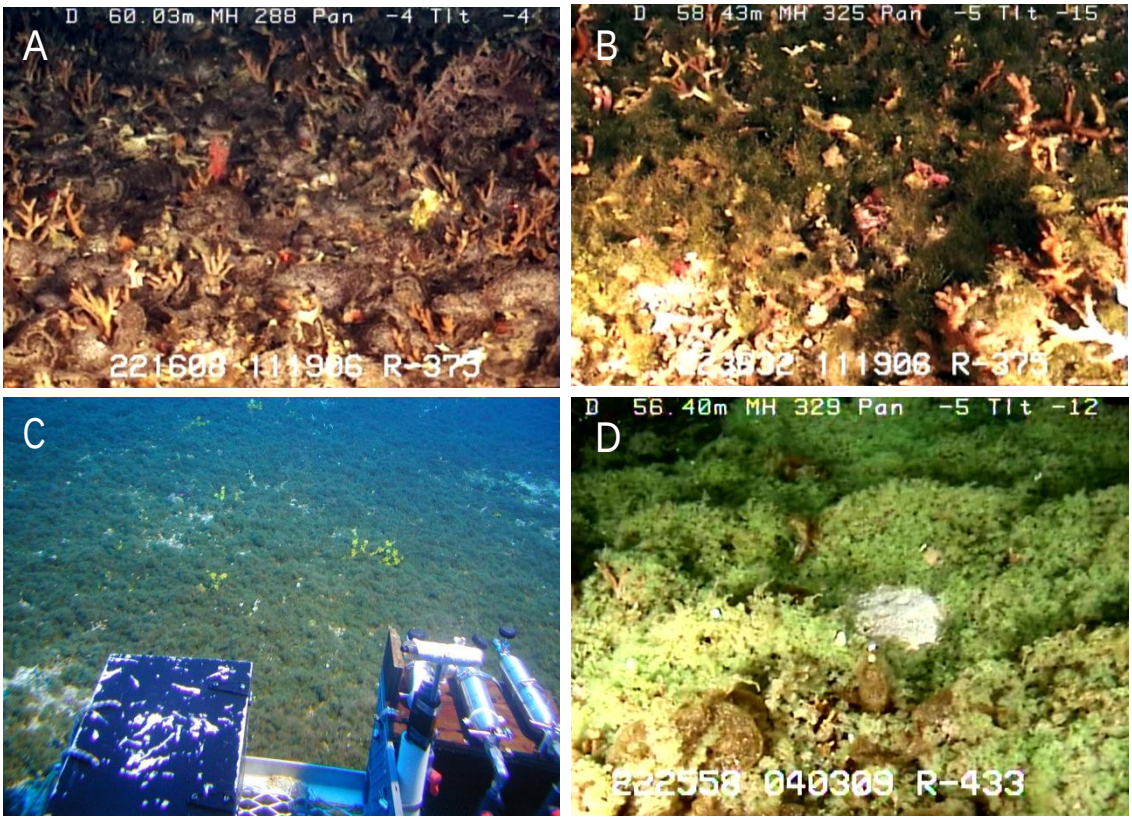

Figure 3. Mesophotic macroalgal assemblages often found with *Montipora capitata* coral reefs (branching morphology) or growing over other substrata off of west Maui. A) *Distromium flabellatum* with *M. capitata* at 60 m. B) *Cladophora* spp. with *M. capitata* at 58 m. C) *Microdictyon umbilicatum* with a few *Halimeda distorta* over carbonate at 70 m. D) *Caulerpa filicoides* growing over *M. capitata* and *D. flabellatum* at 56 m. Photo credit: Hawai‘i Undersea Research Laboratory.

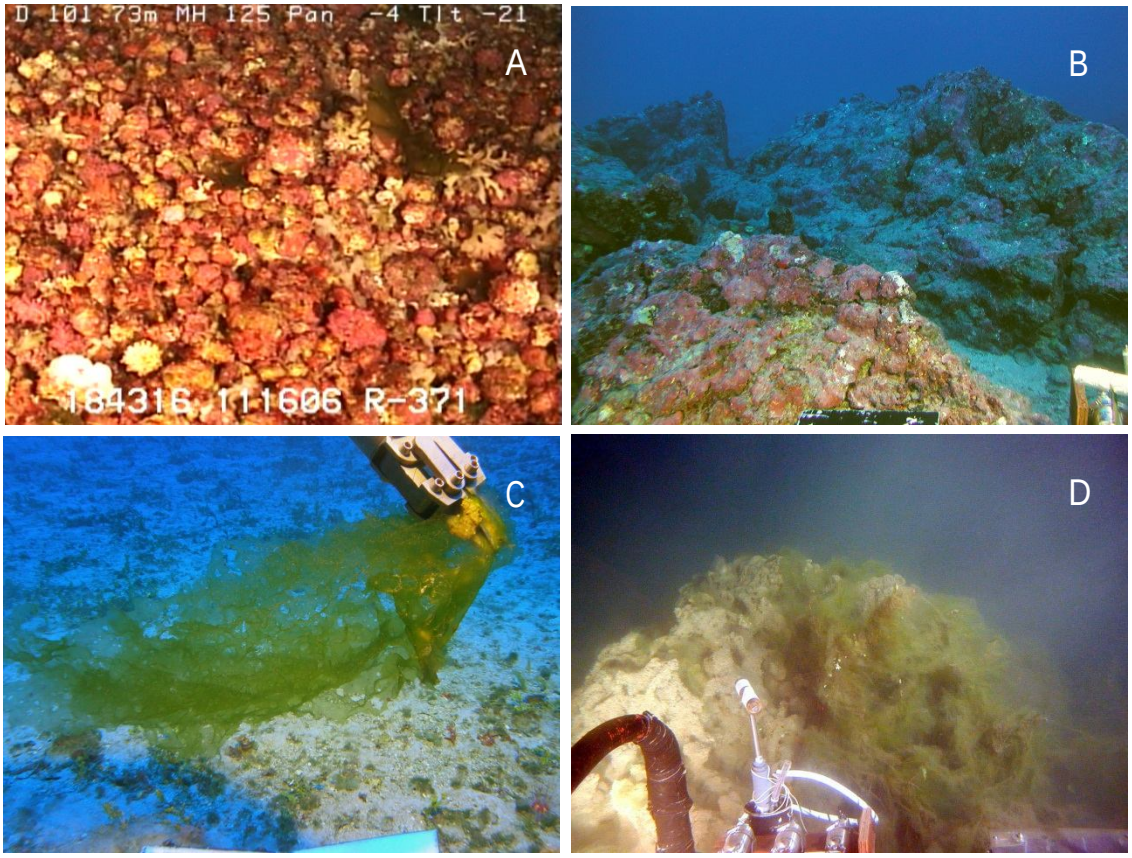

Figure 4. Representative images of mesophotic macroalgal assemblages. A) *Sporolithon* spp. (rhodoliths) with small Ulvales at 101 m. B) Nongeniculate coralline algae at 77 m. C) *Ulva* sp. nov. at 85 m. D) *Cladophora* sp. nov. 1 at 212 m on a rocky outcrop. Photo credit: Hawai‘i Undersea Research Laboratory.

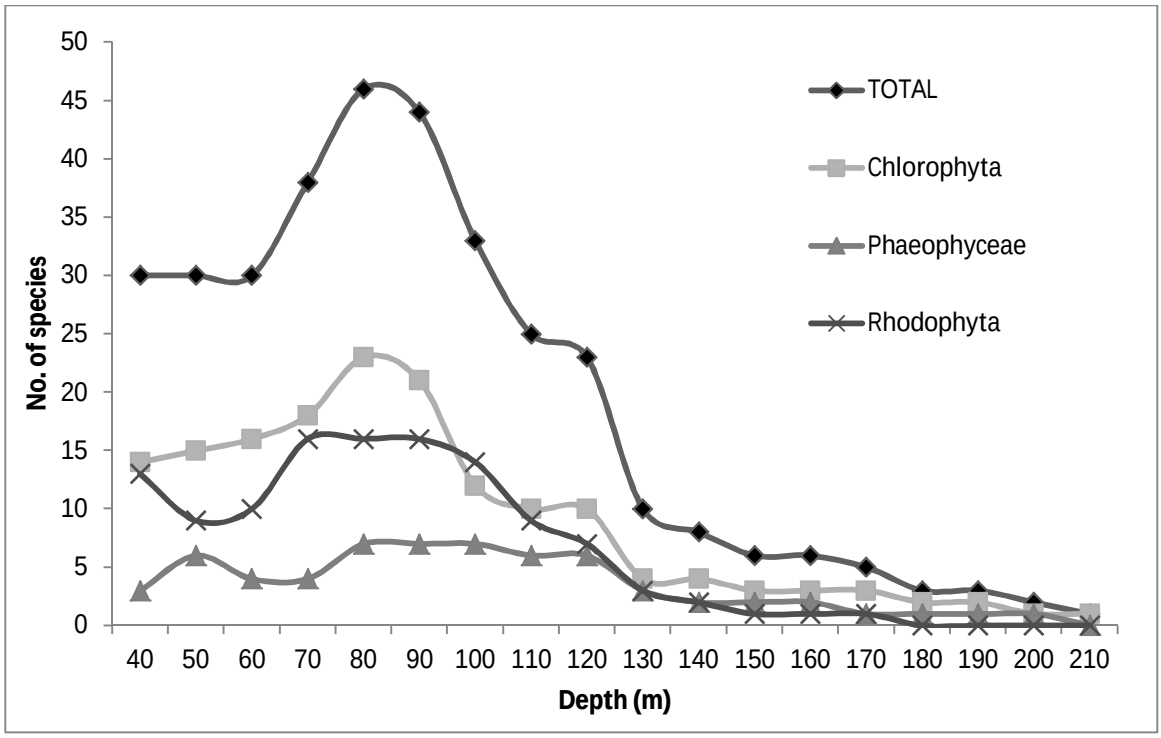

Figure 5. The number of macroalgal species found at each depth surveyed. Depth of occurrence is based upon collections and visual observations when species level identifications were verified.

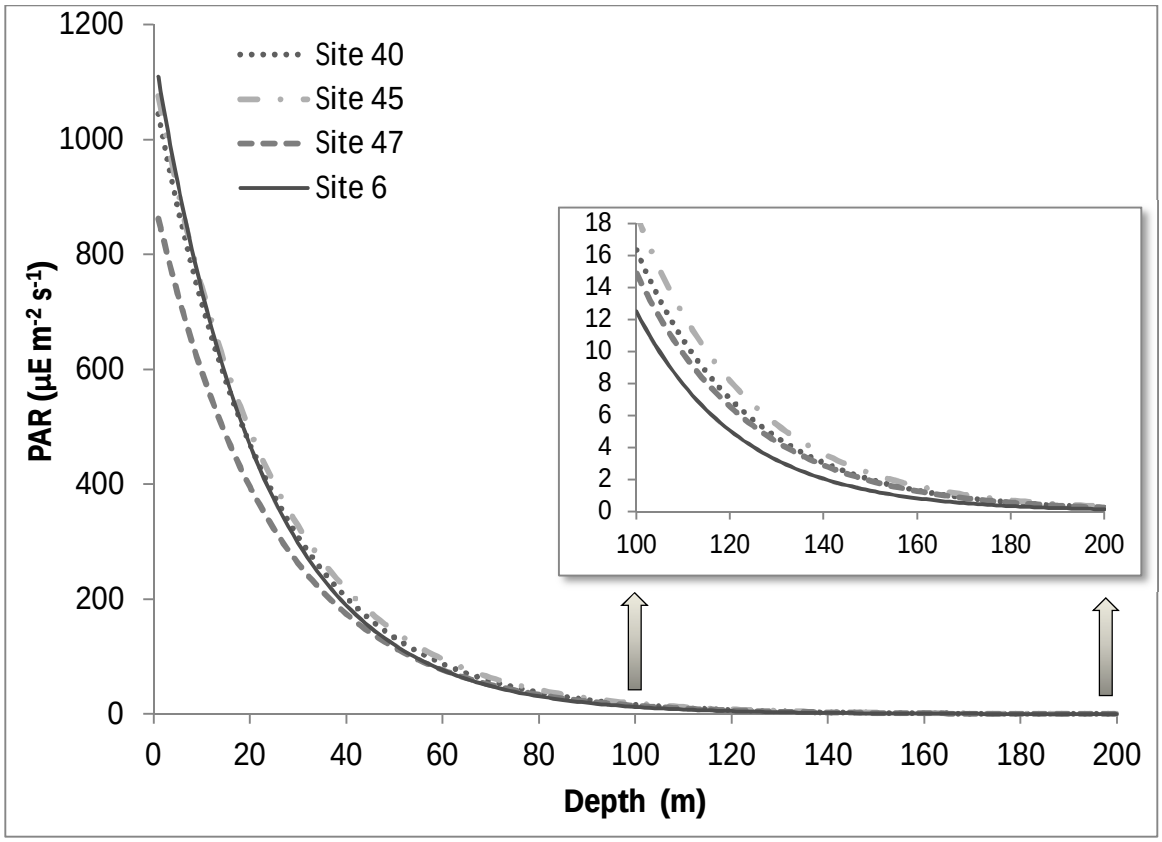

Figure 6. Irradiance (PAR, umol photons m^{-2} s^{-1}) versus increasing depth, based on attenuation coefficients from light profiles through the water column at four sites. Vertical attenuation coefficients provided in 'Results'.

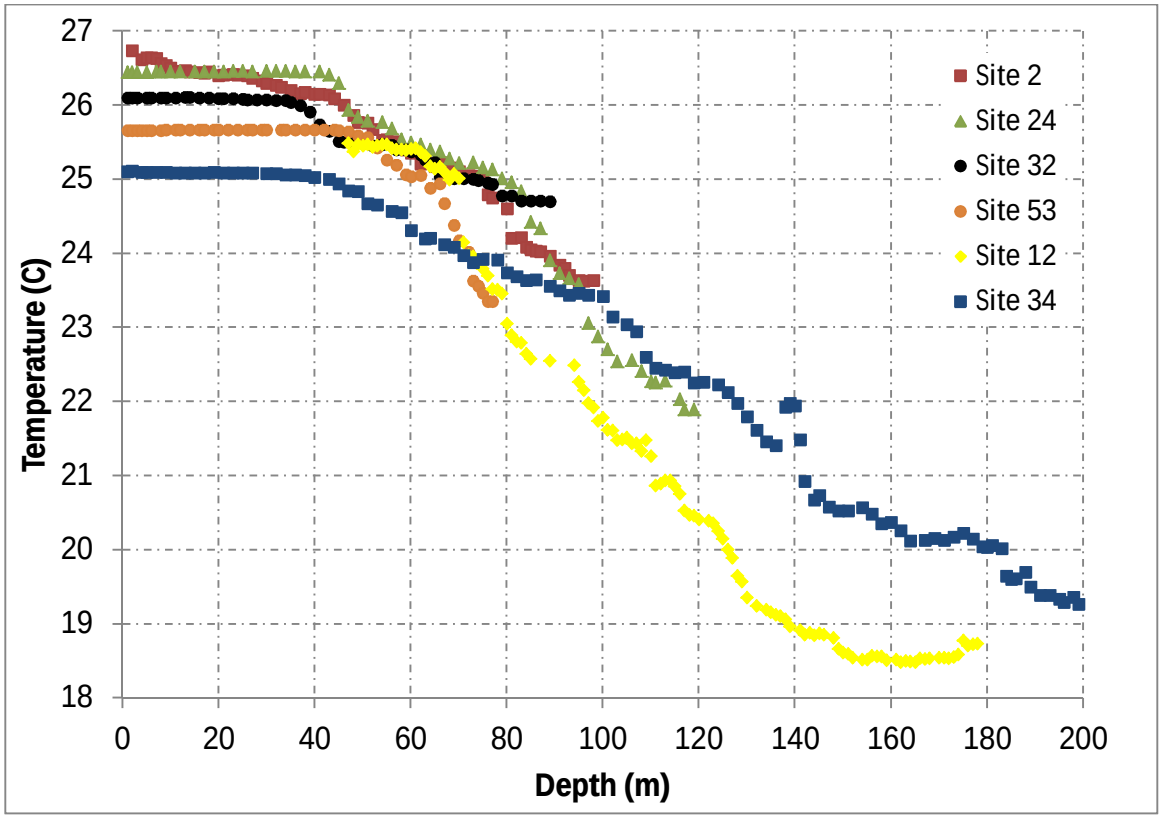

Figure 7. Representative temperature-depth profiles from sites surveyed during November and December 2004. Only temperatures continuously recorded during the submersible descent are shown.

CHAPTER 3:

NATURAL HISTORY AND CALCIUM CARBONATE PRODUCTION OF *HALIMEDA KANALOANA* MEADOWS ALONG AN 85 M DEPTH GRADIENT IN THE MAIN HAWAIIAN ISLANDS

ABSTRACT

Meadows of *Halimeda kanaloana* Vroom cover large portions of the sand-dominated shallow and mesophotic environments in Hawai‘i, yet little is known about their ecology or possible contribution to carbonaceous sediments. To help close this gap, we monitored growth, densities, lifespan, herbivory, quantity of calcium carbonate ($CaCO_3$), and reproduction of dense *H. kanaloana* meadows at multiple locations periodically over a four year period in waters around Maui, Hawai‘i at 10, 20, and 30 m depths. We also collected morphometic and $CaCO_3$ data from 1 to 85 m depths at 32 locations for estimates of $CaCO_3$ across this broad depth gradient. *H. kanaloana* grew rapidly, but growth was highly variable across sampling periods and among depths, with plants at 10 m generally growing faster than those at 20 and 30 m. Abundance (plants m^{-2}) was high, but variable at all depths and sampling periods, with mean densities ranging from 36 to 314 individuals m^{-2}. Individuals were generally long-lived perennials (several years), with low rates of sexual reproduction (less than 1% of the population), and subject to episodic epiphytic bacterial and cyanobacterial blooms. Overall, *H. kanaloana* meadows were highly productive, producing 1756, 1694, and 1320 g $CaCO_3$ m^{-2} yr^{-1} at 10, 20, and 30 m depths at Kahekili Beach Park, Maui, respectively, and 1883 g $CaCO_3$ m^{-2} yr^{-1} when data from its entire depth range (1 to 85 m) in Hawai‘i were considered. Juvenile

Tripneustes gratilla urchins recruited in these meadows, and two Hawksbill turtles (*Eretmochelys imbricata*) were observed regularly using meadows as foraging grounds. The high densities, significant carbonate sand production, and ecological role of *H. kanaloana* in Hawai‘i suggest deep psammophytic algal meadows are an integral and highly productive part of the coral reef ecosystem.

INTRODUCTION

Halimeda meadows are common worldwide in tropical latitudes, and provide critical ecosystem services by providing structural habitat for fish (Lobel & Ogden 1981; Overholtzer & Motta 1999, 2000; Mũnoz et al. 2000; Rossier & Kulbicki 2000; Chittaro 2004), a food source for numerous fish and invertebrate species (Kampfer & Ott 1995), substrate for epiphytic algae (Beach et al. 2003, Vroom et al. 2003) and invertebrates (Naim 1988, Llobet et al. 1991, Coma et al. 1992, Kerr & Paul 1995, Sotka et al. 1999), and essential spawning habitat for fish (Colin & Bell 1991). *Halimeda* abundance at any one site varies seasonally (Bach 1979, Wefer 1980, Multer 1988, Payri 1988, Ballesteros 1991, Garrigue 1991, Vroom et al. 2003) and with depth (Vroom et al. 2003), with variability associated with natural processes that impact nutrient availability, temperature, irradiance, overgrowth from epiphytes, and seasonal disturbance from storms. In regions characterized by deep photic zones or mesophotic regions, *Halimeda* communities present a new dimension to tropical reef biology by providing unexplored depth gradients not often considered by reef ecologists. A fundamental understanding of temporal and spatial variation in densities, morphometrics, lifespan, and growth along a depth gradient

by *H. kanaloana* is needed to best inform ecosystem management about this new coastal ecosystem component.

Halimeda can be an important producer of carbonate sediment on coral reefs and in adjacent lagoons (Elliott 1960, 1965; Bach 1979; Drew 1983; Flugel 1988; Hine et al. 1988; Johns & Moore 1988; Pizzimenti & Silva 1997; Hillis 2001; Milliman 1993; Vroom et al. 2003). The production of sand by macroalgae is often quantitatively more substantial than the carbonate produced by the reef framework itself (Milliman 1974), with species of *Halimeda* often the dominant contributors (Hillis-Colinvaux 1980). Calcium carbonate ($CaCO_3$) production from *Halimeda*, however, may vary over two orders of magnitude depending on the species, depth range, densities, and growth rate (van Tussenbroek & van Dijk 2007). The quantification of growth rate and density for a particular species from a range of depths and locations is essential to properly interpret reef biogenesis, deep-sea sedimentary processes, and the geological record of the tropics (Jensen et al. 1985). I documented the growth rate and densities of *H. kanaloana* from 10 to 30 m depths, and estimated $CaCO_3$ production from 1 to 85 m depths at sites in the Main Hawaiian Islands.

Halimeda meadows are defined as dense, upright algal populations that may exist as a long continuous zone or as a series of discontinuous patches over soft sediments (Friele et al. 1995). Meadows of *Halimeda kanaloana* are only known in the Northern Pacific from Hawai‘i and the Ryukyu Islands (Verbruggen et al. 2006). Originally described as *H. incrassata*, molecular and morphological studies by Verbruggen et. al (2006) split this species into two pseudo-cryptic species plus the original species, describing *H. incrassata* in Hawai‘i as a new species, *H. kanaloana*. Although studies on

H. incrassata provide useful comparisons with *H. kanaloana* for growth and $CaCO_3$ production, *H. kanaloana* meadows in Hawai‘i are unique because they form quasi-monospecific assemblages over a broad depth range (1 to 85 m depths) across several Hawaiian islands (Huisman et al. 2007, Verbruggen et al. 2006), without competition from seagrasses or other *Halimeda* species (HS, pers. obs.). Competition studies between the seagrass *Thalassia testudinum* and *H. incrassata* have found a competitive interaction, with the presence of seagrass negatively affecting *H. incrassata* growth and body size (Davis & Fourqurean 2001), not surprising given the larger stature of the seagrass and possible competitive interactions for light. *H. kanaloana* meadows in Hawai‘i appear to provide a remarkable opportunity to study the ecology of an alga over a broad depth range in monospecific stands.

H. kanaloana meadows in Hawai‘i are currently described as most common around the islands of Moloka‘i, Maui, Lāna‘i, and Kaho‘olawe (Huisman et al. 2007, Verbruggen et al. 2006), known collectively as the Maui Nui complex (Maui Nui, Fig. 1). Maui Nui was connected as a single landmass that reached is maximum areal extent ~1.2 Ma, when it was larger than the current island of Hawai`i (Price and Elliott-Fisk 2004). Subsidence, changes in sea level, and geomorphological processes split the landmass into the current islands, with the islands of Maui and Lāna‘i last connected about 10,000 years ago (Grigg et al. 2002). Limestone bridges composed of sand and sediment plains connect the islands underwater (Grigg et al. 2002), and provide habitat for the formation of *H. kanaloana* meadows. This study focused on *H. kanaloana* meadows in Maui Nui, with limited collections from other Hawaiian islands as new populations of *H. kanaloana* were discovered. My objective was to determine the extent of spatial and temporal

variation in *H. kanaloana*'s distribution, abundance, and growth rate, and use this information to determine $CaCO_3$ production over a broad depth gradient. To do this, I: (1) determined the variability in *H. kanaloana* densities across depths and among sites; (2) described and modeled the variability in *H. kanaloana* morphometrics; (3) examined and compared growth rates across depths and between locations; (4) determined the average lifespan; (5) documented reproductive events and herbivory; and (6) calculated $CaCO_3$ production for specific depths.

MATERIALS AND METHODS

Site descriptions, oceanographic conditions, and other taxa.

Populations of *Halimeda kanaloana* were sampled at 18 locations in the Main Hawaiian Islands (Fig. 1). Sites were selected based on the presence of gently sloping sandy substrate, accessibility by divers from shore or by small boat, and the occurrence of *Halimeda kanaloana*. Kahekili Beach Park (Kahekili BP, site 3; Fig. 1) in west Maui was used for the majority of manipulative experiments because it appeared to contain *H. kanaloana* meadows with densities within the typical range of the meadows observed in other locations in Hawai‘i (HS, pers. obs.), and had populations that were accessible by divers from shore year-round. The 10, 20, and 30 m depth populations at Kahekili BP were ~100, 250, and 325 m from shore, respectively.

The reef at Kahekili BP is characterized by a fringing hermatypic coral reef with low vertical relief to ~10 m depth and gently sloping soft sediments from ~10 to 90+ m depths. Subtidal temperatures at 10 m in this area range from 23.8 to 27.6° C annually (Storlazzi and Jaffe 2008). Oceanographic conditions during summer/fall months are

characterized by consistent northeast trade winds, low rainfall, and small waves with strong alongshore currents, while winter/spring months experience heavy rain, strong winds, strong offshore currents, and large waves with high turbidity (Storlazzi and Jaffe 2008).

Sites 4, 5 and 7 (Fig. 1) contained *H. kanaloana meadows* at 20 m depths that appeared similar to those at Kahekili BP and were used for comparison to Kahekili BP in growth and morphometric studies. These sites were also accessible via kayak or by shore-diving year-round, and had similar exposures as Kahekili BP, suggesting that these sites experienced similar oceanographic conditions. The 20 m depth contour at sites 4, 5 and 7 was located 692, 672, and 63 m from shore, respectively. The 20 m depth at site 7 was much closer to shore because of a steep hermatypic reef drop-off from 2 to 10 m depths.

Sites throughout west and south Maui (Fig. 1) were accessed primarily by divers swimming from shore using enriched air Nitrox on SCUBA or Inspiration® closed circuit rebreathers, with occasional small boat support. Collections of *H. kanaloana* from deeper waters offshore of the Maui Nui complex (Fig. 1) were made with the Hawai‘i Undersea Research Laboratory submersibles *Pisces IV* and *Pisces V,* and used for morphometrics and $CaCO_3$ production measurements. Representative individuals were haphazardly selected for collection based upon their proximity to the submersible at the desired depth, collected with manipulator arms, and placed in covered baskets on the submersible.

Meadows of *H. kanaloana* grow primarily in deep, soft sediments from 10 to 90 m depths, but plants have also been observed growing on hard substrate in less than 1 m depth (Verbruggen et al. 2006). Visually, *H. kanaloana* is the most abundant alga in the meadows, with up to a ~30 cm canopy height. Other rhizophytic algae such as *Caulerpa*

mexicana Sonder ex Kützing and *Codium desultorium* P.C. Silva et M.E. Chacana, sp. ined. were commonly found. The bases of *H. kanaloana* were often encrusted with a diverse assemblage of invertebrates (tunicates, sponges, etc.), a turf of filamentous epiphytic algae, and other small epiphytic macroalgae such as *Dictyota ceylanica* Kützing. Seagrasses (*Halophila* spp.) and other rhizophytic *Halimeda* species were not observed in any of the *H. kanaloana* meadows (H.S. unpublished data).

Density and morphometrics

An individual *Halimeda* was defined as an upright plant having its own distinct holdfast. A 0.25 m^2 quadrat was selected as the optimum size for quantifying the abundance of individuals because it provided precise estimates and allowed for the desired number of replicates at each depth. The number of individuals of *H. kanaloana* was quantified by two divers in 20 randomly placed 0.25 m^2 PVC quadrats along a 25 m transect running parallel to shore along a specific depth contour, e.g. 10, 20, or 30 m depths. Density counts made by different data collectors were calibrated based on counts by HS. Each diver counted the same ten quadrats of *Halimeda,* their counts were compared with counts made by HS, and then adjustments were made by the diver until their counts were consistent with HS. This reduced bias from edge effects or possible confusion in identifying individual *Halimeda* plants.

The height of an individual plant was defined as the length in cm from the bottom of the basal segment to the end of the segment on the tallest axis, and did not include the buried holdfast. An axis was defined as any branch containing at least three segments arising from anywhere along the plant. A segment was defined as a calcified internodal

region separated by uncalcified nodal regions (Hillis-Collinvaux 1980, Vroom et al. 2003).

Because of time limits on dives, five representative individuals of *H. kanaloana* were haphazardly selected within each quadrat and the heights of the longest axes were measured. These data were averaged to generate the mean canopy height for each quadrat. The number of urchins (primarily the collector urchin *Tripneustes gratilla*), reproductive *H. kanaloana*, and observations such as the presence/absence of cyanobacterial or bacterial blooms, were also recorded for each quadrat.

Haphazardly selected *Halimeda* from the growth studies (see below), density measurements, and representative *Halimeda* collected with the submersible (see Table 1 for collection information) were enumerated for morphometrics (height, number of axes per thallus, and number of segments per thallus).

Growth

Growth was measured using an Alizarin Red-S stain technique, modified from Vroom et al. (2003). A 1-ml aliquot of a 1% (w/v) solution of Alizarin Red-S dye was placed in a 1.5 ml Eppendorf tube, and then deposited in a 4-l plastic bag. Twenty individuals were randomly selected along two 25 m transects at least 5 m apart and running parallel to shore along the desired depth contour (e.g. 10, 20, or 30 m depths). Two parallel 25 m transects were used instead of a continuous 50 m transect so that dive buddies were in sight of each other at all times during the dive. A fishing weight with fluorescent flagging tape was placed next to each selected individual for location purposes. The plastic bag with the dye was placed over the individual and gently affixed

around the base of the plant with a cable tie. Dye was then released and carefully distributed throughout the bag by massaging the contents and visually checking each bag for success in attachment and staining. The bags were left attached for 24 h, and then removed. Each stained plant was marked with a loosely attached fluorescent cable tie at its base. Stained plants were harvested after seven to nine days. This time period was found to be the optimum duration to ensure quantifiable growth per day with minimal shedding of axes, senescence, or loss of dye. This method has a negligible effect on *Halimeda* growth (Vroom et al. 2003).

Harvested individuals were placed in a refrigerator (1.6° C) for ~6 hours. The colder temperature caused chloroplasts to retract, easily revealing the stained and unstained portions of plants. The numbers of new and old segments, axes, and height per individual were recorded, and individuals pressed as herbarium specimens for later analyses and voucher specimens.

Longevity

The lifespan, or longevity, of individual *H. kanaloana* was determined by tracking a total of 25 tagged individuals through time at 23 m depth in a dense meadow at Kahekili BP. This depth and location was selected because it contained a continuous meadow with densities (~200 plants m^{-2}) typical of Maui, and was accessible by divers from shore throughout the year. A permanent 25 m transect was established with small subsurface buoys marking the transect's beginning and end. Fluorescent tent stakes were used to mark 11 permanent random numbers along the 25 m transect. Eleven individuals were selected to monitor because this was the maximum number that could be enumerated on a

single dive. To minimize any influence of tent stakes, individuals were selected at least 30 cm from each stake and tagged with a loose fluorescent cable tie around the plant base. Care was taken to select small, epiphyte-free individuals approximately one month of age, estimated via the sizes of individuals from a clearing experiment at the same depth and season (Chapter 4).

A total of 11 marked individuals were initially tracked for the duration of the study. Occasionally, an individual was missing; in these cases, an adjacent small, epiphyte-free individual was tagged and monitored over time. The presence/absence of the plant, number of segments, evidence of herbivory or disturbance, and height of each individual were monitored monthly to semi-annually. Lifespan was calculated as the difference in days between the first and last day the plant could be found in the field + initial age (~30 days).

Reproduction and herbivory

In addition to counts from 0.25 m^2 quadrats (see density studies above), the number of reproductive individuals of *H. kanaloana* and urchins were enumerated on 8 April 2006 and 1 December 2007 in two 50 by 1 m transects running parallel to shore at 18–20 m depths at Kahekili BP. This depth range was chosen for surveys because it contained a dense, continuous meadow of *H. kanaloana* and was accessible by divers. Although the number of urchins and reproductive plants were noted in the quadrat counts, a low incidence of reproductive individuals and urchins indicated that a larger area needed to be surveyed. Surveys were not conducted at deeper depths due to dive time limitations, but we observed similar abundances of reproductive individuals and urchins at 30 and 40 m

depths during the same time frame. The number of reproductive individuals and urchins were enumerated every five meters in a one meter wide path along each transect. Surveys were conducted two days after gamete release. The bright white carbonate skeleton of post-reproductive plants, as compared to the green non-reproductive individuals, was most visible after gamete release. On 11 November 2007, reproductive individuals (~one day prior to gamete release) and non-reproductive individuals were randomly collected along a 25 m transect for morphometric comparisons.

Calcium carbonate production rates

A major axis from each individual from the growth experiments or from collections at other sites (Table 2) was decalcified to determine the average percent $CaCO_3$ and the mass of $CaCO_3$ per segment. The number of segments in each axis was recorded. A major axis was defined as an axis arising within 10 segments of the base of the plant and containing at least 15 segments. The entire individual, versus a major axis, was not used because the majority of carbonate sand production likely occurs from a high turnover of individual axes (van Tussenbroek and van Dijk 2007) rather than turnover of the entire individual. The heavily calcified basal segments are perennial and more heavily calcified than the upper axes (Wiman and McKendree 1975), and would likely contribute to an overestimate of carbonate production.

Each axis selected for analysis was dried at 60° C until a constant weight was achieved (~24 h) with a Sartorius A2005 analytical balance, then decalcified in a 10% HCl solution that was changed every hour until bubbling ceased. Decalcified axes were rinsed with fresh water, dried at 60° C until a constant weight was achieved, and the final

weight was recorded. Percent $CaCO_3$ was calculated as the difference between the initial calcified dry weight and the decalcified dry weight, multiplied by 100%, and then normalized for the number of segments per axes to determine the grams of $CaCO_3$ per segment.

Averaged data from 10, 20, and 30 m depths at Kahekili BP and from 30 other sites and depths (overall mean) were used to model rates of $CaCO_3$ production annually. Mean mass of $CaCO_3$ per segment, mean number of segments per individual, and mean number of individuals per meter were calculated to determine the mass of $CaCO_3$ per meter. Turnover, or renewal of standing stock, was calculated as the mean number of new segments per individual per day multiplied by the mean number of segments per individual. The turnover (in days) was then converted to turnover (in years) and multiplied by the mass of $CaCO_3$ per meter squared for yearly estimates of $CaCO_3$ production per site and overall across the depth distribution for *H. kanaloana* distribution.

Data analysis

All data were analyzed using Minitab® Statistical Software 15.1.30.0 (Minitab Inc.) Two-way analyses of variances (ANOVAs) were used to differentiate the effects of depth and sampling period on *H. kanaloana* densities, canopy height and growth rates, and between depth and morphometrics in reproductive versus non-reproductive individuals at Kahekili BP. One-way ANOVAs were used to differentiate the effects of spatial variation on *H. kanaloana* growth, densities, and canopy height at multiple locations.

Normality, homoscedasticity, and post-hoc multiple comparisons used Minitab® Statistical Software 15.1.30.0. Normality was tested using a Kolmogorov-Smirnov test

with an α of 0.05. Homoscedasticity was evaluated using Bartlett's test with an α of 0.05. Square root and log transformations were used when necessary to satisfy normality and homoscedasticity. All factors were considered fixed. Tukey's post-hoc multiple comparisons were used to detect differences between levels of the factors, if significant in an ANOVA with $\alpha < 0.05$. Linear and polynomial regression analyses were used to analyze the relationships between *H. kanaloana* morphometrics (number of segments, axes, and height) with increasing depth. Density and canopy height data from 10 m on February 2007 were also used for December 2007 at 10 m to balance the statistical design and compensate for a low sample size from December 2007 (n = 1). Inclement weather producing high swells (3 m) and low visibility (< 0.5 m) on December 2007 made density and canopy height data at 10 m impossible to accurately acquire.

RESULTS

Density of plants and canopy heights

Densities of *Halimeda kanaloana* at Kahekili BP varied significantly among sampling periods and depths (Fig. 2, Table 3). When densities from all sampling periods at specific depths were combined, the mean density at 10 m was relatively low (64.7 plants ± 3.2 SE), increased between 20 and 30 m depths (228.7 plants ± 6.9 SE, 262.3 plants ± 6.6 SE respectively), and then decreased at 40 m (136.5 plants ± 7.3 SE). This general depth trend was consistent at Kahekili BP in May 2006 (one-way ANOVA: $F_{3,64} = 43.68$, $P < 0.001$; Fig. 2); however, densities were only measured at 40 m during this one sampling period and subsequent variability in density at this depth over time remains unknown. During all sampling periods, densities ranged from eight individuals m^{-2} at 10 m to 461

individuals m^{-2} at 20 m depth. *H. kanaloana* holdfasts were often exposed (up to ~6 cm) along the 10 m isobath as sand shifted with wave action and surge. Holdfasts from plants at 20 to 40 m depths were completely submerged during all sampling times. Measurements of canopy height at Kahekili BP generally increased with increasing depth, although a significant interaction suggested the effect of "depth" varied with time (Table 3). Canopy heights ranged from 8.0 cm at 10 m to 33.0 cm at 40 m depths (Fig. 2, Table 3). During May 2006, densities were significantly different among all depths at 10, 20, 30, and 40 m (one-way ANOVA: $F_{3,64} = 46.68$, $P < 0.001$), with canopy height increasing with increasing depth. Overall, mean canopy heights (± SE) from 10 to 40 m depths were 12.7 ± 0.2 cm, 18.1 ± 0.2 cm, 19.9 ± 0.2 cm, and 25.1 ± 0.8 cm, respectively.

H. kanaloana densities and canopy heights at Kahekili BP were generally within the range found at other locations surrounding west Maui (Fig. 3). However, densities among some sites and depths varied significantly (Fig. 3), with sites exhibiting similar densities across all depth ranges. For instance, post-hoc tests revealed similar mean (± SE) densities of individuals occurring along the 10 m isobath at site four (159 ± 13 plants m^{-2}), the 20 m isobath at sites two and seven ((151 ± 13 and 166 ± 11 plants m^{-2}, respectively), the 30 m isobath at site six (137 ± 7 plants m^{-2}), and the 40 m isobath at site three (137 ± 7 plants m^{-2}) (Fig. 3). Canopy heights at multiple sites were also significantly different, with height increasing with increasing depth across most sites (Fig. 3).

As found with canopy height measurements at Kahekili BP, heights of plants from multiple locations and depths (Table 2) generally increased with increasing depth from 1 to 85 m depths in a polynomial relationship (Fig. 4). Heights ranged from 6 cm at

a depth of 30 m to 34 cm at a depth of 82 m. No significant trends were found in the number of axes or segments with increasing depth, although a positive linear relationship existed between the number of axes and segments (Fig. 4). Minimum and maximum values ranged from 27 to 1208 segments individual^{-1} and 2 to 151 axes individual^{-1} at 20 and 40 m depths, respectively. On average, individuals across all depths contained168.8 (± 6.3) segments, were 17.9 (± 0.2) cm in height, and possessed 16.9 (+0.7) axes.

Cyanobacterial blooms (*Lyngbya majuscula* (Dillwyn) Harvey)) were observed throughout the year in the meadows surveyed, with mats overgrowing single individuals of *H. kanaloana* or expanding to such an extent that they carpeted large areas (>100 m^2) from 10 to 40 m depths. The longest duration of a large bloom was ~2½ weeks in June 2005 at Kahekili BP. However, *H. kanaloana* densities in June 2005 at Kahekili BP were similar to densities during other sampling periods without cyanobacterial blooms (i.e. July 2006 and December 2007; Fig. 5). The densest bloom observed occurred at Makena Landing in May 2006 at 11 m depth, during which individuals of *H. kanaloana* were completely covered with long, trailing strands of *L. majuscula* (up to 30 cm long). *H. kanaloana* covered entirely by *L. majuscula* had lost pigmentation and fell apart when gently touched by divers. A rust-colored, slimy bloom of unidentified bacteria was also commonly observed covering up to 1 m^2 of the *H. kanaloana* meadow from 10 to 30 m depths at Kahekili BP.

Growth rate

Growth rate, as measured by the number of new segments per individual and percent new growth per individual, at Kahekili BP varied significantly among depths and sampling

periods, but the significant interaction indicated these differences varied depending on sampling period (Fig. 5; Table 4). For instance, there was no difference in the mean number of new segments per day at each depth during July 2006, but significant differences occurred in the number of new segments per day for other sampling periods between 10 and the 20 and 30 m depths. Overall, growth was generally greater along the 10 m isobath than at the 20 and 30 m isobaths, as measured by the number of new segments per individual per day and percent new growth per individual day (Fig. 5; Table 4). The mean (± SE) growth rate of new segments was four times higher for plants at 10 m (5.1 segments ± 0.7, n = 101) than for plants at 30 m (1.3 segments ± 0.1, n = 100). Percent new growth was 1.5 times greater for plants at 10 m than plants at 30 m depths (2.0 ± 0.2, n = 101; 1.2 ± 0.1, n = 100, respectively). Overall, the highest mean (± SE) growth rates occurred in plants along the 10 m isobath during April 2006 (13.7 ± 2.1 segments; 3.5 ± 0.5 % new growth, n = 18) while the lowest rates occurred along the 20 m isobath during April 2008 (0.3 segments ± 0.1; 0.4 % ± 0.1, n = 19). Growth rate (mean ± SE) among sampling periods was highly variable, with no clear trend between winter (April 2006, December 2007, April 2008) and summer (June 2005, May2006, July 2006) months in percent new growth (1.8 % ± 0.1; 1.3 % ± 0.1, respectively) or new segments (2.5 segments ± 0.2; 2.8 segments ± 0.4, respectively). The April 2006 sampling period coincided with an unusual ~40 day storm event associated with high precipitation and turbidity.

Growth rate estimates from this study were based mainly on three depths sampled at one location (Kahekili BP) through time. Modeling the growth rate, and subsequently extrapolating $CaCO_3$ production, from one general location may not be representative of

meadows in other locations, given the variability seen in densities (Fig. 3) and differences in environmental conditions at other locations. Thus, we compared the growth rate of *H. kanaloana* from Kahekili BP at 20 m with three additional locations (Mala, Launiupoko, and Papawai) separated by ~ 30 km of coastline on west Maui during the same time period (April 2008). The growth rate along the 20 m isobath at Kahekili BP, Mala, Launiupoko, and Papawai was significantly different when measured by the mean number of new segments per individual per day, but was similar among locations when measured by percent new growth per individual per day (Fig. 6). The mean number of new segments per individual per day was most similar between Kahekili BP and Mala, and between Launiupoko and Papawai.

Longevity

The estimated lifespan of *H. kanaloana* individuals (n = 19) at 23 m depth ranged from 140 to 1275+ days, with a mean lifespan of 645 days (± 80 SE). Individuals tagged after February 2007 were not considered in this analysis. Four individuals (plants identified as 9, 13, 14, and 17) tagged prior to February. 2007 were still alive at the end of the study; their lifespans were conservatively estimated as their age at the end of study (1245+ days, 797+ days, 797+ days, and 734+ days). Tagged individuals had a mean (± SE) of 60 (10.9) segments per plant and were 14.4 (0.9) cm in height at the start of the experiment. The number of segments per individual varied dramatically, suggesting that segment number alone is not a good indicator of age (Fig. 7). For instance, individuals 9, 13, and 17 consistently had fewer than 100 segments over the time monitored, while individual 7 ranged from 15 to 338 segments (Fig. 7). Height ranged from 1 to 24 cm, with some of

the older individuals decreasing to just a few centimeters in height before disappearing. However, individuals 2 and 4 consistently increased in segment number before disappearing. Herbivory was rarely observed on segments on tagged individuals. The cable ties from the bases of all missing individuals were found intact in their original location, suggesting that sediment disturbance, such as burial from burrowing organisms or storms, was not the cause of death.

Reproduction and herbivory

Reproductive individuals were consistently observed during sampling periods based on their occurrence in density counts. The percent of the population that was reproductive ranged from 0.05 – 0.76% (Table 5). These values are a representative range of the lowest and highest reproductive events observed in the meadows over the course of sampling (June 2005 – April 2008). Sexual reproduction occurred synchronously across depths at a single location (10 to 30 m at Kahekili BP in April 2008) and at other locations at the same depth (Kahekili BP, Mala, Launiupoko, and Papawai at 20 m depth in April 2008). The height, number of axes, and number of segments of all reproductive individuals were significantly different between 10 and 20 m depths (Tables 6, 7). Overall, reproductive individuals at 10 m depths had more axes and segments, but were shorter than the 20 m individuals. There were no significant differences between the height and number of axes in fertile and vegetative individuals. However, fertile individuals had ~1.5x more segments than vegetative individuals (Tables 6, 7).

The collector urchin, *Tripneustes gratilla*, was the most abundant urchin observed in *H. kanaloana* meadows (Table 5). It was uncertain if *T. gratilla* were grazing directly

on *H. kanaloana* or removing epiphytes growing on *H. kanaloana*. However, *T. gratilla* were observed removing individuals of *H. kanaloana* by grazing through the entire basal segment. Recruitment of juvenile *T. gratilla* was observed in *H. kanaloana* meadows from 10 to 40 m depths, with juveniles ranging in size from 18 to 29 mm. *H. kanaloana* harvested for growth studies often had juvenile urchins located at the base of the plants. Fish bites were rarely observed on *Halimeda* plants, except on individuals directly adjacent to small patch reefs with herbivory 'halos'. A large, female Hawksbill sea turtle (*Eretmochelys imbricata*) and a juvenile Hawksbill sea turtle (sex undetermined) were often observed foraging in the *H. kanaloana* meadows at Kahekili BP from 20 to 30 m depths, but were consuming fire worms (*Pherecardia striata*), not *H. kanaloana*. *Pherecardia striata* are very abundant in meadow sediments (Fukunaga 2008).

Calcium carbonate production

H. kanaloana plants were collected from 32 different depths and locations from O'ahu and the Maui Nui complex (Table 2). The quantity of $CaCO_3$ in plants from 1 to 78 m depths was assessed from a total of 3133 segments and 73 individual plants (Table 5). Although the mass of $CaCO_3$ per plant, and its percentage of total plant mass, varied widely within each depth, the means (± SE) of both were fairly consistent across depths (Table 8). The percent $CaCO_3$ (depth sampled) ranged from 48.6% (10 m) to 85.4 % (56 m), while the mass of $CaCO_3$ per plant ranged from 0.0032 g (78 m) to 0.0264 g (10 m) (Table 9). The mean (± SE) percent and mass of $CaCO_3$ per plant across all depths were 70.5% ± 0.9 and 0.0113 g ± 0.0006 (Table 9). The shallower sites had lower sample sizes

because of the rarity of plants in shallow water, while deeper sites had lower sample sizes because of difficulty in accessing those depths (Table 2).

I estimated $CaCO_3$ production for *H. kanaloana* at 10, 20, and 30 m depths by combining data on growth, densities, the number of segments per individual, and the quantity of $CaCO_3$ per segment during 6 sampling periods over a ~4 year span at Kahekili BP. This resulted in depth-specific mean estimates of 1756, 1694, and 1320 grams of $CaCO_3$ per meter squared per year at 10, 20, and 30 m depths, respectively (Table 9). *H. kanaloana* meadows along the 10 m isobath had densities four-fold lower than meadows at 30 m depths, but had a higher $CaCO_3$ production rate because of a greater number of segments per plant, and a higher turnover resulting from a faster growth rate (Fig. 5). Mean $CaCO_3$ production rates varied from 928 to 2986 grams $CaCO_3$ per meter squared per year when considering the lowest and highest mean grams $CaCO_3$ per meter squared and turnover per year from 10 to 30 m depths, thus highlighting the possible range in estimates given the variability of the measurements.

The mean production of $CaCO_3$ at Kahekili BP from *H. kanaloana* generally decreased with increasing depth. However, when mean abundance data from all other sites and morphometric data from 1 to 85 m depths were considered, the mean production of $CaCO_3$ increased to 1883 grams $CaCO_3$ per meter squared per year (Table 9). The overall mean value was greater than values at 10 to 30 m depths at Kahekili BP, primarily due to the higher *H. kanaloana* densities found at other sites. However, the overall mean estimate is meant only as an approximate value because the mean growth rates used were only from 10 to 30 m depths over 4 locations, and the rate of growth at deeper (> 30 m) depths is unknown.

DISCUSSION

To properly evaluate the ecosystem functions and services of a species, fundamental biological data are needed. In the case of many marine macroalgae, and for countless other marine species, these types of critical data are typically lacking (Springer et al. 2010). More specifically, basic research is urgently needed on all aspects of the taxonomy, biology, and functional ecology of calcifying macroalgae, such as *Halimeda* (Nelson 2009). This study provides baseline ecological information for *Halimeda kanaoloana* and suggests that meadows of *H. kanaloana* provide a number of ecosystem services, such as primary production, creation of a perennial biogenic habitat that increases local species diversity and abundance as compared to featureless sand, and the significant production of calcium carbonate sand. The widespread distribution of *H. kanaloana* meadows described here from 1 to 85 m depths at over 32 locations suggests that Hawai‘i has a high concentration of extensive psammophytic algal meadows spanning mesophotic depths, similar to the Great Barrier Reef (Drew and Abel 1988a). Given their distribution and high densities, these meadows should be considered an essential part of the tropical reef ecosystem in Hawai‘i, and must be included in ecosystem-based management schemes.

Density and morphometrics

When compared to densities of psammophytic *Halimeda* from other geographic areas, mean densities of *H. kanaloana* at 20 and 30 m depths in Hawai‘i ranked among the highest reported (Table 10). *Halimeda* densities from other studies generally decreased

with increasing depth (South 1982, Vroom et al. 2003); however, I found a significant increase in densities from 10 m to 20–30 m depths. The higher densities at 20–30 m depths (up to 461 *H. kanaloana* m^{-2}) in my study possibly occurred due to a lack of competition with large seagrasses or other rhizophytic algae. *Halimeda* beds and meadows in other locations typically occur as part of a diverse assemblage of rhizophytic algae and seagrasses (e.g. South 1982, Drew 1983, Drew and Able 1988a, Davis and Fourqurean 2001, Vroom et al. 2003), while *H. kanaloana* meadows in Hawai‘i are largely monospecific stands. Competition with seagrasses has been found to negatively affect the growth of *Halimeda* (South 1982, Davis and Fourqurean 2001), suggesting that release from interspecific competition may increase the growth and densities of *Halimeda* when other factors (i.e. light, nutrients, substrate availability) are not limiting. The decrease in densities from 30 to 40 m depths may arise from light limitation and subsequent intraspecific competition, although additional measurements over a larger temporal scale, and manipulative experiments at 40 m, would be needed to properly gauge this relationship.

Densities of *H. kanaloana* also varied greatly between different sampling periods at Kahekili BP, especially at 20 to 30 m depths. This was surprising; I expected the higher physical disturbance from wave energy at 10 m depth to cause significantly more variation in densities from dislodgement of the holdfasts, while deeper depths were expected to remain less variable due to calmer, more stable conditions with less physical disturbance. It is hypothesized that the episodic occurrence of bacterial and cyanobacterial blooms at deeper depths may have negatively influenced densities as compared to shallower depths. Epiphytic macroalgal overgrowth has been found to

negatively impact the metabolic rate of *Halimeda* by shading and allelopathy (Beach et al. 2003), and has been found to play a significant role in the variability of interannual biomass and density estimates in other *Halimeda* assemblages (Vroom et al. 2003, van Tussenbroek and van Dijk 2007). Epiphytic turf algae and encrusting invertebrates likely had a negligible effect on *H. kanaloana* densities because they were limited to the slower growing, perennial basal segments of the plants and typically covered a small portion of the plant overall. In contrast, the cyanobacteria *Lyngbya majuscula* was found covering entire *Halimeda* plants, appearing to cause the death of numerous *H. kanaloana* at deeper depths in this study. *L. majuscula* is chemically rich and contains secondary metabolites that may impair the growth of other organisms (Nagle and Paul 1999, Kuffner and Paul 2001). Siphonous green algae have the ability to abandon older, epiphytically impaired parts of the plant body (Littler and Littler 1999), but the impact on the alga when the entire plant is covered by cyanobacteria has not been examined. Manipulative studies on the impacts of these blooms on *Halimeda* densities and growth are needed to elucidate their long-term impact on *Halimeda* productivity, particularly because these blooms are expected to increase in frequency with the degradation of water quality in near shore coral reef ecosystems (Hughes 1994, McCook 1999). Loss of individuals from sexual reproduction via holocarpy (Vroom and Smith 2001) did not appear to have a significant effect on densities given the low percentage (< 1%) of the population quantified as reproductive.

The canopy height of *H. kanaloana* significantly increased with increasing depth, with a mean canopy height of 25 cm at 40 m depth, reaching up to 34 cm at 82 m depths. In comparison, Davis and Fourqurean (2001) described *H. incrassata* as 14.9 cm in

height at 7 m depth in a seagrass meadow in Florida. Multer (1988) described individuals of *H. incrassata* up to 30 cm in height in an isolated location at 1 m depth in Nonsuch Bay, Antigua, but these individuals were three times the average height of other individuals in the area and were considered an anomaly with unidentified causes of growth. Canopy height in shallower waters in Hawai‘i may be affected by physical disturbance from wave energy, with increased wave energy cropping axes. Increased canopy height and decreased densities with increasing depth would increase the surface area for light capture, which eventually becomes limiting with increasing depth. Deeper (> 50 m) *H. kanaloana* meadows may represent a more stable community of taller, older individuals of a consistent size class structure, although additional collections and density estimates would be needed to elucidate patterns in size structure with increasing depth. Studies on the physiological ecology of *H. kanaloana* meadows and measurements of segment size along a depth gradient would likely shed light on other factors that may affect the morphology of *Halimeda*.

The number of axes per plant was a useful measure to evaluate the extent of branching in a plant, and to compare changes in morphology with increasing depth. Although the number of axes was greater for fertile and vegetative plants at 10 m depth compared to 20 m depth at Kahekili BP, the number of axes was not significantly different with increasing depth from one to 85 m when data from the rest of the locations were combined. Vroom et al. 2003 also found no difference in the number of axes between a shallow (4–7 m) and deep (15–22 m) population of *H. tuna* in Florida. However, differences in how axes were counted (arising from within 3 segments of the

base of the plant (Vroom et al. 2003), versus arising anywhere along the plant (this study) may preclude comparison.

Growth rate

The growth rate of *H. kanaloana* was highly variable between sampling periods at Kahekili BP, with no apparent difference in growth between summer/fall and winter/spring months. This is in contrast to the large seasonal differences in *Halimeda* growth at other locations (Bach 1979, Wefer 1980, Multer 1988, Ballesteros 1991, Garrigue 1991, Vroom et al. 2003; van Tussenbroek and van Dijk 2007), where higher growth occurred during the warmer summer. Prevailing environmental conditions directly before and during each sampling period might have influenced the variability in *H. kanaloana* growth, as found by Vroom et al. (2003) in *H. tuna* populations from 5 to 21 m depths. For instance, the high growth rates found at the 10 m depth in April 2006 coincided with an unusually large rain event in Maui that occurred for several weeks prior to and during growth measurements. Turbidity from nutrient-rich terrestrial run-off was observed at the 10 m site, but was not at the 20 and 30 m sites. Elevated nutrients may also have resulted from increased sewage input and groundwater intrusion during this period. Manipulative experiments using variable nutrient treatments and continuously monitoring the temperature, irradiance, and currents at each depth would be valuable in further assessing the factors affecting variable growth in *H. kanaloana*. Physical forcing functions such as internal tides, tidal jets, and localized upwelling events must also be considered. These functions have been found to influence the growth of deeper *Halimeda* meadows off of Florida (Leichter et al. 2003, Smith et al. 2004) and the northern section of the Great Barrier Reef (Drew 1983, Wolanski et al. 1988).

Longevity

Literature dealing with the study of *Halimeda* contains little information on life span (the number of days or months an individual lives) as compared to sedimentation rates or growth (Multer and Clavijo 1989). We estimated the mean lifespan of *H. kanaloana* at 23 m depth to be 645 days (± 80 SE), and ranging from 140 to 1275+ days. This is a fairly conservative estimate given that the initial age of the individual was estimated, and four individuals were still living at the end of the experiment. These data, combined with our observations on the extent of epiphytism and long-lived invertebrate species (sponges, tunicates, and the coral *Pocillopora meandrina*) growing on the base of plants, suggest that *H. kanaloana* are fairly long-lived and perennial, with some individuals living over 3 ½ years. Given the consistent size of individuals in deeper (> 40 m) waters, we hypothesize that individuals in deeper depths are likely older. Other species of *Halimeda* from shallower water have lifespan estimates typically less than one year. Observations of six plants of *H. incrassata* and *H. monile* in Antigua suggested an average life span of three months, as compared to a life span of six to twelve months for *H. opuntia* in St. Croix (Multer and Clavijo 1989). The life span of *H. incrassata* and *H. monile* in St. Croix were found to vary according to location and depth, with a longer life span in deeper waters at Salt River Canyon (4–8 months) as compared to shallower depths in Tague Bay (1–4 months, Susan Williams, pers. comm.).

Reproduction and herbivory

Sexual reproduction in *Halimeda* involves holocarpy, the release of the entire protoplasmic contents of the plants as gametes (e.g. Drew and Able 1988b), with

populations usually showing a synchronous spawning of the gametes (Clifton 1997, Hay 1997). Studies on the seasonability and timing of *H. incrassata* sexual reproduction have shown mixed results; Hillis (1959) and Hillis-Colinvaux (1980) report fertility throughout the year in the laboratory, whereas Drew and Able (1988b) reported from field observations a marked seasonality of reproduction, with peak reproduction during 2–3 months varying from early spring (September) to late summer (February) in Australia, with gametes released in late afternoon. I observed *H. kanaloana* to be reproductive throughout the year, with gamete release likely occurring in early morning or pre-dawn hours based on the timing of release from reproductive individuals collected from the field and incubated in the laboratory, and observations on the presence of recently spawned individuals during early morning dives. Reproductive individuals were observed during the same time at multiple sites and depths, suggesting synchronous spawning occurs in this species as well. The percentage of a *H. kanaloana* meadow that was sexually reproductive at any given time (0.05 – 0.76%) was highly variable and was generally low compared to other *Halimeda* populations. For example, Vroom et al. (2003) described 2–5% of the *H. tuna* populations in Florida as fertile during peak reproduction, similar to the findings of Clifton and Clifton (1999) in Panama where 3–5% of the siphonous algal populations were reproductive and synchronously spawned. Drew and Able (1988b) described the simultaneous fertility of up to 25% of *H. discoidea* individuals in the northern Great Barrier Reef. My study provides a useful framework for future studies on *H. kanaloana* reproduction. *H. kanaloana* meadows may be an accessible system for testing what triggers *Halimeda* reproduction in more detail (Clifton

1997, but see Drew and Able 1988) given the frequency of reproduction, high density of individuals, and overall distribution of meadows on a wide spatial and depth gradient.

Although there were no significant differences in the height or number of axes between reproductive and non-reproductive individuals at 10 and 20 m depths, we found sexually reproductive individuals had significantly more segments (~1.5x) than non-reproductive individuals. This is consistent with the observations of Vroom et al. (2003) that the largest individuals (presumably those with the largest number of segments) in a *H. tuna* population were the ones to develop gametangia. However, given that the size, number of segments, or height of individuals of *Halimeda* are likely not an accurate gauge of age, given the fluctuations seen in segment number and height over the lifespan of an individual (Multer and Clavijo 1989), it may not necessarily be the oldest individuals sexually reproducing.

Halimeda kanaloana meadows dominate areas of Hawaiian soft-bottom, benthic communities, providing an expansive habitat for numerous macrofauna through the creation of structural complexity over otherwise featureless expanses of sand. *H. kanaloana* meadows appear to be an important habitat for the recruitment of juvenile urchins (*T. gratilla*), and a foraging ground for Hawksbill sea turtles. A diverse and unique benthic invertebrate community also exists within *H. kanaloana* meadows, with a higher abundance and diversity of organisms found within dense meadows as compared to nearby sandy habitats (Fukunaga 2008). In conjunction with this study, a razor fish (*Cymolutes praetextatus*) commonly found in the *H. kanaloana* meadows was reported for the first time in the Hawaiian Islands (Randall et al. 2006). This species was previously known from East Africa to the Society Islands, but not from east of the

Marshall Islands in the North Pacific. Although species of *Halimeda* contain anti-herbivory compounds (Paul and Van Alstyne 1988, 1992) and are heavily calcified, herbivores such as *T. gratilla* were observed grazing on *Halimeda* and its associated epiphytes. The impact of *T. gratilla* at its current densities is likely minimal in the long-term because *Halimeda* is known to propagate clonally from grazed segments (Walters and Smith 1994, Walters et al. 2002), can regrow plants from their original holdfasts, and has fast growth and turnover rates. On several occasions, we observed a buried individual of *Halimeda* regrowing into several new individuals through the transformation of the original segments into new holdfasts and plants.

Calcium carbonate production

The quantity of $CaCO_3$ in species of *Halimeda* varies substantially both within and among taxa (Table 10). The extent of calcification in *Halimeda* has been suggested to be physiologically mediated through photosynthesis (Stark et al. 1969), with *Halimeda* species more heavily calcified in deep rather than in shallow water (Goreau 1963). Vroom et al. (2003) hypothesized that the increased levels of $CaCO_3$ found in deeper *H. tuna* populations enhance light harvesting by turning internal siphons into bright-white 'integrating spheres" that increase light absorbance. However, we found similar quantities of $CaCO_3$ in *H. kanaloana* from 1 to 78 m depths, with the mean $CaCO_3$ content ranging from 67.2 to 75.0% across all depths. Semesi et al. (2009) found the calcification rate of *Halimeda* in tropical seagrass meadows was enhanced 1.6-fold by the photosynthetic activity of surrounding seagrasses, which in turn increased seawater pH. Determining if the lack of large seagrasses (and thus more constant pH levels) in *H.*

kanaloana meadows in Hawai‘i result in consistent calcification rates across depths merits additional study.

The abundance and plant dynamics of *Halimeda* species vary dramatically in time and space, requiring spatial, seasonal, and interannual variations be considered for a realistic $CaCO_3$ production value (van Tussenbroek and van Dijk 2007). The $CaCO_3$ production of *H. kanaloana* in Hawai‘i is high (1320 – 1883 grams $CaCO_3$ per meter squared per year) compared to *H. incrassata* (Table 10), with the exception of a lagoon in Panama where $CaCO_3$ production was estimated at 2323 gram $CaCO_3$ per meter squared per year (Freile and Hillis 1997). The estimates from Freile and Hillis (1997) were based on two months of data collection, however, with no assessment of seasonal and interannual variation. Otherwise, my estimates are ~twice the rate reported from the Mexican Caribbean (van Tussenbroek and van Dijk 2007). They are the deepest psammophytic *Halimeda* meadows $CaCO_3$ production rates currently available, and suggest that such meadow may be highly productive and significant contributors to carbonate sediments, especially when dominated by a single species. Photosynthesis and calcification rates from four epilithic species of *Halimeda* at 76 m depths also emphasize the importance of deep-growing *Halimeda* species as producers of carbonaceous materials (Jensen et al. 1985), as well as the high abundance of species of *Halimeda* in deep (to 150 m) tropical waters (Freile et al. 1995).

Psammophytic *Halimeda* meadows are an integral component of many shallow and mesophotic tropical ecosystems world-wide, but the impact of climate change and ocean acidification on these meadows is unknown. The response of *H. kanaloana* meadows to increasing ocean temperatures and changes in carbonate saturation levels

will likely be complex due to interactions between lowered pH, the resulting dissolution of the calcium carbonate skeleton and surrounding carbonate sand, and the enhancement of photosynthesis by increased CO_2. Among the calcified macroalgae, most ocean acidification research has focused on nongeniculate (crustose) coralline algae, rhodoliths (or maerl), and a few lithophytic *Halimeda* species (Nelson 2009). Borowitzka & Larkum (1976) found that a decrease of pH from 8 to 7.5 reduced calcification dramatically in *Halimeda tuna*. Ocean acidification may also affect calcification in *H. kanaloana*, though species-specific tests are likely necessary to evaluate possible impacts. Acidification may increase the susceptibility of *Halimeda* to grazing and erosion, and lead to a reduction in carbonate sand production and a significant loss of habitat (Diaz-Pulido et al. 2007). However, mesophotic *Halimeda* meadows whose distributions are influenced by deep ocean currents, such as in the Great Barrier Reef, may be less affected than shallow sites due to the diminishing impacts of decreasing carbonate saturation from surface ocean waters with increasing depth (Diaz-Pulido et al. 2007). Controlled and appropriately replicated mesocosm experiments (such as Kuffner et al. 2007 and Jokiel et al. 2008) and *in situ* experiments on psammophytic *Halimeda* are needed to appropriately gauge the impacts of increasing temperature and fundamental changes in seawater carbonate chemistry due to CO_2 emissions from anthropogenic activities.

Conclusions

Meadows of *H. kanaloana* in Hawai‘i appear superficially static given the dominance of a single species over a wide depth range. However, these are dynamic assemblages with large fluctuations in growth, densities, and morphometrics across a

large depth range and among locations. These deep meadows are highly productive, with fast growth rates and a high turnover, and are significant contributors of calcium carbonate to the sediments. *H. kanaloana* is generally a long-lived (several years) perennial with low rates of sexual reproduction, but appears to recover rapidly from disturbances such as episodic bacterial and cyanobacterial blooms. The high densities, large areal coverage, and ecological role of *H. kanaloana* in Hawai‘i suggest that deep psammophytic algal meadows are an integral part of the coral reef ecosystem.

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Table 1. Sites, sampling dates, depths, and sample sizes (*n*) used for growth studies, density, and height measurements. Depths in boldface were sampled for growth studies, density, and height measurements; depths in regular font were sampled for density and height. Blank spaces under "Sample Size" indicate no data were collected. All individuals from growth studies were also used in morphometric calculations (Table 2).

				Sample Size (*n*)	
Site	Year	Sampling Period	Depths (m)	Growth	Density & Height
Kahekili	2005	Jun 14 – Jun 23	**10, 20, 30**	27, 19, 17	20, 23, 12
Honolua	2005	Jul 07	15		21
Makena Landing	2006	Mar 22	10		9
Kahekili	2006	Mar 27 – Apr 3	**10, 20, 30**	18, 19, 17	16, 25, 28
Olowalu	2006	Apr 9	30		23
Kahekili	2006	May 15 – May 23	**10, 20, 30**, 40	11, 16, 8	10, 20, 24, 15
Honokowai	2006	May 21	10, 20		10, 4
Kahekili	2006	Jul 20 – Jul 28	**10, 20, 30**	19, 18, 18	
Kahekili	2007	Nov 24 – Dec 3	**10, 20, 30**	15, 15, 21	20, 13, 1
Kahekili	2008	Mar 29 – Apr 6	**10, 20, 30**	16, 19, 19	19, 19, 20
Papawai Point	2008	Mar 29 – Apr 6	**20**	20	20
Mala	2008	Apr 1 – 8	10, **20**	20	22, 20
Launiupoko	2008	Apr 1 – 8	**20**	16	20

Table 2. Collection information and sample sizes (*n*) for *H. kanaloana* morphometrics and $CaCO_3$ calculations. Methods starting with "P4" or "P5" indicate collection by the *Pisces IV* or *Pisces V* submersibles and include the dive number; "diver" indicates SCUBA or rebreather diver collections. All plants used in growth studies (Table 1) were also used for morphometrics.

Location	Method	Depth (m)	Date	*n*
Moloka‘i	*P5-563*	85	4-Sep-04	1
West Maui	*P5-565*	56	6-Sep-04	5
KahoolaweKaho‘olawe	*P5-566*	66, 78, 85	7-Sep-04	1, 3, 4
KahoolaweKaho‘olawe	*P4-132*	59, 79	16-Dec-04	4, 5
Moloka‘i	*P4-133*	69	17-Dec-04	2
Mala	Diver	1, 1.5, 4, 7, 9	23-Jun-05, 22-Jun-05, 23-Jun-05, 22-Jun-05, 9-Apr-06	3, 6, 3, 3, 6
Kahekili	Diver	10, 15, 20, 30	8-Jul-05, 7-Jul-05, 29-Jan-06, 29-Jan-06	3, 3, 13, 15
South O‘ahu	Diver	32, 50	9-Dec-05, 19-May-07	3, 3
Puamana	Diver	1, 10, 20	4-Feb-06	6, 8, 11
Olowalu	Diver	27	9-Apr-06	5
Kahekili	Diver	42	16-May-06, 19-May-06	2, 2
Honokowai	Diver	11, 18	21-May-06	5, 5
Makena	Diver	11	22-May-06	8
West Lāna‘i	*P4-185*	40, 63	18-Nov-06	8, 7
East Lāna‘i	*P4-186*	59	19-Nov-06	5
West Maui	*P4-190*	82	29-Nov-06	8
South Maui	*P4-191*	67, 82	30-Nov-06	8, 8

Table 3. Two-way ANOVA results from *H. kanaloana* density (individuals m^{-2}) and canopy height quadrat data at Kahekili Beach Park (Kahekili BP) at 10, 20, and 30 m depths during 2005 - 2008.

	Density		Canopy Height (cm)	
Factor	$F_{5,2,10,324}$	*P*	$F_{5,2,10,324}$	*P*
Sampling date	25.36	< **0.001**	20.03	< **0.001**
Depth	559.08	< **0.001**	364.23	< **0.001**
Interaction	10.12	< **0.001**	4.83	< **0.001**

Table 4. Two-way ANOVA results for *H. kanaloana* growth at 10, 20, and 30 m depths during 2005 - 2008. Statistically significant values are in boldface.

	new segments d^{-1}		% new growth d^{-1}	
Factor	$F_{5,2,10,285}$	*P*	$F_{5,2,10,285}$	*P*
Sampling period	27.18	< **0.001**	24.38	< **0.001**
Depth	58.57	< **0.001**	16.24	< **0.001**
Interaction	7.41	< **0.001**	3.24	**0.001**

Table 5. Densities (individuals m^{-2}) of *H. kanaloana*, reproductive *H. kanaloana*, percentage of reproductive *H. kanaloana*, and densities of the urchin *Tripneustes gratilla* from 50 by 1 m long transects at Kahekili BP . Values are means ± (SE).

Depth (m)	Date	*Halimeda* density	Reproductive *Halimeda* density	% population reproductive	*Tripneustes gratilla* density
18	8-Apr-06	342 (13)	0.16 (0.05)	0.05 %	1.07 (0.83)
20	1-Dec-07	217 (9)	1.64 (0.15)	0.76 %	0.76 (0.13)

Table 6. Characteristics of fertile and vegetative *H. kanaloana* from Kahekili BP at 10 and 20 m depths. Fertile plants were collected on 27 November 2007 and vegetative plants were collected on 03 December 2007. Values are means ± (SE). *n* = 10 per reproductive state and depth. See Table 7 for significant differences.

	Height (cm)		Number of segments individual^{-1}		Number of axes individual^{-1}	
Depth	Fertile	Vegetative	Fertile	Vegetative	Fertile	Vegetative
10 m	14.9 (0.9)	14.5 (0.6)	435 (91)	254 (51)	37.5 (8.4)	29.9 (7.3)
20 m	18.7 (1.1)	20.5 (1.3)	240 (51)	147 (26)	20.9 (5.2)	14.4 (2.4)

Table 7. ANOVA results for morphometrics between fertile and vegetative *H. kanaloana* plants at 10 and 20 m depths. $n = 10$. Statistically significant values are in boldface.

	Height (cm)		Number of axes		Number of segments	
Factor	$F_{1,1,1,36}$	P	$F_{1,1,1,36}$	P	$F_{1,1,1,36}$	P
Depth	22.7	< **0.001**	7.61	**0.009**	8.15	**0.007**
Reproduction	0.43	0.518	1.24	0.272	4.39	**0.043**
Interaction	1.18	0.284	0.01	0.940	0.1	0.753

Table 8. Mean (±SE) percent of $CaCO_3$ and $CaCO_3$ per segment in *H. kanaloana*. See Table 2 for collection locations and specific sample sizes. The *n* values for plants are the number of individual plants used per depth for $CaCO_3$ calculations. The *n* values for segments are the total number of segments used from all plants at that depth for $CaCO_3$ calculations.

	Depth (m)									
	1	**1.5**	**7**	**10**	**15**	**20**	**25**	**30**	**56**	**78**
Mean % $CaCO_3$	70.0 (4.1)	68.5 (6.7)	71.4 (3.1)	75.0 (0.7)	74.4 (3.7)	69.7 (1.7)	76.2 (1.5)	67.2 (2.5)	70.7 (4.1)	72.4 (2.6)
Mean (g) $CaCO_3$ $segment^{-1}$	0.0075 (0.0006)	0.0083 (0.0015)	0.0101 (0.0009)	0.0146 (0.0020)	0.0121 (0.0010)	0.0134 (0.0010)	0.0067 (0.0004)	0.0109 (0.0014)	0.0097 (0.0028)	0.0057 (0.0018)
n = plants	5	3	3	8	3	24	4	15	5	3
n = segments	159	75	134	259	144	951	324	657	234	196

Table 9. Production of $CaCO_3$ from *H. kanaloana* at Kahekili BP from 10, 20, and 30 m depths. "Overall Mean" used data combined from all sites over the entire depth range of *H. kanaloana*. The Min, Max, *n*, and Depth pertain to data used for the Overall Mean. Values shown with parentheses are means ± (SE). See text for explanation of calculations.

	% $CaCO_3$	**g $CaCO_3$ segment^{-1}**	**Segments plant^{-1}**	**g $CaCO_3$ plant^{-1}**	**Plants m^{-2}**	**g $CaCO_3$ m^{-2}**	**New segments plant^{-1} d^{-1}**	**Turnover (d)**	**Turnover yr^{-1}**	**g $CaCO_3$ m^{-2} y^{-1}**
10 m	75.0 (0.7)	0.0146 (0.0020)	223 (19)	3.26	65 (3)	210.94	5.08 (0.66)	43.85	8.32	1755.91
20 m	69.7 (1.7)	0.0134 (0.0010)	117 (7)	1.57	229 (7)	359.62	1.51 (0.15)	77.47	4.71	1694.24
30 m	67.2 (2.5)	0.0109 (0.0014)	105 (6)	1.14	262 (7)	298.83	1.27 (0.14)	82.64	4.42	1319.91
Overall Mean	70.5 (0.9)	0.0113 (0.006)	169 (6)	1.91	174 (5)	332.48	2.62 (0.25)	64.44	5.66	1883.31
Min	48.6	0.0032	26		5		0			
Max	85.4	0.0264	1208		461		31.57			
n	73	73	512		524		303			
Depth (m)	1 – 78	1 – 78	1 – 85		10 – 40		10 – 30			

Table 10. Comparison of *in situ* $CaCO_3$ production for *H. kanaloana* and *H. incrassata*. Values shown are means. "nr" = not reported.

Study	Species	Location	Depth range (m)	Season	% $CaCO_3$	g $CaCO_3$ m^{-2}	Plants m^{-2}	New segments $plant^{-1}$ d^{-1}	Turnover (d)	g $CaCO_3$ m^{-2} y^{-1}
Wefer (1980)	*H. incrassata*	Bermuda	1 – 4	Aug–Sept	nr	6.7	nr	1.00 segment axis d^{-1}	30–52	50
Garrigue (1991)	*H. incrassata*	New Caledonia	11	Year round	72	17.2	nr	0.60 segment axis d^{-1}	150[a]	32
Bach (1979)	*H. incrassata*	Florida	0.95 – 3.6	Year round	26	5.8	nr	nr	32	10
Freile & Hillis (1997)	*H. incrassata*	Panama	0.3 – 7	Sept–Nov	81	211	88	2.90[b]	32	2323
Multer (1988)	*H. incrassata, H. monile*	West Indies	1 – 2.5	April–July	88	nr	26 – 36	1.42 – 2.17	39	97
van Tussenbrock & van Dijk (2007)	*H. incrassata*	Mexican Caribbean	1.5 – 5	Year round	86	nr	99	nr	30	815
This study	*H. kanaloana*	HawaiiHawai'i	10	Year round	75	211	65	5.08	44	1756
This study	*H. kanaloana*	HawaiiHawai'i	20	Year round	70	360	229	1.51	77	1694
This study	*H. kanaloana*	HawaiiHawai'i	30	Year round	67	299	262	1.27	83	1320
This study	*H. kanaloana*	HawaiiHawai'i	1 – 85	Year round	71	332	174	2.62	64	1883

[a]Turnover is the frond population renewal rate. [b]extrapoloated from a mean 93 segment plant and 32 day turnover.

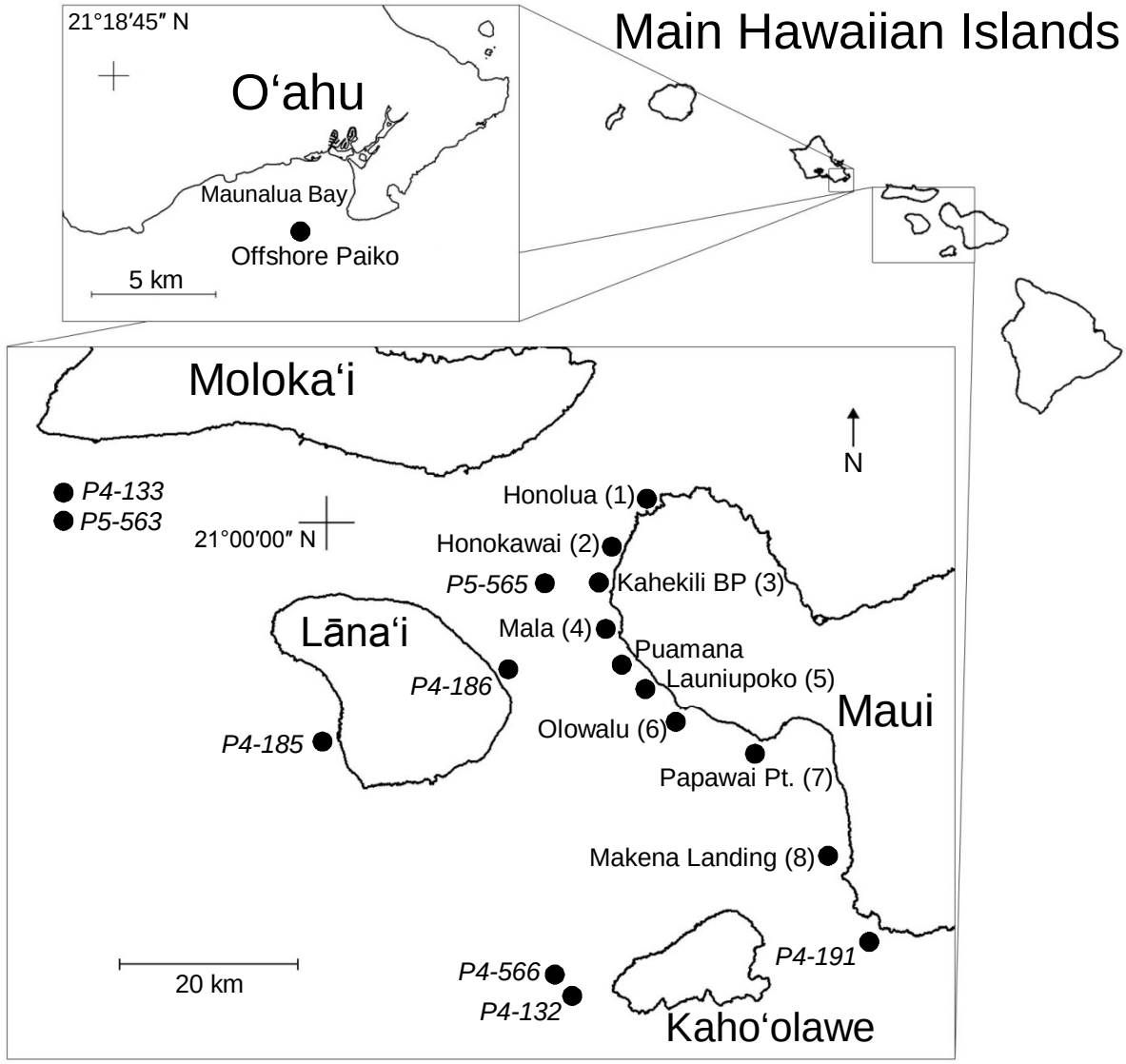


Figure 1. Research sites offshore of Oʻahu (small box) and the Maui Nui complex (large box). The Maui Nui complex is composed of the islands of Molokaʻi, Lānaʻi, Maui, and Kahoʻolawe. The numbers next to sites in west Maui correspond to locations in Fig. 3.

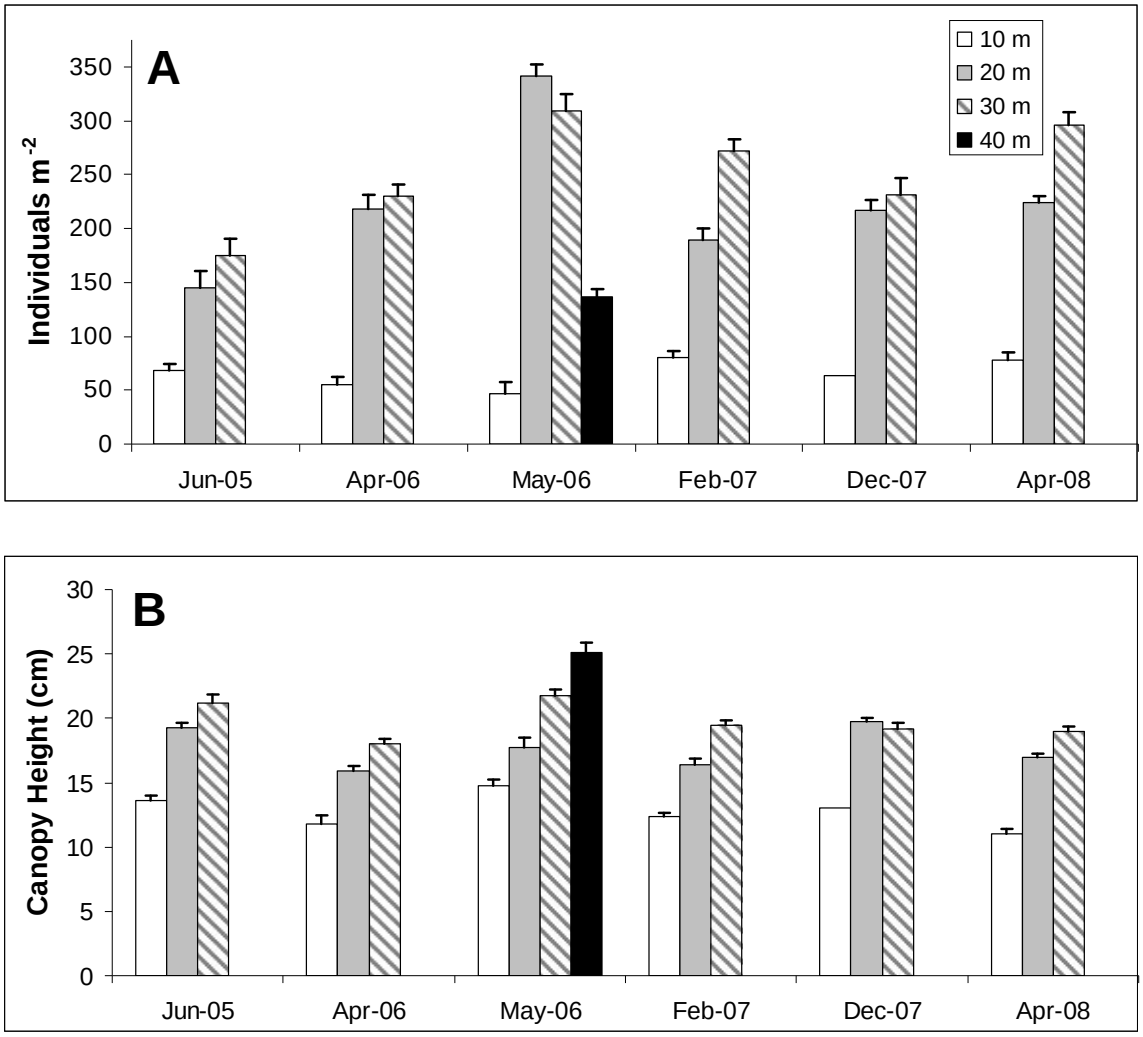


Figure 2. Mean (± SE) densities and heights of *H. kanaloana* at Kahekili BP for 10 – 40 m depths during June 2005 – April 2008. (A) Density (number of individuals m^{-2}) (B) Mean canopy height of five randomly selected individuals from each 0.25 m^2 quadrat used in density measurements. See Table 1 for sample sizes.

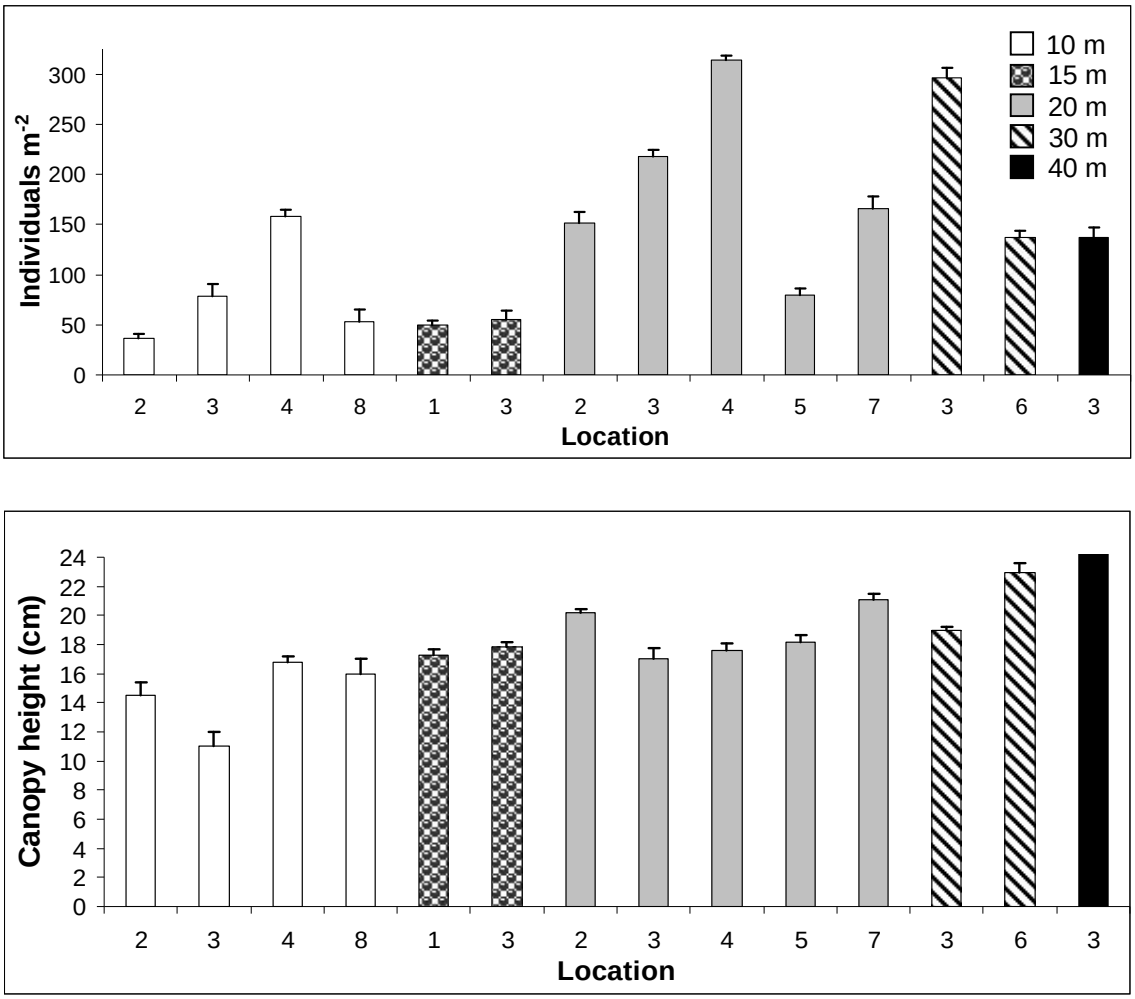


Figure 3. Mean (± SE) densities and heights of *H. kanaloana* at multiple sites from 10 – 40 m depths. One-way ANOVAs showed significant differences among sites in densities ($F_{13, 511} = 107.03$, $P < 0.001$) and heights ($F_{13, 511} = 54.04$, $P < 0.001$). See Figure 1 for locations and Table 1 for sampling dates. Only data from March 29 – April 6, 2008 were used for location 3 (Kahekili BP).

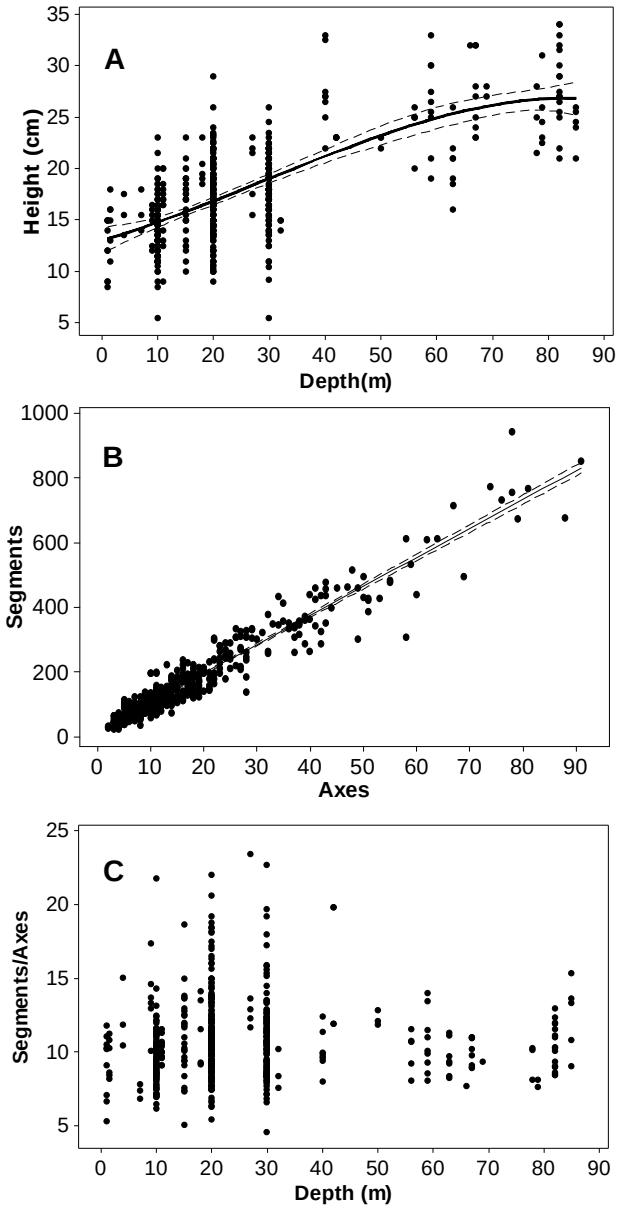


Figure 4. *H. kanaloana* morphometric characteristics. (A) height per individual with increasing depth, R^2 = 49.5%, y = 13.03 + 0.1576 x + 0.002160x^2 - 0.000025x^3, ($F_{3,512}$ = 167.08, $P < 0.001$); (B) relationship between the number of axes per individual and the number of segments per individual, R^2 = 92.6%, y = 18.22 + 8.943x ($F_{1,509}$ = 6415, $P <$ 0.001); (C) number of segments per individual divided by the number of axes per individual with increasing depth, no linear relationship, R^2 = 0.0%, ($F_{1,510}$ = 0.23, P = 0.635). See Table 2 for sampling locations and sample sizes. Dotted lines represent 95% CI.

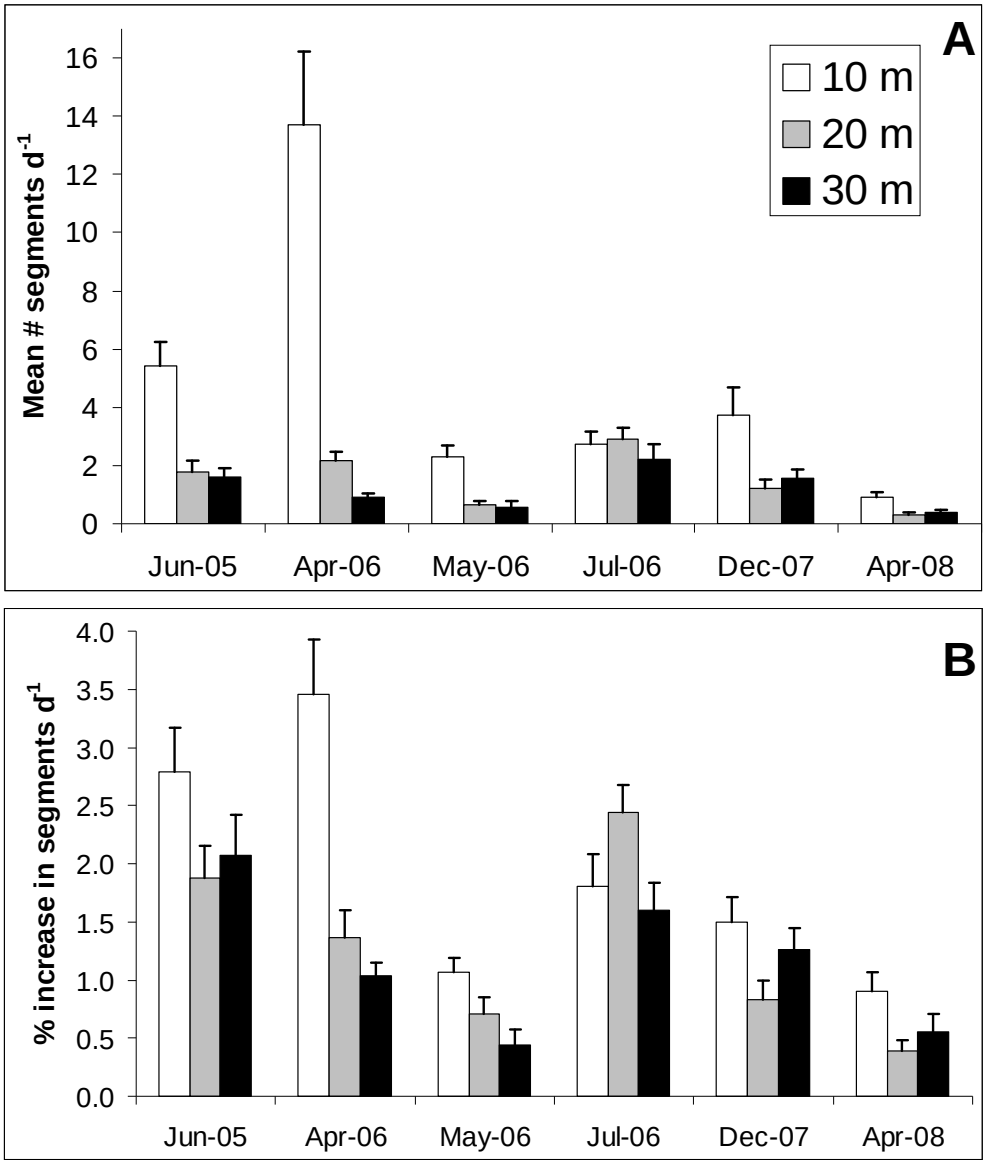
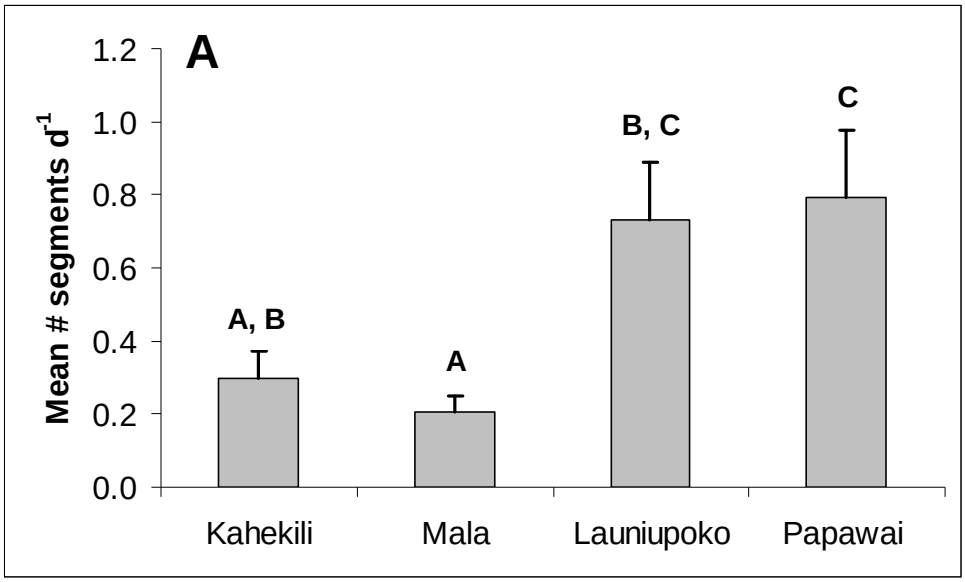


Figure 5. Mean growth rates (± SE) of *H. kanaloana* at 10, 20, and 30 m depths at Kahekili BP from June 2005 – April 2008. (A) mean number of new segments per plant per day, (B) percent increase in new segments per plant per day. See Table 1 for sample sizes.



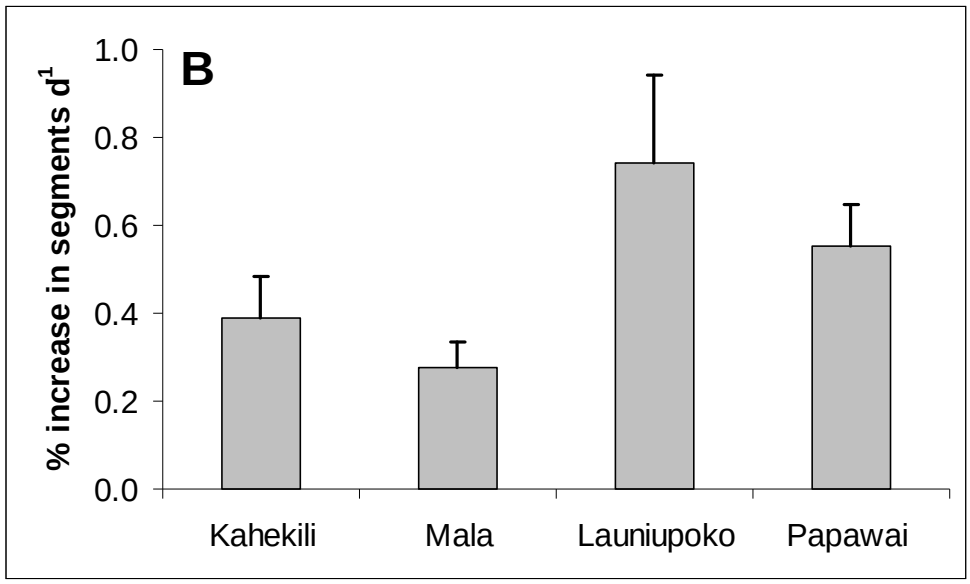


Figure 6. Mean growth rates (± SE) of *H. kanaloana* at 20 m depth at four locations (Fig. 1) in April 2008. (A) number of new segments per day. Common letters indicate no significant difference among sites as judged by one-way ANOVA ($F_{3,71} = 5.47$, $P = 0.002$) and post-hoc tests with $P \leq 0.05$. (B) percent increase in new segments per day. One-way ANOVA showed no significant differences between sites ($F_{3,71} = 2.52$, $P = 0.065$). See Table 1 for sample sizes and collection information.

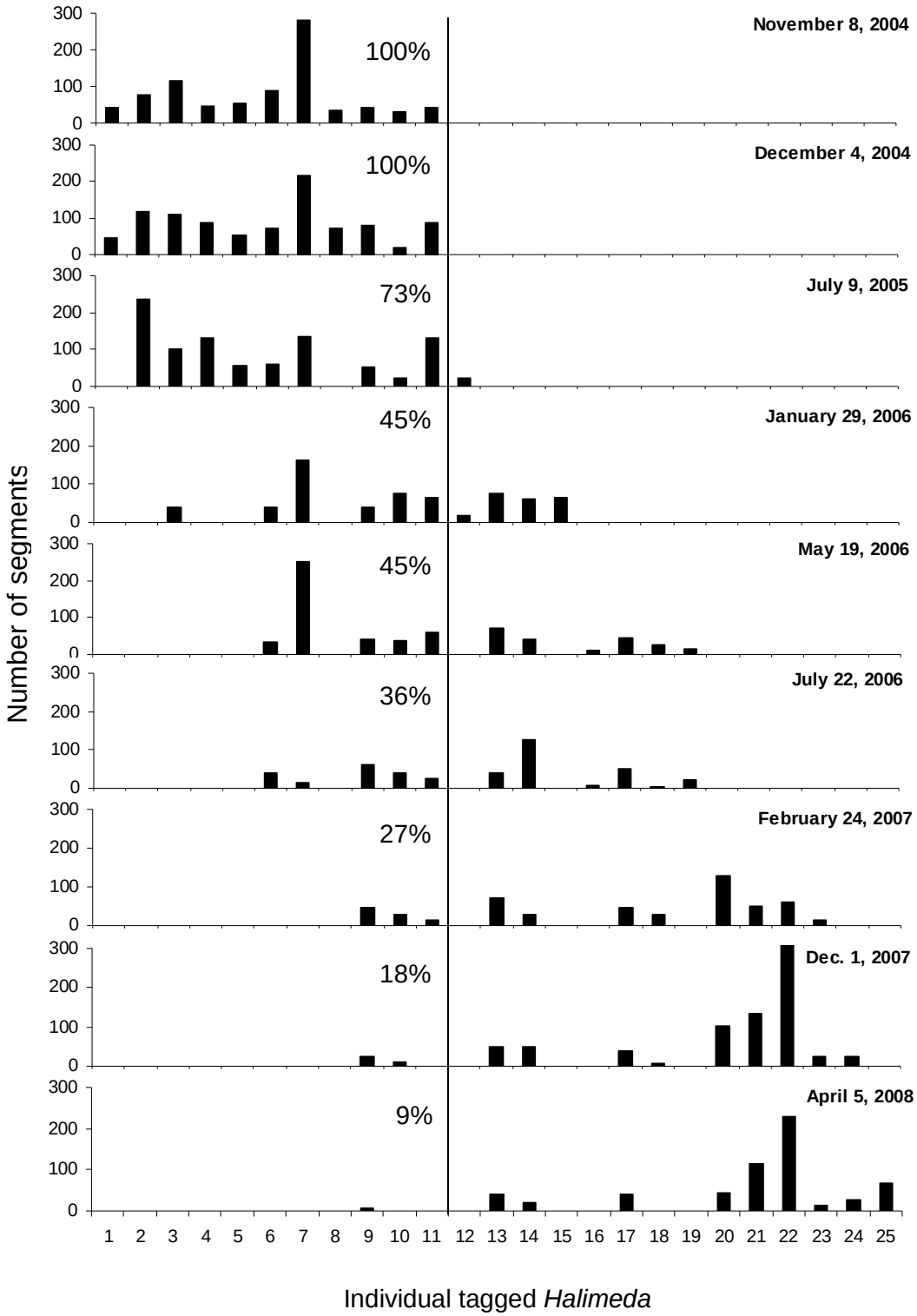


Figure 7. Number of segments per tagged individual *Halimeda* plant during 2004–2008. The line separates the initial 11 tagged individuals, with the % survival of those individuals shown through time.

CHAPTER 4:

RECOVERY FROM DISTURBANCE IN A DEEP ALGAL MEADOW

ABSTRACT

The calcified green alga *Halimeda kanaloana* forms expansive meadows over soft sediments offshore of west Maui, and the meadows are often targeted for anchoring small recreational boats. I monitored the long-term (~2 year) recovery of *H. kanaloana* meadows to a manipulative disturbance experiment mimicking anchor scar damage at a 23 m depth. Cleared treatments removed the entire plant (upright axes and holdfast), while cut treatments removed the upright axes and left the holdfast intact. It took up to 606 days for densities of *H. kanaloana* to return to control levels, while canopy height recovered within 327 days. Cleared treatments generally took longer to recover than cut treatments, suggesting that disturbances removing the entire plant will require a longer recovery time. The phenology of recovery was similar to a near-by anchor scar at 27 m depth created during the same time period, which required up to 734 days to recover to pre-disturbance levels. Forty-nine bioturbating macrofauna were surveyed within the meadows. The most important bioturbators included giant stomatopods, burrowing shrimp and associated gobies, flounder, wrasses, and rays. The recovery rates of deep *H. kanaloana* meadows to disturbances were longer than those previously reported for other psammophytic macroalgae, but very similar to opportunistic, monotypic seagrass meadows. These results suggest that *H. kanaloana* meadows could be damaged by repeated anchoring and that the use of fixed moorings in these areas would be beneficial to the health and stability of deep *Halimeda* meadows.

INTRODUCTION

Marine soft sediment environments are often considered disturbance-dominated, and recovery rates and patterns from disturbance provide important insights into the potential for broad-scale changes in the function of benthic communities (Norkko et al. 2010). Conceptually, disturbance is defined as the partial or total destruction of plant biomass (Grime 1977), which may arise from natural (e.g., herbivory, bioturbation, pathogens, storm surge) or anthropogenic (e.g., anchoring, trawling, eutrophication) mechanisms. Resilience is the potential for recovery from disturbance (Pimm 1991, Thrush et al. 2009), and is also called engineering resilience. In comparison, recovery is the culmination of interactions between species (dominance, inhibition, or facilitation) and extrinsic factors (e.g., colonist supply and environmental setting), making it a direct measurement of engineering resilience (Dayton et al. 1992, Thrush et al. 2009). Disturbance-recovery experiments can reveal processes, feedbacks, and threshold conditions that are important for ecosystem resilience and can thus provide important insights into the functioning and appropriate management of ecosystems (Thrush et al. 2009). This investigation focused on a disturbance-recovery experiment mimicking anchor scar damage in a deep *Halimeda kanaloana* algal meadow over a 2 year period, and provides information that will aid in the management of these soft sediment environments. We also report on the densities of bioturbating macrofauna, which create natural disturbances within these meadows that act in concert with anthropogenic disturbances during the recovery process.

The types of physical anthropogenic disturbances commonly encountered in soft sediment environments may include motor vessel damage (e.g., anchor and mooring

damage, propeller scarring, and hull groundings; Hammerstrom et al. 2007), fishing gear impacts (Jennings et al. 2001, Orth et al. 2002), and even bombing (Meinesz and Lefevre 1984). The majority of literature concerns motor vessel damage in shallow tropical seagrass meadows. Such damage can vary from the shearing of the aboveground canopy to excavation of belowground roots and rhizomes and underlying fine-grained sediments, and is one of the most severe disturbances that can occur in seagrass meadows (Kenworthy et al. 2002, Hammerstrom et al. 2007). Once damage occurs, wind-, wave-, and current-induced erosion may further enlarge the disturbance by undercutting the seagrass at vulnerable scar margins, exacerbating the disturbance and hindering recovery (Hammerstrom et al. 2007). Secondary damage by hurricanes to damaged areas in a high-energy environment may increase the disturbance footprint by more than 100% (Whitfield et al. 2002). However, these abiotic factors are greatly reduced in deep (to 30 m depth) or mesophotic (40 to 150 m depth) algal meadows, such as *H. kanaloana* meadows in Hawai‘i, where wave and storm surge are buffered with increasing depth and distance from shore.

H. kanaloana meadows in Hawai‘i are unique because they form quasi-monospecific assemblages dominated by a single species over a broad depth range (1 to 85 m depths) across several Hawaiian islands (Huisman et al. 2007, Verbruggen et al. 2006), and do not contain seagrasses or other *Halimeda* species (Chapter 4). Located just offshore of the hermatypic reef, these meadows are often targeted for anchoring by small boats or cruise ships. Similar to shallow seagrass meadows, anchoring damage often removes large swaths of upright axes due to anchor scrapping along the sediment surface, and/or removes entire plants (upright axes and holdfast) due anchor penetration into the

sediment. Natural disturbance from bioturbation, such as stomatopod burrows (*Lysiosquillina maculata*) and excavations by rays (*Dasyatis brevis*), may have similar impacts, though the size of these impact appear smaller and less obvious than anchoring damage (HS, pers. observation). These observations, coupled with requests by resource managers to determine the long-term impact of anchoring in *H. kanaloana* meadows, provided the impetus for this study.

The objectives of this research were to determine the recovery rates of *H. kanaloana* to disturbance mimicking two types of anchor scar damage, and to compare this with the recovery of an actual anchor scar in the same area. To accomplish this, we surveyed the densities and canopy height of *H. kanaloana* in experimental treatments over a two year period, and compared results with qualitative surveys of an anchor scar. We also surveyed and determined the relative importance of macrofauna (bioturbators) which naturally disturb the sediment in *H. kanaloana* meadows.

MATERIALS AND METHODS

Site description. The manipulative experiment was conducted in a dense *H. kanaloana* meadow at a 23 m depth, approximately 300 m offshore of Kahekili Beach Park (KBP) on west Maui, Hawai‘i , USA (Fig. 1). This depth was chosen because it is commonly used for anchoring small boats for diving operations, and has extensive *H. kanaloana* meadows. The reef at KBP is characterized by a fringing hermatypic coral reef with low vertical relief to ~10 m depth, and gently sloping soft sediments from ~10 to 90+ m depths. Subtidal temperatures at 23 m depth range annually from 25.3 to 28.7° C (HS, unpubl. data). Oceanographic conditions during summer/fall months are characterized by

consistent northeast trade winds, low rainfall, small waves, and strong alongshore currents, while winter/spring months experience heavy rain, strong winds, strong offshore currents, large waves, and high water turbidity (Storlazzi and Jaffe 2008). *H. kanaloana* dominates the deep sediments in this area, with up to a ~30 cm canopy height and a mean density of 228 plants per meter squared (Chapter 4). *H. kanaloana* forms a continuous meadow from ~10 m to 90 m depths at KBP, with infrequent, small (~5-10 m across) patch reefs scattered throughout. Other rhizophytic algae such as *Caulerpa taxifolia* (M. Vahl) C. Agardh and *Codium desultorium* P.C. Silva et M.E. Chacana, sp. ined. are present in low abundance. Seagrasses and other rhizophytic *Halimeda* species do not occur in the *H. kanaloana* meadows.

Disturbance experiment. The disturbance experiment was initiated in April 2006. Three treatments (cut, cleared, and control) were used to mimic disturbance in the experiment (Fig. 2). The "cut" treatment entailed removing the upright axes of each individual *Halimeda* plant with stainless steel safety shears, leaving 1-2 basal segments on the intact holdfast. This treatment was most similar to the scrapping motion of anchors over the meadows, with the axes often ripped from the holdfast. Initial attempts at ripping the *Halimeda* axes from the holdfast with a small anchor and by hand proved difficult and too time-consuming for the limited dive time available at depth. The "cleared" treatment removed the entire plant (all upright axes and the attached holdfast) of each plant, representing the gouging of the sediments and the resulting unvegetated hole left by anchor scars or sting ray feeding pits. Clearing entire plants resulted in a depression ~5 - 10 cm deep in the sediment. Effort was made to remove the entire holdfast, including

all surrounding rhizoids. Control treatments were left undisturbed. A 0.25 m^2 quadrat was chosen because it was most similar in size to disturbances made by small (< 1 m) anchors in meadows (HS per. obs.). Recreational boats were commonly observed using a large Danforth® or similar type of anchor, and creating impacts that were ~0.25 m^2 in size.

Five replicates of each treatment (n = 15 quadrats total) were randomly located along a 30 m transect at the 23 m depth contour. The transect beginning and end were marked with fluorescent plastic tent stakes and a small buoy. The corners of each 0.25 m^2 quadrat were also marked with tent stakes and plastic cable ties. On day 0 (d0), the transect and quadrat locations were established at depth, and the density and canopy height of *Halimeda* plants were surveyed in each quadrat. An individual *Halimeda* was defined as an upright plant having its own distinct holdfast. The height (cm) of the 5 tallest plants were measured within each quadrat and averaged to generate the mean height for the upper limit of canopy plants (canopy) in each quadrat. The number of urchins (primarily the collector urchin *Tripneustes gratilla*), sexually reproductive *Halimeda*, other macroalgae, and observations such as the presence/absence of cyanobacterial blooms were recorded. On day 1 (d1), the treatments were completed for each quadrat, and the quadrats were re-surveyed for densities and canopy heights. Overall, the density and canopy height of *Halimeda* plants in each quadrat were surveyed seven times: 2 Apr 2006 (d0), 3 Apr 2006 (d1), 15 May 2006 (d41), 23 July 2006 (d110), 25 Feb 2007 (d327), 1 Dec 2007 (d606), and 7 Apr 2008 (d734).

Anchor scar. On 4 Apr 2006, I inadvertently created a large anchor scar at a 27 m depth (Fig. 3), approximately 25 m offshore from the clearing experiment at KBP. The

corners of the anchor scar were marked with orange tent stakes and small buoys, and photographed with a 0.25 m^2 quadrat placed in the center of the anchor scar between the tent stakes. The anchor scar measured ~75 x 85 cm across, and contained a ~30 cm deep pit in the middle where entire *Halimeda* plants (holdfast and upright axes) were removed by the anchor (similar to the cleared treatment). Parallel marks were created where the anchor scraped over the *H. kanaloana* meadow and removed the upright portion of the *Halimeda* axes (similar to the cut treatment). The anchor scar was photographed on 8 Apr 2006 and monitored through time within 1-2 days of the same dates as the disturbance experiment. Monitoring entailed making observations on the extent of recovery and photographing the anchor scar with the 0.25 m^2 quadrat placed in the same position between the permanently marked tent stakes. The corners of the 0.25 m^2 quadrat in the anchor scar were not permanently marked because the sides of the anchor scar were steeply sloped, and any permanent markers could have interfered with *H. kanaloana* recovery in the anchor scar pit.

Bioturbation. During June 2005 and May 2006, SCUBA divers surveyed the macrofauna within the same area of *H. kanaloana* meadows at KBP where the disturbance experiment and anchor scar occurred. Qualitative observations of bioturbating macrofauna were recorded until the completion of the study in April 2008. Only bioturbating macroinvertebrates and fishes inside *H. kanaloana* meadows are reported in this study. Fishes and invertebrates were collected with clove oil using tarpaulins.

The depth of bioturbation (either less than or equal to 1 cm or greater than 1 cm) was determined using a combination of *in situ* observations and literature reviews for each species. One centimeter was chosen to differentiate between low- and high-impact bioturbators because it was commonly observed to be the depth distinguishing between organisms that would disturb just the surface of the sediment during activities such as foraging (less than or equal to 1 cm), versus disturbances such as borrowing into the sediment (greater than 1 cm). Common names followed those published in Hoover (1998), Hoover (2002) and www.fishbase.org (accessed 31 Aug 2011).

Macroinvertebrates. Macroinvertebrate survey methods and density data were adapted from Fukunaga (2008). Macroinvertebrates were surveyed using a combination of epibenthic belt transect protocols and epifaunal tarpaulin enclosures. Five epibenthic belt transect surveys were conducted at both 20 and 30 m depths using a measuring tape. Each transect was 10 x 1 m in length and width and placed randomly inside a *H. kanaloana* meadow. Divers identified and counted solitary macroinvertebrate organisms within the transect. Epifaunal species surveys were conducted using tarpaulin enclosures haphazardly placed in the middle of dense *H. kanaloana* meadows. Epifaunal organisms inside the *Halimeda* vegetation were sampled by applying approximately 500 ml of an anesthetic solution (10% clove oil in ethanol) beneath a 1.5 x 1.5 m weighted plastic tarpaulin. Tarpaulins were lifted five minutes after the application of clove oil. Stupefied organisms floating in the water column were collected using a hand net. In total, four epifaunal tarpaulin surveys were conducted at both 20 and 30 m depths. Macroinvertebrates were preserved in 70% isopropyl alcohol and identified to the lowest

taxon level possible, using a dissecting microscope if necessary. Qualitative observations of macroinvertebrates were also recorded in a 25 x 1 m area at 20 m depth in the *H. kanaloana* meadow during one night dive (24 July 2007).

Fishes. Large-bodied fishes (≥100 mm standard length) were surveyed from 10 to 30 m depths within dense *H. kanaloana* meadows using visual and photographic surveys, and collected with spears and traps. Small-bodied fishes (<100 mm standard length) were sampled from the same epifaunal tarpaulin enclosures as the macroinvertebrates. After the tarpaulin was removed, anesthetized fish floating on the surface of the sediment were gently collected and later identified to the lowest taxon possible. Fish specimens were deposited at the Bishop Museum, Honolulu (BPBM).

Data analysis. The density and canopy height of *H. kanaloana* in quadrats from the disturbance experiment were analyzed with a two-way repeated measures ANOVA using Sigmaplot® 11.0 (Systat Software, San Jose, CA). The assumptions of homogeneity and normality were tested using a Shapiro-Wilk test and Levene Median test, respectively. The canopy height data were square-root transformed to meet the assumption of normality. A Tukey pairwise multiple comparison test was used to detect significant differences between treatments and days when the two-way ANOVA was significant at the $\alpha = 0.05$ level. Observational notes and photographs from the anchor scar at each sampling date were reviewed, and qualitatively compared with the results from the clearing experiment.

RESULTS

Disturbance experiment. Significant differences existed between the cut, cleared, and control treatments, among survey dates, and in the interaction between *H. kanaloana* densities and canopy height over the course of the experiment (Table 1). The mean (± SE) densities in the control treatments varied from 64 (± 8) to 84 (± 8) plants per 0.25 m^2 from 1 to 734 days (Fig. 4), and reflected the natural variation seen in *H. kanaloana* densities annually at KBP (Chapter 4). The density of *H. kanaloana* plants from the cut treatment was greater than the control at 41 days, but was not significantly different from the control at 110 days (Fig. 4; Table 2). The densities in the cleared treatment had a longer recovery rate than the cut treatment, with no significant differences between the clear and control treatments within 327 days (Fig. 4; Table 2). However, there were no significant differences between all treatments after 606 days (Fig. 4; Table 2). Results from the canopy height data were similar between cut and cleared treatments, with no significant differences with the control at 327 days (Fig. 4; Table 2). Overall, canopy height recovered more quickly than the density of plants in disturbed treatments.

Anchor scar. The recovery of *H. kanaloana* in the anchor scar followed a similar trajectory as the disturbance experiment. Areas cleared of just *H. kanaloana* axes (parallel anchor scrapes) were not discernible from the surrounding meadow within 41 days. However, recovery in the anchor scar pit, where the entire plant (upright axes and holdfast) were removed, took substantially longer; at 105 days only 8 plants had regrown into the pit where both the entire holdfast and attached upright axes were removed. By 606 days, roughly 90% of the *H. kanaloana* had regrown into the anchor scar pit (Fig. 3).

Complete recovery of the density of plants within the anchor scar was observed at 734 days, at which point there was no discernable difference observed in densities or canopy height as compared to the surrounding meadow.

Bioturbation. Forty-nine bioturbating macroorganisms (including invertebrates, fishes, and a sea turtle) were documented in the *H. kanaloana* meadow. The densities of bioturbating macroinvertebrates (from Fukunaga 2008) were converted to densities per m^{-2} and combined with observations of macroinvertebrates over the course of the study (Table 3). Overall, macroinvertebrates were not observed uprooting entire plants, but instead would form burrows or would forage around existing plants. The most abundant macroinvertebrates were *Pherecardia striata*, *Tripneustes gratilla*, *Ophiocoma dentata*, and *Stylocheilus striata* (Table 3). Each of these species had a shallow (< 1 cm) depth of bioturbation. The majority of *S. striata* were recorded at the 20 m depth in one survey, which included a large aggregation (hundreds of individuals) on the surface of the sand. It is common for this species to aggregate by the thousands at certain times of the year, forming long chains up to 10 m long (Hoover 1998).

Although their densities were low, the macroinvertebrates causing the largest disturbance to the sediment included the large-bodied *Lysiosquillina maculata*, *Alpheus rapax*, and *Cassis cornuta*. The burrows of *L. maculata* were ~ 5 cm in diameter, and penetrated to ~ 15 cm into the sediment. *A. rapax* and their symbiotic goby partner (*Psilogobius mainlandi*) were more common in sandy patches just outside of the *H. kanaloana* meadows but, when present inside the meadows, were often observed digging burrows and moving sediments. *C. cornuta* was found buried in the sediment with only its horn

tips protruding, and could be seen leaving trails in the sand around the edges of the meadows. The night dive did not reveal any additional bioturbating macroinvertebrates, although 10 unidentified shrimp supposedly of the same species were observed inside the meadows.

Fish data from surveys, collections, and observations of fishes from 10 to 30 m depths were combined (Table 4). Bioturbating fish that created the largest disturbance by residing or burrowing in the sediment included *Cymolutes lecluse, Cymolutes praetextatus*, *Psilogobius mainlandi* (although the burrow was created by the shrimp *A. rapax*), *Bothus pantherinus*, and *Dasyatis brevis* (Table 4). Cylindrical excavation pits from *D. brevis* up to 1 m wide x 25 cm in depth were observed, with uprooted *Halimeda* plants surrounding the pit edges.

Two Hawksbill sea turtles (*Eretmochelys imbricata*) were observed foraging for *Pherecardia striata* in the sediment, and removing large mouthfuls of sediment from inside the *H. kanaloana* meadow.

DISCUSSION

H. kanaloana meadows in Hawai‘i are one of the most abundant communities in soft sediment environments in the Maui Nui Island complex (Kahng and Kelley 2007), and are an important element of the tropical reef ecosystem (Chapter 4). Understanding their resilience to both natural and anthropogenic disturbance are key components of management. Although dense and fast-growing (Chapter 4), we found that *H. kanaloana* takes up to two years to recover from sediment disturbances similar to those caused by small recreational anchors that remove the entire plant. Areas in *H. kanaloana* meadows

that experience repeated anchoring or have larger areas of removal (such as from cruise ship anchoring, vessel groundings, or the installation of deep sea cables) will likely require longer periods to recover. For management purposes, the use of fixed moorings in areas that experience repeated anchoring would likely be beneficial in protecting the integrity of *H. kanaloana* meadows and the novel species found in this ecosystem.

The recovery time of *H. kanaloana* meadows to disturbances mimicking anchor scar damage generally took longer than soft sediment disturbances described in other macroalgal assemblages. Instead, the rate of recovery was similar to some species of monotypic seagrass meadows (Table 5). The 10 cm excavations in the macroalgal understory by Hammerstrom et al. (2007) are most similar in excavation depth to the cleared *H. kanaloana* treatment, but took less than 1 year to recover, as compared to ~20 months for our cleared treatment and about two years for the anchor scar. Surprisingly, *H. incrassata* (which is similar morphologically to *H. kanaloana*) at 20 m depths also has a much quicker recovery rate than *H. kanaloana* (Williams 1988). Kenworthy et al. (2002) determined recovery rates to disturbances in three species of monotypic seagrass meadows. The monotypic assemblages of pioneering and opportunistic seagrass species (*Syringodium filiforme* and *Halodule wrightii*) are most similar to the recovery rates of *H. kanaloana*, while the dominant, climax species (*Thalassia testudinium*), requires substantially longer times to recover (Kenworthy et al. 2002). *H. kanaloana* may be considered an opportunistic species given its fast growth rate, high turnover, and ability to vegetatively propogate, but it's also important to consider its low rate of sexual reproduction and perennial nature, (Chapter 4) which appears to influence its slower recovery as compared to other macroalgae and climax seagrass vegetation.

Observations of the anchor scar created at 27 m depth indicated that *H. kanaloana* required a slightly longer time period to recover, but this was not surprising given its larger size and excavation depth as compared to the treatments in the disturbance experiment. Manipulative experiments using a much larger sample size of anchor scars and different excavation depths would be beneficial in examining recovery dynamics in more detail. However, it is interesting to note that the phenology of recovery between the anchor scar and disturbance experiment was similar, with scraped/cut plants recovering more quickly than removed/cleared plants. The quicker recovery of the scraped/cut plants is likely a function of the importance of the extensive rhizoidal holdfast system of *H. kanaloana* and its ability to regrow from just a few basal segments. A solid, deeply anchored root-rhizome or rhizoid system, combined with a flexible or modular above-ground structure, is an advantageous characteristic to resist perturbation by hurricanes or storms (Cruz-Palacios and van Tussenbroek 2005), and would also aid in recovery from other disturbances. *Udotea flabellum*, another rhizophytic macroalga, recovers more quickly from simulated anchor damage as compared to other macroalgal species without rhizoidal holdfasts (Creed and Filho 1999).

Succession refers to the changes observed in an ecological community following a perturbation that opens up a relatively large space (Connell and Slayter 1977). The cleared space from simulated anchor damage and the anchor scar in this study provided a unique opportunity to observe any possible successional patterns in *H. kanaloana* meadows as a result of disturbance. Although other macroalgal and macrofaunal species were observed growing within the *Halimeda* meadow, we did not observe any change in the species composition or the occurrence of successional species within the cut or

cleared treatments. *Halimeda* was the first to colonize all treatments. In other soft sediment environments, typical successional patterns of recovery from mixed seagrass and macroalgal meadows involve initial colonization by turf-like growth forms, then calcareous species, larger fleshy algae, and seagrasses (Williams 1990, Whitfield et al. 2002). However, where species diversity is low, changes in plant abundance rather than species diversity and composition, may occur following disturbance (Williams 1988). For instance, Williams (1988) reports *Halimeda incrassata* plants buried by storm sediments at a 20 m depth regenerated new upright thalli within one month, and did not observe any change in successional patterns within the *H. incrassata* meadows.

A system is considered stable if it persists despite perturbations, and there will be no succession in such case since there is no change (Connell and Slayter 1977). This study suggests that current *H. kanaloana* meadows are fairly stable communities, but possible changes in species composition, such as from the introduction of invasive species, may change the dynamics of these meadows. Repeated disturbances in *H. kanaloana* meadows, such as repeated anchoring, would likely facilitate the introduction of invasive species by creating large, unvegetated areas in an otherwise densely populated assemblage.

Recovery from anthropogenic sediment disturbances occurs in tandem with recovery from natural, background disturbances, such as macrofaunal activities. *H. kanaloana* meadows contain a diversity of both small and large-bodied bioturbating macroinvertebrates and fishes, but we did not separately measure their impact as compared to the anchor scar experiment. Bioturbating fish likely caused a greater disturbance to the sediment than macroinvertebrates because they appeared to spend

significantly more time in the sediment or created a sizable disturbance when they entered the sediment. Most of the bioturbating fishes were more abundant outside of dense *H. kanaloana* meadows, perhaps because of the difficulty of burrowing in sediments containing a dense *Halimeda* rhizoidal holdfast system. Given that the majority of these bioturbators did not uproot or remove entire plants, their impact as compared to deep anchor scars (similar to the "cleared" treatment) is likely less damaging, with the exception of *D. brevis*. In contrast, Williams et al. (1985) hypothesized that the productivity of species of *Caulerpa* at a 20 m depth in a submarine canyon were likely limited in part by animal-mediated sediment disturbances. Plants experimentally uprooted or buried to simulate the effects of bioturbating macrofauna (conchs, ghost shrimp, hermit crabs, urchins, and rays) had significantly lower stolon elongation, biomass accumulation, and growth rates than controls (Williams et al. 1985). *H. kanaloana* is a calcified, larger plant with a more highly developed and penetrating holdfast system as compared to the species of *Caulerpa*. in the Williams et al. (1985) study, which may make it more resistant to natural sediment disturbances.

The treatments in this study were meant to mimic the physical disturbance incurred by anchor damage, but may also be similar to damage incurred by herbivory. Fish herbivory was rarely observed on *Halimeda* plants over this mostly diurnal study, but the sea urchin *Tripneustes gratilla* was observed occasionally grazing through the basal segments of *Halimeda,* thus removing the entire plant in a manner similar to the "cut" treatment. The surge in *Halimeda* densities from d1 to d41 in the cut treatment could have been overcompensation, in which plants have higher fitness when they are damaged by herbivores compared to related plants that are undamaged (Agrawal 2000).

However, *Halimeda* densities and canopy heights in the cut treatment were similar to controls by 110 and 327 days, respectively, suggesting that the vigorous regrowth by damaged cut plants may be attributable to highly favorable environmental conditions, such as increased light availability from the clearing of the *Halimeda* canopy. Rapid plant regrowth is more likely to have evolved as a strategy to reduce the negative impacts of all types of damage than as a strategy to increase fitness following disturbances similar to herbivory (Belsky et al. 1993).

Conclusions. The results of this study add to our understanding of recovery, succession, and engineering resilience to small disturbances in a deep algal meadow. With only one dominant species forming expansive meadows over a wide depth range, *H. kanaloana* meadows provide the unique opportunity to study ecological processes in a simplified model, as compared to other mixed species, soft-sediment assemblages. The manner of recovery from disturbance in *H. kanaloana* meadows across a wider depth gradient, where light becomes more limiting with increasing depth, would be beneficial to our understanding of recovery in mesophotic soft sediment assemblages.

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TABLES

Table 1. Two-way repeated measures ANOVA results for the *H. kanaloana* disturbance experiment. Treatment levels are cut, cleared and control. Time is the number of days since the start of the experiment. Subject is each 0.25 m^2 quadrat. N = 5 quadrats per treatment. Canopy height data was square-root transformed. *Significant at $p < 0.05$.

Density (plants 0.25 m^{-2})	df	MS	*F*	p
Treatment	2	13032	26.67	<0.001*
Time	6	4645	52.61	<0.001*
Treatment x Time	12	3220	36.47	<0.001*
Subject	12	489		
Residual	72	88		
Canopy height (cm)				
Treatment	2	174124	21.86	<0.001*
Time	6	158202	72.20	<0.001*
Treatment x Time	12	35714	16.30	<0.001*
Subject	12	7965		
Residual	72	2191		

Table 2. Results from Tukey pairwise comparison tests from two-way repeated measures ANOVA (Table 1) for the *H. kanaloana* disturbance experiment. Multiple comparisons are among treatments within each day, but are not among different days. Tests significant at the $\alpha = 0.05$ level. Treatments with the same letter are not significantly different.

	Density (plants 0.25 m^{-2})			Canopy height (cm)		
	Control	Cut	Clear	Control	Cut	Clear
d0	A	A	A	A	A	A
d1	A	B	B	A	B	B
d41	A	B	C	A	B	C
d110	A	A	B	A	B	C
d327	A, B	A	B	A	A	A
d606	A	A	A	A	A	A
d734	A	A	A	A	A	A

Table 3. Invertebrates surveyed in *H. kanaloana* meadows at 20 m and 30 m depths, categorized by depth of bioturbation, if available (≤ 1 cm or > 1 cm sediment depth). "f" = epifaunal survey, "b" = epibenthic survey. Densities <0.01 were infrequently observed outside of epifaunal and epibenthic survey areas. Common names are given, if available. Epifaunal and epibenthic data adapted from Fukunaga (2008).

Phylum	Scientific Name	Common Name	Densities (m^{-2})	≤ 1 cm	> 1 cm
Annelida	*Amphinomidae sp.*	worm	0.06[f]		
	Eunice sp.	worm	0.06[f]		
	Loimia medusa	medusa spaghetti worm	0.02[b]		x
	Pherecardia striata	lined fireworm	0.60[b]	x	
Arthropoda	*Alpheus rapax*	goby shrimp	0.02[b]		x
	Crustacea spp.		0.11[f]		
	Lysiosquillina maculata	giant mantis shrimp	<0.01		x
	Penaeidae sp.1	prawn	0.06[f]		
	Penaeidae sp.2	prawn	0.06[f]		
	Trapezia sp.	crab	0.03[f]		
Echinodermata	*Astropyga radiata*	blue-spotted urchin	0.08[b]	x	
	Echinothrix calamaris	banded urchin	0.18[b]	x	
	Holothuria atra	black sea cucumber	<0.01		x
	Holothuroidae	sea cucumber	0.03[f]		
	Ophiocoma dentata	toothed brittle star	0.28[f]	x	
	Tripneustes gratilla	collector urchin	1.14[b]	x	
Mollusca	*Cassis cornuta*	horned helmet	<0.01		x
	Conidae sp.	cone snail	0.03[f]		
	Melibe pilosa	throw-net nudibranch	0.03[f]	x	
	Mitra mitra	episcopal miter	<0.01		x
	Plakobranchus ocellatus	ringed sap-sucking slug	0.03[f]	x	
	Platydoris formosa	speckled slug	0.03[f]	x	
	Stylocheilus striata	lined sea hare	2.00[b]	x	
	Terebra maculata	marlinspike auger	<0.01		x
Platyhelminthes	*Platyhelminthes spp.*	flatworm	0.06[f]	x	

Table 4. Vertebrates surveyed in *H. kanaloana* meadows from 10 to 30 m depths, categorized by depth of bioturbation, if available (≤ 1 cm or > 1 cm sediment depth). "n" is the total number of individuals surveyed or collected using visual and clove oil surveys. "*" = indicates a likely bioturbator, but not directly observed bioturbating.

Family	Scientific Name	Common Name	n	≤ 1 cm	> 1 cm
Apogonidae	*Foa brachygramma**	weed cardinalfish	5	x	
	Pristiapogon kallopterus	iridescent cardinalfish	1	x	
Balistidae	*Rhinecanthus aculeatus*	lagoon triggerfish	2	x	
Bothidae	*Bothus pantherinus*	panther flounder	4		x
Callionymidae	*Callionymus decoratus*	decorated dragonet	2		x
	Synchiropus spp.	dragonet	10		x
Cheloniidae	*Eretmochelys imbricata*	Hawksbill sea turtle	<0.01		x
Congridae	*Conger cinereus**	mustache conger eel	1		x
Dasyatidae	*Dasyatis brevis*	Hawaiian stingray	<0.01		x
Gobiidae	*Gnatholepis anjerensis*	eyebar goby	20	x	
	Gnatholepis cauerensis	shoulder-spot goby	1	x	
	Psilogobius mainlandi	Hawaiian shrimp goby	2		x
Labridae	*Cheilio inermis**	cigar wrasse	8	x	
	Cymolutes lecluse	Hawaiian knife razorfish	9		x
	Cymolutes praetextatus	knife razorfish	10		x
	Iniistius baldwini	blackside razorfish	1	x	
	Novaculichthys taeniourus	rockmover wrasse	8	x	
	*Oxycheilinus bimaculatus**	twospot wrasse	114	x	
Microdesmidae	*Gunnelichthys curiosus*	curious wormfish	4		x
Pinguipedidae	*Parapercis schauinslandii*	redspotted sandperch	27		x
Scorpaenidae	*Scorpaenopsis diabolis*	devil scorpionfish	1		x
Synodontidae	*Synodus spp.*	lizardfish	4		x
Tetradontidae	*Canthigaster coronata*	crowned toby	2	x	
	Canthigaster jactator	Hawaiian whitespotted toby	23	x	

Table 5. Comparison of macroalgal and seagrass meadow recovery rates from various disturbances in soft sediment environments. "O" = observational studies, "E" = experimental manipulations, "*" = mixed seagrass and macroalgal assemblage. Species in bold are seagrasses; species not in bold are macroalgae. Cut and cleared disturbance factors are consistent with cut and cleared treatments from the *H. kanaloana* disturbance experiment (this study). Depth is the water depth of the study.

Citation	Location	Species	Type of Disturbance	Disturbance Factor	Depth (m)	Recovery Time
Williams et al. (1985)[E]	St. Croix, US Virgin Islands (USVI)	*Caulerpa* spp.	sediment burial	uprooted or buried plants	20	6-7 days
Williams (1988)[O,E]	St. Croix, USVI	*Halimeda incrassata**	Tropical Storm Klaus	sediment burial, cleared plants	20	6 mon
Williams (1988)[O,E]	St. Croix, USVI	***Halophila decipiens****	Tropical Storm Klaus	sediment burial, cleared plants	20	6-8 mon
Creed and Filho (1999)[E]	Abrolhos Marine National Park (AMNP), Brazil	*Udotea flabellum**	simulated anchor damage	cleared plants	2.7	4-5 mon
Creed and Filho (1999)[E]	AMNP, Brazil	Epiphytes on *U. flabellum**	simulated anchor damage	cleared plants	2.7	4 to > 13 mon
Hammerstrom et al. (2007)[E]	Florida Keys National Marine Sanctuary (FKNMS), FL	Macroalgal understory*	manipulative excavations	10, 20, and 40 cm deep excavations	<1.5	1- 5 yrs
Kenworthy et al. (2002)[O,E]	FKNMS, FL	***Thalassia testudinum***	manipulative excavations	cleared plants to ~10-25 cm depth	0.5 - 2	10.5 yrs
Kenworthy et al. (2002)[O,E]	FKNMS, FL	***Syringodium filiforme***	manipulative excavations	cleared plants to ~10-25 cm depth	0.5 - 2	1.5 yrs
Kenworthy et al. (2002)[O,E]	FKNMS, FL	***Halodule wrightii***	manipulative excavations	cleared plants to ~10-25 cm depth	0.5 - 2	1.9 yrs
This study[E]	Maui, Hawai'i	*H. kanaloana*	simulated anchor damage	cut or cleared plants	23	11 to 20 mon
This study[O]	Maui, Hawai'i	*H. kanaloana*	anchor damage	cut and cleared plants	27	up to 2 yrs

FIGURES

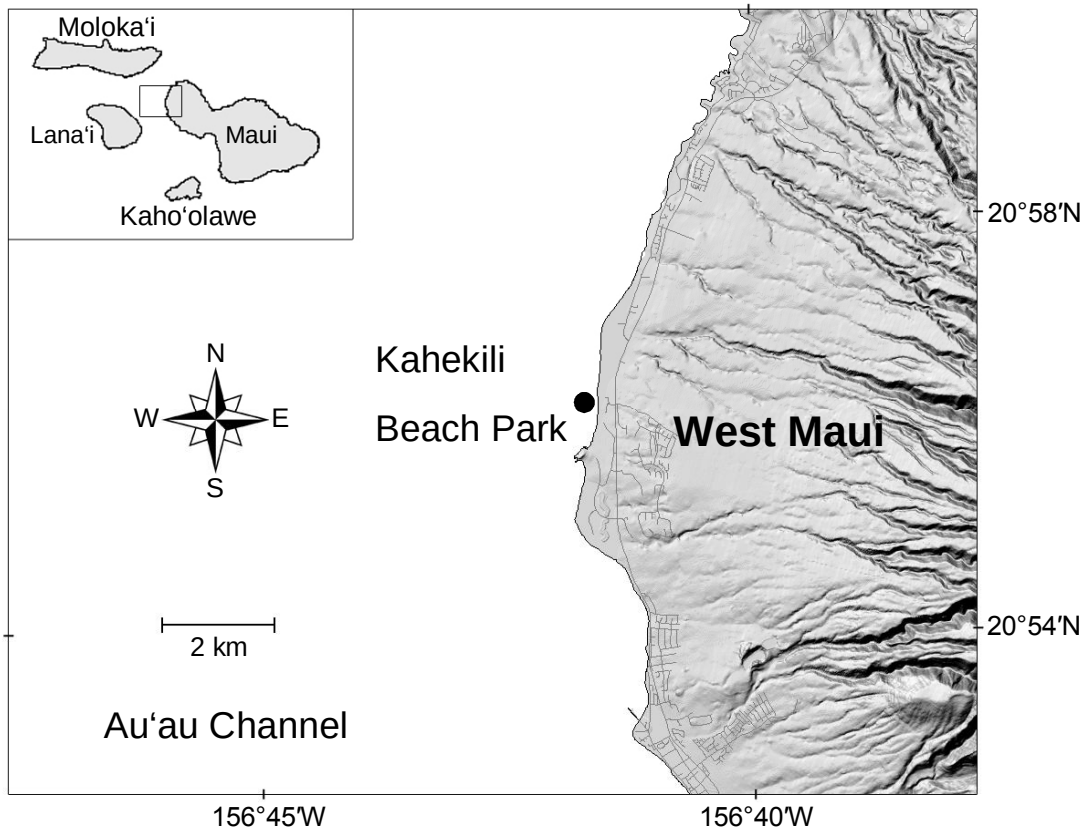

Figure 1. Location of the *H. kanaloana* meadow clearing experiment and the anchor scar in west Maui, Hawai‘i, USA at Kahekili Beach Park. Inset map shows the islands of the Maui Nui complex (Moloka‘i, Lana‘i, Maui, and Kaho‘olawe).

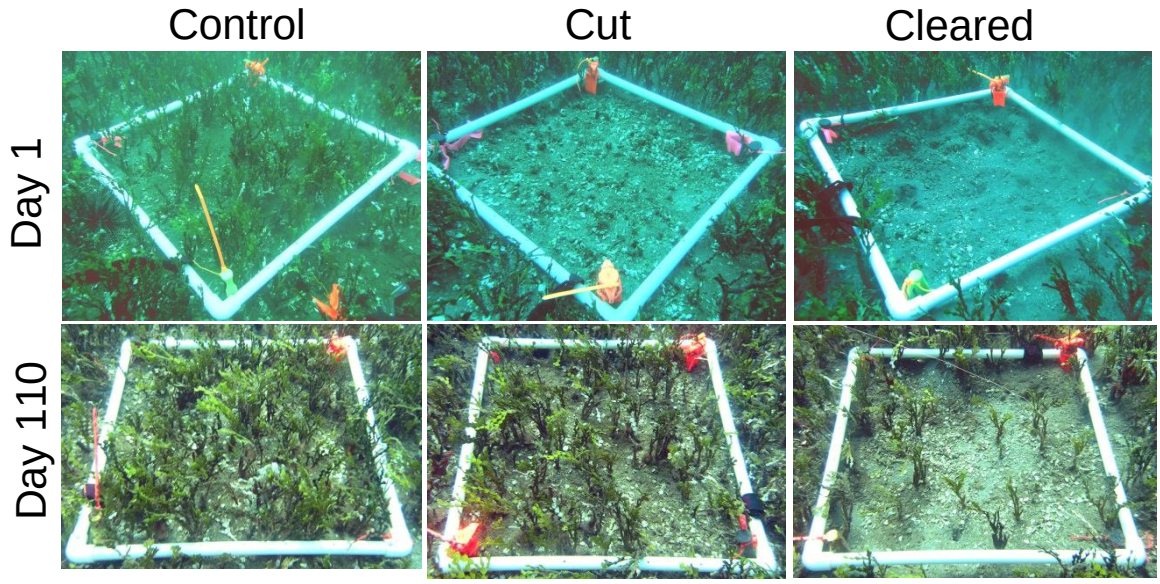

Figure 2. Treatments used in *H. kanaloana* clearing experiments at 23 m depth. Control = no treatment; Cut = upright axes removed, holdfasts intact; Cleared = upright axes and holdfasts removed; Day = days since initiation of treatments.

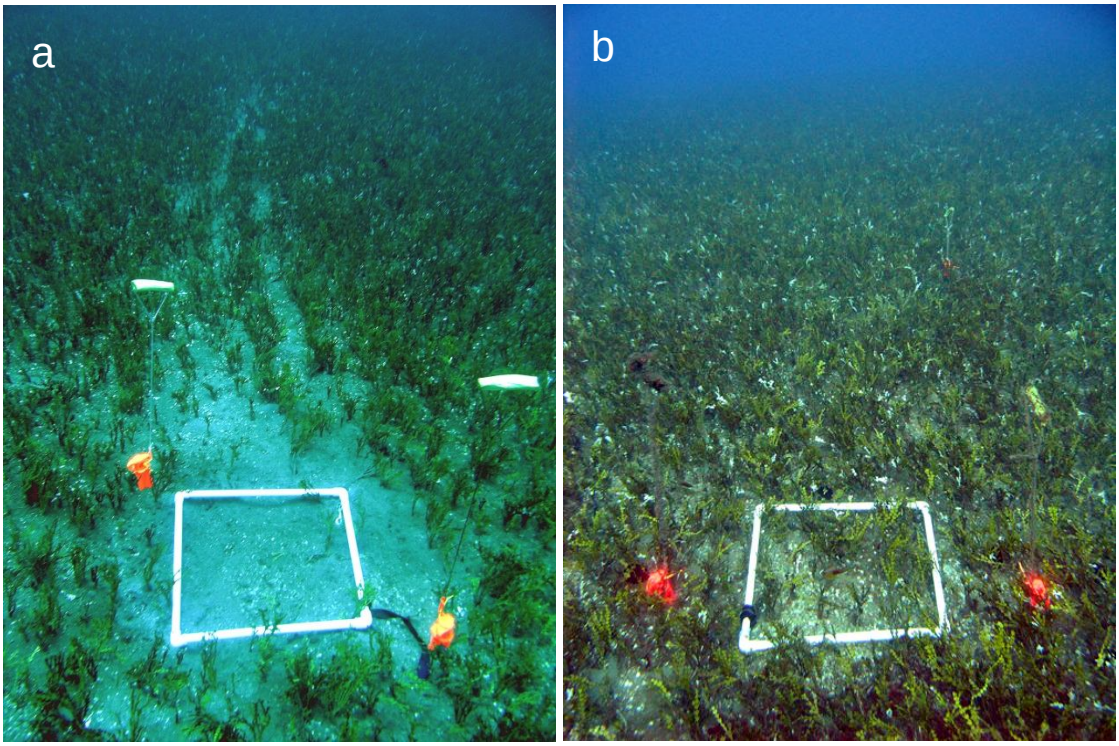

Figure 3. Anchor scar at 27 m depth in a *H. kanaloana* meadow. The white PVC quadrat is 0.25 m^2. (a) One day after the anchor scar was made. Entire *Halimeda* holdfasts were removed from the area inside the quadrat, forming a large pit ~30 cm deep. The lines running through the meadow are areas where the upright axes were scraped off by the anchor. (b) Anchor scar recovery after 606 days.

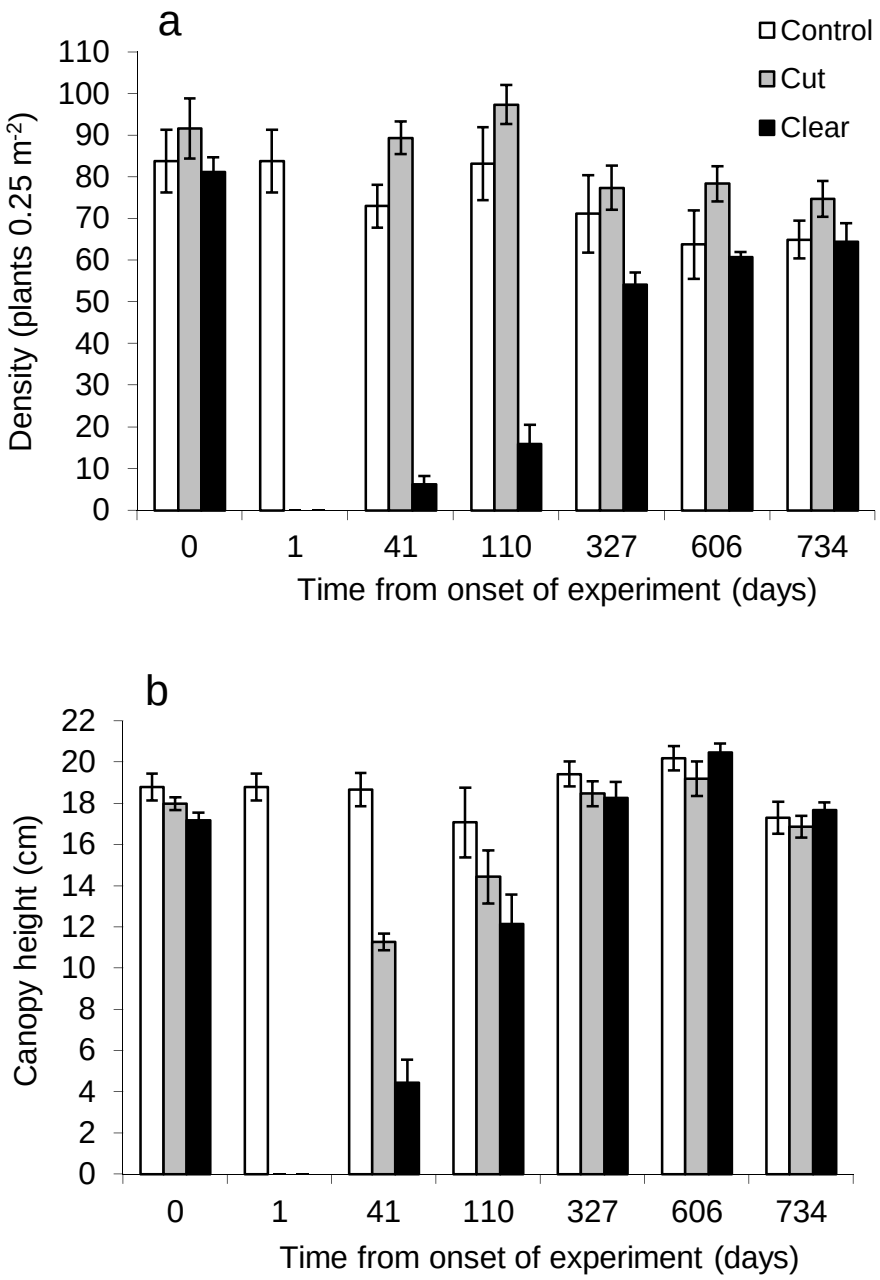

Figure 4. Regrowth in *H. kanaloana* clearing experiments. (a) Density of individual plants (mean ± SE) in each 0.25 m^2 quadrat (n = 5 per treatment). (b) Canopy height (mean ± SE) in each 0.25 m^2 quadrat. See Fig. 2 for explanation of treatments.

CHAPTER V: SYNTHESIS

CONCLUSIONS, SIGNIFICANCE, AND FUTURE RESEARCH

Conclusions and significance

The mesophotic coral ecosystem in the Main Hawaiian Islands (MHI) is an expansive area that hosts a variety of stable apparent climax communities. Macroalgal assemblages inhabit the majority of the mesophotic zone, and also co-occur with corals in the coral-dominated reefs. This calls into question the "coral" in "mesophotic coral ecosystem", suggesting that new terminology is needed when describing this ecosystem. I suggest "mesophotic ecosystem" as the new term to describe this realm, with just a slight re-wording of the definition from Hinderstein et al. (2010):

"Mesophotic ecosystems (MEs) are characterized by the presence of light-dependent communities that are typically found at depths ranging from 30 to 40 m and extending to over 150 m in tropical and subtropical regions. The dominant communities providing structural habitat in the mesophotic zone can be comprised of macroalgae, coral, and sponge species."

The research presented in this dissertation focuses on the distribution and abundance of mesophotic macroalgal in the MHI, with an emphasis on the natural history and recovery dynamics of *Halimeda kanaloana* meadows. A summary of these conclusions follow:

- This dissertation provided a comprehensive survey of mesophotic macroalgal assemblages in the tropics, and revealed a macroalgal-dominated mesophotic system with astounding diversity and spatial complexity.
- While other mesophotic studies typically describe distinct zonation patterns with a few dominant species, we found numerous dominant algal and coral assemblages with overlapping distributions. Twenty-five species of macroalgae were common constituents of the mesophotic assemblage in the MHI with at least 10% cover. Of these, 11 were commonly encountered as a dominant mesophotic assemblage with a distinct flora, and up to 75 - 100% cover ranging from several hundred meters to kilometers square.
- Mesophotic macroalgal assemblages in the MHI contain many new or distinct algae as compared to shallower water, with 45% of the flora only found at depths $\geq$ 40 m.
- The mesophotic flora also contains new records for the Hawaiian flora or new species to science, and will greatly increase rate of endemism for Hawaiian marine algae.
- The mesophotic flora contains genera with tropical, subtropical, and cold-temperate biogeographic affinities.
- The water column above the mesophotic sites sampled was characterized by high clarity and deep light penetration, with a low average attenuation coefficient (-0.042 m^{-1} ± 0.001 SE), although sedimentation from terrigenous sources appeared to decrease macroalgal abundance at a few sites. The sedimentation was from both anthropogenic (run-off from the bombed, denuded island of Kahoʻolawe)

and natural (river run-off) sources. Although the mesophotic zone is generally considered buffered from nearshore processes, it may be impacted by sedimentation when occurring closer to shore.

- The most distinctive changes in diversity and abundance occurred at 80-90 m and 120-130 m depths. This corresponded to ~3% and 0.5% of surface irradiance (SI), respectively, and included depths where large changes in seasonal thermoclines were observed, depending on the site. Thus, irradiance appears to be more important at lower depth limits around 120-130 m depths, while temperature fluctuations may be influential at both 80-90 and 120-130 m depths.
- The discovery of expansive mesophotic populations of the invasive alga *Avrainvillea amadelpha* off O‘ahu stresses the need for understanding the composition mesophotic community if we are to understand the origin and extent of non-indigenous invasive species.
- *Halimeda kanaloana* meadows were the most abundant assemblage in the Maui Nui complex from 40 to 90 m depths. The occurrence of this species from the intertidal to 90 m depths in Maui makes it a model system for studying changes in the biology and physiology of a photosynthetic organism over a broad depth gradient.
- *H. kanaloana* grew rapidly, but growth was highly variable across sampling periods and among depths, with plants at 10 m depths generally growing faster than those at 20 and 30 m depths. Abundance (plants m^{-2}) was also highly variable at all depths and sampling periods, but high with mean densities ranging from 36 to 314 individuals m^{-2}.

- Individuals of *H. kanaloana* were generally long-lived perennials (several years), with low rates of sexual reproduction (less than 1% of the population), and subject to episodic bacterial and cyanobacterial blooms.
- *H. kanaloana* meadows are a significant source of carbonate sediment in the Maui Nui complex. Overall, *H. kanaloana* meadows were highly productive, annually producing 1756, 1694, and 1320 g $CaCO_3$ m^{-2} at 10, 20, and 30 m depths at Kahekili Beach Park, Maui, respectively, and 1883 g $CaCO_3$ m^{-2} when data from its entire depth range (1 to 85 m) in Hawai‘i were considered.
- *H. kanaloana* meadows are important ecologically by providing habitat for fishes, invertebrates, and a foraging ground for Hawksbill sea turtles. Juvenile *Tripneustes gratilla* urchins recruited in these meadows, and two Hawksbill turtles (*Eretmochelys imbricata*) were observed regularly using meadows as a foraging ground. Forty-nine bioturbating macrofauna were surveyed within the meadows, with the most important bioturbators including the giant stomatopod, burrowing shrimp and associated gobies, flounder, wrasses, and rays.
- *H. kanaloana* meadows are targeted for anchoring by small recreational boats, causing anchor scars that uproot *Halimeda* and/or remove the upright plant body. Complete recovery from an experiment mimicking this type of disturbance took ~ 1 ½ years at 20 m depth, while recovery from a near-by anchor scar at 27 m depth took ~2 years.
- Disturbances removing the entire *Halimeda* plant (holdfast and upright portion), will require a longer recovery time than disturbances leaving the holdfast intact.

- The recovery rates of deep *H. kanaloana* meadows to disturbances were longer than those reported for other psammophytic macroalgae, but very similar to opportunistic, monotypic seagrass meadows.
- The use of moorings or avoidance of anchoring in *H. kanaloana* meadows would be beneficial to the health and stability of deep *Halimeda* meadows.

Directions for future research

This dissertation research was both incredibly rewarding and tremendously frustrating. The discovery of each new species or record was met with the excitement of something new, and the exasperation of not being able to identify it. The thrill of discovery starts to wane after the tenth or so new species. It will take a lifetime (or several) to properly identify and describe this breath-taking mesophotic flora, and I suspect there are more new species to be found in the mesophotic zone. In the meantime, below are some suggested directions for future research.

- Are the high abundance of some macroalgal species in the mesophotic region (such as *Distromium flabellatum*, *Caulerpa filicoides*, and *Cladophora* spp.) considered a "bloom", or are they stable constituents of the mesophotic flora? It would be fascinating to monitor these assemblages both seasonally and annually to determine the variability in their distribution and abundance, and determine which factors influence the occurrence of these marine plants.
- How does the benthic productivity of shallow macroalgae compare with similar species in the mesophotic flora? While this type of research has been conducted with mesophotic nongeniculate corallines and *Halimeda* (Littler et al. 1986), it

would be beneficial to consider other abundant assemblages. This would be especially interesting with species belonging to the Ulvales, which are usually considered intertidal and abundant in eutrophic environments.

- What will be the impact of ocean acidification on the distribution and abundance of calcified macroalgal assemblages in the mesophotic zone?
- What types of fish are utilizing macroalgal meadows as habitat, and does this vary with the morphology of the alga (i.e. do flabellate forms like *Udotea* sp. and *Avrainvillea amadelpha* contain a different assemblage of fish than more upright forms like *H. kanaloana*)?
- We found extensive algal meadows of a new genus (*Udotea* sp.) and a highly invasive species (*Avrainvillea amadelpha*) growing in a similar environment in the mesophotic zone. These two species share some similarities morphologically with their size and flabellate form, yet are different in terms of calcification and holdfast morphology. Competition and growth studies between these two species *in situ* would be intriguing, and provide some indication of whether *Avrainvillea* might be displacing *Udotea* sp. Additional questions concerning the invasive traits of *Avrainvillea* and the need to monitor mesophotic populations over time can be found in Peyton (2009).
- How important is herbivory in structuring mesophotic algal populations in *Leptoseris* sp. coral reefs? Manipulative experiments (similar to Brokovich et al. 2010) with *Distromium* and species in the Ulvales would be helpful in determining the importance of bottom-up versus top-down processes in mesophotic coral reefs.

- Long-term oceanographic measurements of temperature and nutrients at 80-90 and 120-130 m depths at multiple sites are also necessary to determine how these factors are affecting the distribution of macroalgal and coral species.
- The majority of studies in the literature report the % surface irradiance (SI) at the lower depth limits of photosynthetic organisms to describe whether light is the limiting factor at this lower depth limit, or compensation point (Kirk 1994). However, there is a wide discrepancy in the literature in how the %SI is calculated, with biologists tending to calculate %SI based upon the actual irradiance *above* the surface of the water (a literal translation), while oceanographers tend to calculate the %SI based on the irradiance just *below* the surface of the water extrapolated from the attenuation coefficient. I have found that irradiance measured both at the surface and just below the surface can be highly variable naturally and due to field conditions, making a reliable measurement of %SI difficult to obtain. I have also found that the %SI extrapolated from the attenuation coefficient to be much lower than what is actually measured in the field, and likely overestimates the %SI at the lower depth limits. I believe that using the actual irradiance levels at depth to assess the lower depth limits of photosynthetic organisms is more biologically relevant, and would eliminate some of the confusion around the %SI measurements in the literature.
- The synthetic oceanographic and ecophysiological modeling used by Graham et al. (2007) to predict and then find mesophotic tropical kelp refugia illustrates the potential to discover other productive areas containing mesophotic macroalgae and corals in the Hawaiian Archipelago. Fundamental physiological

measurements from the dominant macroalgal species and corals are needed to input into this model to determine other areas of potentially high productivity outside of the Maui Nui complex.

- What is the fate of carbonate sediment produced by *H. kanaloana* meadows? We have not observed the large *Halimeda* bioherms described in the mesophotic zone in Australia (Marshall and Davies 1988), India (Rao et al. 1994), or the Caribbean (Hine et al. 1988), suggesting that the sediment produced by *Halimeda* may be transported into shallower or deeper water.
- Why are expansive *H. kanaloana* meadows not found on O‘ahu, despite the availability of gently sloping, soft substrate at the appropriate depths? Existing patches of *H. kanaloana* on O‘ahu may be remnant populations from pre-existing meadows or new introductions within recent years. It’s also possible that *H. kanaloana* meadows exist on O‘ahu, but have not yet been discovered.
- The low occurrence of reproductive individuals observed in the *H. kanaloana* meadows suggests that asexual reproduction, either through fragmentation and/or rhizoid production (Walters and Smith 1994, Walters et al. 2002), may be key to the success of this species in soft sediment environments. What is the degree of clonality in these meadows, and how much connectivity exists from shallow to deep populations? Population studies using molecular analyses would be needed to answer these questions in more detail.

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