ASPECTS OF ECOLOGY AND ALGAL PHYSIOLOGY IN HAWAII'S ROCKY INTERTIDAL ZONES

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ABSTRACT

Intertidal zones are vulnerable to anthropogenic impacts that result from human populations concentrated near coastal waters and while the impact on temperate rocky intertidal systems is much studied, comparatively less is known about these habitats in the tropics. To bolster knowledge and facilitate conservation efforts this dissertation investigates aspects of ecology and algal physiology in Hawaii's rocky intertidal zone. I describe an intertidal zone with a flora and fauna well suited for a tropical environment. Assemblages of tidepool fishes and benthic organisms vary among sites and are vertically structured across narrow shores; consistent with the hypothesis that abiotic or biotic factors vary. A survey of nitrogen concentration and isotopic composition in two species of algae collected in nearshore waters along southwest O'ahu provides evidence that much of this algal community is supported by land-based nutrients. In addition, I use infrared thermography to examine thermal ecology on a tidally driven shore. I found that temperature varies over fine-spatial scales and changes rapidly with tides and waves and organisms either 1) associate with cooler habitats 2) tolerate hotter surroundings or 3) have characteristics allowing them to remain cooler than their surroundings. To examine the affect of temperature on physiology, thermal tolerances of 9 species of reef algae were tested in the laboratory. These species are robust to short doses of heated seawater; maintaining rates of photosynthesis up to 35°C. Results from a photosynthetic survey conducted with pulse amplitude fluoremetry documents an intertidal algal community that is high-light adjusted with the ability to respond to daily fluctuations in supraoptimal irradiances. *In situ* measurements and manipulations reveal that microtidal fluctuations (± 1 m) combined with early morning timing of low tide, limits exposure to intense solar radiation and temperature, and facilitates the physiological performance of common reef algae. This is in contrast to temperate shores where algae experience reduced rates of photosynthesis from extended periods of aerial exposure. This dissertation advances methods in intertidal ecology, provides a baseline on which to measure future change, and has important implications for predicting the impacts of ocean warming and eutrophication on the eco-physiology of tropical reef algae.

TABLE OF CONTENTS

LIST OF TABLES

Page

II. Spatial and temporal patterns of rocky intertidal communities along Hawaii's main islands: a validation of a citizen scientist monitoring program

III. Spatial and vertical patterns in the tidepool fish assemblage on the island of O‘ahu

IV. δ^{15}N values and nitrogen content of reef algae reveal terrestrial derived nitrogen sources that vary over a small landscape scale at ‘Ewa Beach (O‘ahu, Hawai‘i)

V. Thermal ecology on an exposed algal reef- infrared imagery a rapid tool to survey temperature at local spatial scales

VI. Temperature tolerance of nine tropical marine macroalgae

VII. Pulse amplitude fluorescence is used to survey the light harvesting characteristics of diverse reef algae with varying functional forms

VIII. *In situ* measures of stress and performance for tropical reef algae in Hawaii's micro-tidal zones

LIST OF FIGURES

Page

II. Spatial and temporal patterns of rocky intertidal communities along Hawaii's main islands: a validation of a citizen scientist monitoring program

III. Spatial and vertical patterns in the tidepool fish assemblage on the island of O'ahu

IV. δ^{15}N values and nitrogen content of reef algae reveal terrestrial derived nitrogen sources that vary over a small landscape scale at 'Ewa Beach (O'ahu, Hawai'i)

V. Thermal ecology on an exposed algal reef- infrared imagery a rapid tool to survey temperature at local spatial scales

VI. Temperature tolerance of nine tropical marine macroalgae

VII. Pulse amplitude fluorescence is used to survey the light harvesting characteristics of diverse reef algae with varying functional forms

VIII. *In situ* measures of stress and performance for tropical reef algae in Hawaii's micro-tidal zones

Chapter I. Introduction and Background

Introduction to rocky intertidal zones

Rocky Intertidal Zone Model Habitat

Rocky intertidal zones are model habitats to investigate the ecological and physiological implications of stress. Marine organisms that dwell in this habitat are exposed to both terrestrial and marine like conditions with the changing tides. When the tide recedes organisms must cope with increased solar radiation and irradiances, temperature extremes, desiccation, and sporadic inputs of freshwater but when the tide covers the shore, the properties of water acts as a buffer and the surrounding conditions become more consistent. Although tidepools provide some relief from terrestrial like conditions, these shallow water microcosms are also subject to fluctuations in salinity, dissolved oxygen, temperatures, and solar radiation. Thus the ebb and flow of tides results in gradient of terrestrial to marine like conditions across the shore. Striking patterns of species abundance often correlate to these observed gradients. For these reasons intertidal habitats and their organisms have long been of interest to eco-physiologists (Benson 2002; Denny and Gaines 2007).

Physiological Stress in Intertidal Zones

Stress can be defined as external constraints limiting the resource acquisition, growth, or reproduction of organisms (Davison and Pearson 1996). Physiological stress can be further broken down into limitation or disruptive stress. Limitation stress occurs when an organism is limited from a resource while disruptive causes direct damage (Davison and Pearson 1996). Intertidal organisms experience both types of physiological stress. For instance marine algae which rely on water for nutrients and carbon uptake experience limitation stress during low tides and can experience disruptive stress as cellular damage from high temperatures (Davison and Pearson 1996).

Species can vary in their tolerances to stress and in general intertidal species are more robust to fluctuations than subtidal species (Davison and Pearson 1996). Stress is also thought to vary across the shore. Because of the fluctuations in tide the higher elevations are thought to experience more extreme conditions (Harley 2007). Since the ground breaking work of Connell (1961), species upper tidal distributions are known to be controlled by physiological tolerance to harsh conditions. Organisms that occur at high tidal elevations often have adaptations or characteristics that allow them to prevent desiccation stress and tolerate higher temperatures and increased solar radiation (Schonbeck and Norton 1978; Zander et al. 1999; Harley 2007). Mobility will influence the ability to cope with physiological stress during low tides. Benthic organisms are tied to the substratum and have to tolerate their surrounding conditions while crabs, fishes, and other mobile organisms can seek refuge (Huey et al. 2002).

Abiotic factors that shape physiological stress in intertidal zones

Tides and waves influence stress

Tides are driven by the competing pull of the centrifugal force of the earth and gravitational attraction of the moon. Thus the range of the tides or the magnitude in difference in height between the high and low tide varies with moon phases and time of year. Also, the range will vary from one geographic location to another (Denny and Paine 1998; Denny 2007). Tidal ranges can be broken into microtidal (0.0-0.2 m), mesotidal (2.0-4.0 m), macrotidal (4.0-6.0 m), megatidal (>6.0 m) (Bird 2006). There is no global geographic pattern associated with tidal ranges. The surrounding local ocean basin (e.g. coastal shelves or embayments) can influence tidal exchange. The tidal ebb and flow varies not only in magnitude with geographic location but also in frequency. Tides can occur diurnally (one cycle per day), semi-diurnally (two cycles per day), mixed semi-diurnally (two cycles per day) (Denny 2007).

Tidal frequency, magnitude, and timing will influence the duration and extent of exposure and the conditions which organisms experience (Stephenson and Stephenson 1949; Helmuth and Hoffmann 2001; Helmuth et al. 2002; Tomanek and Helmuth 2002; Williams and Dethier 2005; Helmuth et al. 2006). Semi-diurnal tides are likely more stressful as they expose organisms to terrestrial like conditions 2x a day. A larger tidal magnitude will influence the length of the shore exposed to air. In addition, larger tidal differences usually result in longer aerial exposure (Lewis 1964). On temperate shores the timing of low tide effect the temperatures (Schneider and Helmuth 2007) and solar radiation (Hanelt et al. 1993) organisms experience. For instance, tides in southern

California occur in early morning hours and thus have smaller fluctuations in temperature than on Washington and Oregon shores where low tides occur more frequently later in the afternoon (Helmuth and Hoffmann 2001; Schneider and Helmuth 2007). Similarly, low tides that occur in colder months can lead to freezing stress (Davison and Pearson 1996) while tides that occur in summer will lend to heat stress (Somero 2007).

Waves act to modify the duration of aerial exposure and can alter temperatures and irradiances experienced by organisms and add to their mechanical stress (Stephenson and Stephenson 1949; Lewis 1964; Hurd 2000; Helmuth et al. 2002; Hepburn et al. 2007; Schneider and Helmuth 2007). The re-wetting action of waves can relieve desiccation stress and cool, hot shores (Hurd 2000; Helmuth et al. 2002). At temperate latitudes, exposed shores which receive more wave activity are cooler than protected shores (Helmuth et al. 2002). Waves change the wavelength and intensity of light that algae experience by scattering and altering water depth (Cummings and Johnsen 2007). Also waves create more force per unit area on organisms which can rip benthic organisms off the shore or cause direct physical damage (Gaylord et al. 1994). In addition, the compound effect of mechanical stress on already physiological stressed organisms can increase the chances of dislodgement (Denny 1985). Waves are less predictable than tides and the force of the waves and the distance of wave run-up and splash depend upon the topography of the shore, wave height and length (Denny 1985; Davenport and Davenport 2005).

Temperature in intertidal habitats

Because organisms in the intertidal zone are ectotherms, water temperature and air temperature impact their physiology and ecology (Somero 2002; Somero 2007). Temperature can drive important ecological functions such as metabolism and feeding (Somero 2002; Somero 2007). As temperature increases above an organism's thermal limit, proteins loose function and the effects of high temperature can become lethal. For these reasons strong correlations occur between distribution and temperature and occur temporally with temperature changes and organism abundance (Schneider and Helmuth 2007; Somero 2007).

On low tides solar radiation, wind speed, humidity, cloud cover, and angle of the shore to the sun can influence the temperatures an organism experiences. However, air temperature does not necessarily reflect tissue temperature. Some species are known to be 20 °C hotter than their surroundings. Also a wetted organism can have a body temperature lower than air temperature because of the cooling properties of evaporation. However, when submersed an organism's body temperature is similar to the surrounding water (Helmuth 1998; Helmuth 2002; Somero 2002; Schneider and Helmuth 2007; Somero 2007).

In addition to factors that can control climate at the air-water interface, body temperature of an emersed organism is influenced by microhabitat, coloration, physical material property, and behavioral response. Objects and organisms have different specific heat capacities and thus heat differently when exposed to similar air temperatures. Rocks have a low specific heat capacity and heat more readily above air temperature. Benthic organisms, in a manner similar to rocks, conduct heat from the air and surfaces based upon their morphology and material properties (Helmuth 1998; Helmuth 2002; Somero 2002; Schneider and Helmuth 2007; Somero 2007). For example highly dissected thalli heats differently than a blade form (Hay 1981; Bell 1995). Coloration also influences heat capacity. Light colorations reflect solar radiation while dark colorations have the opposite effect. Some microhabitats are shaded or more wetted and remain cooler. For example the underside of rocks can be 14° C cooler than surfaces exposed to the sun (Schneider and Helmuth 2007). In tropical habitats organisms will often compete for these cooler microhabitats (Wethey 1984). Behavioral responses such as avoidance of thermally stressful habitats, aggregations, and retention of water can limit heating (Vermeij 1971; Vermeji 1973). As an example, limpets are known to lift from hot surfaces to remain cool (Vermeij 1971; Vermeji 1973).

Desiccation in the Intertidal

Because organisms that occur in the intertidal have marine ancestry, they experience physiological stress during periods of emersion as water evaporates and cells de-hydrate. Water loss during emersion happens quickly for most marine organisms. This desiccation stress results in decreased metabolism, cell damage, and loss of function and ultimately death. Water loss is increased during periods of activity and under climates that increase rates of evaporation. High or freezing temperatures, low humidity, increased winds, little wave activity, and longer durations of aerial exposure under intense solar radiation will increase desiccation stress (Hand and Menze 2007).

Organisms have different responses to prevent desiccation. In general during emersion metabolic function is reduced or ceases with exposure. Many invertebrates will use anaerobic energy production and cease or limit movement at low tides. Sessile animals reduce surface area exposed to water loss and some sea anemones and sea cucumbers retain water in their body cavity. Snails will move to cooler wetter areas and clamp to the substrate to prevent water loss. Under high stress snails will enter into estivation by pulling their body into shells and cementing mucus along the edge of the aperature and substrate. Fishes can avoid desiccation by moving to wetter microhabitats such as cracks or crevices, or under algal canopies. Only a few amphibious fishes emerge voluntarily from tidepools and for short periods of time. These fish species and those that live higher on the shore have aerial gas exchange mechanisms. Algae increase intracellular osmolytes to prevent water loss (Hand and Menze 2007).

Light in the intertidal habitats

In intertidal habitats, light is important for vision and photosynthesis. The electromagnetic spectrum that stimulates vision and pigments ranges from 300 to 700 nanometers in air. Light underwater is strongly attenuated with depth, dissolved organic matter and suspended solids and light can be filtered through canopies of algae (Cummings and Johnsen 2007).

On low tides organisms experience spectral irradiances similar to terrestrial environments but on high tides the light passes through water which alters the intensity and wavelengths. Water molecules absorb light in the blue-violet and red wavelengths, thus at greater depths the green wavelengths are more prevalent (500-550 nm). Dissolved organic matter and suspended solids have a similar affect on light wavelength as depth. Algae have to cope with this changing light field to optimize photosynthesis while fishes with vision will need to find food, mates and communicate under these fluctuating light conditions (Cummings and Johnsen 2007).

Solar ultra-violet radiation (UV) can have damaging effects on intertidal organisms. Because of its high energy UV damages DNA, protein, and lipids. Nucleotide bases and aromatic amino acids absorb UV and in the process loose structure and function. Therefore UV can damage the photosynthetic apparatus directly or by the formation of highly reactive oxygen radicals, cause skin lesions, kill urchin larvae and damage vision (Shick 2007).

Most UV is absorbed by the ozone and stratospheric oxygen however, UV-A (320-400 nm) and UV-B (280-320 nm) reach intertidal habitats. On high tide, the clarity of water will influence the attenuation of UV light that reaches marine habitats. UV radiation also varies with geographic location due in part to the dissolved organic molecules. UV-A penetrates water deeper than UV-B due to its higher wavelength. Thus, in clear waters with little dissolved organic matter UV can penetrate to several tens of meters (Shick 2007).

Intertidal organisms are known to have a variety of mechanism to repair or prevent UV damage. These include thymine dimer repair via photo reactivation, downward movement in the water column or avoidance, nucleotide excision, and some produce “sunscreen”. Shells or egg capsules can act as screen to prevent UV damage but some intertidal organisms use biochemical sunscreens such as melanin and mycrosporine-like amino acids (MAA). In algae it has been hypothesized that carotenoids and pholortannins also act as sunscreens (Shick 2007).

MAAs are the most common sunscreen in marine organisms. They are natural products of metabolism in algae and are accumulated by consumers via diet. Shallow water corals and algae accumulate higher concentrations of MAAs than deeper dwelling species. Temperate dwelling tidepool sculpin have MAAs with absorption spectra that vary with tidal elevation and geographic latitude (Shick 2007).

Algal physiology in intertidal zones

Algae & Photosynthesis

Marine macroalgae are photosynthetic organisms that are taxonomically diverse spanning three divisions the Ochrophyta, Rhodophyta, and Chlorophyta. These photosynthetic organisms are important to coastal marine ecosystems as they support food webs and provide habitat. Their rates of photosynthesis and production are influenced by abiotic factors, such as irradiance, nutrients, and temperature. Algae growing in the intertidal are tied to the substratum, making intertidal life challenging for photosynthetic production (Davison et al. 1993; Zimmerman 2007).

The requirements for photosynthesis are specific. Marine “plants” need water, carbon dioxide and photosynthetic active radiation to drive light to chemical energy that can be stored. Temperature and pH influence reaction rates whereas nutrients are necessary for pigment production. Photosynthesis can be broken into two parts 1) the light reactions or the harvesting of light energy and 2) the dark reactions. In the dark reactions carbon is fixed and assimilated by the enzyme RuBisCO (Zimmerman 2007).

There are several ways to measure photosynthesis for marine algae (Falkowski and Raven 1997). Measures of O_2 evolution in both light and dark states in an aqueous environment provides a measure of net photosynthesis or overall production. This technique is made possible because O_2 is produced during the splitting of water in the light reactions but consumed during respiration. The drawback of this technique for intertidal algae is samples have to be submersed for extended time to collect the measurements. Other techniques measure CO_2 fixation via tracing isotopically labeled carbon (Falkowski and Raven 1997; Zimmerman 2007). More recent technologies use fluorescence, to gauge the efficiency of photosystem II to convert light energy into the transport of electrons. Fluorescence is an alternate path for excessive energy harvested by chlorophyll *a* (chl a) and it is released proportionally to the amount of energy captured in photosynthesis. Both the oxygen exchange and fluorescence techniques can be used to produce measurements of performance over varying irradiances (Falkowski and Raven 1997; Zimmerman 2007). These measurements and irradiances are used to generate curves that describe the light harvesting ability of algae (White and Critchley 1999; Ralph and Gademann 2005). The advantages of fluorescence techniques are that they can measure performance in intact tissues rapidly in natural settings while other techniques cannot (Longstaff et al. 2002). This is particularly advantageous when examining photosynthesis in intertidal settings where algae are exposed for limited durations to air.

Photosynthetic active irradiance is needed to drive photosynthesis but different light levels occur in a heterogeneous intertidal habitat. Algae which are regularly exposed to terrestrial like conditions are likely to experience excessive irradiances while understory or turf forming species experience low light conditions (Hay 1981; Beach and Smith 1996b; Beach and Smith 1996a; Beach and Smith 1997). Marine algae possess two mechanisms, photoacclimation and chromatic adaptation to cope with dramatic range of light environments (Falkowski and Raven 1997; Smith and Beach 2007).

Algae have evolved different pigments to absorb different wavelengths of light; this is referred to as chromatic adaptation. All macroalgae posses chl *a* for harvesting light energy. However algae from the three divisions have different pigments to harvest specific wavelengths of energy which are then funneled to the chl *a* molecule to drive photosynthesis. In addition to chl *a* the: 1) Rhodophyta have phycobilins and *α*-, *ß*-carotenes, 2) Ochrophyta posses chl *c*, fucoxanthin, and *ß*-carotene 3) Chlorophyta have chl *b*, siphonein, siphonoxanthin, and *ß*-carotene. The phycobilins and fucoxanthin are able to harvest in the green wavelengths that are common at depth or under algal canopies (Falkowski and Raven 1997; Smith and Beach 2007).

Algae can photoacclimate to their environment by altering the pigment ratios and number of photosynthetic reaction centers. Generally high light adapted individuals reduce the light-harvesting pigments and increase the number of photosynthetic reaction centers. The morphology of algae can also alter their abilities to cope with stressful light environments. Some species are parenchymatous and full of pigments to absorb light while other are thin and transmit excessive irradiances. The matt morphology of some algae can allow them to alter their light environment. For instance the intertidal alga *Ahnfeltiopsis concinna* alter pigment ratios in such a way that they have a photo-protective canopy and deeply pigmented light harvesting understory (Falkowski and Raven 1997; Franklin and Forster 1997; Talarico and Maranzana 2000; Smith and Beach 2007).

Intertidal algae experience a wide range of temperatures over the course of tidal ebb and flow which can influence rates of photosynthesis. Generally photosynthesis increases with increasing temperature until an optimum is reached beyond which rates rapidly decline (Berry and Bjorkman 1980). This decline in photosynthetic metabolism can be attributed to changes in the light-use characteristics (Davison 1991; Kubler and Davison 1995), increased rates of photorespiration (Berry and Bjorkman 1980; Raven and Geirder 1988), changes in enzyme activity (Raven and Geirder 1988), or damage to photosystems (PS) (Morris and Kromkamp 2003) and electron transport (Kubler et al. 1991). Damage at PSII and disruption of energy transfer are found to be responsible for short-term declines related to heat stress (Fork et al. 1979; Kubler et al. 1991; Morris and Kromkamp 2003).

Thermal tolerances for photosynthesis vary among species, populations, and life history stages (Luning 1990; Davison 1991). These tolerances are influenced by previous thermal history (acclimation) and confined by genetic adaptation (Lüning 1990; Davison 1991; Kübler et al. 1991). Species or populations with different biogeographic distributions often have different thermal tolerances (Smith and Berry 1986; Eggert and Wiencke 2000) and tolerances have been known to correlate across tidal elevation. Temperature in the local environment can influence photosynthesis. Generally, macroalgae exposed to higher temperatures are found to tolerate or have optimums at higher temperatures (Oates and Murray 1983; Davison 1991; Kubler et al. 1991; Padilla-Gamino and Carpenter 2007; Wernberg et al. 2010). Previous influence of thermal history is apparent in the seasonal shifts in temperature limits observed for a variety of species (Niemeck and Mathieson 1978; Padilla-Gamino and Carpenter 2007).

Exposure duration can influence photosynthesis and growth of marine macroalgae. Exposure to air can cause nutrient and carbon limitation. It can also cause disruptive stress as desiccation (Davison and Pearson 1996; Williams and Dethier 2005).

Macroalgae rely primarily on water for uptake of carbon used in the dark reactions. Dissolved inorganic carbon is plentiful in water and most marine algae extract CO_2 from the pool of bicarbonate. Other marine macroalgae use active transport to uptake bicarbonate into the cell. The diffusion of CO_2 into the cell from water occurs slowly. However CO_2 diffusion into cells in air is rapid. Macroalgae can take up CO_2 in air as long as they remain wetted, but as cells dry they experience desiccation and photosynthesis becomes inhibited (Zimmerman 2007). Thus even the most robust of intertidal species has higher rates of growth and net photosynthesis when submersed (Williams and Dethier 2005).

Macroalgae which unlike plants lack a cuticle are extremely sensitive to water loss and as cells dry photosynthesis decreases. Species can loose up to 90% of water content during a low tide. Therefore, desiccation is considered the environmental driver that controls upper tidal distributions of algae (Beer and Eshel 1983; Smith and Berry 1986; Bell 1995; Matta and Chapman 1995; Beach and Smith 1997).

Species vary in their ability to tolerate and recover photosynthetic rates after desiccation stress. Algae that occur at higher tidal elevations experience longer durations to aerial exposure and are hardier to desiccation stress. These species usually recover rates and have faster recovery than subtidal or lower distributed species (Beer and Eshel 1983; Smith and Berry 1986; Bell 1995; Matta and Chapman 1995; Beach and Smith 1997).

Algal morphology can influence evaporative water loss. Species which minimize surface area have slower rates of water loss. Aggregations of algae can limit water loss and facilitate rates of photosynthesis. Crusts, turf forms or algae that live in cracks or crevices, or under canopies are usually wetter on low tides (Hay 1981; Bell 1995; Dudgeon et al. 1995). The thickness of cells walls can influence water loss and some species increase osmolytes in their cells to help to retain water (Hand and Menze 2007).

Algae uptake nutrients from the water and require nutrients for growth. Under pristine conditions, nitrogen is generally considered limiting for most marine organisms. Nutrients are highly variable in time and space. Waves and tides are important to biomass production as they bring oceanic nutrients to the shore. Algae require active transport to uptake these nutrients and this uptake is subjected to the Michaelis-Menten kinetics (Zimmerman 2007). For some intertidal algal species, rates of nitrogen uptake increase upon submersion to compensate for the limited duration access (Phillips and Hurd 2004). Intertidal zones are also subjected to eutrophication as sewage inputs and other point sources are often focused in coastal areas (Lapointe et al. 1990; Archambault et al. 2001; Lapointe et al. 2004; Pinon-Gimate et al. 2009). For highly competitive species the release of nutrient limitation can lead to nuisance algal blooms (Hughes et al. 1999; Smith and Smith 2003; Teichberg et al. 2008).

Tracing nitrogen inputs into coastal zones

Reef algae are well suited for tracing N in an aquatic environment because many species have simple morphologies that allow uptake of nutrients from the water column and integration into growth or storage. Measurements of algal N content can provide information on the amount and dispersion of nutrients from a source. Laboratory and field studies have tracked changes in N content with enrichment and depletion of ambient dissolved nutrients (Larned 1998; McCook 1999; Schaffelke 1999; Fong et al. 2001; Fong et al. 2003; Lapointe et al. 2004; Lin and Fong 2008; Teichberg et al. 2008). Indeed, Fong et al. (1988) found N content of macroalgae to be more indicative of biologically available nutrient pulses than traditional snapshot water sampling. Yet, there are other studies in tropical environments that have failed to find a correlation between dissolved nutrients and N content (McCook 1999; Schaffelke 1999; Fong et al., 2001). This lack of correlation may be explained by differences in species uptake abilities, a rapid uptake of nutrients, temporal or spatial variable nutrient pulses, or tight coupling between macroalgae and invertebrate physiology (as discussed in Fong et al., 2001). For these reasons, other tools should be coupled with macroalgal N content to monitor nutrient regimes.

The stable isotope ratio of ^{15}N to ^{14}N (expressed as δ-values and referenced to N_2 content in air) of macroalgae is often used to identify and track N sources. The $\delta^{15}N$ values of organisms can be sensitive to N sources and biogeochemical cycling, the species of nitrogen utilized and the extent of utilization of dissolved nitrogen (Peterson and Fry 1987; Kendall 1998). As atmospheric nitrogen (N_2) is fixed and changed to ammonium (NH_4^+ , ammonification) and converted under anaerobic conditions from nitrite to nitrate (NO_3^- , nitrification) by bacteria the N isotopic composition is altered via fractionation, or the differential incorporation of ^{15}N to ^{14}N. Atmospheric fixation has minimal isotopic fractionation thus N_2 fixing bacteria have values of -4 to +4.0 $^0/_{00}$ (Owens 1987; Macko and Ostrom 1994). Natural fertilizer and waste from sewage treatment with increased bacterial processing often have $\delta^{15}N$ values of +6 to +22$^0/_{00}$ (Macko and Ostrom 1994; Kendall 1998). For macroalgae with simple morphologies that utilize nitrate and ammonium incorporation of ^{15}N is nearly complete and thus nitrogen uptake occurs with little fractionation (Peterson and Fry 1987; Gartner et al. 2002; Cohen and Fong 2005) and quickly (within 7 days) (Gartner et al. 2002). Hence, the $\delta^{15}N$ values of macroalgae can reflect the $\delta^{15}N$ values of the source of nitrogen.

Sources, however, can be challenging to identify as the isotopic composition in marine systems is dynamic. Nitrogen not only varies in form but increased N inputs result from upwelling (nitrates), discharge of wastewater (nitrates and ammonium), or natural fertilizer runoff (nitrates and ammonium). Synthetic fertilizers produced by the Haber-Bosch process have δ^{15}N values close to that of atmospheric N_2, which is defined as 0‰ (Macko and Ostrom 1994; Kendall 1998) and are also a possible N source for algae. Mixing of these multiple sources occur under field conditions yet despite these challenges several studies have successfully used bulk δ^{15}N of macroalgae (Sammarco et al. 1999; Schaffelke 1999; Umezawa et al. 2002; Cohen and Fong 2005; Garrison et al. 2007; Lin et al. 2007; Teichberg et al. 2008) to assess land-based N inputs into pristine coastal zones.

Ecological interactions & community patterns in intertidal zones

Structure across the shore

Physiological and ecological factors shape the patterns of species abundance that occur across shores (Doty 1946; Connell 1961; Dayton 1971; Dayton 1975; Bertness 1981; Connell 1985; Harley 2007). With few exceptions, physiological stress is found to control species upper distributional limits while biological interactions are more likely to shape the distribution of species lower on the shore (Harley 2007). However, there are several exceptions to this paradigm and it is often the combination of factors that result in abundance patterns. For instance temperature has been found to control rates of feeding and reproduction (Sanford 1999; Menge 2000a). Earlier in this chapter, we have discussed the environmental factors that can vary across the shore. Biological factors that can contribute to zonation patterns include: predator-prey interactions (Paine 1974; Paine 1995), competition (Connell 1961; Connell 1985), and facilitation (Menge 2000a; Lilley and Schiel 2006; Schiel 2006) (discussed by Bird 2006)

Community structure among shores

Patterns in benthic structure among intertidal communities is often observed to be related to currents and current driven upwelling (Blanchette et al. 2008; Broitman et al. 2008). This is not surprising given that oceanographic conditions are responsible for the delivery of food, nutrients, and propagules to shores. However, distribution and abundance patterns are complex as species vary in their physiological requirements, recruitment patterns (Sutherland 1990; Kendrick and Walker 1994; Menge 2000a; Hutchinson and Williams 2001; Santelices et al. 2003; Walters et al. 2003; Griffiths et al. 2006; Broitman et al. 2008), and ability to colonize and disperse (Kinlan et al. 2005). The topic of overarching community drivers is fiercely debated and often researchers are unable to discern which factors are more important in contributing to community structure, bottom-up or top-down interactions are often cited (Menge 2000b; Menge et al. 2002).

In general, factors such as, patchiness of habitat, dispersal abilities and ocean transport, wave exposure (Bustamante and Branch 1996; Franklin and Forster 1997), and productivity (Menge et al. 1997) can explain patterns in communities across large geographic scales while physical and biological conditions such as predation (Bertness 1981; Menge and Lubchenco 1981), competition (Connell 1961), wave action (Bustamante and Branch 1996), and facilitation (Menge 2000a) influence structure at local scales (see Bird 2006 for in depth discussion).

Describing patterns in abundance and distributions of species is important for discerning between natural or human influenced changes in marine environments. The nearshore coastal environment is particularly vulnerable to human influence. Human traffic and harvesting are concentrated in these coastal zones and these communities often bear the repercussions of terrestrial alterations. Humans are known to alter community structure by transporting species to new locations (Simberloff et al. 2005), contributing to climate change and ocean acidification (Barry et al. 1995; Sagarin et al. 1999), eutrophication (Smith et al. 1999) and sedimentation (Bothner et al. 2006) of nearshore waters, and by harvesting a variety of marine species (Friedlander and DeMartini 2002).

Ecology & algal physiology on Hawaiian Shores

Environmental Conditions

Hawaii's isolated shores experience unique tidal conditions, and substrate types which could influence ecological patterns and physiological stress. The main islands in the archipelago of Hawaiʻi are separated by a maximum distance of ~ 104 km and currents with relatively static marine salinities and temperatures bath the islands. The islands of Hawai‘i are located in the trade wind belt and seasonally directed winds drive wave height and determine which shores (north, south, east, or west) experience wave swell at different times of the year (Gosline 1965; Abbott 1999; Bird 2006). Tides in Hawai‘i are considered microtidal with amplitude of less than 1 m (Gosline 1965; Abbott 1999; Bird 2006). The combination of microtides and surge limit air exposure for intertidal organisms and the vertical span of the intertidal zone are much reduced in comparison to the extensive vertical span of other well studied intertidal shores (Gosline 1965; Abbott 1999). Nonetheless, at peak low tide the nearshore reef regions are exposed and pools are abundant along Hawaiian shores. Spring low tides occur in summer daylight hours when temperatures are at their peak. Additionally, O‘ahu has shorelines that consist of basalt and carbonate rocks (Gosline 1965; Abbott 1999). Basalt shores are often barren of lush macroalgae unlike rough and porous limestone shores (Abbott 1999). Further, tidal benches can be flat raised reef platforms, gently sloped, or cliff-like while boulder to cobble sized fields are made-up of dead coral heads or basaltic rocks (Kay 1979; Abbott 1999). Thus these types of shores may provide different habitats best suited for the survival of different species.

Hawai‘i has sub-tropical to tropical conditions with a unique marine flora and fauna. Hawaiian species have endemic, tropical, and warm-temperate origins (Gosline 1965; Abbott 1999; Abbott and Huisman 2004). These tropical dwelling species experience some of the highest temperatures and irradiances worldwide (Beach and Smith 1996b). In addition, ultraviolet radiation is 2 xs higher in tropical latitudes than at temperate (Beach and Smith 1996a). Although sea surface temperatures are relatively constant and warm, near shore temperatures can fluctuate with weather, currents, and tides.

Past investigations

Only a handful of investigations have been conducted on the ecology and algal physiology in Hawaii’s intertidal zones. Smith (1992) surveyed algae in select intertidal zones to find 100 species of turf and macroalgae and algal community composition did not differ among islands. McDermid (1988) investigated the abundance of algae in plots through time on O‘ahu. She describes a competitive kaleidoscope where no one species is consistently dominant. Beach and Smith (1997) examined the physiological stress for the intertidal alga *Ahnfeltiopsis concinna* on basalt shores. For this tufted red alga irradiance, temperature, and osmotic stress varied from the canopy to the understory, with warmer temperatures and higher irradiances, and less water content experienced at the canopy which had a greater capacity to recover from emersion stress than the understory. For nearshore fishes, Gosline (1965) based on years of observation describes a diverse community that is vertically structured. More recent efforts have focused on community interactions with benthic invertebrates. Zabin (2005) describes pre-emptive competitive interactions between two introduced barnacles. Furthermore she concludes that variability in recruitment determines the barnacle community for a shore. Also she found native *Siphonaria normalis* preferred to occur in areas free of barnacles. Bird (2006) found the functional morphology of algae and the distribution of invertebrate species to correlate with temperatures on a wave driven shore. Furthermore he concluded that, negative or positive interactions among *Cellana* species depended upon population density and productivity. It is clear from these studies that there are complex interactions that shape the communities in Hawaii’s rocky intertidal zones that we are just beginning to understand.

Importance of investigations

The intertidal zone serves as a natural laboratory for researchers interested in ecology, evolution, and conservation. To predict impacts that should result from ocean warming, ocean acidification, eutrophication, sedimentation, and overharvest researchers only need to look to the intertidal zone. Intertidal species have to cope with daily stressors thus understanding the morphological features or mechanisms to cope with stress will allow us to predict which species should thrive under altered conditions. The gradient of conditions across the shore allows researchers to explore the complexity of ecological interactions under varying biological and physiological stress. Ecological paradigms applicable to both marine and terrestrial ecosystems such as keystone species, competitive exclusion, and facilitation were formulated or developed on research conducted in intertidal zones. In addition, our coastal zones are extremely important to indigenous cultures and the economy via harvest of organisms and tourism industry. To conserve these habitats we must understand the physiological and ecological interactions that shape them. For these reasons, this dissertation investigates the ecology and physiology within little studied intertidal zones of Hawai‘i.

Dissertation aims & scope

Overall the purpose of this dissertation is to describe the community structure in Hawaii’s intertidal zones and investigate if the subtropical to tropical environment with intense solar radiation and temperatures, and oligotrophic waters could influence the physiology of organisms and contribute to observed community patterns. Chapters II and III aim to quantitatively describe the tidepool fishes and benthic intertidal communities that occur in Hawai‘i. Also these chapters intend to examine community patterns among shores and examine the vertical composition of species across the shore which would suggest biological or physiological variability. Reef algae in Hawai‘i evolved under relatively oligotrophic conditions; however, humans have potentially contributed substantial nitrogen to nearshore waters, which could alter the abundance of intertidal species. Thus Chapter IV aims to use $\delta^{15}N$ values and N content of algae to examine the nitrogen sources in an intertidal habitat at ‘Ewa Beach, O‘ahu near and away from storm-drains in an area where the surrounding terrestrial land has been altered via agriculture, cesspools, and construction. Because temperature is an important physiological driver of performance and species in the tropics experience some of the highest temperatures worldwide Chapters V and VI explore thermal ecology in Hawaii’s intertidal zones. Specifically, Chapter V uses infrared imagery to describe temperature fluctuations and organism-thermal habitat associations on a tidal driven shore. Chapter VI examines the temperature tolerances of common intertidal algae in the laboratory. The purposes of Chapter VII and VIII are to examine the performance of algae *in situ* to determine if tropical light environment and microtides are physiologically stressful for intertidal reef algae. The light harvesting characteristics of common intertidal reef algae are measured and photosynthetic performances compared when submersed and emersed *in situ*. Then a series of natural experiments are conducted to identify physiological drivers of performance. These investigations (Chapters II-VIII) are pursued in order to provide a baseline on which to measure and predict human related impacts on the ecology and physiology in Hawaii's intertidal zones.

References

Abbott IA (1999) Marine red algae of the Hawaiian Islands. Bishop Museum Press, Honolulu

Abbott IA, Huisman JM (2004) Marine green and brown algae of the Hawaiian Islands. Bishop Museum Press, Honolulu

Archambault P, Banwell K, Underwood AJ (2001) Temporal variation in the structure of intertidal assemblages following the removal of sewage. Marine Ecology Progress Series 222: 51-62

Barry JP, Baxter CH, Sagarin RD, Gilman S (1995) Climate-related, long-term faunal changes in a California rocky intertidal community. Science 267: 672-675

Beach KS, Smith CM (1996a) Ecophysiology of tropical Rhodophytes. I. Microscale acclimation in pigmentation. Journal of Phycology 32: 701-710

Beach KS, Smith CM (1996b) Ecophysiology of tropical Rhodophytes. II. Microscale acclimation in photosynthesis. Journal of Phycology 32: 710-718

Beach KS, Smith CM (1997) Ecophysiology of a tropical Rhodophyte III. Recovery from emersion stresses in *Ahnfeltiopsis concinna* (J. Ag.) Silva et DeCew. Journal of Experimental Marine Biology and Ecology 221: 151-167

Beer S, Eshel A (1983) Photosynthesis of *Ulva* sp. I. Effects of desiccation when exposed to air. Marine Biology Ecology 70: 91-97

Bell EC (1995) Environmental and morphological influences on thallus temperature and desiccation of the intertidal alga *Mastocarpus papillatus* Kutzing. Journal of Experimental Marine Biology and Ecology 191: 29-55

Benson KR (2002) The study of vertical zonation on rocky intertidal shores-a historical perspective. Intergrative and Comparative Biology 42: 776-779

Berry J, Bjorkman O (1980) Photosynthetic response and adaptation to temperature in higher plants. Annual Review of Plant Physiology 31: 491-543

Bertness MD (1981) Predation, physical stress, and the organization of a tropical rocky intertidal hermit crab community. Ecology 62: 411-425

Bird CE (2006) Aspects of community ecology on wave-exposed rocky Hawaiian coasts. PhD. Botany, Honolulu

Blanchette CA, Miner CM, Raimondi PT, Lohse D, Heady KE, Broitman BR (2008) Biogeographical patterns of rocky intertidal communities along the Pacific coast of North America. Journal of Biogeography 35: 1593-1607

Bothner MH, L. RR, Casso MA, Storlazzi CD, Field ME (2006) Quantity, composition, and source of sediment collected in sediment traps along the fringing coral reef off Molokai, Hawaii. Marine Pollution Bulletin 52: 1034-1047

Broitman BR, Blanchette CA, Menge BA, Lubchenco J, Krenz C, Foley M, Raimondi PT, Lohse D, Gaines SD (2008) Spatial and temporal patterns of invertebrate recruitment along the west coast of the United States. Ecological Monographs 78: 403-421

Bustamante RH, Branch GM (1996) Large scale patterns and trophic structure of southern African rocky shores: the roles of geographic variation and wave exposure. Journal of Biogeography 23: 339-351

Cohen RA, Fong P (2005) Experimental evidence supports the use of δ^{15}N content of the opportunistic green macroalga *Enteromorpha intestinalis* (Chlorophyta) to determine nitrogen sources to estuaries. Journal of Phycology 41: 287-293

Connell JH (1961) The influence of intra-specific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. Ecology 42: 710-723

Connell JH (1985) The consequences of variation in initial settlement vs. post settlement mortality in rocky high intertidal communities. Journal of Experimental Marine Biology and Ecology 93: 11-45

Cummings M, Johnsen S (2007) Effects of light. In: Denny MW, Gaines SD (eds) Encyclopedia of Tidepools & Rocky Shores. University of California Press, Berkeley, pp 325-328

Davenport J, Davenport J (2005) Effects of shore height, wave exposure and geographical distance on thermal niche width of intertidal fauna. Marine Ecology Progress Series 292: 41-50

Davison IR (1991) Environmental effects on algal photosynthesis: temperature. Journal of Phycology 27: 2-8

Davison IR, Johnson LE, Brawley SH (1993) Sublethal stress in the intertidal zone: tidal emersion inhibits photosynthesis and retards development in embryos of the brown alga *Pelvetia fastigiata*. Oecologia 96: 483-492

Davison IR, Pearson GA (1996) Stress tolerance in intertidal seaweeds. Journal of Phycology 32: 197-211

Dayton PK (1971) Competition, disturbance and community organization; the provision and subsequent utilization of space in rocky intertidal community. Ecological Monographs 41: 351-389

Dayton PK (1975) Experimental evaluation of ecological dominance in a rocky intertidal algal community. Ecological Monographs 45: 137-159

Denny MW (1985) Wave forces on intertidal organisms: A case study. Limnology and Oceanography 30: 1171-1187

Denny MW (2007) Tides. In: Denny MW, Gaines SD (eds) Encylopedia of Tidepools & Rocky Shores. University of California Press, Berkeley, pp 588-595

Denny MW, Gaines SD (2007) Encyclopedia of Tidepools & Rocky Shores. University of California Press, Berkeley

Denny MW, Paine RT (1998) Celestial mechancis, sea-level changes, and intertidal ecology. Biological Bulletin 194: 108-115

Doty MS (1946) Critical tide factors that are correlated with the vertical distribution of marine algae and other organisms along the Pacific Coast. Ecology 27: 315-328

Dudgeon SR, Kubler JE, Vadas RL, Davison IR (1995) Physiological responses to environmental variation in intertidal red algae: does thallus morphology matter? Marine Ecology Progress Series 117: 193-206

Eggert A, Wiencke C (2000) Adaptation and acclimation of growth and photosynthesis of five Antarctic red algae to low temperatures. Polar Biology 23: 609-618

Falkowski PG, Raven JA (1997) Aquatic photosynthesis. Blackwell Science, Malden

Fong P, Boyer KE, Kamer K, Boyle KA (2003) Influence of initial tissue nutrient status of tropical marine algae on response to nitrogen and phosphorus additions. Marine Ecology Progress Series 262: 111-123

Fong P, Kamer K, Boyer KE, Boyle KA (2001) Nutrient content of macroalgae with differing morphologies may indicate sources of nutrients for tropical marine systems. Marine Ecology Progress Series 220

Fork DC, Murata N, Saton N (1979) Effect of growth temperature on the lipid and fatty acid composition, and the dependence on temperature of light-induced redox reactions of cytochrome f and of light energy redistribution in the thermophilic blue-green alga *Synechococcus lividus*. Plant Physiology 63: 524-530

Franklin LA, Forster RM (1997) Review. The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. European Journal of Phycology 32: 207-232

Friedlander AM, DeMartini EE (2002) Contrasts in density, size, and biomass of reef fishes between the northwestern and the main Hawaiian Islands: the effects of fishing down apex predators. Marine Ecology Progress Series 230: 253-264

Garrison V, Kroeger K, Fenner D, Craig P (2007) Identifying nutrient sources to three lagoons at Ofu and Olosega, American Samoa using $\delta^{15}N$ of benthic macroalgae. Marine Pollution Bulletin 54: 1813-1838

Gartner A, Lavery P, Smit AJ (2002) Use of $\delta^{15}N$ signatures of different functional forms of macroalgae and filter-feeders to reveal temporal and spatial patterns in sewage dispersal. Marine Ecology Progress Series 235: 63-73

Gaylord B, Blanchette CA, Denny MW (1994) Mechanical consequences of size in wave-swept algae. Ecological Monographs 64: 287-313

Gosline WA (1965) Vertical zonation of inshore fishes in the upper water layers of the Hawaiian Islands. Ecology 46: 823-831

Griffiths SP, Davis AR, West RJ (2006) Role of habitat complexity in structuring temperate rockpool ichthyofaunas. Marine Ecology Progress Series 313: 227-239

Hand SC, Menze MA (2007) Desiccation Stress. In: Denny MW, Gaines SD (eds) Encylopedia of Tidepools & Rocky Shores. University of California Press, Berkeley, pp 173-177

Hanelt D, Huppertz K, Nultsch W (1993) Daily course of photosynthesis and photoinhibition in marine macroalgae investigated in the laboratory and field. Marine Ecology Progress Series 97: 31-37

Harley CD (2007) Zonation. In: Denny MW, Gaines SD (eds) Encyclopedia of Tidepools & Rocky Shores. University of California Press, Berkeley, pp 647-654

Hay ME (1981) The functional morphology of turf-forming seaweeds: persitence in stressful marine habitats. Ecology 62: 739-750

Helmuth B (2002) How do we measure the environment? Linking thermal physiology and ecology through biophysics. Integrative and Comparative Biology 42: 837-845

Helmuth B, Harley CDG, Halpin PM, O'Donnell M, Hofmann GE, Blanchette CA (2002) Climate change and latitudinal patterns of intertidal thermal stress. Science 298: 1015-1019

Helmuth B, Mieszokowska N, Moore P, Hawkins SJ (2006) Living on the edge of two changing worlds: forecasting the responses of rocky intertidal ecosystems to climate change. Annual Review of Ecology Evolution and Systematics 37: 373-404

Helmuth BS, Hoffmann GE (2001) Microhabitats, thermal heterogeneity, and patterns of physiological stress in the rocky intertidal zone. Biological Bulletin 210: 374-384

Helmuth BST (1998) Intertidal mussel microclimates: predicting the body temperature of a sessile invertebrate. Ecological Monographs 68: 51-74

Hepburn CD, Holborow JD, Wing SR, Frew RD, Hurd CL (2007) Exposure to waves enhances the growth rate and nitrogen status of the giant kelp *Macrocystis pyrifera.* Marine Ecology Progress Series 399: 99-108

Huey RB, Carlson M, Crozier L, Frazier M, Hamilton H, Harley CD, Hoang A, Kingsolver JG (2002) Plants versus animals: Do they deal with stress in different ways? Integrative and Comparative Biology 42: 415-423

Hughes T, Szmant AM, Steneck R, Carpenter R, Miller S (1999) Algal Blooms on Coral Reefs: What Are the Causes? Limnology and Oceanography 44: 1583-1586

Hurd C (2000) Water motion, marine macroalgal physiology, and production. Journal of Phycology 36: 453-472

Hutchinson N, Williams GA (2001) Spatio-temporal in recruitment of a seasonal, tropical rocky shore: the importance of local versus non-local processes. Marine Ecology Progress Series 215: 57-68

Kay AE (1979) Hawaiian marine shells, reef and shore fauna of Hawaii. Bishop Museum Press, Honolulu

Kendall C (1998) Tracing Nitrogen Sources and Cycling in Catchments Isotope Tracers in Catchment Hydrology. Elsevier, Amesterdam, pp 519-576

Kendrick GA, Walker DI (1994) Role of recruitment in structuring beds of Sargassum spp. (Phaeophyta) at Rottnest Island, Western Australia. Journal of Phycology 30: 200-208

Kinlan BP, Gaines SD, Lester SE (2005) Propagule dispersal and the scales of marine community process. Diversity and Distributions 11: 139-148

Kubler JE, Davison IR (1995) Thermal acclimation of light-use characteristics of *Chondrus crispus* (Rhodophyta). European Journal of Phycology 30: 189-195

Kubler JE, Davison IR, Yarish C (1991) Photosynthetic adaptation to temperature in the red algae *Lomentaria baileyana* and *Lomentaria orcadensis*. British Phycological Journal 26: 9-19

Lapointe BE, Barile PJ, Matzie WR (2004) Anthropogenic nutrient enrichment of seagrass and coral reef communities in the Lower Florida Keys: discrimination of local versus regional nitrogen sources. Journal of Experimental Marine Biology and Ecology 308: 23-58

Lapointe BE, O'Connell JD, Garrett GS (1990) Nutrient couplings between on-site sewage disposal systems, groundwaters, and nearshore surface waters of the Florida Keys. Biogeochemistry 10: 298-307

Larned ST (1998) Nitrogen-versus phosphorus-limited growth and sources of nutrients for coral reef macroalgae. Marine Biology 132: 409-421

Lewis JR (1964) The ecology of rocky shores, London

Lilley SA, Schiel DR (2006) Community effects following the deletion of a habitat-forming alga from rocky marine shores. Oecologia: 672-681

Lin DT, Fong P (2008) Macroalgal bioindicators (growth, tissue N, $\delta^{15}N$) detect nutrient enrichment from shrimp farm effluent entering Opunohu Bay, Moorea, French Polynesia. Marine Pollution Bulletin 56: 245-249

Lin H-J, Wu C-Y, Kao S-J, Kao W-Y, Meng P-J (2007) Mapping anthropogenic nitrogen through point sources in coral reefs using $\delta^{15}N$ in macroalgae. Marine Ecology Progress Series 335: 95-109

Longstaff B, Kildea T, Runcie JW, Cheshire A, Dennison WC, Hurd C, Kana T, Raven JA, Larkum AWD (2002) An *in situ* study of photosynthetic oxygen exchange and electron transport rate in the marine macroalgae *Ulva lactuca* (Chlorophyta). Photosynthesis Research 74: 281-293

Luning K (1990) Seaweeds: their environment, biogeography and ecophysiology. Wiley & Sons, New York

Macko SA, Ostrom NE (1994) Stable Isotopes in Ecology and Environmental Science. Blackwell Scientific Publications, Oxford

Matta JL, Chapman DJ (1995) Effects of light, temperature, and desiccation on the net emersed productivity of the intertidal macroalga *Colpomenia peregrina* Sauv. (Hamel). Journal of Experimental Marine Biology and Ecology 189: 13-27

McCook LJ (1999) Macroalgae, nutrients and phase shifts on coral reefs: scientific issues and management consequences for the Great Barrier Reef. Coral Reefs 18: 357-367

McDermid KJ (1988) Community ecology of some intertidal subtropical algae, and the biology and taxonomy of Hawaiian *Laurencia* (Rhodophyta). Botanical Sciences, Honolulu

Menge B (2000a) Testing the relative importance of positive and negative effects on community structure. TREE 15: 45-46

Menge B, Daley BA, Wheeler PA, Strub T (1997) Rocky intertidal oceanography: an association between community structure and nearshore phytoplankton concentration. Limnology and Oceanography 42: 57-66

Menge B, Olson AM, Dahlhoff EP (2002) Environmental stress, bottom-up effects, and community dynamics: integrating molecular-physiological and ecological approaches. Integrative and Comparative Biology 42: 892-908

Menge BA (2000b) Top-down and bottom-up community regulation in marine rocky intertidal habitats. Journal of Experimental Marine Biology and Ecology 250: 1-2

Menge BA, Lubchenco J (1981) Community organization in temperate and tropical rocky intertidal habitats: prey refuges in relation to consumer prey gradients. Ecological Monographs 51: 429-450

Morris EP, Kromkamp JC (2003) Influence of temperature on the relationship between oxygen and fluorescence based estimates of photosynthetic parameters in a marine benthic diatom (*Cylindrotheca closterium*). European Journal of Phycology 38: 133-142

Niemeck RA, Mathieson AC (1978) Physiological studies of intertidal fucoid algae. Botanica Marina 21: 221-227

Oates BR, Murray SN (1983) Photosynthesis, dark respiration and desiccation resistance of the intertidal seaweeds *Hesperophycus harveyanus* and *Pelvetia fastigiata F. gracilis*. Journal of Phycology 19: 371-380

Owens NPJ (1987) Natural variations in δ^{15}N in the marine environment. Advanced Marine Biology 24: 389-451

Padilla-Gamino JL, Carpenter RC (2007) Thermal ecophysiology of *Laurencia pacifica* and *Laurencia nidifica* (Ceramiales, Rhodophyta) from tropical and warm-temperate regions. Journal of Phycology 43: 686-692

Paine RT (1974) Intertidal community structure: experimental studies on the relationship between a dominant competitor and its principal predator. Oecologia 15: 93-120

Paine RT (1995) A conversation on refining the concept of keystone species. Conservation Biology 9: 962-964

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18: 293-320

Phillips JC, Hurd CL (2004) Kinetics of nitrate, ammonium, and urea uptake by four intertidal seaweeds from New Zealand. Journal of Phycology 40: 534-545

Pinon-Gimate A, Soto-Jimenez MF, Ochoa-Izaguirre MJ, Garcia-Pages E, Paez-Osuna A (2009) Macroalgae blooms and δ^{15}N in subtropical coastal lagoons from the Southeastern Gulf of California: Discrimination among agricultural, shrimp farm and sewage effluents. Marine Pollution Bulletin 58: 1144-1151

Ralph PJ, Gademann R (2005) Rapid light curves: a powerful tool to assess photosynthetic activity. Aquatic Botany 82: 222-237

Raven JA, Geirder RJ (1988) Temperature and algal growth. New Phytologist 110: 441-461

Sagarin RD, Barry JP, Gilman SE, Baxter CH (1999) Climate-related change in an intertidal community over short and long time scales. Ecological Monographs 69: 465-490

Sammarco PW, Risk MJ, Schwarcz HP, Heikoop JM (1999) Cross-continental shelf trends in coral δ^{15}N on the Great Barrier Reef: further consideration of the reef nutrient paradox. Marine Ecology Progress Series 180: 131-138

Sanford E (1999) Regulation of keystone predation by small changes in ocean temperature. Science 283: 2095-2097

Santelices B, Correa JA, Hormazabal M, Flores V (2003) Contact responses between spores and sporelings of different species, karyological phases. Marine Biology 143: 381-392

Schaffelke B (1999) Short-term nutrient pulses as tools to assess responses of coral reef macroalgae to enhanced nutrient availability. Marine Ecology Progress Series 182: 305-310

Schiel DR (2006) Rivets or bolts? When single species count in the function of temperate rocky reef communities. Journal of Experimental Marine Biology and Ecology

Schneider KR, Helmuth B (2007) Patterns of heat and temperature. In: Denny MW, Gaines SD (eds) Encyclopedia of Tidepools & Rocky Shores. University of California Press, Berkeley, pp 263-266

Schonbeck M, Norton TA (1978) Factors controlling the upper limits of fucoid algae on the shore. Journal of Experimental Biology and Ecology 31: 303-313

Shick JM (2007) Ultraviolet stress. In: Denny MW, Gaines SD (eds) Encyclopedia of Tidepools & Rocky Shores. University of California Press, Berkeley, pp 604-609

Simberloff D, Parker IM, Windle PN (2005) Introduced species policy, management, and future research needs. Frontiers in ecology and environments 3: 12-20

Smith CM (1992) Diversity in intertidal habitats: an assessment of the marine algae of select high islands in the Hawaiian archipelago. Pacific Science 46: 466-479

Smith CM, Beach KS (2007) Algal Color. In: Denny MW, Gaines SD (eds) Encyclopedia of Tidepools & Rocky Shores. University of California Press, Berkeley, pp 33-37

Smith CM, Berry JA (1986) Recovery of photosynthesis after exposure of intertidal algae to osmotic and temperature stresses: comparative studies of species with differing distributional limits. Oecologia 70: 6-12

Smith JE, Smith CM (2003) Factors influencing algal blooms on tropical reefs with an emphasis on herbivory, nutrients, and invasive species. PhD dissertation. Department of Botany, Honolulu

Smith VH, Tilman GD, Nekola JC (1999) Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environmental Pollution 100: 179-196

Somero GN (2002) Thermal physiology and vertical zonation of intertidal animals: optima, limits, and cost of living. Integrative and Comparative Biology 42: 780-789

Somero GN (2007) Heat Stress. In: Denny MW, Gaines SD (eds) Encyclopedia of Tidepools & Rocky Shores. University of California Press, Berkeley, pp 266-270

Stephenson TA, Stephenson A (1949) The universal features of zonation between tide-marks on rocky coasts. Journal of Ecology 37: 289-305

Sutherland JP (1990) Recruitment regulates demographic variation in a tropical intertidal barnacle. Ecology 71: 955-972

Talarico L, Maranzana G (2000) Light and adaptive responses in red macroalgae: an overview. Journal of Photochemistry and Photobiology B: Biology: 1-11

Teichberg M, Fox SE, Aguila C, Olsen YS, Valiela I (2008) Macroalgal responses to experimental nutrient enrichment in shallow coastal waters: growth, internal nutrient pools, and isotopic signatures. Marine Ecology Progress Series 368: 117-126

Tomanek L, Helmuth B (2002) Physiological ecology of rocky intertidal organisms: a synergy of concepts. Intergrative and Comparative Biology 47: 771-775

Umezawa Y, Miyajima T, Yamamuro M, Kayanne H, Koike I (2002) Fine-scale mapping of land-derived nitrogen in coral reefs by $\delta^{15}N$ in macroalgae. Limnology and Oceanography 47: 1405-1416

Vermeij GJ (1971) Substratum relationships of some tropical Pacific intertidal gastropods. Marine Biology 10: 315-320

Vermeji GJ (1973) Morphological patterns in high-intertidal gastropods: adaptive strategies and their limitations. Marine Biology 20: 319-346

Walters LJ, Smith CM, Hadfield MG (2003) Recruitment of sessile marine invertebrates on hawaiian macrophytes: do pre-settlement or post-settlement processes keep plants free from fouling? Bulletin of Marine Science 72: 813-839

Wernberg T, Thomsen MS, Tuya F, Kendrick GA, Staehr PA, Toohey BD (2010) Decreasing resilience of kelp beds along a latitudinal temperature gradient: potential implications for a warmer future. Ecology Letters 13: 685-694

Wethey DS (1984) Sun and shade mediate competition in the barnacles *Chthamalus* and *Semibalanus*: A field experiment. Biological Bulletin 167: 176-185

White AJ, Critchley C (1999) Rapid light curves: a new fluorescence method to assess the state of the photosynthetic apparatus. Photosynthesis Research 59: 63-72

Williams SL, Dethier MN (2005) High and dry: variation in net photosynthesis of the intertidal seaweed *Fucus gardneri*. Ecology 86: 2373-2379

Zabin C (2005) Community ecology of the invasive intertidal barnacle *Chthamalus proteus* in Hawai'i. PhD dissertation. Department of Zoology, Honolulu

Zander CD, Nieder J, Martin KLM (1999) Vertical distribution patterns. In: Horn MH, Martin KLM, Chotkowski MA (eds) Intertidal fishes, Life in two worlds. Academic Press, San Diego, pp 26-49

Zimmerman RC (2007) Photosynthesis. In: Denny MW, Gaines SD (eds) Encyclopedia of Tidepools & Rocky Shores. University of California Press, Berkeley, pp 429-435

Chapter II. Spatial and temporal patterns of rocky intertidal communities along Hawaiʻi's main islands: a validation of a citizen scientist monitoring program.

Abstract

Secondary school students quantitatively assessed benthic rocky intertidal communities at 13 sites on Māui, Molokaʻi, Oʻahu, and Hawaiʻi Island between the years 2004-2007. Our goals were 1) to evaluate the quality of data generated by these citizen scientists and 2) to test for differences in invertebrate and macroalgal abundance and composition among sites in order to understand how these tropical communities are organized. In 2007, two independent research teams collected data simultaneously with students at five sites on eight sampling dates. Comparisons of Shannon diversity and Bray-Curtis similarity values computed and simulated from student and researcher collected data revealed that students accurately collect community-level data within the range of researcher biases. Students were, however, likely to misidentify cryptic and rare species. Using student-collected data we found that sites statistically vary and clustered into five groups with 48.2% or greater community similarity. Most sites were included in a cluster that was 55% similar in benthic structure. The introduced macroalga *Acanthophora spicifera* and the brown algal genus *Padina* were major contributors to site similarities. Year-to-year changes in community composition were slight and did not vary as much as the differences among sites. Local scale structure was documented at two sites where molluscs and macroalgae occur in discrete zones across a narrow shore. To our knowledge, this is one of the first descriptions of community level patterns at multiple intertidal habitats along the main islands of Hawaiʻi. These findings can be used as a baseline to better understand ecological processes that shape nearshore tropical habitats as well as provide a protocol to assure future studies with citizen scientists.

Introduction

Systematic monitoring of ecological communities provides a baseline from which to measure human impacts in the long term (Murray et al. 2006) and in the short term allows for early detection and potential eradication of invasive species (Simberloff et al. 2005). In addition, large to mesoscale spatial and temporal community studies have provided much needed ecological information as surveys allow for hypotheses testing and model validation. For example, surveys conducted over 10 to 100s of km over multiple years have found patterns within communities related to global climate change (Barry et al. 1995; Sagarin et al. 1999), recruitment (Broitman et al. 2008), and coastal processes (Blanchette et al. 2008). Furthermore, spatial descriptions of marine communities are needed for adequate design of reserves, as efforts are made to include sites that are representative of, or unique to specific areas (Airame et al. 2003). Intertidal habitats are model systems for examining spatial and temporal structure as physical conditions fluctuate with the tides and abundance and diversity of organisms can vary among and across shores. Also, these habitats are particularly vulnerable to human threats as they lie at the interface between terrestrial and subtidal habitats and thus bear the impact of both terrestrial and marine alterations. For these reasons, meso- to large-scale survey efforts of benthic organisms are being conducted in temperate intertidal areas (Barry et al. 1995; Blanchette et al. 2008; Broitman et al. 2008; Delaney et al. 2008; Nakaoka et al. 2006; Sink et al. 2005). Fewer surveys have been conducted in the tropical intertidal habitats (but see Smith 1992).

Hawaii's nearshore habitats are unique in part because the isolation of the archipelago results in a high number of endemic species. However, these habitats are not insulated from threats. Boat traffic and past interest in aquaculture has resulted in numerous alien species introductions (Eldredge and Smith 2001). Invasions are numerous, with 343 species known to be introduced to Hawaiʻi's waters which include 287 invertebrates and 27 species of macroalgae (Eldredge and Smith 2001). Three species of introduced macroalgae are particularly invasive in shallow water habitats (Smith et al. 2002) and include *Acanthophora spicifiera*, *Gracilaria salicornia,* and *Hypnea musciformis* which are visually abundant in Hawaiʻi's intertidal along with a barnacle *Chthalamus proteus* (Zabin et al. 2007). The origin status of a fourth nuisance alga, *Avrainvillea amaldelpha* is unknown but past records indicate that it was not common in shallow-water habitats. The popularity of Hawaii's shores has attracted human visitation, harvesting of organisms, and development of oceanside resorts. Furthermore, sewage overflows have lead to shallow water macroalgal blooms of nuisance native and introduced species (Stimson et al. 2001; Dailer et al. 2010). However, there have been to our knowledge only few studies that describe both the invertebrate and algal communities for intertidal habitats in Hawaiʻi (Strasburg 1953, Bird 2006, Zabin et al. 2007). These were short-term studies that either did not involve the systematic collection of quantitative data or did not describe the algal community beyond functional level at multiple sites. Thus it is difficult to assess any possible impacts to the community as a whole.

In marine environments, factors such as ocean transport (Blanchette et al. 2008), wave exposure (Bustamante and Branch 1996), and productivity (Menge et al. 1997) can explain patterns in communities across large geographic scales while physical and biological conditions such as predation (Bertness 1981; Menge and Lubchenco 1981), competition (Connell 1961), wave action (Bustamante and Branch 1996), and facilitation (Menge 2000) influence structure at local scales. The main islands in the archipelago of Hawai'i are separated by a maximum distance of ~ 104 km. Current velocity and direction and wave heights are often variable and determined by wind direction (http://www.soest.hawaii.edu/hioos/oceanatlas/).

Hawai'i has a variety of shore types that vary in both composition and profile. Shores can be composed of basalt or limestone substrate and be covered with varying amounts of sand. Tidal benches can be flat raised reef platforms, gently sloped, or cliff-like while boulder- to cobble-sized fields are made up of dead coral heads or basaltic rocks (Abbott 1999; Kay 1979). These variable habitats may suit the survival of different species, resulting in unique communities. Smith (1992) found differences in macroalgal diversity at sites within a single island's intertidal habitat and Zabin et al. (2007) found that intertidal species assemblages at bench sites were more similar to each other than to those found at cobble sites. Other studies on nearshore benthic communities have found that distributions or abundances of benthic organisms can vary with island habitat (Vroom et al. 2010), depth (Littler and Doty 1975), temperature (see Chapter V), and wave exposure (Bird 2006). We might expect similar findings for the benthic intertidal community.

In an effort to bolster ecological information and promote conservation awareness about these intertidal communities, a partnership was formed in Hawai'i between educators and the authors in a monitoring program named Our Project In Hawai'i's Intertidal (OPIHI) after a culturally important limpet, the opihi (*Cellana* spp.) (Baumgartner and Zabin 2006; Baumgartner et al. 2009; Baumgartner and Zabin 2008). OPIHI is a type of citizen science program where trained volunteers perform research related tasks. In our case, the volunteers were secondary school students and were participating as part of their science education. Citizen scientist programs like OPIHI are growing in popularity (see citisci.org) because with volunteers it becomes possible to rapidly survey populations multiple times over large spatial scales (Silvertown 2009). These types of partnerships provide much needed information to scientists and decision-makers and foster a conservation ethic in the general public (Silvertown 2009). Citizen scientist programs have successfully allowed for surveys of birds (Bhattacharhee 2005; McCaffrey 2005), insects (Braschler 2009), subtidal fishes (Pattengill-Semmens and Semmens 2003), and intertidal crabs (Delaney et al. 2008). Well known examples include Aubudon Society's Christmas Bird Count and Reef Check. However, the quality of volunteer generated data is often questioned (Delaney et al. 2008; Silvertown 2009) and if citizen data is not used it can often cause disillusionment, particularly when volunteers are students active in their science education. As part of OPIHI, we partnered with secondary-school students to describe and monitor the benthic intertidal community in Hawai'i to (1) engage this young generation, (2) elucidate ecological patterns, and (3) inform conservation efforts.

The research related goals of this study were twofold; to assess the quality of data generated by OPIHI, and provided that these data are demonstrated to be reliable, describe the benthic intertidal community in Hawai'i. We first specifically asked the question: are students able to identify and describe the abundances of introduced and native species accurately using the OPIHI protocol? We hypothesized that students generate quality community data that is similar to researchers. We examined these data to test three hypotheses: (1) the similarity in invertebrate and macroalgal abundance and composition will vary among sites as would be predicted if biotic or abiotic factors varied among islands and shores and influenced structure; (2) that because physical factors have remained relatively similar during the length of the monitoring program, temporal variation in these communities is limited; (3) that organisms are distributed into discrete bands vertically across shores as would be predicted if localized conditions influenced structure. In addition, we described the presence and abundance of alien species at our sites.

Methods

Site Description

Thirteen rocky intertidal sites located on the islands of Hawai‘i, Māui, Moloka‘i, and O‘ahu were sampled by OPIHI during the years 2004-2007 (Table 2.1, Fig. 2.1). Some sites were sampled multiple times within a year and not every site was sampled each year. Sites were selected to represent a variety of rocky intertidal habitats, but also had to be easily accessible by students. Each site spanned a minimum of 15 m along the coast and was at least 10 m from the top of the littorine zone to the water's edge at mean low water. Five of these sites were haphazardly selected in 2007 for assessment of student-generated data.

Sites can be described as gently sloped basalt or limestone bedrock benches, bedrock surrounded by patches of sand, or boulder- to cobble-sized rocky habitats (Table 2.1). These shores are located on all sides of the islands, and seasonal onshore waves are common at all sites. However, some sites such as Diamond Head have offshore reefs that provide some protection. The Wai‘Opae monitoring site is located within a Marine Life Conservation District where collection of any kind is prohibited. Species richness varies among sites (Zabin 2007).

OPIHI protocol

Students that participated in OPIHI were in secondary grades 6 -12 and enrolled in a science course at both traditional and charter public schools (Table 2.2). Prior to sampling, students participated in core OPIHI curricula. We developed curriculum (Baumgartner and Zabin 2006; Baumgartner et al. 2009; Baumgartner and Zabin 2008) to include science-inquiry and hands-on lessons; these can be found online at www.hawaii.edu/gk-12/opihi.

Students sampled sites on low tides ranging from 0.0 to -0.15 m that occurred in February-June. Students had engaged in prior training in field methods in their science classes (outlined in Baumgartner & Zabin, 2006) and were given laminated identification (ID) cards, data sheets, and guide books to assist in species identifications while in the field. ID cards included species dominant in the Hawaiian intertidal zone and species of special concern, such as invasive species, species that are harvested or collected, and

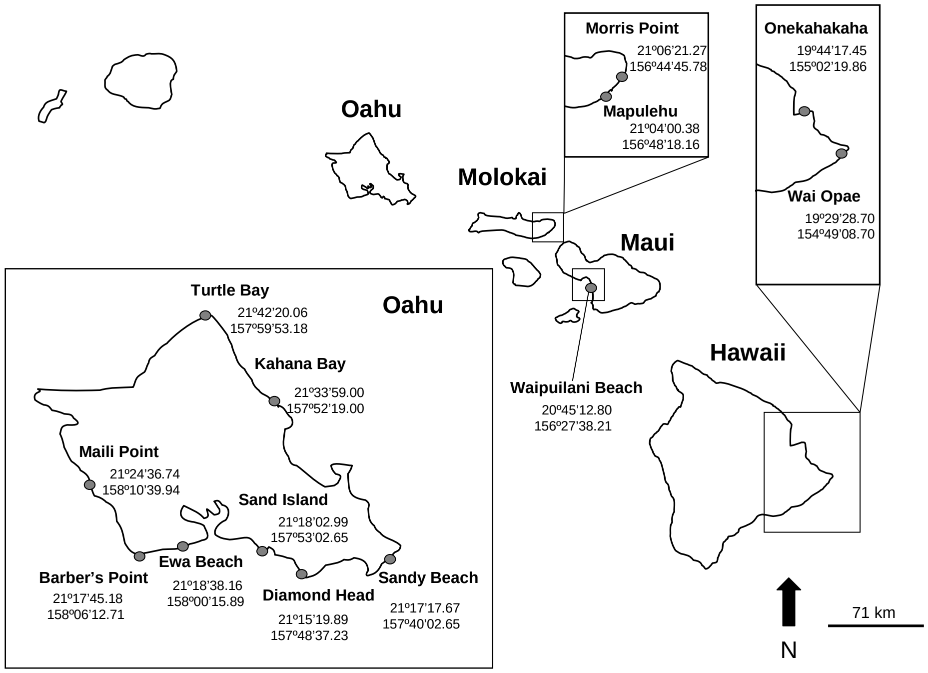

Fig. 2.1 Location of intertidal sites sampled throughout the Main Hawaiian Islands by the citizen scientist monitoring program, OPIHI.

Table 2.1 Site name, description and number of times sampled during the month(s) each year. $^{+}$SB 2007 sampling was not included in community analyses as large waves prevented full access to the exact 2006 monitoring site.

Site Name	**Shore habitat Type**	**Substrate composition**	**# of times sampled in years**				**Sites included in Validity Assessment**
			04	**05**	**06**	**07**	
Barber's Point (BP)	Bench	Limestone		3 Mar-May	2 Apr-May	5 May	*
Diamond Head (DH)	Bench	Limestone & Basalt	2 Feb	4 Feb-Mar	4 Apr	4 Apr	*
'Ewa Beach (EB)	Bench	Limestone			2 May		
Kahana Bay (KB)	Sand & bedrock	Limestone	2 Feb-Mar	2 Feb-Mar			
Maili Point (MPT)	Bench	Limestone				2 Apr	
Mapulehu (M)	Silt & cobble	Basalt			2 Apr		
Morris Point (MP)	Sand & bedrock	Limestone			1 May		
Onekahakaha (O)	Bench	Basalt				2 May	
Sand Island (SI)	Bedrock/Cobble & sand	Basalt		2 Apr-May	2 Apr-May	5 May	*
$^{+}$Sandy Beach (SB)	Sand & bedrock	Basalt			2 May	2 Apr	*
Turtle Bay (TB)	Bench	Limestone		3 May-June			
Waipuilani Beach (WB)	Coral rubble & sand	Limestone		2 May	1 Apr	2 Apr-May	
Wai'Opae (WOP)	Bench	Basalt				2 May	*

those that are plausible indicators of environmental change. Data sheets included the scientific names of common species previously seen at each site and empty areas designated for other identified/unidentifiable species.

Students collected the abundance data for macroalgae and invertebrates using traditional ecological sampling methods. At each site, depending upon the number of students available and the geography of the site, three to seven transect lines were placed ~2 m apart, perpendicular to shore and extended up to 30 m. Between 5 and 12 quadrats (each with 5 horizontal and 5 vertical strings, creating 25 intercepts) were placed at evenly spaced intervals along each transect. The percent cover of algae and invertebrates in each quadrat was sampled by one of two methods: visual estimation or point-contact. Specific methods are described in detail in Baumgartner and Zabin (2006) and a pilot comparison of students sampling the same site on the same low tide with different methodologies (visual estimation or point-contact) revealed similar results. In the point-contact method students recorded the taxa or bare space that occurred under each of the 25 intercepts within the quadrats. In the visual estimation method, the grid was used as a reference to assist students in estimation of the percent cover of each organism or bare space encountered. Students worked in small teams that were supervised by an adult chaperone and had approximately 1.5 hours (the approximate time span of a low tide window) to collect data along one transect line.

Secondary-school students identified species to the lowest taxon possible for their abilities in the field. Bare substrate was recorded as rock or sand along with other categorical data. Categories included “other/unknown algae”, “other/unknown invertebrates”, crustose coralline, brown-colored crust (which includes species of *Ralfsia* and *Peyssonnelia*), cyanobacteria, and algal turf (mixture of macroalgal species 1-2 cm tall). Taxa were often identified to the genus level and those difficult to identify were grouped into genera later by a researcher to ensure proper identification. However, post-hoc groupings were not used in the validity assessment.

Table 2.2 Visual estimate or point-contact method was used by 2 researcher and one student teams to collect percent cover data at each site per visit.

Site Name	# of Sampled visits	Grade(s)	Technique to Determine % Cover
Barber's Point	2*	10–12th	Visual Estimate
Diamond Head	2	9th	Point-Contact
Sand Island	2	10–12th	Visual Estimate
Sandy Beach	1	10–12th	Visual Estimate
Wai'Opae	1	6–10th	Point-Contact

* 1 out of 2 visits only had one research team for comparison

Validity Assessment

In order to examine the validity of data generated by OPIHI, students and researchers using the OPIHI protocol sampled the abundance of benthic macroalgae and invertebrates at five intertidal monitoring sites during the months of April and May 2007 on low tides (< -0.06 m). On each occasion, we used two teams of researchers, each composed of one to two individuals, as a benchmark to evaluate the students and to account for natural variation or biases in data collection which can occur between observers (Benedetti-Cecchi et al. 1996; Dethier et al. 1993). Some sites were visited more than once and during each visit sampling was conducted by different teams of students and researchers, thus we have total of eight student-to-researcher comparisons (Table 2.2). Researchers placed quadrats in the same locations as the students either before or after the students sampled, during the same low-tide window. Researchers were given identical datasheets, ID cards, and quadrats as students. To maintain statistical independence, the researcher teams were not allowed to discuss or interact with each other or with student teams. Teams were also instructed to communicate in quiet voices, so neither students nor the other research team would be influenced by their identifications.

To ensure that quality data would be used in comparisons with student-generated data, researchers had to meet certain criteria to participate in this study. All researchers (n = 9) had completed an upper-level college course in Invertebrate Zoology and/or Phycology and had experience with the standard ecological sampling techniques. Four researchers had served as graduate teaching assistants in Phycology, Invertebrate Zoology, or Marine Biology at the University of Hawai‘i, Manoa, and seven had conducted research on invertebrates and/or marine macroalgae, including two who had focused their doctoral research on intertidal ecology. Prior to data collection, researchers were given online access to the OPIHI curriculum, protocols, datasheets, and species ID cards.

Species composition and abundance data generated by the student and research teams were analyzed for differences using common community measures. First we examined the differences in diversity (Shannon Indices) generated from student and research teams for each site visit. Secondly we computed the similarity in student- and researcher- determined abundance data using the Bray-Curtis Index of similarity and used a bootstap simulation to statistically test the likelihood we would achieve this similarity by chance. Lastly, because community measures can be similar despite slight differences in composition and abundance of species and because we wanted to pinpoint potential student errors, we compared the researcher and student generated list of present species and their given abundances.

Shannon indices were used to calculate diversity values for each site from student and researcher generated data. Because we were interested in comparing variation, for each site we determined the difference between the researcher diversity values and the difference between the averaged researcher and student values. An average diversity value could minimize researcher variation in comparison with students, thus we also plotted raw diversity values to examine any differences. The difference between student and researcher determined Shannon values were initially screened for normality and homogeneity of variance then a paired *t*-test was used to test for significant differences. Statistical results were not adjusted for multiple comparisons as such an adjustment would only bolster support for our hypotheses.

To determine if student-generated abundance data were similar to researcher-generated data we determined the percent cover of benthic organisms per visit for each site for each research and student team. Thus each taxon/bare space had three percent cover values for each visit, one from each research team and one determined by students. We examined the abundance data with and without bare space in our analyses. We generated Bray-Curtis similarity values between the data collected by both researcher teams and between the averaged researchers and students in the statistical package Primer-E (Clarke and Warwick 2001). The Bray-Curtis calculation already down-weights common species however, results were similar when data were transformed, so we used untransformed data. These similarity values were screened for normality and homogeneity of variance. Paired *t*-tests were used to see whether the differences between researcher and student similarity values were statistically significant. In addition, a non-metric multidimensional scaling ordination (nMDS) technique was used to examine the spatial arrangement of data collectors and sites. A two-way crossed multivariate analysis of similarity (ANOSIM, site x data collector) was used to examine whether differences were statistically significant.

We also wanted to know how likely it was that similar values could be generated by chance. Thus we investigated whether our similarity comparisons were not just due to chance alone using a simulated bootstrap technique. Determined abundance values were reshuffled without replacement between taxa found to occur at a site. Monte-Carlo Analysis (in EXCEL pop-tools) was used to simulate the Bray-Curtis similarity values 10,000 times for each researcher-researcher and averaged researcher-student comparison. A 95% confidence interval was determined from the simulated similarity values. The similarity values observed from this validity assessment were considered reliable if they were above the simulated 95% confidence interval. A *p*-value was calculated from the number of simulated values out of 10, 000 that were higher than the determined similarity from field efforts.

To determine possible identification errors made by students we generated a species list for each site that compared both student and researcher generated data. To identify which species students overlooked, we determined which organisms both researcher teams found but students did not for each site visit. We also determined which species students recorded but both researchers did not, as these are most likely species that students misidentified. Here we are assuming student error based on the species lists generated by two researchers.

Large-scale community comparisons

The statistical program Primer-E was used to analyze community data and compare abundance and distribution patterns among sites (Clarke and Warwick 2001). After each sampling trip, an author entered the data and tabulated the abundance of each taxon identified as occurring at a site. Bare substrate was excluded from these analyses as we were interested in describing the biological community. Then a Bray-Curtis similarity matrix among sites and years was generated. A square-root transformation was performed to downweigh the abundant taxa and account for the patchy nature of invertebrate species but keep the emphasis on dominants as students were best able to quantify abundant taxa. A cluster diagram was constructed from the similarity matrix to compare spatial and temporal patterns. Additionally, similarities among sites were compared separately by year to ensure patterns among sites remained similar when additional sites were added to the monitoring program. SIMPER was used to examine which species contributed up to 70% to the similarity values. To test for temporal differences among sites a nested two-way ANOSIM with replicates was performed (year nested within sites). To examine the abundance of introduced species in intertidal habitats the mean abundance of the algal species *Acanthophora spicifera, Avrainvillea amadelpha, Gracilaria salicornia*, and *Hypnea musciformis* were calculated and graphed for each site. Due to the difficulties students had in distinguishing between the introduced and native barnacles we did not include the introduced barnacle *Chthamalus proteus* in these analyses.

Fine-scale distribution & abundance

Two sites (BP and DH) on the south shore of O‘ahu with similar habitat and substrate were selected to examine the distribution of species vertically across the shore. The benthic communities at both sites were sampled over multiple years using the OPIHI protocols. The percent cover of taxa was examined in 5 meter bins (0-4.9, 5.0-9.9, 10.0-14.9, 15.0 -19.9, 20+) as referenced to the water line at low low water. Then for dominant taxa the relative abundance and distributions were calculated within these bins for each year and averaged per site.

Results

Validity Assessment

The difference in Shannon's Index of diversity values indicates that while researchers' diversity values varied more, they were not significantly different than the variation seen between researchers and students (paired *t*-test, n = 7, *p* = 0.15). For four site comparisons students were closer to the averaged researcher diversity than researchers were to each other (Table 2.3, Figure 2.2).

The differences in community similarity values among data collectors were not statistically significant (Table 2.3, paired *t*-test, n = 8, *p* = 0.45). Similarity values were higher when bare substrate was included in the analyses. A bootstrap analysis of similarity values found that researcher and student similarity values were often above the 95% confidence intervals for simulated data. However, when bare space was removed from analyses researchers were not more similar than chance for a one-time sampling at Sandy Beach. There was no statistical difference between community data collected by researchers and students (two-way ANOSIM, R = 0, *p* = 0.58; Fig. 2.3).

The student and researcher species lists do reveal specific discrepancies in identifications. Students missed 10 species that were recorded by both research teams (Table 2.4A). Students also identified 17 species as occurring at sites when researcher did not indicate their presence (Table 2.4B). Six out of 10 taxa not recorded as present by students and 15 of 17 included by students but recorded as absent by researchers were estimated to occur at less than 1% cover. Three of the species missed by students are small (*Dendropoma gregaria*) or cryptic (*Lobophora variegata*, crustose coralline algae). *Montipora fabellata*, a coral species, was also not identified by students. This species as well as *D. gregaria* and *L. variegata* were not on the ID cards. Students did record unknown coral species and "other algae," two categories not utilized by researchers. Three species identified by students as occurring at abundances between 1% and 8% but not by researchers are algal species that are invasive in Hawaii's intertidal: *Acanthophora spicifera*, *Hypnea musciformis*, and *Gracilaria salicornia*.

Table 2.3 Comparison of community measurements (Shannon Diversity value H’ and Bray-Curtis % similarity) collected by student and researcher teams at sampled sites. Values in () are the results from analyses without bare space included as a taxa.

Site	Researcher H’ Difference	Researcher Student H’ Difference	Researcher to Researcher (%) Similarity	Student to Researcher (%) Similarity	Researcher to Researcher Similarity Bootstrap *p*-value	Student to Researcher Similarity Bootstrap *p*-value
BP	0.07	0.18	80.3 (50.6)	70.7 (57.0)	<0.005 (0.05)	<0.001 (<0.01)
DH 1	0.20	0.20	86.1 (74.5)	80.9 (65.1)	<0.001 (<0.001)	<0.001 (<0.001)
DH 2	0.32	0.05	79.6 (74.0)	80.2 (65.0)	<0.001 (<0.001)	<0.001 (<0.001)
SI 1	0.12	0.09	83.9 (83.9)	85.5 (86.5)	0.001 (0.001)	<0.001 (<0.001)
SI 2	0.01	0.02	84.1 (84.1)	87.2 (83.9)	0.005 (<0.001)	0.004 (<0.004)
SB	0.57	0.04	86.6 (24.0)	90.3 (43.4)	<0.001 (0.95)	<0.001 (<0.01)
WOP	0.46	0.18	77.3 (77.3)	72.0 (72.5)	<0.001 (<0.001)	0.02 (0.02)

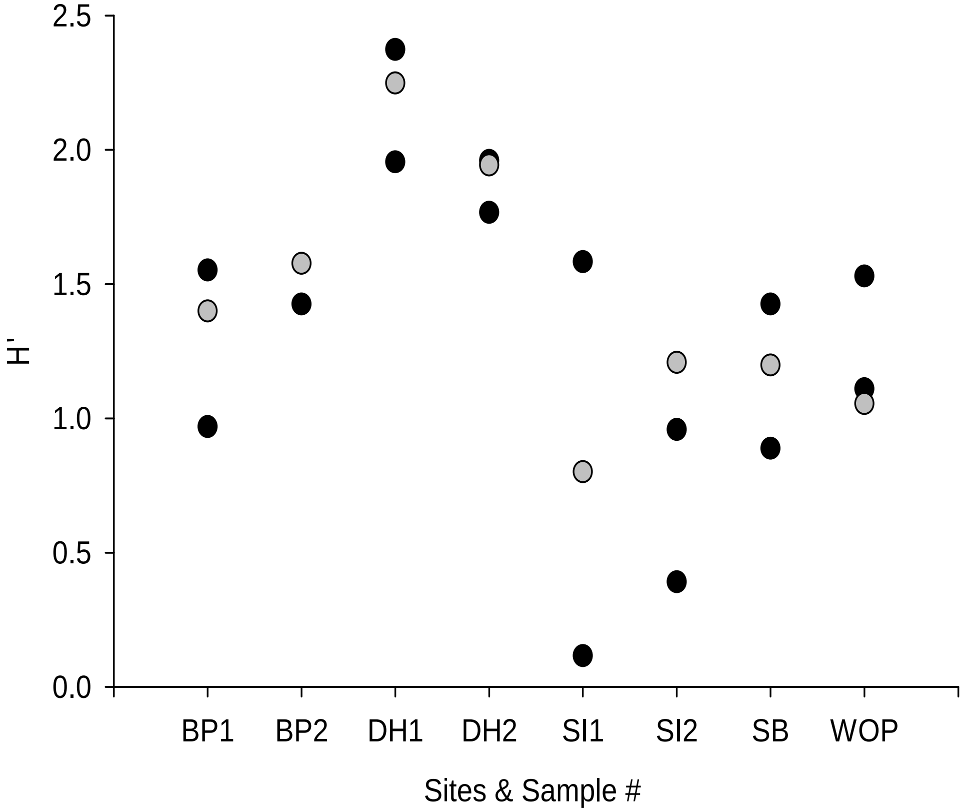

Fig. 2.2 Researchers' (•) and student's (•) Shannon Diversity values (H') for site comparisons.

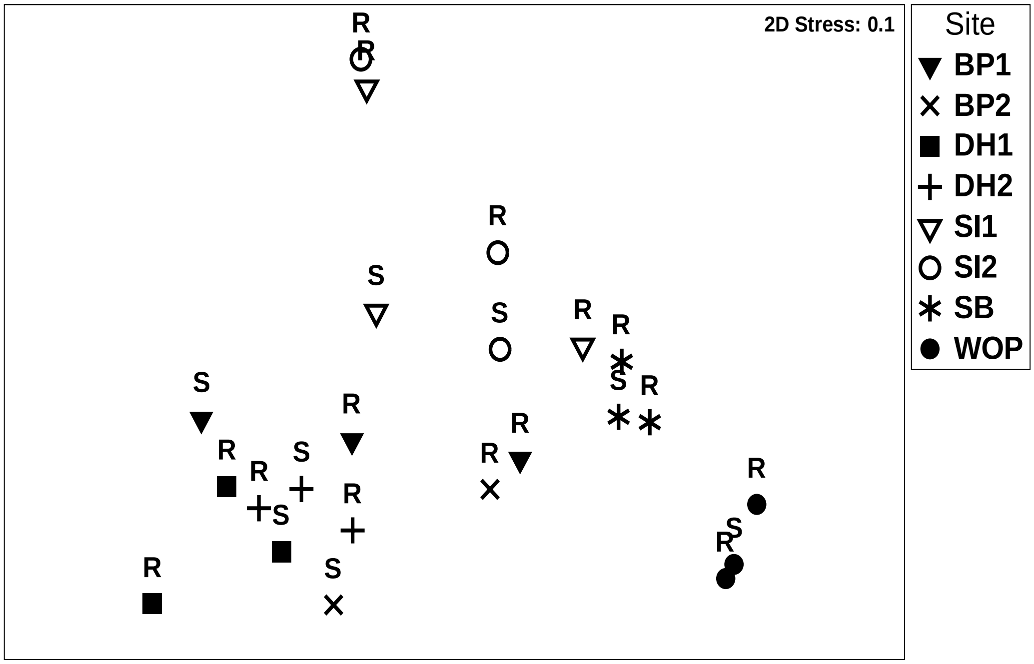

Fig. 2.3 nMDS of similarity values among sites comparing data collected by researchers (R) and students (S)

Table 2.4 Possible taxa mis-identified (A. (top) = taxa both researcher teams found but students did not, B. (bottom) = taxa students found but both researcher teams did not, Invert = Invertebrate)

A.

Taxa	Invert/Algae	Native/Introduced	Location	# of visits	% Cover by Researchers
Dendropoma gregaria	Invert	Native	DH	1	6.7, 7.7
Montipora flabellata	Invert	Native	WOP	1	3.6, 1.3
Dictyota acutiloba	Algae	Native	WOP	1	3.6, 1.3
Lobophora variegata	Algae	Native	SI	1	1.1, 0.2
Crustose Coralline Algae	Algae	Native	DH	1	0.7, 1.2
Microdictyon setchellianum	Algae	Native	DH	1	0.7, 0.2
Dictyosphaeria cavernosa	Algae	Native	DH	1	0.3, 0.7
Barnacle	Invert	Native/Introduced	DH	1	0.2, 0.2
Acanthophora spicifera	Algae	Introduced	SI	2	0.1, 0.3, 0.7, 0.4
Jania spp.	Algae	Native	SI	1	0.1, 0.1

B.

Taxa			Location	# of trips	% Cover by Students
Acanthophora spicifera	Algae	Introduced	DH	1	7.9
Hypnea musciformis	Algae	Introduced	DH	2	1.6, 1.0
Gracilaria salicornia	Algae	Introduced	DH	2	1.5, 1.0
Dictyosphaeria verslusyii	Algae	Native	DH, SI	2	0.9, 0.9
Unidentified coral	Invert	-	WOP, SB	2	0.9, 0.4
Sargassum spp.	Algae	Native	SB	1	0.4
Other algae	Algae	-	DH	1	0.4
Serpulorbis spp.	Invert	Native	SI	1	0.4
Avrainvillea amadelpha	Algae	Introduced	SI	1	0.3
Dictyota spp.	Algae	Native	BP	1	0.2
Echinometra oblonga	Invert	Native	SI	1	0.2
Littoraria pintado	Invert	Native	DH	1	0.2
Asparagopsis taxiformis	Algae	Native	SI	1	0.1
Botrycladia skottsbergii	Algae	Native	DH	1	0.09
Morula granulata	Invert	Native	SI	1	0.08
Echinometra mathaei	Invert	Native	SI	1	0.04
Bornetella sphaerica	Algae	Native	BP	1	0.003

Large-scale community comparisons

Forty-nine macroalgae, 1 or more cyanobacteria, and 31 invertebrate taxa were recorded from the thirteen intertidal sites (Tables 2.5, 2.6). Most sites were dominated by a few macroalgae and molluscs. Common taxa or functional forms included the macroalgae *Padina*, *Sargassum*, *Laurencia*, the introduced *Acanthophora spicifera*, turf forms of algae, and the molluscs *Siphonaria normalis*, *Nerita picea*, and species of littorines. The abundance and composition of taxa varied among sites and years (two-way ANOSIM, Sites: Global R = 0.70, p-value <0.001; Year: Global R = 0.64, *p*-value <0.001). However, year-to-year variation was usually smaller than the variation among sites.

Cluster analyses of Bray-Curtis similarity values generated from the percent cover of taxa showed that many sites had similar community structure (Fig. 2.4). Six clusters of sites (A-F) were determined to be at least 48% similar and a SIMPER analysis revealed the taxa and abundances that contributed to these similarities (Table 2.7). Most sites sampled were included in a cluster that was 55.2% similar (Cluster D) while the communities at WB (Cluster A), WOP (Cluster C), and KB (Cluster E) were at least 65+% dissimilar to any other sites. WOP was unique as corals were dominant while at other sites macroalgae were more common as cover. Two filter feeding invertebrates (the bivalve *Brachidontes crebristriatus* and barnacles) occurred at higher abundances at KB and contributed to differences among sites. WB had a high abundance of the introduced *Hypnea musciformis*. The similarities between sites MPT and M (Cluster F) were lower than the other clusters A-F and this relationship was driven mostly by the abundance of *A. spicifera*. Sand was abundant and varied among sites but did not trend with site similarities (Fig. 2.5). Observations of taxa and sand cover at these thirteen sites are congruent with these analytical findings.

Table 2.5 Percent cover (SE) of macroalgae and cyanobacteria at sites.

Macroalgae & Bacteria Taxa	BP	DH	EB	KB	MPT	M	MP	O	SI	SB	TB	WOP	WB
Acanthophora spicifera	9.4 (9.1)	1.6 (1.0)	16.5	27.5 (22.7)	29.9	23.9	-	-	4.2 (1.3)	0.4	0.6	-	7.5 (2.3)
Asparagopsis taxiformis	-	-	-	-	-	-	-	-	0.4 (0.4)	-	0.2	-	-
Astronema breviarticulatum	0.3 (0.4)	0.1 (0.1)	-	7.4 (2.1)	-	-	-	-	-	-	-	-	0.4 (0.5)
Avranvillea amadelpha	-	0.1 (0.1)	-	-	-	-	-	-	0.1 (0.1)	-	-	-	-
Cyanobacteria	0.3 (0.3)	1.9 (0.9)	-	-	-	31.0	-	-	4.1 (3.3)	-	-	0.3	2.2 (2.7)
Bornetella sphaerica	0.2 (0.2)	-	-	-	-	-	-	-	0.3 (0.4)	-	-	-	-
Botryocladia skottsbergii	-	-	-	-	-	-	-	-	-	-	-	-	-
Brown-Colored Crust	0.5 (0.4)	-	-	-	-	-	5.4	-	0.5 (0.7)	-	-	2.3	0.7 (0.9)
Caulerpa spp.	-	-	0.1	-	-	-	-	-	-	-	-	-	-
Chaetomorpha spp.	-	-	0.3	-	-	-	-	-	-	-	-	-	0.1 (0.1)
Chnoosphora implexa	-	0.1 (0.1)	-	-	-	-	-	-	-	-	-	-	-
Cladophora/Cladophoropsis	0.4 (0.5)	0.5 (0.2)	-	-	-	34.6	3.0	-	0.1 (0.1)	-	0.9	-	0.1 (0.1)
Codium edule	0.2 (0.1)	-	-	-	-	-	-	-	0.5 (0.3)	-	-	-	-
Codium spp.	-	-	-	-	-	-	-	-	0.4 (0.5)	-	-	-	-
Colpomenia sinuosa	-	-	0.1	-	-	-	-	-	0.1 (0.1)	0.8	0.5	-	0.7 (0.6)
Crustose Coralline	1.4 (1.4)	0.3 (0.1)	0.8	1.2 (1.7)	-	0.7	2.2	-	1.4 (1.2)	-	0.2	9.4	27.8 (6.4)
Dictyopteris spp.	0.1 (0.1)	0.5 (0.5)	-	-	-	-	-	-	-	-	-	-	-
Dictyosphaeria cavernosa	0.5 (0.6)	0.1 (0.1)	16.6	-	-	-	-	1.6	-	-	-	-	-
Dictyosphaeria spp.	-	-	-	-	-	-	-	-	0.2 (0.2)	-	-	-	-
Dictyosphaeria verslusyii	3.5 (0.4)	1.5 (1.5)	-	-	-	-	-	-	0.2 (0.2)	1.9	2.0	-	-
Dictyota acutiloba	0.2 (0.2)	0.4 (0.5)	11.8	-	-	-	-	0.5	-	-	-	1.2	0.3 (0.2)
Dictyota sandvicensis	-	0.3 (0.3)	-	-	6.7	0.3	-	-	-	-	-	-	0.2 (0.3)
Dictyota spp.	1.0 (1.3)	4.2 (2.5)	-	-	3.1	-	-	-	-	-	1.3	-	0.1 (0.1)
Galaxaura/Liagora	-	-	-	-	-	-	-	-	0.3 (0.3)	1.7	-	-	-
Gelid	-	0.0 (0.1)	-	-	-	-	-	-	-	3.0	-	-	0.1 (0.1)
Gracilaria salicornia	-	0.4 (0.4)	-	-	-	0.2	-	23.8	-	2.7	-	-	-
Halimeda discoidea	0.1 (0.0)	0.7 (0.7)	1.5	-	-	-	0.2	-	-	-	0.2	-	-
Hydroclathrus clathratus	-	-	-	-	-	-	-	-	-	-	0.8	-	-
Hypnea cervicornis	-	-	1.6	-	-	1.2	-	-	-	-	-	-	-
Hypnea chordacea	0.1 (0.1)	-	-	-	-	-	-	-	-	-	-	-	-
Hypnea musciformis	-	0.3 (0.4)	-	-	-	-	-	-	-	-	-	-	11.9 (7.1)
Laurencia mcdermidiae	0.1 (0.2)	0.3 (0.4)	-	-	0.8	-	-	-	-	-	-	0.2	-
Laurencia spp.	4.7 (1.6)	14.4 (4.6)	17.3	-	7.0	4.3	-	-	2.8 (3.3)	3.4	13.0	1.4	1.5 (1.1)
Lobophora variegata	-	-	-	-	-	-	-	-	0.3 (0.4)	-	-	0.9	-
Martensia spp.	-	0.1 (0.1)	-	-	-	-	-	-	-	-	-	-	-
Microdictyon setchellianum	0.1 (0.1)	0.4 (0.3)	2.1	-	-	-	-	-	-	-	14.2	-	-
Neomeris spp.	0.4 (0.2)	-	-	-	-	-	-	-	0.1 (0.1)	-	-	-	-
other algae	0.9 (0.5)	3.9 (3.2)	-	14.6 (15.5)	33.5	-	-	5.7	4.5 (4.0)	-	6.0	0.1	0.3 (0.2)
Padina spp.	46.3(10.0)	31.3 (2.6)	4.0	1.5 (2.1)	1.0	0.2	9.9	56.0	27.1 (11.2)	13.7	16.6	0.6	2.9 (0.5)
Pterocladiella caeruluscens	0.1 (0.1)	-	-	-	-	-	-	-	-	-	-	-	-
Pterocladiella capillacea	-	0.1 (0.1)	-	-	1.4	-	-	-	-	-	-	-	-
Sargassum spp.	5.6 (0.5)	14.4 (2.6)	9.6	3.2 (4.6)	-	2.8	-	-	1.4 (1.8)	5.7	4.3	-	7.3 (1.6)
Sphacelaria spp.	-	-	-	-	-	-	-	-	-	-	-	-	0.1 (0.1)
Trichogloea requienii	-	-	-	-	-	-	-	-	0.1 (0.1)	-	-	-	0.3 (0.4)
Turbinaria ornata	-	0.4 (0.2)	2.4	-	1.5	0.3	-	-	-	0.8	1.8	-	-
Ulva lactuca	-	-	-	0.6 (0.3)	3.8	-	-	-	-	-	-	-	16.6 (4.8)
Ulva flexuosa	-	0.5 (0.6)	-	-	-	-	-	-	-	-	-	-	-
Ulva reticulata	-	-	-	-	-	-	-	-	-	-	-	-	0.5 (0.2)
Wrangelia elegantissima	-	0.2 (0.2)	-	-	-	-	-	-	-	-	1.1	0.1	-
Turf	17.4 (8.8)	17.7 (6.2)	10.0	13.6 (19.3)	4.1	-	79.3	4.1	48.0 (13.9)	64.5	33.7	59.1	12.4 (7.7)

Table 2.6 Percent Cover (SE) of invertebrate taxa that occurred at sites.

Invertebrate Taxa	BP	DH	EB	KB	MPT	M	MP	O	SI	SB	TB	WO
Actinopyga mauritana	-	-	-	-	0.4	-	-	-	-	-	0.1	-
Anthopleura nigrescens	-	-	-	0.0 (0.1)	-	-	-	-	-	-	-	-
Barnacles	-	0.2 (0.1)	-	14.7 (7.2)	-	-	-	-	-	-	-	-
Brachidontes crebristriatus	-	-	-	3.3 (2.4)	0.5	-	-	3.1	-	-	-	-
Colobocentrotus atratus	-	-	-	-	0.8	-	-	-	-	-	-	-
Conus ebraeu	-	-	-	-	0.2	-	-	-	-	-	-	-
Dendropoma gregaria	3.7 (2.3)	2.0 (1.4)	5.3	-	-	-	-	-	1.3 (1.6)	-	-	-
Echinolittorina hawaiiensis	-	0.2 (0.1)	-	0.0 (0.1)	-	-	-	-	-	-	-	-
Echinometra mathaei	-	-	-	-	1.2	-	-	0.5	0.2 (0.1)	-	1.5	0.5
Echinometra oblonga	-	-	-	-	0.1	-	-	-	0.3 (0.3)	-	0.3	-
Fireworm	-	-	-	-	-	0.2	-	-	-	-	-	-
Holothuria cinerascens	-	-	-	-	-	-	-	-	0.1 (0.1)	-	0.6	-
Holothuria atra	-	0.1 (0.0)	-	-	-	-	-	-	0.2 (0.2)	-	-	-
Isognomon californicum	0.3 (0.1)	-	-	0.9 (1.3)	0.1	-	-	2.1	0.1 (0.1)	-	-	-
Isognomon perna	-	-	-	0.1 (0.2)	-	-	-	-	-	-	-	-
Littoraria pintado	-	0.1 (0.0)	-	0.3 (0.1)	-	-	-	-	-	-	-	-
Montipora capitata	-	-	-	-	-	-	-	-	-	-	-	8.5
Montipora flabellata	-	-	-	-	-	-	-	-	-	-	-	0.7
Morula granulata	0.3 (0.3)	-	-	-	-	-	-	-	0.1 (0.0)	0.4	0.1	-
Nerita picea	0.2 (0.1)	0.1 (0.0)	-	6.9 (5.6)	-	-	-	1.0	-	-	0.1	-
Ophiocoma erinaceus	-	-	-	-	-	-	-	-	-	-	-	0.1
other inverts	-	0.1 (0.1)	-	2.5 (3.2)	0.3	-	-	1.6	0.1 (0.1)	-	-	0.1
Pocillopora damicornis	-	-	-	-	-	-	-	-	-	-	-	0.2
Pocillopora meandrina	-	-	-	-	-	-	-	-	-	-	-	1.8
Porites lobata	-	-	-	-	-	-	-	-	-	-	-	9.5
Serpulorbis	0.6 (0.7)	-	-	-	-	-	-	-	0.5 (0.6)	-	-	2.7
Siphonaria normalis	0.8 (0.4)	0.5 (0.3)	-	1.4 (0.7)	0.2	-	-	-	-	-	-	-
Spirobranhus gigateus	-	-	-	-	-	-	-	-	-	-	-	0.1
Sponge	0.1 (0.1)	-	-	-	-	-	-	-	-	-	-	-
Stomadopod	-	-	-	-	-	-	-	-	0.1 (0.0)	-	-	-
unidentified coral	-	-	-	-	1.1	-	-	-	0.1 (0.1)	1.1	-	0.1

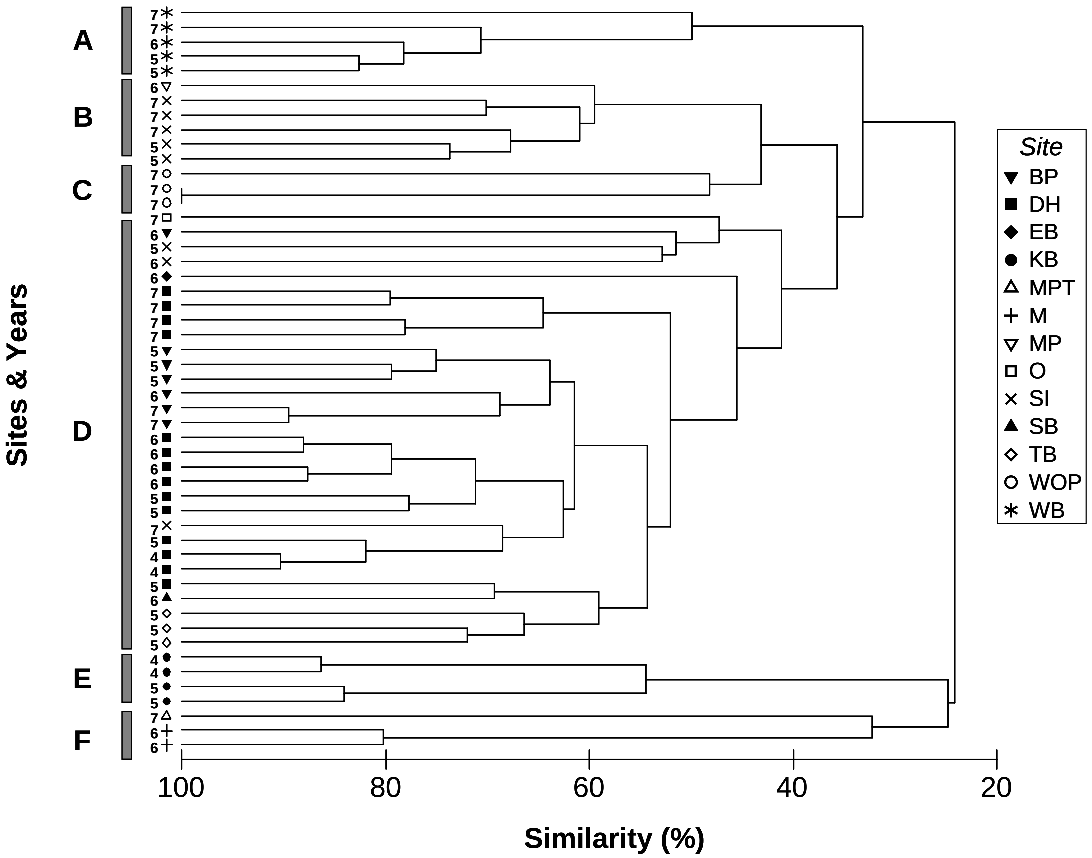

Fig. 2.4 Cluster analysis showing sites with similar community structure. Numbers 4-7 represent years 2004-2007 that the site (see key) was sampled, letter groups and gray bars represent sites that share ~50% or greater similarity in structure. See Table 2.1 for site abbreviations.

Table 2.7 Results from SIMPER analysis showing sites with similar structure and the taxa which contribute (80%) to similarity.

Cluster Groups A-F (% Similar) & Sites included	Abundance % (±SD)	% Contribution
A (65.1) WB		
Crustose Coralline	5.0 (3.1)	22.1
Ulva lactuca	3.9 (2.7)	16.7
Turf	3.5 (4.7)	13.7
Hypnea musciformis	3.2 (1.5)	11.5
Acanthophora spicifera	2.6 (6.2)	11.3
B (49.5) SI, MP		
Padina spp.	5.6 (3.4)	41.1
Turf	5.8 (1.3)	38.3
C (65.4) WOP		
Turf	7.5 (9.6)	53.2
Montipora capitata	4.5 (0.9)	21.5
Laurencia spp.	1.7 (2.3)	10.4
D (55.2) BP, DH, EB, O, SB,TB		
Padina spp.	5.5 (3.2)	30.0
Turf	4.2 (3.0)	20.8
Sargassum spp.	3.0 (2.2)	14.1
Laurencia spp.	2.8 (1.6)	12.0
E (64.7) KB		
Acanthophora spicifera	5.0 (3.0)	24.7
Barnacles	3.8 (5.6)	21.1
Hinskia breviarticulatum	2.8 (7.4)	15.4
Nerita picea	2.5 (2.2)	11.8
Brachidontes crebristriatus	1.7 (2.6)	8.0
F (48.2) MPT, M		
Acanthophora spicifera	5.0 (15.2)	41.0
Cladophora/Cladophoropsis	3.9 (0.6)	17.4
Laurencia spp.	2.3 (8.3)	17.0

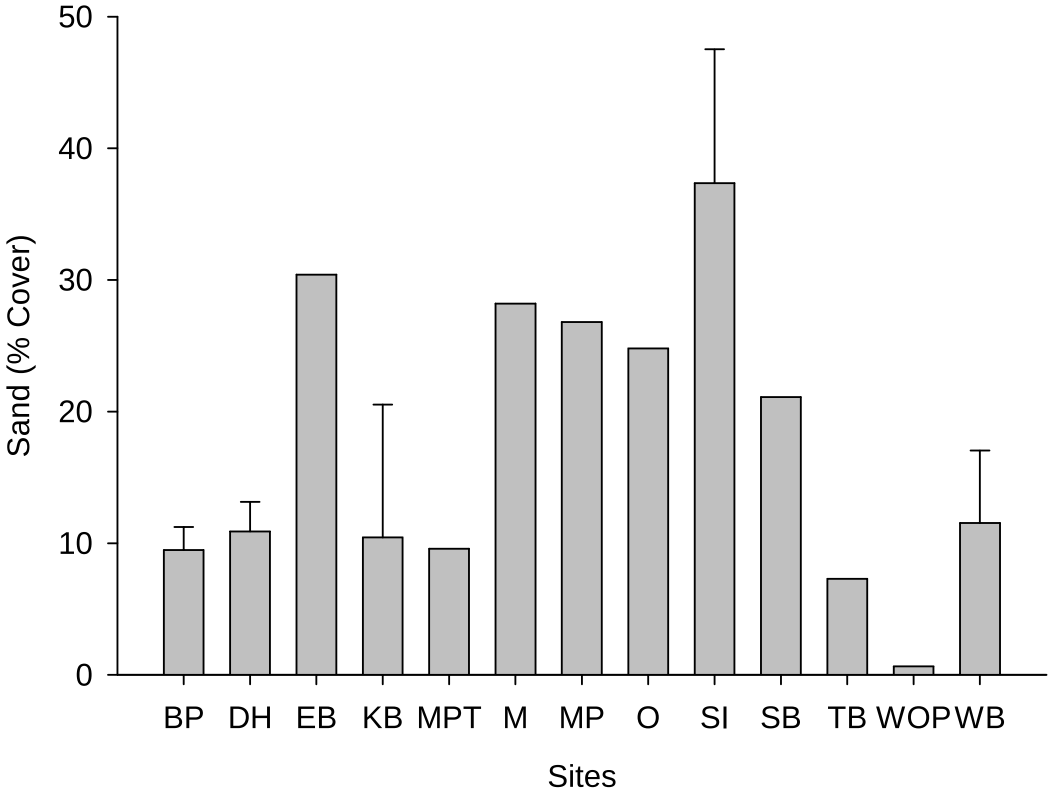

Fig. 2.5 Sand cover at sites sampled.

Introduced or alien macroalgae were found at many intertidal sites and these invaded sites were located on all sampled islands (Fig. 2.6). *Acanthophora spicifera* was the most common introduced species, found at 10 out of 13 sites. This species was also abundant with greater than 10% cover at 5 sites. There were only two sites without introduced benthic species, MP and WOP. *Avrainvillea amadelpha* was found at two sampled intertidal sites (SI and DH) both of which were located on the island of O‘ahu. *Hypnea musciformis* was abundant at WB and *Gracilaria salicornia* was abundant at O. Aside from *C. proteus* (which if found would have been recorded as barnacle), no other introduced benthic invertebrates were found using these methods.

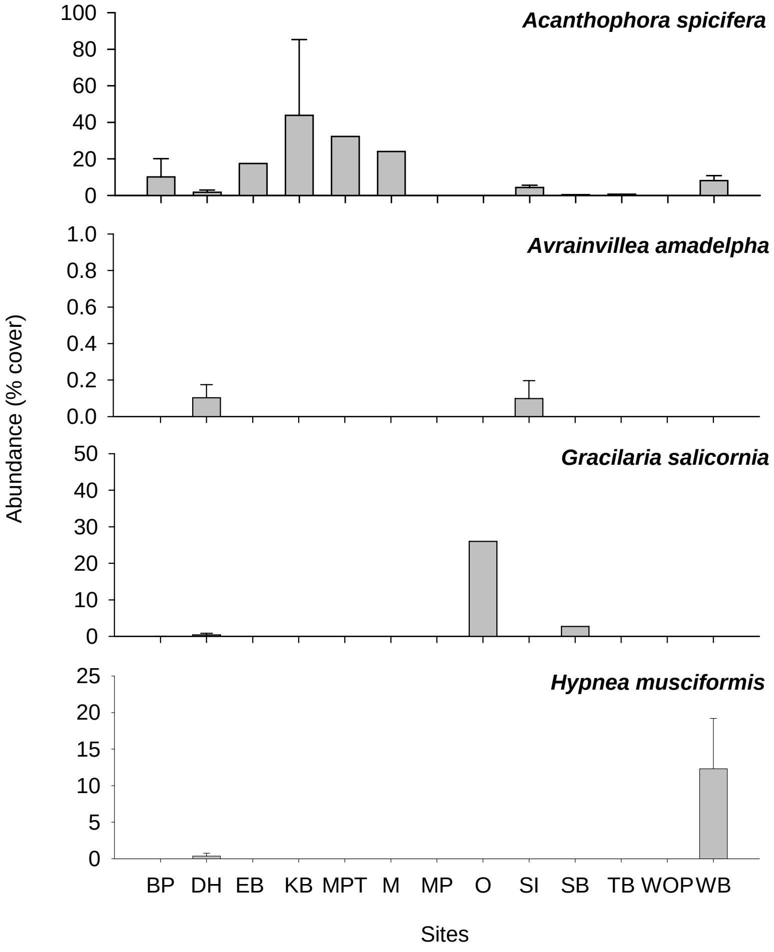

Fig. 2.6 Abundance of invasive macroalgae at sampled intertidal sites.

Fine-scale distributions & abundances

The relative abundances and distributions of dominant taxa varied vertically across the shore and these patterns were consistent between years (Fig. 2.7). The community at both sites revealed similar zonation of macroalgae and molluscs. The macroalgae *Sargasssum* and *Laurencia* were common offshore, close to the submerged subtidal zone; *Padina* was distributed closer to high tide line. The molluscs *Dendropoma gregaria*, *Siphonaria normalis*, *Nerita picea*, and *Echinolittorina Hawai'iensis* dominated the area nearest to the high tide line.

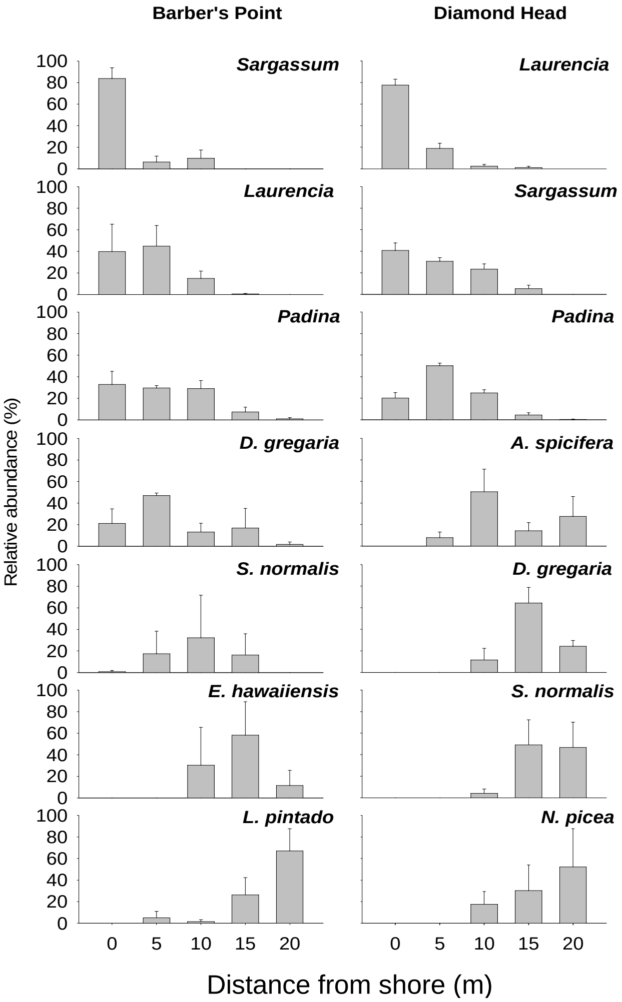

Fig. 2.7 Relative distribution and abundance (±SE) of dominant macroalgae and invertebrates as distance from the subtidal zone for two sites BP (left) and DH (right). Taxa arranged in order of distribution from offshore (top) to onshore (bottom). Macroalgae = *Padina*, *Sargassum*, *Laurencia*, *Acanthophora spicifera*; Molluscs = *Dendropoma gregaria*, *Siphonaria normalis*, *Echinolittoraria hawaiiensis*, *Littoraria pintado, Nerita picea.*

Discussion

Our analyses confirm the validity of student-generated data from the citizen scientist program OPIHI, and therefore these data were used to describe the community structure of benthic organisms at 13 intertidal sites on four of the main islands of Hawai‘i. Community benthic structure statistically varied among sites. The combined evidence from the presence/absence of species, the percent cover of sand, and similarities among communities suggest that local scale physical factors may alter structure and account for these differences among sites. In addition, fine-scale patterns were evident as would be predicted if conditions varied across the shore. Lastly, yearly variation in community structure was found but these changes were not drastic and are congruent with year-to-year stability.

From a research science perspective, there were both benefits and obstacles to using the citizen scientist program for collection of community data (see Dickinson et. al 2010). Citizen scientist allow for collection of more data in a limited amount of time; an important benefit in Hawai‘i where the low tide window only occurs for a few hours and seasonal and sporadic waves often limit safe access to the shore. Therefore without a massive concentrated effort these types of data would be difficult to gather across the island chain. For instance, in this study 20-30 students working in teams were able to sample more quadrats during the low tide window than two teams of researchers. Similarly many other programs benefit from the efforts of coordinating numerous volunteers to collect meso-to large scale data (Bhattacharhee 2005; Braschler 2009; Cohn 2008; Delaney et al. 2008; McCaffrey 2005; Oscarson and Calhoun 2007; Silvertown 2009). Although students in this study were successful in monitoring an entire community, they were prone to misidentify cryptic species, those species occurring at low abundances, or taxa not included on identification cards. This finding is a drawback to using citizen scientist in collection of community level data. Most partnerships focus the efforts of citizen scientists on monitoring one population or assemblage of organisms (Silvertown 2009) as a means to minimize training or the need for taxonomic expertise. Perhaps there is a trade-off in quantity versus quality of data or perhaps certain types of data are going to be difficult for non-scientists to gather. These findings have direct implications on the goals and usage of the monitoring program as the assessment reveals that students using these methods are more likely to misidentify early introductions but are able to monitor the abundances of introduced species once they become established.

A comparison between data generated by different researcher and student teams revealed minimal collector variation that is likely to be present in all ecological sampling and some comparisons had larger observer discrepancies. Few studies have examined collector variation and bias yet in long-term monitoring it is likely that numerous researchers or research teams may be involved in data collection. Benedetti-Cecchi et. al (1996), investigated collector variation in ecological sampling in subtidal habitats and found researcher biases. Dethier et. al (1993) found visual estimation methods of abundances to be more repeatable with less observer variation, however, in this study observer variation tended higher between collectors when visual estimation methods were used. Observer biases were accounted for and efforts made to limit variability as we required trained researchers and used two teams as a baseline to compare with trained and practiced student collectors. Some collector variation was still observed and it could be argued that such bias would exist in studies conducted only by professionals. Therefore, biases need to be considered when assessing long-term ecological change yet, in this study a substantial difference did not occur between researchers and citizen scientists.

Most sites were similar in their community structure. Several other studies examining intertidal structure have found distance (Blanchette et al. 2008; Bustamante and Branch 1996; Nakaoka et al. 2006), currents (Blanchette et al. 2008; Broitman et al. 2008; Bustamante and Branch 1996), and productivity (Menge et al. 1997) to correlate with site similarities. The Main Hawaiian islands, however, are within one biogeographic province with relatively little variation in sea surface temperature and current productivity (Abbott 1999; Kay 1979). In addition, organisms had to cross vast oceanographic barriers to reach these islands (Abbott 1999; Kay 1979). Thus it is not surprising that most sites share a high degree of community similarity that did not diverge with island or geographic distance, however, most sampled sites were located on O‘ahu thus these potential drivers were not explicitly tested. Schoch and Dethier (1996) investigated intertidal communities over a scale similar to this study and found habitat descriptor (example bench vs. cobble habitat) as good indicator of community structure.

Most of the sites we sampled can be described as a bench habitat (7 out of 13) and many (6) were 55.2% similar in macroalgal and invertebrate composition and abundance, however, this hypothesis could not be robustly tested with our current variety and sample size of habitat types. It should be noted that Banks and Skilleter (2007) caution that broad-scale categories of shorelines can fail to capture microhabitat and species diversity that occur at a shore.

Outlier communities, or those that did not group with high similarity to the majority of other sites, may provide insight into local factors that override large scale processes (Blanchette et al. 2008). Based on our results and past site histories we predict that local scale factors such as recruitment, predation, nutrient inputs, wave exposure, and sand scour may alter and maintain these communities. Small scale current patterns along the south shore of O‘ahu can contribute to invertebrate larvae transmitted to different sites and in turn these currents could determine what adult species are present or maintained at a site (Parnell 2000; Zabin 2005). Furthermore sites are speculated to differ in levels of freshwater input, herbivory, and nutrients.

WB, KB, M and MPT differed in structure from many of the sampled sites and may have high nutrients from different sources. Nuisance blooms of macroalgae (*Hypnea musciformis, Acanthophora spicifera,* and *Ulva lactuca*) are common on the reefs off of WB in Kihei, Māui (Smith and Smith 2003) and were found at high abundance in the associated intertidal zone (this study). These blooms have been linked to localized eutrophication of coastal waters from sewage inputs from the nearby injection well (Smith and Smith 2003). This site is also the only coral rubble habitat sampled. KB is the only sampled intertidal site that is located in a relatively clean bay and it is situated near Kahana stream. This stream and submarine groundwater discharge drain water from the Ko‘olau mountain range that surrounds the valley of Kahana (Garrison et al. 2003). Zabin et al. 2007 also found KB to have low species richness compared to other Hawai‘ian intertidal sites. The freshwater input could account for these observed differences in community structure. *Acanthophora spicifera* was dominant at M and MPT but these sites lack other obvious similarities. The benthic substrate at M is silt and mud with cobbles near a fishpond where edible algae is cultivated and sits at the base of Mapulehu Gulch. Terrestrial sedimentary input in shallow water habitats is common on the west side of Moloka‘i, where this site is located (Bothner et al. 2006). MPT is a limestone bench on the west side of O‘ahu located near a large drainage outlet. A homeless camp surrounds the beach where harvesting of marine life is common and sanitation appears to be a problem (personal observations).

The community at WOP is free from introduced macroalgae and corals are in high abundance. WOP also did not share high similarity with any other site. Corals are often associated with warm, low nutrient oceanic waters where herbivory is high (Smith et al. 2001) and WOP is located within a permanent no take Marine Life Conservation District. Perhaps conservation efforts tend towards higher herbivory rates than the other sampled sites. SI and MP were both sites with low biotic cover and were dominated by turf and high percentages of sand. Turf algae are specialized for areas with high physical stress and moderate grazing pressure (Hay 1981) and it is possible that for this shore habitat type, sand scour and predation prevent the cover of lush macroalgae and abundance of small molluscs that occurred at other sites. Further physical measurements and experimental testing should be examined with structure to clarify factors that shape the community over these mesoscales for the main Hawaiian Islands.

Introduced macroalgae were common in intertidal habitats, yet their impact on this type of benthic community have been little studied. Introduced species were found at sites on all islands sampled, with *Acanthophora spicifera* being the most common alien species found. This species is known to have increased growth rates in nutrient rich waters (Fong et al. 2003) and be a preferred food of herbivores (Stimson et al. 2001). Its abundance at sites suggests that herbivores in the intertidal zone are not present in high enough numbers to limit biomass production. *Avrainvillea amadelpha* was only found at sampled sites on O‘ahu. Recent observations of this species in rocky habitats (personal observations TEC) where not previously noted is of concern. Competition between native and introduced species is likely and has been described in Hawaii’s intertidal between an invasive barnacle and a native limpet (Zabin et al. 2007) and suggested to occur between *A. spicifera* and native *Laurencia* species (Russell 1992). Thus, future efforts should be taken to document and monitor any changes in introduced abundances, to quantify any impacts to the community, and to assess the feasibility of removal in rocky intertidal habitats- similar to efforts already undertaken on Hawaii’s reefs.

The zonation patterns observed at two shores in Hawai‘i suggest the importance of local to microhabitat level interactions in shaping communities at finer spatial scales. Molluscs were dominant high on the shore where splash and exposure were common and lush macroalgae occurred lower on the shore in wetted habitats. Other studies conducted in the tropics have posited predation (Bertness 1981; Garrity and Levings 1981; Menge and Lubchenco 1981) and temperature/desiccation (Garrity 1984; Southward 1958; Vermeij 1973) as determinants of distributions. For instance in Panama where many studies on tropical intertidal ecology have been conducted organisms were limited to cracks and crevices as refuge from predation and desiccation (Menge and Lubchenco 1981). In Hawai‘i, Cox and Smith (see Chapter V) describe several species of intertidal molluscs to occur in cooler cracks and crevices but lush algal beds cover the shore. Hawaii’s microtidal regimes and wave action could perhaps limit harsh conditions and account for differences in algal fine scale distributions. In addition, competition and partitioning is likely to alter the distributions of Hawaii’s molluscs and macroalgae as these factors have also been described in Hawai‘i (Zabin 2009) and elsewhere in the tropics (Levings and Garrity 1983; Mak and Williams 1999; Ortega 1985; Sutherland and Ortega 1986). In Panama, nerite species differ in their tolerances to temperature, exposure, and predation and when removed, co-occurring littorines increase in size (Levings and Garrity 1983). Similarily, in Hong-Kong, littorines control the abundance of biofilms and limit algal recruitment from higher zones (Mak and Williams 1999). Along with common neritid *Nerita picea*, Hawai‘i has two common littoraria in intertidal zones, *Echinolittoraria hawaiiensis* and *Litorraria pintado*. *Littoraria pintado* is often found higher on the shore than *E. hawaiiensis* (Kay 1979) and this distribution was observed at Diamond Head. In addition, wave activity and subsequent lower temperatures correlated with the distributions of urchins, molluscs, and macroalgal functional groups at a wave-exposed site on O‘ahu (Bird 2006). Further, fine-scale experiments and investigations are needed to discern which factors contribute to zonation in Hawai‘i.

Describing Hawai‘i’s shores is important for conservation of intertidal habitats as it not only provides a better understanding of the ecological processes that shape these communities but it also provides a baseline from which to measure ecological change.

Eutrophication, climate change, invasions, and marine harvesting are likely to impact these intertidal habitats and results from this study suggest that some sites have already been altered. The impact of seasonality on community structure in marine habitats in Hawai‘i has been little investigated (but see Doty 1971) and further efforts could discern whether year to year differences in composition and abundances were influenced by the February-June samplings. These findings and recommendations stress the importance of citizen scientist programs like OPIHI which foster conservation and ecological awareness in the general public. Moreover, the amount of data we collected could not have been collected in this time period without the efforts of this citizen driven program.

References

Abbott IA (1999) Marine red algae of the Hawaiian Islands. Bishop Museum Press, Honolulu

Airame S, Dugan JE, Lafferty KD, Leslie H, McArdle DA, Warner RR (2003) Applying ecological criteria to marine reserve design: a case study from the California Channel Islands. Ecological Applications 13:170-184

Banks SA, Skilleter GA (2007) The importance of incorporating fine-scale habitat data into the design of an intertidal marine reserve system. Biological Conservation 138:13-29

Barry JP, Baxter CH, Sagarin RD, Gilman S (1995) Climate-related, long-term faunal changes in a California rocky intertidal community. Science 267:672-675

Baumgartner E, Zabin C (2006) Visualizing zonation patterns. Science Teacher 73:60-64

Baumgartner E, Zabin C, Philippoff J, Cox TE, Knope M (2009) Ecological monitoring provides a thematic foundation for student inquiry. In: Yagar R (ed) Inquiry: The Key to Exemplary Science. National Science Teachers Association Press, Arlington

Baumgartner E, Zabin CJ (2008) A case study of project-based instruction in the ninth grade: a semester-long study of intertidal biodiversity. Environmental Education Research 14:97-114

Benedetti-Cecchi L, Airoldi L, Abbiati M, Cinelli F (1996) Estimating the abundance of benthic invertebrates: a comparision of procedures and variability between observers. Marine Ecology Progress Series 138:93-101

Bertness MD (1981) Predation, physical stress, and the organization of a tropical rocky intertidal hermit crab community. Ecology 62:411-425

Bhattacharhee Y (2005) Citizen scientists supplement work of Cornell researchers. Science Magazine 308:1402-1403

Bird CE (2006) Aspects of community ecology on wave-exposed rocky Hawaiian coasts. In: Botany. University of Hawai'i, Honolulu, p 255

Blanchette CA, Miner CM, Raimondi PT, Lohse D, Heady KE, Broitman BR (2008) Biogeographical patterns of rocky intertidal communities along the Pacific coast of North America. Journal of Biogeography 35:1593-1607

Bothner MH, L. RR, Casso MA, Storlazzi CD, Field ME (2006) Quantity, composition, and source of sediment collected in sediment traps along the fringing coral reef off Molokaʻi, Hawaiʻi. Marine Pollution Bulletin 52:1034-1047

Braschler B (2009) Successfully implementing a citizen-scientist approach to insect monitoring in a resource-poor country. Bioscience 59:103-104

Broitman BR et al. (2008) Spatial and temporal patterns of invertebrate recruitment along the west coast of the United States. Ecological Monographs 78:403-421

Bustamante RH, Branch GM (1996) Large scale patterns and trophic structure of southern African rocky shores: the roles of geographic variation and wave exposure. Journal of Biogeography 23:339-351

Clarke KR, Warwick RM (2001) Changes in marine communities: An approach to statistical analysis and interpretation, 2nd edn. Primer-E Ltd, Plymouth

Cohn JP (2008) Citizen science: can volunteers do real research? Bioscience 58:192-198

Connell JH (1961) The influence of intra-specific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. Ecology 42:710-723

Dailer ML, Knox RS, Smith JE, Napier M, Smith CM (2010) Using δ^{15}N values in algal tissue to map locations and potential sources of anthropogenic nutrient inputs on the island of Māui, Hawaiʻi, USA. Marine Pollution Bulletin 60:655-671

Delaney DG, Sperline CD, Adams CS, Leung B (2008) Marine invasive species: validation of citizen science and implications for national monitoring networks. Biological Invasions 10:117-128

Dethier MN, Graham ES, Cohen S, Tear LM (1993) Visual versus random-point percent cover estimations: 'objective' is not always better. Marine Ecology Progress Series 96:93-100

Dickinson JL, Zuckerberg B, Botner DN (2010) Citizen science as an ecological research tool: Challenges and benefits. Annual Review of Ecology Evolution and Systematics 41:149-172

Doty MS (1971) Antecedent event influence on benthic marine algal standing crops in Hawaiʻi. Journal of Experimental Marine Biology and Ecology 6: 161-166

Eldredge LG, Smith CM (2001) A guidebook of introduced marine species in Hawaiʻi. B.P. Bishop Museum an the University of Hawaiʻi, Honolulu

Fong P, Boyer KE, Kamer K, Boyle KA (2003) Influence of initial tissue nutrient status of tropical marine algae on response to nitrogen and phosphorus additions. Marine Ecology Progress Series 262:111-123

Garrison GH, Glenn CR, McMurtry GM (2003) Measurement of submarine groundwater discharge in Kahana Bay, Oʻahu, Hawaiʻi. Limnology and Oceanography 48:920-928

Garrity SD (1984) Some adaptations of gastropods to physical stress on a tropical rocky shore. Ecology 65:559-574

Garrity SD, Levings SC (1981) A predator-prey interaction between two physically and biologically constrained tropical rocky shore gastropods: direct, indirect, and community effects. Ecological Monographs 51:268-286

Hay ME (1981) The functional morphology of turf-forming seaweeds: persistence in stressful marine habitats. Ecology 62:739-750

Kay AE (1979) Hawaiʻian marine shells, reef and shore fauna of Hawaiʻi. Bishop Museum Press, Honolulu

Levings SC, Garrity SD (1983) Diel and tidal movement of two co-occurring neritid snails; differences in grazing patterns on a tropical rocky shore. Journal of Experimental Marine Biology and Ecology 67:261-278

Littler MM, Doty MS (1975) Ecological components structuring the seaward edges of tropical pacific reefs: the distribution, communities, and productivity of *Porolithon*. Journal of Ecology 63:117-129

Mak YM, Williams GA (1999) Littorinids control high intertidal biofilm abundance on tropical, Hong Kong rocky shores. Journal of Experimental Marine Biology and Ecology 233:81-94

McCaffrey RE (2005) Using citizen science in urban bird studies. Urban habitats 3:1541-7115

Menge B (2000) Testing the relative importance of positive and negative effects on community structure. Trends in Ecology and Evolution 15:45-46

Menge B, Daley BA, Wheeler PA, Strub T (1997) Rocky intertidal oceanography: an association between community structure and nearshore phytoplankton concentration. Limnology and Oceanography 42:57-66

Menge BA, Lubchenco J (1981) Community organization in temperate and tropical rocky intertidal habitats: prey refuges in relation to consumer prey gradients. Ecological Monographs 51:429-450

Murray SN, Ambrose RF, Dethier MN (2006) Monitoring rocky shores. University of California Press, Berkeley and Los Angeles

Nakaoka M, Ito N, Yamamoto T, Okuda T, Noda T (2006) Similarity of rocky intertidal assemblages along the Pacific coast of Japan: effects of spatial scales and geographic distance. Ecological Research 21:425-435

Ortega S (1985) Competitive interactions among tropical intertidal limpets. Journal of Experimental Marine Biology and Ecology 90:11-25

Oscarson DB, Calhoun AJK (2007) Developing vernal pool conservation plans at the local level using citizen-scientists. Wetlands 27:80-95

Parnell PE (2000) The effects of circulation, larval planktonic period, adult distribution, and pollution on benthic recruitment in Hawaiian coastal waters. In. University of Hawai‘i, Honolulu

Pattengill-Semmens CV, Semmens BX (2003) Conservation and management applications of the reef volunteer monitoring program. Environmental Monitoring and Assessment 81:43-50

Russell DJ (1992) The ecological invasion of Hawaiian reefs by two marine red algae, *Acanthophora spicifera* (Vahl) Boerg. and *Hypnea musciformis* (Wulfen) J. Ag., and their association with two native species, *Laurencia nidifica* J. Ag and *Hypnea cervicornis* J. Ag. ICES mar. Sci. Symposium 194:110-125

Sagarin RD, Barry JP, Gilman SE, Baxter CH (1999) Climate-related change in an intertidal community over short and long time scales. Ecological Monographs 69:465-490

Schoch GC, Dethier MN (1996) Scaling up: the statistical linkage between organismal abundance and geomorphology on rocky intertidal shorelines. Journal of Experimental Marine Biology and Ecology 201:37-72

Silvertown J (2009) A new dawn for citizen science. Trends in Ecology and Evolution 24:467-472

Simberloff D, Parker IM, Windle PN (2005) Introduced species policy, management, and future research needs. Frontiers in ecology and environments 3:12-20

Sink KJ, Branch GM, Harris JM (2005) Biogeographic patterns in rocky intertidal communities in KwaZulu-Natal, South Africa. African Journal of Marine Science 27:81-96

Smith CM (1992) Diversity in intertidal habitats: an assessment of the marine algae of select high islands in the Hawaiian archipelago. Pacific Science 46: 466-479

Smith JE, Hunter CL, Smith CM (2002) Distribution and reproductive characteristics of nonindigenous and invasive marine algae in the Hawaiian Islands. Pacific Science 56:299-315

Smith JE, Smith CM (2003) Factors influencing algal blooms on tropical reefs with an emphasis on herbivory, nutrients, and invasive species. In: Department of Botany. University of Hawaiʻi at Manoa, Honolulu

Smith JE, Smith CM, Hunter CL (2001) An experimental analysis of the effects of herbivory and nutrient enrichment on benthic community dynamics on a Hawaiʻian reef. Coral Reefs 19:332-342

Southward AJ (1958) A note on the temperature tolerances of some intertidal animals in relation to environmental temperatures and geographic distribution. Journal of Marine Biology 37:49-46

Stimson J, Larned ST, Conklin EJ (2001) Effects of herbivory, nutrient levels, and introduced algae on the distribution and abundance of the invasive macroalga *Dictyosphaeria cavernosa* in Kaneohe Bay, Hawaiʻi. Coral Reefs 19:343-357

Sutherland JP, Ortega S (1986) Competition conditional on recruitment and temporary escape from predators on a tropical rocky shore. Journal of Experimental Marine Biology and Ecology 95:155-166

Vermeij GJ (1973) Morphological patterns in high-intertidal gastropods: adaptive strategies and their limitations. Marine Biology 20:319-346

Vroom PS, Musburger CA, Cooper SW, Maragos JE, Page-Albins KN, Timmers MAV (2010) Marine biological community baselines in un-impacted tropical ecosystems: spatial and temporal analysis of reefs at Howland and Baker Islands. Biodiversity and Conservation 19:797-812

Zabin C (2005) Community ecology of the invasive intertidal barnacle *Chthamalus proteus* in Hawai'i. In: Department of Zoology. University of Hawaiʻi, Honolulu, p 210

Zabin C (2007) Comparison of species composition in two contrasting open-coast, rocky intertidal habitats: California and Hawaiʻi. In: Ecological Society of America, San Jose, California

Zabin C (2009) Battle of the barnacle newcomers: niche compression in invading species in Kaneohe Bay, Oʻahu, Hawaiʻi. Marine Ecology Progress Series 381:175-182

Zabin C, Zardus J, Pitombo FB, Fread V, Hadfield MG (2007) A tale of three seas: consistency of natural history traits in a Caribbean-Atlantic barnacle introduced to Hawaiʻi. Biological Invasions 9:523-544

Chapter III. Spatial and vertical patterns in the tidepool fish assemblage on the island of O‘ahu

Abstract

The microtides, wave regimes, and relative isolation of the Hawaiian archipelago may provide unique environmental and biogeographic effects that shape the structure of tidepool fishes. We sampled fishes across a narrow gradient at low tide from 6 sites on the island of O‘ahu. We tested predictions of the hypotheses that environmental conditions (pool depth, volume, macroalgal cover, temperature, and salinity) would result in a vertically structured tidepool fish assemblage unique to basalt or limestone rocky shores. 343 fish were recorded from 40 pools, and 19 species from 10 families were identified. Tidepool fish diversity (H’: O‘ahu = 2.4; Sites Average = 0.0 – 0.9) was typical for tropical islands, with members from Gobiidae (5 species), Blenniidae (4 species), Pomacentridae (3 species), Acanthuridae (2 species) and Kuhliidae (2 species) among the most common. Endemism (32%) was higher than other well studied assemblages yet similar to Hawaiian reef fishes (25%). Assemblage abundance varied among shores with basalt or limestone substrate, among sites, and vertically among high, mid, and low pools. In general, blenniids occurred at higher proportions on limestone shores and gobiids were more common on basalt shores. High pools were characterized by an abundance of a small sized (29.0 mm median standard length) blenniid *Istiblennius zebra,* while the blenniid *Entomacrodus marmoratus* and wrasses *Thalassoma* spp. were more common in low pools. Temperature was the best environmental predictor of assemblages and this relationship warrants further investigation. Our findings indicate that assemblages can vary across a narrow geographical range and intertidal shore.

Introduction

Intertidal fish assemblages are known to vary in composition across latitudes and continents, between regions, and within individual localities (Gibson and Yoshiyama 1999). Geographic patchiness, dispersal abilities, and evolutionary history explain the distribution of species across latitudes and continents, while abiotic factors often contribute to patterns at regional and local scales (Gibson and Yoshiyama 1999). Vertical gradients in temperature, air exposure, wave action, and salinity can occur across the shore. As water recedes during low tide, fishes that reside in pools (residents) are more tolerant to these variable conditions and the most physiologically tolerant species occur higher on-shore (Yoshiyama et al. 1986, Zander et al. 1999). Substrate type also can contribute to patterns in tidepool fish assemblages. Examples can be found in central California, U.S.A., where stichaeids and pholids are found in tidal boulder fields while heavy vegetated pools are often dominated by cottids and clinids (Yoshiyama et al. 1986) and in the Mediterranean where rock structures affect the species composition (Macpherson 1994). Pool rugosity, volume, and depth can further contribute to tidepool fish community patterns (Griffiths 2003). Isolated oceanic island chains, like the Hawaiian Islands, provide an opportunity to explore the importance of abiotic factors and biogeography in shaping fish assemblage structure in these islands, as has been done for numerous continental shores.

Tidepool fish assemblages are known to exhibit distinct biogeographic affinities resulting from the dispersal abilities of larvae and the degree of geographic connectedness between populations (Prochazka et al. 1999). For example, central California and southern Chile have similar environmental regimes but distinct intertidal fish fauna (Boyle and Horn 2006). Similarly, islands often have different flora and fauna in contrast to nearby mainland populations. The marine waters surrounding the Hawaiian archipelago contain many tropical fish species that co-occur throughout the Indo-West Pacific and presumably these islands serve as a stepping stone for dispersal across a vast oceanic barrier (Randall, 2007). However, the isolation allows for a high number of endemic fish species; 25% of the Hawaiian island marine fish fauna are endemic (Randall, 2007). Therefore, the isolated nature of Hawaiian intertidal zones in combination with the typically harsh environmental conditions may facilitate speciation and result in a unique assemblage of intertidal fishes.

The tropical location and tidal conditions in the state of Hawaiʻi may influence the vertical and spatial patterns of fishes in the intertidal zone. Tides in Hawaiʻi are considered microtidal with an amplitude of less than 1m (Gosline 1965, Abbott 1999). The islands of Hawaiʻi are located in the trade wind belt and seasonally directed winds drive wave height and determine which shores (north, south, east, or west) experience wave swell at different times of the year (Gosline 1965, Abbott 1999, Bird 2006). The combination of microtides and surge limit air exposure for intertidal organisms and the vertical span of the intertidal zone is much reduced in comparison to the extensive vertical span of other well studied intertidal shores (Gosline 1965, Abbott 1999). Nonetheless, pools are abundant along Hawaiian shores (Gosline 1965) and are apparent on spring low tides that occur in summer daylight hours when temperatures are at their peak. Additionally, Oʻahu has both basalt and carbonate based shores (Gosline 1965, Abbott 1999). Basalt shores are often barren of lush macroalgae unlike rough and porous limestone shores (Abbott 1999), thus these types of shores may provide different habitats best suited for the survival of different species of fishes.

During the approximately 35 years since the first observational description of Hawaiian intertidal fishes (Gosline, 1965), much has changed in the near shore environment. Changes include the invasion of palatable and unpalatable alien algae (Stimson et al. 2001, Smith et al. 2002), increased fishing pressure (Friedlander and DeMartini 2002), and altered temperatures and sea level from global warming (Jokiel and Brown 2004). However, it is not known if these changes have impacted the fish assemblages in the intertidal habitat. Additionally, comparable descriptive studies on tropical and temperate intertidal zones focus on quantification of resident fishes found in pools during the low tide (Horn et al. 1999). Gosline (1965) detailed observations of fishes on high and low tides in these coastal zones but robust quantification was not provided.

The aims of this study were to describe the tidepool fish assemblage for the island of Oʻahu and examine fish assemblage structure across and among shores. We tested the hypothesis that tidepool fishes would be vertically distributed. Further, we tested the hypothesis that intertidal fish communities would vary among shores with different substrate type (basalt or limestone). Lastly, the isolation of the Hawaiian archipelago is hypothesized to result in a tidepool fish community for the island of Oʻahu with high species abundance but low richness and high endemism.

Methods

To describe assemblage patterns and abundances of tidepool fishes on Oʻahu, six rocky intertidal sites were chosen for sampling: ʻEwa Beach, Turtle Bay, Nānākuli, Sandy Beach, Makapuʻu and Diamond Head (Fig. 3.1). These sites were selected to ensure a representative sample of fishes and to test the hypothesis that substrate type correlates with structure. Sites are located on the south, east, west, and north shores and included tidal benches composed of basalt or limestone (Table 3.1).

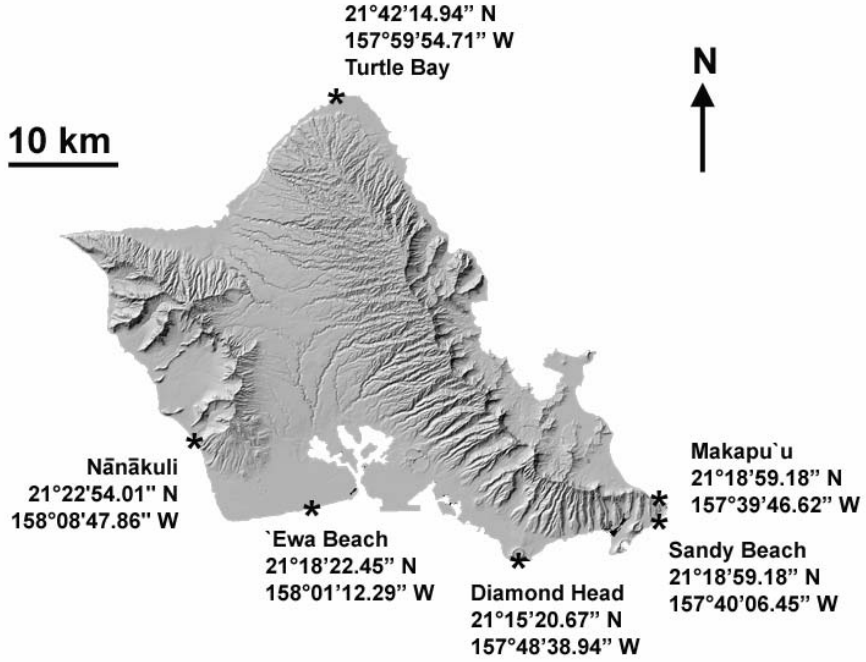

Fig. 3.1 Map of the island of O‘ahu with the location of six intertidal sites

Table 3.1 Site name, substrate type, location and date, tidal height sampled

Sites	Abbreviation	Substrate Type	O‘ahu Shore Location	Date Sampled in 2008	Low Tide Height (m)
Diamond Head	D	Basalt	Southeast	August 1	-0.06
Makapu‘u	M	Basalt	East	June 30	-0.12
Sandy Beach	S	Basalt	East	May 30	-0.09
‘Ewa Beach	E	Limestone	Southwest	June 3	-0.15
Nānākuli	N	Limestone	West	July 2	-0.12
Turtle Bay	T	Limestone	North	June 5	-0.12

To examine the vertical structure of tidepool fishes across the shore, six to eight pools distributed in an on-offshore direction within the intertidal habitat at each site were chosen haphazardly for collection of fishes. Because the Hawaiian Islands have microtides and a limited range of vertical intertidal zonation, tidal height was not obvious, hence pools were sampled as high, mid, or low depending on their location to the water at time of peak low tide. Pools located near the water's edge or subtidal zone and usually covered in fleshy macroalgae were referred to as low, whereas barren pools near terrestrial vegetation and above the gastropod *Nerita* spp. and within the gastropod *Littoraria* spp. zone were referred to as high. Any pools found between the high and low pools were referred to as mid. Pools in the high zone were within the intertidal and not supratidal as these pools are submersed on the incoming high tide (personal observations TEC). Fishes were collected from at least two pools in each position (high, mid, low) at each site for a total of 6-8 pools.

Each site was visited once and sampled for fishes in high, mid, and low pools (Table 3.1). All sampling occurred during the summer months May-August 2008, on a spring low tide. Multiple sites could not be sampled in one day as microtidal conditions quickly limits access to pools. Summer months were chosen for the sampling period because this is when spring low tides co-occur in daylight hours. These spring low tides ranged from -0.12- to -0.06 m. The peak low tides during the sampling period occurred in the morning hours 0600-1011. Sampling began at least one hour prior to peak low tide and continued until high tide prevented accessibility of pools. High, mid and low pools were haphazardly sampled during each site visit.

Non-destructive sampling was preferred as it lessens the impact on the tidepool community and studies on methodology in other intertidal habitats have found similar results regardless of techniques (Gibson 1999, Griffiths 2000). A battery operated submersible bilge pump and various sized buckets were used to drain and bail each pool of seawater. Any fishes present were scooped up by hand or with hand-net. A chopstick or finger was used to probe gently into holes and crevices to ensure the capture of small cryptic fishes. Captured fishes were kept alive and placed in aerated buckets of seawater for identification and measurements.

To determine the abundance and diversity of fishes each individual collected was counted, identified, and measured prior to release. In the field, fishes were anesthetized in buckets of seawater with MS-222 and then identified to the lowest possible taxon using dichotomous keys of Hawaiian Shore Fishes (Randall 2007). A hand lens was used to view any diagnostic features difficult to observe with an unaided eye. Once the species was identified and recorded, the standard length of fishes greater than 15 mm SL was measured. Each individual was assigned an id number, and its size and locality (both site and pool position) recorded. To minimize impact to the tidepool community, fishes were revived in aerated seawater and released to the tidepool from which they were collected after pools were inundated from rising tides or to a nearby location. Fishes were kept in buckets until all sampling had concluded to avoid re-sampling. On rare occasions, fishes were returned while sampling was ongoing but any sampled pools were > 20 meters from release site.

The statistical software package Primer-E (Clarke and Warwick 2001) was used to analyze the spatial distribution and abundance of fishes among and across shores. Because of the difficulty in identifying small *Bathygobius* spp. a conservative approach was taken and in this analysis all *Bathygobius* spp. were grouped into one taxonomic category. However, results did not differ when all *Bathygobius* were grouped by genus or when those identified to species level were considered separately. These counts of fishes were expressed as a proportion of total number of individuals found per pool and each pool was considered a replicate of position (high, mid, or low) nested within a site. Abundances were square-root transformed to down-weight common species and account for the patchy nature of tidepool species (Gibson and Yoshiyama 1999). These data were then used to construct Bray-Curtis similarity matrix between sites and pool position. Dendrograms were used to visualize the similarity of fishes by site, shore substrate type, and pool position. Further, PERMANOVA with pool position nested within sites and sites nested within substrate was used to statistically test the hypothesis that fishes were vertically and spatially distributed. A series of one-way SIMPERs were used to analyze which species contributed to the observed similarity patterns.

To examine if sizes of fishes varied across the shore we compared the standard length (mm) of the most abundant species that occurred on O‘ahu: *Abudefduf sordidus*, *Bathygobius cocosensis*, *Entomacrodus maramoratus*, and *Istiblennius zebra*. Sizes of fishes across sites were pooled for each tide pool position and differences between length medians were tested with Kruskal-Wallis or Mann-Whitney tests.

To describe the assemblage and test diversity hypotheses, species richness (S) and Shannon (H') indices were computed for each pool position at each shore and for the island of O'ahu. For site and position comparisons, each pool was considered a replicate sample and computed values were compared statistically with a two-way ANOVA (sites and position). Prior to testing data were log transformed to meet parametric requirements and alpha values were adjusted to account for multiple comparisons. To determine S and H' for the island of O'ahu all species were pooled from every site and values reported.

To characterize conditions experienced by tidepool fishes in O'ahu, a snapshot sample of physical conditions and surroundings were collected from tidepools during the sampling period. Prior to fish collection, the maximum pool depth, length, and width was measured with a transect tape and were used to calculate a rough estimate of pool volume. Salinity measurements (‰) were collected with a handheld refractrometer, and a visual estimate was made of algal percent cover within and along the edges of pools. The surface water temperature was recorded with 2-3 Hobo temperature loggers placed in sampled and unsampled pools during the low tide window. At some sites measurements were not collected because of instrument failure or observer oversight, thus only sites with all measurements were included in analyses.

In order to test if any of these physical features were related to observed fish assemblage patterns a distance based redundancy ordination analysis (dbRDA) was used in combination with a distance based linear model. The distance based linear model (DISTLM function in PRIMER-E) models the relationship between predictor variables and the multivariate data cloud based on a multiple regression. This routine finds the linear combination of variables that best explains the greatest variation in the data cloud and the amount of variance each covariate explains separately providing a pseudo-F stastical value. dbRDA is an ordination analyses that visualizes these results. Predictors that best explain the data cloud are viewed as vectors in a biplot. The longer the vector the larger the effect of the predictor (Anderson et al. 2008).

Results

A total of 343 fishes were recorded and 327 individuals actually captured from six sites (40 sampled tidepools) on the island of O‘ahu. Fishes that were observed but not captured were often young-of- the-year gobies or blennies. Of the 327 captured, 25 taxa were recorded and 19 species (H’ = 2.5) identified from 10 families (Table 3.2, 3.3). Those taxa identified to only the family or genus level were of small size and belonged to the genera *Bathygobius*, *Entomacrodus*, *Thalassoma*, or Family Creediidae. The most abundant fishes were from 4 families and include (in order of abundance) *Bathygobius cocosensis* (Gobiidae), *Istiblennius zebra* (Blenniidae), *Abudefduf sordidus* (Pomacentridae), *Entomacrodus marmoratus* (Blenniidae), and *Acanthurus triostegus* (Acanthuridae) (Table 3.2 and 3.3).

The nMDS and dendrograms (Fig. 3.2) reveal a large amount of overlap in assemblage similarity among sites and pool position, although the centroid based nMDS plot (Fig. 3.2, top) shows clusters of sites based on substrate type (basalt or limestone). The limestone sites are less clustered than basalt sites as the pool samples from Nānākuli are more distinct. Furthermore, site assemblages differed among high, mid, and low pools (Fig. 3.2, bottom). Results from the PERMANOVA support significant differences among pool positions, sites, and sites with different substrate types (Table 3.4).

Different abundances of the common fishes contribute to the dissimilarity among tested groups (Table 3.2 and 3.5). Although the presence of species was similar among basalt and limestone based shores, there were significant differences in the proportion of blennies and gobies. *Bathygobius* spp. occurred at higher proportions on basalt shores while the blennies *I. zebra* and *E. marmoratus* occurred at higher proportions at limestone shores (Table 3.5). Within the basalt shores (Diamond Head, Makapu‘u, Sandy Beach) roughly 20% of community dissimilarity was accounted for by the differing proportions of *I. zebra* and *Bathygobius* spp. (Table 3.2). *Abudefduf sordidus* was absent from Sandy Beach but abundant at both Diamond Head and Makapu‘u. This species accounts for 30% difference between Sandy Beach and Diamond Head and 12% of the differences between Sandy Beach and Makapu‘u. Assemblages also varied among limestone sites. Nānākuli assemblage was most dissimilar from other limestone sites (84% dissimilar from Turtle Bay, 88% dissimilar from ‘Ewa Beach) as it has a

Table 3.2 Proportion and total # of fish species by family (F) that were collected and identified in the high, mid, and low pools at the 6 sites. *B.* spp = *Bathygobius*, *E.* spp= *Entomarcodus*, *T.* spp = *Thalassoma*; see Table 3.3 for other taxonomic abbreviations

Taxa	**F**	**Diamond Head**			**Makapu‘u**			**Sandy Beach**			**‘Ewa Beach**			**Nānākuli**			**Turtle Bay**			**Total #**
		H	M	L	H	M	L	H	M	L	H	M	L	H	M	L	H	M	L	
B. cocosensis	G	-	0.6	0.6	-	0.6	0.0	-	0.8	0.3	-	-	-	-	-	-	0.3	0.1	0.3	64
I. zebra	B	0.3	-	-	0.7	0.1	0.0	0.1	0.2	0.2	0.2	0.4	-	1.0	-	-	0.2	0.2	0.4	63
A. sordidus	P	0.8	0.3	0.2	-	0.1	0.2	-	-	-	-	0.1	0.1	-	1.0	1.0	-	0.0	-	51
B. spp.	G	-	-	0.2	0.3	-	0.1	0.7	-	0.2	0.5	-	-	-	-	-	0.3	0.2	0.3	46
E. marmoratus	B	-	0.1	0.0	-	-	-	-	-	-	0.1	0.4	0.7	-	-	-	-	0.1	0.1	36
A. triostegus	A	-	-	-	-	0.1	0.2	-	-	0.3	-	-	-	-	-	-	0.1	0.1	-	20
K. sandvicensis	K	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	0.0	-	7
B. coalitus	G	-	-	-	-	-	-	-	-	-	0.2	0.1	0.0	-	-	-	-	0.0	-	6
E. spp.	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.1	-	6
K. xenura	K	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	5
T. purpureum	L	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	0.0	-	5
Creediidae	C	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	3
M. cephalus	M	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	3
B. cotticeps	G	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	2
T. spp.	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	-	2
A. abdominalis	P	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	1
B. gibbifrons	B	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	1
C. lunula	Ch	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	1
C. obscurus	B	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	1
D. griessingeri	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	-	1
G. anjerensis	G	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	1
P. imparipennis	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	1
S. balteata	L	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Total #		12	15	25	6	14	45	7	12	19	11	17	23	18	3	18	16	50	16	327

Table 3.3 Distribution, habitat, and resident status of tidepool species and their families (family abbreviation follows) with a comparison to assemblage determined by Gosline (1965). R = resident species (permanent inhabitants), PR = partial residents (spend part of life in intertidal), T= transients (visitors); + = present - = absent in Gosline (1965) splash zone assemblage. Distribution and habitat according to Randall (2007), definitions follow Gibson (1982)

Family ***Genus species***	**Biogeographic Distribution**	**Habitat**	**Resident Status**
Acanthuridae			
Acanthurus triostegus	Indo-Pacific, Tropical E. Pacific	Juveniles in tidepools, adults shallow water	PR
Blenniidae			
Blenniella gibbifrons	Indo-Pacific	1-3 m	R
Cirripectes obscurus	Hawaiian Islands	1- 6 m	R
Entomacrodus marmoratus	Hawaiian Islands	Tidepools	R
Chaetodontidae			
Chaetodon lunula	Indo-Pacific	1-158 m on coral reefs	T
Creediidae	N/A	15 – 20 m	?
Gobiidae			
Bathygobius coalitus	Indo-Pacific, W. Pacific	Intertidal zone	R
Bathygobius cocosensis	Indo-Pacific	Tidepools	R
Bathygobius cotticeps	Indo-Pacific, W. Pacific	Rocky tidepools, lower intertidal	R
Discordipinna griessingeris	Indo-Pacific	1-37 m	?
Gnatholepis anjerensis	Indo-Pacific	Usually occurs in > 2 m, tidepools	R
Kuhliidae			
Kuhlia sandvicensis	Indo-Pacific	Shallow-water	PR
Kuhlia xenura	Hawaiian Islands	Juveniles occur in tidepools, adults offshore	PR
Labridae			
Stethojulis balteata	Hawaiian Islands & Johnston Island	Shallow-water to 22 m	T
Thalassoma purpureum	Indo-Pacific	Rocky shores shallow-water	T
Mugilidae			
Mugil cephalus	Circumglobal warm-waters	Inshore protected waters	T
Pomacentridae			
Abudefduf abdominalis	Hawaiian Islands & Johnston Island	Young often in tidepools, adults inshore	PR
Abudefduf sordidus	Indo-Pacific	Young often in tidepools, adults inshore	PR
Plectroglyphidodon imparipennis	Indo-Pacific	Reefs usually > 4 m	R

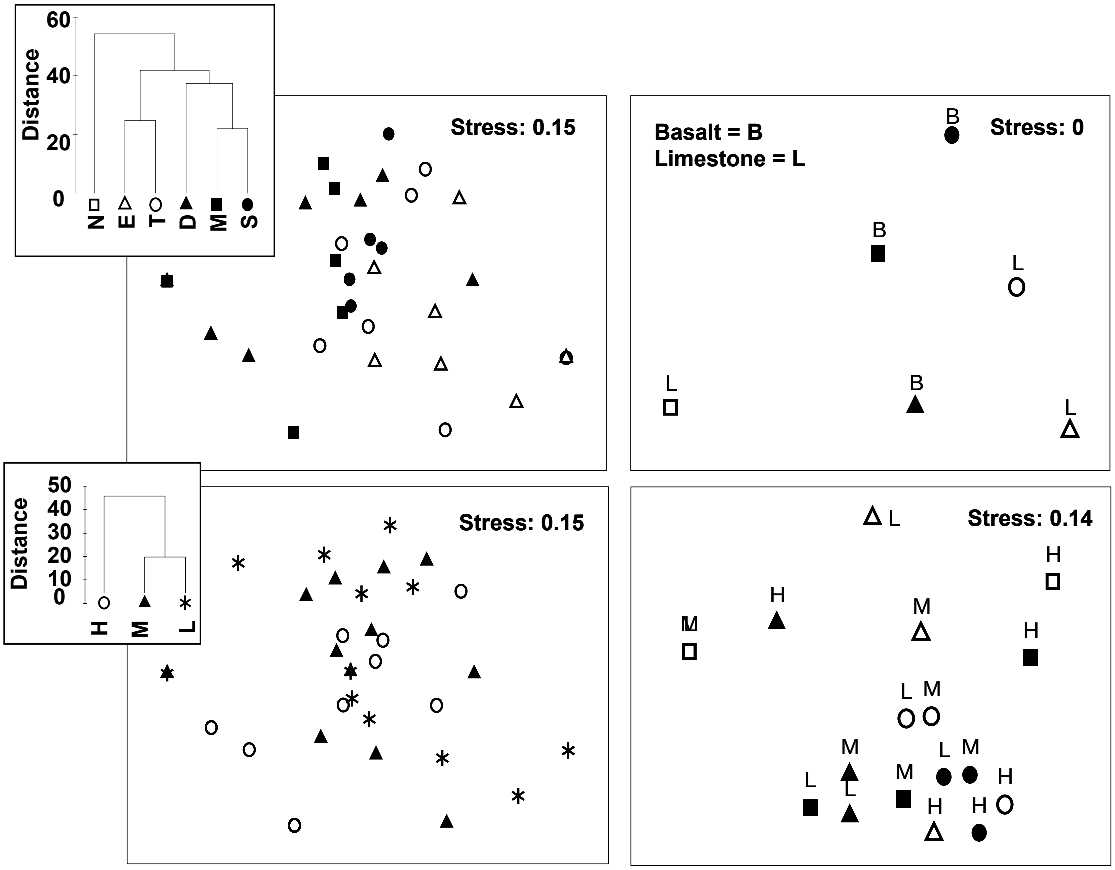

Fig. 3.2 Non-metric multidimensional scaling ordinations (nMDS plots) on the basis of Bray-curtis dissimilarity measure of each pool (top left symbols = sites, see Table 3.1 for abbreviation of site names; bottom left symbols = position) and of centroids (right) of sites (top right) and pool position nested within sites (bottom right symbols = sites). Dendrograms in upper left corners are the similarity distance between the centroids of sites (top left) and pool position (bottom left) and serve as a legend for symbols in nMDS plots. Note the differences shown as distance between basalt and limestone based shores, sites, and pool position

Table 3.4 Results of PERMANOVA showing the significant assemblages of fishes. Results are out of 1,000 permutations

Source of variation	*df*	SS	MS	Pseudo-F	*P* (perm)	Unique Perms
Substrate = Su	1	5742.7	5742.7	3.9	0.01	999
Sites (Su)	4	20119.0	5029.8	3.4	0.001	998
Position (Sites (Su))	12	41222.0	3435.2	2.4	0.001	997
Residual	22	32114.0	1459.7			

higher proportion (0.7) of *I. zebra* (which accounted for ~20% of said dissimilarities). The composition at ‘Ewa Beach and Turtle Bay were only 65% dissimilar and the abundance of *E. marmoratus* accounted for 20% of this dissimilarity.

The composition of fishes varied vertically across the shore (Fig.3.2). Assemblages differences were greatest between high and low pools (Fig. 3.2, Table 3.5). *Istiblennius zebra* was more common in high pools and *E. marmoratus*, *A. triostegus*, and *Thalassoma* spp. were more common in low pools (Table 3.5). Also, out of four common species examined (*A. sordidus*, *Bathygobius* spp., *E. marmoratus*, and *I. zebra*) the median SL (mm) of *I. zebra* was smaller in high pools (Kruskal-Wallis, *p*-value <0.001, Dunn’s Method, *p*-value <0.05) (Fig. 3.3). High pools can therefore be characterized by a high number of small sized individuals of *I. zebra*.

Diversity of fishes measured as species richness and H’ did not statistically (at adjusted α = 0.025) vary across shores but did vary among sites (S: Two-way ANOVA, *p*-value <0.01; H’: two-way ANOVA, *p*-value < 0.001) (Fig. 3.4). Nānākuli (S= 1.0± 0.2 SE, H’ = 0.0 ±0.0 SE) had low species richness and H’ index but was within range of values determined for Diamond Head (S= 2.0 ± 0.3 SE, H’ = 0.5± 0.2) and Sandy Beach (S = 2.3±0.2 SE, H’= 0.7+0.1 SE). Diversity was higher for Makupu`u (S= 3.5± 0.6 SE, H’ = 0.9 ±0.3 SE), Ewa Beach (S = 3.2 ± 1.3 SE, H’ = 0.9 ± 0.2 SE) and Turtle Bay (S= 3.7±0.7 SE, H’ = 0.9±0.2SE).

Measured conditions experienced by fishes during sampling period varied between high, mid, and low pools (Table 3.6). Temperature varied notably as pool temperatures co-varied with substrate type. Basalt shores were warmer than limestone shores. The distance based linear model found temperature to be a significant predictor

Table 3.5 Results from SIMPER analyses showing the species and their proportions contributing to ~80% of the dissimilarity among basalt and limestone shores and across high, mid, and low pools

Dissimilarity = 71.3%	**Basalt**	**Limestone**			
Species	Av. Abund	Av. Abund	Av. Dissimilarity (Stdev)	% Contribution	Cumulative %
Bathygobius spp.	0.6	0.3	16.3 (1.2)	22.9	22.9
Istiblennius zebra	0.3	0.4	14.3 (1.1)	20.1	43.0
Abudefduf sordidus	0.3	0.2	11.8 (0.8)	16.5	59.5
Entomacrodus marmoratus	0.1	0.3	10.3 (0.8)	14.5	74.0
Acanthurus triostegus	0.1	0.1	3.5 (0.5)	4.9	78.9
Dissimilarity = 67.3%	**High**	**Mid**			
Species	Av. Abund	Av. Abund	Av. Dissimilarity (Stdev)	% Contribution	Cumulative %
Istiblennius zebra	0.6	0.3	17.6 (1.2)	26.2	26.2
Bathygobius spp.	0.3	0.6	16.6 (1.2)	24.7	50.9
Abudefduf sordidus	0.2	0.3	12.6 (0.8)	18.6	69.5
Entomacrodus marmoratus	0.0	0.2	6.4 (0.7)	9.5	79.0
Acanthurus triostegus	0.0	0.1	1.0 (0.5)	2.8	81.8
Dissimilarity = 67.1%	**Mid**	**Low**			
Species	Av. Abund	Av. Abund	Av. Dissimilarity (Stdev)	% Contribution	Cumulative %
Bathygobius spp.	0.6	0.5	14.4 (1.1)	21.4	21.4
Entomacrodus marmoratus	0.2	0.3	12.5 (0.9)	18.7	40.1
Abudefduf sordidus	0.3	0.2	11.8 (0.9)	17.6	57.7
Istiblennius zebra	0.3	0.1	9.7 (1.0)	14.4	72.1
Acanthurus triostegus	0.1	0.1	4.6 (0.6)	6.8	78.9
Thalassoma purpureum	0.0	0.1	2.2 (0.5)	3.3	82.1
Dissimilarity = 79.5%	**High**	**Low**			
Species	Av. Abund	Av. Abund	Av. Dissimilarity (Stdev)	% Contribution	Cumulative %
Istiblennius zebra	0.1	0.6	20.9 (1.4)	26.4	26.4
Bathygobius spp.	0.5	0.3	14.6 (1.2)	18.4	44.7
Entomacrodus marmoratus	0.3	0.0	11.5 (0.7)	14.4	59.2
Abudefduf sordidus	0.2	0.2	10.2 (0.8)	12.9	72.0
Acanthurus triostegus	0.1	0.0	4.3 (0.5)	5.4	77.4
Thalassoma purpureum	0.1	0.0	1.7 (0.4)	2.2	79.6

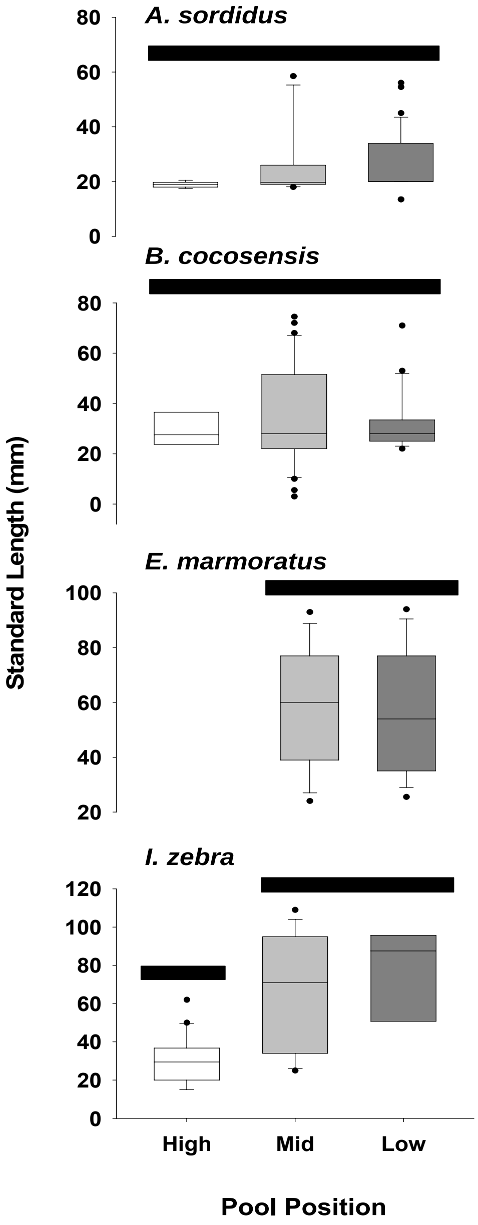

Fig. 3.3 Boxplots showing the distribution (median, quartiles, and outliers) of standard length (mm) for four common taxa *A. sordidus* (n = 51), *B. cocosensis* (n = 63), *E. marmoratus* (n = 34), and *I. zebra* (n = 51) collected from high, mid, and low pools. Bars above boxplots represent similar statistical groupings from Dunn's multiple comparisons test when Kruskal-Wallis or Mann-Whitney test indicated significant differences among pool position

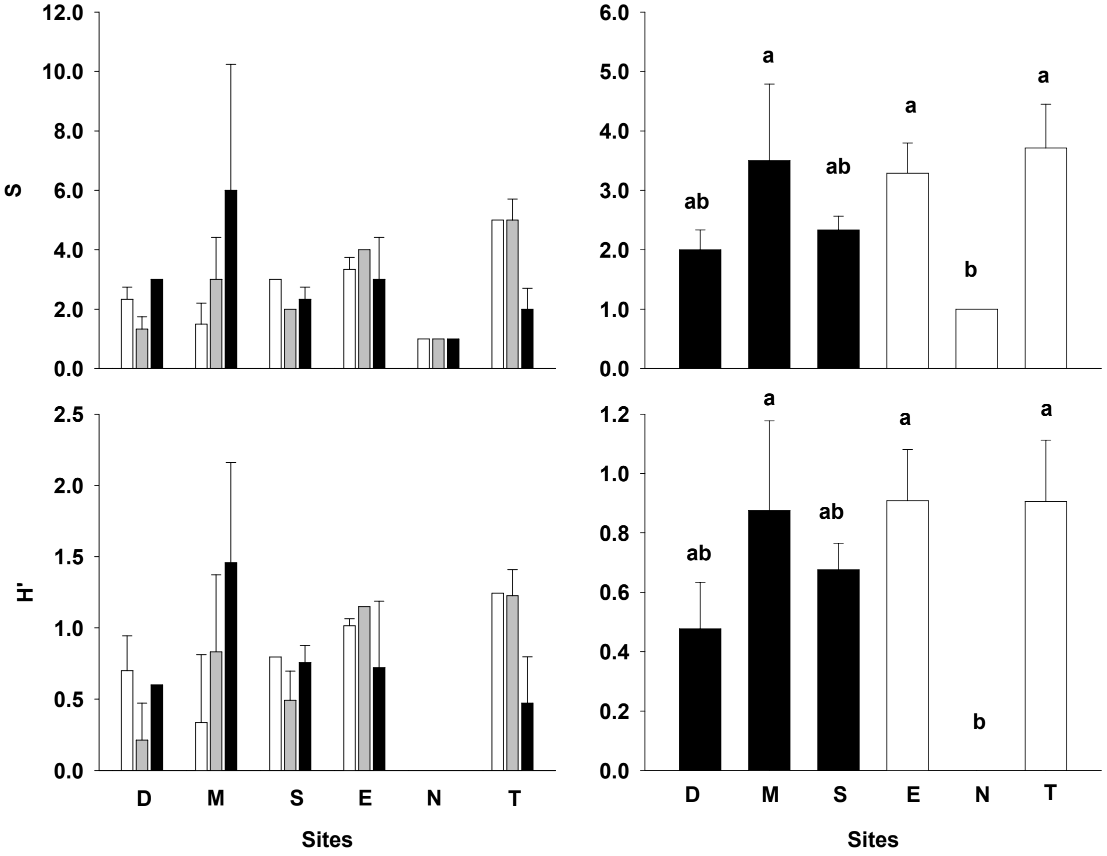

Fig. 3.4 Comparison of species richness (S, top) and diversity (H' bottom) for tidepool fishes among six shores (right: black = basalt, white = limestone shores) and among high (white), mid (gray), low (black) pools nested within site (left) on the island of O'ahu. Letter groups above bars represent similar statistical groups (Two-way ANOVA with an adjusted $\alpha < 0.025$)

Table 3.6 Average (SE) of environmental variables collected across the shore at 6 sites

	Approximate Volume (m^3)	Depth (cm)	Temperature (°C)	Salinity ($^0/_{00}$)	Algal Cover (%)
Diamond Head					
High	0.5 (±0.1)	33.8 (± 2.0)	28.1 (±0.7)	33.2 (±0.7)	22.2 (±14.5)
Mid	0.2 (±0.1)	42.3 (± 5.0)	27.4 (±0.5)	33.5 (±0.3)	60.7 (±16.0)
Low	1.0	40.0	28.1	33.0	100.0
Makapu‘u					
High	0.0 (±0.0)	20.0 (± 0.0)	27.3 (±2.8)	33.2 (±0.7)	-
Mid	0.3 (±0.3)	27.1 (±21.2)	25.0 (±1.0)	32.9 (±1.4)	16.4 (±10.6)
Low	1.9 (±1.3)	37.3 (± 7.1)	25.0 (±0.8)	38.5 (±1.4)	5.3 (±14.1)
Sandy Beach					
High	0.3	30.0	24.2	47.0	-
Mid	1.0 (±0.3)	40.0 (±14.1)	24.2 (±0.3)	40.3 (±6.4)	35.0 (±42.4)
Low	1.1 (±0.7)	29.7 (± 7.4)	24.4 (±0.4)	40.8 (±3.2)	77.2 (± 3.5)
‘Ewa Beach					
High	0.5 (±0.2)	27.3 (± 2.0)	22.7 (±0.0)	36.7	-
Mid	1.0	45.0	23.8	35.0 (±0.4)	20.0
Low	0.1 (±0.1)	20.3 (± 8.9)	24.1 (±0.3)	41.0 (±3.6)	90.0
Nānākuli					
High	0.5 (±0.3)	43.3 (± 2.9)	-	34.7 (±0.3)	0.0 (± 0.0)
Mid	0.4	35.0	-	35.0	0.0 (± 0.0)
Low	0.6	35.0	-	35.0	0.0 (± 0.0)
Turtle Bay					
High	0.2	15.0	25.0	35.0	25.0
Mid	0.5 (±0.2)	18.1 (±12.7)	25.3 (±1.3)	33.1 (±0.7)	59.5 (± 30.1)
Low	0.2 (±0.2)	12.9 (± 2.9)	25.3 (±0.7)	33.6 (±0.4)	74.1 (± 18.7)

(Pseudo-F = 2.9, p-value < 0.001) of assemblages but, the overall best model included all variables (pool volume, depth, salinity, macroalgal cover, and temperature). This can be seen in the dbRDA biplots (Fig. 3.5). The first three axes of the dbRDA explain 94% of the variation of the fitted model but only 25% of the total variation in the data pool. Tidepools at basalt and limestone shores fall along the these axes varying with temperature and macroalgae; basalt shores tended to have a positive correlation with dbRDA axes 1 while limestone shores had a negative correlation with dbRDA axes 1.

Sixty-eight percent of sampled tidepools had a full set of environmental measurements and were included in these multivariate analyses.

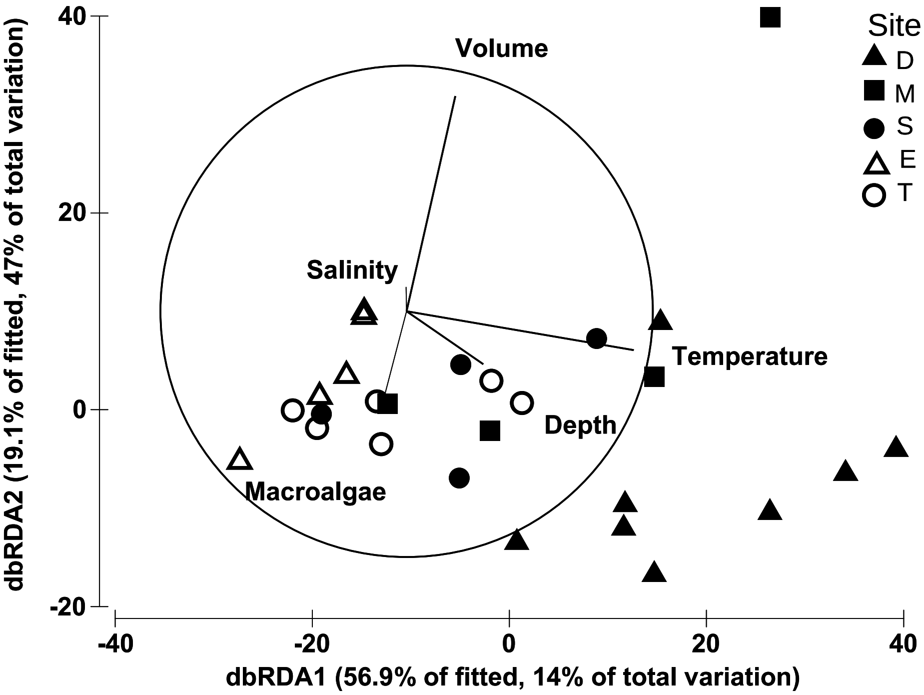

Fig. 3.5 dbRDA ordination plot displays the relationship between environmental predictors that best explain the variation among pools at different sites. The vectors within the circle show the "effect" of the predictor variables included in the model, the longer the vector from the center the larger the "effect". Basalt pools are represented by solid symbols while limestone shores are represented by open symbols. Assemblages sampled from basalt pools tend to positively correlate with temperature while assemblages from limestone shores negatively correlated with temperature and fell along the macroalgae cover vector

Discussion

Our study revealed a tidepool fish assemblage for O'ahu island composed of 19 species from 10 families. Results of this study support the hypothesis that basalt and limestone shores have distinct intertidal fish assemblages and that these assemblages are possibly related to temperature. Results also support the hypothesis that the tidepool fishes are vertically distributed. This vertical distribution coupled with the variation of assemblages among sites and substrate types, reveals a surprising amount of structure within a limited geographical area and across a narrow shore.

The tidepool fish assemblage in O'ahu is similar to other isolated tropical islands which have low species richness and a high number of endemic species, yet assemblage members are from taxonomic families that occur throughout the tropics. The tidepool species on O‘ahu were largely distributed across the Indo-West Pacific (68%) and 32% were endemic to Hawai‘i and Johnston Atoll. This level of endemism is within range of the 25% determined by Randall (2007) for all marine fishes in Hawai‘i and it is higher than percentages recorded for most regions (Prochazka et al. 1999) yet it is lower than 61.7% of endemic rockpool fishes (mostly tripterygiids) that occur in cool temperate New Zealand waters (Paulin and Roberts 1993). The most abundant fish families were Gobiidae (5 species)> Blenniidae (4 species) > Acanthuridae (2 species) > Pomacentridae (3 species), and Kuhliidae (2 species). Most of these families are common in studied tropical intertidal habitats throughout the world except kuhliids which are absent in the west Atlantic (Hiatt and Strasburg 1960, Gosline 1965, Lee 1980, Greenfield and Johnson 1990, Duhart and Ojeda 1994, Mahon and Mahon 1994, Prochazka et al. 1999, Greenfield 2003). Notable differences include the absence of serranids, muraenids, and gobiesocids. Gobiesocids are common in many temperate intertidal regions and are found in a few tropical localities but are absent in Hawai‘i (Prochazka et al. 1999). Serranids are mostly absent in shallow-water collections in Hawai‘i except for a non-indigenous grouper that is common on coral reefs (Randall 2007). In the Indo-West Central Pacific province (where Hawai‘i is located) (Duhart and Ojeda 1994) muraenids are commonly found in tidepool communities. Also muraenids are prominent in tidepool collections from Barbados (Mahon and Mahon 1994) and the Marshall Islands (Hiatt and Strasburg 1960) and have been observed in Hawaiian tidepools. The absence of eels in this study could be because of their high mobility and that they are likely to occur in relatively low densities or are likely to be in larger pools that are only isolated from the ocean on the lowest of low tides. It is also possible that more tidepool species on O‘ahu would be found if sampling occurred across the year, however, the tropics lack strong seasonality and tropical fish assemblages can be stable over short temporal scales (Chang et al. 1973, Castellanos-Gallindo et al. 2005). Similar species richness is observed in tidepools at other isolated island chains. For instance, five species were recorded from a study on Easter Island (Duhart and Ojeda 1994), and 23 species were reported in the Seychelles (Prochazka et al. 1999), where as Taiwan, also located in the Indo-West

Pacific, has 122 species (Prochazka et al. 1999). Thus it is more likely that low species richness is due to the isolated nature of the island chain and the dispersal ability of fishes.

We found many of the same species in O‘ahu’s tidepools that Gosline (1965) observed in the intertidal zone for the main islands of Hawai‘i yet, this study quantifies nearly double the number of species in the intertidal zone. Species richness in exposed rockpools on O‘ahu is likely still higher than we determined as a few species, such as holocentrids, were observed but not sampled. Most of the fishes recorded during this study but not included by Gosline (1965) were transient or cryptic. Differences in methodology and definition of habitat possibly account for the discrepancy in species composition between studies. Gosline (1965) made observations of near-shore fishes for the Hawaiian Islands and refers to a spray, splash, and a surge zone. The splash zone is strictly intertidal and the surge zone he describes can be above or below the mean tidal level. This study quantitatively samples fishes in pools at mean low low water for O‘ahu and thus distinguishes the boundary between intertidal and subtidal zones.

Although the density of fishes varied among limestone and basalt based shores, it is unclear what causes the differing assemblages. In this study basalt shores tended towards higher temperatures and temperature was a predictor of assemblage variation. *Bathygobius* spp., more common on basalt shores, is known to have a high temperature tolerance (thermal maxima ~40°C) (Morina and Ospina 2001). However, a limited set of predictor variables were collected and used in the linear model and temperature alone did not explain a high amount of variation observed in the data pool. Further, *I. zebra* can tolerate conditions on basalt shores as it was present at all shores. Intertidal fishes commonly use holes and crevices for shelter and nesting (Duci et al. 2009). Limestone shores which maybe more easily eroded could provide crevices that suit the recruitment or survival of blenniid species, like *I. zebra* and *E. marmoratus* A similar conclusion was reached by Macpherson (1994) in the Mediterranean as a blenny species was absent in a habitat that lacked crevices in three separate sites. Alternatively, this assemblage difference between basalt and limestone shores could be the result of small number of sites sampled in this study. The addition of assemblages from more basalt and limestone shores could clarify the relationship between substrate and abundance patterns.

The among-site differences observed in this study are surprising given the small geographic distance between sites, the similarity in habitat within substrate groups, and the similar small number of species that occur in pools. These differences may be due to temporal recruitment of juveniles or the variation in the nearby subtidal habitat since the assemblages differed in number of partial resident and transient species. *Acanthurus triostegus*, *A. sordidus,* and *A. abdominalis* are known to recruit to tidepools in summer months. These species grow quickly and move to lower pools and onto reefs as they mature (Randall 2007). Godinho and Lotufo (2010), in Brazil found sites to differ in intertidal fish assemblages and similarly suggested this relationship was due to recruitment differences over a small geographical scale. Seasonality and recruitment at the assemblage level has not been studied for intertidal fishes in Hawai‘i and no conclusive statements can be made.

Similar to several temperate intertidal environments (Zander et al. 1999, Griffiths et al. 2003), the tidepool fish assemblages in O‘ahu were found to be vertically structured and these patterns in abundance are hypothesized to be related to tolerances to harsh conditions that vary across the shore. Diversity as H’ and species richness did not vary statistically across the shore but, relatively small within-site sample sizes likely hindered the ability to detect such differences. For the island of O‘ahu small sized *I. zebra* were abundant in high pools while most other species and larger sized *I. zebra* occurred in mid and low pools. This pattern suggests that harsh conditions are found near shore and that this “high” pool species may have adaptations or morphological features which allow it to survive (Nakamura 1976, Horn and Riegle 1981, Martin 1995, Zander et al. 1999). Indeed another species of *Istiblennius, I. edentulus* which emerges into air in the wild have sense organs suited for intertidal life and behavioral characteristics that prevent rapid desiccation (Zander et al. 1999). In temperate latitudes, temperature, salinity, aerial exposure, ultraviolet radiation, and wave action are abiotic factors that often vary with shore height or pool depth (Metaxas and Scheibling 1992, Denny and Paine 1998, Zander et al. 1999). Hawai‘i’s microtidal regime combined with varying wave heights may alleviate or alter the type of harsh conditions experienced by tidepool fishes, especially in the mid to low pools. However, regardless of microtidal conditions temperature is likely to be a driving environmental factor in tropical localities as organisms experience some of the highest temperatures worldwide.

Biotic conditions, such as predation and competition, can also vary across the shore (Connell 1961) and can contribute to tidepool assemblages (Zander et al. 1999). For example, macroalgae that provides food and shelter for fishes varied in abundance across the sampled shores. Although herbivory tends to be more prominent in the tropics (Horn 1989, Floeter et al. 2005), the diets of many of these tidepool fishes are not known. In addition, macroalgal cover could be structured by similar physical factors and be unrelated to fish distributions. Another biotic condition that can structure communities is predation. Many have suggested that tidepools serve as a nursery for juvenile reef fishes providing a refuge from fish predators (Gibson and Yoshiyama 1999). Indeed, juveniles of the coral reef fishes *Abudefduf sordidus* and *Acanthurus triostegus* were common in mid and low pools. Partitioning through inter-specific competition or by different evolutionary histories could also result in co-occurring species living in different habitats (Zander et al. 1999, Davis 2000). On O‘ahu, species within the genus *Bathygobius* seem to be partitioned into different zones or shores and two blennies *Istiblennius zebra* and *Entomacrodus marmoratus* are also distributed in different areas of the shore (high-mid and mid-low respectively). Additionally intra-specific competition could account for the skewed smaller median size of *I. zebra* in high pools as intra-competition outcomes usually depend on body size (Mayr and Berger 1992). It is also likely that other fishes in this study had skewed body sizes with shore position but this could not be tested with our current sample sizes.

In conclusion, O‘ahu’s tidepool fish assemblage is represented by high endemism and low species richness similar in percentage and number to the general shallow reef fish assemblage in Hawai‘i. Additionally, O‘ahu’s tidepools are dominated by taxonomic families found in other tropical localities (gobiids, blenniids, pomacentrids, and acanthurids). Assemblages varied spatially among sites, among shores, and vertically across shores with pool position. High pool assemblages were the most distinct from low pools and were dominated by small sized *I. zebra*. Future experiments could expand on this study by investigating 1) temporal variation 2) the diets of common species, 3) perform more quantitative measurements of physical conditions in pools 4) identify species tolerances to physical conditions and 5) investigate inter-specific and intra-specific competition.

References

Abbott IA (1999) Marine red algae of the Hawaiian Islands. Bishop Museum Press, Honolulu.

Anderson M.J., Gorley R.N., and Clarke KR. 2008. PERMANOVA+ for Primer: A guide to software and statistical methods PRIMER-E Ltd, Plymouth.

Bird CE (2006) Aspects of community ecology on wave-exposed rocky Hawai'ian coasts. PhD, University of Hawai'i, Honolulu. 255 pp.

Boyle KS, Horn MH (2006) Comparison of feeding guild structure and ecomorphology of intertidal fish assemblages from central California and central Chile. Mar Ecol Prog Ser 319: 65-84.

Chang K-H, Lee S-C, Lee J-C, Chen C-P (1973) Ecological study on some intertidal fishes of Taiwan. Bull Inst Zool Acad Sinica 12:45-50.

Clarke KR, Warwick RM (2001) Changes in marine communities: An approach to statistical analysis and interpretation. Primer-E Ltd, Plymouth.

Connell JH (1961) The influence of intra-specific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. Ecol 42: 710-723.

Davis JL (2000) Spatial and seasonal patterns of habitat partitioning in a guild of southern California tidepool fishes. Mar Ecol Prog Ser 196: 253-268.

Denny MW, Paine RT (1998) Celestial mechancis, sea-level changes, and intertidal ecology. Biol Bull 194: 108-115.

Duci A, Giacomello E, Chimento N, Mazzoldi C (2009) Intertidal and subtidal blennies: assessment of their habitat through individual and nest distribution. Mar Ecol Prog Ser 383: 273-283.

Duhart M, Ojeda FP (1994) Ichthyological characterisation of intertidal pools, and trophic analysis of herbivore subtidal fishes of Easter Island. Med Amb 12: 32-40.

Floeter SR, Behrens MD, Ferreira CEL, Paddack MJ, Horn MH (2005) Geographical gradients of marine herbivorous fishes: patterns and processes. Mar Biol 147:1435-1447

Friedlander AM, DeMartini EE (2002) Contrasts in density, size, and biomass of reef fishes between the northwestern and the main Hawaiian Islands: the effects of fishing down apex predators. Mar Ecol Prog Ser 230: 253-264.

Gibson RN (1999) Methods for studying intertidal fishes. In*:* Horn MH, Martin KLM, Chotkowski MA (ed) Intertidal fishes, Life in two worlds, Academic Press, San Diego

Gibson RN, Yoshiyama RM (1999) Intertidal fish communities. pp. 264-295. In*:* Horn MH, Martin KLM, Chotkowski MA (ed) Intertidal fishes, Life in two worlds, Academic Press, San Diego

Godhino WO, Lotufo TM (2010) Local *v.* microhabitat influences on the fish fauna of tidal pools in north-east Brazil. J of Fish Biol 76: 487-501.

Gosline WA (1965) Vertical zonation of inshore fishes in the upper water layers of the Hawaiian Islands. Ecol 46: 823-831.

Greenfield DW (2003) A survey of the small reef fishes of Kane'ohe Bay O'ahu, Hawaiian Islands. Pac Sci 57: 45-76.

Greenfield DW, Johnson RK (1990) Community structure of western Caribbean blennioid fishes. Copeia 1990: 433-448.

Griffiths SP (2000) The use of clove oil as an anaesthetic and method for sampling intertidal rockpool fishes. J Fish Biol 57: 1453-1464.

Griffiths SP (2003) Rockypool ichthyofaunas of temperate Australia: species composition, residency, and biogeographic patterns. Estuar Coast Shelf Sci 58: 173-186.

Griffiths SP, Davis AR, West RJ (2006) Role of habitat complexity in structuring temperate rockpool ichthyofaunas. Mar Ecol Prog Ser 313: 227-239.

Griffiths SP, West RJ, Davis AR (2003) Effects of intertidal elevation on the rockpool ichthyofaunas of temperate Australia. Environ Biol Fish 68: 197-204.

Hiatt RW, Strasburg DW (1960) Ecological of the fish fauna on coral reefs of the Marshall Islands. Ecol Monogr 30: 65-127.

Horn MH (1989) Biology of marine herbivorous fishes. Oceanogr Mar Biol Ann Rev 27: 162-272.

Horn MH, Martin KLM, Chotkowski, MA (1999) Intertidal Fishes, Life in two worlds. Academic Press, San Diego

Horn MH, Riegle KC (1981) Evaporative water loss and intertidal vertical distribution in relation to body size and morphology of stichaeid fishes from California. J Exp Mar Biol Ecol 50: 273-288.

Jokiel PL, Brown EK (2004) Global warming, regional trends and inshore environmental conditions influence coral bleaching in Hawaii. Global Change Biology 10: 1627-1641.

Lee S-C (1980) Intertidal fishes of a rocky pool of the Sanhsientai, eastern Tawaiian. Bull Inst Zool Acad Sin 19: 19-26.

Macpherson E. (1994) Substrate utilization in a Mediterranean littoral fish community. Mar Ecol Prog Ser 114: 211-218.

Mahon R, Mahon SD (1994) Structure and resilience of a tidepool fish assemblage at Barbados. Environ Biol Fish 41: 171-190.

Martin KLM (1995) Time and tide wait for no fish: intertidal fishes out of water. Environ Biol Fish 44: 165-181.

Mayr M, Berger A (1992) Territoriality and microhabitat selection in two intertidal New Zealand fish. J Fish Biol 40:243-256.

Metaxas A, Scheibling RE (1992) Community structure and organization of tidepools. Mar Ecol Prog Ser 98: 187-198.

Nakamura R (1976) Experimental assessment of factors influencing micorhabitat selection by the two tidepool fishes *Oligocottus maculosus* and *O. synderi*. Mar Biol 37: 97-104.

Paulin CD, Roberts CD (1993) Biogeography of New Zealand rockpool fishes. *In:* B.e. al. (ed.) Proceedings of the second international temperate reef symposium, 7-10 January 1992, National Institute of Water and Atmospheric Research Ltd., Auckland, New Zealand

Prochazka K, Chotkowski MA, Buth DG (1999) Biogeography of rocky intertidal fishes. pp. 332-355. In*:* Horn MH Martin KLM, Chotkowski MA (ed) Intertidal fishes, Life in two worlds, Academic Press, San Diego

Randall JE (2007) Reef and shore fishes of the Hawaiian Islands. University of Hawaii, Sea Grant College Program, Honolulu

Smith JE, Hunter CL, Smith CM (2002) Distribution and reproductive characteristics of nonindigenous and invasive marine algae in the Hawaiian Islands. Pac Sci 56: 299-315.

Stimson J, Larned ST, Conklin, EJ (2001) Effects of herbivory, nutrient levels, and introduced algae on the distribution and abundance of the invasive macroalga *Dictyosphaeria cavernosa* in Kaneohe Bay, Hawaii. Coral Reefs 19: 343-357.

Yoshiyama RM, Sassaman C, Lea RN (1986) Rocky intertidal fish communities of California: temporal and spatial variation. Environ Biol Fish 17: 23-40.

Zander CD, Nieder J, Martin KLM (1999) Vertical distribution patterns. pp. 26-49. In*:* Horn MH, Martin KLM, Chotkowski MA (ed) Intertidal fishes, Life in two worlds, Academic Press, San Diego

Chapter IV. δ^{15}N values and nitrogen contents of reef algae reveal terrestrial derived nitrogen sources that vary over a small landscape scale at ‘Ewa Beach (O‘ahu, Hawai‘i)

Abstract

Proposed construction of a storm-drain along ‘Ewa Beach, Hawai‘i raised concerns over impact of discharge on coastal water quality and abundance of culturally important reef algae. We used δ^{15}N values and nitrogen (N) content of two reef algae, non-indigenous *Acanthophora spicifera* and native *Laurencia nidifica* in the dry and rainy season to investigate nitrogenous sources at replicate sites with and without existing storm-drains. The δ^{15}N values of algae (3.8-17.7‰) and the on-offshore pattern in values were mostly indicative of terrestrial based inputs subjected to active nitrogen cycling. Plant N content and δ^{15}N values were not higher at drain sites, as predicted if discharge delivered N inputs into pristine tropical waters. Values of N in reef algae varied on a local scale and were tied to geographic location. These results reveal a complex interaction between N source, season, and geographic location.

Introduction

Urbanization of coastal areas and associated watersheds is a growing trend in many regions (Smalls and Nicholls, 2003). Currently 10s of millions of people inhabit coasts adjacent to coral reef environments (Salvat, 1997) and this urbanization often has negative ecological consequences to surrounding habitats, in part because tropical reef communities are adapted to oligotrophic nutrient levels (Birkeland, 1988; Larned 1988; Littler et. al., 2006). Dramatic land use changes often accompany coastal urbanization and can include production and cessation of agricultural operations, an increase in development of resorts and neighbors, and a switch from septic tanks to sewer systems. By altering the amount and makeup of runoff, these land use practices can alter and many times increase the level of nitrogen (N) delivered to surrounding coastal waters (Hessen et al., 1997; Smith et al., 1999). Eutrophication of aquatic systems can lead to increased macroalgal production, changes in assemblages of organisms, alteration of food webs, and disruption of nutrient cycling (Valiela et al., 1992; Walsh 2000; Choi et al., 2007; Cole 2003). In tropical pristine waters where nutrient levels are low, addition of nutrients can be particularly detrimental. Eutrophication of oligotrophic waters is known to be a catalyst for community phase shifts from coral to algal dominated reefs (McCook, 1999). In rain events, storm-drains have the potential to act as conduits, collecting and focusing N enriched runoff into coastal systems. In addition, increased anthropogenic water use could extend the delivery of N into dry seasons, yet very little is known about impacts or the fate of nutrients that accompany storm-drain effluent in tropical oligotrophic waters. The few attempts to quantify and measure stormwater nutrient inputs have been made in estuaries on continental landmasses (Lapointe and Matzie 1996; Cohen and Fong 2006; Dillon and Chanton 2008) and may not be applicable to this study conducted in a marine habitat that surrounds an oceanic high island with a porous substrate and a benthic community well adapted to low macro-nutrient concentrations.

In ‘Ewa Beach, a coastal community on the island of O‘ahu, Hawai‘i land use change may have altered N inputs to oligotrophic waters. The land in ‘Ewa Beach area once used for agriculture is currently being developed into neighborhood subdivisions (Schaefers, 2008). Although climate in ‘Ewa Beach is tropical and dry, episodic rainfall events in winter months often results in coastal low-land floods. The average precipitation between 1949-2001 was 508 mm but, 381 mm fell during October-March 1949-2001 (Otkin and Martin, 2004). To prevent flooding and continue development, a developer has been taxed with constructing a storm-drainage system (Schaefers, 2006; Bernardo, 2008) that would discharge into nearby intertidal habitat. The shallow coastal area along southwest O‘ahu has historically been an area of high algal abundance and is culturally important to Hawaiians for collection of edible limu (macroalgae) (Abbott, 1996; Leone 2004; Ohira 2005). Longtime residents familiar with the ‘Ewa Beach area recall the ability to collect burlap bags full of edible macroalgae (Abbott, 1996). The recent macroalgal community appears to be different from past descriptions and the local perception is the edible limu is in decline. In the past two decades, sugar production has ceased and residents have switched from septic tanks to sewer. Nutrients from past agricultural and waste management may explain the wide availability of limu in the ‘Ewa Beach area in the past and the coincident reef algal decline with changes in land use.

Blooms of non-indigenous, nuisance marine species are common in Hawai‘i, particularly in areas adjacent to sewage inputs. Several of these species are able to out-compete native algae and corals (Banner 1974; Russell 1992; Stimson et al., 2001; Smith et al. 2002; Smith et al., 2004; Smith et al. 2005). For these reasons, local residents opposed to the construction of the storm-drain are concerned that the addition of effluent from this drainage system will degrade marine habitats leading to further declines in edible reef algae abundance and a phase shift to non-native species (Bernardo, 2008). Arguably, pulsed N inputs from drain effluent or low-land flooding could be enough to support short-lived production and may provide an advantage to species that are able to rapidly acquire and use nutrient sources (McCook, 1999; Kamer et al., 2001; LaPointe, 2004).

The sources of nutrients that support the reef algae community in ‘Ewa Beach area are not well known Potential sources include groundwater seeps, anthropogenic inputs such as sewage and runoff, atmospheric deposition, and upwelling. In addition, long shore currents and waves have the potential to rapidly mix and transport nutrients. Identifying nutrient sources and fates, particularly N because reef algae are generally N limited, can help to predict and measure potential impacts from storm-drain discharge on water quality and reef algal physiology and abundance.

Reef algae are well suited for tracing N in an aquatic environment because many species have simple morphologies that allow uptake of nutrients from the water column and integration into growth or storage. Measurements of algal N content can provide information on the amount and dispersion of nutrients from a source. Laboratory and field studies have tracked changes in N content with enrichment and depletion of ambient dissolved nutrients (Larned 1998; McCook 1999; Schaffelke 1999; Fong et al., 2001; Fong et al., 2003; Lapointe et al., 2004; Lin and Fong 2008; Teichberg et al., 2008). Fong et al. (1988) found N content of macroalgae to be more indicative of biologically available nutrient pulses than traditional snapshot water sampling. Yet, there are other studies in tropical environments that have failed to find a correlation between dissolved nutrients and N content (McCook 1999; Schaffelke 1999; Fong et al., 2001). This lack of correlation may be explained by differences in species uptake abilities, a rapid uptake of nutrients, temporal or spatial variable nutrient pulses, or tight coupling between macroalgae and invertebrate physiology (as discussed in Fong et al., 2001). For these reasons, we have investigated the use of nitrogen stable isotopic compositions to investigate the coupling between macroalgal N content and nutrient regime.

The nitrogen isotopic composition of macroalgae can be used to identify and track the sources of N. The $\delta^{15}N$ values of macroalgae vary as a function of N sources, the biogeochemical cycling of nutrients, species of nitrogen utilized, and the extent of utilization of dissolved nitrogen (Peterson and Fry 1987; Kendall 1998; Robinson 2001). As atmospheric nitrogen (N_2) is fixed and changed to ammonium (NH_4^+ , ammonification) and converted under anaerobic conditions from nitrite to nitrate (NO_3^- , nitrification) by bacteria the N isotopic composition is altered via fractionation, or the differential incorporation of ^{15}N to ^{14}N. Atmospheric fixation has minimal isotopic fractionation thus N_2 fixing bacteria have $\delta^{15}N$ values of -4 to +4.0 $^0/_{00}$ (Owens 1987; Macko and Ostrom 1994). Manure and waste from sewage treatment with increased bacterial processing often have $\delta^{15}N$ values of +6 to +22$^0/_{00}$ (Macko and Ostrom 1994; Kendall 1998). For macroalgae with simple morphologies that completely utilize the available pool of nitrate and ammonium the incorporation of ^{15}N occurs with little fractionation (Peterson and Fry 1987; Gartner et al, 2002; Cohen and Fong 2005) and quickly (within 7 days) (Gartner et al, 2002). Hence, the $\delta^{15}N$ values of macroalgae can reflect the $\delta^{15}N$ values of the source of nitrogen.

Sources, however, can be challenging to identify as the isotopic composition in land to marine systems is dynamic. Nitrogen not only varies in form but increased N inputs result from upwelling (nitrates), discharge of wastewater (nitrates and ammonium), or natural fertilizer runoff (nitrates and ammonium). Synthetic fertilizers produced by the Haber-Bosch process have $\delta^{15}N$ values close to that of atmospheric N_2, which is defined as 0‰ (Macko and Ostrom 1994; Kendall 1998) and are also a possible N source for algae. Mixing of these multiple sources occur under field conditions yet despite these challenges several studies have successfully used bulk $\delta^{15}N$ values of macroalgae (Sammarco et al., 1999; Schaffelke 1999; Umezawa et al., 2002; Cohen and Fong 2005; Garrison et al., 2007; Lin et al., 2007; Teichberg et al., 2008) to assess land-based N inputs into pristine coastal zones.

We used the N content and the $\delta^{15}N$ values of two species of reef algae to identify the potential source and fate of N along ‘Ewa Beach, O‘ahu and these N measurements were used to determine the potential impact of storm-drain discharge on water quality and reef algal physiology. We hypothesize that N sources for reef algae in the ‘Ewa Beach area are anthropogenic in origin and that these sources (such as runoff, fertilizer input, etc.) may vary over a small spatial scale, related to geographic location. Additionally, we tested the hypothesis that storm-drain discharge delivers seasonal pulse

N to intertidal and subtidal habitats where reef algae grow. To test these hypotheses we sampled reef algae in an on-offshore direction in areas near and removed from storm-drain outlets in two seasons. Support for these hypotheses would be indicated by a spatial effect of reef algae with enriched N concentration and ^{15}N content in near-shore macroalgae (e. g. closer to anthropogenic sources), and at sites with a storm-drain, after large rain events.

Methods

Site Description

Ten sites (numbered 1-10 from west to east) were selected along the southwest shore of O‘ahu, Hawai‘i to examine the N sources of reef algae and to determine the extent of storm-drain discharge in contributing to these sources (Fig. 4.1). Sites were located along ~2.13 km of coastline extending from One‘ula Beach Park (21° 18’36.59”N -158° 0’ 27.52 W) to ‘Ewa Beach proper (N 21°18’ 42.17” N -158°0’ 15.66 W). To assess the impact of discharge from a proposed storm-drain on reef algae, these sites are located directly to the east and west of a future storm drain site (Control/ Proposed Drain 2). Four sites (Sites 4, 6, 8, 9) each contain an existing large storm-drain that directly discharges accumulated residential runoff into the intertidal zone. These sites are referred to as “Drain sites”. “Control sites”, (Sites: 1, 2, 3, 5, 7, 10), were selected for comparison and are located in areas with similar topography, limestone and sand substrate, and shore direction-interspersed between sites with large storm-drains. Control sites 1-3 are not associated with a nearby drainage outlet and were sampled for comparison and as a baseline to assess potential impact when the proposed drain is constructed.

The large existing storm-drains were constructed in late 1960s to early 1970s and have been in use for over 30 years (C. Morgan, Planning Solutions, *personal communications*). The drains at Drain 4, 6, and 8 are concrete lined and have one or two circular outlets that are exposed during low tides and submerged during high tides. Drain 9 is sand lined. A large sand berm blocks the standing water from the intertidal zone. To enter intertidal habitat, discharge from this drain has to either erode the berm (which happens with some high tide and storm events) or seep through the sand. Storm-drains 4, 6, and 8 are drainage outlets for a limited watershed (approximately 16-31 acres) while

Drain 9 is the ocean outlet for a larger watershed that extends more than 420 acres (C. Morgan, Planning Solutions, *personal communications*).

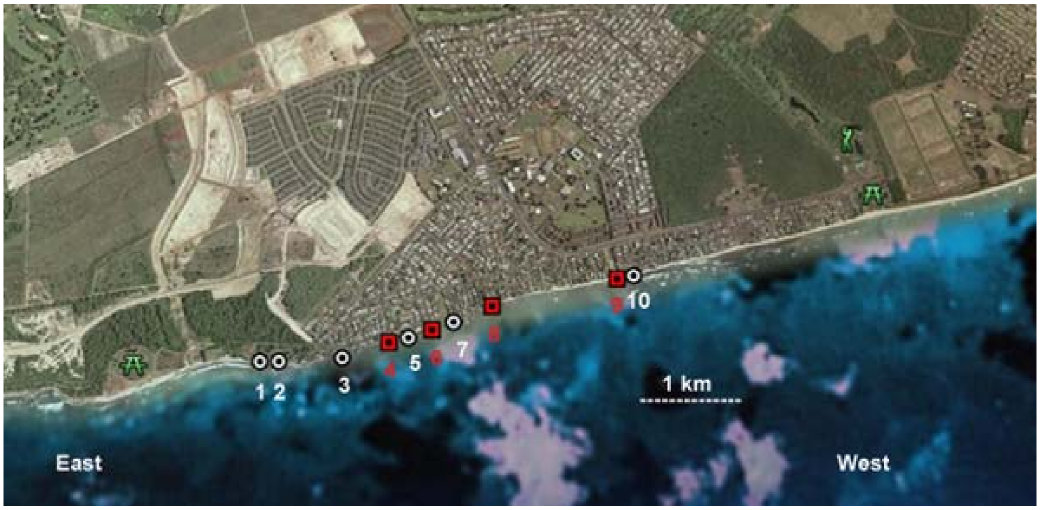

Fig. 4.1 Aerial image of Ewa Beach area (Google Earth©) showing sites 1-10 (white circles are control sites with no drain present, black squares are drain sites, i.e. drain present), nearby parks (picnic tables), and golf course (golfer).

The intertidal region at these sites consists of carbonate platforms covered with benthic reef algae and invertebrates while sand and reef platforms extend into the shallow subtidal zone. All sites are located in an area with a dry climate (~46 cm rain/year). Heavy rains (usually several inches in one event) occur sporadically, often associated with large waves during the rainy season (September-March). On these occasions, the surrounding coastal water becomes turbid and a visible sedimentation plume extends out 100+ m (TEC, *personal observations*). The surrounding coast line is an urban area with two small recreational parks. A golf course is located in close proximity to sites 9 &10.

The algal community in the ‘Ewa Beach area is diverse and most sites are dominated by red reef algae. Two closely related reef algae, *Acanthophora spicifera* and *Laurencia nidifica* (Order Ceramiales, Family Rhodomelaceae) were sampled for this study because they are abundant in the intertidal and shallow subtidal habitats and have a simple morphology that should allow rapid incorporation of the $\delta^{15}N$ values of nitrogenous nutrients. *A. spicifera*, an introduced species, in Hawai’i (Russell 1992), is known to fragment readily (Kilar and McLachlan, 1986), exhibits increased growth in N enriched waters and has been previously used successfully to identify anthropogenic and natural nitrogenous sources (Lin and Fong, 2008). Small (1-2 cm^2) discrete clumps consisting of several whole thalli of *A. spicifera* or *L. nidifica* were collected at sites 1-10. *Laurencia nidifica* was rare at sites 8-10 and few or no individuals were collected.

Sampling Protocol & Analyses

Sampling protocol assumes that influence of storm-drains on the nitrogenous content of seawater should be highest at the point of discharge, and diminish with distance offshore. High wave action limited access to shallow waters and hence sampling occurred from the upper edge of the intertidal zone or storm-drains to a distance of approximately 20 m (1-3 m depth) from shore. One algal sample of each species was collected, when encountered, at set intervals along the transect line. If both species did not occur, the available species was collected. Thus the n values in Table 4.1 represent the number of samples of each species collected along the transect tape for each site per season.

Replicate algal samples were collected once in dry (July-August) and rainy (September-October) months in 2007 and 2008 to account for seasonality and discharge events. Sampling conducted in rainy seasons occurred within 5-7 days after rain events with rainfall amounts greater than 0.1 inches to ensure drain flow but less than 3.0 inches. Sites 1-6 were sampled in 2007 while Sites 7-10 were sampled in 2008.

To determine the nutrient inputs delivered by storm drain flow and water column nutrients, at time of algal collection in the dry season, 80 ml water samples (n=1/ site) were collected from near-shore or at the mouth of drains. On a large rain event in 2007, samples of flowing water were collected from street storm grates that empty directly into Drain 4 and 6. Because large waves and high tides prevented access to the mouth of these drains, these samples were used as a proxy for storm-drain nutrient source. For comparison, on the same rain event, water was collected from the ocean at Control 3. In the dry season in 2008, an 80 ml water sample was collected from standing water that occurs in the drain located at Drain 9. Because groundwater in Hawaiʻi can have high nutrient contents (Laws et. al, 1999), two 80 ml water samples were collected from a nearby non-potable irrigation well that taps into groundwater as a water source. This well is located at a nearby golf course that is less than a mile from sites 9 and 10. These irrigation well samples are a proxy for groundwater source.

Algal samples were cleaned of epiphytes, rinsed in de-ionized water to remove sand and salt, and placed into an oven at 60˚C until dried. Dried samples were ground into a fine powder with a mortar and pestle, stored in glass scintillation vials under desiccant until further analyses. Water samples were filtered (0.22 µm) and stored frozen (-20°C) until analyzed.

Carbon and nitrogen isotope compositions of reef algae were determined using an on-line carbon-nitrogen analyzer coupled with an isotope ratio mass spectrometer (Finnigan ConFlo II/Delta-Plus). Isotope values are reported in standard δ-notation relative to an international standard. Standards are V-PDB and atmospheric N_2 for carbon and nitrogen, respectively. Glycine reference compounds with well-characterized isotopic compositions were used to ensure accuracy of all isotope measurements. Several samples were measured in duplicate or triplicate and the reproducibility associated with these measurements was typically $\leq$0.2‰

Analyses of water samples for dissolved inorganic nutrients (NH_4^+, NO_2^-, NO_3^-, PO_4^-, SiO_4^-) were performed by the Analytical Lab at the Marine Science Institute, University of California, Santa Barbara using a continuous flow technique with a Quick Chem 800 Flow Injection Analyzer manufactured by Lachat Instruments, Inc.

To determine if reef algae N values differed at storm-drain sites from the control sites and to examine if values for algae changed with season and distance from shore, statistical analyses were performed on total N content and $\delta^{15}N$ values (separately) for each species. Specifically for each species, a two-way analysis of variance (ANOVA) was used to compare reef algal values between drain and control sites in the dry and rainy season. Data were screened and met the requirements for normality and homogeneity of variance. Multiple linear regressions were used to examine the relationship between $\delta^{15}N$ values of *L. nidifica* and *A. spicifera* and distance from shore. Each site was examined separately because of unique values at each site. Season and an interaction of Distance x Season were included in the model as the seasonality of rain could alter delivery of N to the shore. Regressions were not performed on %N in algae because of the correlation with $\delta^{15}N$ values.

Because nutrient inputs, such as runoff, groundwater seeps etc. have the potential to act on a local scale, statistical analyses examined the geographic affinity of total N content and $\delta^{15}N$ values for these two reef algae. Separate analyses were performed for total N content and $\delta^{15}N$ values for each species in both seasons. Using Euclidean distance, a similarity matrix was constructed between each value at every site. A separate, similarity matrix of geographical distance between sites also was constructed. RELATE in PRIMER-E (Clarke and Warwick 2001), a Mantel-like test, was used to examine the statistical relationship between the two types of similarity matrices for both species in both dry and rainy season. RELATE performs Spearman Rank correlation to determine Rho with 999 permutations to determine statistical significance.

Results

The average $\delta^{15}N$ values of reef algae ranged from 4.8 - 14.7 ‰ (Table 4.1) and plant %N ranged from 1.1 to 2.6 % (Table 4.1). The nutrient levels in water column are summarized (Table 4.2). Drain water had higher nitrites and nitrates than well water which as expected was high in silicates (Table 4.2).

Although the $\delta^{15}N$ values of *A. spicifera* differed among sites, differences were not significant between drain and control sites and did not differ between season (Fig. 4.2) (Two-Way ANOVA, drain vs control *p*-value = 0.45, season *p*-value = 0.41, drain x season *p*-value = 0.89). The $\delta^{15}N$ values of *A. spicifera* from Control 3, 5, and 10 were consistently lower than the other nearby drain and control sites. Drains 4, 6, and 8 had *A. spicifera* with similarly high values to Controls 1, 2, and 7. $\delta^{15}N$ values of *A. spicifera* samples collected from Drain 9 were higher than many samples collected from the nearby Control 10 and both sites had reef algae with lower values than those that occurred Sites 1-8

The total N content of *A. spicifera* differed among sites but varied little with season and values found at control and drain sites did not significantly differ (Fig. 4.2) (Two-way ANOVA, drain vs control p = 0.42, season p = 0.92, drain x season p = 0.63). Sites with a storm-drain present often had individual samples of *A. spicifera* with increased percentages of N but the variation around the samples collected from these sites were statistically similar to the variation observed at nearby control sites. In both season

Table 4.1 Plant mean ± SE Total Nitrogen as % dry weight and δ^{15}N values for *A.spicifera* and *L. nidifica* in dry and rainy seasons at sites 1-10.

		Dry Season			Rainy Season		
Site Name	**Species**	**n**	**Total N (%)**	**δ^{15}N**	**n**	**Total N (%)**	**δ^{15}N ($^{0}/_{00}$)**
Control 1	*A. spicifera*	5	2.6 ±0.1	14.0 ±0.5	3	1.6 ±0.1	8.2 ±0.7
	L. nidifica	2	2.6 ±0.3	14.7 ±0.9	3	1.6 ±0.1	9.5 ±1.1
Control/Proposed Drain 2	*A. spicifera*	6	2.1 ±0.1	11.6 ±0.2	1	2.3	10.8
	L. nidifica	4	2.1 ±0.1	12.3 ±0.1	4	2.1 ±0.4	11.4 ±0.3
Control 3	*A. spicifera*	5	1.8 ±0.1	6.8 ±0.1	3	1.8 ±0.1	7.0 ±0.0
	L. nidifica	4	2.1 ±0.1	6.9 ±0.1	4	2.0 ±0.2	7.3 ±0.1
Drain 4	*A. spicifera*	5	2.5 ±0.2	12.2 ±0.4	5	2.5 ±0.1	12.0 ±0.4
	L. nidifica	5	2.6 ±0.2	13.3 ±0.7	4	2.1 ±0.3	12.0 ±0.7
Control 5	*A. spicifera*	5	2.1 ±0.1	9.2 ±0.2	7	1.9 ±0.2	8.5 ±0.1
	L. nidifica	7	2.1 ±0.2	9.5 ±0.2	6	2.0 ±0.2	8.7 ±0.1
Drain 6	*A. spicifera*	8	2.7 ±0.1	11.3 ±0.4	8	2.4 ±0.1	10.5 ±0.2
	L. nidifica	6	2.5 ±0.2	11.9 ±0.2	6	2.5 ±0.1	11.3 ±0.3
Control 7	*A. spicifera*	6	1.8 ±0.1	11.4 ±0.4	5	1.7 ±0.1	12.5 ±0.7
	L. nidifica	4	1.5 ±0.3	11.0 ±0.5	-	-	-
Drain 8	*A. spicifera*	6	1.7 ±0.2	12.4 ±1.5	5	2.3 ±0.1	10.4 ±1.5
	L. nidifica	1	1.3	8.8	-	-	-
Drain 9	*A. spicifera*	6	1.1 ±0.1	7.5 ±0.5	5	1.2 ±0.0	5.3 ±0.5
	L. nidifica	-	-	-	-	-	-
Control 10	*A. spicifera*	6	1.1 ±0.0	4.8 ±0.3	5	1.4 ±0.1	5.3 ±0.3
	L. nidifica	-	-	-	-	-	-

Table 4.2 Phosphate, Silicate, Nitrite, Nitrate, and Ammonia as ppm values from water samples collected inshore at Sites 1-10, storm-drain grates, and well. n =1 unless otherwise specified, n>1 values are expressed as mean ± SE. Values with an * are below detectable values.

	Dry Season (μM)					**Rainy Season** (μM)				
Site Name	**PO_4^{3-}**	**SiO_2**	**NO_2^-**	**NO_3^-**	**NH_3**	**PO_4^{3-}**	**SiO_2**	**NO_2^-**	**NO_3^-**	**NH_3**
Control 1	-	-	-	-	-	0.10*	7.9	0.20	0.96	1.18
Control/Proposed Drain 2	-	-	-	-	-	0.09*	12.7	0.38	2.53	2.02
Control 3	0.15	10.0	0.22	1.03	0.81	0.10	7.0	0.29	1.53	0.94
Drain 4	0.23	10.9	0.18	2.88	2.71	0.12	6.6	0.29	2.57	1.86
Control 5	0.15	3.3	0.18	0.72	0.54	0.07*	8.2	0.26	1.29	0.34
Drain 6	0.07	16.5	0.14	7.93	7.79	0.07*	13.5	0.37	1.71	0.87
Control 7	-	-	-	-		-	-	-	-	-
Drain 8	0.08*	3.7	0.20	1.02	0.82	0.08	3.7	0.20	0.82	3.10
Drain 9	0.09*	2.6	0.13	0.59	0.46	-	-	-	-	-
Control 10	-	-	-	-		0.09	12.7	0.38	2.53	2.02

	n	**PO_4^{3-}**	**SiO_2**	**NO_2^-**	**NO_3^-**	**NH_3**
Drain Water	2	26.9 ±9.4	103.1 ±55.8	6.0 ±0.8	76.7 ±45.2	29.7 ±3.2
Water In Drain 9	2	1.4 ±0.1	515.6 ±13.8	3.1 ±0.0	96.0 ±0.3	15.6 ±0.1
Well water, proxy for groundwater	2	2.5 ±0.1	574.2 ± 20.0	0.8 ±0.0	23.5 ±0.4	10.1 ±0.0

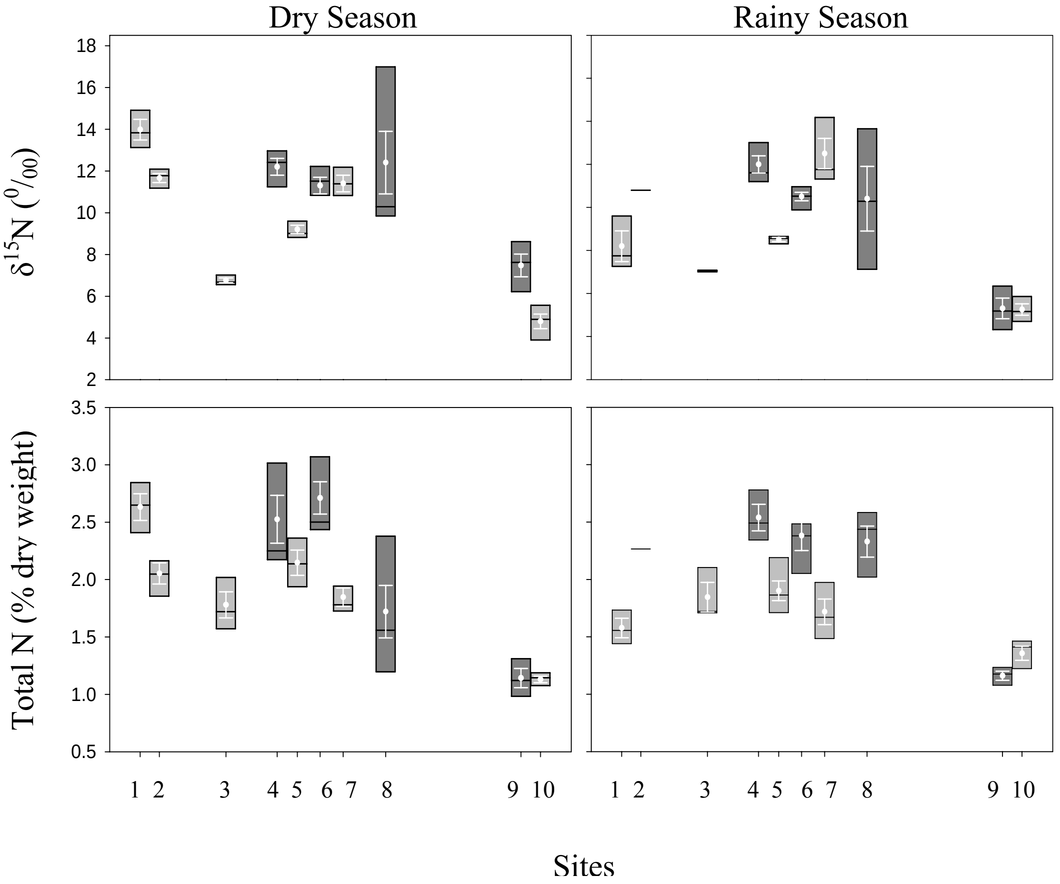

Fig. 4.2 **δ^{15}N (‰)** (top) and total N (% of dry weight) values (bottom) for *A. spicifera* in the dry and rainy season at drain (dark gray) and control sites (light gray). Boxplots represent 1st , 2nd , and 3rd quartiles. Sites are arranged to represent geographic distance. The mean and SE are represented by a white point and ±error bars. However, when n<2 the mean and SE were not graphed.

samples of *A. spicifera* collected from Sites 9 and 10 had lower total N than other drain and control sites.

Site average δ^{15}N values for *L. nidifica* are similar to patterns observed in δ^{15}N values site averages of *A. spicifera* (Fig. 4.3). The δ^{15}N values of *L. nidifica* varied among sites but statistical differences using a Two-way ANOVA were not found between values for drain and control sites (*p*-value = 0.21), rainy or dry season (*p*-value = 0.45), nor between an interaction of site type (drain or control) and season (*p*-value = 0.33).

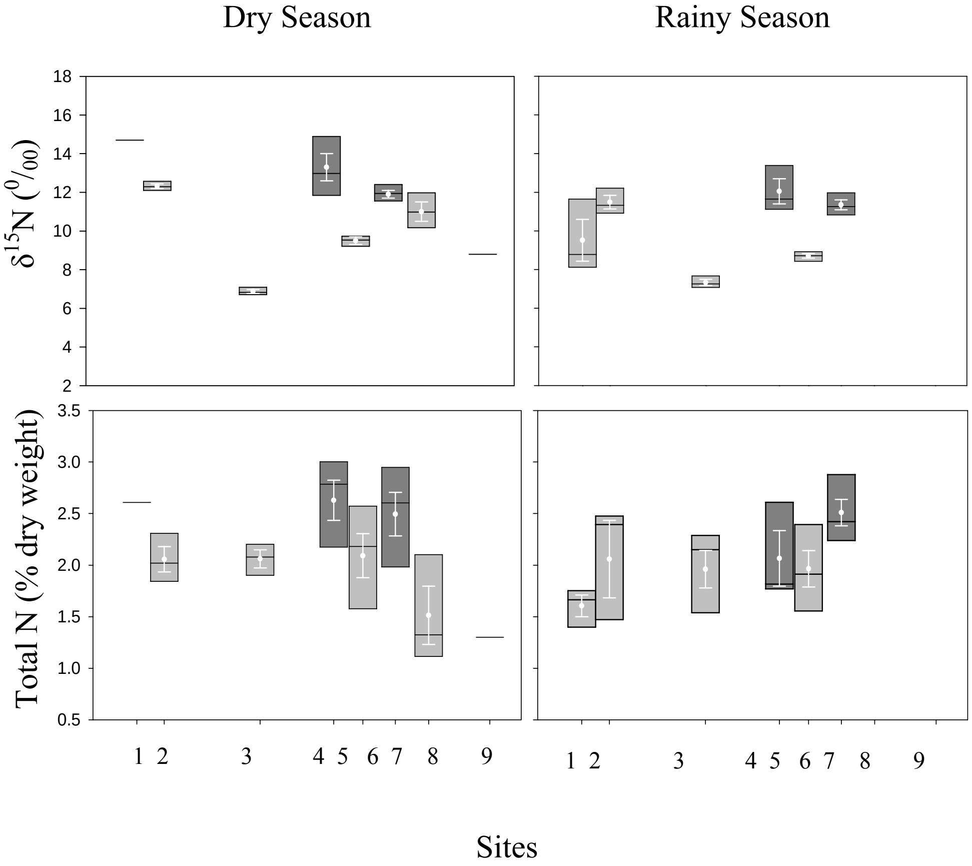

Fig. 4.3 δ^{15}N (‰) (top) and total N (% of dry weight) values (bottom) for *L. nidifica* in the dry and rainy season at drain (dark gray) and control sites (light gray). Boxplots represent 1st, 2nd, and 3rd quartiles. Sites are arranged to represent geographic distance. The mean and SE are represented by a white point and ±error bars. However, when n<2 the mean and SE were not graphed.

When *L. nidifica* was present and sampled, Controls 3, 5, and Drain Site 8 had algae with lower values than those collected at Controls 1, 2, 7 and Drains 4 and 6.

Although *L. nidifica* at Drains 4 and 6 had increased % N in both seasons, the values at these sites were statistically similar to Controls 1, 2, 3, 5, 7 and the one algal sample collected in the dry season at Drain 8 (Two-way ANOVA, drain vs. controls, *p*-value = 0.40, drain x season *p*-value = 0.53). In addition values of %N in *L. nidifica* show a similar pattern in the dry and rainy season (Fig. 4.3) (*p*-value = 0.98). Several

Drain and Controls Sites had *A. spicifera* and *L. nidifica* with higher δ^{15}N values nearshore with lower values further from shore and this on-offshore pattern was seen in both seasons (Fig. 4.4). In 9 out of 10 sites distance from shore was a significant factor in a multiple regression model used to determine δ^{15}N values of *A. spicifera* (Table 4.3). In both seasons, *A. spicifera* at Drain Site 8 had about 10 ‰ lower δ^{15}N value within a 20 m distance from shore. In contrast, Control Site 3 the values in *A. spicifera* changed by as little as 0.5 ‰ over a similar distance. Season was also often a significant predictor in the regression models for Control Site 5, Drain Site 6, Drain Site 8, and Drain Site 9 (Table 4.3). Again, Drain Site 8 had *A. spicifera* samples with the largest change in δ^{15}N values from the dry to rainy season (about ~4 ‰ lower value) while other sites the variation with season was much smaller (~0.3 ‰ lower value). Values of δ^{15}N tended to be lower in the rainy season and for most sites did not alter the slope of δ^{15}N values with distance from shore. However, an interaction of distance and season was observed in samples of *A. spicifera* at three sites: Control 1, Control 3, and Control 5 (Table 4.3).

The δ^{15}N values of *L. nidifica* differed at some sites with distance from shore and varied for some sites with season (Fig. 4.4). Three sites out of six sampled showed significant variation in δ^{15}N values of *L. nidifica* with distance. Distance was a significant predictor of δ^{15}N values at Drain Site 4, Control Site 5, and Drain Site 6. For these sites the δ^{15}N values were lower with distance from the shoreline. Season was a significant predictor of *L. nidifica* δ^{15}N values for Control Site 1, Control Site 3, and Control Site 5. Control Site 1 had the largest difference (1-3 ‰) in *L. nidifica* δ^{15}N values between seasons. At two of these sites, values were lower during the rainy season while Control Site 3 values of δ^{15}N were higher in the rainy season. Drain Site 4 was the only regression model that had a significant interaction between distance from shore and season (Table 4.3).

δ^{15}N values of reef algae were related to the geographic location where collection occurred. Average δ^{15}N values of reef algae varied among sites (4.8 - 14.7 ‰, Table 4.1) and this range of variation was greater than the largest variation that occurred between seasons (5.8 ‰ difference), the species *A. spicifera* and *L. nidifica* at a site within a season (1.3 ‰ difference), and individuals (3.8 ‰ difference) at a site within a season.

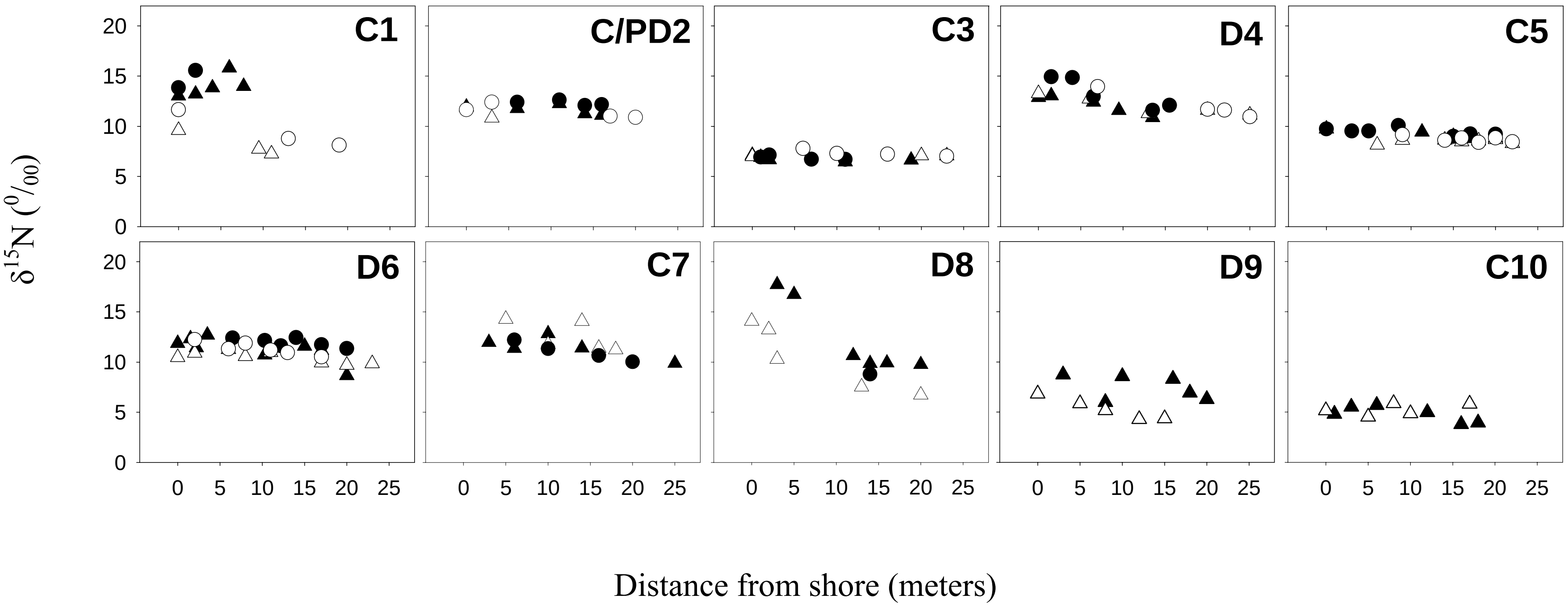

Fig. 4.4 δ^{15}N (**$^{0}/_{00}$**) values of *A. spicifera* ▲ and *L. nidifica* ● in the dry (closed) and rainy (open) season as distance from shore (meters) for sites.

Table 4.3 Multiple linear regressions show the relationship between δ15N of *A. spicifera* and *L. nidifica* with distance from shore, season, and the interaction of terms at drain (D) & control (C) sites (rel=direction of relationship, NS= not significant at α = 0.05, NA= not applicable).

	Acanthophora spicifera						***Laurencia nidifica***					
Site	**Terms**	**rel**	**df**	**F**	***p*-value**	**R^2 (x100)**	**Terms**	**rel**	**Df**	**F**	***p*-value**	**R^2 (x100)**
C1	Distance, p = 0.010 Season, NS DistancexSeason, p = 0.011	- - +	7	27.8	0.004*	95.4	Distance, NS Season, p = 0.043 DistancexSeason, NS	 -	4	11.3	0.043	79.4
C2	Distance, NS Season, NA DistancexSeason, NA		5	0.6	0.488	12.7	Distance, NS Season, NS DistancexSeason, NS		7	1.1	0.433	39.6
C3	Distance, p = 0.032 Season, NS DistancexSeason, p = 0.041	- - +	8	20.6	0.085	70.6	Distance, NS Season, p = 0.021 DistancexSeason, NS	 +	8	9.3	0.021	61.6
D4	Distance, p = 0.001 Season, NS DistancexSeason, NS	- - +	9	22.4	0.001	73.6	Distance, p = 0.002 Season, NS DistancexSeason, p = 0.019	- - +	8	37.1	<0.001	95.7
C5	Distance, p = 0.010 Season, p = <0.001 DistancexSeason, p = 0.016	- - +	11	6.2	<0.001*	85.9	Distance, p = 0.003 Season, p = 0.045 DistancexSeason, NS	- -	12	21.4	<0.001*	81.1
D6	Distance, p = 0.017 Season, p = <0.001 DistancexSeason, NS	- -	15	14.3	<0.001*	68.8	Distance, p = 0.021 Season, NS DistancexSeason, NS	-	11	9.4	0.021	48.4
C7	Distance, p = 0.046 Season, NS DistancexSeason, NS	-	10	5.30	0.046*	37.4	NA					
D8	Distance, p = <0.001 Season, p = 0.009 DistancexSeason, NS	- -	10	18.0	0.001*	81.8	NA					
D9	Distance, p = <0.040 Season, p = 0.002 DistancexSeason, NS	- -	10	10.8	0.005	73.0	NA					
C10	Distance, p = 0.044 Season, NS DistancexSeason, NS	-	10	2.7	0.128	53.4	NA					

Significant relationships were found between geographic location and δ^{15}N values of *A. spicifera* for both seasons (RELATE: dry season, Rho = 80.9%, p-value = 0.001; rainy season, Rho = 65.4%, p-value = .001). The δ^{15}N values of *L. nidifica* were less correlated but significantly related to geographic location for the dry season (RELATE test: , Rho = 18.9%, p-value = 0.02), but not in the rainy season (Rho = 3.5%, p-value = 0.20).

Total N content of *A. spicifera* was related to geographic location where collection occurred but for *L. nidifica*, sampled only at sites 1-8, the relationship was not evident. There was a significant relationship between the similarity matrices of geographic location and *A. spicifera* total N content in dry (Rho = 36.4%, p = 0.001) and rainy (Rho = 36.3%, p-value = 0.001) season. However there was not a relationship between geographic location and *L. nidifica* N content for either season (RELATE: dry season, Rho = -4.0%, p-value = 0.69; wet season, Rho = 3.0%, p-value = 0.29).

Discussion

Overall the results of this study suggest that N sources for reef algae at ‘Ewa Beach area are partially derived from land as N content and δ^{15}N values in algae were often higher nearest to shore and varied on a small spatial scale related to geographic location. There was little to no evidence to support the hypothesis that storm-drain effluent influences the N content and δ^{15}N values of macroalgae at ‘Ewa Beach. Also N content and δ^{15}N values in reef algae did not increase with season as predicted. Instead our results reveal a complexity in the interaction of nutrient source, season, and spatial location along southwest O‘ahu.

Elevated δ^{15}N values (>6 ‰) and N contents (>2 %) in both species of macroalgae in the ‘Ewa Beach area indicate a high degree of nitrogen cycling as these values, particularly δ^{15}N values fall above the range commonly cited for animal waste (Macko and Ostrom 1994; Kendall 1998). Furthermore, the $\delta\ ^{15}$N values and N contents of reef algae fall within range from studies in other locations that investigated anthropogenic enrichment in tropical and subtropical waters. Published values of δ^{15}N of various warm water macroalgae range from +1.0 - +5.5 ‰ in the Florida Keys and were interpreted as being indicative of agricultural runoff (LaPointe et al., 2004), in waste water influenced locations of South Central Florida +5.0 - +13.0 ‰ (Barille 2004); +7.7 -

+11.4 ‰ in southeastern Gulf of California were plausible sources included sewage, agriculture, and shrimp farms, a mean of -0.5‰ in Hanalei Bay, Kauai island, Hawai‘i concluded to be indicative of synthetic fertilizers from nearby Resorts (Derse et al., 2007), +2 - +8 ‰ in Ishigaki, Japan (Umezawa et al., 2002), and +1.0 - +4.8 ‰ in Ofu, Samoa concluded to be indicative of non-anthropogenic sources (Garrison et al., 2007). Recent studies by Dailer et al. 2010 on Maui have shown remarkably elevated values (>40 ‰) for field collected materials. In this study a single clear anthropogenic source, such as an injection well or shrimp farm, was not evident within the ‘Ewa Beach area and groundwater seeps are common. The surrounding aquifer is likely to have an active microbial community where nitrogen can undergo a high degree of biogeochemical cycling prior to being discharged into nearshore waters. Further investigations are needed to distinguish whether the high nitrogen values in reef algae result from the incorporation of a land based anthropogenic or natural nitrogenous source.

There was not statistical evidence to support the hypothesis that elevated $\delta^{15}N$ and N values in reef algae result from storm drain discharge. It was predicted that storm-drains deliver enriched nutrients to sites, however, reef algae from drain sites were not consistently higher in $\delta^{15}N$ values and total N content relative to reef algae collected from controls. Some evidence for a drain effect is evident in the trend in higher total N values of *A. spicifera* and *L. nidifica* collected from few drain sites in both seasons. Thus, total N content may be a better tool to track rapid pulses of nutrients delivered by drain flow. Macroalgae, like those used in this study, have rapid nutrient uptake and thus %N could reflect N levels quickly (over a few hours) compared to $\delta^{15}N$ values which may take up to 7 days to be incorporated by algae. It is surprising that a drain effect trend was observed in the dry season when drain flow should be minimal. Many Hawaiian species of reef algae can deplete tissue stored N within 10 -11 days in un-enriched seawater (Larned, 1998). Therefore, drain discharge would need to occur prior to algal collection to observe increased N content in reef algae. Alternately, an additional or other source supplies N to reef algae and maintains enrichment.

For most sites $\delta^{15}N$ and N values in reef algae were elevated nearshore and were lower offshore suggesting terrestrial nitrogen sources but, algae could also be supported by sub-surface marine and terrestrial mixing. Storm-drains are predicted to add N to N limited waters by collecting and focusing runoff, thus we hypothesized that the reef algae at the mouth of drains and nearshore at drain sites should have higher δ^{15}N values and N contents than other samples –unless synthetic fertilizers are commonly applied which would tend to lower δ^{15}N values in algae. This was not consistently found. For instance, the reef algae at Drain site 8 shows the predicted drain effect of elevated N values nearshore and substantially lower values offshore. However, N values in reef algae at control Site 1 (no drain present) had a similar pattern. Therefore it seems conservative to conclude that nitrogen is at least somewhat terrestrial derived either via runoff or as groundwater for reef algae. Similar nearshore enrichment has been observed in other studies that investigated N values of reef algae in coastal waters (Sammarco et al., 1999; Umezawa et al., 2002).

The geographic affinity of δ^{15}N values and %N of reef algae reveal that sources act on a local scale and reveal two overall nutrient patterns. Reef algae at Sites 1-8 have nutrient sources with higher δ^{15}N values while reef algae at Sites 9-10 have sources with lower δ^{15}N values. Possible sources for sites 1-8 with high nitrogenous values could include storm-drain discharge, runoff, naturally or anthropogenic enriched groundwater seeps, natural fertilizers, or sewage waste. Water column nutrient values did not facilitate the identification of terrestrial derived sources. A residential area is concentrated near Sites 4-8 where reef algae with higher N values occurred and there are a higher proportion of storm-drains. Sites 1-3 have reef algae with similar N values but lack nearby storm-drains and concentrated anthropogenic activity. Currents could potentially bring nutrient enriched water from the east sites to the western sites (Foster and Cox, 2006). However, a gradual decrease in algae and water nutrient values from east to west was not observed. Groundwater seeps are commonly observed along Sites 1-8 and perhaps this could account for the localized reef algae values. Large green areas, ‘Ewa Beach Golf Course (~1.47 km away from sites), and a State Park (~1.47 km away from sites) occur near Sites 9-10 which have reef algae with lower δ^{15}N values. These values possibly result from onshore currents bathing algae in low nitrogen oceanic waters, lowered runoff and anthropogenic activity, an absence of groundwater seeps, or manufactured fertilizer applied to course and/or park lawns.

Because δ^{15}N values are tied to geographic location, perhaps a more appropriate analysis to determine drain effect would be to limit the comparison of reef algae N values from drain sites to those from the most adjacent control sites. Results from this post-hoc analysis (not shown) provided little evidence for a drain effect and trends are similar to reported findings. In addition, this post-hoc analysis is hindered statistically by small sample sizes (e.g. comparison limited to Drain 9 and Control 10) and is not realistic in the field setting where confounding variables are difficult to account for. Future research on N values in reef algae should be conducted in a controlled laboratory setting where nutrient regimes can be altered and pulsed effluent can be added.

In conclusion, our study revealed a complex pattern of nutrient sources that is inconclusive for ‘Ewa Beach area reef algae. It is likely that nitrogen sources for reef algae are partially terrestrial derived and tied to geographic location but, point sources of N were difficult to identify. Furthermore, nitrogen source varied substantially over a small geographic area. Conclusive statements about drain effects cannot be made from these results as it is difficult to determine a drain effect in an area with enriched N sources. If there are drain impacts, they appear to be localized and vary with discharge events. To better determine the effects of drain discharge future research should measure the amount and timing of discharge and analyze reef plants grown in effluent. However, it seems unlikely that discharge amount impacted study outcome, since reef algae N values did not correlate with upland acreage drained. Results from this study will be valuable in assisting in decisions about how to manage water flow and can be used as baseline to measure future alterations to nutrient regimes in the ‘Ewa Beach area.

References

Abbott, I. A., 1996. Limu, an ethnobotanical study of some Hawaiian seaweeds. National Tropical Botanical Garden. 39 pp.

Banner, A.H., 1974., Kane'ohe Bay, Hawaii: Urban pollution and a coral reef ecosystem. In: Proceedings 2nd international coral reef symposium. 2, 685-702.

Bernardo, R., 2008. Limu delays project to ease Ewa flooding. Star Bulletin, Honolulu. http://archives.starbulletin.com/2008/01/28/news/story04.html

Birkeland, C. 1988. Second order ecological effects of nutrient input to coral communities. Galaxea 7, 91-100.

Choi, W.-J., Han, G.-H., Lee, S.-M., Lee, G.-T., Yoon, K.-S., Choi, S.-M., Ro, H.-M., 2007. Impact of land-use types on nitrate concentration and δ^{15}N in unconfined groundwater in rural areas of Korea. Agriculture, Ecosystems & Environment 120, 259-268.

Clarke, K.R., Warwick, R.M., 2001. Changes in marine communities: An approach to statistical analysis and interpretation, Primer-E Ltd, Plymouth.

Cohen, R.A., Fong, P., 2005. Experimental evidence supports the use of δ^{15}N content of the opportunistic green macroalga *Enteromorpha intestinalis* (Chlorophyta) to determine nitrogen sources to estuaries. Journal of Phycology 41, 287-293.

Cohen, R.A., Fong, P., 2006. Using opportunistic green macroalgae as indicators of nitrogen supply and source to estuaries. Ecological Applications 16, 1405-1420.

Cole, M.L., 2003. Detection of eutrophication in aquatic ecosystems: nitrogen stable isotopes in macrophytes and groundwater. Boston University, Boston. PhD Dissertation

Dailer, M.L., Knox, R.S., Smith, J.E., Napier, M., Smith, C.M., 2010. Using δ^{15}N values in algal tissue to map locations and potential sources of anthropogenic nutrient inputs on the island of Maui, Hawai‘i USA. Marine Pollution Bulletin 60, 655-671.

Derse, E., Knee, K.L., Wankel, S.D., Kendall, C., Berg, C., Paytan, A. 2007. Identifying sources of nitrogen to Hanalei Bay, Kauai utilizing the nitrogen isotope signature of macroalgae. Environmental Science and Technology 41, 5217-5223

Dillon, K.S., Chanton, J.P., 2008. Nitrogen stable isotopes of macrophytes assess stormwater inputs to an urbanized estuary. Estuaries and Coasts 31, 360-370.

Fong, P., Boyer, K.E., Kamer, K., Boyle, K.A., 2003. Influence of initial tissue nutrient status of tropical marine algae on response to nitrogen and phosphorus additions. Marine Ecology Progress Series 262, 111-123.

Fong, P., Kamer, K., Boyer, K.E., Boyle, K.A., 2001. Nutrient content of macroalgae with differing morphologies may indicate sources of nutrients for tropical marine systems. Marine Ecology Progress Series 220, 137-152.

Foster, M.S., Cox, T.E., 2006. The effects of ocean discharge from storm water drains on intertidal seaweeds (limu) in the Ewa Beach Area., for: Morihara Lau & Fong LLP, Honolulu.

Garrison, V., Kroeger, K., Fenner, D., Craig, P., 2007. Identifying nutrient sources to three lagoons at Ofu and Olosega, American Samoa using δ^{15}N of benthic macroalgae. Marine Pollution Bulletin 54, 1813-1838.

Gartner, A., Lavery, P., Smith, A.J., 2002. Use of δ^{15}N signatures of different functional forms of macroalgae and filter-feeders to reveal temporal and spatial patterns in sewage dispersal. Marine Ecology Progress Series 235, 63-73.

Hessen, D.O., Hindar, A., Holtan, G., 1997. The significance of nitrogen runoff for eutrophication of freshwater and marine recipients. Ambio 26, 312-320.

Kendall, C. 1998. *Tracing Nitrogen Sources and Cycling in Catchments*. In: Isotope Tracers in Catchment Hydrology. Elsevier, Amesterdam, pp. 519-576.

Kamer, K., Boyle, K. A., Fong, P., 2001. Macroalgal bloom dynamics in a highly eutrophic southern California estuary. Estuaries and Coasts 24, 623-635.

Kilar, J.A., McLachlan, J.L., 1986. Ecological studies of the alga, *Acanthophora spicifera* (Vahl) Boerg. (Ceramiales: Rhodophyta): Vegetative fragmentation. Journal of Experimental Marine Biology and Ecology 104, 1-21.

Lapointe, B.E., Barile, P.J., Matzie, W.R., 2004. Anthropogenic nutrient enrichment of seagrass and coral reef communities in the Lower Florida Keys: discrimination of local versus regional nitrogen sources. Journal of Experimental Marine Biology and Ecology 308, 23-58.

Lapointe, B.E., Matzie, W.R., 1996. Effects of stormwater nutrient discharges on eutrophication processes in nearshore waters of the Florida Keys. Estuaries 19, 422-435.

Larned, S.T., 1998. Nitrogen-versus phosphorus-limited growth and sources of nutrients for coral reef macroalgae. Marine Biology 132,409-421.

Laws, E.A, Ziemann, D., Schulman, D., 1999. Coastal water quality in Hawaii: the importance of buffer zones and dilution. Marine Environmental Research 48, 1-21.

Leone, D., 2004. Ewa ban on limu picking proposed. Honolulu Star Bulletin, Honolulu. http://archives.starbulletin.com/2004/01/12/news/index2.html

Lin, D.T., Fong, P., 2008. Macroalgal bioindicators (growth, tissue N, δ^{15}N) detect nutrient enrichment from shrimp farm effluent entering Opunohu Bay, Moorea, French Polynesia. Marine Pollution Bulletin 56, 245-249.

Lin, H.-J., Wu, C.-Y., Kao, S.-J., Kao, W.-Y., Meng, P.-J., 2007. Mapping anthropogenic nitrogen through point sources in coral reefs using δ^{15}N in macroalgae. Marine Ecology Progress Series 335, 95-109.

Littler, M.M., Littler, D.S., Banks, B.L. 2006. Harmful algae on tropical coral reefs: Bottom-up eutrophication and top-down herbivory. Harmful Algae 5, 565-585.

Macko, S.A., Ostrom, N.E., 1994. Stable Isotopes in Ecology and Environmental Science, Blackwell Scientific Publications, Oxford.

McCook, L.J., 1999. Macroalgae, nutrients and phase shifts on coral reefs: scientific issues and management consequences for the Great Barrier Reef. Coral Reefs 18, 357-367.

Ohira, R., 2005. Hau Bush reunion evokes memories of Ewa Beach's jewel. Honolulu Advertiser,Honolulu. http://the.honoluluadvertiser.com/article/2005/Jun/23/ln/ln27p.html

Otkin, J.A., Martin, J.E., 2004. A synoptic climatology of the subtropical kona storm. Monthly Weather Review 132, 1502-1517.

Owens, N.P.J., 1987. Natural variations in δ^{15}N in the marine environment. Advanced Marine Biology 24, 389-451.

Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18, 293-320.

Robinson,D., 2001. δ^{15}N as an integrator of the nitrogen cycle. Trends in Ecology and Evolution 16, 153-162.

Russell, D.J., 1992. The ecological invasion of Hawaiian reefs by two marine red algae, *Acanthophora spicifera* (Vahl) Boerg. and *Hypnea musciformis* (Wulfen) J. Ag., and their association with two native species, *Laurencia nidifica* J. Ag and *Hypnea cervicornis* J. Ag. ICES Marine Science Symposium 194, 110-125.

Sammarco, P.W., Risk, M.J., Schwarcz, H.P., Heikoop, J.M. 1999. Cross-continental shelf trends in coral δ^{15}N on the Great Barrier Reef: further consideration of the reef nutrient paradox. Marine Ecology Progress Series 180, 131-138.

Salvat, B., 1997. *Coral reefs-A challenging ecosystem for human societies*. In: The human impact reader, readings and case studies, Ed. Andrew Goudie. Blackwell Publisers, Ltd Malden pp. 394-400.

Schaefers, A., 2006. Ocean pointe drainage plan under attack. Star Bulletin, Honolulu. http://archives.starbulletin.com/2006/12/04/business/story02.html

Schaefers, A., 2008. New luxury Ewa Beach resort community opens for viewing. Star Bulletin,Honolulu. http://archives.starbulletin.com/2008/04/18/business/story02.html

Schaffelke, B., 1999. Short-term nutrient pulses as tools to assess responses of coral reef macroalgae to enhanced nutrient availability. Marine Ecology Progress Series 182, 305-310.

Small, C., Nicholls, R. J., 2003. A global analysis of human settlement in coastal zones. Jounral of Coastal Research 19, 584-599

Smith, J.E., Hunter, C.L., Conklin, E.J., Most, R., Sauvage, T., Squair, C., Smith, C.M., 2004. Ecology of the invasive red alga *Gracilaria salicornia* (Rhodophyta) on Oahu Hawaii. Pacific Science, 58, 325-343.

Smith, J.E., Hunter, C.L., Smith, C.M., 2002. Distribution and reproductive characteristics of nonindigenous and invasive marine algae in the Hawaiian Islands. Pacific Science 56, 299-315

Smith, J.E., Runcie, J.W., Smith, C.M., 2005. Characterization of a large-scale ephemeral bloom of the green alga *Cladophora sericea* on the coral reefs of West Maui, Hawaii. Marine Ecology Progress Series 302, 77-91.

Smith, V.H., Tilman, G.D., Nekola, J.C. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environmental Pollution 100, 179-196.

Stimson, J., Larned, S.T., Conklin, E.J., 2001. Effects of herbivory, nutrient levels, and introduced algae on the distribution and abundance of the invasive macroalga *Dictyosphaeria cavernosa* in Kaneohe Bay, Hawaii. Coral Reefs 19, 343-357.

Teichberg, M., Fox, S.E., Aguila, C., Olsen, Y.S., Valiela, I., 2008. Macroalgal responses to experimental nutrient enrichment in shallow coastal waters: growth, internal nutrient pools, and isotopic signatures. Marine Ecology Progress Series 368, 117-126.

Umezawa, Y., Miyajima, T., Yamamuro, M., Kayanne, H., Koike, I., 2002. Fine-scale mapping of land-derived nitrogen in coral reefs by $\delta^{15}N$ in macroalgae. Limnology and Oceanography 47, 1405-1416.

Valiela, I., Foreman, K., LaMontagne, K., Hersh, D., Costa, J., Peckol, P., Demeo-Andreson, B., D'Avanzo, C., Babione, M., Sham, C., Brawley, J., Lajtha, K., 1992. Couplings of watersheds and coastal waters. Sources and consequences of nutrient enrichment in Waquoit Bay, Massachusetts. Estuaries 15, 443-457.

Walsh, C.J., 2000. Urban impacts on the ecology of receiving waters: a framework for assessment, conservation and restoration. Hydrobiologia 431, 107-114

Chapter V. Thermal ecology on an exposed algal reef - infrared imagery a rapid tool to survey temperature at local spatial scales

Abstract

We test the feasibility of infra-red (IR) thermography as a tool to survey *in situ* temperatures in intertidal habitats. We employ this method to describe aspects of thermal ecology for an exposed algal reef in the tropics (O‘ahu, Hawai‘i). In addition, we compare temperatures of the surrounding habitat as determined by IR thermography and traditional waterproof loggers. Images of reef organisms (6 macroalgae, 9 molluscs, 1 anthozoan, and 2 echinoderms), loggers, and landscapes were taken during two diurnal low tides. Analysis of IR thermographs revealed remarkable thermal complexity on a narrow tropical shore as habitats ranged from 18.1 to 38.3°C and surfaces of organisms that ranged from 21.1 to 33.2°C. The near 20°C difference between abiotic habitats and the mosaic of temperatures experienced by reef organisms across the shore are similar to findings from temperate studies using specialized longterm loggers. Further, IR thermography captured rapid temperature fluctuations that were related to tidal height and cross-correlated to wave action. Finally, we gathered evidence that tidal species were associated with particular temperature ranges and that two species possess morphological characteristics that limit thermal stress. Loggers provided similar results as thermography but lack the ability to resolve variation in fine scale spatial patterns. Our results support the utility of IR thermography in exploring thermal ecology and establishing baseline conditions in a changing and heterogeneous environment.

Introduction

Nearshore coastal habitats with fluctuating tidal conditions and discrete zones of organisms has long been recognized as model system to study the influence of abiotic factors on species distributions (Stephenson and Stephenson 1949; see Benson 2002). Since the groundbreaking research of Connell (1961), it is recognized that physical abiotic stress often determines intertidal species upper distributional limits while biological interactions are more influential closer to subtidal zones. Recently this paradigm has been expanded to incorporate how physical factors such as temperature can alter biological interactions like predation and competition (Wethey 1984; Hoegh-Guldberg and Pearse 1995; Sanford 1999; Schneider and Helmuth 2007; Pincebourde et al. 2008). Indeed temperature which influences a wide variety of physiological processes is often cited as the single most important abiotic factor impacting the ecology of marine organisms (Helmuth 2002; Somero 2002) and increasingly marine environments are subjected to that stress (IPCC 2007).

Temperature is likely a more important ecological driver in the tropics than at other latitudes (Tewksbury et al. 2008). Tropical marine habitats can be abiotically stressful for organisms (Moore 1972) as tropical species not only experience some of the highest temperatures and irradiances worldwide (Beach and Smith 1996) but they also can be acclimated to narrow thermal regimes (Stillman and Somero 1996; Tewksbury et al. 2008). For example, an increase of a few degrees above normal conditions in shallow marine waters results in coral bleaching (Brown 1997; Jokiel and Brown 2004; IPCC 2007). Thermal stress could be greater for intertidal reef organisms as the pool of water buffer drains away and many tropical habitats in which numerous species reside lack shade from canopy-forming algae (Abbott and Huisman, 2004 but see Beach and Smith 1996). Few examples of intertidal habitats in tropical areas confirm this hypothesis as organisms reside in cooler cracks and crevices (Menge and Lubchenco 1981), invertebrates exhibit cooling behaviors and morphologies (Vermeij 1971, 1973; Garrity 1984; Williams and Morritt 1995) , and even species compete for cooler microhabitats (Wethey 1984). Along the subtropical to tropical Main Islands of Hawaiʻi, where micro-tidal conditions persist for a marine fauna with temperate and tropical ancestry (Kay 1979; Abbott 1999), very little is known about nearshore thermal conditions nor the ecological influence of temperature. Bird (2006) shows that temperature and distributional patterns of animal and algae by functional groups are correlated on wave dominated shores in Hawaiʻi. The thermal variability and fine-scale thermal distribution patterns have yet to be examined.

Thermal ecology is difficult to investigate in any intertidal zone (Helmuth 2002; Tomanek and Helmuth 2002; Fitzhenry et al. 2004). This difficulty lies in the complexity of the environment as temperature can vary over different temporal and spatial scales (Helmuth and Hoffmann 2001; Helmuth et al. 2006a, 2006b). Tides, waves and even local weather act to rapidly alter temperatures and their magnitudes and timing can dictate the degree of heating or cooling (Helmuth et al. 2002). Habitat structure and location alter temperatures as the very nature of incoming and outgoing tides results in hotter temperatures for organisms found at higher tidal elevations (Somero 2002). However, angle of the shore and crevices can provide cool microhabitats and afford high zone species thermal protection (Helmuth 1998). Thus, to capture and account for this level of variation requires numerous efforts and large numbers of traditional waterproof loggers and probes.

The success of technologies can be gauged by their application to answer novel scientific questions. Thermography is clearly a useful approach in measuring sea surfaces temperatures collected by aerial infrared (IR) techniques, further thermal data from offshore surface buoys are combined with these data to characterize thermal stress and to predict the impacts of global climate change (Schneider and Helmuth 1997). Our approach is to employ this established technology to ascertain the localized temperatures experienced by reef organisms on low tides. Recent local scale efforts with IR technology have been useful in a laboratory setting to describe temperatures of captive sea stars (Pincebourde et al. 2009) and casually in the field to examine habitats (Schneider and Helmuth 1997; Helmuth 2002). This approach has yet to be applied rigorously to quantitatively assess temperatures in an intertidal reef.

The purpose of this study is to explore the feasibility of using IR thermography in an intertidal system to study aspects of thermal ecology. We use this technique to describe *in situ* temperatures that occur on diurnal low tides in a little studied intertidal reef, O‘ahu, Hawai‘i. Specifically, we examine 1) if reef organisms are associated with particular thermal habitats 2) if these organisms have characteristics that allow them to remain cooler than their surroundings and 3) the spatial and temporal temperature heterogeneity that occurs during spring diurnal low tides. Lastly, to test the efficacy of our methods we compare temperatures from IR thermography to traditional waterproof loggers and discuss the feasibility of this technique to rapidly assess temperatures at varying spatial scales.

Methods

Site selection

We examined shore temperatures during low tides at Diamond Head, O‘ahu (21°15’20.67” N, 157°48’38.94” W). The exposed reef platform at Diamond Head is composed of limestone with a small offshore reef that protects this intertidal zone from large onshore waves. The community is observably zonate (Kay 1979; Abbott 1999; Hoover 2002; Abbott and Huisman 2004) with small mollusks (*Echinolittorina* *hawaiiensis, Nerita picea,* and *Siphonaria normalis*) occurring closest to the high tide line, followed by a narrow zone of the mollusk *Dendropoma gregaria,*, and ending in a wetter habitat dominated by the reef macroalgae *Padina sanctae-crucis* (TEC personal observations).

Tidal selection

Sampling for this study occurred on 20th Jul 2009 and 21st Jul 2009, two days with a negative low tide of -0.12 m occurring at 0744 and 0847 hrs (respectively). These times are typical for Hawaii spring tides in summer months and a -0.12 m tide is close to the maximum negative height for mean low low water for the island of Oahu (max = -0.15 m for 2009, Honolulu Tidal Station, NOAA).

Habitat temperature ranges during low tides

On the two sampling days from the hrs of ~0730 (an hr after dawn) to 1030 (when tidal inundation prevented access to the shore) we used a Handy Thermo TVS-200 EX IR camera (NEC Avio IR Technologies, Tokyo, Japan) to capture digital images and video clips of the shore and resident biota. This camera captures temperature changes at 1/60 s from a distance range of 30 cm to infinity and has an accuracy of ± 2.0 °C with precision better than 0.08°C. All images were taken within a defined area of shore, from the terrestrial vegetation to the subtidal zone (~ 8-15 m) and a horizontal area that was ~ 20 m parallel to the water.

Infrared energy is converted to temperature by the following equation:

$$\text{Infrared energy} = \text{sigma} * e * T^4$$

where sigma is the Stefan-Boltzmann constant, e is emissivity, and T is Temperature (°K). The emissivity of biological organisms varies between 0.96-0.99 averaged across the long-wave infrared waveband (Tracy 1978, Nussear et al. 2000) and the organisms and rocks in the intertidal zone are estimated to vary similarly (Table 5.1). However, emissivity of objects can vary with weathering, composition of materials, and temperature (Rivard et al. 1995). Most organisms and habitat in the intertidal zone were wetted, likely increasing emissivity to values close to 1.0. Therefore the camera correction setting for emissivity was set to 1.0 and some error in determined temperatures

Table 5.1 Summarized emissivity values averaged over the long wave infrared band gathered from the literature for common surfaces found in Hawaii’s intertidal zones.

Surfaces Encountered	**Measured Emissivity**	**Reference**
Basalt	0.96	Rivard et al. 1995
Biological organisms	0.96-0.99	Tracy 1978, Nussear et al. 2000
Calcium carbonate (in shells, reef, spp. of algae)	0.96	Rivard et al. 1995
Photosynthetic surfaces	0.98	Samson and Lemeur 2000
Sand	0.90	Commercial tables online
Seawater	0.99	Charnell 1967

is expected. However, this error is likely not to exceed the ± 2.0 °C accuracy of the camera and logger recorded temperatures were similar to the temperatures observed in images.

Thermal analyses of photos were conducted using ImageJ software (www.nih.gov). The IR camera and its associated thermography software saved three images from each photo that were used in analyses: 1) color jpeg of the subject 2) thermal image in false color with a temperature scale bar and 3) a composite image of the normal jpeg and thermal image. Each thermal image or frame was in false color, thus images were converted with plugin script written and developed by William Winter (pers. comm.) to an indexed image so that each combination of red, blue, and green had one value from 0-364 that was linearly related to the thermal image scale bar. Using the linear option in the calibration function in ImageJ, it was then possible to determine the average, minimum, and maximum temperatures of the image or of a defined area within the image.

Organismal temperature ranges during low tides

To capture the temperature range that reef organisms experience during a typical diurnal low tide at Diamond Head, we periodically walked the defined area of shore and captured images (<0.5 m^2) of organisms in their habitats. These images were captured perpendicular to the substrate from a similar height to minimize any parallax error. An effort was made to not resample or take repetitive pictures of the same individuals. Images were analyzed as described above. Organisms were outlined in images to determine their average surface temperature. These reported temperatures reflect the temperatures that organisms endure in the field and do not necessarily reflect core body temperatures. In addition, we examined the conventional jpeg image to confirm species identifications. Composite images were used as a guide to determine surface temperature of the species in the photos.

Photos of organisms in their representative habitats and microhabitats were used to determine the typical range of temperatures encountered and were used to investigate if organisms differed in surface temperature from their surroundings. A grid of 56 randomly assigned points was generated and the point-intercept methodology used to determine the average temperature in a habitat. Points that fell upon organisms were excluded from the average to ensure independence. In images of invertebrate species, we also quantified the average temperature of the microhabitat (area directly next to the individuals). Differences in temperature were compared via paired t-tests as data met normality requirements.

Within habitat temporal temperature variation

To determine the variation in temperature that occurs during these typical low tides, we took landscape photos of the exposed reef within the *Padina sanctae-crucis* habitat, taken from roughly the same location at a similar angle, and determined the temperature of six locations for 900 s intervals throughout the day. These images captured an area of shore that was approximately 3 m horizontal and 8 m vertical in distance, as determined from a known reference, making use of topographic and introduced landmarks to re-locate sites.

Separate analyses were conducted over the two days, thus a different set of six locations were used each day. The temperatures from each sampling were averaged together by 60 s intervals and then averaged over 900 s intervals. These data were regressed with tidal height to examine the relationship between temperature and tidal cycle.

To explore the effect of waves at low tide on the temperature of the shore, we captured the thermal consequences of two series of wave events (1 series of events day^{-1}). One wave series was captured at 08:20 (less than an hour after peak low tide) on 20-Jul-2009 with video using the IR camera with a rate of 40 frames s^{-1} and lasted for 75 s. The second wave series was documented with a series of digital stills on the 21-Jul-2009 at 09:02 (less than an hour after peak low tide) and lasted for 63 s. The average temperature of the shore was determined using ImageJ (as described earlier) every 0.3 s throughout the filmed wave series (153 frames) and for each digital still (~ 1 to 5 images s^{-1} = 16 total images). Also, for each photo and frame the total number of pixels exposed to air were measured with ImageJ by outlining the area of shore directly ahead of the water line and quantifying pixels. The pixels exposed to air were plotted with the temperature and cross correlation was used to statistically investigate the relationship between temperature and wave events.

Within habitat spatial temperature variation

To investigate spatial variation of temperature and to determine the number of temperature loggers needed to answer a specific research question, we investigated the horizontal (parallel to the shore) and vertical variation (perpendicular to the shore) in temperature across different distances within one habitat (Fig. 5.1). Five landscape photos captured from a similar location on day 1 during the low tide were haphazardly selected. Using a visible known distance in indexed images, the number of pixels to m was calibrated to create virtual transect lines. Initially two transect lines placed on raised surfaces (tidepools, cracks, and crevices were avoided) were created to extended across the horizontal and vertical distance of the image. The lengths of the initial horizontal lines varied slightly among pictures (2.5 - 3.4 m); while the vertical transect distances were approximately 8 m from the landward edge of the *Padina* zone to the incoming tide. Temperatures along the transect lines were plotted and the interquartile distance among temperatures determined. The interquartile distance provides an estimate of variation between the 25% and 75% quartile and it is robust to outliers thus it is a conservative measurement of overall variation. Then using the known pixel to distance ratio the transect lines were sub-sampled. Horizontal transect lines were sub-sampled into 1.00, 0.5, 0.25, and 0.15 m transects. Because of the limited number of pixels (less than 3 pixels for 0.25 meters transects) vertical transect lines were sampled into 1.00 and 0.50 m lengths. Mean interquartile distances were determined for each transect length on each image. Initial screening did not reveal statistical differences among images thus these interquartile values were averaged across photos (N = 5). . Because we sub-sampled the initial transect length data lacked independence and 95% confidence intervals were used to compare means within horizontal and vertical lines.

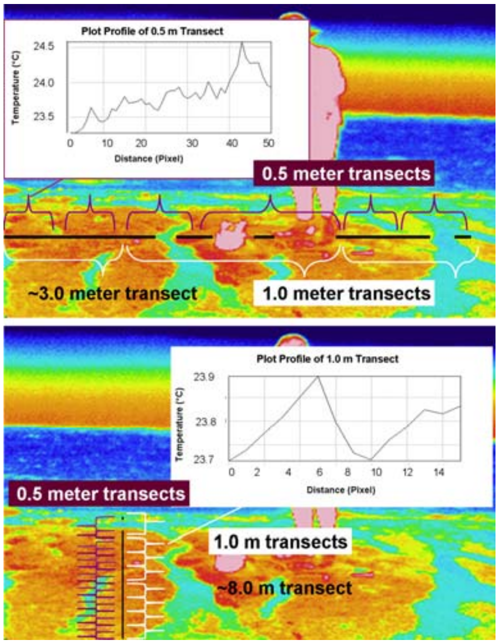

Fig. 5.1 Method used to determine horizontal (panel A) and vertical (panel B) temperature variation within raised surfaces of *Padina sanctae-crucis* habitat. A horizontal transect of ~3.00 m (black) was sub-sampled into 1.00 (white), 0.50 (violet), 0.25 (not shown), 0.15 (not shown) distances while a vertical transect of ~8.00 m (black) was sub-sampled into 1.00 (white) and 0.50 m (violet) distances. Plots of show the temperature plotted over the set number of pixels within the 0.50 or 1.00 m transect.

Comparison of methodologies

To compare methodologies, we examined the temperatures determined from IR images to the temperature determined by HOBO$^{©}$ waterproof loggers. Loggers were placed in white rubber covers to mimic the white colored limestone shore. One logger was placed near shore, one mid shore, and one close to the submerged reef on both days sampled. Between 07:00 hrs and 10:30, hrs photos of the temperature loggers were taken that included the nearby shore. An area of equal size to the logger was analyzed in photos for an average temperature. The temperature loggers recorded temperature every 900 s and temperature as determined via the analyzed images was compared to temperature from the nearest logger. Sample comparison between IR and loggers were never more than ± 300 s apart. Each location on the shore had 2 to 15 sampling intervals for comparison. An average temperature was calculated for each methodology, each day, at each location and pooled together for a total of six average comparisons (three per day). Data were initially screened for normality and homogeneity of variance and a paired *t*-test was used to determine if there were any statistical differences between daily average temperatures determined from the two methods.

Results

Over the two days during the low tide events 339 thermal images and three videos (4735 frames) of aerial exposed reef organisms and habitats were collected. Temperatures in these photos were determined to range from 18.3 to 38.3°C. However, the range of temperature that the organisms experienced (organism surface and microhabitat temperature only) was narrower, 21.1 to 33.2 °C (Table 5.2).

Organism surface and habitat temperatures did not correlate with distribution patterns. The gastropod, *Nerita picea*, which was distributed above the macroalgae and *Dendropoma gregaria* band (Kay 1979), had the lowest average surface temperature of any invertebrate or macroalgae examined despite being in the hottest zone. Similarly, *Siphonaria normalis*, a mollusk that commonly occurs close to the high tide line (Kay 1979), exhibited an outer shell temperature cooler than organisms from habitats closer to the low tide water line. Finally, the mollusk *Dendropoma gregaria* commonly occurred above the subtidal zone (Kay 1979), yet exhibited the hottest surface temperatures.

Surface temperatures of organisms were often different than their surrounding habitats (Figs. 5.2, 5.3, and 5.4). Macroalgae were found to be 0.4 to 1.8 °C cooler than the surrounding habitat, with species of the red alga *Liagora* (paired *t*-test, n = 5, *P* = 0.02) and the brown alga *Padina sanctae-crucis* (paired *t*-test, n = 7, *P* = 0.03) being significantly cooler than their habitats. Species of *Liagora* had the largest difference in temperature from the overall habitat, which was often bare of other macroalgae. Several molluscs were encountered with variable temperature differences from their overall habitat and their nearby microhabitat, suggesting movement or settlement preference to cooler microhabitats. Individuals of *Siphonaria normalis* (paired *t*-test, n = 10, *P* = 0.03) had cooler surface temperatures than their habitats, but their surfaces were warmer than the nearby microhabitat. Images of species of *Morula*, *Isognomon californicum* and

Table 5.2 Average ±SE, minimum, and maximum temperature (°C) of the surface of organisms and their habitats during low tides. Zone specifies the area of shore (Splash (S)> High (H)>Mid (M)>Low (L)) that organism is known to commonly occur based upon Kay 1979, Abbott 1999 & 2004, Hoover 2002. Area is expressed as sampled pixels for seaweeds while # of individuals are expressed for invertebrates.

Species	Authority	Zone (S,H,M,L)	# of Ind. or Area	# of Pictures	Organism Temp.	Min	Max	Habitat Temp.	Min	Max
Seaweeds										
Acanthophora spicifera	(Vahl) Borgesen	L	17076	4	25.3 ±1.3	22.1	26.3	26.2 ±1.7	22.1	28.1
Hydroclathrus clathratus	(C. Agardh) Howe	H,M,L	4383	1	25.5	-	-	26.4	-	-
Laurencia mcdermidiae	Abbott	L	2420	2	25.5 ±0.5	25.2	25.9	25.9 ±0.3	25.7	26.1
Liagora spp.		N/A	76125	5	26.5 ±0.7	25.1	28.8	28.0 ±0.9	25.3	29.5
Padina sanctae-crucis	Børgesen	L	65016	7	24.8 ±0.3	22.5	26.2	25.4 ±0.4	22.5	27.4
Sargassum echinocarpum	J. Agardh	L	9757	1	25.0	-	-	25.3	-	-
Molluscs										
Cypraea mauritiana	Linnaeus	M,L	2	1	23.2	-	-	22.5	-	-
Drupa ricina	Linnaeus	N/A	1	1	25.1	-	-	25.7	-	-
Morula spp.		N/A	4	3	27.4 ±1.2	26.4	29.8	27.9 ±0.9	27.9	29.3
Nerita picea	Recluz	S	51	5	23.3 ±0.8	21.7	25.9	23.0 ±0.6	21.7	24.4
Echinolittorina hawaiiensis	Rosewater and Kadolsky	S	192	8	25.2 ±0.8	21.4	27.7	25.2 ±0.7	21.1	26.9
Siphonaria normalis	Gould	S	81	9	28.5 ±0.3	26.3	32.3	29.4 ±0.5	26.4	33.2
Thais armigera	Link	L	1	1	24.3	-	-	24.1	-	-
Dendropoma gregaria	Hadfield and Kay	L	<200	3	28.6 ±0.5	28.1	19.1	28.0	-	-
Isognomon californicum	Conrad	H	5	1	24.5	-	-	25.0	-	-
Other invertebrates										
Anthopleura nigrescens	Verrill	L	15	2	27.5 ±0.2	27.3	27.7	27.6 ±0.1	27.6	27.7
Actinopyga mauritiana	Quoy and Gaimard	N/A	1	3	25.4	24.3	26.0	25.4	24.2	26.2
Echinometra oblonga	De Blainville	L	4	2	25.3 ±0.3	25.1	25.6	25.5 ±0.9	24.9	26.1

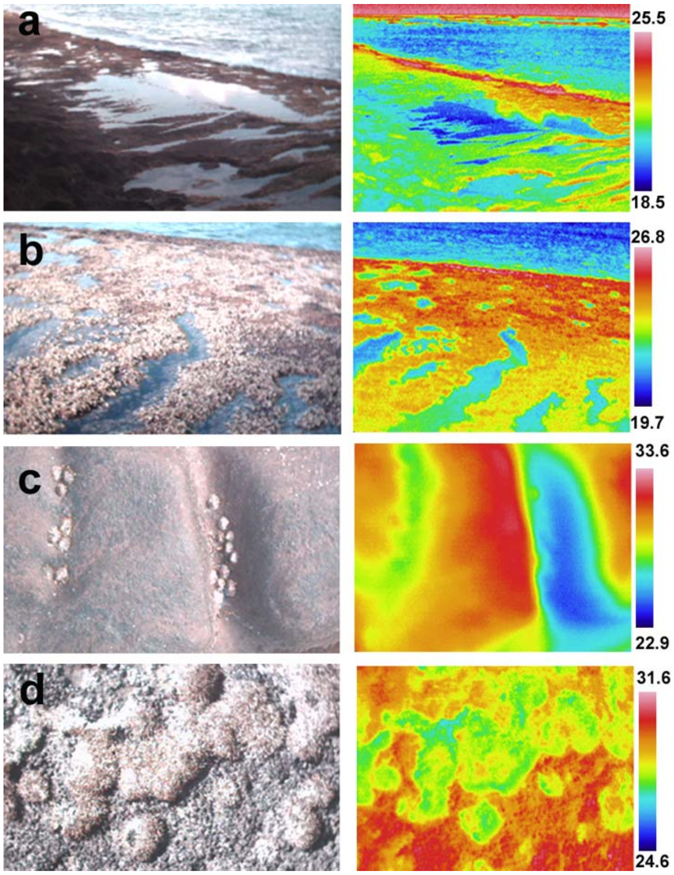

Fig. 5.2 Select thermal images; panels a and b show the habitat thermal heterogeneity and panels c and d show the surface temperatures of reef organisms. *Siphonaria normalis* (2.0 cm in size) occurs in sunken depressions (green and blue color) that are ~6°C cooler than nearby raised surfaces and (bottom) *Liagora* sp. (individuals up to 5 cm in size) with cooler surface temperatures than the nearby habitat. Images on the left are taken instantaneously with the right images which are calibrated (°C) in infrared.

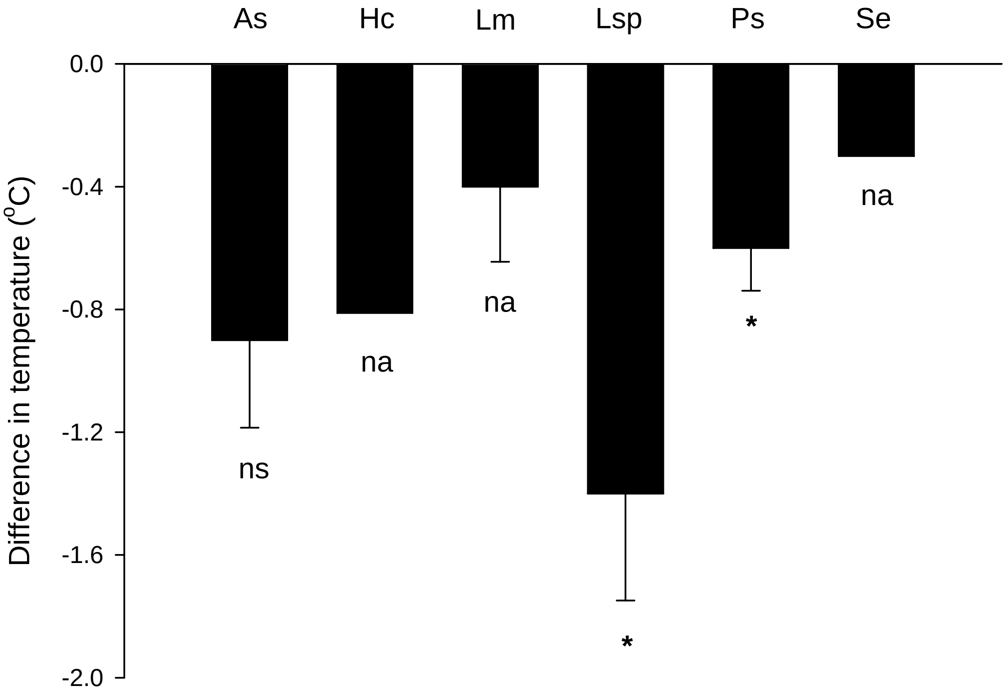

Fig. 5.3 Mean (± SE) difference in temperature (°C) between macroalgae and their overall reef habitat. Text below bars represents the results of paired t-tests between organism and the overall habitat temperatures; abbreviations are as follows: na = not applicable due to small sample size, ns = not significant, * <0.05. Abbreviations for seaweeds are as follows As = *Acanthophora spicifera*, Hc = *Hydroclathrus clathratus*, Lm = *Laurencia mcdermidiae*, Ps = *Padina sanctae-crucis*, Se = *Sargassum echinocarpum*

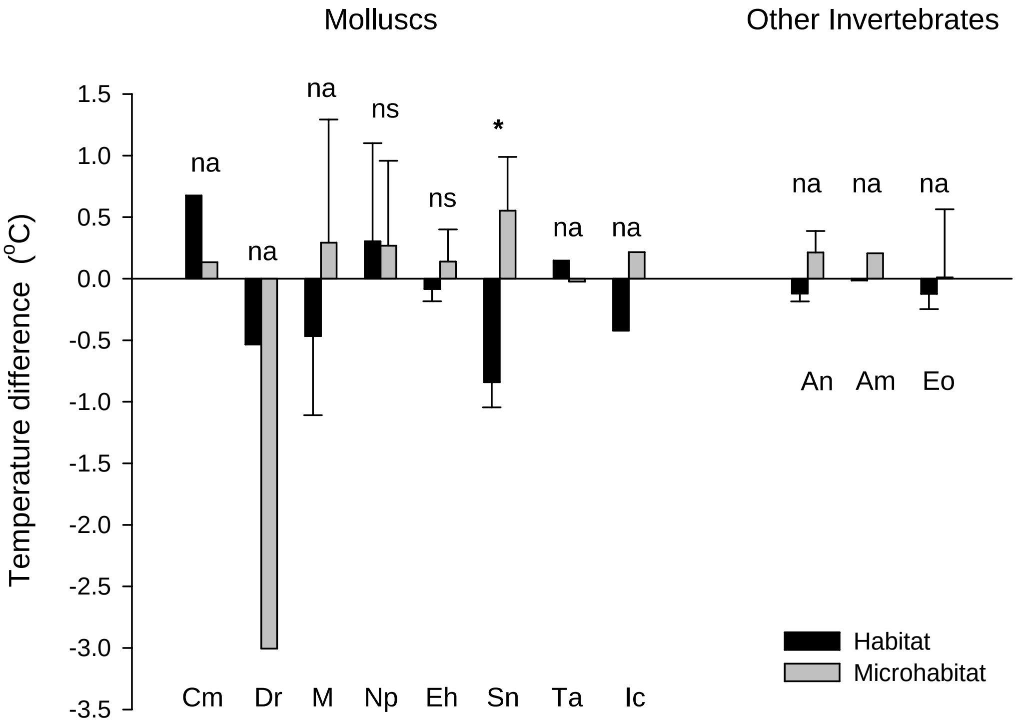

Fig. 5.4 Mean (± SE) difference in temperature (°C) between invertebrates and their habitat and microhabitat. Text above bars are the results of paired t-tests: na = not applicable due to small sample size, ns = not significant, * < 0.05. Abbreviations for invertebrates are as follows: Cm = *Cypraea mauritiana*, Dr = *Drupa ricina*, M = *Morula* spp., Np = *Nerita picea*, Eh = *Echinolittorina hawaiiensis*, Sn = *Siphonaria normalis*, Ic = *Isognomon californicum*, An = *Anthopleura nigrescens*, Am = *Actinopyga mauritiana*, Eo = *Echinometra oblonga*

Echinolittorina hawaiiensis (paired *t*-test, n = 8, *P* = 0.50) exhibited a similar trend, but either had too few images taken for statistical comparison or no statistical differences were detected using the current sample size. The surface temperature of *Nerita picea* did not tend to differ from the overall habitat or nearby microhabitat (paired *t*-test, n = 4, *P* = 0.58). Interestingly *Drupa ricina* was 3.0°C cooler than its microhabitat but was similar in temperature to the overall habitat. The echinoderms *Actinopyga mauritiana* and *Echinometra oblonga* and the anemone *Anthopleura nigrescens* were found in sunken depressions or crevices but surprisingly, did not differ in temperature from their respective habitats.

Temperature varied temporally and was related to tidal cycles (Day 1, cubic relationship, $P = 0.01$, $R^2 = 66\%$; Day 2, cubic relationship, $P = 0.04$, $R^2 = 75\%$) (Fig. 5.5). On both days, the peak average temperature of the exposed reef occurred approximately ~40 minutes after mean low low water. Day 2, with a similar but later peak low tide, averaged warmer temperatures (21.4 to 27.3 °C) than Day 1 (19.4 to 28.4). The difference between the minimum and maximum temperature recorded within this habitat over the low tide was determined to be 9°C on Day 1 and 6°C on Day 2.

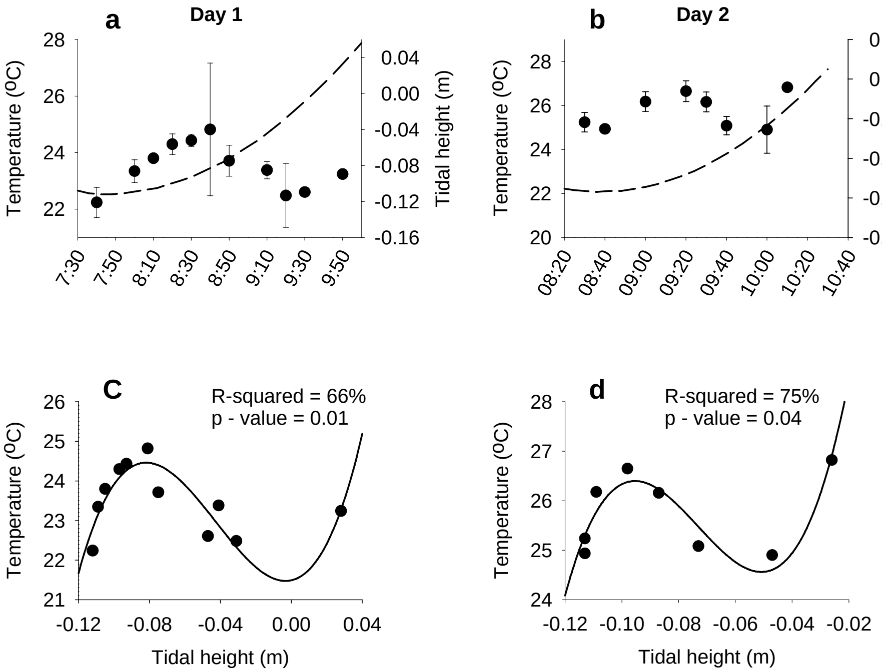

Fig. 5.5 Relationship between temperature and tidal height for two days (Day 1 = panel a and c; Day 2 = panel b and d), habitat temperature = circles ± SE plotted with tidal height = long dashed line (panels a and b) and a fitted line plot showing the cubic relationship (panels c and d).

Wave events lowered the temperature on the exposed intertidal reef (Figs. 5.6 and 5.7). A cross correlation between shore temperature and wave events revealed that temperature changes lag behind incoming waves by two seconds. Temperature was 66% correlated to wave events.

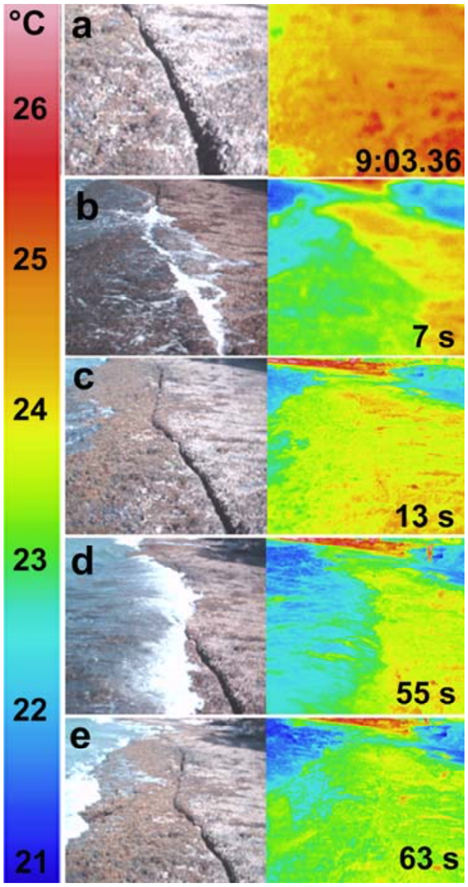

Fig. 5.6 Rapid temperature fluctuations during a series (panels a-e) of wave events captured with IR thermography (IR calibrated image on right simultaneously taken digital image on left). Coarse, pixel color calibrated to °C scale on right. Time stamp in corner of images is seconds that have elapsed from first image captured at 0903.36.

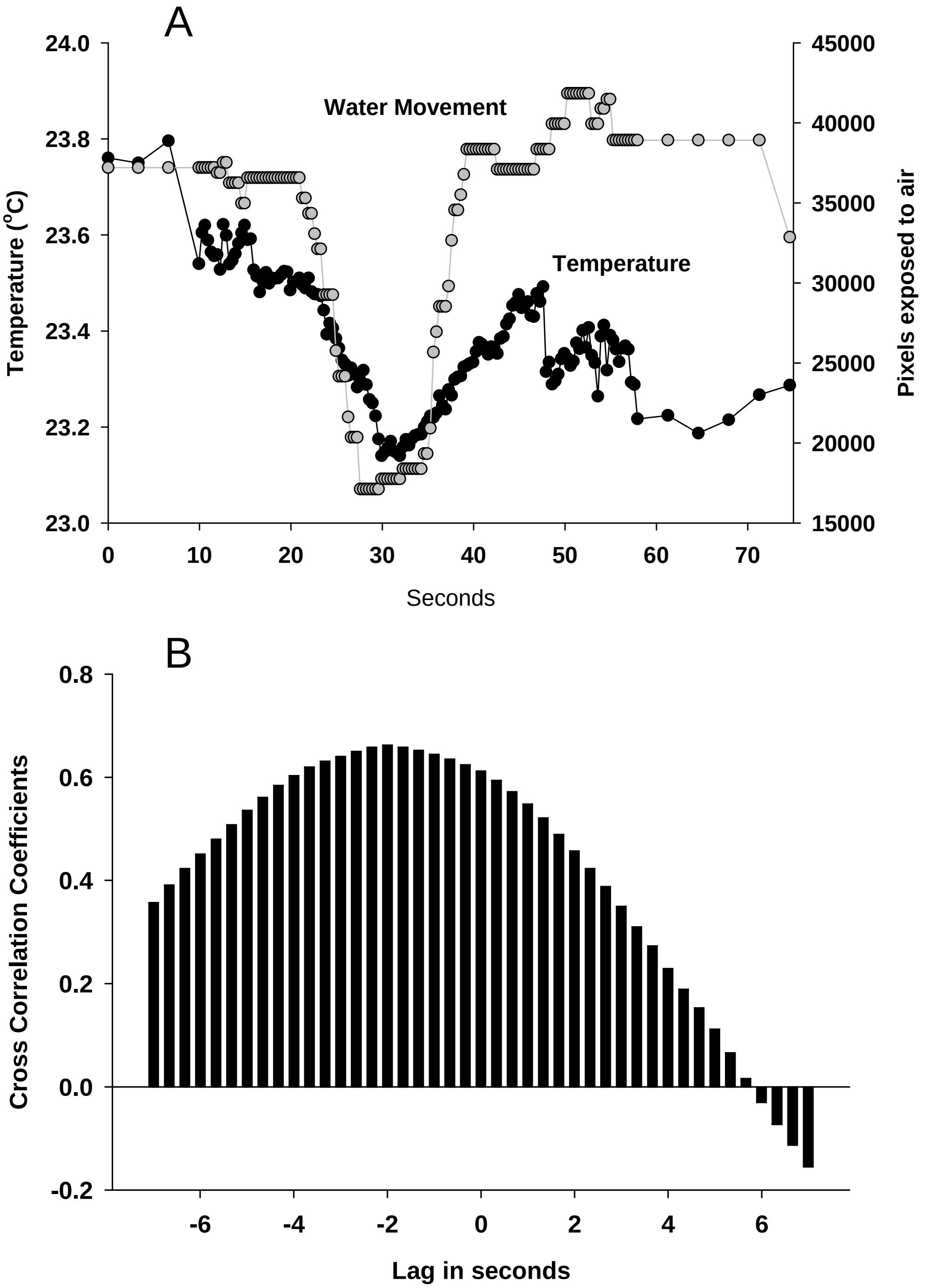

Fig. 5.7 Relationship between temperature and wave events. Panel A is a plot of temperature with wave movement (measured as pixels exposed to air) in time. Panel B shows the results of a cross correlation. Note temperature is 66% correlated to wave movement and that temperature lags behind the wave by 2 seconds.

IR determined temperatures did not differ from those logged by conventional devices; however, spatial variation in temperature within the *Padina* habitat was detected (Fig. 5.2 and Fig. 5.8). Temperature varied in the horizontal direction over the varying transect lengths from mean interquartile distances of 0.29-0.35°C. Horizontal and vertical variation tended to decrease with smaller sampled transect lengths and pixel number yet, the 95% confidence intervals around means often overlapped. Temperature varied in the vertical direction over varying transect lengths from mean interquartile distances of 0.19-0.40°C. Variation, measured as interquartile distance, was highest within the 8.0 meter vertical transect which had a similar pixel range as the 1.0 m horizontal transects. Yet the mean interquartile distance within the 1.0 m vertical transect was lower than the average value determined for 0.15 m horizontal transect and both of these transects had a similar sampled pixels.

Discussion

For just two diurnal tidal events that represent typical low tides in mid day, IR thermography rapidly revealed surprisingly fine scale perspectives. Tidal species were associated with particular thermal habitats and exhibited thermal heating, thermal cooling and thermal neutral patterns. The range of temperatures that occur in intertidal reef habitats in Oʻahu, is surprisingly complex and dynamic for a shore that experiences small tidal ranges (Beach et al. 2006) and relatively warm constant air and water temperatures (Tewksbury et al. 2008). Habitat heterogeneity contributes to temperature variability, and IR images allowed for visualization and quantitative assessment of algae and invertebrates that occupy these hot and cold microhabitats. Thus, contrary to predictions, organisms living closest to the high tide line were not necessarily hotter than those situated near the subtidal reef. Further, IR thermography indicated that temperature varies temporally and changes rapidly with the tidal height and wave events. These findings support the utility of IR thermography as a useful to tool in exploring thermal ecology in a changing, heterogeneous environment and documenting baseline thermal stress levels with the realization of climate change.

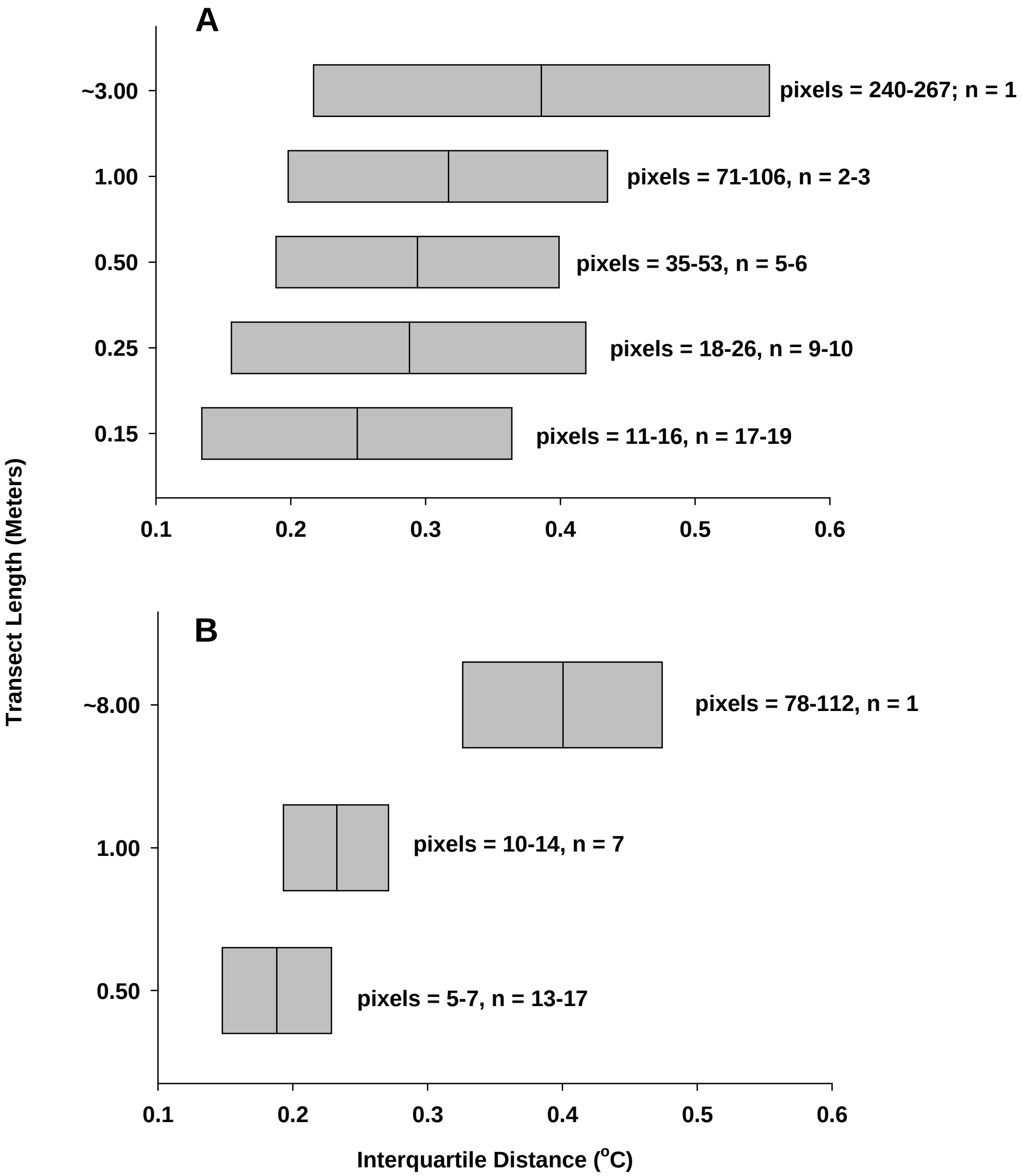

Fig. 5.8 Spatial variation in temperature found within *P. sanctae-crucis* habitat, expressed as interquartile distance, across horizontal (panel A) and vertical (panel B) transects of varying length on a low tide. Boxplots represent the 95% confidence interval around the mean interquartile distance determined from temperatures captured in 5 photos. Note the number of pixels sampled vary with distance and due to parallax, pixel range varies between horizontal and vertical analyses. n represents the number of transects sampled per photo.

Analyses of thermal images show that habitat temperature in the tropics can vary by as much as 20°C across a narrow intertidal reef yet, the difference in temperature that organisms experience at the microhabitat level may differ by as little as 12°C. This discrepancy is the result of the presence of cool and hot microhabitats. For instance, the calcified brown alga *Padina sanctae-crucis* is commonly intermixed with pockets of habitat-forming *Dendropoma gregaria* colonies, yet the alga maintain cooler surface temperatures than the molluscs. Similarly, the mollusc *Siphonaria normalis* was distributed in hot habitats near the high tide line but occurs in sunken depressions that were up to 6°C cooler than nearby raised rock surfaces. Garrity (1984) records similar findings for tropical gastropods in Panama, and Wethey (1984) also reports barnacles to favor cooler depressions. In Hawaiʻi, habitat heterogeneity and other factors such as angle of the sun may have more of an impact on organism's temperature and its thermal stress than distance from high tide line- a finding that would be difficult to determine with loggers or probes.

IR thermography captured rapid temperature fluctuations that are related to the tide and waves and it is likely greater variation would be observed with repeated temporal sampling. During diurnal low tides, temperature was related to tidal height with the hottest temperatures occurring after peak low tide but before high tide. Similar findings are found along the Pacific coast of United States with loggers that mimic mussels. Shores with low tides that coincided with mid-day experience hotter temperatures (Helmuth et al. 2002) and shores with more wave action are cooler (Helmuth et al. 2006a). Daily temperatures in mussel beds in temperate areas fluctuate by ~11 °C (Helmuth et al. 2006a), yet in Hawaiʻi, *P. sanctae-crucis* exposed reefs experience less temperature variation (up to 9 °C). Further investigations that combine the rapid thermal assessment provided by IR thermography to the more long term conditions captured by mimic loggers could enhance our predictions of thermal stress under hotter climates predicted to occur as a result of global climate change.

IR thermography reveals that despite the hot conditions experienced by organisms on Hawaiian shores, select algal, and invertebrate species occur in intertidal reefs and either 1) tolerate excessive temperatures (e.g. *Echinolittorina hawaiiensis*), 2) possess physical characteristics that limit thermal stress (e.g. calcified species of *Liagora* spp. and

Padina sanctae-crucis) that allow them to remain cooler that their immediate environment, or 3) or occur in cooler microhabitats (e.g. *Siphonaria normalis*) that perhaps optimize performance. Macroalgae are commonly cooler than their habitats, possibly because of the retention of water and subsequent evaporative cooling of thalli. Taxa that are consistently cooler than their habitats, *P. sanctae-crucis* and species of *Liagora* possess whitish deposits of reflective calcium carbonate (Abbott 1999; Abbott and Huisman 2004; Beach et al. 2006) that may limit solar heating. Alternately, the clumping morphology of species of *Liagora* and *Hydroclathrus clathratus* and the tightly growing monospecific stands of *P. sanctae-crucis* may limit desiccation and allow for evaporative cooling (Bell 1995). Few studies have documented energy budget parameters for intertidal algae and morphological characteristics such as calcification can alter the amount of light absorbed by 23 % lowering rates of maximum photosynthesis but defending against photoinhibition (Beach et al. 2006) and perhaps heat stress. Contrary to macroalgae and many other molluscs, it appears that the gastropods *Nerita picea, Cypraea mauritiana* and the echinoderms *Actinopyga mauritiana* and *Echinometra oblonga* endure tropical temperatures by favoring cooler habitats. Surfaces temperatures do not necessarily reflect the actual body temperatures of organisms (Lewis 1963), however, surface values provide insight into the types of temperatures these organisms must withstand. Further field and laboratory examinations with IR thermography at different shores combined with body temperatures may reveal more thermal specialists able to tolerate high temperatures or species with features that limit thermal stress.

Temperatures from IR thermography are similar to temperatures from waterproof sensors, yet data loggers are unable to match the spatial pixel scale detected by image based thermography. Additionally, numerous loggers and expense would be needed to replicate these thermography findings. Our spatial investigation into the variation over set distances, within a habitat at this site, reveal that to confidently reduce deviation to an approximate difference of ± 0.4 °C, loggers should be placed 1.0 m apart. Therefore to precisely quantify temperature across a similar area as examined in this study (20 m x 8 m = 160 m^2), at least 160 loggers (160 m^2 / 1.0 m = 160) are needed. Further quantitative sampling of aerial photos perpendicular to the shore with similar pixel to distance comparisons are needed to clarify observed differences in temperature variation among horizontal and vertical areas of shore. Finally, thermography also allows for quick measurements of temperature over a vast area and the visualization of cooler and hotter microhabitats as wells as the co-occurrence of wave events without much effort. Previous studies interested in thermal “preferences” (Vermeij 1971; Garrity 1984; Wethey 1984) would physically measure the temperature with a probe across the habitat surrounding an organism, requiring more physical efforts. Our calculations and observations underscore the potential of IR thermography for rapid temperature assessments and the need to have an adequate number of loggers to precisely and accurately answer fine-scale to landscape scale research questions.

These data clearly demonstrate the potential of IR thermography as tool for ecologists. Not only can IR thermography document baseline conditions in the light of global climate change but it also could be used to investigate natural or other anthropogenic thermal disturbances. For instance, the rapidity and fine scale nature of this tool could allow scientists to asses the thermal impacts from wave altering jetties or piers, heating of habitats and organisms that result from oil spills, and the impacts to a community when cooling canopy is removed via herbivory, human harvest, or wave events.

IR thermography while useful does have some limitations. Emissivity of infrared energy is difficult to account for in a heterogeneous environment like the intertidal zone. Further efforts to ground-truth emissivity of organisms and habitats could add to the utility of thermography for marine ecologists. IR thermography is also best suited for rapid assessments and it is not ideal for long-term monitoring. Surface temperatures captured in images do not necessarily reflect body temperatures. In addition, while images are relatively easy to collect, parallax error needs to be accounted for and analyses, and post processing can be time-consuming . Despite these limitations, this tool provides exciting results. IR thermography allowed us to take a new look at thermal ecology on a tropical intertidal reef.

References

Abbott IA (1999) Marine red algae of the Hawaiian Islands. Bishop Museum Press, Honolulu

Abbott IA, Huisman JM (2004) Marine green and brown algae of the Hawaiian Islands. Bishop Museum Press, Honolulu

Beach KS, Borgeas HB, Smith CM (2006) Ecophysiological implications of the measurement of transmittance and reflectance of tropical macroalgae. Phycologia 45:450-457

Beach KS, Smith CM (1996) Ecophysiology of tropical rhodophytes. II. Microscale acclimation in photosynthesis. J Phycol 32:710-718

Bell EC (1995) Environmental and morphological influences on thallus temperature and desiccation of the intertidal alga *Mastocarpus papillatus* Kutzing. J Exp Mar Biol Ecol 191:29-55

Benson KR (2002) The study of vertical zonation on rocky intertidal shores-a historical perspective. Intergr Comp Biol 42:776-779

Bird CE (2006) Aspects of community ecology on wave-exposed rocky Hawaiian coasts. Ph D. Dissertation, Botany. University of Hawai'i, Honolulu, p 255

Brown BE (1997) Coral bleaching: causes and consequences. Coral Reefs 16:129-138

Charnell RL (1967) Long-wave radiation near the Hawaiian Islands. J Geophysical Res 72:489-495

Connell JH (1961) The influence of intra-specific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. Ecol 42:710-723

Fitzhenry T, Halpin PM, Helmuth B (2004) Testing the effects of wave exposure, site, and behavior on intertidal mussel body temperatures: applications and limits of temperature logger design. Mar Biol 145:339-349

Garrity SD (1984) Some adaptations of gastropods to physical stress on a tropical rocky shore. Ecol 65:559-574

Helmuth B (2002) How do we measure the environment? Linking thermal physiology and ecology through biophysics. Integr Comp Biol 42:837-845

Helmuth B, Broitman BR, Blanchette CA, Gilman S, Halpin PM, Harley CD, O'Donnell M, Hofmann G, Menge B, Strickland D (2006a) Mosaic patterns of thermal stress in the rocky intertidal zone: implications for climate change. Ecol Monogr 76:461-479

Helmuth B, Harley CDG, Halpin PM, O'Donnell M, Hofmann GE, Blanchette CA (2002) Climate change and latitudinal patterns of intertidal thermal stress. Science 298:1015-1019

Helmuth B, Mieszokowska N, Moore P, Hawkins SJ (2006b) Living on the edge of two changing worlds: forecasting the responses of rocky intertidal ecosystems to climate change. Ann Rev Ecol Evol Syst37:373-404

Helmuth BS, Hoffmann GE (2001) Microhabitats, thermal heterogeneity, and patterns of physiological stress in the rocky intertidal zone. Biol Bull 210:374-384

Helmuth BST (1998) Intertidal mussel microclimates: predicting the body temperature of a sessile invertebrate. Ecol Monogr 68:51-74

Hoegh-Guldberg O, Pearse JS (1995) Temperature, food availability, and the development of marine invertebrate larvae. Am Zool 35:415-425

Hoover JP (2002) Hawaii's sea creatures: a guide to Hawaii's marine invertebrates. Mutual Publishing, Honolulu

Intergovernmental Panel on Climate Change (2007) Climate Change 2007: Synthesis Report Pachauri RK, Reisinger A (eds) Intergovernmental panel on climate change, Geneva, Switzerland

Jokiel PL, Brown EK (2004) Global warming, regional trends and inshore environmental conditions influence coral bleaching in Hawaii. Global Change Biol 10:1627-1641

Kay AE (1979) Hawaiian marine shells, reef and shore fauna of Hawaii. Bishop Museum Press, Honolulu

Lewis JB (1963) Environmental and tissue temperatures of some tropical intertidal marine animals. Biol Bull 124:277-284

Menge BA, Lubchenco J (1981) Community organization in temperate and tropical rocky intertidal habitats: prey refuges in relation to consumer prey gradients. Ecol Monogr 51:429-450

Moore HB (1972) Aspects of stress in the tropical marine environment. Adv Mar Biol 10:217-269

Nussear KE, Simandle ET, Tracy CR (2000) Misconceptions about colour, infrared radiation, and energy exchange between animals and their environment. Herpetological J 10:119-122

Pincebourde S, Sanford E, Helmuth B (2008) Body temperature during low tide alters the feeding performance of a top intertidal predator. Limnol Oceanogr 53:1562-1573

Pincebourde S, Sanford E, Helmuth B (2009) Natural History Note, An intertidal sea star adjusts thermal inertia to avoid extreme body temperatures. Am Nat 174:890-898

Rivard B, Thomas PJ, Giroux J (1995) Precise emissivity of rock samples. Remote Sens Environ 54:152-160

Samson R, Lameur R (2000) The role of surface temperature in the simulation of forest canopy photosynthesis. In: Ceulmans RJM, Veroustaete F, Gond V, Van Rensbergen JBHF(eds) Forest ecosystem modeling, upscaling, and remote sensing. Academic Publishing, by the Hague, Netherlands pp 69-86

Sanford E (1999) Regulation of keystone predation by small changes in ocean temperature. Science 283:2095-2097

Schneider KR, Helmuth B (1997) Patterns of heat and temperature. In: Denny MW, Gaines SD (eds) Encyclopedia of tidepools and rocky shores. University of California Press, Los Angeles, p 265

Schneider KR, Helmuth B (2007) Spatial variability in habitat temperature may drive patterns of selection between an invasive and native mussel species. Mar Ecol Prog Ser 339:157-167

Somero GN (2002) Thermal physiology and vertical zonation of intertidal animals:optima, limits, and cost of living. Integr Comp Biol 42:780-789

Stephenson TA, Stephenson A (1949) The universal features of zonation between tide-marks on rocky coasts. J Ecol 37:289-305

Stillman JH, Somero GN (1996) Adaptation to temperature stress and aerial exposure in congeneric species of intertidal porcelain crabs (Genus *Petrolisthes*): correlation of physiology, biochemistry, and morphology with vertical distribution. J Exp Biol 199:1845-1855

Tewksbury JJ, Huey RB, Deutsch B (2008) Putting the heat on tropical animals. Science 320:1296-1297

Tomanek L, Helmuth B (2002) Physiological ecology of rocky intertidal organisms: a synergy of concepts. Intergr Comp Biol 47:771-775

Tracy CR (1978) Comments on emissivity: a little explored variable. In: Burt EH (ed) The behavioral significance of color. Garland Publishers, New York, pp 28-32

Vermeij GJ (1971) Substratum relationships of some tropical Pacific intertidal gastropods. Mar Biol 10:315-320

Vermeij GJ (1973) Morphological patterns in high-intertidal gastropods: adaptive strategies and their limitations. Mar Biol 20:319-346

Wethey DS (1984) Sun and shade mediate competition in the barnacles *Chthamalus* and *Semibalanus*: A field experiment. Biol Bull 167:176-185

Williams GA, Morritt D (1995) Habitat partitioning and thermal tolerance in a tropical limpet, *Cellana grata*. Mar Ecol Prog Ser 124:89-103

Chapter VI. Temperature tolerance of nine tropical marine macroalgae

Abstract

With our changing oceans, temporal differences in altered abundance of algae and coral in reef systems must be examined from both the physiological and ecological perspectives, particularly responses to thermal stress. To gain insight into the physiological sensitivity or tolerance of tropical macroalgae to thermal increases in coastal waters, we surveyed photosynthetic metabolism of nine (native, endemic, and introduced) species that are common on Hawaii's nearshore reefs. Fluorescence and oxygen exchange techniques were compared and used to measure net photosynthesis and relative maximum electron transport rates before and after individuals were exposed to short term doses (10 min) of warmed (25, 30, 35, or 40°C) seawater. The two methods provided and revealed similar results; reef algae can photosynthesize and recover from remarkably high short term temperatures doses (between 35 and 40°C). Species differed in their photosynthetic response. The non-indigenous invasive, *Acanthophora spicifera*, was sensitive to elevated temperatures, but maintained rates of photosynthesis that were competitive with more thermally robust species. These findings underscore the importance of a thermal tolerance assay to screen candidate invasive species, but also results provide a predictive framework for identifying physiologically competitive species that could be associated with range expansions via warmer water marine environments.

Introduction

Temperature is a main factor controlling photosynthesis (Davison 1991), growth (Gerard and Du Bois 1988), and the distribution of marine macroalgae (Lüning 1990). Algal diversity and distribution patterns globally correspond with ocean temperatures (Lüning 1990) and on a local scale where temperature fluctuates algal abundances also vary (Oates and Murray 1983; Kübler and Davison 1993).

Macroalgae have an optimum range for photosynthesis (Davison 1991). Generally, photosynthesis increases with increasing temperatures until an optimum is reached beyond which rates rapidly decline (Berry and Bjorkman 1980). This decline in photosynthetic metabolism can be attributed to changes in light-use characteristics (Davison 1991; Kübler and Davison 1995), increased rates of photorespiration (Berry and Raison 1981; Raven and Geirder 1988), changes in enzyme activity (Raven and Geirder 1988), or damage to photo-systems (PS) (Morris and Kromkamp 2003) and electron transport (Kübler et al. 1991). Damage to photo-system II (PSII) and disruption of energy transfer are known to be responsible for short-term declines related to heat stress (Fork et al. 1979; Kübler et al. 1991; Morris and Kromkamp 2003).

Thermal tolerances for photosynthesis vary among species, populations, and life history stages (Lüning 1990; Davison 1991) are influenced by previous thermal history (acclimation) and confined by genetic adaptation (Lüning 1990; Davison 1991; Kübler et al. 1991; Kübler et al. 1991; Kübler and Davison 1993; Kübler and Davison 1995). Species or populations with different biogeographic distributions often have different thermal tolerances (Smith and Berry 1986; Eggert and Wiencke 2000). Temperature in the local environment or laboratory can influence photosynthesis. Generally, macroalgae exposed to higher temperatures are found to tolerate or have optima rates of photosynthesis at higher temperatures (Oates and Murray 1983; Davison 1991; Kübler and Davison 1993; Padilla-Gamino and Carpenter 2007; Wernberg et al. 2010). Previous influence of thermal history is apparent in the seasonal shifts in temperature limits observed for a variety of species (Niemeck and Mathieson 1978; Padilla-Gamino and Carpenter 2007). The islands of Hawaiʻi have a diverse marine flora with temperate and tropical origins that experience relatively warm constant water temperatures and small tidal fluctuations (Abbott 1999; Abbott and Huisman 2004). Introduced algal species from tropical localities are often observed on Hawaiian reefs and can form nuisance blooms (Smith 2003). This phenomenon provides the impetus for our research; given the sub-tropical to tropical environment and the diverse marine flora how sensitive are reef algae in Hawaiʻi to high temperatures and thermal stress?

In tropical marine habitats, that experience some of the highest temperatures worldwide (Beach and Smith 1996), the ability of reef algae to photosynthesize at high temperatures and recover from heat stress may contribute to growth and abundance as well as be beneficial for survival. Although sea surface temperatures are relatively constant and warm, near shore temperatures can fluctuate with weather, currents, and tides. On reefs in Waikiki the biomass of select species change with season (Doty 1971) but in narrow intertidal habitats temperature fluctuates over fine spatial scales (Cox and Smith, *in review*). Bird 2006 found the distribution of reef algae by functional group to correlate with temperature on wave dominated shores. In the face of rising temperatures brought about by global climate change we may expect temperature sensitive species to decline while robust species start to thrive and possibly become invasive.

To understand changes in abundances and growth of macroalgae on our tropical reefs we must have comparable tools and techniques that allow measurements of species sensitivity and photosynthetic response to temperatures. Assays that are rapid and procedural provide an objective technique to compare responses. The rapid light curve function in Diving-pulse amplitude modulated fluorometer (PAM) measures the efficiency of PSII to covert light energy to electron transport over set range of irradiances (White and Critchley 1999; Ralph and Gademann 2005). This tool is amenable to field conditions, takes measurements rapidly, and produces curves similar to the traditional photosynthetic-irradiance (P-I) curves (White and Critchley 1999). Photosynthetic versus irradiance (P-I) curves are produced via oxygen exchange and they can be directly related to carbon gain (Littler 1979). However, it is difficult to relate previous oxygen evolution studies to new fluorescence based measurements. Therefore, we investigated the relationship between fluorescence and oxygen exchange since there has been much debate over use and limitations of this tool in application with marine algal production (Genty et al. 1989; Hanelt and Nultsch 1995; Rascher et al. 2000; Longstaff et al. 2002; Beer and Axelsson 2004; Herlory et al. 2007; Ihnken et al. 2010).

The purpose of this research was to survey the photosynthetic sensitivity of Hawaiian reef algae to high heat to gain insight into their ecology and physiology. We used a dosage assay to survey nine species from different taxonomic groups collected from nearshore habitats. We measured the aspects of their photosynthetic response via chlorophyll fluorescence and oxygen exchange to 10 minute doses of temperatures that were within and above those that occur in natural settings. Species more tolerant to high temperatures were expected to be able to maintain rates of photosynthesis and the maximum relative electron transport rates ($r\mathrm{ETR}_{\mathrm{max}}$) while sensitive species were expected to exhibit decline rates. Lastly, we compare the relationship between measured electron transport rates and O_2 production for these species. Results of this study provide a baseline assessment and a framework for further field experiments exploring coral reef health as well as the physiological ecology of reef algae.

Methods

Species Collection & Preparation

Experimental trials to assess the photosynthetic sensitivity of common reef algae were conducted between 1200-1700 hrs in the months of July-September 2010. We selected species that are commonly encountered in shallow reef settings and collected individuals daily from two sites along the south shore of O‘ahu at 0.5-2.0 m depths between 600-800 hrs (Table 6.1 & 6.2). The two sites, Diamond Head (21°15'18.68 N, 157°48'39.48 W) and Black Point (21°15'25.21 N, 157°47'49.39 W), are approximately 1.5 km from each other. Species were kept in seawater, and were transported in a darkened cooler to the laboratory.

Algae were immediately sampled by creating tissue segments or disks with a 2 cm^2 cork borer or razor blades, avoiding the meristems (Table 6.2). Similarly, a second sample was cut from the fresh algal material to produce a control. Samples were placed in separate seawater filled chambers and kept on a shaker table underneath a 12-h cyclic light bank (PAR, 200 μmoles m^{-2} s^{-1}) where they were allowed to wound heal for ~4-6 hours. The seawater in holding chambers (used for wound healing and for 24-h recovery) was collected from near shore with the macroalgae. The temperature in the chambers was maintained by a surrounding water bath that slowly fluctuated from an overnight low of 23°C to a daylight temperature of 25°C (±0.5). After wound healing but prior to treatments, initial photosynthetic measurements were made.

Experimental Setup: Short-term effect

After initial values of photosynthesis had been measured, experimental samples were transferred into separate water-jacketed Clark electrode chambers kept under dim ambient light and exposed to a heated seawater treatment of 25, 30, 35, or 40±0.2 °C for 10 min. These treatment temperatures resembled (25, 30°C) or were above (35, 40°C) conditions experienced in natural settings. On occasional events, such as a low tide on a hot day, macroalgae may experience a 30°C environment but 35 and 40 °C temperatures are likely above natural field based condition experienced and are included to test the

Table 6.1 A description of the species arranged alphabetically within Divisions. Table includes the taxonomy, distribution, habitat as referenced from A from Abbott 1999, Abbott and Huisman 2004, and Huisman et al. 2007

Species	Taxonomy (Division/Class, Order)	Description of Adult Form	Distribution	Habitat
Acanthophora spicifera	Rhodophyta, Ceramiales	Upright cylindrical axes with spine like branches	Introduced to Hawaii, Tropical to warm temperate	Intertidal to shallow subtidal
Laurencia mcdermidiae	Rhodophyta, Ceramiales	Bright green, erect fronds, grows in clumps	Hawaiian Islands	Low intertidal exposed areas
Dictyosphaeria cavernosa	Chlorophyta,Cladophorales	Hollow cushions, single layer of cells	Tropical & subtropical seas	Generally shallow subtidal
Dictyosphaeria verslusyii	Chlorophyta, Cladophorales	Solid cushions, many cell layers thick	Tropical & subtropical seas	Intertidal to subtidal
Ulva lactuca	Chlorophyta, Ulvales	Blade, 2 cell layers thick	Widespread warmer seas	Intertidal & shallow subtidal
Lobophora variegata	Phaeophyceae, Dictyotales	Fan shaped, attached, to 12 cells thick	Tropical to warm temperate	Intertidal to subtidal
Padina sanctae-crucis	Phaeophyceae, Dictyotales	Fan shaped, calcified, 2 cells layers thick	Widespread warmer seas	Dominant in intertidal to subtidal
Sargassum aquifolium	Phaeophyceae, Fucales	Complex with stipe, blades, holdfast	Hawaiian Islands	Dominant in mid -low intertidal, shallow water
Stypopodium flabelliforme	Phaeophyceae, Dictyotales	Fan shaped, iridescent, non-calcified	Warm waters of the Indian & Pacific Oceans	Low intertidal to subtidal

Table 6.2 Summary of collection and sampling of algae.

Species	Sampled Area	Collection Location	Habitat Sampled From
A. spicifera	Below apical meristem, 3 cm tall with 3 branches	Black Point	Intertidal
L. mcdermidiae	Below apical meristem, Clump ~3 cm tall	Diamond Head	Low intertidal
D. cavernosa	Center to edge of cushion	Diamond Head	Shallow subtidal
D. verslusyii	Center to edge of cushion	Diamond Head	Low intertidal to shallow subtidal
U. lactuca	Blade inside of meristem	Black Point	Intertidal
L. variegata	Inside of meristem	Diamond Head	Low intertidal
P. sanctae-crucis	Inside of meristem, calcified surface to light	Diamond Head	Intertidal
S. aquifolium	Flattened blades avoiding edges	Diamond Head	Low intertidal
S. flabelliforme	Blade inside of meristem	Diamond Head	Subtidal

sensitivity or tolerance of species to extreme heat stress. Seawater in all Clark electrode chambers was collected from the south shore of O‘ahu, filtered (2 μm), and replaced between sampling periods. After a 10 min exposure to heated seawater, samples were immediately transferred back to 25°C (measuring chambers) and photosynthesis and respiration rates collected via oxygen exchange followed by fluorescence measurements.

Experimental trials were conducted over a 3 month period. For each day when testing was conducted, multiple trials were run with fresh samples. For each trial, treatment temperature and species sampled were varied in combinations of four in a haphazard manner until each species had reached a sample size of three or more at each temperature level (25, 30, 35, or 40°C). In addition, each day the order of the temperature treatments and the species combinations were varied. This haphazard sampling accounted for any confounding day to day or hourly effects on the response to stress. Therefore, a species' sensitivity to heat was determined as the average response from the field conditions over the sampling period and comparable to the other tested species.

Experimental Setup: Long-term effect

To examine the long-term effect of the heat dosage on photosynthesis we allowed samples a "recovery" period where they were kept in holding chambers overnight. After ~24 h, photosynthetic rates of control and experimental samples were measured at a similar time of day as the previous measurements. Control samples did not receive any heat treatment and their rates were used as a benchmark to account for changes that may result from laboratory conditions.

O_2 Measurements of Photosynthesis and Respiration

Gross photosynthetic (GP) and respiration rates were measured as oxygen exchange using calibrated, water-jacketed Clark-type oxygen electrodes, following the methods of Beach et al. (1996). GP was corrected for respiration to provide net photosynthetic rates. These rates were standardized to surface area and reported as μmoles O_2 m^{-2} s^{-1}. Because respiration rates can differ with illumination, oxygen consumption was measured in the dark for 5 min before and after illumination and averaged prior to adjusting for net photosynthesis (NP). For all measurements, temperature in chambers was maintained at 25 ± 0.2°C. Constant illumination (250 μmoles quanta m^{-2} s-^{1}) was provided by slide projectors with tungsten bulbs. This illumination is within the range of the saturation irradiance (E_k) for these species during summer months (see Chapter VII). All samples were handled similarly and were attached via clear small bands to a screen platform connected to chamber lids.

Rapid Light Curves

The pre-programmed rapid light curve (RLC) routine in the Diving-Pulse Amplitude Modulated (Diving-PAM, Walz Inc.) fluorometer was used to determine the photosynthetic characteristics of the macroalgal species tested (as described in White and Critchley 1999; Ralph and Gademann 2005). Individuals were placed one layer thick into the standard leaf clip held at a set distance from the end of the PAM fiber optic cable. Eight increasing irradiances were delivered to each macroalga over a 90 s interval. Irradiances ranged between 0-1800 μmoles m^{-2} s^{-1}. With each increasing irradiance a saturating pulse was applied to compare the minimum (F) and maximum quantum yield (F'_m) in light adapted algae. Then these values were used to calculate the effective quantum yield (Φ_{PSII}) as the indication of the amount of energy used in the photochemistry of PSII (Genty et al. 1989).

$$\Phi_{PSII} = \Delta F / F'_m = (F'_m - F) / F'_m$$

From Φ_{PSII} the approximation of the rate of electrons (*r*ETR) pumped through the photosynthetic chain (Beer et al. 2001) was calculated using the following equation:

$$rETR = \Phi_{PSII} \times 0.5 \times AF$$

The 0.5 is assuming that half the photon energy absorbed by photosynthetic pigments was diverted into photosystem II and AF is the standard absorption factor 0.84.

The *r*ETR values were plotted against the measured irradiances to produce a RLC. The intensity of the eight irradiances was adjusted for each species to optimize the curves in such a way that the *r*ETR values plotted against irradiance ramped to an asymptote and then slightly leveled off at higher irradiances. These curves were fitted with the exponential model which includes the dynamic photo-inhibition parameter proposed by Platt et al. (1980) using the following Levenberg–Marquardt regression algorithm:

$$P = P_s\,[1 - \exp(-\alpha E/P_s)]\,\exp(-\beta E/P_s)$$

P_s is a scaling parameter defined as the maximum potential *r*ETR; α is the photosynthetic efficiency measured by the initial slope of the RLC before the onset of saturation; E is the downwelling photon flux density; β is the negative slope of the RLC for high irradiances. In the absence of dynamic photo-inhibition (β=0), the function becomes a standard rectangular hyperbola, with an asymptotic maximum *r*ETR value (Harrison and Platt 1986), and the equation loses $\exp(-\beta E/P_s)$. The parameters $r\mathrm{ETR}_{max}$, the relative maximum electron transport rate, and E_k were estimated using the following equations:

$$r\mathrm{ETR}_{max} = P_s\,[\alpha/(\alpha + \beta)]\,[\beta/(\alpha + \beta)]^{\beta/\alpha}$$

$$E_k = r\mathrm{ETR}_{max}/\alpha$$

Statistical comparisons

A treatment effect within species was determined from differences in net photosynthetic rates and the $r\mathrm{ETR}_{max}$ values. For each species and treatment, a repeated measures ANOVA was used to compare values collected from experimental individuals initially, immediately after the treatment, and after the 24 h recovery. Then Tukey's multiple comparison was used to determine whether initial values statistically differed from short-term measurements. A *t*-test was used to compare the control and experimental values determined after the 24 h recovery. Data were screened for normality and homogeneity of variance and transformed when necessary to meet parametric requirements. A short-term effect from the heat treatment was considered to occur if the initial values statistically differed from the short-term measurements, whereas a long-term effect was consider to occur if control values statistically differed from the experimental long-term measurements. If any short-term or long-term effects were observed within the 25°C treatment this was considered a handling effect and any other differences that occurred were not contributed to heat stress. The other RLC parameters were reported but not statistically tested as they lacked independence from the $r\mathrm{ETR}_{\max}$.

We examined the contribution of respiration and GP to NP for each species at each treatment level. The respiration and gross photosynthetic rates were averaged and plotted for each sampling interval within each treatment by species.

We compared, among species, the percent reduction or recovery in photosynthetic values after exposure to 35 and 40°C treatments. Short-term measurements were expressed as a percent of the initial values and reported as percent reduced for each species. The long-term experimental measurements were expressed as the percent of the control measurements and reported as percent recovered for each species.

Lastly, we statistically compared the relationship between *r*ETR values and GP. For each RLC, the *r*ETR value at 250 μmoles m^{-2} s^{-1} was determined via a back-calculation using the exponential model (see earlier equation) and the derived α, ß, and P_s parameters. Then for each species a multiple regression was used to determine the relationship between GP and *r*ETR. We included the temperature treatment to which the macroalga was exposed and an interaction term in the model. Terms were removed in a stepwise manner but GP (the dependent variable), was always included. Data that did not meet normality or homogeneity of variance were transformed to meet parametric requirements. A sequential Bonferroni was used to correct statistical α for the 5 (2 RM-ANOVAs, 2 *t*-tests, and 1 multiple regression) comparisons made with each sample (Holm 1979; Rice 1989).

Results

O_2 Measurements of Photosynthesis and Respiration

Macroalgae have varying initial rates of NP (Table 6.3). When rates where standardized to surface area *A. spicifera* had the highest rates followed by

Table 6.3 Initial rates of net photosynthesis at irradiance of 250μmoles m^{-2} s^{-1}

Species	**N**	**μmoles O_2 m^{-2} s^{-1}**	**μmoles O_2 g fresh wt^{-1} min^{-1}**	**μmoles O_2 g dry wt^{-1} min^{-1}**
A. spicifera	21	7.03±0.40	-	-
L. mcdermidiae	20	5.26±0.39	-	-
D. cavernosa	15	0.85±0.08	0.05±0.00	0.68±0.08
D. verslusyii	17	0.82±0.06	0.03±0.00	0.39±0.03
U. lactuca	23	1.83±0.12	2.45±0.15	7.34±0.46
L. variegata	21	1.19±0.08	0.37±0.02	1.78±0.12
P. sanctae-crucis	20	1.29±0.09	1.11±0.08	1.73±0.12
S. aquifolium	24	1.46±0.03	0.33±0.03	2.50±0.22
S. flabelliforme	19	1.10±0.09	0.63±0.05	3.29±0.27

L. mcdermidiae. Two species of *Dictyosphaeria* exhibited similar rates that were lower than all other species examined. Phaeophyceaen algae varied in rate and the order of variation (highest to lowest) depended upon whether the rates were standardized to surface area, grams of wet weight, or grams of dry weight.

Macroalgae were able to maintain rates of NP similar to initial or control samples within the 25°C treatments (Figs. 6.1, 6.2). Thus manipulating or handling the algae did not affect NP. However, initially measured net photosynthetic rates were often statistically different than those determined from the long-term controls. These differences were likely due to laboratory conditions that may not perfectly mimic natural settings.

All species maintained net photosynthetic rates comparable to initial or long-term control rates after exposure to 30°C treatment, except for *A. spicifera*. Short-term measurements of NP after 30°C in *A. spicifera* were statistically lower than initial measurements (RM-ANOVA, $p \leq 0.001$; Tukey's, p = 0.04) but, these values were on average 84.6 ($\pm$2.2) % of the initial value (Table 6.4). Also, NP recovered to values determined for long-term controls.

Most of the red and brown algal species tested exhibited NP in short-term measurements after exposure to 35°C treatment (Figs. 6.1, 6.2). Four species were found to have statistically lower net rates after the statistical alpha had been corrected sequentially for multiple comparisons. These affected species included the red algae *A. spicifera* (RM-ANOVA, $p \leq 0.05$; Tukey's, $p = 0.03$) and *L. mcdermidiae* (RM-ANOVA, $p \leq 0.001$; Tukey's, $p \leq 0.01$), and the brown algae *L. variegata* (RM-ANOVA, $p \leq 0.001$; Tukey's, $p = 0.02$) and *S. flabelliforme* (RM-ANOVA, $p \leq 0.01$; Tukey's, $p \leq 0.01$). The long-term values of NP for these species recovered to rates measured for controls (Figs. 6.1, 6.2).

Although these species (*A. spicifera*, *L. mcdermidiae*, *L.variegata*, and *S. flabelliforme*) had values that were affected in the short-term from the 35°C dosage, the percent reduction in NP was similar to or less than the reductions that occurred for other species (Table 6.4). For example, short-term measurements of NP in the affected species, *A. spicifera*, were on average 83.2$\pm$3.8% of the initial measurements while *P. sanctae-crucis,* which was not statistically affected after a 35°C dosage, had short-term NP values

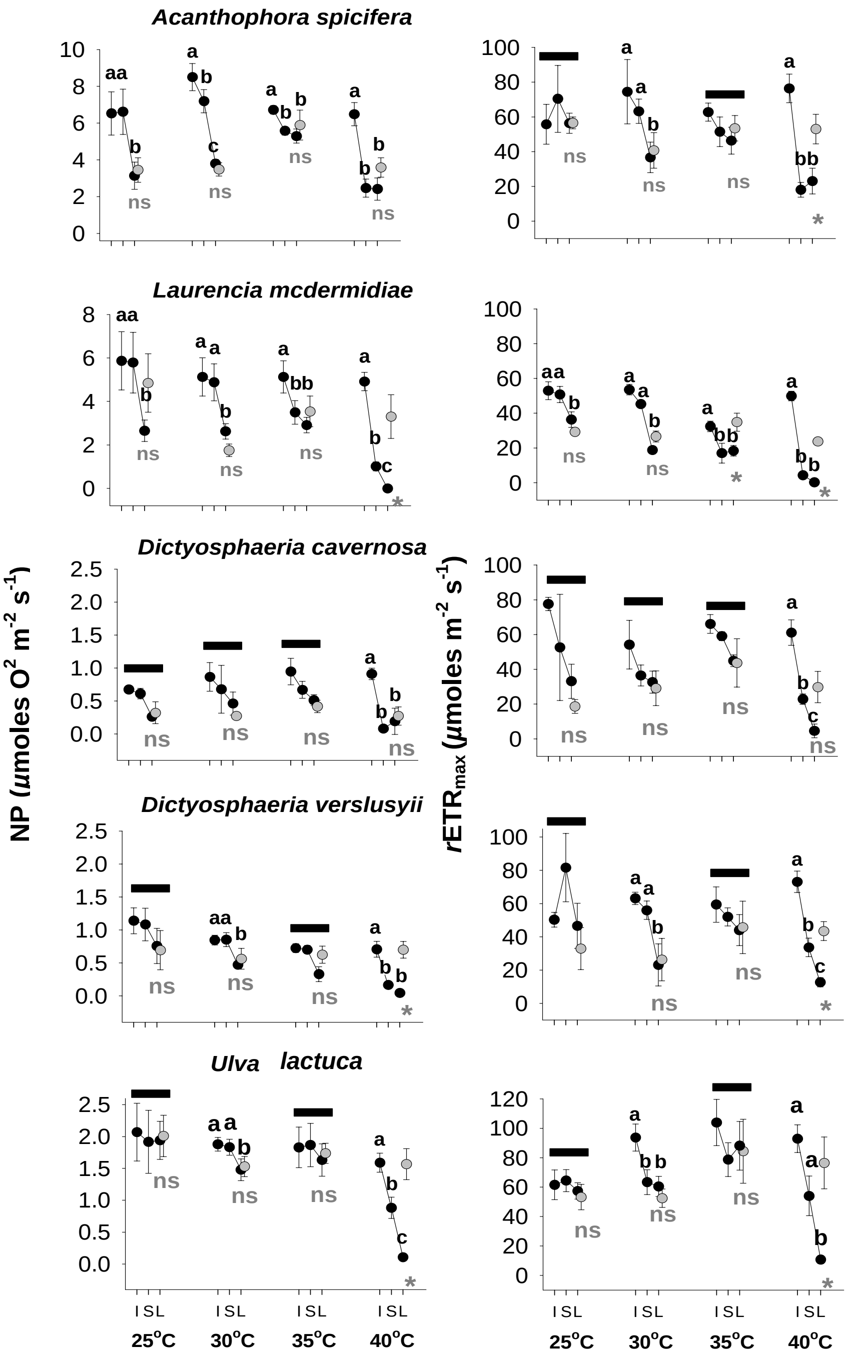

Fig. 6.1 The effect of temperature treatments on mean ± SE (left) net photosynthesis and (right) $r\mathrm{ETR}_{\mathrm{max}}$ of tested red and green macroalgae. Rates are measured initially after wound healing (= I), in the short-term immediately following heat exposure to 25, 30, 35, and 40°C = S, and after a 24-h long-term recovery (= L). Heat treated samples are in black and control samples to account for natural 24-h fluctuations are in gray. Letter groupings above black circles show statistically similar groups based on multiple comparison tests while bars represent similarity based upon one-way RM-ANOVAs. Results from longterm comparisons are below circles, ns= not significant and * = significance based on a two-tailed *t*-test.

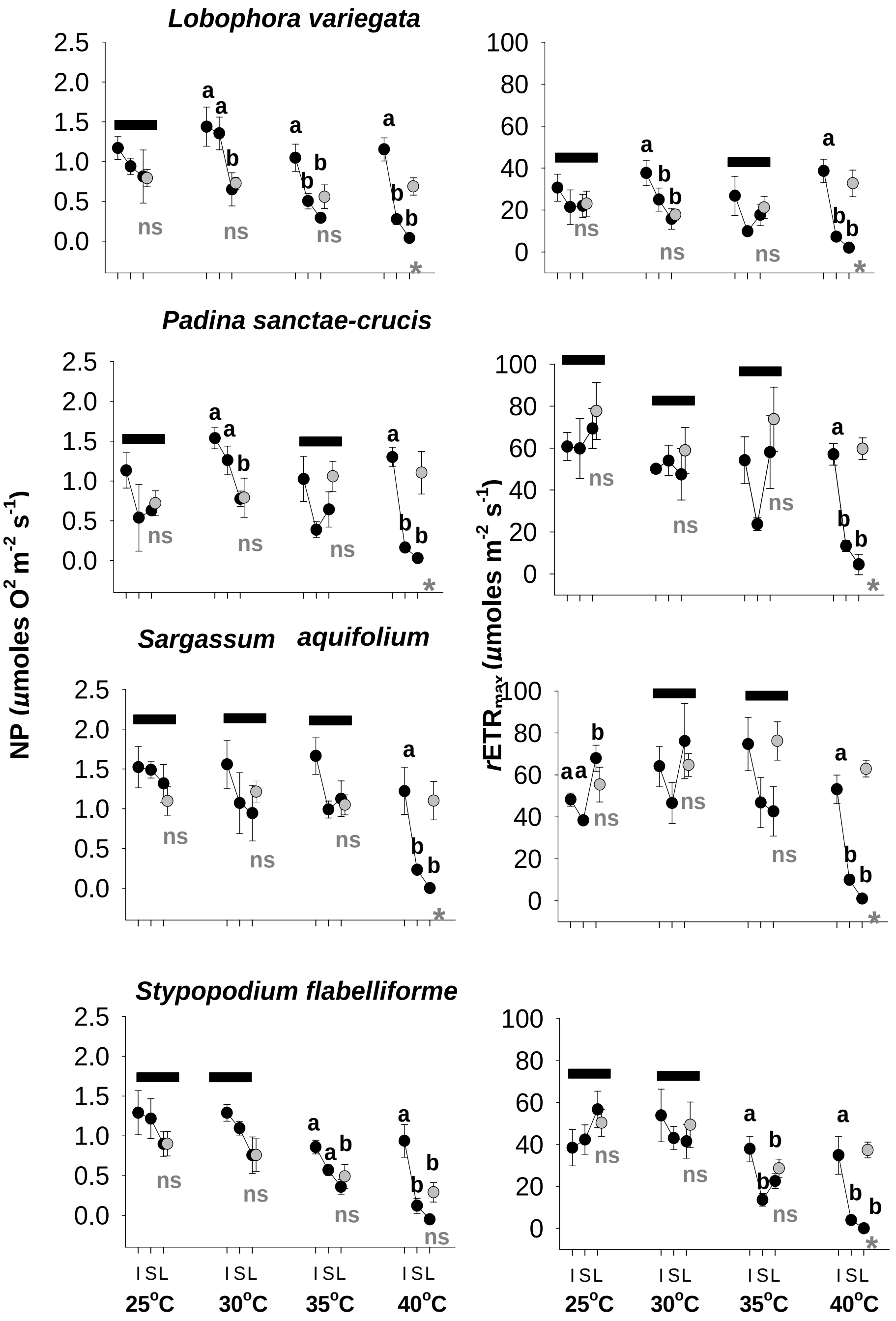

Fig. 6.2 The effect of temperature treatments on mean ± SE net photosynthesis and *r*ETR$_{max}$ of tested brown macroalgae. Rates are measured initially after wound healing (= I), in the short-term immediately following heat exposure to 25, 30, 35, and 40°C = S, and after a 24-h long-term recovery (= L). Heat treated samples are in black and control samples to account for natural 24-h fluctuations are in gray. Letter groupings above black circles show statistically similar groups based on multiple comparison tests while bars represent similarity based upon one-way RM-ANOVAs. Results from longterm comparisons are below circles, ns= not significant and * = significance based on a two-tailed *t*-test.

Table 6.4 Percent net photosynthesis (NP) and maximum relative electron transport rates ($r\mathrm{ETR}_{\mathrm{max}}$) are reduced (mean ± SE) in short-term measurements (S) (as expressed as % of initial values) for each species after 35°C and 40°C treatments. * designate the species that have significant reductions after the treatment.

	35°C % Reduced (S as % of initial value)		40°C % Reduced (S as % of initial value)	
	NP at 250 μmols m^{-2} s^{-1}	$r\mathrm{ETR}_{\mathrm{max}}$	NP at 250 μmols m^{-2} s^{-1}	$r\mathrm{ETR}_{\mathrm{max}}$
Acanthophora spicifera	83.2± 3.8*	82.1±13.3	37.3±4.8*	24.5± 6.1*
Laurencia mcdermidiae	68.1± 2.7*	48.8±13.4*	20.3±2.8*	8.5± 2.0*
Dictyosphaeria cavernosa	74.9±21.2	90.7±10.9	8.2±4.2*	38.1± 5.1*
Dictyosphaeria verslusyii	99.5±13.2	93.5±12.1	25.4±5.1*	45.3± 5.0*
Ulva lactuca	101.9± 5.5	76.4± 3.1	56.4±9.4*	56.2±10.6*
Lobophora variegata	52.5±13.9*	44.5± 8.1	26.3±6.6*	19.0± 1.3*
Padina sanctae-crucis	42.1± 9.8	51.1±13.8	12.2±3.4*	23.2± 3.2*
Sargassum aquifolium	62.8±10.5	62.7±14.6	23.2±6.4*	17.8± 2.9*
Stypopodium flabelliforme	68.9± 9.0*	40.5± 9.7*	11.0±6.8*	14.8±10.4*

that were 42.1$\pm$9.8% of initial measurements. This discrepancy is due to variable rates of NP in species such as *P. sanctae-crucis.*

NP in all tested species was statistically lowered in the short-term by a 40°C dosage and these declines tended to continue in long-term measurements (For all species: RM-ANOVA, $p \leq 0.001$, Tukey's $p \leq 0.01$) (Figs. 6.1, 6.2). However, long-term NP values were not statistically different (after sequential Bonferroni correction) between experimental and control samples for these species treated with 40°C seawater: *A. spicifera*, *D. cavernosa*, and *S. flabelliforme* (for all significantly reduced species at long-term measurements: *t*-test, $p \leq 0.01$)

Even though many of the species could not recover net rates after the 40°C dosage, the short-term reductions measured as a percent of the initial rates varied among species (Tables. 6.4, 6.5). *Ulva lactuca* and *A. spicifera* had the smallest mean reductions in NP whereas *D. cavernosa, P. sanctae-crucis, and S. flabelliforme* had the most reduced mean, short-term net photosynthetic rates.

GP for all algal species responded in a similar manner as NP, while respiration rates often remained steady except when metabolic function ceased in long-term measurements after 40°C (Figs. 6.3, 6.4). Significant differences among NP values are mostly the result of changes that occur in light harvesting and are less related to dark respiration.

Rapid Light Curves

The $r\mathrm{ETR}_{max}$ of algae responded in a similar manner as NP (Figs. 6.1, 6.2). The maximum rates remained similar to initial or control maxima within the 25°C treatment for all species. Thus a handling effect on *r*ETR was not apparent from these results. Long-term measurements of $r\mathrm{ETR}_{max}$ from control and experimental samples were often different from initial measurements; therefore, lab conditions are likely to vary from those that occur in a natural setting.

Table 6.5 Percent recovery (mean ± SE) of net photosynthesis (NP) and maximum relative electron transport rates ($r\mathrm{ETR}_{max}$) as expressed L(E)= long-term experimental value as % of L(C) = long-term control value. N/A is reported for species that were not significantly reduced after 35°C and for species that did not show recovery after 40°C treatments.

	35°C % Recovered (L(E) as % of L (C) value)		40°C % Recovered (L(E) as % of L (C) value)	
	NP at 250 μmoles m^{-2} s^{-1}	$r\mathrm{ETR}_{max}$	NP at 250 μmoles m^{-2} s^{-1}	$r\mathrm{ETR}_{max}$
Acanthophora spicifera	92.7± 6.9	N/A	72.4±19.1	N/A
Laurencia mcdermidiae	91.7±18.5	54.2± 5.8	N/A	N/A
Dictyosphaeria cavernosa	N/A	N/A	37.8±45.5	20.8±18.8
Dictyosphaeria verslusyii	N/A	N/A	N/A	N/A
Ulva lactuca	N/A	N/A	N/A	N/A
Lobophora variegata	111.6±10.2	N/A	N/A	N/A
Padina sanctae-crucis	N/A	N/A	N/A	N/A
Sargassum aquifolium	N/A	N/A	N/A	N/A
Stypopodium flabelliforme	73.4±27.9	88.1±19.6	-8.7±18.5	N/A

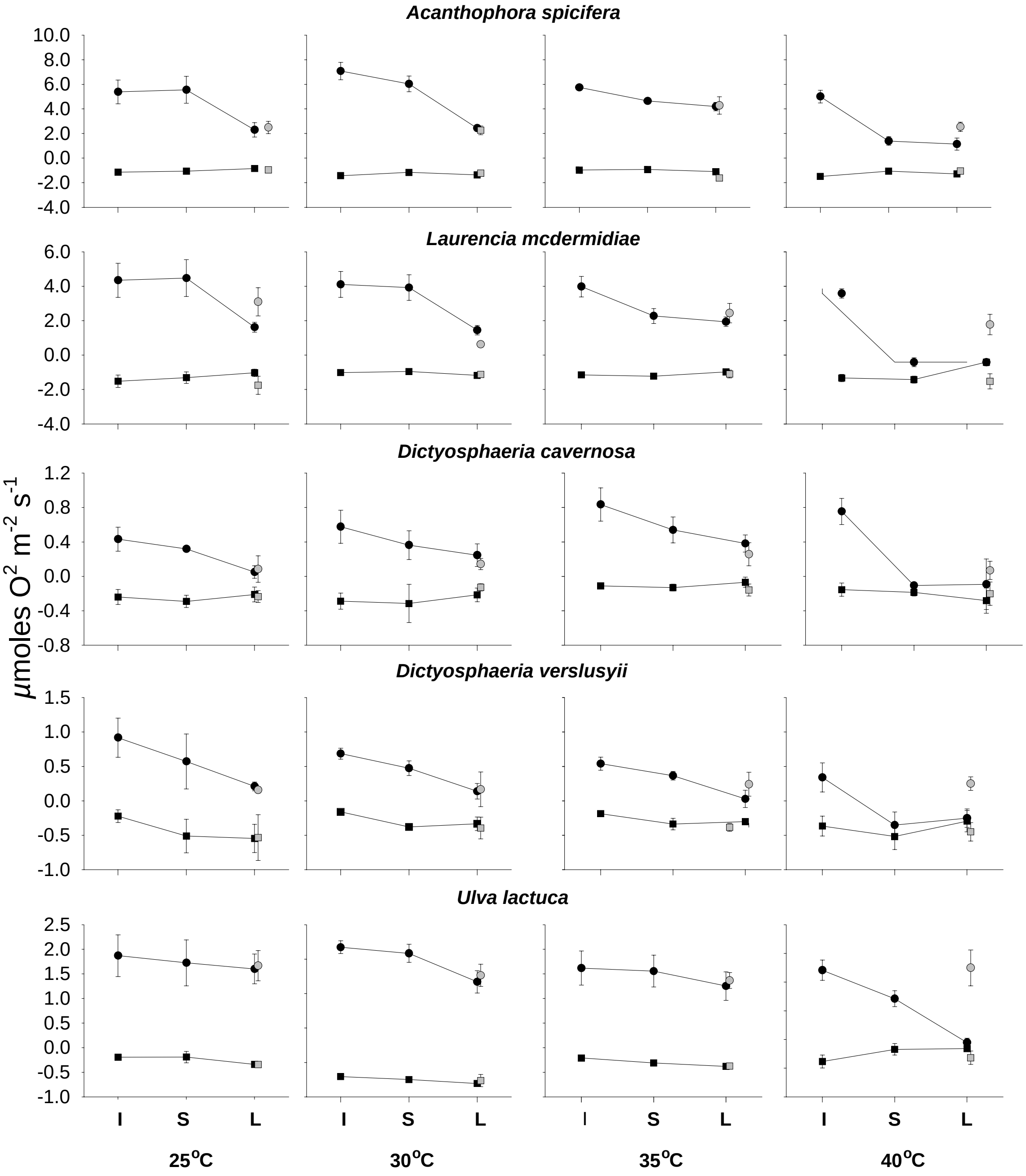

Fig. 6.3 The effect of temperature treatments on mean ± SE (circles) gross photosynthesis and (squares) respiration of tested red and green macroalgae. Rates are measured initially after wound healing (= I), in the short-term immediately following heat exposure to 25, 30, 35, and 40°C = S, and after a 24-h long-term recovery (= L). Heat treated samples are in black and control samples to account for natural 24-h fluctuations are in gray.

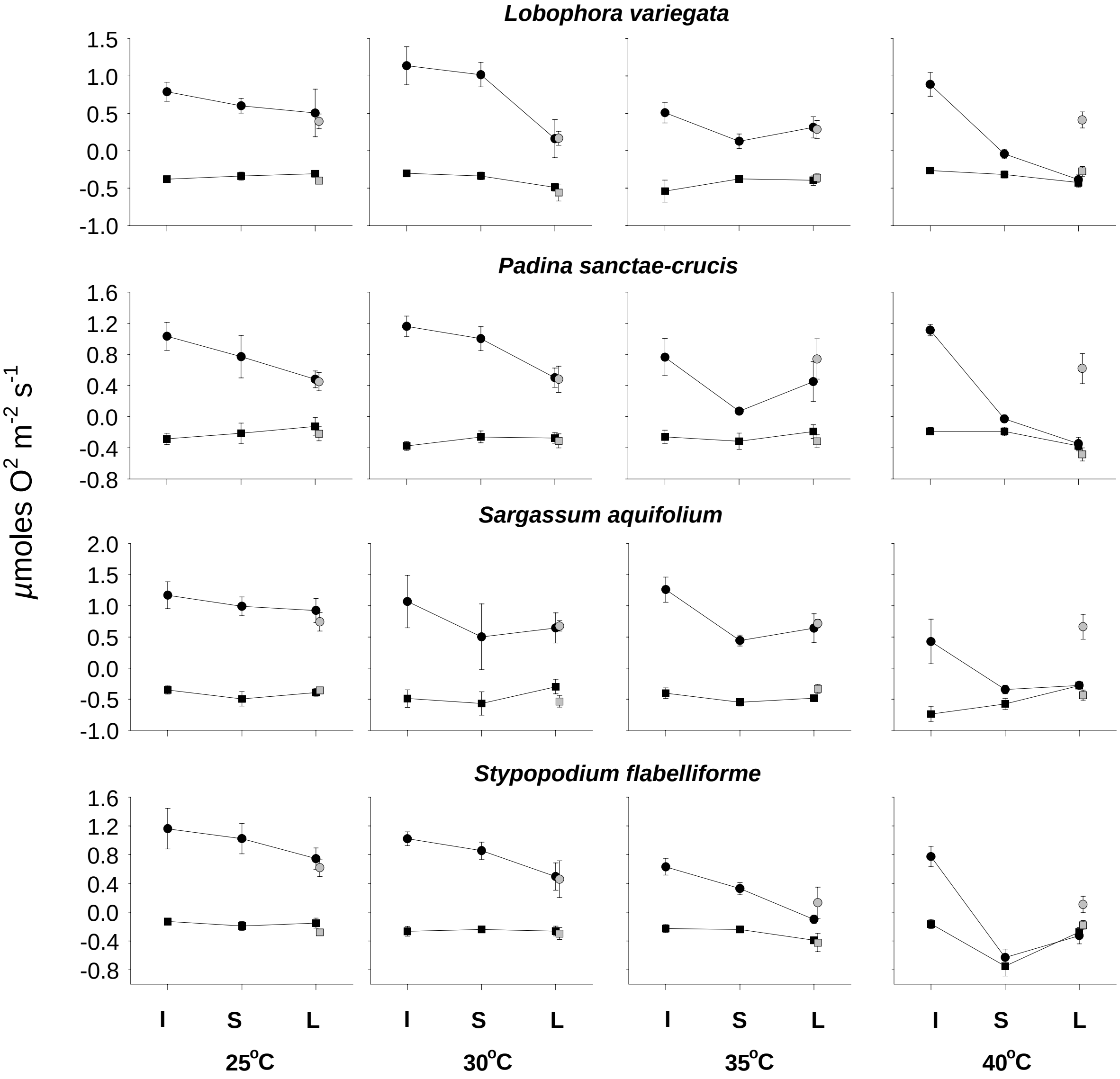

Fig. 6.4 The effect of temperature treatments on mean ± SE (circles) gross photosynthesis and (squares) respiration of tested brown macroalgae. Rates are measured initially after wound healing (= I), in the short-term immediately following heat exposure to 25, 30, 35, and 40°C = S, and after a 24-h long-term recovery (= L). Heat treated samples are in black and control samples to account for natural 24-h fluctuations are in gray.

Unlike measurements of NP, two species showed a short-term effect in the *r*ETR$_{max}$ after a 30°C treatment: *U. lactuca* (RM-ANOVA, $p \leq 0.001$, Tukey's $p \leq 0.01$) and *L. variegata* (RM-ANOVA, $p \leq 0.001$, Tukey's $p \leq 0.001$) (Figs. 6.1, 6.2). These species were both reduced to a mean of 66.6±8% of the initial values (Table 6.4). In addition, the effect on *r*ETR$_{max}$ was only short-term as rates were able to recover to those recorded for long-term controls (Table 6.5). After 35°C dosage, macroalgae tended to have lower *r*ETR$_{max}$ and two species had significantly lower maxima: *L. mcdermidiae* (RM-ANOVA, $p \leq 0.001$, Tukey's $p \leq 0.001$) and *S. flabelliforme* (RM-ANOVA, $p \leq 0.01$, Tukey's $p \leq 0.01$) (Figs. 6.1, 6.2). Most brown macroalgal species were reduced to a similar percentage of the initially determined rate yet their short-term determined *r*ETR$_{maxs}$ did not statistically differ from initial measurements. *Laurencia mcdermidiae* and *S. flabelliforme* were also able to recover from the heat stress, as *r*ETR$_{maxs}$ in experimental samples were similar to control values (Table 6.5).

All species exhibited lower *r*ETR$_{max}$ after the 40°C treatment and experimental values tended to be even lower at the long-term sampling (*S. flabelliforme*: RM-ANOVA, $p = 0.01$; Tukey's, $p = 0.02$; *t*-test, $p \leq 0.001$; all other species except *U. lactuca*: RM-ANOVA, $p \leq 0.001$, Tukey's $p \leq 0.001$) (Figs. 6.1, 6.2). Individuals of *U. lactuca* had variable response to the 40°C heat and the average *r*ETR$_{max}$ in the short-term samplings was lower than initial measurements but not statistically different. However, by the long-term sampling the average *r*ETR$_{max}$ was substantially lower than the average control values (*t*-test $p \leq 0.001$; for all other species except *D. cavernosa*, $p \leq 0.001$). In general, the *r*ETR$_{max}$ of the sampled green algae and *A. spicifera* were reduced less as percent of initial values than the brown algae (Table 6.4). *Dictyosphaeria cavernosa* was the only species that showed recovery in *r*ETR as the maxima were similar to those measured in the control samples (*t*-test, $p = 0.03$, not significant with a statistical α of 0.25) (Table 6.5). These control samples had very low *r*ETR.

The other parameters of the rapid light curve (α, E_k, ß) tended to vary with exposure to temperature treatments. The general trend in the tested red, green (Table 6.6) and brown macroalgae (Table 6.7) was when the *r*ETR$_{max}$ decreased the α, E_k tended to also decrease and the ß tended to increase. Thus the light harvesting characteristics of the PSII tended to be affected by treatments.

Table 6.6 Rapid light curve parameters for red and green taxa before and after brief exposure to temperature treatments (I = initial, S = short-term, L (E) = long-term experimental sample L (C) = long-term control sample measurements).

	α				**E_k**				**ß**			
°C	**I**	**S**	**L (E)**	**L (C)**	**I**	**S**	**L (E)**	**L (C)**	**I**	**S**	**L (E)**	**L (C)**
A. spicifera												
25, n=6	0.18 (0.03)	0.24 (0.02)	0.18 (0.03)	0.22 (0.01)	335 (53)	304 (85)	322 (43)	274 (35)	108 (13)	104 (9)	106 (7)	92 (21)
30, n=5	0.24 (0.02)	0.26 (0.02)	0.15 (0.02)	0.16 (0.01)	309 (67)	241 (29)	244 (35)	247 (41)	109 (11)	119 (5)	121 (8)	120 (9)
35, n=5	0.26 (0.02)	0.24 (0.02)	0.17 (0.01)	0.18 (0.02)	240 (19)	215 (31)	272 (35)	300 (26)	119 (4)	126 (9)	114 (7)	108 (4)
40, n=5	0.23 (0.01)	0.08 (0.01)	0.10 (0.03)	0.22 (0.03)	329 (40)	223 (30)	217 (20)	243 (25)	104 (7)	126 (9)	126 (5)	118 (6)
L. mcdermidiae												
25, n=5	0.23 (0.01)	0.22 (0.01)	0.19 (0.02)	0.17 (0.01)	226 (19)	228 (22)	185 (7)	169 (5)	122 (4)	122 (5)	134 (3)	139 (2)
30, n=5	0.21 (0.00)	0.24 (0.00)	0.12 (0.01)	0.17 (0.02)	252 (13)	187 (5)	159 (7)	160 (12)	117 (3)	131 (1)	143 (2)	143 (4)
35, n=5	0.20 (0.02)	0.13 (0.02)	0.14 (0.02)	0.21 (0.02)	170 (23)	124 (24)	140 (27)	177 (46)	143 (6)	161 (11)	151 (12)	136 (10)
40, n=5	0.22 (0.01)	0.05 (0.01)	0.00 (0.00)	0.17 (0.01)	231 (8)	98 (13)	31 (24)	145 (14)	121 (2)	184 (6)	86 (60)	147 (5)
D. cavernosa												
25, n=3	0.19 (0.01)	0.15 (0.04)	0.12 (0.00)	0.08 (0.03)	406 (23)	323 (113)	285 (87)	240 (36)	92 (4)	107 (31)	114 (10)	121 (4)
30, n=5	0.17 (0.02)	0.15 (0.01)	0.13 (0.02)	0.11 (0.02)	300 (60)	235 (18)	242 (36)	246 (43)	109 (9)	121 (5)	121 (8)	121 (10)
35, n=3	0.21 (0.01)	0.18 (0.02)	0.13 (0.02)	0.13 (0.03)	323 (31)	339 (36)	341 (29)	319 (52)	104 (5)	102 (6)	101 (4)	105 (9)
40, n=4	0.19 (0.02)	0.11 (0.00)	0.02 (0.01)	0.14 (0.02)	321 (35)	215 (33)	101 (76)	206 (24)	104 (5)	122 (10)	68 (46)	129 (6)
D. verslusyii												
25, n=3	0.16 (0.03)	0.20 (0.04)	0.17 (0.01)	0.14 (0.02)	324 (50)	440 (155)	277 (89)	229 (81)	104 (7)	92 (17)	115 (16)	127 (21)
30, n=4	0.20 (0.01)	0.18 (0.03)	0.10 (0.02)	0.11 (0.01)	321 (33)	314 (22)	208 (64)	219 (79)	105 (5)	105 (4)	133 (15)	133 (18)
35, n=5	0.19 (0.02)	0.16 (0.01)	0.14 (0.01)	0.14 (0.04)	322 (56)	329 (29)	307 (51)	292 (49)	107 (9)	103 (4)	110 (11)	112 (10)
40, n=5	0.22 (0.02)	0.11 (0.01)	0.06 (0.01)	0.15 (0.01)	339 (30)	313 (45)	233 (31)	290 (37)	102 (5)	107 (9)	123 (8)	111 (7)
U. lactuca												
25, n=5	0.23 (0.02)	0.25 (0.01)	0.17 (0.02)	0.17 (0.01)	266 (28)	258 (21)	341 (8)	302 (36)	114 (5)	116 (4)	101 (1)	108 (6)
30, n=7	0.28 (0.02)	0.23 (0.01)	0.16 (0.02)	0.15 (0.03)	337 (37)	274 (25)	367 (25)	370 (39)	90 (17)	96 (18)	83 (15)	84 (16)
35, n=5	0.28 (0.03)	0.28 (0.03)	0.20 (0.03)	0.20 (0.03)	378 (66)	290 (42)	429 (51)	415 (66)	98 (10)	111 (8)	90 (7)	93 (9)
40, n=5	0.28 (0.01)	0.19 (0.02)	0.04 (0.01)	0.22 (0.04)	335 (30)	277 (46)	277 (48)	349 (54)	102 (5)	115 (10)	113 (8)	99 (7)

Table 6.7 Rapid light curve parameters for brown taxa before and after brief exposure to temperature treatments (I = initial, S = short-term, L (E) = long-term experimental sample L (C) = long-term control sample measurements).

	α				**E_k**				**ß**			
°C	**I**	**S**	**L (E)**	**L (C)**	**I**	**S**	**L (E)**	**L (C)**	**I**	**S**	**L(E)**	**L(C)**
L. variegata												
25, n=5	0.14 (0.01)	0.10 (0.02)	0.09 (0.01)	0.10 (0.01)	235 (53)	204 (33)	230 (37)	230 (36)	126 (13)	111 (18)	105 (25)	124 (10)
30, n=4	0.15 (0.04)	0.12 (0.02)	0.07 (0.02)	0.09 (0.02)	274 (48)	199 (15)	202 (26)	211 (21)	114 (9)	130 (5)	107 (27)	127 (6)
35, n=5	0.11 (0.02)	0.06 (0.00)	0.08 (0.02)	0.11 (0.02)	224 (29)	173 (21)	227 (39)	202 (34)	125 (7)	121 (19)	125 (9)	132 (10)
40, n=6	0.16 (0.02)	0.08 (0.01)	0.02 (0.01)	0.15 (0.03)	241 (36)	90 (13)	59 (26)	209 (30)	122 (8)	177 (11)	114 (60)	130 (9)
P. sanctae-crucis												
25, n=5	0.23 (0.03)	0.23 (0.02)	0.23 (0.03)	0.25 (0.03)	285 (64)	261 (56)	315 (52)	307 (34)	114 (12)	114 (14)	107 (8)	107 (6)
30, n=4	0.22 (0.03)	0.25 (0.02)	0.20 (0.03)	0.21 (0.03)	239 (37)	218 (39)	254 (67)	289 (63)	120 (8)	126 (11)	121 (15)	111 (10)
35, n=5	0.21 (0.04)	0.13 (0.01)	0.17 (0.04)	0.25 (0.03)	262 (29)	185 (20)	336 (79)	290 (36)	116 (6)	124 (6)	106 (11)	111 (7)
40, n=4	0.24 (0.02)	0.12 (0.01)	0.02 (0.02)	0.28 (0.04)	241 (33)	113 (16)	77 (65)	227 (42)	120 (7)	168 (60)	168 (79)	122 (8)
S. aquifolium												
25, n=6	0.24 (0.02)	0.19 (0.02)	0.23 (0.02)	0.21 (0.02)	220 (18)	211 (21)	296 (24)	275 (23)	121 (7)	127 (6)	107 (5)	113 (4)
30, n=4	0.25 (0.02)	0.20 (0.01)	0.21 (0.04)	0.19 (0.01)	258 (27)	235 (37)	349 (40)	340 (37)	116 (8)	123 (12)	94 (4)	97 (2)
35, n=5	0.29 (0.02)	0.16 (0.03)	0.25 (0.03)	0.24 (0.03)	263 (38)	277 (64)	177 (41)	325 (29)	115 (7)	119 (15)	107 (4)	104 (4)
40, n=7	0.23 (0.01)	0.09 (0.01)	0.01 (0.01)	0.21 (0.03)	226 (23)	111 (12)	104 (99)	319 (35)	123 (5)	166 (5)	84 (50)	106 (6)
S. flabelliforme												
25, n=5	0.22 (0.04)	0.23 (0.03)	0.21 (0.04)	0.20 (0.02)	170 (18)	181 (18)	276 (12)	250 (15)	138 (6)	134 (5)	112 (2)	117 (3)
30, n=5	0.23 (0.03)	0.19 (0.02)	0.20 (0.05)	0.25 (0.06)	237 (44)	242 (51)	235 (45)	215 (25)	123 (10)	122 (9)	123 (10)	125 (5)
35, n=6	0.19 (0.02)	0.10 (0.02)	0.12 (0.02)	0.14 (0.03)	207 (37)	140 (7)	212 (41)	206 (21)	130 (8)	160 (13)	131 (11)	129 (5)
40, n=3	0.14 (0.01)	0.05 (0.04)	0.00 (0.00)	0.12 (0.01)	238 (49)	83 (25)	0 (0)	324 (62)	122 (12)	184 (16)	0 (0)	105 (9)

Relationship between GP & rETR

Multiple regression results revealed that GP and *r*ETR at a PAR of 250 µmoles m^{-2} s^{-1} were related (Fig. 6.5, Table 6.8). GP and heat stress were highly significant factors in predicting *r*ETR for most species. However, temperature stress did not explain the variation between *r*ETR and GP for *D. cavernosa* and *D. verslusyii*. GP and *r*ETR were positively correlated for 5 species while for 4 species these parameters were inversely related. This inverse relationship was driven by the significant interaction of GP with temperature. At high temperature stress the relationship between *r*ETR and GP is strongly linear but, at 25 and 30°C the relationship between these parameters is more variable. Furthermore, simple linear regressions, without temperature included, revealed an overall positive relationship between *r*ETR and GP for all species. Individual effects were also significant in predicting the strength of the relationship for *U. lactuca* and *L. variegata*.

Discussion

Overall, these results provide evidence that tropical macroalgae have remarkable photosynthetic capabilities to cope with brief exposures to high heat up to 35°C and that species vary in their thermal sensitivity. As expected, most macroalgae were able to maintain rates of photosynthesis and electron transport after being treated briefly with temperatures most similar to those that occur in natural settings (25 and 30°C). However, a brief exposure to extreme heat (40°C) for most species appears to cause un-repairable damage to photosynthesis and dark respiration. In addition, the results from this study raise issues about the eco-physiological definition of sensitivity or tolerance. The fluorescence technique (RLC) and oxygen exchange measurements revealed somewhat similar patterns of reduction after heat stress and were found to be related. These results have important implications for the ecology of reef macroalgae and the advancement of *in situ* physiological measurements of production and stress.

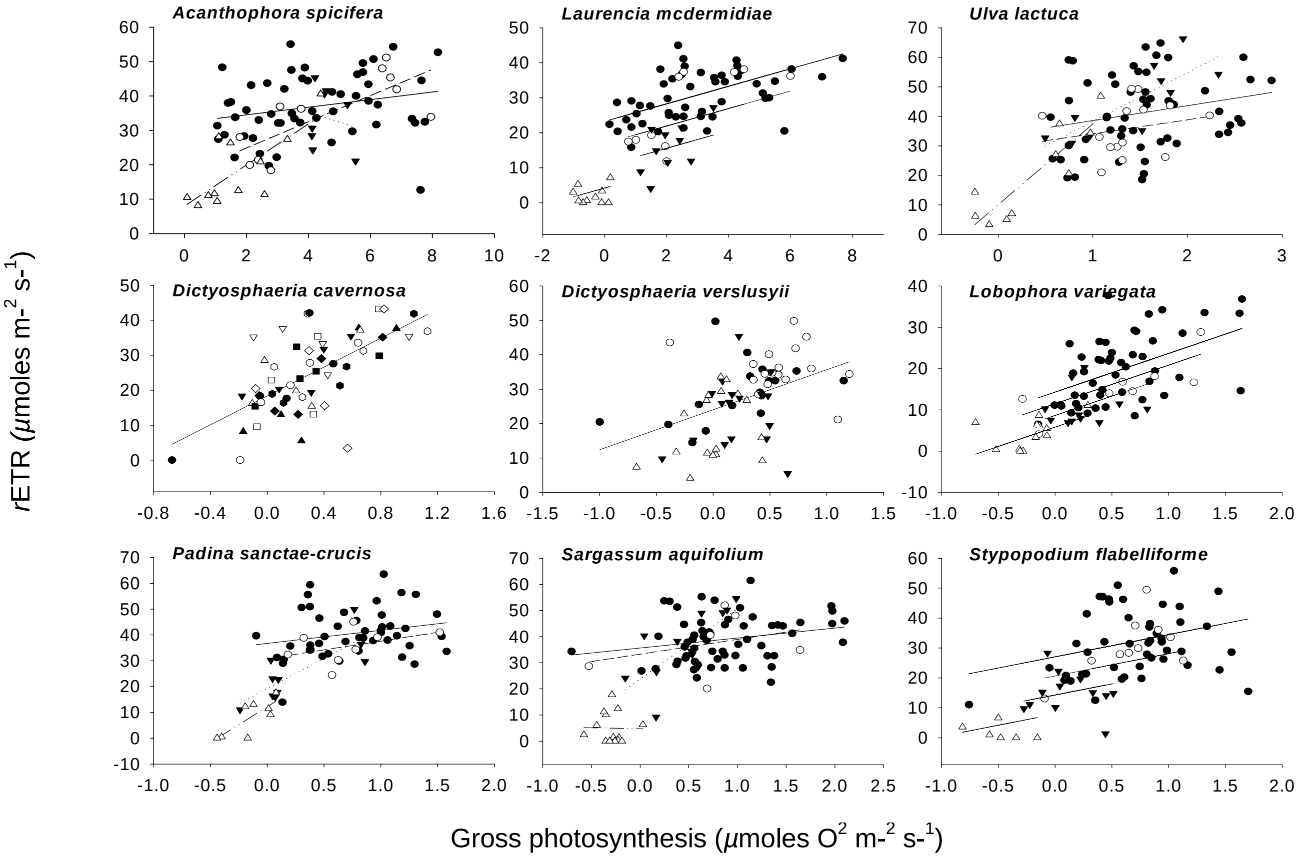

Fig. 6.5 The relationship between *r*ETR and gross photosynthesis for marine macroalgae exposed to heat treated seawater. Symbols represent the results for individuals exposed to different temperatures and lines are the slopes determined from multiple regressions (solid circles and solid line = 25°C, open circles and dotted line = 30°C, closed triangles and dashed line = 35°C, open triangles and solid-dotted line = 40°C).

Table 6.8 Statistical results from multiple regressions showing the relationship between gross photosynthesis and *r*ETR backcalcuated for an irradiance at 250 μmoles m^{-2} s^{-1} for each species; n = number of samples, N = number of individuals sampled.

Species	**n**	**N**	**Model terms**	**Slopes**	***p*-values**	**R^2 (%)**
A. spicifera	83	41	Gross photosynthesis (Gp)	-5.6 (2.7)	0.043	45.0
			Temperature treatment (Tt)	-1.5 (0.3)	<0.001	
			Gp x Tt		0.005	
L. mcdermidiae	80	39	Gross photosynthesis	2.5 (0.4)	<0.001	73.4
			Temperature treatment	-1.3 (0.2)	<0.001	
D. cavernosa	60	29	Gross photosynthesis	18.5 (3.0)	<0.001	40.5
D. verslusyii	67	33	Gross photosynthesis	11.6 (3.0)	<0.001	18.8
U. lactuca	88	43	Gross photosynthesis	-20.8 (9.0)	<0.001	49.8
			Temperature treatment	-1.7 (0.4)	<0.001	
			Individual	0.4 (0.1)	<0.001	
			Gp x Tt		<0.001	
L. variegata	79	39	Gross photosynthesis	9.6 (1.6)	<0.001	62.3
			Temperature treatment	-7.5 (0.1)	<0.001	
			Individual	0.2 (0.1)	<0.001	
P. sanctae-crucis	71	36	Gross photosynthesis	-31.0 (13.0)	0.02	66.5
			Temperature treatment	-1.7 (0.2)	<0.001	
			Gp x Tt		0.003	
S. aquifolium	92	45	Gross photosynthesis	-44.3 (9.3)	<0.001	65.9
			Temperature treatment	-2.2 (0.3)	<0.001	
			Gp x Tt		<0.001	
S. flabelliforme	74	37	Gross photosynthesis	7.4 (2.7)	0.007	49.5
			Temperature treatment	-1.3 (0.3)	<0.001	

The results of this study provide evidence that tropical species have acclimated or are adapted to high temperatures. It has been noted that species that are grown at warmer temperatures have higher temperature tolerances or optima than those that are grown at cooler temperatures (Oates and Murray 1983; Davison 1991; Kübler et al. 1991; Kübler and Davison 1993; Padilla-Gamino and Carpenter 2007; Wernberg et al. 2010). For *Chondrus cripsus,* warm water acclimated individuals had better ability to recover from thermal stress than those grown at a lower temperature (Kübler and Davison 1993). The photosynthetic metabolism of many of these tested species were able tolerate exposures to temperatures up to 35°C and those affected were able to recover rates within 24 h. There was evidence for a few species (*A. spicifera*, *D. cavernosa, S. flabelliforme*) that recovery occurred after 40°C treatments; yet, this evidence was weak as the control measurements were either variable or low. Studies on the influence of temperature on photosynthesis for marine macroalgae are often conducted on species with temperate and arctic distributions (Smith and Berry 1986; Gerard and Du Bois 1988; Kübler et al. 1991; Henley et al. 1992; Kübler and Davison 1993; Eggert and Wiencke 2000; Padilla-Gamino and Carpenter 2007). Therefore, ranges or tolerance for photosynthetic metabolism are not tested much above 30°C and when examined to or over 35°C, rates tend to be significantly reduced (Henley et al. 1992; Padilla-Gamino and Carpenter 2007; Li et al. 2009). Most determined tolerance limits were between 35-40°C but, it is unclear whether adaptation or acclimation accounted for differences in tolerance.

Species collected from near shore and during summer months, were observed to have different photosynthetic sensitivity (reduced rates significantly below initial) to high heat. Different responses occurred among Phyla and within the same Order. Temperature tolerances and optima are known to vary for temperate species or populations that occur across different tidal elevations and with varying biogeographic distributions (Oates and Murray 1983; Smith and Berry 1986; Gerard and Du Bois 1988; Padilla-Gamino and Carpenter 2007; Wernberg et al. 2010). The finding that there are species-level differences in photosynthetic temperature tolerances was surprising in this study given the similar warm water distributions (Abbott 1999; Abbott and Huisman 2004), consistent sea surface temperatures that occur in summer (Abbott 1999; Abbott and Huisman 2004), the close proximity of the two shores where collection occurred, the small tidal fluctuations ($\geq$ 1m) (Abbott 1999), and the shallow depths from which individuals were collected. If previous thermal history influenced the observed difference in rate response to temperature than temperature would need to vary over this same fine spatial scale. Temperature can vary over fine spatial scales on Hawaiian shores (Cox and Smith, *in review*) but species such as *P. sanctae-crucis*, *L. mcdermidiae*, and *D. verslusyii* that are common (and were collected) from intertidal habitats were not more tolerant to high temperature doses than those that are more common (and were collected) in presumably consistent and cooler subtidal habitats such as *S. flabelliforme* and *D. cavernosa*.

Sensitivity to heat was defined by this study as reduced rates of net production and $r\mathrm{ETR}_{max}$ after a heat treatment but, sensitivity could be also defined as the percent rates are reduced in comparison among other species. For instance, after 35°C treatment four species had significantly reduced NP and 2 species had significantly reduced $r\mathrm{ETR}_{max}$ yet the mean percent reduction for these species was similar to or less than the mean percent reductions that were observed for “unaffected” species. *Acanthophora spicifera* is sensitive to heat at 30°C but initially individuals had high rates and when reduced by heat, rates were still physiologically competitive; they were within the range of rates measured for other common macroalgae. Additionally, if affected species have the ability to recover from heat stress and rates are restored quickly to healthy levels, these species will gain the physiological competitive advantage. If we re-define sensitivity as percent reduced in comparison among species we see a different trend in tolerances. The tested Chlorophyta taxa and *A. spicifera* would be more robust to heat stress and the Phaeophyceae and *L. mcdermidiae* would be similarly tolerant and more sensitive. To clarify definitions, further ecological studies *in situ* should be designed to test the physiological capabilities among species under conditions of thermal stress.

It is unclear from our results which part of metabolism is most sensitive to heat stress. Overall NP and $r\mathrm{ETR}_{max}$ decreased after heat stress but, dark respiration rates remained stable. Photorespiration, not measured in this study, could account for lower NP because the enzyme affinity for O_2 increases with increasing temperature (Berry and Bjorkman 1980). CO_2-concentrating mechanisms have been suggested for some marine algae but are not well documented for a variety of species (Raven 1991). It is plausible that some of the species tested have CO_2-concentrating mechanisms which could facilitate and account for differences in species tolerances. PSII is often considered the most thermolabile component of photosynthesis (Fork et al. 1979). In red algae the disruption in transfer of energy between the phycobillisomes and PSII is observed when temperatures increase above optimum (Kübler et al. 1991). In this study if PSII was impacted more than PSI or other components of photosynthesis we may expect to see *r*ETR_{max} to be significantly lowered in a similar manner as NP. Although the patterns of reduction were similar there were slight discrepancies. For instance, NP was significantly reduced for 4 species after 35°C doses of heated seawater and only 2 of these 4 had significantly reduced $rETR_{max}$, whereas 40°C treatments reduced the efficiency of PSII and overall photosynthesis for all tested species. Dark respiration is known to increase with temperature (Berry and Bjorkman 1980) but, few studies with marine macroalgae have found rates to be relatively stable in the short-term (Oates and Murray 1983; Kübler and Davison 1993; Padilla-Gamino and Carpenter 2007). We found dark respiration to remain relatively stable except when metabolic function ceased in long-term measurements after 40°C treatments. Further experiments which probe the different photosystems, dark respiration, and the ability to withstand photorespiration when exposed to heat could clarify the physiological changes that occur for these species.

Although these assays were not designed to specifically test the relationship between O_2 production and *r*ETR collected via fluorescence, a comparison of the measurements reveals that results from the two techniques are related. PAM has been used extensively with land plants where the relationship between CO_2 fixation and ETR has been well documented (Schreiber et al. 1986; Bolhar-Nordenkampf et al. 1989; Krause and Weis 1991). Although there has been much discussion on the relationship and limitations of fluorescence measurements (Genty et al. 1989; Hanelt and Nultsch 1995; Rascher et al. 2000; Longstaff et al. 2002; Beer and Axelsson 2004; Herlory et al. 2007; Ihnken et al. 2010), Genty et al. 1989 found the two parameters to be correlated. Beer and Axelsson (2004) discuss the limitation of fluorescence at high irradiance. Furthermore, Longstaff et al. (2002), found for *Ulva* at moderate irradiances that the two techniques were highly related. Our results support their findings as we found positive relationship between rates of GP and relative electron transport when measured at irradiances of 250 μ moles m^{-2} s^{-1} for all nine tested species. It is unclear why the relationship becomes stronger when some species are exposed to higher temperatures. Perhaps there is less variability in photosynthetic metabolism as the macroalgae becomes stressed.

Our results demonstrate that temperature will affect species of reef algae in different ways. The physiological response to temperature could play a role in ecological abundances and we can begin to form testable hypotheses based on our findings. We would expect *A. spicifera*, *D. verslusyii*, and *U. lactuca* to thrive in hot, thermally variable habitats and *L. mcdermidiae* and perhaps *S. flabelliforme* to be sensitive to changing thermal conditions. Two of the three thermally robust species form nuisance blooms in shallow water reefs (Stimson et al. 2001; Smith et al. 2002) and can be found in the intertidal zone (Abbott 1999; Abbott and Huisman 2004; Vermeij et al. 2011) whereas the other two mentioned sensitive species occur in submersed zones (Abbott 1999; Abbott and Huisman 2004) and are not as common (*personal observation*). This assay could also be used as a screening process for macroalgae in the aquarium or aquaculture trade to assist in the identification of thermally tolerant species as potential invasive candidates for shallow water reefs.

We caution that these responses to heat are not directly related to reef algal abundances in the field without more testing. Often temperature tolerances are above optima for growth and a short-term response to heat does not necessarily reflect long-term responses as macroalgae have the ability to acclimate (Kübler et al. 1991). However, our results could indicate taxa more likely to be successful in ocean warming range expansions. Macroalgae have complex life histories and different stages may have varying thermal sensitivity (Lüning 1990). Furthermore, in natural settings abiotic factors are variable and physiological capabilities to acquire CO_2, nutrients, or harvest light will likely vary among these species. Other biotic interactions will reduce and alter abundances. For instance *A. spicifera* is a preferred food of many reef fishes (Smith 2003). Despite these caveats, the findings from this study have provided a baseline to understand the ecology and physiology of shallow water species in the tropics.

References

Abbott IA (1999) Marine red algae of the Hawaiian Islands. Bishop Museum Press, Honolulu

Abbott IA, Huisman JM (2004) Marine green and brown algae of the Hawaiian Islands. Bishop Museum Press, Honolulu

Beach KS, Smith CM (1996) Ecophysiology of tropical rhodophytes. II. Microscale acclimation in photosynthesis. Journal of Phycology 32: 710-718

Beer S, Axelsson L (2004) Limitations in the use of PAM fluorometry for measuring photosynthetic rates of macroalgae at high irradiances. European Journal of Phycology 39: 1-7

Beer S, Bjork M, Gademann R, Ralph PJ (2001) Measurements of photosynthesis in seagrasses. In: Short FT, Coles R (eds) Global Seagrass Research Methods. Elsevier Publishers, The Netherlands, pp 183-198

Berry J, Bjorkman O (1980) Photosynthetic response and adaptation to temperature in higher plants. Annual Review of Plant Physiology 31: 491-543

Berry JA, Raison JK (1981) Response to the Physical Environment. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds) Encyclopedia of Plant Physiology New Series. Springer-Verlag, Berlin, pp 277-338

Bird CE (2006) Aspects of community ecology on wave-exposed rocky Hawai'ian coasts. PhD. Botany, Honolulu

Bolhar-Nordenkampf HR, Long SP, Baker NR, Oquist G, Shreiber U, Lechner EG (1989) Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. Functional Ecology 3: 497-514

Davison IR (1991) Environmental effects on algal photosynthesis: temperature. Journal of Phycology 27: 2-8

Doty MS (1971) Antecedent event influence on benthic marine algal standing crops in Hawaii. Journal of Experimental Marine Biology and Ecology 6: 161-166

Eggert A, Wiencke C (2000) Adaptation and acclimation of growth and photosynthesis of five Antarctic red algae to low temperatures. Polar Biology 23: 609-618

Fork DC, Murata N, Sato N (1979) Effect of growth temperature on the lipid and fatty acid composition, and the dependence on temperature of light-induced redox reactions of cytochrome f and of light energy redistribution in the thermophilic blue-green alga *Synechococcus lividus*. Plant Physiology 63: 524-530

Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochemistry Biophysics Acta 990: 97-92

Gerard VA, Du Bois KR (1988) Temperature ecotypes near the southern boundary of the kelp *Laminaria saccharina*. Marine Biology 97: 575-580

Hanelt D, Nultsch W (1995) Field studies of photoinhibition show non-correlations between oxygen and fluorescence measurements in the arctic red alga *Palmaria palmata*. Plant Physiology 145: 31-38

Harrison WG, Platt T (1986) Photosynthesis-irradiance relationships in polar and temperate phytoplankton populations. Polar Biology 5: 153-164

Henley WJ, Lindley ST, Levavasseur G, Osmond CB, Ramus J (1992) Photosynthetic response of *Ulva rotundata* to light and temperature during emersion on an intertidal sand flat. Oecologia 89: 516-523

Herlory O, Richard P, Blanchard GF (2007) Methodology of light response curves: application of chlorophyll fluorescence to microphytobenthic biofilms. Marine Biology 153: 91-101

Holm S (1979) A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics 6: 65-70

Ihnken S, Eggert A, Beardall J (2010) Exposure times in rapid light curves affect photosynthetic parameters in algae. Aquatic Botany 93: 185-194

Krause GH, Weis E (1991) Chlorophyll fluorescence and photosynthesis: the basics. Annual Review of Plant Physiology 42: 313-349

Kübler JE, Davison IR (1993) High-temperature tolerance of photosynthesis in the red alga *Chondrus crispus*. Marine Biology 117: 327-335

Kübler JE, Davison IR (1995) Thermal acclimation of light-use characteristics of *Chondrus crispus* (Rhodophyta). European Journal of Phycology 30: 189-195

Kübler JE, Davison IR, Yarish C (1991) Photosynthetic adaptation to temperature in the red algae *Lomentaria baileyana* and *Lomentaria orcadensis*. British Phycological Journal 26: 9-19

Kübler J, Davison IR, C. Y (1991) Photosynthetic adaptation to temperature in the red algae *Lomentaria baileyana* and *Lomentaria orcadensis*. European Journal of Phycology 26: 9-19

Li D, Wang G, Chen L, Lu F (2009) Effects of irradiance and temperature on the photosynthesis and vegetative propagation of *Caulerpa serrulata*. Journal of Integrative Plant Biology 59: 147-154

Littler MM (1979) The effects of bottle volume, thallus weight, oxygen saturation levels, and water movement on apparent photosynthetic rates in marine algae. Aquatic Botany 7: 21-34

Longstaff B, Kildea T, Runcie JW, Cheshire A, Dennison WC, Hurd C, Kana T, Raven JA, Larkum AWD (2002) An *in situ* study of photosynthetic oxygen exchange and electron transport rate in the marine macroalgae *Ulva lactuca* (Chlorophyta). Photosynthesis Research 74: 281-293

Lüning K (1990) Seaweeds: their environment, biogeography and ecophysiology. Wiley & Sons, New York

Morris EP, Kromkamp JC (2003) Influence of temperature on the relationship between oxygen and fluorescence based estimates of photosynthetic parameters in a marine benthic diatom (*Cylindrotheca closterium*). European Journal of Phycology 38: 133-142

Niemeck RA, Mathieson AC (1978) Physiological studies of intertidal fucoid algae. Botanica Marina 21: 221-227

Oates BR, Murray SN (1983) Photosynthesis, dark respiration and desiccation resistance of the intertidal seaweeds *Hesperophycus harveyanus* and *Pelvetia fastigiata F. gracilis*. Journal of Phycology 19: 371-380

Padilla-Gamino JL, Carpenter RC (2007) Thermal ecophysiology of *Laurencia pacifica* and *Laurencia nidifica* (Ceramiales, Rhodophyta) from tropical and warm-temperate regions. Journal of Phycology 43: 686-692

Platt T, Gallegos CL, Harrison WG (1980) Photoinhibition of photosynthesis in natural assemblage of marine phytoplankton. Journal of Marine Research 38: 687-701

Ralph PJ, Gademann R (2005) Rapid light curves: a powerful tool to assess photosynthetic activity. Aquatic Botany 82: 222-237

Rascher U, Liebig M, Luttge U (2000) Evaluation of instant light-response curves of chlorophyll fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field. Plant Cell Environment 23: 1397-1405

Raven JA (1991) Physiology of inorganic C acquisition and implications for resource use efficiency by marine phytoplankton: relation to increased CO_2 and temperature. Plant, Cell and Environment 14: 779-794

Raven JA, Geirder RJ (1988) Temperature and algal growth. New Phytologist 110: 441-461

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43: 223-225

Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new modulation fluoremeter. Photosynthesis Research 10: 51-62

Smith CM, Berry JA (1986) Recovery of photosynthesis after exposure of intertidal algae to osmotic and temperature stresses: comparative studies of species with differing distributional limits. Oecologia 70: 6-12

Smith JE, Hunter CL, Smith CM (2002) Distribution and reproductive characteristics of nonindigenous and invasive marine algae in the Hawaiian islands. Pacific Science 56: 299-315

Smith JE (2003) Factors influencing algal blooms on tropical reefs with an emphasis on herbivory, nutrients, and invasive species. PhD dissertation. Department of Botany, Honolulu

Stimson J, Larned ST, Conklin EJ (2001) Effects of herbivory, nutrient levels, and introduced algae on the distribution and abundance of the invasive macroalgae *Dictyosphaeria cavernosa* in Kaneohe Bay, Hawaii. Coral Reefs 19: 343-357

Vermeij MJA, Dailer M, Smith CM (2011) Crustose coralline algae can suppress macroalgal growth and recruitment on Hawaiian coral reefs. Marine Ecology Progress Series 422: 1-7

Wernberg T, Thomsen MS, Tuya F, Kendrick GA, Staehr PA, Toohey BD (2010) Decreasing resilience of kelp beds along a latitudinal temperature gradient: potential implications for a warmer future. Ecology Letters 13: 685-694

White AJ, Critchley C (1999) Rapid light curves: a new fluorescence method to assess the state of the photosynthetic apparatus. Photosynthesis Research 59: 63-72

Chapter VII. Photosynthetic light harvesting characteristics of diverse reef algae with varying functional forms.

Abstract

The photosynthetic light harvesting parameters of 16 species of reef algae (n = 1-8) were sampled *in situ* on two days during similar hours using the rapid light curve function of a diving pulse amplitude fluorometer. These reef algal species span three phyla, five functional forms, and varied in internal anatomy. Most displayed characteristics typical of sun tolerant producers, having high maximum rates of electron transport (mean range, 12.9 -112.2 μmoles e^- m^{-2} s^{-1}) and saturation irradiances (mean range 131.8 - 659.7 μmoles m^{-2} s^{-1}). Light harvesting as measured by pulse amplitude modulation was dynamic as species differed in light harvesting parameters and these differences varied between days. Filamentous, thin forms and the iridescent foliose brown alga, *Stypopodium flabelliforme,* tended to have lower absorption than other tested species. Lastly, a comparison of absorption values revealed a range of absorption more similar to reported values determined with *in vivo* absorbance than the standard used coefficient of 84%. This study is unique in that it investigates a variety of macroalgae within a community and reveals efforts that should be taken to account for the dynamic nature of light harvesting and variable absorption factors to prevent misinterpretation of eco-physiological findings.

Introduction

Sub-tropical to tropical reefs are dynamic ecosystems where photosynthetic organisms experience high levels of photosynthetic active radiation (PAR) and ultraviolet radiation (UV) while exposed to a tropical-like atmosphere and warm seawater temperatures (Franklin and Forster 1997; Beach 1995). Irradiances in the tropics at noon are known to reach 5x the amount needed to saturate photosynthesis in macroalgae (Beach et al. 1995; Kirk 1994). Excessive irradiances can damage photosystems resulting in photoinhibiton (Falkowski and Raven 1997) or death (Fejtek et al. 2011). Despite this harsh environment, reef algal communities are diverse, are able to photosynthesize and grow on shallow reefs and in intertidal zones, attaining significant stature (Beach et al. 1995; Beach and Smith 1996b; Gorbunov et al. 2001; Beach et al. 2003).

To survive and grow in potentially stressful high light settings, macroalgae employ a variety of physiological adjustments to adjust or even optimize photosynthesis in variable or stressful light environment (Henley et al. 1992; Franklin and Forster 1997; Figueroa et al. 2003b; Fairhead and Cheshire 2004; Ekelund et al. 2008; Israel et al.

2008; Hanelt and Roleda 2009). Species are able to change the ratio of pigments and alter the photosynthetic unit size. For example, *Ahnfeltiopsis concinna* and other red algae are able to decrease shade pigments and increase carotenoids which function in photo-protection (Beach and Smith 1996b; Beach and Smith 1996a; Talarico and Maranzana 2000; Israel et al. 2008). Although these abilities to adjust or photo-adapt occur over long time scales (weeks to months), species that live in shallow water habitats must have the ability to adjust to rapid supraoptimal fluctuations in irradiance that can occur with changing cloud cover and water movements (Falkowski and Raven 1997; Gorbunov et al. 2001). This can be achieved in producers via the down-regulation of reaction centers in PSII or thermal dissipation of excess energy via the xanthophyll cycle. Both of these tactics decrease the absorption cross-section in macroalgae (Falkowski and Raven 1997; Gorbunov et al. 2001).

The form of macroalgae also alters the absorption cross section and allows for varying abilities to adjust to high light (Beach and Smith 1996a; Johannesson and Snoeijs 2002; Israel et al. 2008). The optical properties of photosynthetic pigments are altered by the cell morphology and plant structure (Dubinsky 1992). Meristematic tissue, aplanospores, and male and female cells of the same species have different abilities to harvest and cope with light stress due to scattering, denser packing of pigments, and the path of light within the cell (Israel et al. 2008; Fejtek et al. 2011). In addition, macroalgae have different adult morphology which influence absorption (Beach and Smith 1996b; Beach and Smith 1996a) and photosynthetic productivity (Littler and Arnold 1982). For instance, flexible or canopy-forming morphologies can self shade or move with the waves to increase light scattering, reflecting, and absorbing (Beach and Smith 1996b; Vergara et al. 1997; Israel et al. 2008). Sheet forms with increased surface-to-volume ratios are known to have high productivity (Littler and Arnold 1982). In contrast, rigid cushion or crust forms of algae are fixed and must cope physiologically with their surroundings.

Physiological adjustments are shaped by the environment and confined by genetics. Species may be more shade or sun tolerant and have various abilities to adjust photosynthetic capacity to cope with different light environments (Boardman 1977). Compared to shade tolerant producers, species that are accustomed to high photon flux densities require higher irradiances to saturate photosynthetic capacity, and have lowered quantum efficiencies. In addition at saturating irradiances, sun adapted plants usually have higher maximum rates of photosynthesis or electron transport (Boardman 1977). These sun to shade adjustments have been measured for net photosynthesis with O_2 exchange (Beach and Smith 1996b; Fairhead and Cheshire 2004; Choo et al. 2005) and for PSII with pulse amplitude fluorescence (Ekelund et al. 2008).

Pulse amplitude modulated fluorometry has allowed researchers a third method to quantify photosynthesis with the benefit of *in situ* measurements via yield and light response curve estimates (Ralph and Gademann 2005). This technology has mostly been used to assess the physiology of a single species in different regions, habitats, or physiological states. In addition, most studies (except Gomez et. al 2004) are conducted on economically important or model macroalgal species (e.g. *Porphyra*, *Fucus*, kelps, *Ulva*) (Longstaff et al. 2002; Figueroa et al. 2003a; Beer and Axelsson 2004; Ekelund et al. 2008; Edwards and Kim 2010; Ihnken et al. 2010). In the sub-tropics a number of studies have examined the photosynthetic responses of invasive or bloom algae (Smith et. al 2005, Beach et. al 2006a) yet the broader reef community has remained understudied. The rapid light curve (RLC) function in the pulse amplitude fluorometer allows for quick measurements and produces similar photosynthetic parameters as the traditional P-I (photosynthesis vs. irradiance) curves, referred to as P-E curves (White and Critchley 1999; Ralph and Gademann 2005). Further measurements in natural settings and on a variety of taxa will provide insight into photosynthetic capacity of communities, the variability of macroalgal physiology to changing field conditions, and the limitations and usefulness of this more recent technology.

On Hawaii's shallow subtropical to tropical reefs, with relatively consistent water temperatures and high irradiances, one might expect all reef macroalgae to possess similar high light or sun adapted P-E parameters (quantum efficiency of PSII or α, relative electron transport maximum or $r\mathrm{ETR}_{\mathrm{max}}$, the saturation irradiance of PSII or E_k, and the dynamic photoinhibition parameter ß). To test this hypothesis, we used RLCs to survey the light harvesting characteristics of a diverse reef algal community in the afternoon hours on two summer days. This survey provides insight into the types of strategies used by an array of reef algae with different forms use to cope with tropical irradiances. It also provides physiological information on PSII that will allow us to design more ecologically relevant experiments in the field and laboratory using fluorometry techniques. Lastly, these parameters determined via pulse amplitude fluorescence are placed into a broader context to gain insight into the light harvesting abilities of macroalgae.

Methods

Sampling Design

We used two Diving-Pulse Amplitude Modulated fluorometers (PAM) to sample the light harvesting characteristics of reef macroalgae on June 4 and June 11, 2010 between the hours of 1300-1630. One to eight individuals of each species were collected from Diamond Head (21°15'18.68 N, 157°48'39.48 W), located on the south shore of O‘ahu, Hawai‘i within >0.5 m depths. Samples were collected, immediately put into separate containers, covered with seawater, and sampled at the site within 20 min from time of collection. To account for natural variation that may occur with time of day, individuals were sampled in a haphazard manner and efforts were made to sample species across the afternoon hours on the two days.

Species selection

We selected species that are common on Hawaiian reefs but vary in taxonomy and morphology (Table 7.1). Species span three taxonomic Phyla and several Orders. They vary in morphology and functional form. Some species are flexible and upright while others may be encrusting or rigid. Internal structure of the plant also differs. Many of the species have a colorless medulla surrounded by pigmented cells. However, *Microdictyon setchellianum* and *Chaetomorphora antennina* are one layer thick and lack a cortex, medulla or medullary filaments (Abbott and Huisman 2004). In addition, several species are iridescent or calcified which can influence PAR scattering, reflection, or light harvesting capabilities (Beach et al. 2006a).

Table 7.1 Species descriptions, internal anatomy (Abbott, 1999; Abbott and Huisman 2004), and functional form (Littler and Arnold, 1982) arranged alphabetically within form. T = Taxonomy, P = Phaeophyceae, C = Chlorophyta, R = Rhodophyta.

Species	T	Brief Description	Functional Form	Internal Anatomy
Dictyota acutiloba	P	Dichotomously divided, flat narrow and spirally twisted branches	Sheet	Parenchymatous
Dictyota sandvicensis	P	Dichotomously divided, iridescent, branches off main branches	Sheet	Parenchymatous
Lobophora variegata	P	Fan-shaped attached to substrate, many cell layers thick	Sheet	Parenchymatous
Padina sanctae-crucis	P	Fan-shaped, calcified, 2 cell layers thick	Sheet	Parenchymatous
Stypopodium flabelliforme	P	Fan-shaped, iridescent blue, many cell layers thick	Sheet	Parenchymatous
Chaetomorpha antennina	C	Filamentous, large cells, unbranched and tufted	Filamentous	Uniserate
Microdictyon setchellianum	C	Flattened fronds, filaments form netlike structure	Filamentous	Uniserate
Acanthophora spicifera	R	Upright axes, cylindrical with spine-like branches	Coarsely branched	Psuedoparenchymatous
Gracilaria salicornia	R	Terete branches with regular constrictions	Coarsely branched	Psuedoparenchymatous
Laurencia mcdermidiae	R	Erect with terete axes, bright green, clumps	Coarsely branched	Psuedoparenchymatous
Liagora sp.	R	Erect dichotomously divided, calcified, bushy	Coarsely branched	Medullary filaments
Codium reediae	C	Spongy, dichotomously branched and upright	Thick-leathery	Medullary siphons
Dictyosphaeria verslusyii	C	Bright green hollow cushions	Thick-leathery	Psuedoparenchymatous
Hydroclathrus clathratus	P	Light brown, saccate, convoluted with perforations or netlike	Thick-leathery	Psuedoparenchymatous
Sargassum aquifolium	P	Structurally complex with stem, leaf-like branches, wide leaves	Thick-leathery	Parenchymatous
Halimeda discoidea	C	Upright, calcified, broad flattened segments	Jointed Calcareous	Medullary siphons

Sampling Conditions

The weather conditions were stable before and between sampling periods (Table 7.2). The average PAR values measured on site with a Li-Cor 4 pi sensor and LI-1400 data logger were similar. Sampling occurred when tidal heights were positive, however, the tidal cycle in morning hours varied from positive low tide heights from May 31-June 6 to spring tides with negative tide heights from June 7-June 11.

Table 7.2 Sampling dates and conditions during the sampling period.

Date Sampled	Hours Sampled	Mean Air Temperature (°C)	Mean Irradiance
June 4, 2010	1300-1600	26.1	1648 μmoles m^{-2} s^{-1}
June 11, 2010	1300-1630	26.1	1818 μmoles m^{-2} s^{-1}

Rapid Light Curves

The pre-programmed RLC routine in the Diving PAMs was used at the site to determine the photosynthetic characteristics of reef algae (as described in White and Critchley 1999; Ralph and Gademann 2005). Reef algae were placed one layer thick into the standard leaf clip held at a set distance from the end of the PAM fiber optic cable. Eight increasing irradiances were delivered to the reef alga over 90 s. Irradiances ranged between 0-1800 µmoles m^{-2} s^{-1}. With each increasing irradiance a saturating pulse was applied to compare the minimum (F) and maximum quantum yield (F'_m) in light adapted algae. Then these values were used to calculate the effective quantum yield (Φ_{PSII}) as the indication of the amount of energy used in the photochemistry of PSII (Genty et al. 1989).

$$\Phi_{PSII} = \Delta F / F'_m = (F'_m - F) / F'_m$$

From Φ_{PSII} the approximation of the rate of electrons (*r*ETR) pumped through the photosynthetic chain (Beer et al. 2001) was calculated using the following equation:

$$r\text{ETR} = \Phi_{PSII} \times 0.5 \times AF$$

The 0.5 is assuming that half the photon energy absorbed by photosynthetic pigments was diverted into photosystem II and AF is the fraction of incident light that is absorbed.

The relationship between *r*ETR values and measured irradiances were plotted to produce a RLC. The intensity of the eight irradiances was adjusted for each species to optimize the curves in such a way that the *r*ETR values plotted against irradiance ramped to an asymptote and then slightly leveled off at higher irradiances. These curves were fitted with the exponential model which includes the dynamic photo-inhibition parameter proposed by Platt et al. (1980) using the following Levenberg–Marquardt regression algorithm:

$$P = P_s\,[1-\exp(-\alpha E/P_s)]\,\exp(-\beta E/P_s)$$

P_s is a scaling parameter defined as the maximum potential *r*ETR; α is the photosynthetic efficiency measured by the initial slope of the RLC before the onset of saturation; E is the photon flux density; β is the negative slope of the RLC for high irradiances. In the absence of dynamic photo-inhibition (β=0), the function becomes a standard rectangular hyperbola, with an asymptotic maximum *r*ETR value (Harrison and Platt 1986), and the equation loses $\exp(-\beta E/P_s)$. The parameters $r\mathrm{ETR}_{max}$, the relative maximum electron transport rate, and E_k were estimated using the following equations:

$$r\mathrm{ETR}_{max} = P_s\,[\alpha/(\alpha + \beta)]\,[\beta/(\alpha + \beta)]^{\beta/\alpha}$$

$$E_k = r\mathrm{ETR}_{max}/\alpha$$

Absorption Factor

The AF in the *r*ETR (Φ_{PSII} x 0.5 x AF) equation was determined in the field and compared to the literature. Genty (1989) used the plant standard absorption factor of 0.84 and Beach et al. (2006a) determined the *in vivo* absorbance spectra for common Hawaiian reef macroalgae (Table 7.3). We determined absorption values following the methods described in Beer and Bjork (2000) for seagrass. Absorption was measured in seawater onsite by placing a one layer thick sample over the miniature irradiance sensor of the

Diving-PAM. The reading before and after the plant blocked incident light was expressed as fraction of absorbed irradiance. Reflection was assumed to be negligible. Measurements of absorption were conducted on day 2 of the survey for 1-6 individuals and averaged for each species.

Table 7.3 The mean (SE) absorption factor for species as measured in the field and reported in Beach et al. 2006a. Taxa are arranged alphabetically within functional form.

Species	**N**	**Functional Form**	**Absorption Factor**	**Absorption Factor Beach et al. 2006a**
D. acutiloba	4	Sheet	0.85 (0.02)	0.83
D. sandvicensis	4	Sheet	0.73 (0.10)	-
L. variegata	3	Sheet	0.86 (0.18)	-
P. sanctae-crucis	4	Sheet	0.75 (0.08)	0.75
S. flabelliforme	3	Sheet	0.56 (0.27)	0.64
C. *antennina*	2	Filamentous	0.64 (0.15)	-
M. setchellianum	2	Filamentous	0.53 (0.02)	-
A. spicifera	1	Coarsely branched	0.76	0.89
G. salicornia	1	Coarsely branched	0.92	0.84
L. mcdermidiae	3	Coarsely branched	0.88 (0.08)	0.83
Liagora sp.	3	Coarsely branched	0.79 (0.10)	-
C. reediae		Thick-leathery	-	0.96
D. verslusyii	6	Thick-leathery	0.71 (0.06)	0.82
H. clathratus	1	Thick-leathery	0.86	-
S. aquifolium	3	Thick-leathery	0.88 (0.09)	-
H. discoidea	6	Jointed Calcareous	0.75 (0.08)	0.83

Statistical Comparisons

The fluorescence parameters were compared separately by day and a series of parametric or non-parametric ANOVAs were used to compare values among species. Two-way ANOVAs were used to compare the values of $r\mathrm{ETR}_{\mathrm{maxs,}}$ E_{k}, and α among reef species on both day 1 and day 2. The values of ß on day 1 did not meet parametric requirements therefore a Kruskal-Wallis was used to compare values among species while on day 2 data were analyzed with a Two-way ANOVA. Tukey's or Dunn's multiple comparison tests were used to determine which species were similar or different in $r\mathrm{ETR}_{\mathrm{max}}$, E_{k}, ß, and α.

A nested-ANOVA (species nested within comparisons) was used to investigate if the absorption factors varied among taxa and the extent to which differently derived factors among studies also varied. Specifically, we tested whether spectral absorbance derived values, standard plant values, or values measured directly in this study differed within species. If these factors did differ than it was assumed that AF can significantly alter *r*ETR$_{max}$, α, E$_k$ and ß parameters which are calculated using these varying absorption values and are reported.

The ability of a producer to respond to irradiance (quantum efficiency or α) should be related to absorption of light. Therefore to test this hypothesis, for each day a linear regression was used to investigate the relationship between the mean determined absorption factor (this study) and α. A sequential Bonferroni was used to correct the statistical alpha for multiple comparisons (6) made using the same data (Rice 1989).

Results

Variation in Photosynthetic Light Harvesting Parameters Among Reef Algae

The *r*ETR$_{max}$ for reef algae in this study ranged from 12.9 to 112.2 μmoles e^{-} m^{-2} s^{-1} and significantly varied among species on day 1 (One-way ANOVA, df = 9, F= 7.9, $p < 0.001$) while on day 2 values ranged from 17.4 to 86.9 μmoles e^{-} m^{-2} s^{-1} and did not vary among species (Table 7.4). For some species, the *r*ETR$_{max}$ was similar among days but, for other species, the maximum rates were variable from day 1 to day 2. The brown alga, *H. clathratus* sampled on day 1 had the highest measured *r*ETR$_{max}$ (112.2 μmoles e^{-} m^{-2} s^{-1}) and differed significantly in maximum rates from *C. anteninna*, *D. verslusyii*, *S. flabelliforme*, *Liagora* sp., *L. variegata,* and *H. discoidea*. *Dictyota acutiloba* also had a mean maximum that was higher than those measured for other species but it was not significantly different after correcting the statistical alpha (α<0.01) for multiple comparisons.

The quantum efficiency of PSII (α) in reef algae varied from 0.07 to 0.26 over the two days sampled and significantly varied among species (Day 1: ANOVA, df = 9, F =10.5, $p<0.001$; Day 2: ANOVA, df = 7, F = 8.0, $p<0.001$). The efficiency of light harvesting tended to be higher for reef algae on day 1 of sampling. For both days the mean α measured for brown algae, *H. clathratus, D. acutiloba, S. aquifolium, P. sanctae-crucis* tended to be higher than most reef species (0.13-0.26) while *S. flabelliforme*, *C. antennina*, and *M. setchellianum* had lower efficiencies of light harvest (0.07-0.13). The

Table 7.4 The light harvesting parameters of species sampled on day 1 and day 2. Genus and species names are abbreviated by first initial. Letter groups following the parameter column signify significantly similar groups as determined by Tukey's or Dunn's multiple comparisons when statistical differences were found. The statistical alpha was sequentially corrected for multiple comparisons and is below the parameter

	Day 1							**Day 2**							
Species	**n**	**$r\mathrm{ETR}_{max}$ $(\alpha<0.01)$**		**α $(\alpha<0.008)$**		**E_k**	**ß $(\alpha<0.013)$**	**n**	**$r\mathrm{ETR}_{max}$**	**α $(\alpha<0.008)$**		**E_k $(\alpha<0.013)$**		**ß $(\alpha<0.01)$**	
D. a.	4	88.5 (15.4)	ab	0.26 (0.03)	ac	352.5 (77.4)	70.9 (27.9)	4	28.8 (2.9)	0.13 (0.02)	ab	232.2 (24.0)	a	121.6 (5.6)	ac
D. s.	1	59.2	ab	0.19	abc	385.8	95.5	5	48.1 (17.1)	0.13 (0.02)	a	310.3 (83.9)	a	97.2 (11.4)	abc
L. v.	3	51.2 (14.8)	a	0.20 (0.05)	abc	259.9 (75.6)	120.3 (21.2)	1	21.2	0.13	ab	161.2	a	146.0	ac
P. s.	4	72.4 (13.4)	ab	0.24 (0.02)	ac	317.5 (67.2)	95.8 (10.2)	5	59.8 (13.2)	0.20 (0.03)	ab	291.3 (36.9)	a	99.7 (6.5)	abc
S. f.	3	36.8 (11.4)	a	0.13 (0.02)	abc	274.9 (64.7)	70.4 (10.4)	3	26.0 (7.4)	0.09 (0.02)	a	298.2 (41.2)	a	75.9 (5.4)	ab
C. a.	1	12.9	a	0.09	abc	148.2	101.1	1	20.5	0.09	ab	226.7	ab	93.6	abc
M. s.	1	51.6	ab	0.13	abc	398.0	58.6	1	17.4	0.07	ab	260.1	ab	72.8	abc
A. s.	-	-	-	-	-	-	-	1	23.9	0.14	ab	181.4	a	83.0	abc
G. s.	1	34.7	ab	0.12	abc	249.6	117.1	-	-	-	-	-	-	-	-
L. m.	5	56.4 (4.7)	ab	0.20 (0.02)	abc	288.2 (11.3)	114.1 (2.4)	1	33.2	0.13	ab	247.5	ab	122.8	abc
*L.*sp.	4	50.8 (11.9)	a	0.16 (0.03)	abc	305.3 (27.4)	100.6 (4.7)	4	43.7 (26.6)	0.15 (0.02)	ab	314.7 (71.9)	a	96.2 (9.6)	abc
*C. r.**	1	36.3		0.24		131.8	154.7	-	-	-	-	-	-	-	-
D. v.	3	31.8 (4.8)	a	0.19 (0.01)	abc	323.9 (86.7)	88.7 (10.4)	3	86.9 (8.8)	0.13 (0.01)	ab	659.7 (59.1)	ab	59.0 (3.4)	abc
H. c.	4	112.2 (9.8)	b	0.27 (0.00)	a	435.9 (22.7)	88.4 (3.0)	-	-	-	-	-	-	-	-
S. a.	5	54.5 (5.7)	ab	0.24 (0.01)	ac	223.4 (15.4)	122.3 (7.5)	3	76.3 (14.7)	0.26 (0.02)	ab	292.8 (29.2)	a	113.8 (5.4)	ac
H. d.	2	37.5 (25.8)	a	0.17 (0.01)	abc	219.1 (127.5)	118.5 (33.7)	6	22.2 (2.9)	0.13 (0.02)	a	177.0 (21.3)	a	114.6 (3.9)	ac
Mean	15	54.8 (6.6)		0.18 (0.02)		293.3 (19.2)	98.3 (5.4)	13	41.8 (6.1)	0.14 (0.01)		289.5 (32.5)		100.5 (6.4)	

green alga, *C. reediae* had a high value of α but because AF was not measured directly it was not included in statistical analyses.

The saturation irradiance (E_k) of reef algae varied from 131.8 - 435.9 μmoles m^{-2} s^{-1} on day 1 and 161.2 – 659.7 μmoles m^{-2} s^{-1} on day 2. *D. verslusyii* and *H. clathratus* had high values of E_k, saturating at a mean irradiances above 300 μmoles m^{-2} s^{-1} and several species had E_k values ~ 200 to 300 μmoles m^{-2} s^{-1}. Although the values of E_k did not significantly differ among species on day 1, those values did differ on day 2 (ANOVA, df = 7, F = 8.7, $p<0.001$). The phycocyanin-rich *L. mcdermidiae*, and green algae *M. setchellianum*, *C. antennina*, and *D. verslusyii* were statistically similar.

The values of the decline in $r\mathrm{ETR}_{\mathrm{max}}$, ß, for reef algae were determined to range from 58.6 - 154.7. There were significant differences among species on day 2 (ANOVA, df = 7, F = 12.3, $p<0.001$) but no significant differences occurred among species on day 1 (statistical α < 0.001, Kruskal-Wallis, df =13, H =25.8, $p = 0.02$). Most species had mean ß ranged from 100 to 130. The values of α, $r\mathrm{ETR}_{\mathrm{max}}$, and E_k, reported from the literature for macroalgae were variable (Table 7.5A, B, C). Parameters varied among species in the temperate and subtropics, among studies conducted in the laboratory or *in situ*, with depth, temporally among seasons, days, hours sampled, and even within individual plants.

Comparison of Absorption Factors

AFs varied significantly among species and a comparison of values to those in the literature also varied but this variation was not significant when the alpha was corrected for multiple comparisons (statistical α = 0.017) (nested-ANOVA: reported value comparison, $p = 0.019$; species, $p = 0.007$). Values of AF from this study ranged from 0.53-0.92 (Table 7.3). Beach et al. (2006a) made measurements using *in vivo* absorbance and found a somewhat similar range (0.63-0.89). When comparing the results from the two studies absorption varied as much as 11% for a single species yet this percentage is within the range of standard error determined for several species in this study.

The standard applied plant AF of 0.84 was within the range of absorption determined for many species in this study, but *C. antennina*, *S. flabelliforme*, and *M.*

Table 7.5 A The dynamic variability in light harvesting abilities of green (A), brown (B), and red (C) macroalgae as reported from the literature.

Species	**Distribution**	**α**	**rETR$_{max}$**	**E$_k$**	**Study**	**Lab / *in situ***	**Duration Measured Over**
Chaetomorpha antennina	Subtropical	0.09	12.9-20.5	148.2-226.7	*this study*	*in situ*	2 days during similar hours of day
Chaetomorpha linum	Temperate	0.15	66.2	431.4	Gomez et al. 2004	Lab	2 days during similar hours of day
Cladophora sericea	Subtropical	~0.36-0.42	~100.0-140.0	~250.0-350.0	Smith et al. 2005	*in situ*	varying depth, nutrients
Cladophora sericea	Subtropical	0.29-0.35	38.5-61.02	139.2-180.4	Smith et al. 2005	Lab	enriched nutrients for 10 days
Codium fragile	Temperate	0.35	6.9	19.0	Ihnken et al. 2010	Lab	10 s intervals between light pulses
Codium fragile	Temperate	0.33	7.3	22.0	Ihnken et al. 2010	Lab	90 s intervals between light pulses
Codium reediae	Subtropical	0.24	36.3	131.8	*this study*	*in situ*	2 days during similar hours of day
Dictyosphaeria cavernosa	Subtropical	0.17-0.21	54.2-77.6	299.8-406.4	Cox & Smith in prep.	Lab	after 4-6 hours in the Lab
Dictyosphaeria verslusyii	Subtropical	0.13-0.19	31.8-86.9	323.9-659.7	*this study*	*in situ*	2 days during similar hours of day
Dictyosphaeria verslusyii	Subtropical	0.16-0.22	50.3-73.1	321.1-338.6	Cox & Smith in prep.	Lab	After 4-6 hours in the Lab
Enteromorpha intestinalis	Temperate	0.26	86.6	324.4	Gomez et al. 2004	Lab	controlled conditions
Halimeda discoidea	Subtropical	0.13-0.17	22.2- 37.5	177.0-219.1	*this study*	*in situ*	2 days during similar hours of day
Microdictyon setchellianum	Subtropical	0.07-0.13	17.4-51.6	260.1-398.0	*this study*	*in situ*	2 days during similar hours of day
Ulva costata	Temperate	0.26	16.5	78.5	Gomez et al. 2004	Lab	controlled conditions
Ulva lactuca	Subtropical	0.23-0.28	61.4-103.9	266.1-378.0	Cox et al. in prep	Lab	after 4-6 hours in the Lab
Ulva lactuca	Temperate	-	11-84	-	Longstaff et al. 2002	*in situ*	over 22 hours
Ulva rigida	Temperate	0.22	38.2	167.5	Gomez et al. 2004	Lab	controlled conditions
Ulva rotundata	Temperate	0.28-0.38	78.5-186.6	-	Figuero et al. 2003	Lab	varying light qualities
Ulva sp.	Temperate	0.03	37.0	114.0	Ihnken et al. 2010	Lab	10 s intervals between light pulses
Ulva sp.	Temperate	0.41	51.0	125.0	Ihnken et al. 2010	Lab	90 s intervals between light pulses
Ulva sp. (summer)	Temperate	-	5.2-20.6	6.0-23.7	Saroussi & Beer2007	*in situ*	3x a day 1x a month
Ulva sp. (winter)	Temperate	-	49.7-68.8	75.8-85.6	Saroussi & Beer2007	*in situ*	3x a day 1x a month

Table 7.5 B The dynamic variability in light harvesting abilities of green (A), brown (B), and red (C) macroalgae as reported from the literature.

Species	**Distribution**	**α**	**rETR$_{max}$**	**E$_k$**	**Study**	**Lab / *in situ***	**Duration Measured Over**
Desmarestia lingulata	Temperate	0.25	36.1	144.8	Gomez et al. 2004	Lab	controlled conditions
Dictyota acutiloba	Subtropical	0.13-0.26	28.8-88.5	232.2-352.5	this study	*in situ*	2 days during similar hours of day
Dictyota menstrualis	Subtropical	0.12-0.4	~2.0-90.0		Beach et al. 2006b	*in situ*	3 days in August over 3 depths
Dictyota sandvicensis	Subtropical	0.13-0.19	48.1-59.2	310.3-385.8	*this study*	*in situ*	2 days during similar hours of day
Ecklonia radiate	Temperate	0.26	3.0	13	Ihnken et al. 2010	Lab	10 s intervals between light pulses
Ecklonia radiate	Temperate	0.26	9.0	13	Ihnken et al. 2010	Lab	90 s intervals between light pulses
Fucus vesiculosus (depth)	Temperate	0.19-0.20	-	66.0-85.0	Eukland el al. 2008	*in situ*	1x on 3 different days
Fucus vesiculosus (depth, control)	Temperate	0.16-0.28	-	70.0-88.0	Eukland el al. 2008	Lab	initial & 24 hrs after high light stress
Fucus vesiculosus (depth, stressed)	Temperate	0.09-0.12	-	167.0-348.0	Eukland et al. 2008	Lab	response to high light stress
Fucus vesiculosus (surface)	Temperate	0.08-0.21	-	110.0-183.0	Eukland el al. 2008	*in situ*	1x on 3 different days
Fucus vesiculosus (surface, control)	Temperate	0.21-0.29	-	61.0-112.0	Eukland el al. 2008	Lab	initial & 24 hrs after high light stress
Fucus vesiculosus (surface, stressed)	Temperate	0.03-0.08	-	289.0-454.0	Eukland el al. 2008	Lab	response to high light stress
Hydroclathrus clathratus	Subtropical	0.27	112.2	435.9	*this study*	*in situ*	2 days during similar hours of day
Kelp recruits (transplanted)	Temperate	-	18.0-26.0	-	Wernberg et al. 2010	*in situ*	multiple spp. transplanted
Laminaria saccharina	Temperate	-	18.0-51	146.0-440.0	Gévaert et al. 2003	*in situ*	varying tide while alga submersed
Lessonia variegata	Temperate	0.31	13.0	44.0	Ihnken et al. 2010	Lab	10 s intervals between light pulses
Lessonia variegata	Temperate	0.40	13.0	44.0	Ihnken et al. 2010	Lab	90 s intervals between light pulses
Lobophora variegata	Subtropical	0.13-0.20	21.2-51.2	161.2-259.9	*this study*	*in situ*	2 days during similar hours of day
Lobophora variegata	Subtropical	-	11.2-20.6	-	Cox & Smith in prep.	*in situ*	2 days with varying tidal exposure
Lobophora variegata	Subtropical	0.11-0.16	26.8-37.7	223.9-274.2	Cox & Smith in prep.	Lab	after 4-6 hours in the Lab
Macrocystis pyrifera	Temperate	0.22	72.3	327.4	Gomez et al. 2004	Lab	2 days during similar hours of day
Macrocystis pyrifera (old blades-depth)	Temperate	0.76-0.88	50.3-77.4	64.5-94.7	Edwards & Kim 2010	*in situ*	3x/ 3 days in March and June
Macrocystis pyrifera (old blades-mid)	Temperate	0.81-0.93	48.1-128.1	60.4-148.4	Edwards & Kim 2010	*in situ*	3x/ 3 days in March and June
Macrocystis pyrifera (old blades-surface)	Temperate	0.58-0.93	57.2-147.4	85.0-178.5	Edwards & Kim 2010	*in situ*	3x/ 3 days in March and June
Macrocystis pyrifera (scimitars-depth)	Temperate	0.73-0.90	18.7-76.3	24.8-84.8	Edwards & Kim 2010	*in situ*	3x/ 3 days in March and June
Macrocystis pyrifera (scimitars-mid)	Temperate	0.75-0.93	38.5-90.7	49.7-102.8	Edwards & Kim 2010	*in situ*	3x/ 3 days in March and June
Macrocystis pyrifera (scimitars-surface)	Temperate	0.55-0.98	22.1-124.6	61.8-144.0	Edwards & Kim 2010	*in situ*	3x/ 3 days in March and June
Padina sanctae-crucis	Subtropical	0.20-0.24	59.8-72.4	291.3-317.5	this study	*in situ*	2 days during similar hours of day
Padina sanctae-crucis	Subtropical	-	9.2 - 59.3	-	Cox & Smith in prep.	*in situ*	4 days varying tides 2 sites
Padina sanctae-crucis	Subtropical	0.21-0.24	50.1-60.7	240.7-285.0	Cox & Smith in prep.	Lab	after 4-6 hours in the Lab
Petalonia fascia	Temperate	0.34	17.0	50.9	Gomez et al. 2004	Lab	controlled conditions
Sargassum aquifolium	Subtropical	0.24-0.26	54.5-76.3	223.4-292.8	this study	*in situ*	2 days during similar hours of day
Sargassum aquifolium	Subtropical	-	21.3-47.2	-	Cox & Smith in prep.	*in situ*	4 days with varying tidal exposure
Sargassum aquifolium	Subtropical	0.23-0.29	50.3-74.7	220.4-262.6	Cox & Smith in prep.	Lab	after 4-6 hours in the Lab
Stypopodium flabelliforme	Subtropical	0.09-0.13	26.0-36.8	274.9-298.2	this study	*in situ*	2 days during similar hours of day
Stypopodium flabelliforme	Subtropical	0.14-0.23	34.8-53.8	169.5-236.8	Cox & Smith in prep.	Lab	after 4-6 hours in the Lab

Table 7.5 C The dynamic variability in light harvesting abilities of green (A), brown (B), and red (C) macroalgae as reported from the literature

Species	Distribution	α	rETR$_{max}$	E$_k$	Study	Lab / *in situ*	Duration Measured Over
Acanthophora spicifera	Subtropical	0.14	23.9	181.4	*this study*	*in situ*	2 days during similar hours of day
Acanthophora spicifera	Subtropical	-	23.1-49.4	-	Cox & Smith in prep.	*in situ*	4 days with varying tidal exposure
Acanthophora spicifera	Subtropical	0.18-0.26	55.7-76.4	240.3-335.5	Cox & Smith in prep.	Lab	after 4-6 hours in the Lab
Ahnfeltiopsis durvillaei	Temperate	0.22	31.3	138.6	Gomez et al. 2004	Lab	controlled conditions
Callophyllis variegata	Temperate	0.02	11.2	81.9	Gomez et al. 2004	Lab	controlled conditions
Gelidium lingulatum	Temperate	0.34	80.9	335.6	Gomez et al. 2004	Lab	controlled conditions
Gelidium sesquipedale	Temperate	0.08-.123	16.7-24.2	-	Silva et al. 1998	*in situ*	10-22 m depths
Gracilaria chilensis	Temperate	0.15	28.2	182.8	Gomez et al. 2004	Lab	controlled conditions
Gracilaria salicornia	Subtropical	0.12	34.7	249.6	*this study*	*in situ*	2 days during similar hours of day
Gracilaria salicornia	Subtropical	-	0.0-30.0	-	Smith et al. 2004	Lab	varying temperature and salinity
Grateloupia doryphora	Temperate	0.21	21.2	104.0	Gomez et al. 2004	Lab	controlled conditions
Gymnogongrus furcellatus	Temperate	0.16	20.7	117.7	Gomez et al. 2004	Lab	controlled conditions
Hildenbrandia rubra	Temperate	0.12	8.0	67.0	Kim & Garbary 2006	Lab	within 24 hours
Hildenbrandia rubra (within a plant)	Temperate	-	2.0-5.3	20.0	Kim & Garbary 2006	Lab	within 24 hours
Jania rubens (summer)	Temperate	-	40.2-123.9	56.4-176.1	Saroussi & Beer2007	*in situ*	3x a day 1x a month
Jania rubens (winter)	Temperate	-	48.0-87.5	81.2-124.1	Saroussi & Beer 2007	*in situ*	3x a day 1x a month
Laingia hookerii	Temperate	0.13	33.8	256.1	Gomez et al. 2004	Lab	controlled conditions
Laurencia mcdermidiae	Subtropical	0.13-0.20	33.2-56.4	247.5-288.2	*this study*	*in situ*	2 days during similar hours of day
Laurencia mcdermidiae	Subtropical	-	16.4-41.8	-	Cox & Smith in prep	*in situ*	4 days varying tidal exposure 2 sites
Laurencia mcdermidiae	Subtropical	0.20-0.23	37.1-66.3	269.9-226.3	Cox & Smith in prep	Lab	after 4-6 hours in the Lab
Laurencia nidifica (summer)	Subtropical	-	~0.0-20.0	-	Padilla-Gamino & Carpenter 2007	Lab	varying temperatures (10-35°C)
Laurencia nidifica (winter)	Subtropical	-	~0.0-30.0	-	Padilla-Gamino & Carpenter 2007	Lab	varying temperatures (10-35°C)
Laurencia pacifica (summer)	Temperate	-	~0.0-40.0	-	Padilla-Gamino & Carpenter 2007	Lab	varying temperatures (10-35°C)
Laurencia pacifica (winter)	Temperate	-	~0.0-20.0	-	Padilla-Gamino& Carpenter 2007	Lab	varying temperatures (10-35°C)
Liagora sp.	Subtropical	0.15-0.16	43.7-50.8	305.3-314.7	*this study*	*in situ*	2 days during similar hours of day
Mazzaella laminarioides	Temperate	0.06	14.1	237.2	Gomez et al. 2004	Lab	controlled conditions
Polysiphonia sp.	Temperate	0.14	33.8	237.4	Gomez et al. 2004	Lab	controlled conditions
Porphyra columbina (shade)	Temperate	0.14	25.6	179.9	Gomez et al. 2004	Lab	controlled conditions
Porphyra columbina (sun)	Temperate	0.2	20.6	136.5	Gomez et al. 2004	Lab	controlled conditions
Porphyra leucosticte	Temperate	-	2.3-20.7	-	Figuero et al. 2003	Lab	varying light quantities
Porphyra leucosticte	Temperate	0.07-.015	5.09-28.1	-	Figuero et al. 2003	Lab	varying light qualities
Sarcothalia crispate	Temperate	0.13	24.8	181.7	Gomez et al. 2004	Lab	controlled conditions
Ulva olivascens	Temperate	0.21-0.44	13.1-106.2	-	Figuero et al. 2003	Lab	varying light qualities

setchellianum had substantially lower values (0.53-0.64) with a maximum difference as much as 31% for a single species. The two red algal species, *A. spicifera* and *L. mcdermidiae,* had determined PAR absorptions (this study) more similar to 84% than to those reported in the Beach et al. 2006a.

Absorption Factor Related to Quantum Efficiency

Although not significant, the species average absorption factors determined in this study explained 30-40% of the variation in the average quantum efficiency of light harvest for species sampled on day 1 and day 2 (Linear Regressions: Day 1, $R^2 = 30\%$, $p = 0.04$; Day 2, $R^2 = 40\%$, $p = 0.02$, not significant at statistical $\alpha < 0.016$) (Fig. 7.1). For both days the planar mesh species, *M. setchellianum*, the uniseriate filamentous *C. antennina* and the iridescent subtidal species *S. flabelliforme* had relatively low mean absorption and quantum efficiencies. On the other end of the spectrum, more structurally complex species common in intertidal habitats, such as *P. sanctae-crucis*, *S. aquifolium*, *L. mcdermidiae*, *D. acutiloba* exhibited higher absorption values and quantum efficiencies.

Discussion

Estimates of photosynthesis via PAM fluorometry reveal that this shallow water algal community exhibited light harvesting characteristics typical of sun tolerant producers yet these characteristics are dynamic and vary among species. In addition, our results reveal that absorption used to calculate relative electron transport differs among species and tends to be lower for filamentous species with uniserate anatomy. We also demonstrate that absorption factors can be estimated quickly in the field and we recommend for best accuracy direct measurements absorption should be made on sampled plants, as these values effect the calculated parameters of brown, red, and green macroalgae.

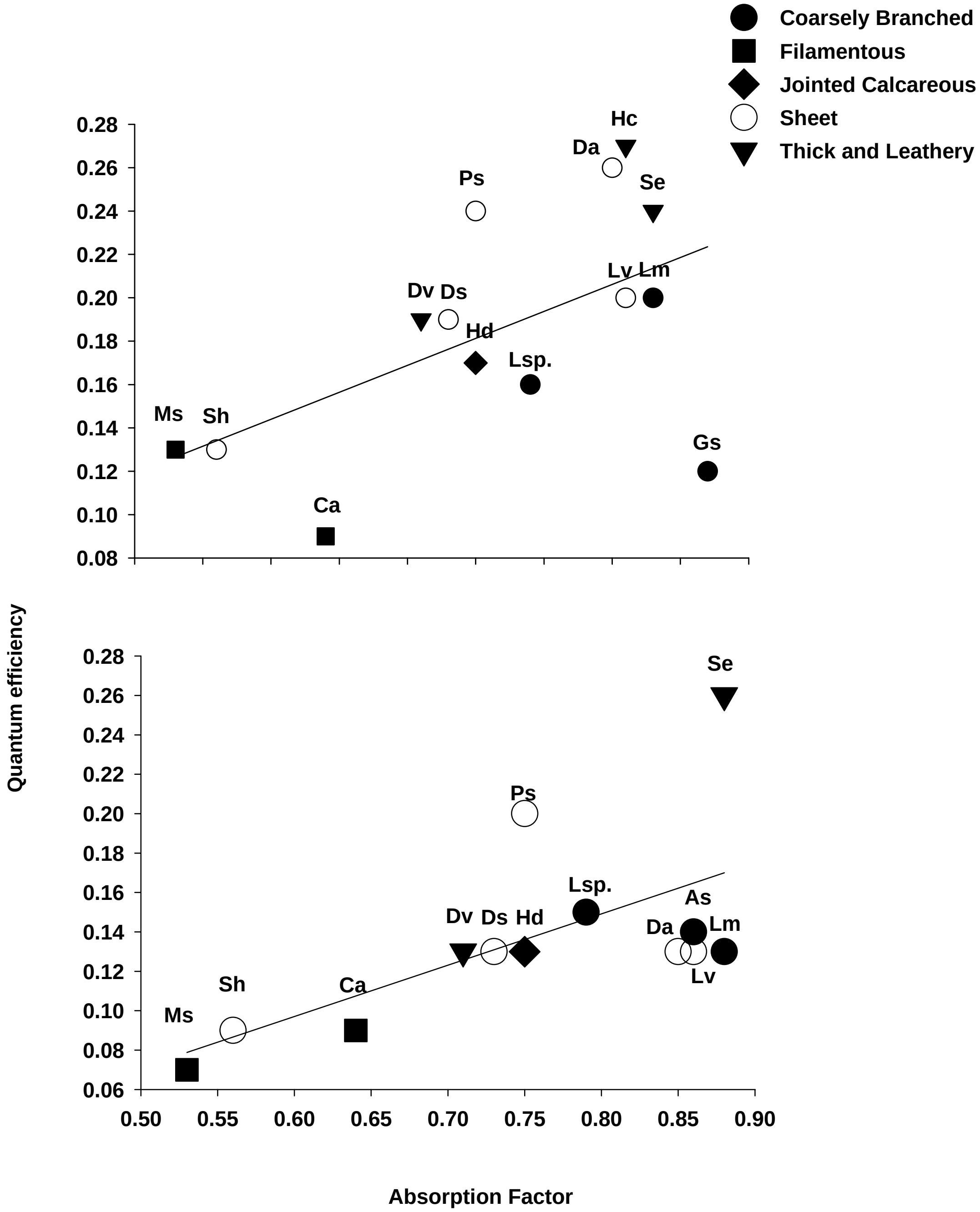

Fig. 7.1 The linear relationship between absorption and quantum efficiency for species sampled on days 1 (top) and 2 (bottom) of sampling. Genus and species names are abbreviated by the first initial. Note the two filamentous species *C. antennina* and *M. setchellianum* and the iridescent subtidal *S. flabelliforme* have lower values.

Most reef algae collected from a tropical shallow reef tended to have light harvesting characteristics that typify sun tolerant producers. The maximum *r*ETRs (mean = 54.8 µmoles e^- m^{-2} s^{-1}) and the irradiances necessary to saturate rates were high (mean = 293.3 *µ*moles quanta m^{-2} s^{-1}). High rates of electron transport and saturation irradiances have been determined for *Fucus* (Ekelund et al. 2008), *Gelidium* (Silva et al. 2005), *Porphyra* (Israel et al. 2008), and *Ecklonia* (Fairhead and Cheshire 2004) grown at shallower depths, and for zooxanthallae growing at the tips of corals which receive more light than shaded polyps (Helmuth et al. 1997). Despite the differences in techniques, the E_k values determined from this study were similar to I_k measured for canopy and understory tissue in intertidal Hawaiian red algae (Beach and Smith 1996) and saturation values are well below the irradiances these reef species experience in a tropical setting (Williams and Carpenter 1990; Beach and Smith 1996b). Sampled species also showed similar declines in electron transport after reaching an optimum, even extended plateau. The apparent ability of reef algae to experience high rates of electron transport on two days suggests that these plants are able to rapidly adjust to supraoptimal irradiances.

Macroalgae can rapidly alter their characteristics of light harvesting by changing the absorption cross-section of PSII. The summary of values reported from the literature (Table 7.5 A-C) and from this study reveal that these marine plants are dynamic and flexible in their ability to respond to different environments. This dynamic variability in light harvesting parameters may allow algae to remain competitive under different conditions. Rapid alterations in light harvesting ability can be achieved by altering the connectivity or function of PSII or through non-photochemical quenching. For example, a decrease in PSII absorption cross section via non-photochemical quenching which occurs under high irradiances can shift the E_k to substantially lower values. These types of changes happen on the time scale of cloud movements (Falkowski and Raven 1997; Gorbunov et al. 2001). Combined with photoacclimation (which occurs over weeks) this allows macroalgae to adjust to changing irradiances on multiple scales (Gantt 1990). The dynamic nature of light harvesting needs to be accounted for when designing comparative ecophysiological studies. Best efforts should be made to sample parameters simultaneously *in situ* and studies conducted in the lab or after long delays and applied to a natural setting should be interpreted cautiously.

The mesh or filamentous, uniserate forms had lower absorption factors and lower quantum efficiencies. This finding is likely the result of the effective absorption cross section. More light can transmit through a thin layer of cells than a thick layer (Beach et al. 2006a). Most species in this study have a pigmented layer of cells that surround a colorless medulla or thick medullary filaments (Abbott 1999; Abbott and Huisman 2004; Beach et al. 2006a). These species exhibited higher and more variable absorption values and quantum efficiencies. It is likely that the ultrastructure of the surrounding cells and thickness impacts the amount of pigment per unit area and the ability to absorb light. However, in a natural setting filamentous species occur in tufts or in turfs which alters PAR absorption. *Stypopodium flabelliforme* is known to have higher values of reflectance and transmittance than other brown algae with similar form because of its iridescent habit (Beach et al. 2006a), which could explain the low value of absorption for this species.

Absorption by reef algae can vary from the standard applied value of 84% used by Genty et al. (1989) and should be measured directly to eliminate bias when making ecophysiological comparisons. Beach et al. (2006a) documented a range of plant absorptance of PAR from 16-98%. These values were calculated from absorbance spectra and different values were recorded for the same species examined in this study. The largest discrepancies between the species determined absorption values occurred for red algae. Red algae are able to photoacclimate over microscales (>10 cm) within the same plant on tropical reefs (Beach et al. 2006a); therefore it seems likely that absorption would be more spatially and temporally variable for these taxa. It is unclear whether methodology or physiological differences in pigment composition account for discrepancies between studies yet, it is clear that accurate values are necessary to make ecophysiological comparisons.

A different value of absorption alters the determined physiological parameters when using both O_2 (Beach et al. 2006a) and chlorophyll fluorescence for red, brown, and green macroalgae (this study, Table 7.6). As expected, *C. antennina*, *M. setchellianum*, and *S. flabelliforme* with substantially lower absorption factors from the plant standard of 0.84 have the largest discrepancies among calculated P-E parameters. For example, for *C. antennina* there is a 5.2 μmoles e^{-} m^{-2} s^{-1} difference in maximum

Table 7.6 The light harvesting parameters *r*ETR$_{max}$, α , ß calculated with different absorption factors (AF). Columns refer to sample size (n), and to the AF used in calculations (standard = 0.84 from Genty (1989), absorbance = Beach et al. (2006a), and field = this study Table 7.3).

		***r*ETR$_{max}$**			**α**			**ß**		
Species	**n**	**standard**	**absorbance**	**field**	**standard**	**absorbance**	**field**	**standard**	**absorbance**	**field**
D. acutiloba	8	58.1 (13.6)	57.6 (13.5)	58.7 (13.7)	0.19 (0.03)	0.19 (0.03)	0.20 (0.03)	94.9 (15.8)	94.0 (15.7)	95.8 (16.0)
D. sandvicensis	7	58.3 (13.2)	-	50.7 (11.3)	0.17 (0.02)	-	0.14 (0.02)	106.6 (10.2)	-	92.7 (8.9)
L. variegata	4	42.9 (12.9)	-	43.8 (13.1)	0.19 (0.04)	-	0.19 (0.04)	124.5 (15.7)	-	126.8 (16.0)
P. sanctae-crucis	9	73.0 (9.6)	65.5 (8.6)	65.4 (8.6)	0.24 (0.02)	0.24 (0.02)	0.24 (0.02)	108.8 (5.4)	97.5 (4.8)	97.4 (4.8)
S. flabelliforme	6	47.1 (9.1)	35.9 (6.9)	31.4 (6.1)	0.16 (0.02)	0.12 (0.02)	0.11 (0.02)	109.4 (7.3)	83.4 (5.6)	73.4 (4.9)
C. antennina	2	21.9 (7.1)	-	16.7 (5.4)	0.12 (0.00)	-	0.09 (0.00)	127.8 (7.0)	-	97.3 (5.3)
M. setchellianum	4	34.1 (18.7)	-	21.5 (11.8)	0.15 (0.07)	-	0.10 (0.05)	104.0 (15.8)	-	65.7 (10.0)
A. spicifera	1	26.1	27.7	23.9	0.14	0.13	0.15	83.1	76.0	88.0
G. salicornia	1	31.0	31.0	34.0	0.12	0.12	0.14	117.1	117.1	128.7
L. mcdermidiae	7	50.3 (5.5)	49.8 (5.4)	52.5 (5.7)	0.18 (0.02)	0.18 (0.02)	0.19 (0.02)	110.8 (2.4)	109.5 (2.3)	115.6 (2.5)
Liagora sp.	8	50.1 (8.9)	-	47.2 (8.5)	0.16 (0.02)	-	0.15 (0.02)	104.5 (5.0)	-	98.4 (4.7)
C. reediae	1	31.8	36.3	-	0.24	0.28	-	154.7	176.8	-
D. verslusyii	6	70.0 (16.6)	67.8 (15.8)	59.4 (14.1)	0.14 (0.01)	0.13 (0.01)	0.12 (0.01)	87.0 (10.0)	85.0 (9.7)	73.9 (8.5)
H. clathratus	4	113.4 (7.5)	-	115.6 (7.6)	0.26 (0.01)	-	0.26 (0.01)	86.8 (3.0)	-	88.5 (3.1)
S. aquifolium	8	60.0 (6.1)	-	62.7 (6.8)	0.24 (0.01)	-	0.25 (0.01)	114.0 (4.4)	-	119.2 (4.9)
H. discoidea	8	29.1 (5.6)	28.7 (5.5)	26.0 (5.0)	0.15 (0.01)	0.15 (0.01)	0.14 (0.01)	129.0 (6.2)	127.6 (6.2)	115.5 (5.6)

rates of electron transport, 0.3 difference in α, and 30.4 difference in ß when comparing values derived from field measurements to those derived using the plant standard AF. Although these differences seem small when compared within a species, the differences have a larger impact when comparing physiology and ecology among species.

Our findings have direct implications for the design and interpretation of field studies and on our understanding of shallow water algal physiology. Shallow water algal communities are dynamic and ecophysiological parameters vary daily, and likely even over hours or minutes. We provide evidence that algae with different morphologies and taxonomic affinities have varying strategies to adjust to high light environments. Thin species may transmit and absorb less PAR while thicker species may rely on pigment ratios for photoprotection. An estimate of absorption in natural settings with flexible forms where wave and self shading occurs will be a challenge for ecophysiologists. Pulse amplitude fluorescence requires accurate measurements of absorption to compare physiological traits among species. To ensure absorption values are accurate, measurements should be conducted on sampled plants in the field with high sample sizes to ensure precision.

References

Abbott IA (1999) Marine red algae of the Hawaiian Islands. Bishop Museum Press, Honolulu

Abbott IA, Huisman JM (2004) Marine green and brown algae of the Hawaiian Islands. Bishop Museum Press, Honolulu

Beach KS, Borgeas HB, Smith CM (2006a) Ecophysiological implications of the measurement of transmittance and reflectance of tropical macroalgae. Phycologia 45: 450-457

Beach KS, Walters LJ, Borgeas HB (2006b) Irradiance and nutrient limitation of *Dicytota* spp. populations on Conch Reef, Florida Keys, USA. Journal of Experimental Marine Biology and Ecology 329: 101-112

Beach KS, Smith CM (1996a) Ecophysiology of tropical Rhodophytes. I. Microscale acclimation in pigmentation. Journal of Phycology 32: 701-710

Beach KS, Smith CM (1996b) Ecophysiology of tropical rhodophytes. II. Microscale acclimation in photosynthesis. Journal of Phycology 32: 710-718

Beach KS, Smith CM, Michael T, Shin H (1995) Photosynthesis in reproductive unicells of *Ulva lactuca* and *Entermorpha flexuosa* implications for ecological success. Marine Ecology Progress Series 125: 229-237

Beach KS, Walters L, Vroom PS, Smith CM, Hunter CL (2003) Variability in the ecophysiology of *Halimeda* spp. (Chlorophyta, Bryopsidales) on Conch Reef, Florida Keys, USA. Journal of Phycology 39: 633-643

Beer S, Axelsson L (2004) Limitations in the use of PAM fluorometry for measuring photosynthetic rates of macroalgae at high irradiances. European Journal of Phycology 39: 1-7

Beer S, Bjork M (2000) Measuring rates of photosynthesis of two tropical seagrasses by pulse amplitude modulated (PAM) fluorometry. Aquatic Botany 66: 69-76

Boardman K (1977) Comparative photosynthesis of sun and shade plants. Annual Review of Plant Physiology 28: 355-377

Choo KS, Nilsson J, Pedersen M, Snoeijs P (2005) Photosynthesis, carbon uptake and antioxidant defense in two coexisiting filamentous green algae under different stress conditions. Marine Ecology Progress Series 292: 127-138

Dubinsky Z (1992) The optical and functional cross-sections of phytoplankton photosynthesis. In: Falkowski PG, Woodhead AD (eds) Primary productivity and biogeochemical cycles in the sea. Plenum, New York, pp 31-35

Edwards MS, Kim YK (2010) Diurnal variation in relative photosynthetic performance in giant kelp *Macrocystis pyrifera* (Phaeophyceae, Laminariales) at different depths as estimated using PAM fluorometry. Aquatic Botany 92: 119-128

Ekelund NGA, Nygard CA, Nordstrom R, Gylle AM (2008) In situ study of relative electron transport rates in the marine macroalga *Fucus vesiculosus* in the Baltic Sea at different depths and times of year. Journal of Applied Phycology 20: 751-756

Fairhead VA, Cheshire AC (2004) Seasonal and depth related variation in the photosynthesis-irradiance response of *Ecklonia radiata* (Phaeophyta, Laminariales) at West Island, South Australia. Marine Biology 145: 415-426

Falkowski PG, Raven JA (1997) Aquatic photosynthesis. Blackwell Science, Malden

Fejtek SM, Edwards MS, Kim YK (2011) Elk Kelp, *Pelagophycus porra*, distribution limited due to susceptibility of microscopic stages to high light. Journal of Experimental Marine Biology and Ecology 396: 194-201

Figueroa FL, Conde-Alvarez R, Gomez I (2003a) Relations between electron transport rates determined by pulse amplitude modulated chlorophyll fluorescence and oxygen evolution in macroalgae under different light conditions. Photosynthesis Research 75: 259-275

Figueroa FL, Escassi L, Perez-Rodriquez E, Korbee N, Giles AD, Johnsen G (2003b) Effects of short-term irradiation on photoinhibition and accumulation of mycosporine-like amino acids in sun and shade species of the red algal genus *Porphyra*. Journal of Photochemistry and Photobiology B: Biology 69: 21-30

Franklin LA, Forster RM (1997) Review. The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. European Journal of Phycology 32: 207-232

Gantt E (1990) Pigmentation and photoacclimation. In: Cole KM, Sheath RG (eds) Biology of Red Algae. University Press, Cambridge, Cambridge, pp 203-220

Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochemistry Biophysics Acta 990: 97-92

Gévaert F, Créach A, Davoult D, Migné A, Levavasseur G, Arzel P, Holl A-K, Lemoine Y (2003) *Laminaria saccharina* photosynthesis measured *in situ*: photoinhibition and xanthophyll cycle during a tidal cycle. Marine Ecology Progress Series 247: 43-50

Gomez I, Figueroa FL, Ulloa N, Morales V, Lovengreen C, Huovinen P, Hess S (2004) Patterns of photosynthesis in 18 species of intertidal macroalgae from southern Chile. Marine Ecology Progress Series 270:103-116

Gorbunov MY, Kolber ZS, Lesser MP, Falkowski PG (2001) Photosynthesis and photoprotection in symbiotic corals. Limnology and Oceanography 46: 75-85

Hanelt D, Roleda MY (2009) UVB radiation may ameliorate photoinhibition in specific shallow-water tropical marine macrophytes. Aquatic Botany 91: 6-12

Harrison WG, Platt T (1986) Photosynthesis-irradiance relationships in polar and temperate phytoplankton populations. Polar Biology 5: 153-164

Helmuth BST, Timmerman BEH, Sebens KP (1997) Interplay of host morphology and symbiont microhabitat in coral aggregations. Marine Biology 130: 1-10

Henley WJ, Lindley ST, Levavasseur G, Osmond CB, Ramus J (1992) Photosynthetic response of *Ulva rotundata* to light and temperature during emersion on an intertidal sand flat. Oecologia 89: 516-523

Ihnken S, Eggert A, Beardall J (2010) Exposure times in rapid light curves affect photosynthetic parameters in algae. Aquatic Botany 93: 185-194

Israel A, Stambler N, Dubinsky Z, Friedlander M, Malik Z, Rothmann C, Katz S (2008) Light absorption and fluorescence, and photoacclimation features in the marine macroalga *Porphyra leucosticta* (Rhodophyta). Israel Journal of Plant Sciences 56: 61-68

Johannesson G, Snoeijs P (2002) Macroalgal photosynthetic responses to light in relation to thallus morphology and depth zonation. Marine Ecology Progress Series 244: 63-72

Kim YK, Garbary DJ (2006) Fluorescence responses of photosynthesis to extremes of hyposalinity, freezing and desiccation in the intertidal crust *Hildenbrandia rubra* (Hildenbrandiales, Rhodophyta). Phycologia 45: 680-686

Kirk JT (1994) Light and photosynthesis in aquatic ecosystems. 2nd edition, Cambridge University Press, United Kingdom

Littler MM, Arnold KE (1982) Primary productivity of marine macroalgal functional-form groups from southwestern North America. Journal of Phycology 18: 307-311

Longstaff B, Kildea T, Runcie JW, Cheshire A, Dennison WC, Hurd C, Kana T, Raven JA, Larkum AWD (2002) An *in situ* study of photosynthetic oxygen exchange and electron transport rate in the marine macroalgae *Ulva lactuca* (Chlorophyta). Photosynthesis Research 74: 281-293

Padilla-Gamino JL, Carpenter RC (2007) Thermal ecophysiology of *Laurencia pacifica* and *Laurencia nidifica* (Ceramiales, Rhodophyta) from tropical and warm-temperate regions. Journal of Phycology 43: 686-692

Platt T, Gallegos CL, Harrison WG (1980) Photoinhibition of photosynthesis in natural assemblage of marine phytoplankton. Journal of Marine Research 38: 687-701

Ralph PJ, Gademann R (2005) Rapid light curves: a powerful tool to assess photosynthetic activity. Aquatic Botany 82: 222-237

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43: 223-225

Saroussi B, Beer, S (2007) Acclimations of macroalgae as reflected in photosynthetic parameters derived from PAM fluorometry, and possible implications for abundance patterns. Marine Ecology 28:377-383

Silva J, Santos R, Calleja ML, Duarte CM (2005) Submerged versus air-exposed intertidal macrophyte productivity: from physiological to community-level assessments. Journal of Experimental Marine Biology and Ecology 317: 87-95

Smith JE, Hunter CL, Conklin EJ, Most R, Sauvage TP, Squair S, Smith CM (2004) Ecology of the Invasive Red Alga *Gracilaria salicornia*(Rhodophyta) on O'ahu, Hawai'i. Pacific Science 58: 325-343.

Smith JE, Runcie JW, Smith CM (2005) Characterization of a large-scale ephemeral bloom of the green alga *Cladophora sericea* on the coral reefs of West Maui, Hawaii. Marine Ecology Progress Series 302: 77-91

Talarico L, Maranzana G (2000) Light and adaptive responses in red macroalgae: an overview. Journal of Photochemistry and Photobiology B: Biology: 1-11

Vergara JJ, Perez-Llorens JL, Hernandez I, Niell FX (1997) Seasonal variation of photosynthetic performance and light attenuation in *Ulva* canopies from Palmones River estuary. Journal of Phycology 33: 773-779

Wernberg T, Thomsen MS, Tuya F, Kendrick GA, Staehr PA, Toohey BD (2010) Decreasing resilience of kelp beds along a latitudinal temperature gradient: potential implications for a warmer future. Ecology Letters 13: 685-694

White AJ, Critchley C (1999) Rapid light curves: a new fluorescence method to assess the state of the photosynthetic apparatus. Photosynthesis Research 59: 63-72

Williams SL, Carpenter RC (1990) Photosynthesis/photon flux density relationships among components of coral reef algal turfs. Journal of Phycology 26: 36-40

Chapter VIII. *In situ* measures of stress and performance for tropical reef algae in Hawaii's micro-tidal zones

Abstract

Minimal tidal conditions with fluctuations <1 m coupled with high tropical temperatures and irradiances were predicted to have severe physiological consequences on intertidal species in the Hawaiian Archipelago. To explore survival mechanisms of macroalgae in tropical intertidal environments, a pulse amplitude fluorometer was used investigate physiological performance for common intertidal macroalgal species at two sites over back-to-back tides of varying heights on the island of O'ahu. Surprisingly, the reef algal species monitored maintained or increased electron transport maxima during negative tidal heights with the exception of one sampling period when reef algae had been exposed to elevated mid-morning irradiances and air temperatures for up to 6 hours. In particular, for individuals of *Padina sanctae-crucis* measured across the shore, no single intertidal zone (high, mid, or low) consistently accommodated individuals with higher rates of electron transport. Instead the optimal position varied among shores and with the timing and magnitude of the low tide. Further manipulations and measurements provided evidence that aerial exposure in daylight, while limited of duration (0-6 hrs), could cause desiccation in *P. sanctae-crucis* but, the aggregations of individuals in monospecific stands combined with the timing and magnitude of low tides limited water loss and facilitated optimal temperatures for elevated photosynthetic performances. These results are in contrast to those for temperate systems where intertidal algae experience prolonged exposure stress and our findings suggest warm temperatures that should result from global climate change could affect algal performance.

Introduction

Intertidal habitats are usually considered one of the most stressful environments for marine primary producers because of heterogeneity of habitat and rapidly changing physical and biotic conditions. Intertidal macroalgae that are anchored to the substratum rely on water flow to acquire limiting nutrients and CO_2, and must tolerate diurnally and rapidly fluctuating irradiances and temperatures, and cope with periods of desiccation that accompany tidal changes (Davison and Pearson 1996). Such physiological stresses are known to alter distribution patterns (Doty 1946; Connell 1961; Paine 1974; Schonbeck and Norton 1978) and impact primary production (Silva et al. 2005; Williams and Dethier 2005).

Our understanding of stress for rocky intertidal macroalgae comes primarily from laboratory studies (Johnson et al. 1974; Dring and Brown 1982; Smith and Berry 1986; Bell 1995; Dudgeon et al. 1995; Matta and Chapman 1995; Hunt and Denny 2008) or, to a lesser extent, from field studies conducted at temperate latitudes (Foster 1982; Oates and Murray 1983; Davison et al. 1993; Ganzon-Fortes 1997; Wright et al. 2004; Dethier et al. 2005; Williams and Dethier 2005; Dethier and Williams 2009). In general, stress varies across the shore with a species' upper distribution linked to tidal elevation and tolerance to physical factors (Doty 1946; Connell 1961; Paine 1974). Species vary in their ability to withstand and recover from stress (Smith and Berry 1986; Matta and Chapman 1995; Davison and Pearson 1996; Beach and Smith 1997). Intertidal algae are often more tolerant of harsh emersion conditions than subtidal species (Davison and Pearson 1996). However, even the most robust intertidal brown algae (e.g. fucoids) exhibit higher rates of photosynthesis when submersed (Chapman 1995).

Temperate algae experience extended exposure to air (Williams and Dethier 2005) and photosynthesis becomes inhibited as cells overheat and dry (Davison and Pearson 1996). Therefore, desiccation has been considered the driver of primary producer performance in intertidal habitats (Schonbeck and Norton 1978; Davison et al. 1993). Desiccation rates can vary with solar irradiance, air temperature, wind speed, algal morphology, humidity, and varying tidal heights (Bell 1995; Dudgeon et al. 1995; Matta and Chapman 1995; Beach and Smith 1997; Schaeffelke and Deane 2005; Hunt and Denny 2008). For example, turf algae which are diminutive in size and aggregated tend to be more resistant to desiccation stress than larger macrophytes (Hay 1981).

While effects of irradiance, temperature, and desiccation on algal performance have been well studied in the laboratory for temperate and some tropical species, we know less about the actual stress intertidal algae experience in natural settings (Bell 1995; Davison and Pearson 1996; Williams and Dethier 2005). Because it is difficult to mimic natural conditions in laboratory settings, comparisons between field and laboratory measurements of production can reveal discrepancies in photosynthetic rates (Tait and Schiel 2010). For instance, in the lab, investigators often isolate macroalgae from natural surroundings that may facilitate stress reduction (Bertness et al. 1999; Molina-Montenegro et al. 2005). More recent fluorescence technologies allow researchers to rapidly measure *in situ* performance (White and Critchley 1999; Ralph and Gademann 2005). Without reliable field measurements of physiological capacities, it is difficult to identify which abiotic and biotic factors control growth, abundance and distributions of marine algae (e.g. Bell 1995).

It is expected that stress experienced by reef algae in Hawaii's tropical intertidal environments differs from the models provided by temperate systems. Tropical reef algae experience some of the highest temperatures and irradiances worldwide (Beach and Smith 1996b) with ultraviolet radiation 2x higher than in temperate regions (Beach and Smith 1996a) and the diurnal timing of spring low tides coincides with daylight hours during warmer months (Jan-July) (www.tidesandcurrents.noaa.gov). However, to potentially compensate for these extremely stressful conditions, tides in the islands fluctuate less than 1 m and seasonal wave heights can swamp exposed shores (Gosline 1965; Kay 1979; Abbott 1999). Past studies focusing on one Hawaiian intertidal alga growing high on basaltic shores, *Ahnfeltiopsis concinna,* found that physiological stress and response varied over microscales from the canopy to understory (Beach and Smith 1996b), likely allowing the alga to deal with the unique stressors found in tropical locations. In other Hawaiian macroalgal taxa, species distribution varies over shores (see Chapter II) and functional forms are known to coincide with temperature zones (Bird 2006) suggesting that physiological or biological variability exists among tidal algae, even in tropical settings.

The few studies providing relevant data for stress coping mechanisms of marine algae in tropical intertidal zones (Beach and Smith 1996b, Bird 2006) provide tantalizing hints of how these plants survive in harsh environments, and also provide the opportunity to ask additional fundamental questions: (1) will common tropical reef algae experience *in situ* physiological stress on low tides less than -1.0 m?; (2) if so, which factors drive changes in performance?; (3) do intertidal reef algae retain water in order to maintain or enhance performance at low tide?; and (4) will increased irradiance, temperature and desiccation hinder photosynthetic performance when exposed?

To address these questions, this study used pulse amplitude fluorescence *in situ* to consecutively measure the performance of five common reef algal species before, during, and after peak low tides at two sites on the south shore of O‘ahu, Hawai‘i. To examine variation with tidal phasing, performance was measured over several consecutive days experiencing varying low tide tidal heights over differing times of the day. In particular, we sampled one tropical algal species, *Padina sanctae-crucis*, extensively across the shore to determine the extent to which photosynthetic performances varied over narrow (< 15 m wide) tidal benches. Additionally, we simultaneously documented environmental variables such as temperature, irradiance, exposure duration, and wind speed. Finally experiments on the dominant intertidal alga *P. sanctae-crucis* were conducted in the laboratory and field to isolate which environmental factors affected performance.

Methods

Physiological response with tides

Site Description

Reef algae were sampled at two sites on the south shore of O‘ahu, the intertidal reefs of Diamond Head (21º17’45.18, 158º06’13.42) and Kalaeloa (21º15’19.89, 157º48’37.23) (Fig. 8.1). Both sites possess limestone solution benches/ platforms with similar diversity and abundance of reef algal species (see Chapter II). Diamond Head has an intertidal region which is ~15 m wide at mean low, low water and contains a nearshore depression that allows water to pool during low tides with little to no tidal fluctuation. The tidal reef at Kalaeloa is a ~30 m wide bench with an area midshore that is slightly elevated with respect to sea level.

Species Selection

Common red and brown algae in intertidal zones were selected for performance comparisons (Table 8.1). On both shores, *P. sanctae-crucis* (a tropical cosmopolitan species), forms monospecific stands in the high and mid intertidal zones and, although less abundant, extends lower into the subtidal habitat (see Chapter II; Abbott and Huisman 2004). *Sargassum aquifolium* and *Laurencia mcdermidiae,* two Hawaiian endemics, occur low on the shore in wave washed areas (Cox et al. *in prep*; Abbott 1999; Abbott and Huisman 2004; Huisman et al. 2007) but *S. aquifolium* is often absent at Kalaeloa (see Chapter II). The introduced red alga, *Acanthophora spicifera*, is common in the high intertidal zone at Kalaeloa and *Lobophora variegata,* a brown alga, commonly occurs in sunken crevices in the mid and low intertidal zones at Diamond Head (*personal observations*).

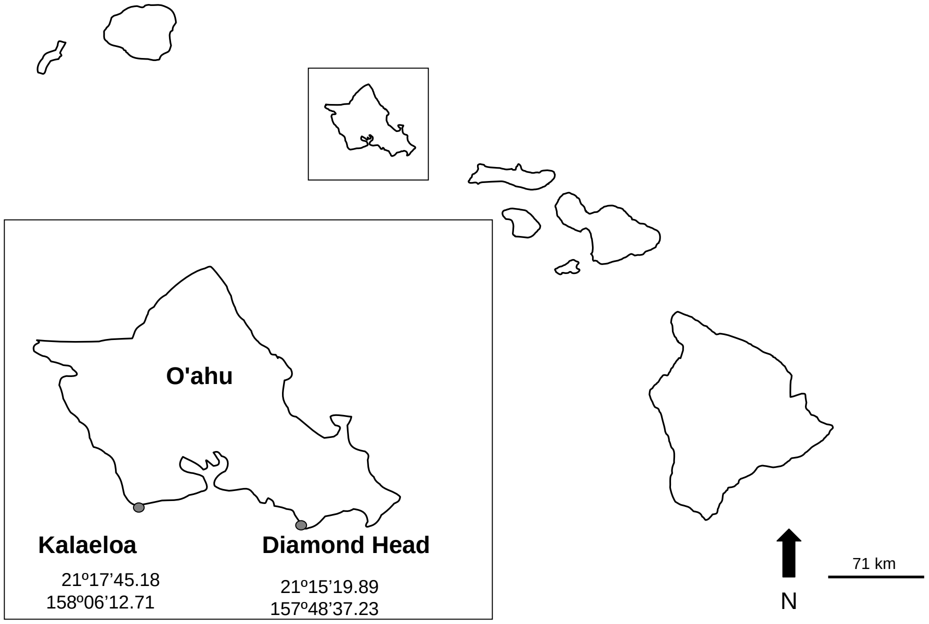

Fig. 8.1 Map of sites on the island of O‘ahu.

Table 8.1 Common red and brown reef algae selected for comparisons.

Species	Division/Class	Site Sampled	Position	Description
Padina sanctae- crucis	Phaeophyceae	Diamond Head & Kalaeloa	High, Mid, Low	Fan-shaped, calcified, 2 cell layers thick
Lobophora variegata	Phaeophyceae	Diamond Head	Crevices in Low	Fan-shaped attached to substrate, many cell layers thick
Sargassum aquifolium	Phaeophyceae	Diamond Head	Low	Complex with stem, leaf-like branches, wide leaves
Acanthophora spicifera	Rhodophyta	Barber's Point	High	Upright axes, cylindrical with spine-like branches
Laurencia mcdermidiae	Rhodophyta	Diamond Head & Kalaeloa	Low	Erect with terete axes, bright green, clumps

Survey Protocol

These reef algae were sampled for relative electron transport maximum ($r\mathrm{ETR}_{\mathrm{max}}$) near to *in situ* with the pre-programmed rapid light curve routine in the diving-Pulse Amplitude Modulated Fluorometer (Diving PAM; Walz Co) on May 4-11, 2008 between 0700-1530 hrs. Sampling at sites occurred during some of the lowest, low tides of the year for O‘ahu, Hawai‘i (www.tidesandcurrents.noaa.gov). An effort was made to sample reef algae continuously before, during, and after the peak low tide. When low tides occurred in early morning hours (on May 4 at 08:34 and May 5 at 09:07), the reef algae were sampled near to low tide and sampling continued periodically until the shore was fully submersed. Reef algae were sampled at each site for four days (Diamond Head = May 4, 7, 8, 11; Kalaeloa = May 5, 6, 9, 10) for a total of eight consecutive days of sampling (Table 8.2).

Upon arrival at each site, one to three individuals of *Padina sanctae-crucis* were collected in haphazard order at varying distances from the low tide line, and were subsequently labeled as occurring in high (near vegetation), mid, and low intertidal zones (near subtidal habitat). Samples were immediately placed into separate, water-filled containers, brought to shore and fluorescence measurements collected in a haphazard order. While individual measurements were made, the remaining samples were maintained in shaded areas. Then new, fresh samples were collected and the protocol repeated until the shore was submersed for at least one hour.

To compare photosynthetic responses among other macroalgal species, individuals *of S. aquifolium, L. mcdermidiae, A. spicifera*, and *L. variegata* (see Table 8.1) were collected and sampled along with *P. sanctae-crucis* at haphazard sampling intervals. During these samplings, one individual of *P. sanctae-crucis* and two to three individuals of additional species were collected from each zone where they occurred. $r\mathrm{ETR}_{\mathrm{max}}$ in each set of samples was measured with the diving-PAM within approximately 30 minutes from time of collection. A pilot experiment revealed that within this 30 minute interval, $r\mathrm{ETR}_{\mathrm{max}}$ for collected samples did not significantly change from initial value (*unpublished data*).

Table 8.2 Tidal height and time of day for MLLW that occurred for each day sampled shown here with the results of two-way ANOVAs. The results for within *P. sanctae-crucis* comparisons are on the left while results for the species comparisons are on the right. NS = not significant (α = 0.025).

Site	Tidal Height of MLLW (meters)	Time of MLLW	Time of Day	Position (H, M, L)	Time x Position	Species	Time of Day
Diamond Head							
Day 1, May 4, 2008	-0.15	08:34	p=0.01	NS	NS	NS	NS
Day 2, May 7, 2008	-0.12	10:24	p<0.001	p=0.003	NS	p<0.001	p<0.001
Day 3, May 8, 2008	-0.09	11:10	NS	p=0.01	p=0.002	p=0.002	NS
Day 4, May 11,2008	0.12	14:46	NS	NS	NS	p=0.02	P<0.001
Kalaeloa							
Day 1, May 5, 2008	-0.15	09:07	p=0.003	NS	NS	p=0.002	p=0.001
Day 2, May 6, 2008	-0.15	09:44	NS	NS	NS (p = 0.04)	p =0.001	NS
Day 3, May 9, 2008	-0.03	12:03	NS	NS	NS	p<0.001	p=0.003
Day 4, May 10, 2008	0.03	13:11	NS	NS	NS	P=0.005	NS

For each day of sampling, a two-way ANOVA was used investigate the effect of time of day and shore position (high, mid, low) on *P. sanctae-crucis* performance. An interaction of time and position was included in ANOVA models. Tukey's multiple comparison tests were used to determine any statistical groupings.

To investigate if species performed differently each day with height of the tide, a series of two-way ANOVAs were used. Prior to statistical testing, the mean $r\mathrm{ETR}_{\max}$ determined for high, mid, and, low *Padina* samples were averaged to produce one value of *Padina sanctae-crucis* performance per sampling interval. Thus at each sampling interval we could compare the average performance among sampled species. Tukey's multiple comparison tests were then used to determine statistical groupings. The statistical alpha was adjusted with a Bonferroni correction for multiple comparisons made with *Padina* performance ($\alpha = 0.025$).

Environmental variables

Environmental variables were measured during sampling periods at each site. One Hobo© Water Temp ProV2 temperature logger was placed in each intertidal zone (low, mid, and high) and temperatures were logged every 60 s. Irradiances were measured near the intertidal with a Li-Cor 4 pi sensor and a LI-1400 data logger, calibrated for air measurements. Measurements of wind speed were collected with a handheld wind gauge every hour. Temperature and irradiance were averaged by hour. Landscape photographs were taken every 15 minutes to estimate aerial exposure. Each intertidal area was considered exposed until every portion was submersed. Wave heights for the south shore of O‘ahu (www.ndbc.noaa.gov/Station 51204) were gathered from NOAA's National Buoy Data Center.

Investigations into tidal stress

Relative Water Content of Padina *with Tides*

To examine the effect of low tide on algal desiccation, the relative water content (RWC) of *P. sanctae-crucis* was measured on June 22-25, 2009 at 800, 1100, and 1400 hours at Diamond Head using the methods of Smith and Berry (1986). These times were before, during, or after exposure from low tides. The mean low, low water ranged from -

0.03 m to -0.15 m and occurred from 0841 to 1115 hour. For each sampling, six to seven individuals were collected from the high and low zone and the fresh weight obtained. Samples were placed overnight in seawater filled containers and allowed to rehydrate. The difference between fresh and rehydrated wet weight was standardized to dry weight and reported.

For each day a two-way ANOVA was used to determine if RWC of *P. sanctae-crucis* varied with sampling interval and position on shore. An interaction of sampling interval and shore location was included. Tukey's multiple comparison tests were used to determine similar statistical groupings.

Padina *performance in pools or on raised surfaces*

During a -0.12 m low tide that occurred on April 26, 2009 at 1002, the maximum rates of electron transport of *Padina* from two habitats was measured and compared. A 1 m^2 quadrat was haphazardly placed twelve times in the intertidal in such a manner that the quadrat covered a pool and a nearby raised, aerial exposed surface. In each quadrat, $r\mathrm{ETR}_{\mathrm{max}}$ from one submerged individual of *P. sanctae-crucis* in the pool was sampled and also from one exposed individual located on the nearby raised surface. The temperature of both habitats was also measured. Sampling occurred from the hours of 0940 to 1130.

The performance of *P. sanctae-crucis* in pools was compared to the performance of those nearby on a raised exposed surface with a two-tailed *t*-test. The temperature of pools and raised surfaces was compared with a two-tailed *t*-test.

Padina *response to simulated tidal exposure and tissue desiccation*

On June 11, 2009, an experiment was conducted to examine the response of *P. sanctae-crucis* to air exposure. Because desiccation could potentially be experienced differently for isolated versus tightly growing populations, 12 individuals and 12, 7 cm clumps of three or more individuals of *P. sanctae-crucis* were collected at 0830 hour. Clumps of *Padina* were connected via holdfasts and possessed the *Vaughniella*-stage growing loosely around the bases. Samples were divided into 6 control "individual" and "clumped" samples and 6 experimental "individual" and "clumped" samples, and placed into 24 separate shallow containers of water (5 cm depth) and covered with a shade cloth. Samples were exposed to natural outdoor temperatures and irradiances, which ranged from 26.7-29.0°C and reached 250 μmoles m^{-2} s^{-1} under the shade cloth, respectively.

Prior to exposure, initial $r\text{ETR}_{\text{max}}$ measurements were collected from six experimental and control samples. Experimental samples were then placed into an empty container and exposed to air for 40 min, about the length of time or shorter than would occur during a -0.1 m low tide. After 40 min experimental samples were placed back into the seawater filled containers for 10-min to gauge whether they could quickly recover from desiccation stress. Fluorescence measurements were collected from experimental and control samples at 10 and 40 min intervals of exposure and after 10 min recovery period.

The initial measurements and exposure to air were haphazardly staggered in time so that two clump and one single or two single and one clump experimental and control samples were conducted within the same 10 min period using two PAM fluorometers, until all 24 samples had been measured over ~3 hrs. Control and experimental samples were sampled simultaneously and fresh seawater was periodically added to submersed samples to maintain ample ambient levels of carbon and nutrients, and to maintain ambient temperatures.

A repeated measures two-way ANOVA (sampling interval nested within treatment) was used to determine if performance changed for experimental single or clumped samples of *P. sanctae-crucis* after 10 and 40 min durations of air exposure. We further evaluated if photosynthetic performance was able to recover to initial and control values upon submersion. A Holm-Sidak comparison was used to determine similar statistical groupings.

Padina *response with temperature and exposure*

The tissue temperature and $r\text{ETR}_{\text{max}}$ of *P. sanctae-crucis* were measured simultaneously at Diamond Head during aerial exposure that resulted from a -0.12 m magnitude low tide that occurred at 07:07 on June 20, 2009. Fluorescence measurements were collected with PAM as described above; tissue temperatures were determined using thermal imaging. A Handy Thermo TVS-200 EX IR camera (NEC Avio IR

Technologies, Tokyo, Japan) was used to capture digital images of the sampled thalli immediately prior to PAM measurements. This camera captures temperature changes at 1/60 s from a distance range of 30 cm to infinity and has an accuracy of ± 2.0 °C with precision better than 0.08°C. Infrared energy is converted to temperature by the following equation:

$$\text{Infrared energy} = \text{sigma} * e * T^4$$

where sigma is the Stefan-Boltzmann constant, e is emissivity, and T is temperature (°K). An emissivity of 1.00 was assumed because *P. sanctae-crucis* was wetted and seawater has an emissivity of 0.99. Thermal analyses of *P. sanctae-crucis* in images were conducted using ImageJ software (www.nih.gov) following the methods of Cox and Smith (*in review*).

A linear regression was used to investigate the relationship between temperature and performance for *P. sanctae-crucis*. For all statistical comparisons data were initially screened for parametric requirements and transformed if needed.

Rapid Light Response Curves (RLCs) to determine *r*ETR$_{max}$

The Rapid Light Response Curves (RLC) routine in the Diving PAM was used to determine several parameters of the photosynthetic response of intertidal reef algae (White and Critchley 1999; Ralph and Gademann 2005). For this study we chose to focus on the maximal rate of relative electron transport as this parameter indicates the maximum performance of an individual can be measured rapidly and is used as a gauge to indicate a change in performance.

Sample reef algae were placed one layer thick into the standard leaf clip held at a set distance from the end of the PAM fiber optic cable. Eight increasing irradiances were delivered to the reef alga over 90 s. Irradiances ranged between 0-1200 µmoles m^{-2} s^{-1}. With each increasing irradiance, a saturating pulse was applied to compare the minimum (F) and maximum quantum yield (F'_m). These values were then used to calculate the effective quantum yield (Φ_{PSII}) as the indication of the amount of energy used in the photochemistry of PSII (Genty et al. 1989).

$$\Phi_{PSII} = \Delta F / F'_m = (F'_m - F) / F'_m$$

From Φ_{PSII}, an approximation of the rate of electrons (*r*ETR) pumped through the photosynthetic chain (Beer et al. 2001) was calculated using the following equation:

$$r\text{ETR} = \Phi_{PSII} \times 0.5 \times \text{AF}$$

where the factor 0.5 accounts for the assumption that half the photon energy absorbed by photosynthetic pigments was diverted into photosystem II; AF is the fraction of incident light that is absorbed. Table 8.3 lists the values used for each alga.

Table 8.3 The absorption factors for sampled reef algae measured in summer months at Diamond Head as reported in Cox et al. *in prep*.

Species	**Absorption Factor**
A. spicifera	0.76
L. mcdermidiae	0.88
L. variegata	0.86
P. sanctae-crucis	0.75
S. aquifolium	0.88

The *r*ETR values were plotted against the measured irradiances to produce a RLC. The intensity of the eight irradiances was adjusted for each species to optimize the curves in such a way that the *r*ETR values plotted against irradiance ramped to an asymptote and then leveled off at higher irradiances. These curves were fitted with the exponential model which included the dynamic photo-inhibition parameter proposed by Platt et al. (1980) using the following Levenberg–Marquardt regression algorithm:

$$P = P_s\,[1-\exp(-\alpha E/P_s)]\,\exp(-\beta E/P_s)$$

Where P_s is a scaling parameter defined as the maximum potential *r*ETR; α is the photosynthetic efficiency measured by the initial slope of the RLC before the onset of saturation; E is the photon flux density; β is the negative slope of the RLC for high irradiances. In the absence of dynamic photo-inhibition (β=0), the function becomes a standard rectangular hyperbola, with an asymptotic maximum *r*ETR value (Harrison and

Platt 1986), and the equation loses exp(−βE/P_s). The parameter $r\mathrm{ETR}_{max}$, or the maximum rate of relative electron transport, was estimated using the following equation:

$$r\mathrm{ETR}_{max} = P_s\,[\alpha/(\alpha + \beta)]\,[\beta/(\alpha + \beta)]^{\beta/\alpha}$$

Results

Physiological response with tides

There was little evidence of exposure stress as measured by $r\mathrm{ETR}_{max}$ for *P. sanctae-crucis* (Fig. 8.2, Tables 8.2, 8.4). For both sites, individuals of *P. sanctae-crucis* exhibited similar to higher rates of $r\mathrm{ETR}_{max}$ during the potential exposure windows (when tidal heights were negative) as when samples were submersed (on positive tidal heights). The $r\mathrm{ETR}_{max}$ for *P. sanctae-crucis* at Diamond Head during the four days of measurement ranged from 9.2 - 47.3 μmoles e^- m^{-2} s^{-1} and the maximum rates measured at Kalaeloa ranged from 16.2- 59.3 μmoles e^- m^{-2} s^{-1}.

Possible signs of stress were observed after ~4 hrs of aerial exposure to intense mid-morning solar radiation and temperatures. At Diamond Head, on day 2, the electron transport rates maximum measured for the low samples of *Padina* at 1100 hr after experiencing many hours of exposure to mid-day irradiances, were significantly reduced from values that were collected at 0800, 0900, 1300, and 1400 hrs (Tables 8.2, 8.4). Similarly the mid-tidal samples of *P. sanctae-crucis* had reduced rates of $r\mathrm{ETR}_{max}$ on day 2 in the hour after the peak low at Kalaeloa when samples experienced ~5 hours of mid-morning aerial exposure.

Performance of individuals of *P. sanctae-crucis* was more variable among high, mid, and low intertidal zones during exposure. This variation in performance among intertidal zones was more pronounced on days with extended exposure duration that occurred later in the day (Tables 8.2, 8.4). For instance on day 2 of sampling at Diamond Head with a longer exposure duration occurring later in the day, high zone *P. sanctae-crucis* was significantly different from low zone samples. Then on day 3 at Diamond Head with a similar tide there was a significant interaction of time and position. Although not significant at an alpha of 0.025, performance varied the most among *P. sanctae-crucis* from different zones on day 2 with the lowest, low tide sampled for Kalaeloa. In

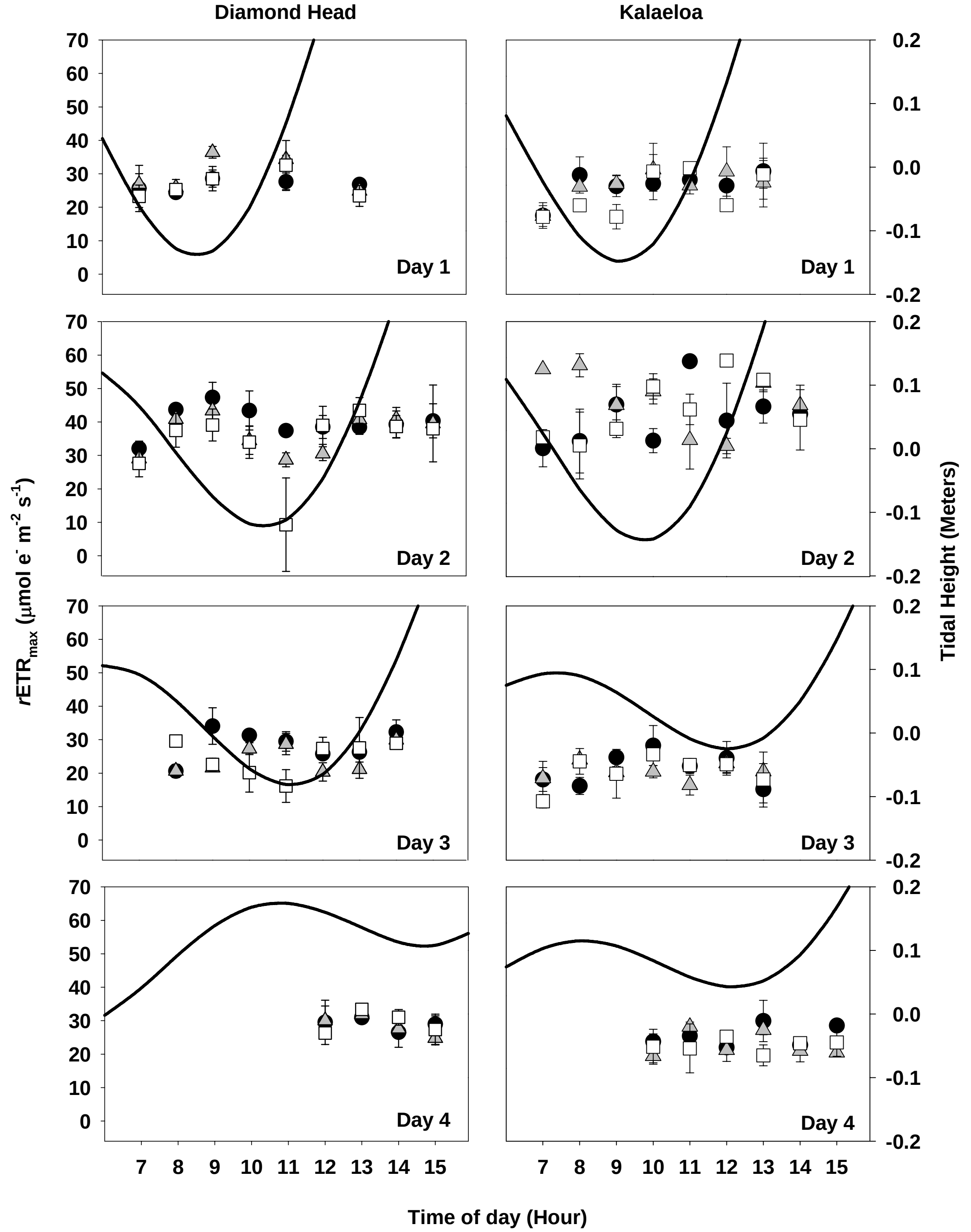

Fig. 8.2 Mean (SE) $r\mathrm{ETR}_{max}$ by hour for *P. sanctae-crucis* over four days (top to bottom). The results from two sites are shown, Diamond Head (left) and Kalaeloa (right), for individuals found in three zones (high = black circles, mid = gray upright triangle, low = white squares). The black line represents the tidal height at each hour.

Table 8.4 The results from Tukey's multiple comparisons test for significant factors at two sites Diamond Head (D) and Kalaeloa (K). Letter groups in columns represent statistically similar groups. The left portion of the table shows the similar groupings of *r*ETR$_{max}$ that occurred among hours sampled while the lower portion of the table shows the differences among *r*ETR$_{max}$ by zone (left) or by species (right).

Padina Position Comparison				**Species Comparisons**						
	D		**K**		**D**			**K**		
Time	**Day 2**	**Day 3**	**Day 1**	**Time**	**Day 2**	**Day 3**	**Day 4**	**Day 1**	**Day 2**	**Day 3**
07:00	AD		AB	07:00	A		AB	AB		AB
08:00	BD		ABC	08:00	A		AB	AB		AB
09:00	BCD		ABC	09:00	B		AB	AB		AB
10:00	ACD		BC	10:00	A		AB	AB		A
11:00	ABD		BC	11:00	B		AB	A		AB
12:00	ABCD		ABC	12:00	A		AB	AB		AB
13:00	BCD		ABC	13:00	A		AB	AB		AB
14:00	BCD			14:00	A		A	AB		
15:00				15:00	A		AB	AB		
Zone				**Species**						
High	A	A		P.s.	A	A	A	A	A	A
Mid	A	B		S.e.	A	A	A	-	-	-
Low	B	B		L.m.	A	A	A	B	B	B
				L.v.	B	B	-	-	-	-
				A.s.	-	-	-	B	B	B

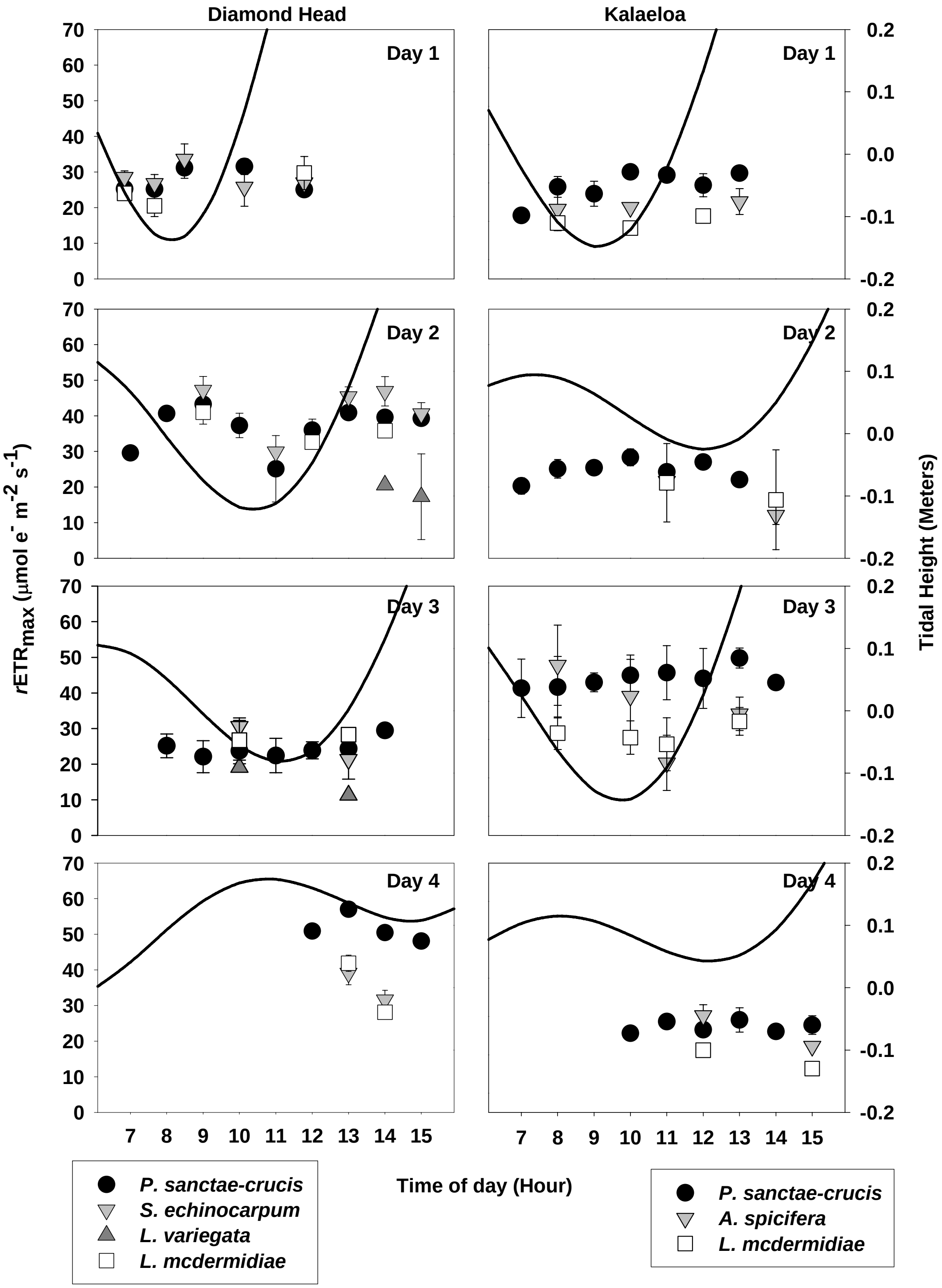

Fig. 8.3 Mean (SE) $r\mathrm{ETR}_{\mathrm{max}}$ by hour for common red and brown seaweeds (see legend) measured over 4 days (top to bottom). The results from two sites are shown, Diamond Head (left) and Kalaeloa (right). The black line represents the tidal height at each hour.

contrast, there was little variation in performance when samples were fully submersed or for days when the exposure window (negative tidal heights) occurred before 0900 hr.

The performance of other brown and red seaweeds varied by hour and with tidal height in a manner similar to *P. sanctae-crucis* (Fig. 8.2, Tables 8.2, 8.4). These species had similar to higher rates of $r\mathrm{ETR}_{\mathrm{max}}$ during exposure windows as when submersed. In addition, species differed in maximum rates and these differences varied among sites and by day. At Diamond Head, *Lobophora variegata* was sampled on days 2 and 3 and exhibited rates significantly lower than *P. sanctae-crucis*, *Sargassum aquifolium*, and *Laurencia mcdermidiae*. Although the $r\mathrm{ETR}_{\mathrm{max}}$ of *P. sanctae-crucis* and *L. mcdermidiae* did not significantly differ at Diamond Head, on days 1-3 at Kalaeloa, *P. sanctae-crucis* exhibited rates that were higher than those measured for *L. mcdermidiae*.

The environmental conditions during the sampling period were typical for tropical shores (Table 8.5). The mean irradiance (± SE) during the sampling period was 1565±96 μmoles m^{-2} s^{-1} at Diamond Head and 1408±175 μmoles m^{-2} s^{-1} at Kalaeloa and varied predictably across the course of the day (Fig. 8.4). Wind speeds were variable from average of 1.3 to 3.4 on the Beaufort Scale. Wave heights were similar among shores and between days with average values ranging from 0.7 to 0.9 m. The average temperature in the intertidal zone was 25.8±0.5°C at Diamond Head and 26.0±0.3°C at Kalaeloa. Even though the mean temperatures were similar among shores (Table 8.5), temperatures were variable among high, mid, and low zones. Habitat temperatures were also more variable among zones on days with longer aerial exposure and during the exposure windows (Fig. 8.5). Exposure to air differed across the zones of the shore (Table 8.5). At Diamond Head the low zone was often exposed more than other zones but, at Kalaeloa the mid zone was exposed more than the high and low zone. Despite the longer length of shore at Kalaeloa, the reef algae at Diamond Head were exposed longer at low tide.

Table 8.5 Environmental variables measured at two sites, Diamond Head and Kalaeloa, over 4 days with different mean low, low water (MLLW). The mean (SE) are reported when possible, and wind speed is reported in Beaufort Scale (BS).

Site	MLLW (m)	Time of MLLW	Irradiance (μmoles m^{-2} s^{-1})	Intertidal Temp (°C)	Wave Height (m)	Wind Speed (BS)	Exposure to Air (Maximum # of Hours)
Diamond Head							
May 4, 2008 Day 1	-0.15	08:34	1367.0±180.2	25.0±0.2	-	1.7±0.0	High=4, Mid=4, Low=5
May 7, 2008 Day 2	-0.12	10:24	1383.2±171.3	25.2±0.3	0.8	2.3±0.2	High=4, Mid=4, Low=6
May 8, 2008 Day 3	-0.09	11:10	1576.9± 54.3	24.7±0.4	0.7	3.4±0.3	High=4, Mid=4, Low=5
May 11, 2008 Day 4	0.12	14:46	1783.2±110.4	25.5±0.0	0.8	3.1±0.3	High=0, Mid=0, Low=0
Kalaeloa							
May 5, 2008 Day 1	-0.15	09:07	1020.5±110.8	25.9±0.8	0.9	1.6±0.8	High=0, Mid=4, Low=2
May 6, 2008 Day 2	-0.15	09:44	1314.9±121.2	25.8±0.5	0.9	1.3±0.5	High=3, Mid=5, Low=3
May 9, 2008 Day 3	-0.03	12:03	1628.2±121.5	26.9±1.1	0.9	2.9±0.3	High=0, Mid=1, Low=0
May 10, 2008 Day 4	0.03	13:11	1669.0± 83.3	25.4±0.2	0.8	2.4±0.2	High=0, Mid=0, Low=0

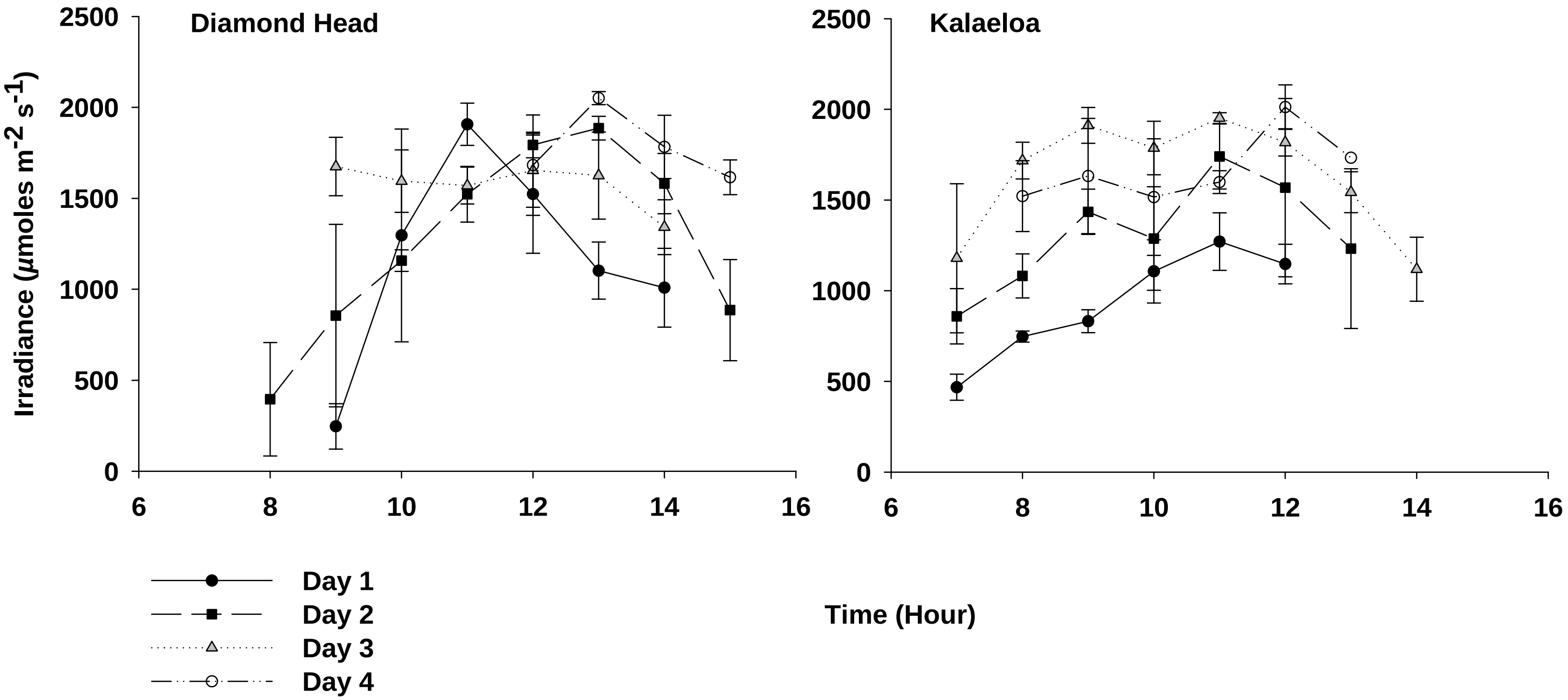

Fig. 8.4 Mean (SE) irradiances by hour over 4 days (see legend) and two sites, Diamond Head (left) and Kalaeloa (right).

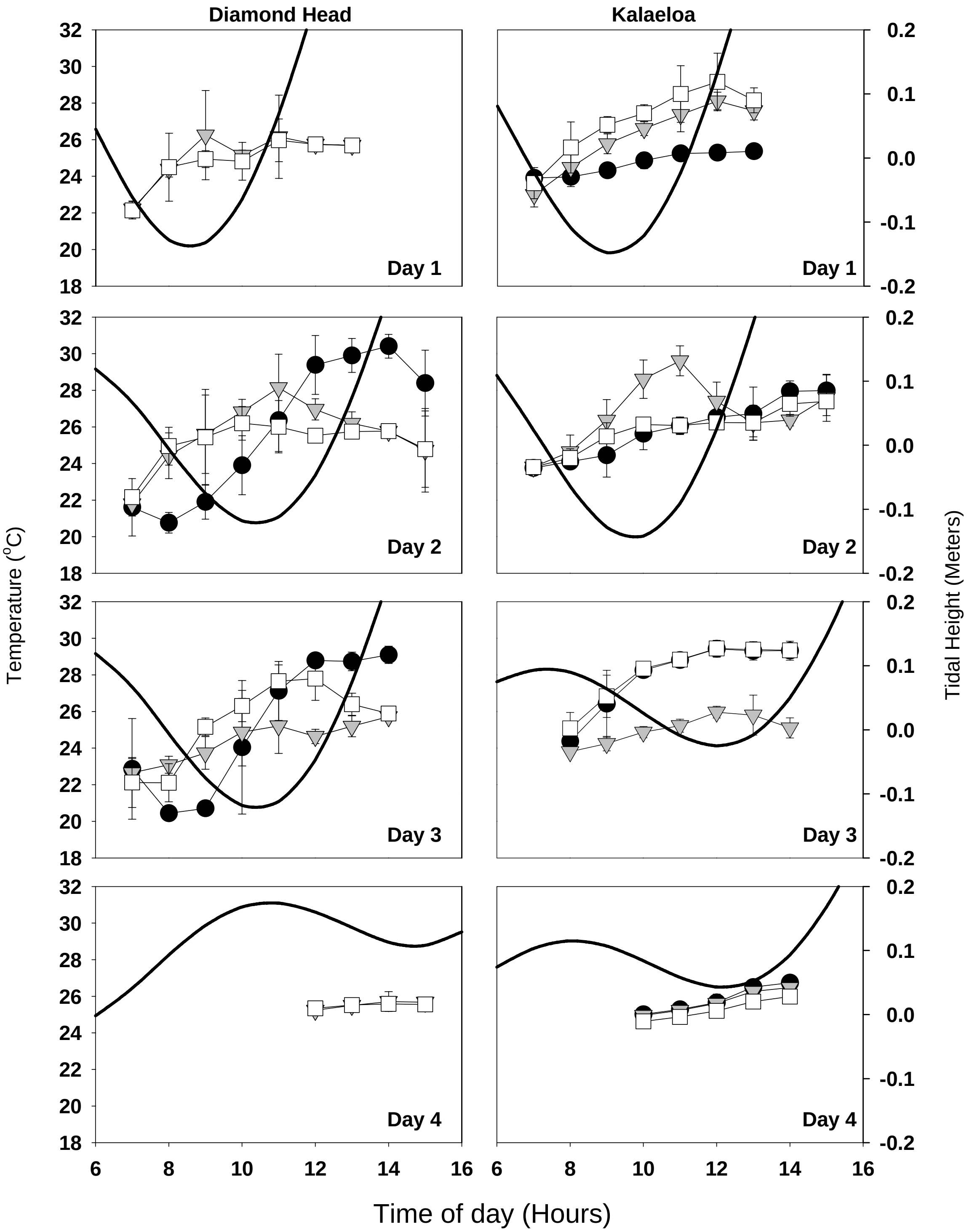

Fig. 8.5 Mean (SE) temperature (°C) by hour across the shore (high = black circles, mid = gray triangles, low = white squares) over four days (top to bottom). The results from two sites are shown, Diamond Head (left) and Kalaeloa (right). The black line represents the tidal height at each hour.

Investigations into tidal stress

Even though the magnitude of low tide varied by day, the water content of *P. sanctae-crucis* at Diamond Head changed little among the three sampling intervals with varying tidal heights (Fig. 8.6). The RWC of *P. sanctae-crucis* sampled repeatedly over a four day interval ranged from 65-120%. On the day with the lowest low tide (-0.15 m) *P. sanctae-crucis* sampled from the low zone where the slope is slightly higher had lost significantly more water during a negative tidal height than individuals sampled high on the shore. These low (or closer to the subtidal) individuals continued to have significantly lower water content even after the tide submersed the shore (two-way ANOVA, position, df =1, F = 15.9, $p < 0.001$). On days 2 and 3 with negative low tides occurring later in the day, the water content of *P. sanctae-crucis* tended to be lower during sampling intervals with negative tidal heights. However, on day 4 with little tidal difference water content was similar for both the high and low zones samples.

The $r\text{ETR}_{\text{max}}$ of *P. sanctae-crucis* varied among habitats with different aerial exposure and temperature (Fig. 8.7). *P. sanctae-crucis* submersed in a warmer habitat had significantly higher measurements of performance (*t*-test, df = 31.0, T-value = 2.09, $p = 0.45$). Tidepools were on average 2°C warmer than the exposed nearby surfaces and tidepool dwelling individuals of *Padina* had maximum rates that were on average 8 μmoles e^{-} m^{-2} s^{-1} higher than those dwelling on raised surfaces. This difference in temperature between pools and surfaces was significantly different (*t*-test, df =31.0, T-value = 3.2, $p < 0.001$).

The $r\text{ETR}_{\text{max}}$ of *P. sanctae-crucis* was impacted by substantial exposure to air and this effect was different for isolated versus clumped individuals (Fig. 8.8) (RM-ANOVA: type, df =3, F = 8.13, $p<0.001$; sampling interval, df = 3, F = 6.8, $p<0.001$). Rates were significantly reduced after at least 10 mins of aerial exposure for isolated individuals and tightly growing clumps of individuals. However, clumped individuals were able to recover maximum rates similar to the measurements at initial and 10 min intervals. Control samples did not significantly vary in maximum rates over the duration of the experiment and the variation in clump treatments did not differ from the variation observed within clump controls.

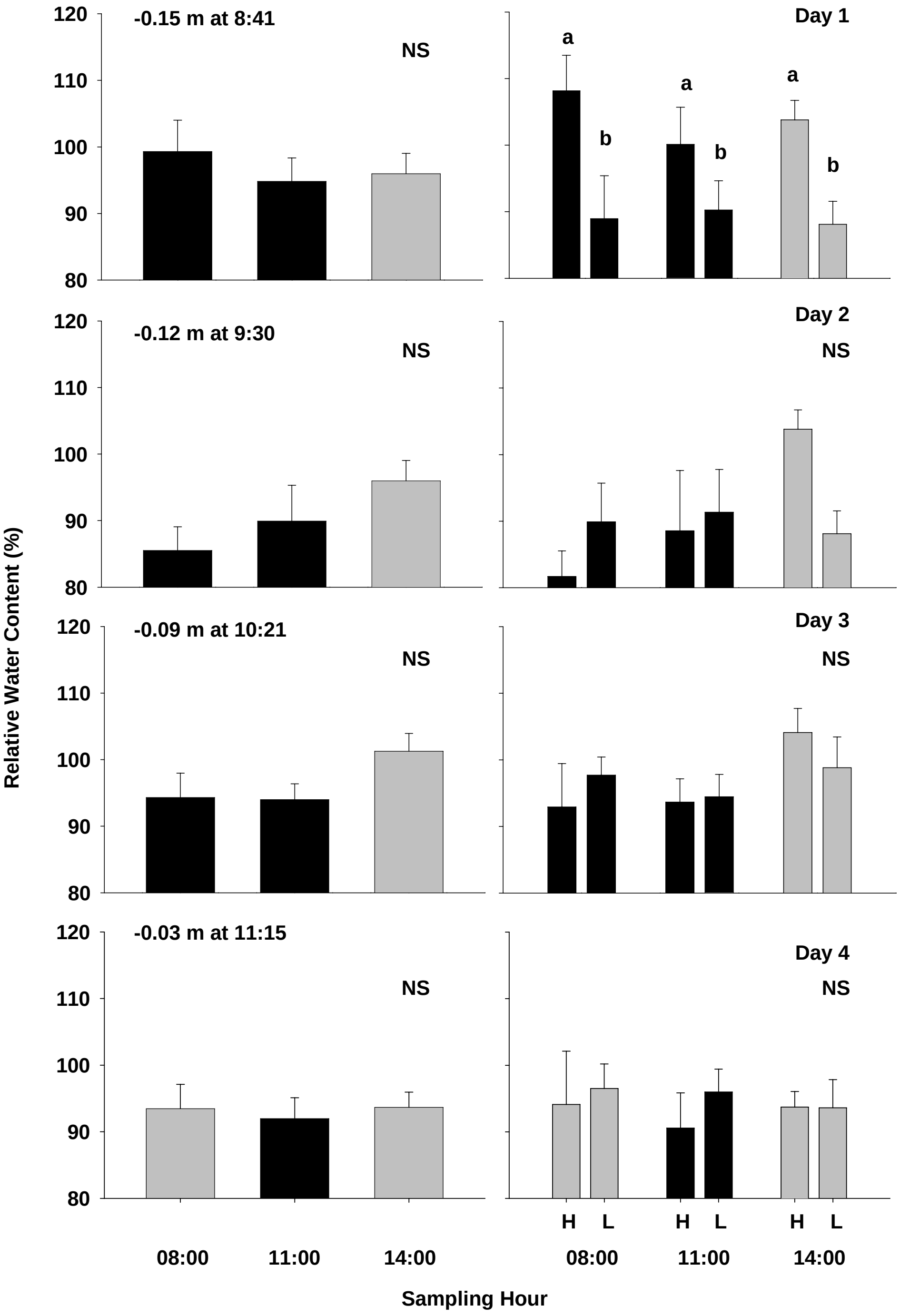

Fig. 8.6 Relative water content of *P. sanctae –crucis* reported as the mean (SE) of high and low samples (right) and the overall mean (SE) (left) at different sampling intervals on 4 days with different tidal heights. Black bars represent samplings that occurred on negative tidal heights. Letters represent significantly similar groups and NS = no statistical differences.

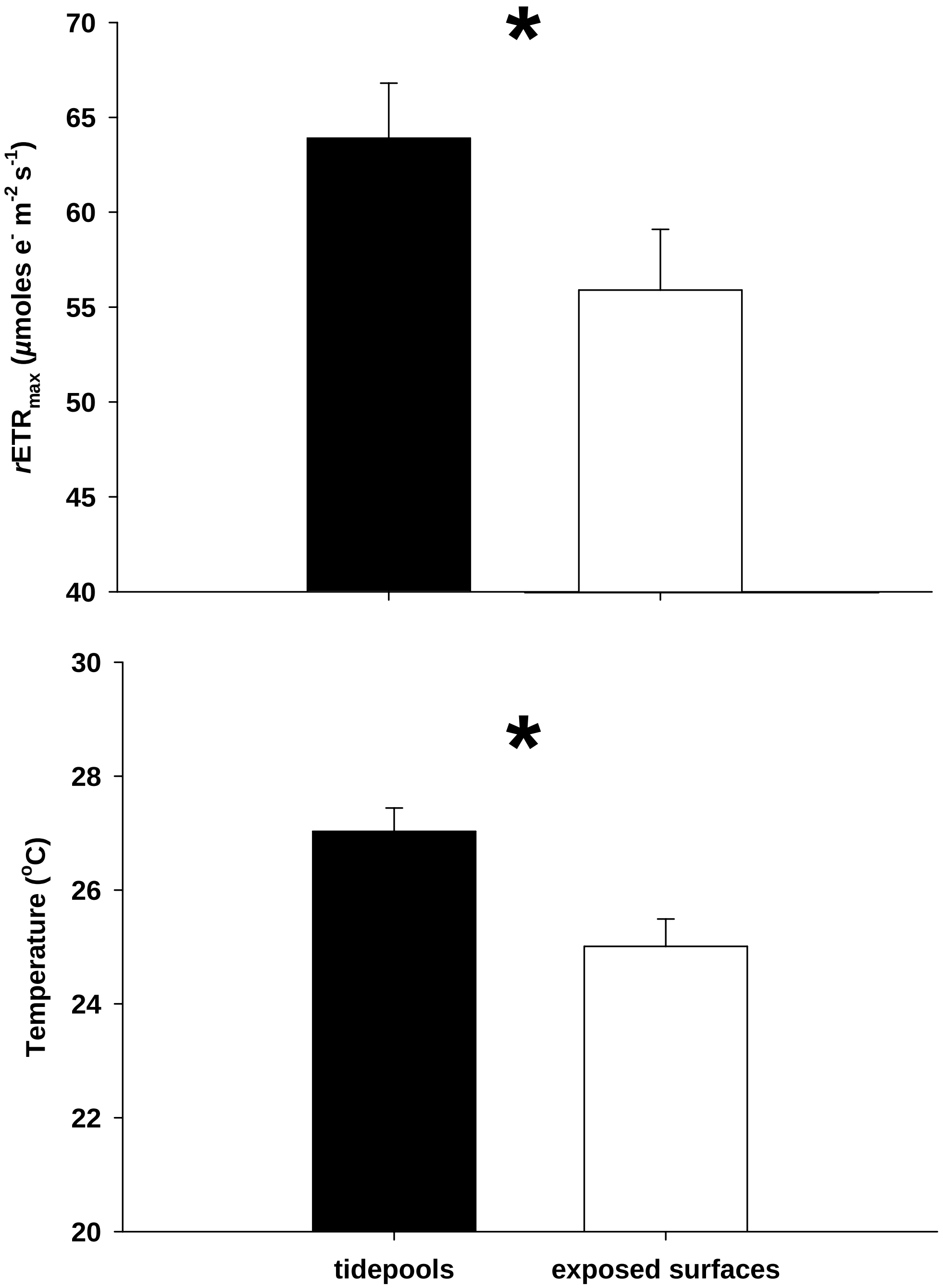

Fig. 8.7 Mean (SE) rETR$_{max}$ of *P. sanctae-crucis* (top) and mean (SE) temperature (bottom) measured from two habitats, tidepools and raised exposed surfaces at Diamond Head. * represent significant differences ($\alpha = 0.05$) as determined from a two-tailed *t*-test.

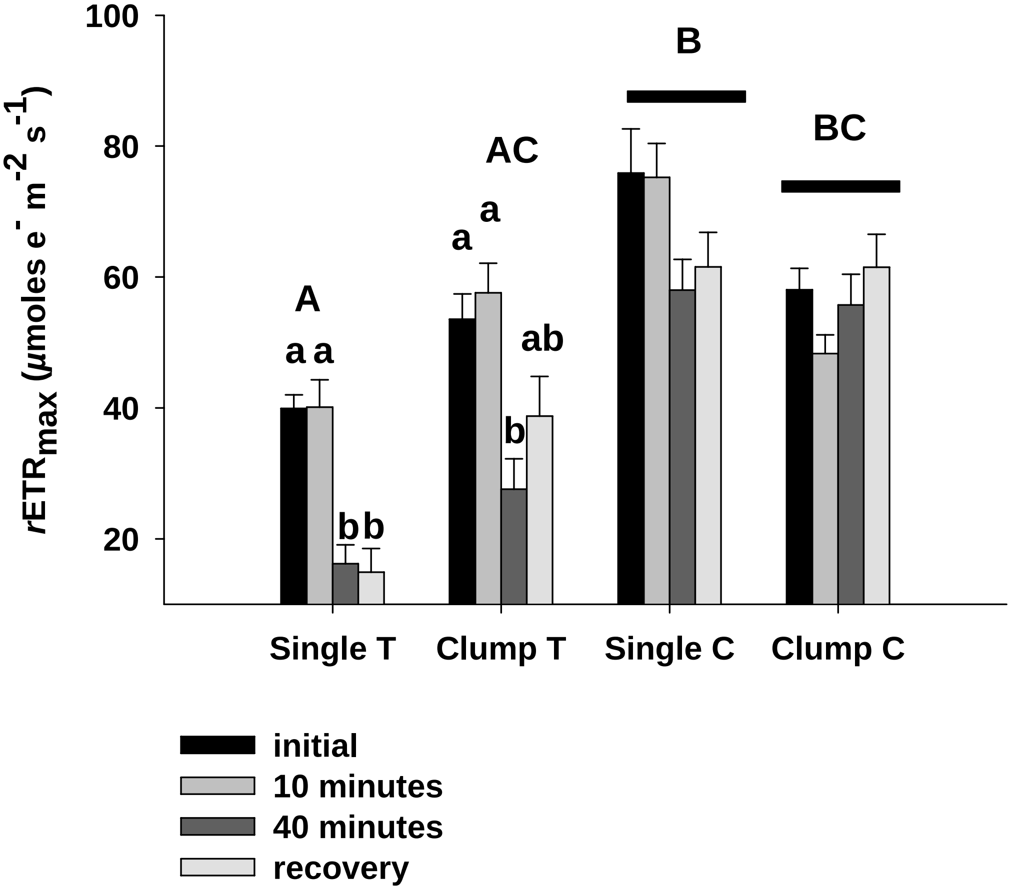

Fig. 8.8 Results of desiccation experiment for single and clumped samples of *P. sanctae-crucis*. Mean (SE) of rETR$_{max}$ measured at initial, 10 minutes, 40 minutes, and recovery intervals are shown (see key). Control samples (C) remained submersed in seawater while treatment samples (T) were exposed to air and then submersed for 10 minutes to recover. Capital letters and lower case letters above bars represent significantly similar groupings as determined by Holm-Sidak comparison when repeated-measures two-way ANOVA found significances, solid bars represent groups that did not differ.

There was a significant relationship between *r*ETR$_{max}$ of *P. sanctae-crucis* and temperature (Linear Regression, p = 0.03). Temperature could explain 29.8% of the variation in *r*ETR$_{max}$ for exposed *P. sanctae-crucis* samples (Fig. 8.9).

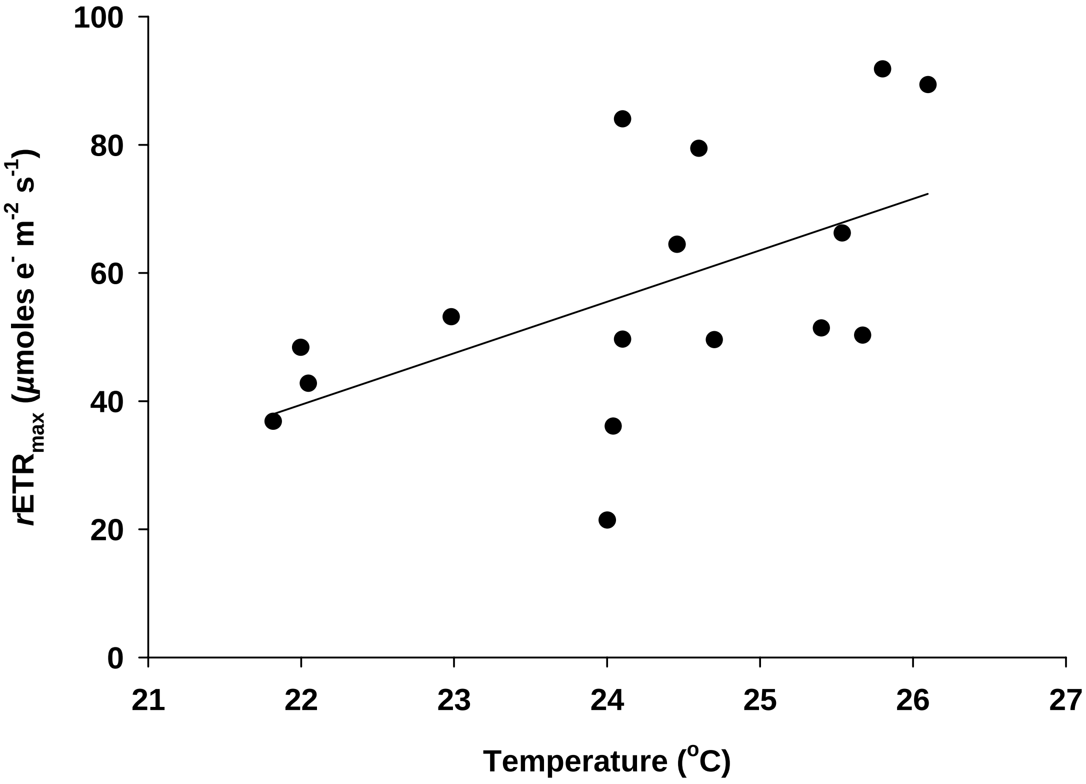

Fig. 8.9 Relationship between temperature (°C) and *r*ETR$_{max}$ (μmoles e^{-} m^{-2} s^{-1}).

Discussion

This study provides a working model of performance for Hawaiian intertidal algae that differs from temperate systems. As opposed to cold water macroalgae, common tropical intertidal species were often able to maintain or increase rates of electron transport during negative tidal heights, and daily changes in performance were observed for *P. sanctae-crucis* across a remarkably small spatial scale (a few meters). Lab and field investigations suggest temperature can explain observed patterns in performance. Furthermore, loosely woven monospecific stands of intertidal algae may reduce desiccation stress during moderate tides. Finally, species which are distributed in different areas of the shore have different rates of maximum electron transport; it is unclear if tidal ebb/rise drives these species differences in responses.

Photosynthetic performance of reef algae on Hawaiian shores does not support the traditional temperate paradigms of stress in intertidal systems. These paradigms include: (1) macroalgae experience stress and lower photosynthesis during low tides (Davison and Pearson 1996), and (2) that stress varies predictably across a tidal gradient from high to low elevation (Doty 1946; Connell 1961; Paine 1974). Even though seaweeds were exposed to air from anywhere from 0-6 hours during daylight hours, their performance was maintained or increased when compared to rates measured in submersed individuals. This finding is remarkable given that a field examination of one of the hardiest intertidal genera, *Fucus*, from 14 shores in Washington had net photosynthesis 2 orders of magnitude lower when exposed to air (Williams and Dethier 2005). This difference could be explained by exposure duration. *Fucus* typically experiences much longer tidal exposures than intertidal seaweeds in Hawai‘i where microtides limit exposure duration. Secondly, Hawai‘i has relatively little tidal elevation, although rates of electron transport for *Padina* were found to vary over just a few meters; the optimal position varied from day to day with tidal phasing and diurnal solar insulation. Thus rates of electron transport were not predictable or consistent. Recent studies have cast similar doubts on the validity of the widely held paradigm that a tidal elevation gradient of stress is directly correlated to photosynthetic performance (Wright et al. 2004; Williams and Dethier 2005; Dethier and Williams 2009).

It is plausible that warming influences from high tropical irradiance during tidal exposures could benefit photosynthetic performance. For producers, photosynthesis increases with temperature until an optimum is reached (Davison 1991). The tropical algal species examined during this study can tolerate short-term exposure to temperatures up to 35°C (a temperature well above any recorded during this study) before net photosynthesis and $r\mathrm{ETR}_{\mathrm{max}}$ are reduced (see Chapter VI). Irradiance and water depth/exposure can act synergistically and affect the temperature of an alga (Bell 1995; Hunt and Denny 2008), thus we argue that temperature appears to be the ultimate driver of performance for these common species during microtidal fluctuations. Furthermore, field measurements of performance regressed with temperature and individuals occurring in warmer tidepools exhibited higher performance. Coarse measures of temperature (one logger in each zone) did not mimic performance but, temperatures are known to vary over fine spatial scales on shores (see Chapter V, Helmuth and Hoffmann 2001) under canopies of algae (Molina-Montenegro et al. 2005), or even from holdfast to canopy within a species (Beach and Smith 1997). There was a similar pattern of high temperatures on days with increased $r\mathrm{ETR}_{\mathrm{max}}$ and both measurements were more variable among zones during the exposure windows.

Padina sanctae-crucis in Hawaiʻi did not show signs of desiccation stress or even substantial water loss during emersion. The RWC of *P. sanctae-crucis* measured over three different tides at three different sampling intervals showed little variation. In comparison, species in the green algal genus *Ulva* are known to dry 3x faster at noon than at other sampling intervals (Beer and Eshel 1983). RWCs in algae studied here were fairly high (65-120%, mean values range from 81-99%) in comparison to the Hawaiian red alga *Ahnfeltiopsis concinna* isolated on basaltic shores (canopy: mean 66%, lowest 18%, understory: mean 76%, lowest 36% (Beach and Smith 1997)), *Porphyra* which desiccates to 15% RWC in 3 hours (Smith and Berry 1986), or *Fucus* which was measured to have a water content from 40-100% (Williams and Dethier 2005). In an outdoor experiment (*this study*), 40 mins of aerial exposure severely reduced $r\mathrm{ETR}_{\mathrm{max}}$ while 10 minute durations did not alter performance. Thus it is apparent that *P. sanctae-crucis in situ* did not commonly experience desiccation stress, despite repeated exposures. An exception may have occurred on day 2 at Diamond Head when rates were severely reduced at the 1100 hr sampling. Individuals of *Padina* occurring in the low intertidal zone had experienced up to 6 hrs of exposure at high irradiances. The RWC on a day with a similar tide also showed significant reduction in water content in individuals from low intertidal areas as opposed to high intertidal areas of the shore (low area is slightly raised while the high area is inverted).

Even though tidal fluctuations are small in Hawai‘i, we hypothesize that reef algae in the nearshore experience tidal exposure long enough (0-6 hrs) to cause desiccation (40 mins) but other environmental factors prevent water loss. Exposure durations during a week with spring tides ranged from 0 to 50% of daylight hours. However, year round intertidal reef algae remain submersed or have limited exposure to air in daylight. Spring tides occur in daylight almost exclusively in Jan-July but, the tides with maximum fluctuations occur in earlier hours when irradiances and temperatures are lower. For instance, on day 1 at Kalaeloa and Diamond Head with the most negative MLLW sampled, the $r\mathrm{ETR}_{\mathrm{max}}$ varied little and the peak low tide occurred before or near to 0900 hr. Then on day 2 irradiances at MLLW were higher and $r\mathrm{ETR}_{\mathrm{max}}$ values were more variable. In addition, aggregations of seaweeds are known to reduce stress (Hay 1981; Bell 1992; Bertness et al. 1999; Molina-Montenegro et al. 2005) and we clearly show that *P. sanctae-crucis* growing in clumps had a different response to aerial exposure than isolated individuals. Aggregations of loosely woven *P. sanctae-crucis* blades with the *Vaughniella* stage at their bases may allow this species to retain water and thus avoid desiccation under tropical irradiances. The calcium carbonate impregnating the blade surface of *P. sanctae-crucis* has also been hypothesized to reflect excessive irradiances (Beach et al. 2006) and lower thermal heating (Cox and Smith *in review*) which could limit water loss.

Although wave height was consistent during this study, water movement is likely to alter aerial exposure. Waves can reduce temperature by several degrees over ~ 1 min in Hawai‘i (see Chapter V) and are known to alter predator-prey interactions (Bird 2006). Therefore any model used to predict stress must include this physical factor.

PAM measurements offer a third independent method to gauge photosynthesis by assessing the rates of electron transport. It is possible that an intertidal species that remains hydrated *in situ* could absorb carbon into a wetted cell wall, if not fix carbon via photosynthesis in air. Select temperate intertidal species increased photosynthesis 6x in air when compared to submersed individuals when measured via gas exchange in a laboratory; this may be a case where carbon is absorbed into the cell wall as there was no isotopic labeling to confirm fixation (Johnson et al. 1974). Additionally, rapid air-water CO_2 diffusion, as compared to slow diffusion in seawater, is hypothesized to result in an immediate increase of performance in air (Silva et al. 2005). However, such a rapid air-water CO_2 diffusion hypothesis would not explain why tidepool dwelling individuals of *Padina* exhibited higher rates of $r\mathrm{ETR}_{max}$ than exposed individuals. The increased temperatures during low tides better explain observations. Respiration rates could also be increasing under tidal exposure (Matta and Chapman 1991; Beach and Smith 1997), yet for tested species $r\mathrm{ETR}_{max}$ is known to be directly related to net photosynthesis (see Chapter VI). Thus we do not expect respiration to significantly alter our findings.

Species differences in performance were observed at both sites. Individuals of *Padina sanctae-crucis* have a similar morphology and are evolutionarily related to *Lobophora variegata*, yet rates of electron transport varied significantly. Similar differences were recorded by Beach et al. (2006) in the laboratory for tropical Hawaiian representatives of the brown algal order Dictyotales. Differences varied among shores, *P. sanctae-crucis* exhibited $r\mathrm{ETR}_{max}$ similar to *L. mcdermidiae* at Diamond Head, but these two species exhibited different rates from each other for 3 out 4 days at Kalaeloa. Differences did not appear to be tidally driven yet distributional patterns varied across the narrow intertidal shore. Ability to cope with the high irradiances that are well above saturation could possibly account for these differences (Beach et al. 2006). For instance, the calcium carbonate on the surface of *Padina* is known to reflect light (Beach et al. 2006) while *Lobophora* is more obviously pigmented and preliminarily appears to be more shade-adjusted. In addition, all three species occur in different microhabitats where irradiance and temperature may vary (see Chapter II).

Conclusions

Our investigation of photosynthetic performance of marine algae in tropical intertidal ecosystems provides exciting results- intertidal reef algae can perform as well or better when emersed despite experiencing high tropical temperatures and irradiances.

The combination of algal aggregations, waves, and small tidal fluctuations during early morning hours virtually eliminates desiccation stress for *P. sanctae-crucis*, although *Ahnfeltiopsis concinna* occurring high on basaltic shores may experience comparatively more water loss. Lastly, temperatures in tropical environments are an important physiological driver effecting electron transport rates, which will have implications for predicting the impact of global climate change on coastal species.

References

Abbott IA (1999) Marine red algae of the Hawaiian Islands. Bishop Museum Press, Honolulu

Abbott IA, Huisman JM (2004) Marine green and brown algae of the Hawaiian Islands. Bishop Museum Press, Honolulu

Beach KS, Borgeas HB, Smith CM (2006) Ecophysiological implications of the measurement of transmittance and reflectance of tropical seaweeds. Phycologia 45: 450-457

Beach KS, Smith CM (1996a) Ecophysiology of tropical Rhodophytes. I. Microscale acclimation in pigmentation. Journal of Phycology 32: 701-710

Beach KS, Smith CM (1996b) Ecophysiology of tropical Rhodophytes. II. Microscale acclimation in photosynthesis. Journal of Phycology 32: 710-718

Beach KS, Smith CM (1997) Ecophysiology of a tropical Rhodophyte III. Recovery from emersion stresses in *Ahnfeltiopsis concinna* (J. Ag.) Silva et DeCew. Journal of Experimental Marine Biology and Ecology 221: 151-167

Beer S, Bjork M, Gademann R, Ralph PJ (2001) Measurements of photosynthesis in seagrasses. In: Short FT, Coles R (eds) Global Seagrass Research Methods. Elsevier Publishers, The Netherlands, pp 183-198

Beer S, Eshel A (1983) Photosynthesis of *Ulva* sp. I. Effects of desiccation when exposed to air. Marine Biology Ecology 70: 91-97

Bell EC (1992) Consequences of morphological variation in an intertidal macroalga: physical constraints on growth and survival of *Mastocarpus papillatus* Kutzing., Standford

Bell EC (1995) Environmental and morphological influences on thallus temperature and desiccation of the intertidal alga *Mastocarpus papillatus* Kutzing. Journal of Experimental Marine Biology and Ecology 191: 29-55

Bertness MD, Leonard GH, Levine JM, Schmidt PR, Ingraham AO (1999) Testing the relative contribution of positive and negative interactions in rocky intertidal communities. Ecology 80: 2711-2726

Bird CE (2006) Aspects of community ecology on wave-exposed rocky Hawaiian coasts. PhD. Botany, Honolulu

Chapman ARO (1995) Functional ecology of fucoid seaweeds: twenty-three years of progress. Phycologia 32: 197-211

Connell JH (1961) The influence of intra-specific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. Ecology 42: 710-723

Davison IR (1991) Environmental effects on algal photosynthesis: temperature. Journal of Phycology 27: 2-8

Davison IR, Johnson LE, Brawley SH (1993) Sublethal stress in the intertidal zone: tidal emersion inhibits photosynthesis and retards development in embryos of the brown alga *Pelvetia fastigiata*. Oecologia 96: 483-492

Davison IR, Pearson GA (1996) Stress tolerance in intertidal seaweeds. Journal of Phycology 32: 197-211

Dethier MN, Williams SL (2009) Seasonal stresses shift optimal intertidal algal habitats. Marine Biology 156: 555-567

Dethier MN, Williams SL, Freeman A (2005) Seaweeds under stress: manipulated stress and herbivory affect critical life-history functions. Ecological Monographs 75: 403-418

Doty MS (1946) Critical tide factors that are correlated with the vertical distribution of marine seaweeds and other organisms along the Pacific Coast. Ecology 27: 315-328

Dring MJ, Brown FA (1982) Photosynthesis of intertidal brown seaweeds during and after periods of emersion: a renewed search for physiological causes of zonation. Marine Ecology Progress Series 8: 301-308

Dudgeon SR, Kubler JE, Vadas RL, Davison IR (1995) Physiological responses to environmental variation in intertidal red seaweeds: does thallus morphology matter? Marine Ecology Progress Series 117: 193-206

Foster MS (1982) Factors controlling the intertidal zonation of *Iridaea flaccida* (Rhodophyta). Journal of Phycology 18: 285-294

Ganzon-Fortes ET (1997) Influence of tidal location on morphology, photosynthesis and pigments of the agarophyte, *Gelidiella acerosa*, from Northern Philippines. Journal of Applied Phycology 9: 525-532

Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochemistry Biophysics Acta 990: 97-92

Gosline WA (1965) Vertical zonation of inshore fishes in the upper water layers of the Hawaiian Islands. Ecology 46: 823-831

Harrison WG, Platt T (1986) Photosynthesis-irradiance relationships in polar and temperate phytoplankton populations. Polar Biology 5: 153-164

Hay ME (1981) The functional morphology of turf-forming seaweeds: persistence in stressful marine habitats. Ecology 62: 739-750

Helmuth BS, Hoffmann GE (2001) Microhabitats, thermal heterogeneity, and patterns of physiological stress in the rocky intertidal zone. Biological Bulletin 210: 374-384

Huisman JM, Abbott IA, Smith CM (2007) Hawaiian Reef Plants. University of Hawaii Seagrant College Program

Hunt LJH, Denny MW (2008) Desiccation protection and disruption: a trade-off for an intertidal marine alga. Journal of Phycology 44: 1164-1170

Johnson WS, Gigon A, Gulmon SL, Mooney HA (1974) Comparative photosynthetic capacities of intertidal seaweeds under exposed and submerged conditions. Ecology 55: 450-453

Kay AE (1979) Hawaiian marine shells, reef and shore fauna of Hawaii. Bishop Museum Press, Honolulu

Matta JL, Chapman DJ (1991) Photosynthetic responses and daily carbon balance of Colpomenia peregrina: seasonal variations and differences between intertidal and subtidal populations. Marine Biology 108: 303-313

Matta JL, Chapman DJ (1995) Effects of light, temperature, and desiccation on the net emersed productivity of the intertidal macroalga *Colpomenia peregrina* Sauv. (Hamel). Journal of Experimental Marine Biology and Ecology 189: 13-27

Molina-Montenegro MA, Munoz AA, Badano EI, Morales BV, Fuentes KM, Cavieres LA (2005) Positive associations between macroalgal species in a rocky intertidal zone and their effects on the physiological performance of *Ulva lactuca*. Marine Ecology Progress Series 292: 173-180

Oates BR, Murray SN (1983) Photosynthesis, dark respiration and desiccation resistance of the intertidal seaweeds *Hesperophycus harveyanus* and *Pelvetia fastigiata F. gracilis*. Journal of Phycology 19: 371-380

Paine RT (1974) Intertidal community structure: Experimental studies on the relationship between a dominant competitor and its principal predator. Oecologia 15: 93-120

Platt T, Gallegos CL, Harrison WG (1980) Photo-inhibition of photosynthesis in natural assemblage of marine phytoplankton. Journal of Marine Research 38: 687-701

Ralph PJ, Gademann R (2005) Rapid light curves: a powerful tool to assess photosynthetic activity. Aquatic Botany 82: 222-237

Schaeffelke B, Deane D (2005) Desiccation tolerance of the introduced marine green alga *Codium fragile* spp. *tomentosoides*-clues for likely transport vectors. Biological Invasions 7: 557-565

Schonbeck M, Norton TA (1978) Factors controlling the upper limits of fucoid seaweeds on the shore. Journal of Experimental Biology and Ecology 31: 303-313

Silva J, Santos R, Calleja ML, Duarte CM (2005) Submerged versus air-exposed intertidal macrophyte productivity: from physiological to community-level assessments. Journal of Experimental Marine Biology and Ecology 317: 87-95

Smith CM, Berry JA (1986) Recovery of photosynthesis after exposure of intertidal seaweeds to osmotic and temperature stresses: comparative studies of species with differing distributional limits. Oecologia 70: 6-12

Tait LW, Schiel DR (2010) Primary productivity of intertidal macroalgal assemblages: comparison of laboratory and *in situ* photorespirometry. Marine Ecology Progress Series 416: 115-125

White AJ, Critchley C (1999) Rapid light curves: a new fluorescence method to assess the state of the photosynthetic apparatus. Photosynthesis Research 59: 63-72

Williams SL, Dethier MN (2005) High and dry: variation in net photosynthesis of the intertidal seaweeds *Fucus gardneri*. Ecology 86: 2373-2379

Wright JT, Williams SL, Dethier MN (2004) No zone is always greener: variation in the performance of *Fucus gardneri* embryos, juveniles and adults across tidal zone and season. Marine Biology 145: 1061-1073

Chapter IX. Synthesis & Significance

Despite the tropical temperatures and intense solar radiation Hawaii's micro-intertidal zones are lush with algae, dense with invertebrates and tidepool fishes that appear to be well suited for tropical life.

Synthesis of findings

Hawaii's rocky intertidal zones are dominated by a few reef algae and molluscs that occur in discrete zones across the shore. Molluscs tend to occur closer to the high tide line while reef algae tend to be closer to the subtidal habitats.
Common taxa include the macroalgae *Padina*, *Sargassum*, *Laurencia*, turf, and introduced species *Acanthophora spicifera* and the molluscs *Siphonaria normalis*, *Nerita picea*, and species of littorines. The introduced macroalgae *Acanthophora spicifera* is a member of most intertidal communities.

The tidepool fish community in Hawai‘i is fairly diverse with tropical members that are vertically structured across the shore. 343 fishes were recorded from 40 tidepools, and 19 species from 10 families were identified. Tidepool fish diversity (H': O‘ahu=2.4; Sites Average=0.0–0.9) was typical for tropical islands, with members from Gobiidae (5 species), Blenniidae (4 species), Pomacentridae (3 species), Acanthuridae (2 species) and Kuhliidae (2 species) among the most common. Endemism (32%) was higher than other well studied assemblages yet similar to Hawaiian reef fishes (25%). Tidepool fish assemblage abundance varied among shores with basalt or limestone substrate, among sites, and vertically among high, mid, and low pools. In general, blenniids occurred at higher proportions on limestone shores and gobiids were more common on basalt shores. High pools were characterized by an abundance of a small sized (29.0 mm median standard length) blenniid *Istiblennius zebra*, while the blenniid *Entomacrodus marmoratus* and wrasses *Thalassoma* spp. were more common in low pools.

Intertidal communities in the tropics can vary over meters (across the shore) and kilometers (among shores) yet these differences do not correlate with island nor shore direction. Instead, intertidal benches or sites with bedrock substrate have more similar benthic intertidal communities while shores with known elevated nutrients and freshwater input were structured differently that most. The differences among communities are surprising given similar biogeographic province and that organisms had to travel vast distances to colonize islands. Perhaps local factors such as temperature, sand scour, currents, anthropogenic influence, or predation account for these differences.

The on-shore pattern in nitrogen contents and δ^{15}N values for two species of reef algae at ‘Ewa Beach, Hawai‘i suggests that Hawaii’s algal communities are at least partially supported by land-based nutrients. These values of nitrogen incorporation and assimilation were associated with geographic location. For much of the ‘Ewa Beach area these marine plants had elevated nitrogen contents and δ^{15}N values that indicate active nitrogen cycling. Furthermore, the nitrogen contents and δ^{15}N values sampled from algae in front of drains did not differ from those removed from storm-drains. Thus, more investigations are needed to clarify whether anthropogenic nutrients support this marine community.

Temperature at one tropical shore varied unexpectedly and rapidly over fine-scales with the tides and waves. Habitats ranged from 18.1 to 38.3°C and surfaces of organisms that ranged from 21.1 to 33.2°C. Hot and cold spots on the shore, due to angle of the shore and differing heat capacities, resulted in a mosaic of thermal habitats. Organisms were either 1) associated with cooler habitats 2) tolerated hotter surroundings or 3) had characteristics allowing them to remain cooler than their surroundings. Calcification was indicated as a morphological feature that could allow organisms to reflect solar radiation and remain cooler than their surroundings. Also evaporation may allow species to remain cooler than the air while wetted.

Physiologically reef algae differ in their ability to tolerate tropical temperatures and intense solar radiation but generally can maintain photosynthesis and recover from remarkably high temperature doses (between 35 and 40°C). The alien invasive, *Acanthophora spicifera*, was sensitive to heat (rates reduced within species) but was able to maintain rates that were competitive with other thermally robust species. These findings suggest that reef algae, particularly invasive algae with tropical origins may be more tolerant to high temperatures than thermally sensitive corals.

Common reef algae are adjusted physiologically to high irradiances and evidence suggests that they have the ability to rapidly adjust to supraoptimal light fluctuations that can occur among days. The light harvesting parameters of sixteen reef algae from three divisions and five functional forms and varying in internal anatomy displayed characteristics typical of sun tolerant producers, having high maximum rates of electron transport (mean range, 12.9 -112.2 μmoles e^{-} m^{-2} s^{-1}) and saturation irradiances (mean range 131.8 - 659.7 μmoles m^{-2} s^{-1}). Light harvesting was dynamic in shallow water community as species differed in parameters and these differences varied between days. Filamentous, thin forms and the iridescent brown alga, *Stypopodium hawaiiensis,* tend to have lower absorption than other parenchymatous species. This finding may reflect a different tactic to cope with high irradiances. Perhaps, thin species can transmit more light while thicker parenchymatous species will absorb more light and rely on photoprotective pigments or a xanthophyll cycle to deal with excessive light conditions.

Evidence from this research suggests that aggregations of algae in monospecific stands combined with the timing and minimal tidal difference in Hawaii limit water loss and facilitate optimal temperatures for photosynthetic performances. Algae in the intertidal habitats were exposed to aerial conditions for up to 6 hours or 50% of daylight, but often maintained or increased electron transport maximums during aerial exposure. During the course of these studies, reef algae only exhibited photosynthetic stress *in situ* after exposure to elevated mid-morning irradiances and air temperatures for an extended duration. These results are surprising and in sharp contrast to temperate latitudes where exposure to air is lengthy and it is assumed algae will be stressed during emersion.

Results suggest that calcium carbonate impregnated in the surfaces of some algal thalli may be a mechanism to tolerate tropical life. The calcified species *Padina sanctae-crucis* and *Liagora* sp. were significantly cooler than their surroundings. Furthermore, during low tides *Padina sanctae-crucis* lost relatively little water content. The reflective properties of calcium may limit excessive heating which could prevent water loss.

Advancement of methods

Although not an overall aim, this dissertation advances the use of technologies and methodologies to assess a community, physiological stress, and the environment. I will briefly discuss these advancements below:

With the growing popularity of community involvement in scientific endeavors, this study provides the framework for how to incorporate and ensure the accuracy of citizen scientist generated data. Student assistance allowed us to increase our sampling in a limited amount of time; a necessity when sampling is limited to the exposure duration of low tides. Student assistance also allowed us to survey multiple sites on multiple islands. Further other efforts revealed that students gain knowledge about ecology and conservation by participating. Comparisons of benthic intertidal community data generated by researchers and secondary students as citizen scientists revealed that students collect accurate community level data that is within the range of researcher biases, however, students were likely to misidentify cryptic species or those occurring in low abundances.

Chapter V details the use of thermal imaging to document temperature in intertidal habitats. Temperature loggers provide similar results as thermography but cannot match the pixel strength of the camera to explore fine-scale variation. Therefore, our results support the utility of high resolution IR thermography in exploring thermal ecology and establishing baseline conditions in a changing and heterogeneous environment.

By using fluorescence technology this dissertation documents its usefulness and limitations when measuring photosynthesis in reef algae. Because the results in Chapter VI reveal that oxygen and fluorescence measurements are related for many common reef algae, this portable and rapid technology may be used *in situ* to measure productivity. In Chapter VII, the pulse amplitude modulated flourometer (PAM) is used to measure the light harvesting parameters of reef algae *in situ*- revealing the dynamic ability of reef algae to respond to light conditions. Thus, provides a cautionary message for interpretation of photosynthetic measurements taken out of context or after an extended period in the laboratory. Furthermore, a comparison of reef algal absorption values used when analyzing PAM results revealed a range of absorption more similar to those determined with *in vivo* absorbance than the commonly used standard 84%. Therefore, it is necessary to use the proper absorption to eliminate mis-interpretation of PAM determined eco-physiological findings.

Lastly, Chapter VI outlines a temperature sensitivity assay which can be used quickly to screen for candidate invasive species, and provides a framework for identifying physiologically competitive species in warm water environments.

Significance

This dissertation provides significant advances in understanding community ecology in tropical intertidal zones and adds to our broader understanding of eco-physiology of algae. Results provide a baseline upon which to measure future human impacts to these coastal communities and have important implications for predicting the impacts of ocean warming. Finally, this dissertation contributes fundamental new insight for tropical tidal zones and reefs and advances the use of citizen scientists and technologies to assess a community's physiological stress, new tools that will likely emerge as critical to the study of our changing ocean and its biota.