POTENTIAL INFLUENCES OF SUBMARINE GROUNDWATER DISCHARGE, NUTRIENTS, AND HERBIVORY ON HAWAIIAN REEF ALGAE

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ABSTRACT

Phase shifts have occurred on tropical reefs throughout the world. In Hawaii, phase shifts have been attributed to eutrophication, herbivore reduction, and alien algae. The first study from this dissertation examines the environmental relationships of Submarine Groundwater Discharge (SGD), a likely source of anthropogenic nutrients to Hawaii's reefs. The second study analyzes the relative and simultaneous effects of nutrient enrichment and reduced herbivory on algal succession in contrast to control conditions. The third study assesses the potential of indigenous herbivorous fish to control alien algae.

The fine scale salinity profiles in the first study established differences between the average salinity values of three sample events, and correlations between SGD and low tide and, SGD and depth. Water quality samples displayed correlations with SGD and concentrations of numerous chemical constituents. Nitrate from water quality samples at Waiopae were up to 3.8 times greater than samples from upland wells suggesting that anthropogenic sources of nitrate are intruding the aquifer. This study exemplifies the dynamics of SGD and nutrient intrusion.

Results from the second study in Kealakekua Bay show that the effects of nutrient enrichment were minor, herbivore reduction were considerable, and nutrient enrichment combined with herbivore reduction were major in governing algal biomass and cover. Coralline crustose algae dominated surfaces under control and nutrient enrichment. In herbivore reduction treatments turf algae dominated. Nutrient enrichment had a greater effect at one site, and herbivore reduction had a greater effect at another site, demonstrating that a single solution may not be equally effective for all Hawaiian reefs.

Feeding assays from the third study documented that two herbivorous fish species were potential biological control agents for managing alien algae. These fish can achieve different objectives in an effort to manage alien algae. *Acanthurus triostegus* is suited to control attached populations, *Kyphosus vaigiensis* is crucial in reducing dispersal by fragments.

Results from this dissertation show that nutrient concentrations enter the near shore environment quite rapidly on Hawaii. With sustainable land use practices and increasing herbivore pressure by enhancing select herbivore species, we can improve the resilience of Hawaiian reefs and minimize the possibility of phase shifts.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER 1. GENERAL INTRODUCTION

This dissertation attempts to better understand the drivers to algal phase shifts in Hawaii. A phase shift occurs when an ecological community changes from one stable state to another, as observed by changes in the species that comprise the dominant cover (Scheffer *et al.* 2001), and are known from reefs throughout the tropics (Done 1992; Hughes 1994; McManus and Polsenberg 2004; Rogers and Miller 2006; Hughes *et al*. 2007; Bruno *et al*. 2009; Norstrom *et al*. 2009). Hawaiian reefs of Kaneohe Bay, Waikiki, and West Maui are also shifting from reef builders such as corals and coralline crustose algae (CCA) to turf and macro-algae (Banner 1974, Smith *et al*. 1981; Smith *et al.* 2004a; Smith *et al*. 2005; Dailer *et al*. 2010). There is concern among the scientific and natural resource management community that other Hawaiian reefs could undergo such adverse transformations. These shifts from reef builders to turf and macro-algae have been largely attributed to eutrophication, a reduction in herbivore pressure, and alien algae (Banner 1974; Smith *et al*. 1981; Smith *et al.* 2004; Smith *et al*. 2005; Conklin 2007; Dailer *et al*. 2010). These three factors will be examined by studies within this dissertation.

Although tropical reefs have developed and persist in coastal areas with naturally turbid water and substantial nutrient concentrations and fluxes, an unnatural increase in nutrients from anthropogenic sources can and will eventually degrade tropical reefs (McCook 1999).

One of the earliest studies assessing the effects of increased nutrient concentrations on tropical reefs occurred in Kaneohe Bay, Hawaii. Release of domestic sewage into shallow waters of Kaneohe Bay elevated nutrients and led to overgrowth of corals by *Dictyosphaeria cavernosa* (Banner 1974). On the reefs of Molokai significant positive correlations exists between anthropogenic ammonium concentrations and biomass production of *Gracilaria parvispora* (Glenn *et al.* 1999). At Puako, a reef on the west coast of Hawaii Island, experimentally increased nutrient concentrations and restricted herbivory result in elevated algal biomass (Smith *et al.* 2001) and alter benthic succession (Smith *et al.* 2010) on experimental settlement tiles. On the tropical reefs of Guam growth of *Padina tenuis*, and *Dictyota bartayresiana* increases with nitrate and iron additions to seawater, while growth of *Lyngbya majuscula* increases with phosphate additions (Kuffner and Valerie 2001).

In Moreton Bay, Australia, *L. majuscula* productivity increases with soil extract additions, which were high in phosphorus, iron, and organic carbon. Populations of *L. majuscula* from the Great Barrier Reef have similar responses with comparable nutrient additions. These results suggest that Australia's *L. majuscula* blooms are a result of enrichments delivered through natural and anthropogenic mechanisms (Albert *et al.* 2005).

On a reef at the Florida Keys enrichments of nitrogen and phosphorus both, individually, increase the growth rate of *Gracilaria tikvahiae* during winter, but during the summer, only phosphorus increases this alga's growth (Lapointe 1987). Results from Florida Bay's reefs show that the effects of phosphorous on growth are greater compared to nitrogen in field experiments with *Laurencia poitei, Gracilaria tikvahiae, Sargassum pteropleuron*, and *Sargassum polyceratium*. These results suggest that phosphorous, not nitrogen limits algal productivity in the Florida Bay area (Lapointe 1989); in other words, nitrogen levels were already elevated. Variable results such as those mentioned above encourage further studies that analyze the relationship between algae and nutrients.

At Carrie Bow Cay, Belize *Dictyota divaricata* and *Acanthophora spicifera* from a barrier reef site significantly increases their productivity with additions of inorganic nitrogen and phosphorus enrichments, but these enrichments have no effect on the same species of algae collected from mangrove sites (Lapointe *et al*. 1987). At West Maui, Hawaii, the photosynthetic activity of *Cladophora sericea* increases with nutrient enrichment at deeper sites where background nutrient levels are relatively low, but nutrient enrichment has no effect at shallower sites where background nutrient levels are elevated (Smith *et al*. 2005). Similar results exist with *Halimeda tuna* at Key Largo, Florida (Smith *et al*. 2004b). These results suggest that an experimental increase in nutrient concentrations will have different effects on algae from different nutrient regimes, based on the recent history of the habitat and algal species studied.

Not surprisingly, a number of laboratory experiments also display the positive relationship between nutrients and algal growth. In the laboratory, exposure to six different nitrogen and phosphorus concentrations increases growth of *Enteromorpha intestinalis* proportionally to enrichment across the lower four of the six treatments. However, nutrient tissue concentrations decreases at the four lower nutrient concentrations due to dilution by growth, but increases in the highest concentrations partially due to a lack in growth, but storage by the alga. These results suggest that this bloom-forming alga is adapted to pulses of nutrients. When nutrient pulses are intense, *E. intestinalis* can delay growth in favor of saving energy to maximize nutrient uptake and storage (Fong *et al.* 2004). Another laboratory experiment shows that nitrogen alone can enhance growth of *E. intestinalis*. Results suggest that *E. intestinalis* abundance in the field is due to nitrogen availability, indicating that blooms will likely continue to proliferate in Southern California estuaries unless nutrient loading is reduced (Kamer and Fong 2001).

In laboratory experiments growth of *Gracilaria foliifera* and *Neoagardhiella bailiyi* correlates positively with nutrients. However, ammonium enrichments produce higher growth rates than nitrate. As expected, the lowest growth rate occurs in the chambers receiving un-enriched control seawater (DeBoer *et al*. 1978).

Another laboratory experiment shows *Ulva lactuca* increases growth rate and photosynthetic efficiency with an increase of nitrogen in a high light environment, but the same is not true in a low light environment (Lapointe and Tenore 1981).

Results from an Australian laboratory that used six algal species from the Great Barrier Reef for nutrient enrichment assays shows that *Sargassum baccularia* has a 50% higher net photosynthetic rate and 40% higher tissue nutrient concentrations after enrichment. The net photosynthetic rates of *Chnoospora implexa*, *Hydroclathrus clathratus*, and *Padina tenuis* increase by 30 to 50% and tissue nutrient levels increase by 10 to 20% after a single nutrient pulse. *Chlorodesmis fastigiata* and *Turbinaria ornata*, has no photosynthetic responds to nutrient additions, but accumulates 15 to 20% more tissue nutrients than untreated specimens. In this study, nutrients increase growth in four of six algal species and increases tissue nutrient in all species (Schaffelke 1999). In sum, all of these experiments demonstrate that algal responses to nutrient enrichment differ among species, based on habitat and recent nutrient history.

At the community level, increases in nutrient concentration can alter the composition of benthic primary producers, although it is important to note that biological interactions are usually not considered in these studies. A nutrient concentration gradient created by an urban sewage outfall off the coast of Spain shows *Ulva* to be dominant at sites close to the nutrient source, *Corallina* dominates communities at an intermediate distance, and *Cystoseira* dominates communities farthest away from the nutrient source (Arevalo *et al.* 2007). Biological surveys along a nutrient concentration gradient at the Great Barrier Reef, show macro-algae of the divisions Rhodophyta and Chlorophyta increases with increasing nutrients, but Phaeophyta populations remain constant (Fabricius 2005).

The inter-tidal algal community of Marseille, France was documented from 1972-1974, pre sewer outfall and from 1995-1996, post sewer outfall. Since the sewer outfall has been installed, there has been an increase in algal species and ephemeral turfs are taking the place of *Corallina elongata* (Soltan *et al*. 2001).

A study from Belize uses both inorganic fertilizer and organic fertilizer enrichment methods to analyze the relationship between nutrients and algae. In general, all forms of nutrient enrichment may potentially increase algal biomass. However, different enrichment methods can result in different algal communities. *Enteromorpha prolifera* and *Digenia simplex* dominates the inorganic fertilizer treatment and combined treatments of inorganic fertilizer and organic fertilizer. The organic fertilizer treatment has greater cover of *P. sanctae* and *D. cervicornis* than the control. While, brown frondose algae *Lobophora variegata* dominates the control, but *Padina sanctae* and *Dictyota cervicornis* were also present (McClanahan *et al*. 2005).

To investigate the seasonality of algal assemblages associated with nutrient rich upwelling on a Colombian reef, percent cover of algal species and functional groups were monitored over one year (1994-1995). When upwelling is not occurring, benthic communities have more turf algae and lower macro-algal cover. In contrast, during periods of upwelling macro-algae dominates benthic communities. Cover of *Dictyota* spp. declines from 34% during the upwelling to 5% in the non-upwelling rainy seasons. This study suggests that naturally occurring nutrient enrichment events can cause algal populations to shift from turf to macro-algae (Diaz-Pulido and Garzon-Ferreira 2002).

Nutrients can also promote shifts in sea-grass meadows. On a Florida Key reef flat, there are four naturally occurring nutrient regimes. Sea-grasses at the two sites with the highest nutrient concentrations have low shoot densities; low shoot production rates, low biomass values, and low production rates, but high levels of attached epiphytes, and mat forming macro-algae. Sea-grasses at the site with the lowest nutrient values has the highest shoot densities, highest biomass values, highest production rates, and typically the lowest or second lowest epiphyte levels of all nutrient regimes. This study suggests that nutrient enrichment from land-based activities increases biomass of attached epiphytes and macro-algae, which attenuate light, reduce dissolved oxygen, and leads to a shift from sea-grass to macro-algae (Lapointe *et al.* 1994).

To better understand the role of nutrients and interactions of herbivory on tropical reefs; the third chapter analyzes the effects of nutrients on algal succession under ambient and reduced herbivory, at two sites, one where the take or marine organisms is restricted and another where the take of marine organisms is allowed.

Submarine groundwater discharge (SGD) occurs when nutrient laden freshwater seep from land into the costal oceans (Moore 1999). SGD can occur via diffuse seepage or point source springs (Cable *et al.* 1996). Freshwater contributed as SGD represents 0.01 to 10% of all freshwater from land entering the ocean (Church 1996). SGD poses a potentially rich source of nutrients to tropical reefs (Johannes 1980; Oberdorfer *et al.* 1990; Corbett *et al.*1999; Glenn *et al.* 1999; Umezawa *et al.* 2002; Garrison *et al*. 2003; Talbot *et al.* 2003; Hwang *et al*. 2005b; Johnson *et al*. 2008; Parson *et al.* 2008).

Spatially SGD can decreases with distance from shore, for continental regions. This was evident at Buzzards Bay, Massachusetts (Valiela *et al.* 1990); Chesapeake Bay (Staver and Brinsfield 1996; Robinson *et al.* 1998); St. George Sound, Florida (Cable *et al.* 1997); as well as Kahana Bay, Hawaii (Garrison *et al.* 2003). SGD has also been shown to negatively correlate with depth, for example on the west coast of Barbados (Lewis 1987). The negative relation of SGD to distance from shore and depth in estuarine settings may exist because rates of SGD can be greatest just inland of the freshwater saltwater interface, as was the case at Yatsushiro Sea, Japan (Taniguchi *et al.* 2006). The pattern of salinity negatively correlating with depth (Lewis 1987) opposes open ocean salinity profiles from tropical marine regions where evaporation persist, resulting in greater salinity values at the surface or a negative relationship between salinity and depth, at least for the top layer of the halocline (Pinet 2003). These differences can have profound effects on physical, chemical, and biological processes.

Often depth profiles and distance transects that detect salinity and other constituents of water are used to demonstrate the spatial dynamics of SGD. The resolution of depth profiles and transects that run perpendicular to shore are dependent on the distance between sampling points. Studies from Hawaii at Honokohau (Johnson *et al.* 2008), the north shore of Kauai (Knee *et al.* 2008), Honokohau and Kealakekua (Parsons *et al.* 2008) applied distances between profile points in the range of ~0.17 – 0.25 m, 0.1 – 0.9 m, and 0.5 – 6 m respectively. Transect points from these studies ranged between ~5 – 200 m, 3 – 22 m, and ~50 m respectively, while surface samples were in depth of 0 – 0.5 m. On the leeward shores of Molokai, Maui, and Hawaii transect points ranged between ~13 – 25 m and surface samples were 0-0.5 m deep (Street *et al.* 2008). The level of resolution should be set by the research question, acknowledging tradeoff between fine scale studies and large areas to be examined.

Ocean dynamics such as pressure driven by tides have been shown to be another influence on SGD. Studies from the near shore environment of the Florida Keys have negatively correlated tide height with SGD (Lapointe *et al.* 1990). At Buzzards Bay, Massachusetts, groundwater flow was not constant but greatest during the ebb and low tide (Valiela *et al.* 1990). At Nauset Marsh, Massachusetts SGD occurred exclusively during low tide (Portnoy *et al*. 1998). Instantaneous SGD rates were inversely correlated to tidal elevation at Chesapeake Bay, Maryland (Robinson 1998) and Ria Formosa, Portugal (Rocha *et al.* 2009). A study from the Waquoit Bay, Cape Cod, Massachusetts estuary demonstrated that SGD rates were inversely related to tidal heights in the near shore zone, but patterns were not as evident in the middle and far from shore zone (Michael *et al.* 2003). Another study from Cape Cod demonstrated that tidal fluctuations affected the magnitude, location, timing, and salinity of SGD. The greatest rate of SGD occurred during the early to mid ebbing tide (Urish and McKenna 2004). At Suruga Bay, Japan the semidiurnal changes of SGD was due to tidal effects along with other factors

(Taniguchi *et al.* 2005). Salinity was significantly lower at low tide than at high tide on a Beach at Honokohau (Street *et al.* 2008). Generally, SGD seems greatest during ebb and low tides, although few studies from oceanic islands have examined this relationship thoroughly.

A theoretical model has demonstrated that other dynamic ocean processes such as waves can have an effect on SGD (Li *et al.* 1999). This model was supported in the field at the West Atlantic (Riedl *et al.* 1972) and Cockburn Sound, Australia (Smith *et al.* 2009) where pumping from waves influenced the rate of SGD.

Temperature is another physical feature that has been correlated with and used to detect SGD. In temperate regions, SGD is often warmer than surrounding seawater, as was the case at Plymouth Sound, United Kingdom (Roxburgh 1985); Cape Henlopen, Delaware Bay (Miller and Ullman 2004); Aberdeen Proving Ground, Maryland (Banks *et al.* 1996); and Nauset Marsh, Massachusetts (Portnoy *et al.* 1998). In the tropics, SGD is normally cooler than the surrounding seawater. On the island of Hawaii, aerial thermal infrared imagery has been used to detect SGD a number of times. In 1964, researchers used thermal images to identify more than 25 major points of SGD. Often these points were cooler than seawater, but a few were warmer, especially those adjacent to the northeast and southwest rift zones of Kilauea (Fischer *et al.* 1964). At Kaloko fishpond, Kona on Hawaii Island patterns of SGD was more apparent in aerial thermal infrared images than in ground truth data, because of a thin cool surface layer formed by SGD (Duarte *et al.* 2006). Low altitude thermal infrared imagery has identified the input locations and fine scale mixing structure of cool SGD at more than 30 points along the coast of Kona, Hawaii Island (Johnson *et al.* 2008). On the island of Oahu at Kahana

Bay, temperature profiles revealed a three layered salinity structure (Garrison *et al.* 2003). Although temperature can be a practical measure of SGD, preliminary field data must be collected to establish a relation between SGD and temperature.

The lack of salinity and conductivity are the most common chemical relations used to detect and define SGD that is fresh in origin. At the tropical and sub-tropical Atlantic sites of Jamaica, Southeast Florida (Lapointe 1997), and Barbados (Lewis 1987) salinity has been shown to negatively correlate with SGD. In Nauset Marsh, Massachusetts, salinity surveys were used to identify high-velocity costal seeps (Portnoy *et al.* 1998). In Kahana Bay, Hawaii, conductivity profiles revealed a three-layer water structure (Garrison *et al.* 2003). Salinity signals have also been used to detect SGD at Honokohau and Kealakekua, Hawaii (Parson *et al.* 2008)

Salinity is often used to represent a gradient of groundwater concentrations that are used to demonstrate relationships between SGD and water quality factors that are physical and chemical in nature. Off the coast of Kona, Hawaii salinity has been used to establish relationships between SGD and temperature (Duatre *et al.* 2006; Johnson *et al.* 2008); and between SGD and nutrients (Parson *et al.* 2008; Street *et al.* 2008).

Other chemicals that have been used to detect and estimate salinity values, including bromide (Whittemore 1988; Johnson and Coletti 2002) and silica (Garrison *et al.* 2003; Hwang *et al.* 2005b; Street *et al.* 2008). However, silica has also been shown to fuel diatom blooms (Brzezinski 2001), which incorporates a biological factor that may affect silica concentrations. Constituents of seawater such as chlorine, sodium, sulfur, magnesium, calcium, and potassium (Pidwirny 2006), are another set of chemicals that could potentially be used to detect and estimate salinity values.

SGD entering the ocean in large quantities has altered the understanding of chemical mass balance in the marine setting (Church 1996). Nutrients are a group of groundwater constituents that has received much attention. Nutrient input through SGD can rival river inputs in certain regions and can play a more significant role in coastal oceans than rivers (Slomp and Cappellen 2004). SGD has been shown to contribute greater nutrient concentrations to surrounding coastal waters, this is evident for nitrate at Perth, Australia (Johannes 1980); Shiraho, Japan (Umezawa *et al.* 2002); Waquoit Bay, Massachusetts (Talbot *et al.* 2003); and for phosphorus at Molokai, Hawaii (Glenn *et al.* 1999). However, more often than not this pattern holds true for both nitrogen and phosphorus, this was the case at Tomales Bay, California (Oberdorfer *et al.* 1990) Florida Bay (Corbett *et al.*1999); Kahana Bay, Hawaii (Garrison *et al*. 2003); Yeoja Bay, Korea (Hwang *et al*. 2005b); and Kona, Hawaii (Johnson *et al*. 2008; Parson *et al.* 2008).

Nevertheless, at times it can be difficult to establish a relation between SGD and ammonia. Ammonia was moderately negatively correlated with salinity at Jawaharlal Nehru Port Trust, India (Gupta *et al.* 2005); while on the north shore of Kauai, Hawaii ammonia had a significant inverse relationship with salinity at only two out of five sites (Knee *et al*. 2008). On the other hand, no relation was found between ammonia and salinity or SGD at Guanabara Bay, Brazil (Paranhos *et al*. 1998) and Molokai, Hawaii (Glenn *et al*. 1999). This lack in relation maybe because ammonia is present on tropical reefs mainly as a transient chemical that is quickly taken up by algae or oxidized to nitrite then nitrate by bacteria (Glenn *et al*. 1999). Another reason for the weakness in relation is that the source of ammonia is not only from land, but from fish excretion as well (Randell and Wright 1987).

If one is not aware of potential fluctuations in aquatic nutrient levels, it can be difficult to relate nutrients and SGD. Diurnal patterns in nutrient concentrations due to light and biological activity have been found in the coastal environment at the Bay of Brest, France with silica (NiLongphuirt *et al.* 2009). This pattern was also evident in the freshwater setting of River Taw, United Kingdom with nitrate, nitrite, and phosphorus (Scholefield *et al*. 2005). A researcher that is unaware of these processes may have faulty conclusions when it comes to the relation of nutrients and SGD.

Although at times SGD can naturally have greater nutrient levels than surrounding marine waters, these nutrient levels can be exacerbated by anthropogenic influences. More atmospheric nitrogen is fixed by humans than all other natural terrestrial process combined (Vitousek *et al.* 1997). The highest values of nitrogen and phosphorus in groundwater are typically found shallow below agriculture land and in sewage plumes (Slomp and Cappellen 2004). Groundwater has been shown to deliver nutrients from agricultural lands and residential developments into the costal environment at the Florida Keys (Lapointe *et al.* 1990); Ontario, Canada (Robertson *et al.* 1991); Buttermilk Bay, Massachusetts (Harris 1995); Chesapeake Bay (Gallagher *et al*. 1996; Reay 2004); Nauset Marsh, Massachusetts (Portnoy *et al.* 1998); and West Maui (Dailer *et al*. 2010). Increasing coastal populations and un-sustainable land use practices can enhance anthropogenic nutrient loading in SGD contributing to reef degradation (Paytan *et al*. 2006; Swarzenski *et al.* 2005). Such anthropogenic effects can have a substantial impact on the near shore environment (Garrison *et al.* 2003). These realizations have increased the awareness and interest in the influence that SGD can have on near shore marine environments, especially phase shifts.

Biologically SGD creates unique localized habitats that allow estuarine species to exist in marine settings, which can increase overall biodiversity. Nutrients from these seeps can also fuel biological productively, increasing overall biomass (Miller and Ullman 2004). At Cape Henlopen, Delaware SGD creates localized regions of reduced salinity, stable temperature, and increased nutrients fueling high micro-algal abundances (Miller and Ullman 2004). On the reef flats of Molokai, ammonia provided by SGD has been shown to increase the algal biomass of *Gracilaria parvispora* (Glenn *et al.* 1999). Other algal species that consistently inhabit the estuarine surroundings of SGD in Hawaiian marine environments include *Enteromorpha prolifera* (Abbott 1996), *Ulva lactuca*, (Abbott and Huisman 2004) and *Grateloupia filicina* (Abbott 1999).

The estuarine setting created by SGD may also serve as a nursery for fish (Boesch and Turner 1984; Beck *et al*. 2001; Islam and Tanaka 2006). In Sendai Bay, Japan the stone flounder, *Platichthys bicoloratus* had greater growth rates, fuller guts, and greater densities in an estuary than an adjacent near shore marine setting (Yamashita *et al*. 2003). An experiment analyzed the survivorship of the juvenile sea lamprey *Petromyzon marinus* undergoing metamorphic transformation in a gradient of salinities. The results showed that survivorship at higher salinities increased with increased degree of metamorphosis, while those in earlier stages of metamorphosis had lower survivorship at equivalent salinity levels (Reis-Santos *et al.* 2008), suggesting that juveniles can only survive at lower salinities. Cobias, *Rachycentron canadum* spends its adult life in a marine setting. Experiments showed that juvenile cobias were able to tolerate salinity levels of 2ppt for 24hrs (Burkey *et al*. 2007). Such results demonstrate the potential of juvenile cobias to inhabit estuarine settings. In Hawaii fish such as *Kuhlia sandvicensis* spend a portion of their juvenile life in estuaries and later move into or near coral reefs as adults (Benson and Fitzsimons 2002). *Mugil cephalus* is another estuarine species from Hawaii that spends its juvenile life in estuaries than later moves offshore as adults to breed (Nishmoto *et al*. 2007). These studies and observations demonstrate the importance of estuaries created by SGD to the life cycle and biological process of numerous aquatic organisms.

As previously mentioned a number of physical, chemical, and biological parameters influences or are influenced by SGD throughout the world. In this dissertations second chapter, an attempt is made to determine if these relations exist in a small basaltic lagoon on the island of Hawaii, in the interest of the nutrients that SGD can provide to these near shore environments.

Herbivory is an essential top-down pressure that regulates macro-algae, mediates competition between macro-algae and corals, and provides resilience for tropical reefs following disturbances (Burkepile and Hay 2010). On tropical reefs, herbivorous fish and urchins often remove the majority of algal production, maintaining an environment of small, highly productive, filamentous and turf algae that in turn support the herbivorous population (Carpenter 1986; Carpenter 1988; Hatcher and Larkum 1983; Burkepile and Hay 2010). A reduction of herbivores due to disease or overfishing often results in an abundance of macro-algae and coral mortality (Lewis 1986; Smith *et al.* 2001; Burkepile and Hay 2006; Hughes *et al.* 2007; Burkepile and Hay 2010).

One of the first studies that experimentally reduce herbivory on tropical reefs occurs at Kealakekua Bay, Hawaii. In this experiment, a cage over natural substrate reduces herbivore interactions with substrate and results in a 50-750% increase in algal height after two months (Randall 1967). Herbivores also influence benthic succession on Hawaiian Reefs. On the reefs of Kaneohe Bay, under ambient levels of herbivory, CCA replaces early succession species of algae. Succession under cages has early communities of fine green and brown algae, a mid community of thin and finely branched red filamentous algae and a late community of blades and coarsely branched red filamentous algae. This study from Kaneohe Bay also shows that grazing by different groups of fish had substantial and different effects on the rate and trajectory of benthic algal succession (Hixon and Brostoff 1996). On the relatively healthy tropical reef of Puako, Hawaii the interaction of ambient levels of herbivory on settlement tiles result in nearly bare surfaces, setting the stage for coral recruitment, while turf algal communities persist on settlement tiles in herbivore reduction treatments (Smith *et al*. 2001, Smith *et al.* 2010).

On a Guamanian reef a reduction in herbivory increases cover of palatable algae such as *Padina tinuis* and *Tolypiocladia glomerulata* on natural reef substrate (Thacker *et al*. 2001). Another experiment from Guam that uses settlement tiles shows that herbivory reduces fleshy algal biomass and sediment load, while increasing CCA cover (Belliveau and Paul 2002). A feeding assay from Guam shows that the herbivorous fish *Siganus rostratus* and *S. spinus* consistently consume only ten of 45 algal genera offered. Of these ten preferred genera, eight are filamentous algae and two are flesh algae (Tsuda and Bryan 1973).

On the Great Barrier Reef, an abundant herbivore population buffers and protects reef communities against increases in algal production fueled by anthropogenic nutrients (McCook 1999). At Great Palm Island, Great Barrier Reef herbivory prevents the overgrowth of *Porites cylindrica* by *Lobophora variegata* (Jompa and McCook 2002). At Rib Reef, Great Barrier Reef herbivores reduce the density and growth of *Lobophora variegata* and *Sargassum fissifolium* recruits (Diaz-Pulido and McCook 2003). Within three bays at Orpheus Island, Great Barrier Reef a diverse array of 45 herbivorous fish is present, however only *Siganus canaliculatus, S. javus,* and *Kyphosus vaigiensis* consume *Sargassum* transplants at experimental sites (Cvitanovic and Bellwood 2009).

A study from Key Largo shows that on carbonate settlement plates, both turf and macro-algae increases when herbivorous fish are excluded while CCA decline (Miller *et al.* 1999). On a reef at Key Largo a reduction of herbivory interacting with natural substrate results in an increase of algal cover and biomass, and a decrease in CCA cover; while in the presence of herbivores there in an increase in the survival rate of corals such as *Porites porites* and *P. asteroids* (Burkepile and Hay 2009). On settlement tiles at Key Largo herbivory reduces the cover of frondose algae, turf algae, and cyano-bacteria, and increases CCA cover and bare space (Miller *et al.* 1999). At Pickles Reef, Florida a reduction of herbivory on coral tiles increases the cover of *Dictyota, Dasycladus, Hypnea*, *Amphiroa*, and *Jania*; while decreasing the cover of CCA and turfs (Sotka and Hay 2009). On tropical reefs, select fish species can play different roles in benthic community composition. At Key Largo, new substrate undergoing primary succession exposed to *Acanthurus bahianus* and *Scarus taeniopterus* supports communities of short filamentous algae and CCA. However, *A. bahianus* and *S. taeniopterus* allows an increase of macro-algae on substrate with established communities not undergoing primary succession. In contrast, new substrate undergoing primary succession exposed to *Sparisoma aurofrenatum* results in an accumulation of tall turf algae then an increase in macro-algae, but *S. aurofrenatum* reduces macro-algae on substrate with established communities not undergoing primary succession (Burkepile and Hay 2010).

A mass mortality of *Diadema antillarum* and a reduction of herbivorous fish due to trapping results in extensive algal blooms on Jamaican reefs (Hughes 1994). A set of experiments from Discovery Bay, Jamaica show that reductions in herbivory lead to an increase in filamentous algae and decrease in CCA, demonstrating that herbivores play a major role in generating and maintaining the benthic pattern of algal distribution (Morrison 1988).

On a back reef of Belize, experimental reductions of herbivorous fish alter existing patterns of benthic species composition and abundance on natural substrate. After 10 weeks, macro-algae abundance increases significantly in herbivore exclusion plots relative to controls. The increase of macro-algae negatively correlates with cover of available space, several turf species, CCA, and corals. The overall result of herbivorous fish reductions is a decline in biodiversity (Lewis 1986). On Belizean reefs herbivory promotes the growth of corals and CCA by reducing the presence of fleshy algae (Littler *et al*. 2006). This is evident on settlement tiles at Glover's Reef, Belize where herbivory negatively influences algal biomass and cover (McClanahan *et al.* 2003). However, on Belizean reefs with high macro-algae cover, macro-algae overwhelms the ability of herbivorous fish to reduce macro-algae cover, in contrast to reefs with low macro-algae cover where herbivorous have the ability to sustain low macro-algae cover (Williams *et al*. 2001).

A study from the Virgin Islands that examines the different size classes of herbivores on tropical reefs show that amphipods and gastropods treatments result in macro-algae communities. Urchin treatments result in communities of epilithic and endolithic filamentous and crustose algal species. While the turf species, *Sphacelaria tribuloides* is the primary cover within herbivorous fish treatments (Carpenter 1986).

A study that incorporates the Caribbean sites of Barbados, Belize, Cayman Islands, Cuba, and Jamaica show that herbivorous fish biomass negatively correlates with macro-algae cover and positively correlates with bare, turf, and CCA cover on mid-depth reefs (Williams and Polunin 2001).

On the tropical reefs of Kenya, algal development and composition of benthic communities on experimental coral plates depends on whether the dominate grazer is fish or urchins. On reefs with urchins settlement plates have filamentous algal turfs and succession is complete in <50 days. On reefs with herbivorous fish settlement plates do not complete succession until >120 days. Communities exposed to herbivorous fish pass through more successional stages and develop into communities with greater diversity and more distinct seasonal changes (McClanahan 1997). On natural substrate at Kenyan reefs a reduction in herbivory increases the biomass of *Enteromorpha* spp., *Caulerpa spp*., *Dictyota* spp., *Actinotrichia fragilis*, and *Amphiroa rigida* (Mork *et al.* 2009).

The combined subjects of nutrient enrichment and herbivory are explored in two chapters by this dissertation. In chapter three, the role of herbivory on benthic succession is assessed on ambient and nutrient enriched settlement tiles at two sites within Kealakekua Bay, Hawaii. In chapter four, the preference of two Hawaiian herbivorous fishes are evaluated by simultaneously offering 14 algal species in multiple-choice feeding assays.

Alien species are one the primary threats to our planets natural environment (Vitousek *et al*. 1997). On a global scale alien species are a major threat to biodiversity (Clout and DePoorter 2005; Riley 2005) and ecosystem functions and services (Schaffelke *et al*. 2006). Hawaii is no exception. On land, an array of alien plant species are changing the three dimensional structure of native rainforests (Asner *et al*. 2008). In Hawaii's ocean, alien algae are taking over reefs and sea-grass meadows (Smith *et al*. 2002). These marine alien species are not only a threat to the natural environment, but also to industries such as fishing and tourism (Bax *et al*. 2003). In Kaneohe Bay, Hawaii, several species of alien algae are overgrowing and killing coral species (Conklin and Smith 2005). On the island of Maui, alien algae blooms causes the County of Maui losses of more than $20 million per year from lower hotel occupancy rates, lower property value, and the cost of removing foul odor alien algae mats that wash up on beaches (Beukering and Cesar 2004).

On land, methods to manage alien species generally fall into the categories of chemical, physical, or biological. Compared to the terrestrial setting the control of alien species in the ocean is a relatively young field, but a few examples do exist. In Agua Hedionda Lagoon, California *Caulerpa taxifolia* has been effectively managed with the use of sodium hypochlorite (Anderson 2005) a form of chemical control. In Kaneohe Bay, Hawaii, a number of methods have been experimented with in an effort to control species in the *Eucheuma-Kappaphycus* complex. Efforts include physical methods such as manual removal by hand (Conklin and Smith 2005) and most recently an underwater vacuum that sucks algae off the reef then deposits it top side on a small craft (Conklin 2007). This suction device is also being applied on the reef flats of Molokai, Hawaii

(Walsh 2009). Biological controls have also been experimented with at Kaneohe Bay. In small experimental enclosures, *Tripneustes gratilla* a native urchin has been shown to be effective on species in the *Eucheuma-Kappaphycus* complex (Conklin and Smith 2005). At Koloko-Honokohau National Historic Park, a number of methods have been experimented with in an effort to control *Acanthophora spicifera*. Physical efforts included manual removal and shading. A biological effort involved the establishment of rock shelters as a means to enhance local herbivorous fish populations. Of the three methods experimented with at Koloko-Honokohau National Historic Park the enhancement of herbivore populations was the most promising (Weijerman *et al*. 2008).

Recent evidence has demonstrated that fishing is diminishing target fish populations throughout the Hawaiian Islands (Williams *et al*. 2008). Such realizations have influenced the State of Hawaii's Department of Land and Natural Resources, Division of Aquatic Resources to establish the Kahekili Herbivore Fisheries Management Area on the island of Maui. Herbivorous fish and urchins are protected at this Fisheries Management Area in an effort to control alien algae. Although the state of Hawaii has been proactive in the effort to protect herbivorous fish as a mean to control alien algae, marine natural resource managers in Hawaii seem apprehensive. Before the state continues to move forward on this issue, more must be learned about the ecological services provided by herbivorous reef fish in Hawaii.

If marine natural resource managers want to use herbivorous fish as a means to control alien algae, the primary matter of concern is what do these herbivorous fish eat? Although numerous studies have analyzed the diet and preference of herbivorous fish in the tropics and warmer temperate waters (Tsuda and Bryan 1973; Bryan 1975; Fishelson *et al*. 1987; Wylie and Paul 1988; Schupp and Paul 1994; Clements and Choat 1997; McClananan 1997; Choat *et al*. 2002), little work has been carried out in Hawaii. In 1961 (Randell) conducted multiple-choice algal feeding experiments with juvenile *Acanthurus triostegus* from Hawaii. In 1968 (Jones) published the most comprehensive study of herbivorous fish diets in Hawaii. However, both of these studies happened before Hawaii had any major algal issues and before the vast majority of alien algae species were introduced. A more recent study used pair wise feeding trials of alien algae and their native counterparts with an assortment of Acanthurids and groups of non-descript juvenile Scarids to determine if herbivorous fish prefer native or alien algae (Smith *et al*. 2004). Although these studies have provided the foundation in the effort to better understand what herbivorous fish eat on Hawaii's reef, the information at present is not enough to assist in the management of herbivorous fish as a means to control alien algae in Hawaii.

A general assumption is that native herbivores prefer native to alien algae, and are likely to be ineffective at controlling alien algae (Williams and Smith 2007). However, experiments and observations from Hawaii (Conklin and Smith 2005; Conklin 2007; Arthur and Balaz 2008; Weijerman *et al*. 2008; Vermeij *et al*. 2009) seem to suggest that these relationships are more complex, at least for select alien algae and herbivorous species.

In an effort to determine the feasibility of employing herbivorous fish as a means to control alien algae, my final research chapter experimentally determines algal preference and consumption rates for two herbivorous reef fish, *Acanthurus triostegus* and *Kyphosus vaigiensis,* potential candidates for biological controls of *Acanthophora spicifera* and *Gracilaria salicornia*.

The goals of this dissertation are to address Hawaii's algal issues at a watershed scale, by tracking the connections as water and nutrients move from land into the ocean. In particular, how are nutrients transferred from land to the ocean? What are the effects that nutrients can have on algal communities? What are the contributions of herbivores to the control of detrimental algal communities? This dissertation presents potential solutions that may be applied by natural resource managers in an effort to ease the algal woes of Hawaii.

CHAPTER 2. THE RELATIONSHIP OF SUBMARINE GROUNDWATER DISCHARGE AND ENVIRONMENTAL PROCESSES IN A SMALL HAWAIIAN LAGOON

2.1 ABSTRACT

As reef degradation increases throughout the world, identifying the effect of reef stressors has become increasingly important. Submarine Groundwater Discharge (SGD) and its constituents is one such stressor. Although numerous studies characterizing SGD have been conducted throughout the world and Hawaii, limited work has been conducted on the east coast of Hawaii Island where the greatest rates of SGD may exist and intrudes coastal regions with unknown effects. This study selected a small basalt lagoon on the east coast of Hawaii Island to characterize the influences of SGD on the structuring and mixing of that water body which has a resident algal and coral community. Both salinity profiles and water quality samples were used to characterize SGD at Waiopae where we conducted an exceptionally fine scale sampling, to reveal both periods of striking stratification and dynamic mixing. Salinity profiles established a significant difference between the salinity values of three sample events, a significant positive correlation between SGD and low tide, and a significant negative correlation with SGD and depth. Water quality samples demonstrated a significant positive correlation with SGD and nitrate, ortho-phosphate, total-nitrogen, total-phosphate, and silica; and a significant

negative correlation with SGD and ammonia, bromide, and sulfate. Nitrate from water quality samples at Waiopae were up to 3.8 times greater than upland water quality samples from wells, suggesting an anthropogenic source of nitrate may be intruding the aquifer between the wells and Waiopae. This study exemplifies the dynamic nature of SGD and nutrient intrusion at Waiopae. More studies should be conducted with a finer scale and at sites with greater SGD, which can be used as models in determining the potential effects of SGD to the near shore environment.

2.2 INTRODUCTION

Groundwater from land directly entering the marine environment is submarine groundwater discharge (SGD) (Moore 1999). SGD is characterized by diffuse seepage over a wide range or by percolation from a point source spring (Cable *et al*. 1996). SGD represents at least 0.01%, but up to 10% of freshwater from land entering the ocean (Church 1996). Many aspects of SGD have been characterized including, variations in spatial differences, temperature differences, impacts generated by tides and waves, land based contributions, and influences on biological distributions. However, none of these relations has been established on the east coast of Hawaii Island where the greatest rates of SGD may exist (Fischer *et al*. 1964).

In the near shore environment SGD decreases with depth (Lewis 1987) and distance from shore (Valiela *et al*. 1990; Staver and Brinsfield 1996; Cable *et al*. 1997;

Robinson *et al*. 1998; Garrison *et al*. 2003). This inverse relation of SGD with depth and distance from shore may exists because SGD is greatest just inland of the freshwater lens which is often located at the shoreline (Taniguchi *et al*. 2006).

Pressure created by tidal flux influences SGD. Numerous studies throughout the world show that SGD is greatest during the ebb and low tide (Lapointe *et al.* 1990; Valiela *et al*. 1990; Portnoy *et al*. 1998; Robinson 1998; Michael *et al*. 2003; Urish and McKenna 2004; Street *et al*. 2008; Rocha *et al*. 2009). A theoretical model demonstrates that waves can also create pressure that influences SGD (Li *et al*. 1999). This model is supported in the field by Riedl *et al*. (1972) and Smith *et al*. (2009).

In temperate regions, SGD is warmer than surrounding seawater (Roxburgh 1985; Banks *et al*. 1996; Portnoy *et al*. 1998; Miller and Ullman 2004), while in the tropics, SGD is generally cooler than surrounding marine waters (Fischer *et al*. 1964; Duarte *et al*. 2006; Johnson *et al*. 2008; Garrison et al. 2003), but there are exceptions. In the tropics on the east side of Hawaii Island SGD can be warmer then surrounding seawater due to heating from volcanic activity (Fischer *et al.* 1964; Scholl *et al*. 1996).

The lack of salt or conductivity is used to detect SGD that is fresh in origin (Lewis 1987; Lapointe 1997; Portnoy *et al*. 1998; Garrison *et al*. 2003). Salinity contours can represent a gradient of groundwater concentrations (Duarte *et al*. 2006; Parson *et al.* 2008; Street *et al.* 2008) and land based components (D’Elia *et al.* 1981; Johnson *et al.* 2008). Other chemicals used to detect and estimate salinity include bromide (Whittemore 1988; Johnson and Coletti 2002) and silica (Hwang *et al.* 2005b; Street *et al.* 2008). However, silica values may be confounded by diatoms that absorb silica for cell division (Brzezinski 2001). Components of seawater such as chlorine, sodium, sulfur, magnesium, calcium, and potassium (Pidwirny 2006), are another set of chemicals that could potentially be used to detect and estimate salinity values.

SGD including dissolved inorganic nutrients enter the ocean and influence the chemical makeup of near shore waters (Church 1996). Nutrient input through SGD can be greater than that of rivers and streams (Slomp and Cappellen 2004). The contribution of greater nutrient concentrations by SGD into coastal waters has been shown for nitrate (Johannes 1980; D'Elia *et al*. 1981; Umezawa *et al*. 2002; Talbot *et al*. 2003), and phosphorus (Glenn *et al*. 1999). More often than not, this pattern holds true for both nitrogen and phosphorus simultaneously (Oberdorfer *et al*. 1990; Corbett *et al*.1999; Garrison *et al*. 2003; Hwang *et al*. 2005b; Johnson *et al*. 2008; Parson *et al*. 2008). However, it can be difficult to establish a relation between SGD and ammonia (Paranhos *et al*. 1998; Glenn *et al*. 1999; Gupta *et al*. 2005; Knee *et al*. 2008). This lack of relation on tropical reefs may exist because ammonia is quickly assimilated by photosynthetic organisms or ammonia is quickly oxidized to nitrate by bacteria (Glenn *et al*. 1999). Another reason for this lack in relation may be that the source of ammonia in near shore settings is not only from land via SGD or surface flow, but fish excretion as well (Randell and Wright 1987). Diurnal fluxes in silica concentrations are also attributed to biological activities influenced by irradiance in coastal environments (NiLongphuirt *et al*. 2009). These diurnal fluxes of nutrients are also evident in freshwater settings (Scholefield *et al*. 2005).

Although at times SGD can naturally have greater nutrient concentrations than surrounding marine waters, these nutrient concentrations can be amplified by anthropogenic activities (Lapointe *et al.* 1990; Robertson *et al.* 1991; Harris 1995;

Gallagher *et al*. 1996; Portnoy *et al*. 1998; Reay 2004). The highest values of nitrogen and phosphorus in groundwater are typically found below agricultural lands and in sewage plumes (Slomp and Cappellen 2004). Fertilizers from golf courses can also increase the concentration of nutrients in groundwater (Dollar and Atkinson 1992). The increase of nutrient concentrations in SGD has significant impacts on the near shore environment including altering the function and structure of marine communities (Dollar and Atkinson 1992) ultimately leading to reef degradation (Swarzenski *et al*. 2005; Paytan *et al*. 2006).

From a biological perspective, SGD increases biomass and biodiversity by fueling productively and allowing estuarine species to persist in marine settings (Miller and Ullman 2004). Ammonia provided by SGD increases the biomass of *Gracilaria parvispora* on the reef flats of Molokai (Glenn *et al*. 1999); other marine algal species that inhabit the estuarine surroundings created by SGD in Hawaii include *Enteromorpha prolifera*, *Ulva lactuca* (Abbott 2004), and *Grateloupia filicina* (Abbott 1999). The estuarine setting created by SGD may also serve as nurseries for fish (Beck *et al*. 2001; Islam and Tanaka 2006). Some fish seem adapted to low salinity environments (Burkey *et al*. 2007), where growth rates (Yamashita *et al*. 2003) and survivorship (Reis-Santos *et al*. 2008) are increased. In Hawaii, economically important fish utilize estuarine habitats created by SGD (Benson and Fitzsimons 2002; Nishimoto *et al*. 2007).

This chapter will examine the short term, fine scale physical, chemical, and biological parameters that influence or are influenced by SGD in a small basaltic lagoon on the east side of Hawaii Island.

2.3 METHODS AND MATERIALS

2.3.1 Waiopae, Puna, Hawaii

Waiopae, Puna, is located on the island of Hawaii (Figure 1). The island of Hawaii is the youngest oceanic island of the Hawaiian Islands, with the greatest distance of basaltic coastline in the archipelago. Waiopae sits on the northeast rift zone of an active volcano (Scholl *et al*. 1996) and is just 3 km south of Cape Kumakahi, the most eastern point of the Hawaiian Archipelago, which is exposed to the persistent northeast trade winds (Juvik and Juvik 1998). Due to the persistent winds and orographic rainfall, Puna can receive up to 460 cm of rain per year. However, there are no perennial streams entering the ocean (Juvik and Juvik 1998). The young basalt substrate of this district is extremely porous and highly conductive, resulting in limited surface flow and extensive aquifers (Scholl *et al*. 1996). Because of Puna's young substrate, one expects relative substrate and aquifer nitrogen values to be low and phosphorus values to be high compared to the rest of the Hawaiian Islands (Vitousek and Farrington *et al*. 1997).

Waiopae possess a network of semi-enclosed lagoons and tide pools. Live coral is the greatest benthic cover at Waiopae (Williams 2009). In addition, an algal flora of 59 algal species occurs here (data not included 2000 report).

At Waiopae, the lagoons and tide pools (Figure 1) that are most inland are most likely to be subjected to SGD. Layering and separation of groundwater from marine water is easily observed by eye. A single inland lagoon has been selected to characterize SGD in this area (Figure 2). The dimensions of this lagoon are 170 m by 25 m with a west to east long axis. The north side is directly bordered by six residences. The majority of this lagoon is one to two m deep. The deepest part (three m) is adjacent to the channel that connects this lagoon to open ocean. *Montipora capitata* is the most abundant coral species in this lagoon even though it suffered an episode of dieback in 2008 that was investigated by employees from the Division of Aquatic Resources (DAR) (Walsh 2009).

Although a majority of the watershed upland of Waiopae is undeveloped tropical forest, anthropogenic influences do exist. An adjacent and upland of community of approximately 90 homes exists. The occupants of these homes vary from permanent residence to vacation renters. Five km away but further upland an extensive papaya orchard can be found, and the subdivisions of Nanawale and Leilani are 10 km upland of Waiopae. Pahoa is one of the two urban centers of Puna and is located 16 km upland of Waiopae. There are no public wastewater treatment facilities in the entire district of Puna, so all residences are on cesspools or septic tanks with leach fields. Moreover, substantial areas of the upland watershed have been invaded by the alien *Falcataria moluccana*, a fast growing nitrogen fixing tree (Hughes and Uowolo 2006) which is thought to contribute nitrogen into aquifers.

During 2004, the County of Hawaii Department of Water Supply wells in the district of Puna had a maximum nitrate value of 5.76 µM, and of the four out of five wells that had detectable levels of nitrate, the average value was 5.37 µM. The years following 2004 also have comparable nitrate values. The closest of these wells is 13 km away, while the furthest is 27 km away from Waiopae (Figure 1).

2.3.2 Salinity Profiles

Salinity profiles were established during three days: sample events 1, 2, and 3 (Table 1). No rain occurred during any sample event. Salinity profiles were conducted at low and high tide. The low tide level during these sample events were ≤ 0.0 m, while high tide levels were ≥ 0.69 m. Salinity profile samples were collected within two hours, one hour before, and one hour after the low or high tide. Nine points were strategically selected throughout the lagoon to characterize the entire lagoon (Figure 2). Eleven depths were sampled at each point. Sample depths were 0 (surface), 5, 10, 15, 20, 25, 30, 40, 50, 60, and 70 cm.

All water samples for salinity profiles were extracted from the lagoon with the least possible disturbance to maintain salinity stratification. Samples were taken while standing on shore, wading in shallow water, or floating on a surfboard. To further minimize disturbance samples were extracted with a narrow bore, long glass pipette. Deeper depths were accomplished by connecting multiple pipettes with surgical tubing. An 85 g fishing lead was attached near the tip of the pipette to compensate for the buoyancy of multiple pipettes and surgical tubing. Salinity values were determined with a refractometer (Vee Gee STX-3).

2.3.3 Water Quality

Water quality samples were collected during three days: sample events 4, 5, and 6 (Table 1). No rain occurred during sample events. Salinity values for all water quality sample events were determined with an YSI 30M water quality sensor that has a resolution of 0.1 ppt and an accuracy of ± 0.1 ppt. The YSI was also used to detect temperature during sample event 4. All water samples were collected within two hours, one hour before, and one hour after the low or high tide. After the water samples were collected, they were immediately placed on ice and delivered to the appropriate laboratory within 24 hours.

Methods for sample events 4 and 5 were in accordance with the Dionex manual (1998) which involved collecting samples in 15 mL glass test tubes with polyethylene covers. Tubes and covers had been thoroughly cleaned and rinsed with de-ionized water prior to collection. Strong acids and detergents were not used to clean tubes and covers to avoid contamination of ions that could interfere with the water analysis. Water samples from events 4 and 5 were analyzed by the Dionex DX-120 Ion Chromatograph Auto Sampler at the University of Hawaii at Manoa, Water Resources Research Center. This auto sampler's respective detection limits for bromide, nitrate, and sulfate were 2.5, 1.6, and 2.1 µM. Samples for event 4 were taken during low (10:19 am, 0.0 m) and high (2:05 pm, 0.64 m) tide. Samples for event 5 were collected during low tide (5:00 am, 0.03 m). Only low tide was sampled during sample event 5 due to the lack of SGD detected during the high tide of sample event 4.

Samples for event 6 were collected in accordance to the United States, Environmental Protection Agency's standards. Samples were collected in new one L rectangular amber high-density polyethylene containers. Water samples from this event were analyzed by the State of Hawaii's Department of Health, Laboratories Division, Environmental Health Analytical Services Branch, Water Pollution Section at Pearl City, Hawaii. The methods used by Hawaii's Department of Health for analyzing ammonia, chlorophyll-a, nitrate, ortho-phosphorus, total-nitrogen, total-phosphorus, and silica has detection limits of 0.11 µM, 0.06 µM, 0.02 µM, 0.02 µM, 0.03 ppm, 0.005 ppm, and 8.3 µM respectively. Samples from event 6 were collected during low tide (6:02 pm, 0.0 m).

Sample points for water quality were strategically selected throughout the lagoon to characterize the entire lagoon (Figure 2). During sample events 4 and 5 eight points were sampled. Two depths (top and bottom) were sampled during event 4 and 5. Only six of the most inland points were sampled during sample event 6. Although only six points were sampled during sample event 6, the points that were sampled were so with greater detail. During sample event 6 one or two mid samples were collected at each sample point depending on depth, in addition to surface and bottom samples. This alteration in sample event 6 was to better understand the inland portion of the lagoon were SGD was more evident during previous sample events.

2.3.4 Biological Surveys

Biological observations were conducted during sample events and other visits. Species of algae, coral, and fish were recorded along with their relative location within the lagoon. Estimates of relative density were made for algal and coral species. The number and sizes of fish species were recorded. Observations of other noteworthy organisms were also recorded.

2.3.5 Data Analysis

The MINITAB 14 statistical program was used to conduct *t*-tests between low and high tide values from salinity profiles. To compare the salinity values between sample events 1, 2, and 3 an ANOVA and Tukey's pair wise comparison was conducted in

MINITAB 14. The MINITAB 14 program was also used to create salinity contour graphs with salinity profile data. Microsoft Excel 2003 was used to create graphs displaying the relationship between salinity and spatial characters from salinity profile data, and salinity and chemical component concentrations from water quality data. EXCEL 2003 was also used to add trend lines, calculate trend line equations, and calculate the r^2-value. MINITAB 14 was then used to calculate the r-value and p-value of correlations between salinity and spatial characters, and salinity and chemical component concentrations. Significance level for all analyses was alpha of 0.05.

2.4 RESULTS

2.4.1 Salinity Profiles

The mean value from all salinity profile sample events (1, 2, and 3) combined was 28.35 ppt. Data ranged from 6-50 ppt, ± 0.32 Standard Error of the mean (SE), n = 594. The salinity value means for sample events 1, 2, and 3 (low and high tide combined); were 23.01, 35.54, and 26.52 ppt respectively; ranged from 6-37, 31-50, and 10-36 ppt respectively; with a ± 0.54, ± 0.13, and ± 0.47 SE respectively, with an n = 198 for each sample event. The salinity values from sample events 1, 2, and 3 were all significantly different from each other ($p < 0.001$).

A pooled comparison between low and high tide salinity values of sample events 1, 2, and 3 combined determined that low tide salinity values were significantly lower than high tide (low tide mean = 24.89, SE = ± 0.53, n = 297; high tide mean 31.82, SE = ± 0.23, n = 297; $p < 0.001$). Salinity values between low and high tide were also compared for each sample event. Low tide salinity values were significantly ($p < 0.001$) lower than high tide for sample events 1 (low tide mean = 17.18, SE = ± 0.55, n = 99; high tide mean = 28.83, SE = ± 0.41, n = 99) and 3 (low tide mean = 21.67, SE = ± 0.55, n = 99; high tide mean = 31.37, SE = ± 0.30, n = 99). However, low tide salinity values were significantly ($p = 0.030$) higher than high tide for sample event 2 (low tide mean = 35.85, SE = ± 0.23, n = 99; high tide mean = 35.25, SE = ± 0.13, n = 99).

A pooled comparison of distance from shore and salinity of both low and high tide salinity values from sample event 1, 2, and 3 combined did not have a significant correlation, but a similar pooled comparison for depth and salinity did have a significant positive correlation (Table 2). When salinity profile data were analyzed individually by each event during each tide, distance from shore and salinity had a significant positive correlation only during low tide of sample event 1 (Table 2). However, depth and salinity had a significant positive relationship during both tides of all salinity profile sample events, except during the high tide of sample event 2 (Table 2).

Salinity contour plots from sample events 1 (Figures 3 - 8) and 3 (Figures 15 - 20) showed greater layering and lower salinity values during low tide when compared to high tide. The general pattern for sample events 1 and 3 was an increase in salinity from the surface down through the water column. However, salinity contours from sample event 2 are different, there is little to no layering during either low or high tide. The salinity values found on contour plots from sample event 2 (Figures 9 - 14) had a narrow range when compared to plots from sample event 1 and 3 (Figures 3 - 8 and 15 - 20).

2.4.2 Water Quality

Ten water quality components were analyzed. Ammonia had a positive significant relationship with salinity during sample event 6 (Figure 22, Table 3). Bromide had a positive significant relationship with salinity during sample event 4, 5, and when the data were pooled (Table 3). Chlorophyll-A content was not related with salinity during sample event 6 (Table 3). Nitrate had a negative significant relationship with salinity during sample event 4, 5, 6, (Figure 21, Table 3) and when the data were pooled (Table 3). Ortho-phosphate had a negative significant relationship with salinity during sample event 6 (Figure 24, Table 3). Temperature did not show a relationship with salinity during sample event 4 (Table 3). Total-nitrogen (Figure 23, Table 3), total-phosphorus (Figure 25, Table 3), and silica had a negative significant relationship with salinity during sample event 6 (Table 3). Sulfate had a positive significant relationship with salinity during sample event 6 (Table 3).

2.4.3 Biological Observations

Distribution patterns were observed for some of the organisms within the lagoon being investigated. Algal cover was greatest toward the back (most inland) of the lagoon (Table 4), while coral cover was greatest toward the front (closest to the open ocean) (table 5). Although abundant, juvenile parrotfish (Family: Scaridae) were difficult to identify in the field, thus these juveniles were grouped when observed. Some fish species were abundant and found throughout the lagoon while others had relative densities that were greater toward the front of the lagoon (Table 6). If a fish was observed just once or only a few times at various parts of the lagoon it was difficult to establish a pattern, thus it was considered to have no pattern observed (Table 6). One mega-fauna species, a green sea turtle *Chelonia mydas* was observed twice at the front of the lagoon (Table 7).

2.5 DISCUSSION

Although there are numerous studies that characterizes SGD in Hawaii (Fischer *et al*. 1964; Dollar and Atkinson 1992; Garrison *et al*. 2003; Duarte *et al*. 2006; Johnson *et al*. 2008; Knee *et al*. 2008; Parsons *et al*. 2008; Street *et al*. 2008), only one has previously detected SGD in the district of Puna on the east side of Hawaii Island (Fischer *et al*. 1964). This is somewhat of a paradox considering that Puna's young highly porous substrate and high amount of rainfall (Juvik and Juvik 1998) may facilitate the greatest rates of SGD in the Hawaiian Islands. Rates of SGD up to 378,541 m^3 per day have been estimated at sites in the district of South Hilo (Fischer *et al*. 1964) which is adjacent and just north of Puna. The substrate and rainfall in the southern vicinity of South Hilo is comparable to Puna (Juvik and Juvik 1998), making similar rates of SGD conceivable and a remarkable opportunity for this study.

The size of the lagoon being characterized in this study is relatively small. The distance between depth profile samples of this study is at least two times smaller than other studies from Hawaii, while the distance between transect points are comparable to studies with the finest scale from Hawaii (Johnson *et al*. 2008; Knee *et al*. 2008; Parsons *et al*. 2008; Street *et al*. 2008). This scale allowed for substantial detail as highlighted in the resolution of the salinity contours. This high resolution was critical to distinguish salinity layers at Waiopae, where strata can be only five cm thick. If complex patterns such as detected at Waiopae were not profiled appropriately, then other regions may have not detected their highest concentrations of SGD. This level of resolution also increases our understanding of interactions between SGD and biological species, especially the stress of fluctuating salinity that intertidal and benthic species that could fit in a single stratum, must tolerate.

Salinity contours of Waiopae demonstrated a strong gradient of groundwater throughout the lagoon, such as during sample event 1 and 3. Although supported by just one significant example, contour plots show groundwater concentrations are greatest toward the back of the lagoon and decreases when moving seaward as found with a number of estuaries (Valiela *et al*. 1990; Staver and Brinsfield 1996; Cable *et al*. 1997; Robinson *et al*. 1998; Garrison *et al*. 2003). The lack in significance between distance from shore and salinity maybe due to the lagoons relative small size, the fact that it is nearly enclosed, and multiple sites of seepage are likely to exist (Figure 5).

Contour plots showed that groundwater decreases with depth like the west coast of Barbados (Lewis 1987). This pattern was supported by numerous significantly inverse correlations between the presence of groundwater and depth at Waiopae. This maybe apparent because freshwater is less dense than saltwater of the same temperature (Taniguchi *et al*. 2006).

Similar to other estuaries (Lapointe *et al.* 1990; Valiela *et al*. 1990; Portnoy *et al*. 1998; Robinson 1998; Michael *et al*. 2003; Urish and McKenna 2004; Street *et al*. 2008; Rocha *et al*. 2009) SGD was influenced by tidal pressure at Waiopae. When values of sample events 1, 2, and 3 were pooled evidence of SGD displayed by lower salinity values was significantly greater at low tide when compared to high tide. This difference was also evident in low and high tide salinity contour plots from sample event 1 and 3. Although sample event 2 did have a significantly greater average salinity at low tide when compared to high tide, it is likely that this contradiction is due to evaporation (Pinet 2003), tides, and the absence of SGD. During low tide, the intrusion of seawater from the open ocean into the lagoon is limited, making the lagoon more isolated during low tide. This isolation may exacerbate affects of evaporation, making salinity values greatest at low tide when SGD is not occurring. Salinity contour graphs exemplify how the presence or absence of SGD can influence the hydrological dynamics of salinity in this lagoon.

Unlike temperate sites (Roxburgh 1985; Miller and Ullman 2004; Banks *et al*. 1996; Portnoy *et al*. 1998) and other Hawaiian sites (Garrison *et al*. 2003; Duarte *et al*. 2006; Johnson *et al*. 2008) SGD at Waiopae did not show any relation with temperature (table 3). This lack of relation may have arisen as temperature values were taken in the mid-morning (between 9:19 am and 11:19 am), late enough in the day for heating from the sun to override the temperature differences between marine and groundwater. Heating may be increased by the lagoons shallow waters, lack of flow, and the black basalt substrate. The time between late evening and predawn may be best when establishing relations between SGD and temperature at this site. However, groundwater flow into Waiopae may be heated by geothermal activities of Kilauea’s northeast rift zone, as documented in other coastal regions of Puna (Fischer *et al*. 1964; Scholl *et al*. 1996).

Throughout this section, a correlation that is positive with salinity will be assumed to be a positive correlation with marine water, while a negative correlation with salinity will be assumed to be a positive correlation with SGD (Lapointe 1997; Lewis 1987; Portnoy *et al*. 1998; Parson *et al*. 2008).

The significant and positive correlation of bromide and sulfate with marine water, along with the high *r*-value of these relations suggest that both bromide and sulfate could be used as tracers for marine water (Whittemore 1988; Johnson and Coletti 2002; Pidwirny 2006) at Waiopae. The significant positive correlation of silica with groundwater along with the high *r*-value suggests that it could be used as tracer for groundwater (Hwang *et al*. 2005b; Street *et al.* 2008) at Waiopae. However, some biologist may be apprehensive about using silica as a tracer because silica can be absorbed by biologic processes once it enters the marine environment (Garrison *et al*. 2003).

Nitrate values from all water quality sample events significantly and positively correlated to SGD similar to other estuary settings (D'Elia *et al*.1981; Johnson *et al.* 2008; Parson *et al*. 2008). However, when comparing correlation equations between nitrate and salinity the slope was much steeper and the y-intercept much greater for sample event 5, relative to sample event 4 and 6. This may be due to anthropogenic factors or because sample event 5 was pre dawn while sample events 4 and 6 were during the day. It is expected that if all other factors affecting nitrate concentration were to remain the same throughout a diurnal cycle, the greatest concentration of nitrate would be found just before sunrise, the time of day when nitrate concentrations can build up to their greatest before being absorbed by photosynthetic organisms, similar to what happens to nutrients in other estuarine (NiLongphuirt *et al*. 2009) and freshwater (Scholefield *et al*. 2005) settings. This is especially true of nitrate, a chemical that is absorbed at greater rates when irradiance is increased (Lobban and Harrison 1997). This flux of nitrate questions the generalization of correlations between SGD and nitrate that have been established by D'Elia *et al*. (1981), Johnson *et al*. (2008), and Parson *et al*. (2008). These relations established by previous studies may only represent the minimal level of nitrate associated with SGD. It may be that the nitrate values from the field sites of D'Elia *et al*. (1981), Johnson *et al*. (2008), and Parson *et al*. (2008) would have greater concentrations of nitrate if samples were taken during pre dawn.

Like nitrate, total-nitrogen had a significant and positive correlation with groundwater, but the same could not be said for ammonia. Ammonia values from Waiopae were much less than other sites in Hawaii such as the south shore of Molokai (Glenn *et al*. 1999) and north shore of Kauai (Knee 2008). This along with ammonia's positive significant correlation with marine water at Waiopae suggests that ammonia is oxidized to nitrite and nitrate by bacteria (Glenn *et al*. 1999) in the aquifer before being released into the marine setting. The data also suggest that the ammonia that is present at Waiopae is from fish excretion (Randell and Wright 1987) and other biological activities.

Both ortho-phosphorus and total-phosphorus positively and significantly correlated to SGD. This relationship between phosphorus and SGD pattern was also true at other estuarine sites (Johnson *et al*. 2008; Parson *et al*. 2008).

Although numerous studies (Johannes 1980; Oberdorfer *et al*. 1990; Glenn *et al*. 1999; Corbett *et al*.1999; Umezawa *et al*. 2002; Garrison *et al*. 2003; Talbot *et al*. 2003; Hwang *et al*. 2005b) have demonstrated that SGD can deliver greater concentrations of nitrogen and phosphorus to the surrounding near shore environment few (D'Elia *et al.*1981; Johnson *et al.* 2008; Parson *et al.* 2008) have attempted to or have established significant correlations between concentrations of SGD and nutrient concentrations as this study has.

When comparing nutrient values from similar salinity concentrations throughout the Hawaiian Islands one would suspect nitrogen values to be greater from older substrate and less from younger substrate, while phosphorus values should be greater from younger substrate and less from older substrate (Vitousek and Farrington 1997). Nitrate values from Waiopae were comparable to those from the reef flats of Molokai (Glenn *et al.* 1999) and brackish ponds of Kona, Hawaii (Johnson *et al.* 2008). Nitrate values from Waiopae were less than open ocean samples from Kona, Hawaii (Johnson *et al.* 2008). Phosphate values from Waiopae (figure 24) were less than those from Molokai (Glenn *et al.* 1999) and one study from Kona, Hawaii (Johnson *et al.* 2008). However, another study from Kona, Hawaii (Parson *et al.* 2008) had phosphate values that were less than at Waiopae. The pattern relating age of substrate to nutrient concentration (Vitousek and Farrington 1997) was not supported by this study. This may be because anthropogenic influences are amplifying groundwater nutrient concentrations (Slomp and Cappellen 2004) at various levels throughout the state of Hawaii.

Before groundwater at Waiopae mixes with marine water nitrate values can reach 46.6 µM. When comparing nitrate values, the nitrate values in groundwater at Waiopae can be 3.8 times greater than the nitrate values found in all of the County of Hawaii Department of Water Supply wells in Puna. As water moves through the aquifers between the wells and coastline, nitrogen from cesspools and septic tanks leach fields and agricultural lands such as papaya and flower farms may accumulate. Further, the nitrogen fixer *Falcataria molussana* may also contribute to the nitrogen concentration of groundwater at Waiopae. Chronic exposure to such increased nutrients and other chemicals will upset the balance of benthic species at Waiopae, leading to reef degradation, such as has already been demonstrated, by the *Montipora capitata* mortality event in 2008 at this study site. Mortality was greatest with highest concentration of groundwater in the lagoon (Walsh 2009).

Observations from Waiopae support the concept that SGD can influence the distribution of marine organisms, similar to other marine settings exposed to SGD (Glenn *et al*. 1999; Miller and Ullman 2004). The algal trend may be fueled by nutrients provided by SGD (Glenn *et al*. 1999), however corals had an opposing trend, which may have been influenced by the *Montipora capitata* dieback in 2008 (Walsh 2009). Fish are mobile relative to algae and corals so defining patterns can be more difficult, yet the distribution of some fish species seem to be influenced by SGD. Adult species of marine fish that may be able to tolerate higher concentrations of groundwater include *Abudefduf abdominalis*, *Abudefduf sordidus*, *Acanthurus triostegus*, and *Thalassoma duperrey*. Juvenile species of fish that inhabit the estuarine setting of Waiopae include *A. triostegus*, *T. duperrey*, and species from the family Scaridae. These fish may possess life cycles comparable to other fish (Benson and Fitzsimons 2002; Yamashita *et al*. 2003; Nishmoto *et al*. 2007). The range of salinity values and chemical concentrations found at Waiopae highlights the tolerance of the organisms especially benthic species such as algae and corals observed within the lagoon of interest at Waiopae. Salinity patterns and distribution of benthic species suggest that algae are more tolerant to stress induced by

SGD when compared to corals. At times SGD can increase overall biodiversity (Miller and Ullman 2004), but the reverse maybe true when pollutants and elevated nutrient values are present.

2.6 CONCLUSION

Results from this study revealed that the estuarine setting at Waiopae is an extremely dynamic environment. On a diurnal scale, SGD can fluctuate significantly, driven by tides. Nutrient concentrations may also fluctuate on a diurnal scale. Nutrient levels are expected to be greatest just before dawn and lowest just before dusk due to biological activities. Seasonally, SGD values are likely to vary significantly, especially as upland precipitation changes thru the year.

Nutrients delivered to Waiopae by SGD include nitrogen, phosphorus, and silica; all of these constituents have the potential to influence marine benthic communities.

More research in smaller water bodies should be conducted, because finer scales resulting in greater detail is feasible. Results could be used as a model to assist in the management of larger water bodies. More research should also be conducted in areas where high rates of SGD are known to exist. This will help determine the potential effects of SGD on the marine environment and the true extent to which SGD remains a stress for marine species.

When considering the significance of this study at Waiopae, this dynamic estuary has marked gradients in biota that would likely be tied to gradients in physical factors. Better understanding of major processes at this site is likely to require permanent continuous monitoring over longer time periods.

CHAPTER 3. THE RELATIVE AND SYNERGISTIC EFFECTS OF NUTRIENTS AND HERBIVORY ON ALGAL SUCCESSION AT TWO SITES WITHIN KEALAKEKUA BAY, HAWAII

3.1 ABSTRACT

Throughout the tropics, marine benthic communities of reef building species such as corals and crustose coralline algae (CCA) shift into communities dominated by turf and macro-algae. Most phase shifts have been attributed to impacts of increased nutrient concentrations and reduced herbivore pressure. This study conducts a multi factorial experiment, analyzing the relative and simultaneous effects of nutrient enrichment and reduced herbivory on algal succession at two reefs within Kealakekua Bay, Hawaii. Results from this experiment showed that nutrient enrichment had a minor effect, herbivore reduction had a considerable effect, and nutrient enrichment combined with herbivore reduction had a major effect on algal biomass and percent cover. In general, control and nutrient treatments were dominated by CCA, and turf algae dominated herbivore reduction treatments, with and without nutrient enrichment. When comparing the effects of treatments between sites nutrient enrichment had a greater effect at one site, while a reduction in herbivore pressure had a greater effect at another site. Reductions in nutrient concentrations and increases in herbivore pressure are likely to benefit Hawaiian

reefs, but a single solution is not expected to be equally effective for all Hawaiian reefs or even within a single bay such as Kealakekua.

3.2 INTRODUCTION

Multiple stable states occur when more than one type of community persists in a single environment (Knowlton 1992). Such changes in stable states in coral reef communities are known as "phase shifts" (Bruno *et al.* 2009). In the marine environment, phase shifts from one functional form to another e.g. sea-grass to macro-algae (Valiela 1990; Lapointe *et al.* 1994) or from one dominate algal species to another (Soltan *et al.* 2001; Diaz *et al.* 2002; Diaz-Pulido and Garzon-Ferreira 2002; Arevalo *et al.* 2007) have been documented in benthic communities. However, phase shifts from coral to macro-algae on tropical reefs have received the greatest attention (Done 1992; Hughes 1994; McManus and Polsenberg 2004; Rogers and Miller 2006; Hughes *et al.* 2007; Bruno *et al.* 2009; Norstrom *et al.* 2009). At times, these phase shifts to macro-algae seem irreversible because the presence of certain macro-algae can suppress the settlement and survivorship of coral (Hughes *et al.* 2007).

Decreases of corals and increases in macro-algae on tropical reefs can be attributed to a variety of factors such as bleaching (Glynn 1993), disease (Aronson and Precht 2001), destructive fishing methods (McManus 1997), ocean acidification (Anthony 2008), predation by *Acanthaster planci* (Chesher 1969), storms (Rogers and

Miller 2006), sedimentation (Fields 2008), alien species (Russell 1983), eutrophication (Lapointe 1997), and a reduction in herbivores (Hughes 1994). Although the majority of these factors are undisputed, identifying the relative and synergistic roles of bottom-up (eutrophication, increased nutrients) and top-down (reduced herbivore pressure) forces pertaining to phase shifts from coral to macro-algae have been a topic of debate (Lapointe 1997; Hughes and Szmant 1999), and critical in understand the ecology of tropical reefs (Burkepile and Hay 2006).

Algae in tropical settings, quickly respond to nutrient enrichment with high rates of nutrient uptake (Wallentinus 1984). The result is often an increase in productivity and biomass, a phenomenon referred to as bottom-up control due to nutrient additions (Lapointe 1997). The increase in algal growth rates and biomass has been demonstrated in the laboratory (Lapointe and Tenore 1981; Schaffelke 1999; Fong *et al.* 2004) and field (Lapointe 1997; Glenn *et al.* 1999; Smith *et al.* 2004) with tropical algal species. These studies confirm that tropical algae often persist in nutrient limited settings, and that an increase in nutrient concentrations will amplify growth rates (Lapointe 1997).

In Hawaii, a well document phase shift from corals to algae occurred at Kaneohe Bay. During the 1950s through 1977, Kaneohe Bay received millions of gallons of primary treated sewage from three wastewater treatment plants. The result was blooms of the macro-algae *Dictyosphaeria cavernosa*, and to a lesser extent *Acanthophora spicifera* and *Gracilaria salicornia*. These algal species overgrew coral and remain the dominant or very common benthic cover (Banner 1974; Smith *et al.* 1981). More recently, on Maui, filamentous algal blooms of *Cladophora sericea* have been fueled by land-based nutrients (Smith *et al.* 2005). Macro-algal blooms of *Hypnea musciformis* and

Ulva lactuca are strongly associated with coastal intrusion of effluent from injection wells (Dailer *et al.* 2010). Changes in benthic community structure with increased nutrients from land-based pollution has also occurred at The Great Barrier Reef (Fabricius *et al.* 2005), Argentina (Diaz *et al.* 2002), Massachusetts (Valiela 1990), France (Soltan *et al.* 2001), and Spain (Arevalo *et al.* 2007).

Herbivory is an important top-down force that regulates macro-algae, mediates competition between macro-algae and corals, and provides resilience for tropical reefs following disturbances such as hurricanes and coral bleaching (Burkepile and Hay 2010). On tropical reefs, herbivorous fish and urchins often remove the majority of algal production, maintaining an environment of small, highly productive, filamentous, and turf algae that in turn support the population of herbivores (Carpenter 1986; Carpenter 1988; Hatcher and Larkum 1983; Burkepile and Hay 2010). A reduction of herbivores from disease or over fishing often results in coral mortality and an abundance of macro-algae (Lewis 1986; Smith *et al.* 2001; Burkepile and Hay 2006; Hughes *et al.* 2007; Burkepile and Hay 2010). Therefore sustaining high grazing rates is critical in negating the impact of macro-algae on coral recruitment, coral growth, and coral survivorship; and may assist in facilitating the recovery of tropical reefs (Edmunds and Carpenter 2001; Hughes *et al.* 2007; Mumby *et al*. 2007). CCA are known to facilitate the settlement of some coral species (Morse and Morse 1996, Harrington *et al.* 2004). The recruitment and survival of CCA, like corals, are also suppressed by macro-algae and promoted by herbivory (Lewis 1986; McCook *et al.* 2001; Jompa and McCook 2002; Burkepile and Hay 2006).

Both natural and anthropogenic reductions in herbivory can lead to a large-scale phase shifts away from corals and CCA. Without the urchin *Diadema antillarum*, and with the continued depression of herbivorous fish because of over fishing, the entire reef system of Jamaica and other reefs throughout the Caribbean has undergone a regional phase shift that began in 1983, and has continued for over a decade (Lessios 1988; Hughes 1994). Such an event was anticipated by studies prior to the mass mortality of sea urchins (Ogden *et al.* 1973; Sammarco *et al.* 1974; Carpenter 1981). However, by the year 2000, populations of *D. antillarum* began to recover at select Jamaican reefs. The result of this recovery is a decrease in macro-algae cover and an increase in coral recruitment (Carpenter and Edmunds 2006), a unique and encouraging example of phase shift reversal on tropical reefs.

One of the first studies that experimentally reduced herbivory on tropical reef occurred in Kealakekua Bay, Hawaii in 1955. In this single factor experiment, herbivory is reduced by placing a cage over natural substrate. The result is a 50-750% increase in height of algal thalli after two months (Randall 1967). A more recent comparable study from Puako, Hawaii displays an increase in algal biomass on experimental tiles when herbivory is reduced along with increases in nutrients and finally synergistic interactions with both factors (Smith *et al.* 2001). Analyses of species percent cover on these tiles show that under natural levels of herbivory, CCA dominate, but when herbivory is reduced, turf algae dominate (Smith *et al.* 2010).

Although a number of multi-factorial experiments (Miller *et al.* 1999; Jompa and McCook 2002; Diaz-Pulido and McCook 2003, Sotka and Hay 2009) and a review of multi-factorial experiments (Burkepile and Hay 2006) have deemed the influence of herbivore reduction to be greater than nutrient enrichment, these results are equivocal. An increase in nutrient concentrations has been shown to enhance algal growth in the laboratory (DeBoer *et al.* 1978; Lapointe and Tenore 1981; Schaffelke 1999; Fong *et al.* 2004) and field (Glenn *et al.* 1999; Smith *et al.* 2004). Studies have also shown that eutrophication has been primarily responsible for numerous phase shifts (Banner 1974; Smith *et al.* 1981; Valiela 1990; Soltan *et al.* 2001; Diaz *et al.* 2002; Fabricius *et al.* 2005; Smith *et al.* 2005; Arevalo *et al.* 2007; Dailer *et al.* 2010).

With the recent evidence of increased nutrient levels entering the Hawaiian marine environment (Parson *et al.* 2008; Dailer *et al.* 2010) and a reduction of herbivorous fish throughout the world (Hughes 1994) and in Hawaiian waters (Williams *et al.* 2008), the threat of phase shifts from coral to macro-algae is now greater than ever before. Although natural resource managers are ready to act in the best interest of Hawaii's reefs, they are uncertain of the most effective action. Therefore, the resolution of the debate between the relative importance of nutrients and herbivory to phase shifts remains significant. In response to this debate and the urgency created by the increasing nutrient concentrations and depleted herbivorous fish stocks in Hawaiian waters, a three-way factorial design testing the affects of nutrients, herbivory, and site was implemented within Kealakekua Bay, a well managed reef in Hawaiian waters.

3.3 METHODS AND MATERIALS

3.3.1 Site

Kealakekua Bay is located in the district of South Kona on the west side of Hawaii Island (Figure 26). The island of Hawaii is the youngest island in the Hawaiian archipelago.

Two sites within Kealakekua Bay, Kaawaloa and Kahauloa were selected within Kealakekua Bay to conduct a replicated multi factorial experiment (Figure 27). The presence of submarine groundwater discharge has been established at both sites by select samples that are lower (<10 ppt) than typical Hawaiian marine water (Doty 1968, Parson *et al.* 2008). Kaawaloa is located within a major cove at the north end of Kealakekua Bay. The slope of this reef is extremely steep, nearing an angle of 45°. Although there are no residences or other development adjacent to this reef, the site is visited daily by tens of tourists via large snorkel tour catamarans, and kayaks. This site is the location of Captain Cook's monument and within a Marine Life Conservation District (MLCD), restricting the take of any marine life.

The Kahauloa site is located off a minor cove and minor point within the south side of Kealakekua Bay. The slope of this reef is moderate. Unlike Kaawaloa, there is no management district or protection for the marine life at this site. Just upland and directly adjacent to Kahauloa is the community of Napoopoo where waste is disposed by cesspools and septic tanks with leach fields.

Although one might suspect differences between nutrient concentrations and algal δ^{15}N values between these two sites from the differential extents of development and waste disposal, no such differences were detected (Parson *et al.* 2008), yet samples throughout the bay show an increase of nitrogen, but no change in phosphate values between 1968 and 2005 (Doty 1968, Parson *et al.* 2008). Nevertheless, significant differences were present between the benthic communities. Kaawaloa has a significantly greater percent cover of healthy corals (Kaawaloa = 62%, Kahauloa = 35%), CCA (Kaawaloa = 14%, Kahauloa = 7.7%), fleshy algae (Kaawaloa = 1.5%, Kahauloa = 0.5%), and greater coral diversity (Kaawaloa = 1.0 H′, Kahauloa = 0.4 H′), while Kahauloa has a significantly greater percent cover of turf algae (Kaawaloa = 14%, Kahauloa = 40%) (Parson *et al.* 2008). Kaawaloa has both a significantly greater number (Kaawaloa = 1.54 specimens/m^2, Kahauloa = 1.07 specimens/m^2) and biomass (Kaawaloa = 104.48 g/m^2, Kahauloa = 66.54 g/m^2) of herbivorous fish than Kahauloa (Williams 2009). Kahauloa receives greater water motion than Kaawaloa, because Kaawaloa reef is located in a cove and Kahauloa is located just offshore of Manini Point, a more exposed area of the Bay (Parson *et al.* 2008). Finally, when high surf is directed to the west coast of Hawaii Island the Kahauloa reef becomes a highly esteemed surf break known as “Maninis” (C. Okano, pers. comm., 2003), suggesting substantial disturbance to the benthic community especially during the winter season.

3.3.2 Experimental Design

The approach to this work follows Smith *et al.* (2001) in almost all aspects of the design and implementation with the modification of a split plot design, which reduced the number of racks placed on the reef, therefore minimizing the potential of disturbing the well-managed reef system within Kealakekua Bay. Like Smith *et al.* (2001) this experiment tests the impact of increased nutrients and reduced herbivory independently and simultaneously, but unlike Smith *et al.* (2001), this experiment was replicated at two adjacent reefs, with contrasting land use and residential densities.

The experimental design consisted of racks framed by white poly vinyl chloride (PVC) tubes. Poultry netting with a mesh size of 2.8 cm coated with green PVC was fitted on the frame and served as the platform for these racks. Experimental settlement tiles were then secured to the netting with zip ties. Tiles were composed of flat gray PVC (Hixon and Brostoff 1985; Smith *et al.* 2001) with an area of 50 cm^2 (Smith *et al.* 2001). Tiles were exposed to three levels of caging: no cage, partial cage, and full cage. The no cage treatment exposes tiles to ambient levels of herbivory, while full cage treatment limits tiles exposure to macro-herbivores. Partially caged treatments had caging material only on the top with sides exposed to herbivory. The partially caged treatment served as cage control (Thacker *et al.* 2001; Belliveau and Paul 2002; Diaz-Pulido and McCook 2003). Tiles that were fully caged and not caged were exposed to both ambient and enriched concentrations of nutrients. Partially caged tiles were only exposed to ambient nutrient concentrations. Fouling organisms were removed from cages monthly.

Enriched nutrient concentrations were achieved by placing 1.4 kg of United Horticultural Supply lawn fertilizer (11% NH_4, 10% NO_3, 7% P_2O_5) into unglazed terra cotta flowerpots, 20 cm diameter at their widest. The drainage hole at the bottom of the pots was plugged with a cork, while at their widest dimension, pots were fixed to PVC plates with silicone sealant. Pots were then secured on the racks with zip ties when deployed. Fertilizer pots were replaced monthly. All tiles exposed to enriched nutrient concentrations were < 50 cm from fertilizer pots, well within the usual 1 m enrichment plume (Smith *et al.* 2001).

Two types of racks were placed on the reef. The first type of rack was the nutrient enrichment rack, which included a fertilizer pot with tiles under the fully caged and no cage treatments. The dimension of first type of rack was 80 by 50 cm. The second type of rack was the non-nutrient enrichment rack. It possessed tiles under all three levels of caging with no fertilizer source. The dimension of second type of rack was 100 by 50 cm. The racks were systematically alternated on a 10 m depth contour (Belliveau and Paul 2002; Smith *et al.* 2010) parallel to shore. A distances of 10 m separated racks. Five of each rack type was placed at Kaawaloa and Kahauloa for a total of ten racks per site. Racks were secured to the reef with zip ties on dead coral coated with turf algae or CCA.

Racks were attached to the reef on 3/19/04, the first set of tiles was collected on 6/18/04, the second on 8/19/04, and the third on 10/21/04. During each collection event, two tiles were randomly recovered from each rack for biomass quantification and percent cover determinations. After recovering tiles from the field, they were placed in plastic bags and frozen until analysis.

3.3.3 Quantifying Tile Parameters

Prior to biomass analysis, invertebrates were detached and tiles were rinsed with freshwater to remove all visible sediment. Corals were not detected on any of the tiles. Then, all algal matter was scraped from the top of tiles with a metal scraper and placed into pre-weighed coffee filters. Coffee filters were placed in a funnel that allowed water to escape. Samples within coffee filters were then dried at 60° C in a drying oven. Samples remained in the drying oven until a consistent dry weight was attained.

Algal percent cover was determined with a mini double-strung 8.7 x 8.7 cm quadrat containing 10 evenly spaced strings: five vertical and five horizontal, forming 25 points within the quadrat. The quadrat was then fitted over settlement tiles and 20 of the 25 points were randomly selected to be sampled. Under each of the 20 selected points, algal specimens were identified to the lowest taxonomic level possible.

3.3.4 Statistical Analysis

Prior to analysis, biomass data were log transformed so that they would conform to the requirement of homogeneity of variance. In addition, with the aim of potentially simplifying analysis, algal biomass in cage-control treatments was compared to biomass in control treatments within sites during each time period using *t*-tests. In all cases, differences between control and cage-control were highly non-significant indicating no effect of cage-controls on algal biomass. Therefore, cage-control treatments were removed from subsequent analysis. Remaining treatments (control, nutrient, cage, nutrient-cage) and experimental configuration were suitable for analysis as a split-plot design. Split plot analysis was conducted using the Fit Model platform in JMP 7.0 with Nutrient and Cage as main factors, and Block (nutrient) as a random factor. Interaction terms were Cage * Nutrient and Cage * Block (nutrient). Analyses were separately conducted for each time period and location. Post hoc tests of difference between treatments were conducted using Tukey's multiple comparison tests. To determine if differences existed between sites *t*-tests were used to compare the treatments of each time period. A *t*-test for each time period was appropriate because only two data sets were being compared. The time period and treatment were the same in these comparisons; the only difference was the site. *T*-tests were carried out in MINITAB 14.

The PRIMER 5 program and the ANOSIM analysis was used to determine the differences between algal species percent cover of treatments within sites, and to determine the difference within each treatment within each time period between sites. The ANOSIM analysis in PRIMER is a pair wise comparison. To determine significance ANOSIM produces a sample statistic or *r*-value and significance level of sample statistic or *p*-value. The SIMPER analysis was then used to determine the contribution of each algal species to the similarity within each treatment. In this case, significant differences were found between the control and cage controls at Kahauloa during the five and seven month time period. Therefore, the cage control treatments were included in this analysis.

Significance levels for all analyses was alpha ≤ 0.05

3.4 RESULTS

3.4.1 Cage-Control Treatment, Biomass

No significant difference was detected between the algal biomass of control and cage-control treatments within each site after all time periods ($0.090 < p < 0.985$).

3.4.2 Cage-Control Treatment, Cover

No significant difference was evident between the percent cover of control and cage-control treatments at Kaawaloa after any time period (Tables 9 - 11). However, a significant difference was detected between the percent cover of control and cage-control treatments at Kahauloa after five (Table 13) and seven months (Table 14). These differences can be attributed to increases in cover of *Ralfsia expansa* in the cage-control treatment (Table 16).

3.4.3 Nutrient Enrichment Treatment, Biomass

Nutrients alone had a limited affect on algal biomass in this experiment at Kealakekua Bay (Figures 28 and 29). At Kaawaloa, the algal biomass in the nutrient enrichment treatment was significantly greater than the control after three months, but not after five and seven months (Figure 28). After three months, the biomass of the nutrient treatment was 2.3 greater than the control at Kaawaloa. At Kahauloa, the nutrient treatment did not have a significant effect on algal biomass after any time period when compared to the control (Figure 29).

3.4.4 Nutrient Enrichment Treatment, Cover

A significant difference existed between the percent cover of the control and nutrient treatments at each site for the majority of time (Tables 9 - 14). At Kaawaloa, nutrients significantly altered the percent cover after three (Table 9) and seven months (Table 11), but not after five months (Table 10). Differences in percent cover between control and nutrient treatments after three and seven months at Kaawaloa were attributed to an increase in *Ulvella* spp. and loss of open space by the nutrient treatment when compared to the control (Table 15).

At Kahauloa, nutrients significantly altered the percent cover after three (Table 12) and seven months (Table 14), but not after five months (Table 13). Differences in algal species percent cover between control and nutrient treatments after three months at Kahauloa may be attributed to differences in non-calcified crustose algal species cover (Table 16). Although, CCA and open space were the top two functional groups contributing to similarity of both control and nutrient treatments after three months at Kahauloa, *Ulvella* spp. was the third contributing algal species to similarity within the control (Table 16). While, *Ralfsia expansa* was the third contributing algal species to similarity within the nutrient treatment at Kahauloa after three months (Table 16). Differences in percent cover between control and nutrient treatments after seven months at Kahauloa appear to be tied to the differences in CCA and *Sphacelaria* spp. CCA were the top functional group contributing to similarity within the control, and *Sphacelaria* spp. was the top species contributing to similarity within the nutrient treatment at Kahauloa after seven months (Table 16).

3.4.5 Caging (Reduced Herbivory) Treatment, Biomass

Caging alone did have an effect on algal biomass at Kealakekua Bay (Figures 28 and 29). Caging significantly increased algal biomass after all time periods at Kaawaloa (Figure 28). Relative to the control, the biomass of the cage treatment was 12.8 times greater after three months, 7.5 times greater after five months, and 2.7 times greater after seven months at Kaawaloa. At Kahauloa, cage treatments did not have a significant effect on algal biomass in early months, but did significantly increase algal biomass after seven months (Figure 29). The biomass of the cage treatment was 2.8 times greater than the control after seven months at Kahauloa.

3.4.6 Caging (Reduced Herbivory) Treatment, Cover

Percent cover comparisons between control and cage treatments resulted in significant differences at both sites after every time period (Tables 9 - 14). The major contributing species to similarity within the control treatment at Kaawaloa after all time periods were CCA; the major contributing species to similarity within the cage treatment at Kaawaloa after all time periods was *Jania pumila*, a calcified articulated coralline alga that is typically considered a turf species (Table 15).

Although CCA were the major contributor to similarity for the control and cage treatments at Kahauloa early months, open space was the second major contributor to similarity within the control after three months and *Calothrix* spp. was the second major contributor to similarity within the control after five months (Table 16). *Sphacelaria* spp. was the second major contributor to similarity within the cage treatment after three and five months at Kahauloa (Table 16). After seven months, CCA were again the top contributor to similarity within the control, but *Jania pumila* was the top contributor within the cage treatment after the same time period at Kahauloa (Table 16).

3.4.7 Nutrient-Cage Treatment, Biomass

To explore the potential synergistic interactions of increased nutrients and reduced herbivory, nutrient and cage treatments were employed simultaneously at both sites during all time periods with significant results when compared to the control (Figures 28 and 29). At Kaawaloa, algal biomass of the nutrient-cage treatment was 9.2 times greater after three months, 11.3 times greater after five months, and 8.7 times greater after seven months when compared to the control. At Kahauloa, algal biomass of the nutrient-cage treatment was 6.6 times greater after three months, 2.8 times greater after five months, and 4.9 times greater after seven months when compared to the control.

When compared to the nutrient treatment the nutrient-cage treatment was significantly greater during all time periods at both sites (Figures 28 and 29). At Kaawaloa, algal biomass of the nutrient-cage treatment was 4.0 times greater after three months, 8.1 times greater after five months, and 5.4 times greater after seven months when compared to the nutrient treatment. At Kahauloa, algal biomass of the nutrient-cage treatment was 6.9 times greater after three months, 2.5 times greater after five months, and 3.1 times greater after seven months when compared to the nutrient treatment.

The nutrient-cage treatment was also significantly greater than the cage treatment at Kahauloa after all time periods (Figure 29), but at Kaawaloa, the nutrient-cage was significantly greater than the cage treatment only after seven months (Figure 28). At Kahauloa, algal biomass of the nutrient-cage treatment was 2.6 times greater after three months, 1.5 times greater after five months, and 2.3 times greater after seven months when compared to the cage treatment. At Kaawaloa, algal biomass of the nutrient-cage treatment was 3.1 greater after seven months.

3.4.8 Nutrient-Cage Treatment, Cover

The nutrient-cage treatment had a significant effect on percent cover at both sites after every time period when compared to the control (Tables 9 - 14). At Kaawaloa and Kahauloa, differences in form and functional groups of major contributing species are driving the difference between the algal species percent cover of the control and nutrient-cage treatments (Tables 9 - 11). At Kaawaloa, the top three major contributing species to similarity within the control after all time periods were crustose species such as CCA, *Calothrix* spp., and *Ulvella* spp., or open space (Table 15). The top three major contributing species to similarity within the nutrient-cage treatment at Kaawaloa during all time periods was turf species such as the calcified *Jania pumila*, *Centroceras* spp., *Sphacelaria* spp. and the calcified *Amphiroa* spp., but CCA were the third major contributing group to similarity within the nutrient-cage treatment at Kaawaloa after three months (Table 15). At Kahauloa, the top three major contributing species to similarity within the control after all time periods were either crustose species such as CCA, *Calothrix* spp., and *Ulvella* spp. or open space. However, *Sphacelaria* spp. was the third major contributing species to similarity within the control at Kahauloa after three months (Table 16). The top three major contributing species to similarity within the nutrient-cage treatment at Kahauloa after all time periods was turf species such as the calcified *Jania pumila*, Vaughaniella stage, *Sphacelaria* spp., and *Dictyota friabilis*. Open space was the second most contributing cover to similarity within the nutrient-cage treatment at Kahauloa after five months (Table 16).

When compared to the nutrient treatment the algal species percent cover of the nutrient-cage treatment was significantly different within both sites after every time period (Tables 9 -14). At Kaawaloa, the major contributing species to similarity for the nutrient treatment was open space and crustose species such as CCA, *Calothrix* spp., and *Ulvella* spp.; while the major contributing species to similarity for the nutrient-cage treatment was turfs such as *Jania pumila*, *Centroceras* spp., *Amphiroa* spp., and *Sphacelaria* spp. (Table 15). However, CCA was the third major contributing species to similarity for the nutrient-cage treatment at Kaawaloa after three months (Table 15). At Kahauloa CCA was the top major contributing species to similarity for the nutrient treatment after three and five months, and *Sphacelaria* spp. after seven months; while *Jania pumila* was the top major contributing species to similarity for the nutrient-cage treatment after all time periods (Table 16).

When compared to the cage treatment, algal cover of the nutrient-cage treatment was significantly different within both sites after every time period (Tables 9 - 14). At Kaawaloa after three months, *Jania pumila* was top the major contributing species to similarity for the cage treatment, while *Centroceras* spp. was the top major contributing species to similarity for the nutrient-cage treatment. Although *Jania pumila* was the top major contributing species to similarity for both cage and nutrient-cage treatments after five and seven months, CCA were the second major contributing species to similarity after five months for the cage treatment and *Centroceras* spp. was the second major contributing species to similarity after five months for the nutrient-cage treatment. After seven months, *Chondria* spp. was the third contributing species for the cage treatment and *Amphiroa* spp. was the third contributing species for the nutrient-cage treatment. At Kahauloa, CCA was the top major contributing species to similarity within the cage treatment after three and five months, while *Jania pumila* was the top major contributing species to similarity within the nutrient-cage treatment after three and five months. After seven months, the *Vaughaniella* stage was the second major contributing species to similarity for the cage treatment and *Ceramium* spp. was the second major contributing species to similarity for the nutrient-cage treatment.

3.4.9 Trajectories of Succession

The Kaawaloa MDS plot displayed two trajectories for development of the benthic community on settlement tiles. One trajectory illustrates changes in species treatments that were caged; the other trajectory reflects species composition when exposed to ambient levels of herbivory (Figure 30). Trajectories at Kahauloa were different (Figure 31). The control and cage control trajectories were close to each other and seemed to nearing a state of equilibrium at Kahauloa (Figure 31). The trajectories of the cage and nutrient-cage treatments were similar to each other and different from the control and cage control trajectories (Figure 31). The assemblage of species that develop under nutrient enrichment was intermediate (Figure 31). After three and five months, the nutrient treatment trajectory resembled the control and cage-control treatments (Figure 31). Although the point representing the species percent cover of the nutrient treatment after seven months was closer to the points of the control and cage-control treatments after seven months, the path of the nutrient treatment trajectory after seven months looks more like the cage and nutrient-cage treatments at Kahauloa (Figure 31).

3.4.11 Between Site Comparisons of Identical Treatments, Biomass

When comparing the accumulation of algal biomass under the same experimental treatment between two sites after each time period, the algal biomass of the control was significantly different and 1.4 – 7.9 times greater at Kahauloa after all periods (Table 8). The same was true for the nutrient treatment that was 1.4 – 3.3 times greater at Kahauloa. There was no difference in algal biomass between cage treatments at of Kaawaloa and Kahauloa after any time period (Table 8). When comparing the algal biomass of the nutrient-cage treatment, Kahauloa was significantly different and 5.7 times greater only after three months. No significant differences were displayed after five and seven months (Table 8).

3.4.12 Between Site Comparison of Nutrients and Herbivory, Biomass

When comparing results of nutrient enrichment and reduced herbivore pressure on algal biomass between Kaawaloa and Kahauloa the effects of nutrient enrichment with natural herbivore pressure was slightly greater at Kaawaloa, but the effects of nutrient enrichment when herbivore pressure was reduced was much greater at Kahauloa (Figures 28 and 29). The effects of reduced herbivory with ambient nutrient concentrations were greater at Kaawaloa (Figures 28 and 29).

3.4.13 Between Site Comparison of Nutrients and Herbivory, Cover

The effects of the cage-control on algal species percent cover were greater at Kahauloa than Kaawaloa. The effects of increased nutrients with ambient herbivory, ambient nutrient with reduced herbivory, and increased nutrients with reduced herbivory on algal species percent cover was comparable at both sites (Table 9 - 14).

3.5 DISCUSSION

This multi factorial experiment was simultaneously run at two sites and has the power in teasing out the relative and synergistic roles of nutrients and herbivory (Belliveau and Paul 2002) on reefs with different management strategies.

The influence that herbivores have on tropical reef succession (Hixon and Brostoff 1996) is exemplified by the successional trajectories displayed in the MDS plot of percent cover at Kaawaloa in contrast with data from Kahauloa. These plots showed that the relative influence of nutrient enrichment on algal succession is minimal when compared to the influence of herbivory. However, a comparison of trajectories suggests that nutrients may have a greater effect on percent cover at Kahauloa when compared to Kaawaloa. The nutrient treatment trajectory at Kahauloa implies that the impact of nutrients on algal species cover does occur, but is delayed relative to the effects of reduced herbivory. If this is generally applicable elsewhere, tropical reefs may degrade faster when herbivory is reduced relative to when eutrophication occurs. Nevertheless, trajectories from Kahauloa suggest that over time both herbivore reduction and nutrient enrichment may degrade reefs equally.

An assessment of biomass results revealed that lessening herbivory by caging had a greater effect at Kaawaloa, while increase nutrient concentration had a greater effect at Kahauloa especially when herbivory was reduced. These results support the premise that ambient levels of herbivory and nutrients are greater at Kaawaloa than Kahauloa (Parson *et al.* 2008; Williams 2009), suggesting that within Kealakekua's reefs there are different levels of resilience. Different strategies would have to be applied to different regions of the bay for effective management. A reduction in nutrient concentration would be more beneficial for Kaawaloa, while an increase in herbivory would be more beneficial for Kahauloa. These differences highlight the importance of managing tropical reef on a reef-by-reef basis and that a single strategy will not be equally effective in an effort to improve the resiliency of reefs throughout the tropics or even within a bay such as Kealakekua.

Although a reduction in herbivore pressure resulted an increase of algal biomass (Randall 1967; Hatcher and Larkum 1983; Miller *et al.* 1999; Smith *et al.* 2001; Belliveau and Paul 2002; Burkepile and Hay 2009; Vermeij *et al.* 2009) at both sites within Kealakekua Bay, Kaawaloa has significantly greater numbers and biomass of herbivorous fish when compared to Kahauloa (Williams 2009), treatments exposed to ambient levels of herbivory had a greater algal biomass at Kahauloa when compared to Kaawaloa.

In general, studies from Hawaiian reefs with herbivorous fish populations greater than 40 g/m^2, such as Puako (Smith *et al.* 2010) and Kealakekua Bay, show that early succession results in high CCA cover in both ambient and enriched nutrient regimes. Results suggest that herbivores prefer to graze on genera such as *Centroceras*, *Distromium*, *Jania*, and *Sphacelaria*, which represented the majority of cover in caged treatments at Kealakekua Bay.

When compared to the cage treatments of Randall (1967) which was also conducted at Kealakekua Bay, but at a shallower depth (~2 m), cage treatment similarities were at the species level. Across 50 years of land-based development for this area, *Jania* spp. and *Sphacelaria* spp. were two of the top four species within the cage treatment of Randall (1967), and remain major contributors to cover within this study's cage treatment.

Studies outside of Hawaii that quantify algal species percent cover also show differences between control and cage treatments (Miller *et al.* 1999, Belliveau and Paul 2002; McClanahan *et al.* 2003; Burkpile and Hay 2009). Some studies such as those from Guam (Belliveau and Paul 2002) and the Florida Keys (Burkpile and Hay 2009) even show similarities in algal species of cage treatments when compared to Puako (Smith *et al.* 2010) and Kealakekua. Other studies from the tropics show differences in algal species of cage treatments when compared to Kealakekua (Miller *et al.* 1999; McClanahan *et al.* 2003; Sotka and Hay 2009). At Kealakekua and the numerous sites mentioned above, a reduction in herbivory generally results in increases of turf and macro-algae cover and decreases in CCA and open space. This suggests that herbivory sets the stage for coral recruitment and may increase coral survivorship (Morse and Morse 1996; Harrington *et al.* 2004; Hughes *et al*. 2007).

Algal species percent cover of nutrient-cage treatment at Kealakekua suggest that if a phase shift was to occur due to reduced herbivory and increased nutrient concentrations within this bay the first signs of it would be an increase in cover by algal species such as *Amphiroa* spp., *Centroceras* spp., *Ceramium* spp., *Dictyota friabilis*, *Jania pumila*, and *Sphacelaria* spp. All of these algal species are common throughout the reefs of Hawaii (Abbott 1999; Abbott and Huisman 2004), cycle thru an isomorphic alternation of generations, and a number of these species have been documented within Kealakekua Bay since 1955 (Randall 1967). Therefore, the cover of these algal species may serve as an indicator of reef resilience at Kealakekua Bay.

The effects of nutrient additions on biomass when herbivory was reduced differed between sites at Kealakekua. At Kaawaloa, nutrients had a limited effect on algal biomass when herbivory was reduced (Miller *et al.* 1999; Belliveau and Paul 2002; Burkepile and Hay 2009), while at Kahauloa nutrients significantly increase algal biomass when herbivory was reduced (Smith *et al.* 2001; McClanahan *et al.* 2003; Littler *et al.* 2006).

Differences in the effect of nutrients on algal biomass when herbivory is reduced between Kaawaloa and Kahauloa may be due to water quality, water motion, and herbivore pressure. Kaawaloa had a trend of greater ambient nutrient concentrations when compared to Kahauloa (Parson *et al.* 2008). Elevated nutrient concentrations have been shown to negate the effect of nutrient additions elsewhere in the tropics (Smith *et al.* 2004; Smith *et al.* 2005). Water motion is greater at Kahauloa than at Kaawaloa (Parson *et al.* 2008). The lower level of water motion at Kaawaloa may not reduce the boundary layer enough to replenish nutrient concentrations needed by algal communities (Wheeler 1980).

Although excess nutrients have been shown to increase algal biomass on tropical reefs (Banner 1974; Lapointe 1997; Glenn *et al.* 1999; Kuffner and Valerie 2001; Smith *et al.* 2001), nutrients alone had a limited effect on algal biomass throughout Kealakekua, similar to other studies from the tropics (Drew 1983; Grigg 1995; McCook 1996; Miller *et al.* 1999; Thacker *et al.* 2001; Belliveau and Paul 2002; Jompa and McCook 2002; Szmant 2002; Diaz-Pulido and McCook 2003; Burkepile and Hay 2009; Vermeij *et al.* 2009).

The limited effect of nutrients at Kealakekua may be due to elevated ambient nutrient levels. The greatest nitrate + nitrite value during the duration of this study at Kealakekua was ~165 µM (Parson *et al.* 2008), which is a 4.1 times increase from the greatest values detected in 1968 (Doty 1968). This increase in ambient nutrient levels suggests that the algal community at Kealakekua is nutrient replete, similar to results from Key Largo (Smith *et al.* 2004b) and Maui (Smith *et al.* 2005). This could be especially true at Kaawaloa where nutrients did not increase algal biomass in treatments exposed to herbivory and had a limited effect in treatments that were not exposed to herbivory.

Another consideration is that nutrient enrichment may have increased alga growth at Kealakekua. However, herbivores are generally nitrogen limited (Miller *et al.* 1999), which may make algae enriched with nutrients more palatable, resulting in a greater consumption rates on enriched algae when compared to algae grown in ambient conditions (Jimenez *et al.* 1996; Diaz-Pulido and McCook 2003; Boyer *et al.* 2004; Burkepile and Hay 2009). This interaction may have occurred at Kahauloa because nutrient enrichment did not increase algal biomass on tiles when exposed to herbivory, but increased nutrient concentrations did increase algal biomass when herbivory was limited.

The various responses that nutrient enrichment experiments have on algal biomass can be attributed to variations in spatial scale, ambient nutrient concentrations, nutrient enrichment concentrations, flow, and time (Smith *et al.* 2010). However, nutrient enrichment experiments are also not uniform. Twelve published papers use eight different methods to achieve 'nutrient enrichment' (Miller *et al.* 1999; Smith *et al.* 2001; Thacker *et al.* 2001; Belliveau and Paul 2002; Jompa and McCook 2002; Diaz-Pulido and McCook 2003; McClanahan *et al.* 2003; McClanahan *et al.* 2005; Littler *et al.* 2006; Burkepile and Hay 2009; Mork *et al.* 2009; Sotka and Hay 2009) despite the knowledge that different nutrient enrichment methods may produce different results in algal biomass, growth, and percent cover (McClanahan *et al.* 2005).

At Kealakekua CCA were the dominant cover for the control and nutrient treatments. Comparable results are shown by other experiments throughout the tropics (Miller *et al.* 1999; Burkepile and Hay 2009; Sotka and Hay 2009; Smith *et al.* 2010).

Although significant differences were found between the percent cover of control and nutrient treatments, the differences were revealed as changes in relative cover of species. The control treatment often had greater CCA cover and open space, while the nutrient treatment had greater cover of turf and non-calcified crusts. These differences may facilitate a decrease of coral recruitments, due to a reduction in CCA cover and open space at nutrient enriched sites relative to ambient sites (Morse and Morse 1996, Harrington *et al.* 2004).

The diverse effects that nutrient enrichment has on various algal species (Schaffelke 1999) can help explain the development of different algal communities. At Kealakekua Bay, the presence of species such as *Ralfsia expansa*, *Sphacelaria* spp., and

Ulvella spp. in nutrient enrichment treatments suggest that they are better at sequestering access nutrients and using it for growth relative to other algal species.

Difference between the percent cover of control and cage-control treatments at Kahauloa imply that caging artifacts such as water motion, chemicals released from the cage, light intensity, and limits on largest herbivores may have an influence on percent cover.

It is unlikely that water motion or chemicals released from cages was a factor that contributed to the differences in percent cover between the control and cage-control treatments. Control and cage-control treatments were adjacent, placing treatments in similar water motion (Smith *et al.* 2001; McClanahan *et al.* 2003; Mork *et al.* 2009) and chemical regimes. Caging was present on the top of cage controls placing the control and cage control treatment under similar irradiance levels.

Restriction of the largest herbivores is the most difficult caging artifact to dismiss. Similar to this experiment, Diaz-Pulido and McCook (2003) also detects minor differences between control and cage-control treatments. In their study, the cage-control is considered an intermediate herbivore level that allows the majority of herbivores to interact with the substrate in question, while restricting the largest herbivores (Diaz-Pulido and McCook 2003). Similar interactions may have occurred at Kahauloa, resulting in differences between the percent cover of the control and cage-control treatments. Partial caging may have restricted large specimens of *Chlorurus perspicillatus, Scarus rubroviolaceus,* and *Naso unicornis* (Williams 2009) from interacting with tiles.

At Kealakekua Bay, a reduction of herbivory often significantly increased algal biomass, while nutrient enrichment often did not (Miller *et al.* 1999; Thacker *et al.* 2001; Jompa and McCook 2002; Diaz-Pulido and McCook 2003; Burkepile and Hay 2009). Reductions in herbivory also had greater effects on cover by altering algal communities at the form and function level, but nutrients had less of an effect by altering communities at the species level. The results of this study suggest the herbivory has a greater influence on algal biomass and cover than nutrients, in particular within this short term, seven-month time frame at Kealakekua Bay.

3.6 CONCLUSION

Although the results from numerous studies have consistently demonstrated the adverse effects of herbivore reduction on tropical reefs, the same cannot be said for eutrophication. More studies need to be conducted to resolve the effects of increased nutrient concentrations on tropical reefs. In general, reductions in nutrient concentration and increases in herbivory enacted simultaneously should benefit all tropical reefs. However, the benefits are not likely to be equally exhibited by all reefs as suggested by the results of this study from two sites within Kealakekua Bay. The best solution would be to manage tropical reefs as individual units by determining the needs of each reef in an effort to increase resilience and minimize the threat of phase shifts.

CHAPTER 4. THE POTENTIAL TO USE INDIGENOUS HERBIVOROUS REEF FISH AS BIOLOGICAL CONTROL AGENTS TO MANAGE ALIEN ALGAE IN HAWAII

4.1 ABSTRACT

Alien, non-indigenous algae have invaded Oahu's reefs and spread across the high islands of Hawaii at alarming rates, thereby creating severe threats to coastal communities. The consumption of alien algae by herbivorous fish has been suggested as means to control alien algae in Hawaii. To evaluate this option multiple-choice feeding assays using detached algae as well as no-choice feeding assays using attached algae in a laboratory setting determined that the indigenous herbivorous reef fishes *Acanthurus triostegus* and *Kyphosus vaigiensis* are potential biological control agents to manage the alien algae *Acanthophora spicifera* and *Gracilaria salicornia*. These fish can also achieve different objectives in an effort to manage alien algae. *A. triostegus* was more suited to control attached algal populations, and *K. vaigiensis* was more effective in reducing dispersal by floating algal fragments. This limited analysis of herbivorous fish has resulted in two biological control agents to manage alien algae in Hawaii, yet a broader analysis of Hawaiian herbivorous fish could reveal an abundance of approaches to control alien algal overgrowth in coastal Hawaiian communities.

4.2 INTRODUCTION

Alien species are one of the primary threats to our planet's natural environment (Vitousek *et al*. 1997). Alien species are a threat to biodiversity (Clout and DePoorter 2005; Riley 2005) and ecosystems (Schaffelke *et al*. 2006). In Hawaii, alien algae are a realized threat, taking over reefs (Rogers and Cox 1999; Smith *et al*. 2002; Smith *et al*. 2004; Conklin and Smith 2005; O'Doherty and Sherwood 2007), sea-grass meadows (Smith *et al*. 2002), and culturally significant fishponds (Weijerman *et al*. 2008). These marine alien species are not only a threat to natural and cultural resources, but also to commerce, including fishing, tourism, and real estate value (Bax *et al*. 2003; Smith *et al*. 2004). Hawaii has five extremely invasive alien algae: *Acanthophora spicifera, Avrainvilla amadelpha*, *Gracilaria salicornia, Hypnea musciformis*, and the *Eucheuma-Kappaphycus* complex (Smith *et al*. 2002).

There have been numerous studies analyzing methods to control alien algae species. In Kaneohe Bay, Hawaii, the effectiveness of controlling species in the *Eucheuma-Kappaphycus* complex via manual removal, biological control by *Tripneustes gratilla*, and use of an underwater vacuum have been explored (Conklin and Smith 2005; Conklin 2007). At Kaloko-Honokohau National Historic Park, Hawaii, the effectiveness of manual removal, shading, and the establishment of rock shelters as a means to enhance herbivorous fish populations have been tested in an effort to control *A. spicifera*. The enhancement of herbivorous fish populations by establishing rock shelters was the most successful method at Kaloko-Honokohau National Historic Park (Weijerman *et al*. 2008).

Recent evidence demonstrates that over fishing has depleted target fish populations in the Hawaiian Islands (Williams *et al*. 2008). Such realizations, along with expanding alien invasive algal populations (Rogers and Cox 1999; Smith *et al*. 2004; Conklin and Smith 2005) have led the State of Hawaii's Department of Land and Natural Resources, Division of Aquatic Resources (DAR) to establish the Kahekili Herbivore Fisheries Management Area (FMA) on the island of Maui. Herbivorous fish and urchins are protected at this FMA in an effort to control alien algae. However, before DAR can manage herbivorous populations by establishing additional herbivore FMAs or through restrictions on the take of herbivores, more fundamental information is critical to understand the ecological services provided by herbivorous reef fish.

To determine if enhancing herbivorous fish populations could be an appropriate means of controlling alien algae, one needs to know that the fish in question will graze the targeted alien algae. Although a number of studies have analyzed the diet and preference of herbivorous fish in the tropical Pacific and warmer temperate waters (Tsuda and Bryan 1973; Bryan 1975; Wylie and Paul 1988; Schupp and Paul 1994; Clements and Choat 1997; Choat *et al*. 2002), limited research has been conducted in Hawaii. In 1961, Randall conducted multiple-choice algal feeding experiments with juvenile *Acanthurus triostegus*. In 1968, Jones published the most comprehensive study of herbivorous fish diets to date. Both of these studies (Randall 1961; Jones 1968) occurred before Hawaii had experienced significant algal overgrowth on reefs and sea-grass habitats. A more recent study used pair wise feeding trials, which included alien algae and their native counterparts. During the pair-wise feeding trials two species of algae were offered to an assortment of Acanthurids and juvenile Scarids to determine if herbivorous fish prefer native or alien algae (Smith *et al.* 2004a). Although these studies have provided the foundation to better understand what herbivorous fish eat on Hawaii's reefs, the state of knowledge is not sufficient to manage herbivorous fish species as a means to control alien algae.

Some believe that herbivores prefer native to alien algae, and are likely to be ineffective at controlling alien algae (Williams and Smith 2007). However, experiments and observations from Hawaii (Conklin and Smith 2005; Conklin 2007; Arthur and Balaz 2008; Weijerman *et al.* 2008; Vermeij *et al.* 2009) suggest that alien algae and herbivore interactions are difficult to predict.

In an effort to determine if it is feasible to use herbivorous fish as a means to control alien algae, this study will experimentally determine the algal preference and consumption rates of two indigenous herbivorous reef fish. The two alien algae analyzed in this study are *A. spicifera* and *G. salicornia*, while the two fish species sampled in this study are *Kyphosus vaigiensis* and *A. triostegus*.

A. spicifera is the most widespread alien algae in Hawaii, occurring on the islands of Kauai, Oahu, Molokai, Lanai, Maui, and Hawaii (Smith *et al.* 2002) and typically inhabits intertidal regions and tide-pools where this alga displaces native benthic species (Smith *et al.* 2002). Although *A. spicifera* reproduces sexually and asexually, it is unclear as to which method is employed most often in Hawaii (O'Doherty and Sherwood 2007). Of the five most common alien algae in Hawaii, *A. spicifera* is the most palatable to Acanthurids and juvenile Scarids (Conklin 2007). On reef flats, *A. spicifera* is preferred over natives such as *Ulva lactuca* and *Sargassum* spp. (Vermeji *et al.* 2009). A correlation of *A. spicifera* densities with herbivorous fish biomass results in a significant negative relation (Conklin 2007). Fish such as *Naso lituratus*, *Naso unicornis* (Jones 1968), *Zebrasoma flavescens* (Wylie and Paul 1988), and the green sea turtle, *Chelonia mydas* (Arthur and Balaz 2008) prefer or consume *A. spicifera*; but the urchin *T. gratilla* prefers other algae over *A. spicifera* (Stimson *et al*. 2007).

G. salicornia has become a broadly distributed, dominant algal species on many reefs in Hawaii (Smith *et al*. 2002), where *G. salicornia* impacts benthic ecology, community dynamics, and biodiversity (Smith *et al*. 2004a) on Oahu, Molokai, and Hawaii (Smith *et al*. 2002). *G. salicornia* almost exclusively reproduces asexually via fragmentation (Nishimura 2000; Smith *et al*. 2002). The results of a pair wise preference test with Acanthurids and juvenile Scarids concluded that the native *Gracilaria coronopifolia* is preferred over *G. salicornia* (Smith *et al*. 2004a). Additional analysis from preference tests with Acanthurids and juvenile Scarids determined that *G. salicornia* is intermediate in preference when compared to five alien and five native algal species (Conklin 2007). A correlation of *G. salicornia* densities with herbivorous fish resulted in no clear relationship (Conklin 2007). Feeding trials with the urchin *T. gratilla* shows other algal species are preferred over *G. salicornia* (Stimson *et al*. 2007).

K. vaigiensis, brassy chub, or nenue inhabits a diverse array of habitats including reef crests (Galzin and Legendre 1987; Russ 1989), reef flats (Russ 1989; Hoey and Bellwood 2009), reef slopes (Russ 1989), reef bases, patch reefs (Hoey and Bellwood 2009), sea-grass beds, (Gell and Whittington 2002), estuaries (Bugalho *et al*. 2008), and transit habitats such as drifting fish aggregation devices (Taquet 2007). *K. vaigiensis* is often referred to as exclusively herbivorous (Russ1989; Clement and Choat 1997; Choat *et al*. 2002; Willmott *et al*. 2004; Mantyka and Bellwood 2007), but *K. vaigiensis* is also classified as herbivorous planktivore (Taquet 2007). Gut analysis of *K. vaigiensis* concluded that they feed on large thallate algae, often Chlorophyta and Heterokontophyta, but Rhodophyta are also consumed (Clements and Choat 1997; Choat *et al*. 2002; Choat *et al*. 2004; Crossman *et al*. 2005). A *Sargassum* translocation study showed that *K. vaigiensis* is one of three fish species that plays a significant role in *Sargassum* removal (Cvitanovic and Bellwood 2009), while another *Sargassum* translocation study shows no significant relationship between algal biomass removal and *K. vaigiensis* populations (Hoey and Bellwood 2010). An additional *Sargassum* transplant study shows that *N. unicornis* is the primary consumer of *Sargassum*, and *K. vaigiensis* is a distant second (Hoey and Bellwood 2009).

A. triostegus, convict surgeonfish, or manini is the most abundant Acanthurid in Hawaii (Randall 1961). *A. triostegus* inhabits a diverse array of habitats including intertidal reef crests, sub-tidal rock pavements, sub-tidal reef slopes (Robertson and Gaines 1986), fringing reefs, channels, barrier reefs, outer reef slopes (Galzin and Legendre 1987), estuaries (Bugalho *et al*. 2008), bays, harbors, exposed reefs, shallow waters, depths of 0 - 33 m (Randall 1961), and reefs disturbed by anthropogenic activities (Chabanet *et al*. 1997). Temperature tolerances of *A. triostegus* are between 16.2 – 35.1 ° C in the field and 13 - 36.5 ° C in the laboratory, while chlorinity tolerances are between 3.62 – 21.30 ‰ in the field (Randall 1961). Although *A. triostegus* inhabits a variety of habitats and has the ability to tolerate a wide array of temperature and chlorinity values, *A. triostegus* has a small home range (Randall 1961), and high site fidelity when foraging (Conklin 2007). Genetic studies also indicate that exchange of individuals mainly occurs between neighboring populations, while long distance migrations are rare (Planes 1993a;

Planes 1993b; Planes *et al*. 1996; Planes *et al*. 1998). A multiple-choice feeding experiment conducted with juvenile *A. triostegus* determined that the algae *Enteromorpha* sp. and *Polysiphonia* sp. are at the highest level of preference, while the next tier of preference included filamentous or turf algae along with a few macro-algae such as *Dasya* sp., *Gracilaria* sp., *Grateloupia* sp., and *Hypnea* sp. (Randall 1961). Another experiment that offers *A. triostegus* a carrageen matrix with variable levels of calcium found *A. triostegus* avoids carrageen with calcium compared to carrageen without calcium (Scupp and Paul 1994). Gut contents of *A. triostegus* from Hawaiian and Johnston islands contains mostly filamentous algae (Jones 1968), whereas gut contents of *A. triostegus* from Aldabra Atoll, India contains *Lyngbya* sp., *Gelidium* sp., and *Laurencia* sp. (Robertson and Gains 1986). Field observations from the Seychelles found that *A. triostegus* prefers epilithic or turf algae over other algae and often avoids macro-algae (Ledlie *et al*. 2007). A stable isotope study from the estuaries of Kosrae determined that the sea-grass *Enhalus acoroides* and the mangrove *Lumnitzera littorea* makes up $\geq$ 40% of *A. triostegus*'s diet (Bugalho *et al*. 2008).

This study will assess the ability of two indigenous herbivorous reef fishes, *K. vaigiensis* and *A. triostegus* to control two alien algae, *A. spicifera* and *G. salicornia* by analyzing multiple-choice feeding assays using detached algae and no-choice feeding assays using attached algae in the laboratory.

4.3 METHODS AND MATERIALS

4.3.1 Fish Husbandry

To conduct these experiments approval from the University of Hawaii Institutional Animal Care and Use Committee was required. A mortality rate of < 10 % was maintained throughout this study. Fish were never used more than once for the same treatment. All fish were returned to the wild after experiments.

K. vaigiensis were captured using two different methods during daylight hours. The first method involved rod and reel. Bait was offered about two m below the surface with a floater. Flour and blended canned fish were mixed to a doughy consistency and used for bait. The second method used to capture *K. vaigiensis* was a surround net. *A. triostegus* were captured while snorkeling at night. *A. triostegus* often lie motionless on the reef at night. *A. triostegus* were caught with a scoop nets when sleeping. After capture, both *K. vaigiensis* and *A. triostegus* were quickly placed in a 19 L bucket with seawater and carried to a waiting truck outfitted with an aerated 91 L storage container filled with seawater. The fish were then transported to the DAR Fisheries Research Station in Hilo within three hours after capture.

From June 2008 to June 2009, fish were housed and experiments were conducted at the DAR Fisheries Research Station in Hilo, Hawaii. The station is located within the Wailoa State Park alongside the Wailoa Estuary. Water is piped in from the Wailoa Estuary to the DAR Fisheries Research Station to sustain fish. The salinity within the tanks at the station varied from 10 to 34 ppt depending on tide and rainfall. The fish were housed in four m diameter fiberglass tanks. Fishes were acclimated at the station for at least two weeks prior to an experiment. Continuous flow was maintained while fish were kept in tanks. Fish were sustained on a varied diet consisting of fresh romaine lettuce, prepared dry nori (*Porphyra* spp.), the diatom *Melosira nummuloides*, Silver Cup (© 2009, All Rights Reserved) floating trout feed, and biological matter that naturally occurred in tanks. Flow into tanks was stopped prior to experiments to assure salinity was > 30 ppt throughout the duration of experiments. Feeding was restricted 24 hours prior to experiments. The depth of the water in the tanks was 40 to 45 cm during experiments.

4.3.2 Multiple-Choice Feeding Assays

A total of 14 algal species were offered simultaneously during the multiple-choice feeding assays. The algae were collected from intertidal regions and tide pools one to two days prior to experiments. The algae were aerated and kept under diffuse sunlight at the DAR station. Algae were offered to fish early in the morning, pre dawn when possible, and left in the tanks for 24 hours (Smith *et al*. 2004; Davis *et al*. 2005; Wessels *et al*. 2006). Each alga was offered to the fish by inserting them between the braids of yellow polypropylene rope, following Peyton (2009). The rope was about 20 cm long and weighted down with 89 g of lead on each end. Each species of algae was offered on its own piece of rope. The 14 algae were offered in a circular formation (Davis *et al*. 2005) about one m from the edge of the tank. The position of each alga in the circle was randomly assigned in each trial (Capper *et al*. 2006; Wessels *et al*. 2006). Controls were required for each trial to complete the consumption calculation. The weights of all algal specimens were determined using a balance (ACCULAB, VICON) with an accuracy of

0.001 g, before and after each experiment. Prior to placing algal specimens on the balance to determine weight they were patted dry with cotton towel to remove excess water. Within each experiment, effort was made to equalize the weight of algal specimens being offered. Two to five *K. vaigiensis* used for each multiple-choice feeding assay. The number of *A. triostegus* used for each multiple-choice feeding assay varied between six and 11. The number of fish used for each trial depended on the quantity of fish captured prior to each experiment. The weight of every experimental fish used in the multiple-choice feeding assays was determined after the trial, prior to release. A total of five multiple-choice feeding assay trials were achieved for each species of fish.

The consumption calculation applied in this study was based on the Wessels *et al.* (2006) Consumption Calculation as detailed below:

Consumption = $(T_i \times C_f/C_i) - T_f$

T_i = treatment algal weight prior to exposure to fish

T_f = treatment algal weight after being exposed to fish for 24 h

C_i = control algal weight at the start of a 24 h cycle

C_f = control algal weight at the end of a 24 h cycle

Although the multiple-choice feeding assays were limited by the algal species consistently available in the field, an attempt was made to assemble a diverse array of species. The algal array included species that are considered palatable, calcified, chemically defended, and structurally defended (Abbott 1999, Abbott and Huisman 2004). A total of 14 algal species were offered. Besides the two alien species, the algal array included, *Anfeltiopsis concinna*, *Amansia glomerata*, *Asparagopsis taxiformis*, *Dichotomaria marginata*, *Grateloupia filicina*, and *Pterocladiella capillacea*, from the division Rhodophyta; *Chnoospora implexa*, *Padina sanctae-crucis, Sargassum polyphyllum*, and *Turbinaria ornata* from the division Heterokontophyta; and *Chaetomorpha antennina* and *Ulva lactuca* from the division Chlorophyta.

4.3.3 No-Choice Feeding Assays

The no-choice feeding assays involved offering the alien algae *G. salicornia* and *A. spicifera*. The algae were attached to two different types of rocks to simulate the range of substrate encountered by herbivorous fish on Hawaiian reefs. The first type of rocks naturally occurred in the field. These were basalt rocks coated with a veneer of crustose coralline algae. The dominant cover was *G. salicornia*. An attempt was made to collect rocks without cracks and crevices that would be difficult for fish to access, and had a *G. salicornia* cover of $\geq$ 50 %. These rocks ranged in diameter from five to 15 cm. Other macro-algae that occurred on these rocks with low cover included *Cladophoropsis membranacea*, *Hypnea pannosa*, *Phyllodictyon anastomosans*, and *P. capillacea*. After this first type of rock was collected, they were housed at the DAR Fisheries Research Station in 1136 L plastic agricultural water troughs. Salinity was maintained between 30 to 35 ppt. Diffuse light, aeration, and appropriate nutrient concentrations were provided for these basalt rock communities. Water was changed every two weeks. Basalt rock treatments were offered to fish within a month of collection.

The second type of rocks offered to the fish was dried, bleached, beach collected skeletons of *Porites lobata* that ranged in diameter from 10 to 25 cm. As before, an attempt was made to collect rocks without cracks and crevices that would be difficult for fish to access. Coral skeletons were then cut in half with an electric circular miter saw to form a flat surface, making them stable and easier to attach to caging material. *A. spicifera* and *G. salicornia* were then fastened to the rocks with rubber bands. These coral skeletons were caged to limit herbivore interaction and placed on a reef flat in Hilo Bay, Hawaii. This second type of rock was left on the reef for three months prior to being offered to fish. After three months a majority of the rocks had *A. spicifera* and *G. salicornia* attached. Attachment was confirmed by the removal of rubber bands and testing for plant detachment. After at least three months, coral skeletons were collected from the field, transported to the DAR Fisheries Research Station at Hilo, and housed similarly to basalt rocks. The coral skeleton rocks were used in trials within two weeks of collection.

Rocks were offered to the fish by securing them to a 5.25 kg hollow tile brick with cable ties and wire mesh. During each experiment, an attempt was made to balance the algal area offered to the fish biomass. The rocks with alien algae attached to them were offered to the fish for 48 hours.

Algal percent cover was determined with a mini double-strung 8.7 x 8.7 cm quadrat containing 10 evenly spaced strings: five vertical and five horizontal, forming 36 square sample units within the quadrat. The quadrat was placed on the top and sides of rocks during quantification. If an alga was present within a sample unit, the alga was scored present. If no algae except coralline crustose algae were found, algae were scored absent. Percent cover was determined before and after rocks were offered to fishes. Controls were used to assure algal removal from rocks exposed to fish, was due to fish consumption.

The mass of a portion of the experimental fish population was determined prior to experimentation to control for variability among fish mass throughout the no-choice feeding assays. Two *K. vaigiensis* were used for each no-choice feeding assay. The number of *A. triostegus* used for each no-choice feeding assay varied from seven to 10. The number of fish used for each trial depended on the quantity of fish captured prior to each experiment. A total of three no-choice feeding assays were achieved per treatment. The treatments included, *K. vaigiensis* feeding on *G. salicornia* attached basalt substrate; *K. vaigiensis* feeding on *A. spicifera* and *G. salicornia* attached coral skeleton substrate; *A. triostegus* feeding on *G. salicornia* attached to basalt substrate; and *A. triostegus* feeding on *A. spicifera* and *G. salicornia* attached coral skeleton substrate.

4.3.4 Data Analysis

To determine if fish preferred one algal species over another data from the multiple-choice feeding assay were analyzed by the Friedman's nonparametric repeated measures test (Theodorsson-Norheim 1987). This test was used instead a traditional ANOVA, because data from multiple-choice feeding assays are non-independent (Davis *et al*. 2005). Consumption rate data were transformed by ranking prior to analysis. The Tukey's multiple comparisons procedure was conducted after significance was determined (Elliott 2007). The Friedman's test and Tukey's comparison were calculated in MINITAB 14. To determine if *K. vaigiensis* and *A. triostegus* had different algal preferences during multiple-choice feeding assays a multi variant dimensional scaling (MDS) plot followed by an ANOSIM and SIMPER analyzes, these analyzes were completed in PRIMER 5. To determine significance ANOSIM produces a sample statistic or *r*-value and significance level of sample statistic or *p*-value. Comparison of consumption rates from no-choice feeding assays was conducted using an ANOVA and Tukey's pair-wise comparison in MINITAB 14. Raw data were calculated to percentages then a log transformation was applied prior to analysis. This statistical analysis included all four treatments. Significance level for all analyses was alpha of 0.05.

4.4 RESULTS

4.4.1 Multiple-Choice Feeding Assays

A. spicifera was the most preferred alga by both *K. vaigiensis* (Figure 32) and *A. triostegus* (Figure 34) during the multiple-choice feeding assays. *G. salicornia* was fourth most preferred by *K. vaigiensis* and among the least preferred by *A. triostegus*. Fish displayed highly significant differences in feeding preference among the algae compared ($p < 0.01$). *K. vaigiensis* significantly preferred *A. spicifera* over *P. sanctae-crucis, U. lactuca, A. taxiformis, C. antennina*, *A. glomerata, P. capillacea,* and *D. marginata*. In addition, *K. vaigiensis* significantly preferred *G. salicornia* over *A. taxiformis, C. antennina, A. glomerata*, *P. capillacea,* and *D. marginata* (Figure 33). *A. triostegus* significantly preferred *A. spicifera* over *D. marginata*, *A. glomerata, G. salicornia*, *P. capillacea*, and *T. ornata* (Figure 35).

There was a significant difference in algal preference between *K. vaigiensis* and *A. triostegus* during multiple-choice feeding assays (PRIMER ANOSIM, sample statistic or *r* = 0.94, significance level of sample statistic or *p* = 0.008, Figure 36). The top six species driving the difference in consumption rates between *K. vaigiensis* and *A. triostegus* was *T. ornata*, *G. salicornia*, and *S. polyphyllum*, which were consumed at greater rates by *K. vaigiensis*; and *C. antennina*, *A. taxiformis,* and *G. filicina,* which were consumed at greater rates by *A. triostegus*. There was little difference in the consumption rates of *A. spicifera* between the two herbivorous fishes (PRIMER SIMPER).

4.4.2 No-Choice Feeding Assays

A. triostegus had a significantly greater consumption rate on *G. salicornia* attached to basalt rocks when compared to *K. vaigiensis* (*p* = 0.017, Figure 37). Although not significant, *A. triostegus* had a greater consumption rate on *A. spicifera* and *G. salicornia* attached to coral skeletons compared to *K. vaigiensis* (Figure 37). Further *K. vaigiensis* had a greater consumption rate on *A. spicifera* and *G. salicornia* attached to coral skeletons when compared to *G. salicornia* attached to basalt rocks (Figure 37). There was little difference in consumption rates of *A. triostegus* between *G. salicornia* attached to basalt rocks and *A. spicifera* and *G. salicornia* attached to coral skeletons (Figure 37).

4.5 DISCUSSION

Alien algae are a problem throughout the world (Schaffelke *et al*. 2006; Williams and Smith 2007), as land-use and overfishing alter nutrient loading and grazing pressures, which are exemplified in eutrophic communities adjacent to urbanized developments (Waddell and Clarke 2008). Although there have been only a few efforts that have successfully eradicated alien algae (Anderson 2005), a number of studies have been carried out in an effort to manage these problematic species (Smith *et al*. 2004, Conklin and Smith 2005, Weijerman 2008). This study examined the potential of herbivorous reef fish to control alien algae, by analyzing multiple and no-choice feeding experiments. Such an experimental effort to control alien algae is novel and has broad implications for tropical reef management.

The results from these multiple-choice feeding assays and other studies (Wylie and Paul 1988; Boyer *et al*. 2004; Vermeji *et al*. 2009) demonstrate that *A. spicifera* is highly palatable to herbivorous fishes and is thus likely to be an excellent candidate for biological control by indigenous herbivorous fishes. When considering a fish for use as a biological control for *A. spicifera*, the fish must not only consume *A. spicifera*, but also inhabit the same environment. *A. spicifera* typically inhabits intertidal regions, tide-pools, and harbors (Smith *et al*. 2002). *K. vaigiensis* is a fish that prefers *A. spicifera* and inhabits a diverse array of marine habitats including those inhabited by *A. spicifera* (Galzin and Legendre 1987; Russ 1989; Gell and Whittington 2002; Taquet 2007; Bugalho *et al*. 2008; Hoey and Bellwood 2009). The same is true for *A. triostegus* (Randall 1961; Robertson and Gaines 1986; Galzin and Legendre 1987; Chabanet *et al.*

1997; Bugalho *et al*. 2008), making these two fish potential agents of biological control for *A. spicifera*.

The results from the multiple-choice feeding assays demonstrate that *G. salicornia* is not as palatable as *A. spicifera*. The consumption rates of *A. triostegus* were significantly greater for *A. spicifera* than *G. salicornia*. However, differences in palatability were less clear-cut for *K. vaigiensis*. *G. salicornia* consumption rates by *K. vaigiensis* in multiple-choice feeding trials were less than *A. spicifera*, but that difference was not significant. In this case, *K. vaigiensis* may be a more effective biological control agent for *G. salicornia* than *A. triostegus*. Besides being a consumer of *G. salicornia, K. vaigiensis* also inhabits the same habitat as *G. salicornia* (Galzin and Legendre 1987; Russ 1989; Abbott 1999; Gell and Whittington 2002; Taquet 2007; Bugalho *et al*. 2008; Hoey and Bellwood 2009), making *K. vaigiensis* a potential agent of biological control for *G. salicornia*.

Multiple-choice feeding trials indicate that *K. vaigiensis* had a preference for thicker more robust algae such as *T. ornata, G. salicornia*, and *S. polyphyllum*; whereas *A. triostegus* had a greater preference for softer and more delicate algae such as *C. antennina, A. taxiformis*, and *G. filicina*. This difference in preference has confirmed that different species of herbivorous fish prefer to consume different algal species (Randall 1961; Jones 1968; Clements and Choat 1997; Choat *et al*. 2002; Choat *et al*. 2004; Smith *et al*. 2004; Crossman *et al*. 2005; Ledlie *et al*. 2007; Cvitanovic and Bellwood 2009; Hoey and Bellwood 2009). These results support the need to confirm if all herbivorous populations are effective at controlling alien algae species.

The results from the no-choice feeding assays show that *A. triostegus* had a significantly greater consumption rate on *G. salicornia* attached to basalt rock than *K. vaigiensis*. Although the difference was not statistically significant, *A. triostegus* also had higher consumption rates on *A. spicifera* and *G. salicornia* attached to coral skeleton rock than *K. vaigiensis*. *A. triostegus* also inhabits the same habitat as *A. spicifera* and *G. salicornia* (Randall 1961; Robertson and Gaines 1986; Galzin and Legendre 1987; Chabanet *et al*. 1997; Abbott 1999; Bugalho *et al*. 2008) making *A. triostegus* a better potential agent of biological control for *A. spicifera* and *G. salicornia* compared to *K. vaigiensis*.

At first, it seems that the results of the multiple-choice and no-choice feeding assays were contradictory. In the multiple-choice feeding assay, *K. vaigiensis* consumption rate on *G. salicornia* was greater than *A. triostegus*, while the opposite was true for the no-choice feeding assay, but methods offering the algae were different between the multiple and no-choice feeding assays. During the multiple-choice feeding assays, algae were offered by inserting them between the braids of rope. These algae were detached and fish may perceive algae offered by this method to be algae in drift. During the no-choice feeding assays, the algae offered to fish were attached to substrate. The differences in consumption rate on *G. salicornia* demonstrate by *K. vaigiensis* and *A. triostegus* when comparing the results of the multiple and no-choice feeding assays suggest that *K. vaigiensis* prefers to consume detached algae in drift, while *A. triostegus* prefers to feed on algae attached to substrate.

Besides being classified as an herbivore (Clement and Choat 1997; Russ1989, Willmott *et al*. 2004; Mantyka and Bellwood 2007) *K. vaigiensis* is also been classified as an herbivorous planktivore (Taquet 2007). Its classification as an herbivorous planktivore not only acknowledges what *K. vaigiensis* eats, but also where it feeds.

The feeding behavior of *K. vaigiensis* (Taquet 2007) explains the differences between this studies multiple-choice and no-choice feeding assays, moreover anomalies from other observations and experiments can also be explained by *K. vaigiensis*'s feeding behavior. Preliminary studies and observations conducted by authors of this study have found the guts of *K. vaigiensis* filled with benthic algae, but *K. vaigiensis* had rarely been observed feeding on algae attached to substrate. It may be that the algae found in the guts of *K. vaigiensis* had been acquired from detached benthic algae, eliminating the need for *K. vaigiensis* to interact with substrate.

The likelihood of *K. vaigiensis* feeding in the water column because of its herbivorous planktivore classification (Taquet 2007) may also explain why fishermen offer bait in the water column when fishing for *K. vaigiensis*, as the authors of this study have when fishing for *K. vaigiensis* for the feeding assays.

Although not significant, during this study's no-choice feeding assay, *K. vaigiensis* had a greater feeding rate on algae attached to coral skeletons than algae attached to basalt rock. The reason for this pattern may be because when *K. vaigiensis* is only offered attached algae it prefers to feed off the less dense coral skeleton rather than the denser basalt rock. *K. vaigiensis* feeding on coral skeleton rather than basalt rock may reduce teeth damage caused by feeding on algae attached to substrate. On the Great Barrier Reef *K. vaigiensis* does not feed on in situ algae naturally attached to substrate (Fox and Bellwood 2008). Thus, *K. vaigiensis* plays a significant role in biomass removal of transplanted algae secured to substrate with a rubber band or wire (Cvitanovic and Bellwood 2009). In this case *K. vaigiensis* may prefer to feed on algae secured to substrate relative to algae attached, where the potential for *K. vaigiensis* to pull the algae off the substrate prior to consumption exist, rather than feed on algae that is actually attached to substrate where the necessity to interact with substrate is increased.

These feeding strategies limit the interactions between *K. vaigiensis* and substrate. The results from this study, other studies, and field observations suggest that *K. vaigiensis* prefers feeding on drifting algae. This may be why *K. vaigiensis* is observed in areas of high energy (Galzin and Legendre 1987; Hoey and Bellwood 2009) where drifting algae is much more likely to be encountered.

As for *A. triostegus*, results from the multiple-choice and no-choice feeding assays indicated that *A. triostegus* prefers algae attached to substrate. Large schools of *A. triostegus* can be observed descending on Hawaiian reefs and vigorously feeding. Such observations support the hypothesis that this fish is accustomed to feed on algae attached to substrate.

Consistencies in studies of *K. vaigiensis*'s diet show that *K. vaigiensis* often feeds on large macro-algae from the divisions of Chlorophyta and Heterokontophyta (Clements and Choat 1997; Choat *et al*. 2002; Choat *et al*. 2004; Crossman *et al*. 2005). In the multiple-choice feeding assay of this study, two of the top three algae consumed were from the division Heterokontophyta. Although none of the algae from the division Chlorophyta was preferred, the two that were offered (*C. antennina* and *U. lactuca*) are not typical large algae. However, one of the inconsistencies that must be considered is that the most preferred alga from these multiple-choice assays was *A. spicifera*, an alien alga from the division Rhodophyta.

Observations and experiments of *A. triostegus*'s diet are somewhat consistent. *A. triostegus* prefers smaller algae (Randall1961; Jones 1968; Ledlie *et al*. 2007), but also readily consumes species of algae with larger bodies (Randall1961; Robertson and Gains 1986) as well as mangrove and sea-grass (Bugalho *et al*. 2008). Although this multiple-choice feeding assay supports the premise that *A. triostegus* generally prefers algae with small or soft thalli (*G. filicina*, *C. antennina*, *C. implexa*, *A. taxiformis*, *P. sanctae-crucis*, and *U. lactuca*); the most preferred algae was *A. spicifera*. *A. triostegus* preferring *A. spicifera* does not agree with the general pattern. Such a diverse diet indicated by the cited studies suggest that *A. triostegus* consumes the majority of algae it encounters.

In an effort to manage alien algae, *K. vaigiensis* and *A. triostegus* can be regulated to attain different objectives. The most common way that alien algae in Hawaii reproduce is fragmentation (Smith *et al*. 2002). These fragments may be considered drift algae. Feeding by *K. vaigiensis* may therefore prevent dispersal via fragmentation. However, enhancing the population size of *A. triostegus* may have a greater impact on local biomass of existing attached alien algae populations.

When attempting to enhance the populations of *K. vaigiensis* and *A. triostegus* management by area schemes, such as no take zones may be effective for *A. triostegus,* because of their small home range (Randall 1961) and high site fidelity when foraging (Conklin 2007). As for *K. vaigiensis*, more studies need to be conducted to determine if area management would be effective in enhancing *K. vaigiensis*'s population. Nevertheless, in both cases, regulating take of *K. vaigiensis* and *A. triostegus* may serve as an effective strategy in enhancing these herbivore populations.

4.6 CONCLUSION

Further trials would be necessary to determine whether *A. triostegus* and *K. vaigiensis* are the best candidate biological control agents for *A. spicifera* and *G. salicornia*, but the trials described here have demonstrated that both species consume alien algae. Enhancing their populations could be an effective control measure to better manage coastal regions. In addition, different species may be well suited for different roles, with *A. triostegus* most suited to control attached algal populations, and *K. vaigiensis* potentially able to reduce dispersal of floating fragments of algae. However, before decisions are made to enhance populations of *A. triostegus* and *K. vaigiensis* as a means to control alien algae more must be learned about the interactions between these herbivorous fish and alien algae. One issue is the potential of these fish spreading alien algae. It is plausible that these herbivores may be spreading the algae via incomplete digestion, especially for those algae that sexually reproduce, such as *A. spicifera*. This concern should be addressed as the "next step" in harnessing herbivory to control alien algae.

CHAPTER 5. CONCLUSION

With stressors increasing on Hawaiian reefs (Friedlander 2005) and the economic benefits that reefs provide for the state (Cesar and van Beukering 2004), more effort should be spent in managing this vital resource. Events such as sedimentation (Field *et al.* 2008) and ship groundings (Kolinski *et al.* 2007) can have localized impacts on Hawaii's reefs. However, as the population grows in Hawaii the potential of eutrophication (Smith *et al*. 1981; Parson et al. 2008; Dailer *et al*. 2010) and reductions in herbivore pressure (Smith *et al.* 2001; Williams *et al.* 2008; Smith *et al.* 2010) to have detrimental effects on Hawaiian reefs increases. To remedy this problem Hawaii must comprehend the negative effects of eutrophication and reduced herbivore pressure on Hawaii's reefs.

To assist natural resource managers in swaying Hawaii's population to take steps in applying strategies to improve the status of degraded reef and increase the resiliency of healthy reefs this dissertation has made three novel contributions to better understand the algal issues of Hawaii. The first study examined the spatial features of SGD at very fine scales to aid in the understanding of how and where marine benthic communities interact with nutrient rich SGD. Although studies have analyzed the relative and simultaneous effects of increased nutrient concentrations and a reduction in herbivore pressure on the west coast of Hawaii Island (Smith *et al.* 2001; Smith *et al.* 2010), no study has analyzed these effects on two reefs at the same time as the second study in this dissertation has. The results of this study emphasize the importance to manage Hawaii's reefs on a reef-by-reef basis and that one solution is not likely to benefit all of Hawaii's reefs equally.

The third study examined the potential of herbivorous fish to control alien algal species by conducted multiple-choice feed experiments that offered 14 algal species to herbivorous fish simultaneously. No other study has been conducted with this intensity in an effort to control alien algae in Hawaii. The results of this study have endorsed two candidates in the effort to control alien algae in Hawaii. In the following paragraphs, results of this dissertation are used to influence managers in their endeavor to sustain Hawaii's reefs.

Chapters from this dissertation and other studies have demonstrated that SGD can increase near shore nutrient concentrations and that nutrients can have adverse effects on tropical reefs by increasing algal populations. Although most agree that SGD can increase near shore nutrient levels, there seems to be a disagreement within Hawaii's scientific community on the effect that nutrient enrichment has on Hawaii's reefs (Grigg 1995; Smith *et al.* 2001; Dailer *et al*. 2010). However, as more studies examine the effects of nutrients on Hawaii's reefs I feel that its detrimental impacts will become evident. As for Hawaii's general population a small portion seems to understand the adverse affect of nutrients on Hawaii's reefs and less seem to visualize a solution. If the output of nutrients on Hawaii's reefs is to be reduced, it has to be carried out at an individual's basis. People have to adjust their personal waste disposal systems and pressure needs to be applied on politicians to develop proper waste disposal facilities. More outreach and education needs to occur in an effort to show people the detrimental effect of nutrient on Hawaii's reefs. Currently scientist and managers have a small arsenal of studies (Banner 1974; Smith *et al*. 1981; Smith *et al.* 2001; Smith *et al.* 2005; Parson *et al*. 2008; Dailer *et al*. 2010; Smith *et al.* 2010) to carry out this endeavor. More such studies conducted throughout the state on all islands would make this issue undisputable; thus having a greater chance in persuading the people of Hawaii to address the detrimental effect on eutrophication on their reefs.

Chapters from this dissertation and other studies have displayed the importance of herbivore populations in sustaining the health of tropical reefs. Most of the scientific and management community in Hawaii agrees on the detrimental effect of herbivore reduction by overfishing on Hawaii’s reefs (Williams *et al*. 2008). However, this is a touchy subject for Hawaii’s general population, especially for those who consume reef fish. Although, most scientist and managers in Hawaii understand that by sustaining herbivorous population, the health of tropical reefs are sustained and therefore the fisheries are sustained, this concept has not been quantified in Hawaii and can be difficult for the lay person to comprehend. Such an effort to quantify this concept would show fishers that sustaining herbivore populations is in their best interest. One such opportunity presents itself on Molokai.

The recent outreach and education effort conducted by the DAR, funded by National Fish and Wildlife Foundation NFWF (C. Walsh pers. comm., 2010) has set the stage for an effort to quantify and display the benefits of enhancing herbivore populations on desecrated reefs. Due to the efforts of DAR and NFWF, the Molokai community understands the negative impacts of alien algae and the labor involved to physically remove these problematic species, making biological control a potentially viable option. The reef flat of Molokai may be an influential place for DAR to enhance herbivore populations in an effort to teach people how herbivores can benefit the entire fisheries population. Ways that herbivore populations can be increased is by persuading community leaders to propose the establishment of FMAs similar to Kahekili, Maui or by releasing *Tripneustes gratilla* from DARs urchin breeding project (R. Nishimoto personal communication, 2010). If successful, such an effort may have an influence throughout the state.

Similar to the rest of the world (Bruno *et al.* 2009), the majority of Hawaii's reef are still intact. However, with the increasing threats from overfishing (Williams *et al*. 2008) and eutrophication (Dailer *et al*. 2010) they may not remain in a resilient state for long. The time to act is now, before it is too late.

APPENDIX 1. TABLES

Table 1. Summary of Sample Events
This table below summarizes the time and tide of salinity profile sample events and water quality sample events. This table also shows the facility samples were processed at and what constituents were analyzed.

Sample Event (Date)	**Tide (Time)**	**Analyzed By**	**Analyzed For**
-salinity profile- event #1 (03/22/05)	low (8:11a) high (1:35p)	UH Botany Department	salinity
-salinity profile- event #2 (06/09/05)	low (9:15a) high (2:10p)	UH Botany Department	salinity
-salinity profile- event #3 (08/21/05)	low (10:00a) high (4:25p)	UH Botany Department	salinity
-water quality- event #4 (03/23/04)	low (10:19a) high (2:05p)	UH Water Resource Center	bromide, nitrate, salinity
-water quality- event #5 (06/28/04)	low (5:00a)	UH Water Resource Center	bromide, nitrate, salinity, sulfate
-water quality- event #6 (11/27/05)	low (6:02p)	Department of Health	ammonia, chlorophyll-a, nitrate, ortho-phosphate, salinity, total-nitrogen, total-phosphorus, silica

Table 2. Salinity Profile Values and Correlations

This table summarizes the analyses of salinity values and correlations from salinity profile data. Salinity values are reported in parts per thousand (ppt, ‰), “distance” refers to distance from back of lagoon, n = 99, except for pooled comparisons were n = 594.

Salinity Profile Values and Correlations					
sample event (date & tide)	**salinity average**	**standard error**	**comparison**	***r*-value**	***p*-value**
1 (03/22/05, low)	17.18	0.55	distance & salinity	0.275	0.006
“	“	“	depth & salinity	-0.833	<0.001
1 (03/22/05, high)	28.83	0.41	distance & salinity	0.125	0.218
“	“	“	depth & salinity	-0.837	<0.001
2 (06/09/05, low)	35.85	0.23	distance & salinity	0.112	0.269
“	“	“	depth & salinity	0.259	0.010
2 (06/09/05, high)	35.25	0.13	distance & salinity	-0.029	0.774
“	“	“	depth & salinity	0.094	0.356
3 (08/21/05, low)	21.67	0.55	distance & salinity	0.089	0.382
“	“	“	depth & salinity	-0.878	<0.001
3 (08/21/05, high)	31.37	0.30	distance & salinity	0.153	0.129
“	“	“	depth & salinity	-0.758	<0.001
pooled	28.35	0.32	distance & salinity	0.067	0.103
“	“	“	depth & salinity	-0.301	<0.001

Table 3. Water Quality Correlations

This table summarizes correlations between salinity and water quality components. The n values for sample event 4, 5, and 6 were 28, 14, and 20 respectively. The n value for the pooled comparisons of salinity and bromide was 42, while the n value for the pooled comparisons of salinity and nitrate was 62.

Water Quality Correlations			
comparison (sample event)	***r*-value**	***p*-value**	**equation**
ammonia & salinity (6)	0.838	<0.001	amm = 0.0119(sal) + 0.0069
bromide & salinity (4)	0.956	<0.001	bro = 16.778(sal) + 149.33
bromide & salinity (5)	0.999	<0.001	bro = 35.572(sal) + 29.016
bromide & salinity (pooled)	0.880	<0.001	bro = 14.898(sal) + 236.22
chlorophyll-a & salinity (6)	-0.267	0.255	chl-a = -0.0037(sal) + 0.391
nitrate & salinity (4)	-0.950	<0.001	nit = -0.3741(sal) + 12.823
nitrate & salinity (5)	-0.991	<0.001	nit = -2.5342(sal) + 46.631
nitrate & salinity (6)	-0.758	<0.001	nit = 0.1667(sal) + 6.7945
nitrate & salinity (pooled)	-0.706	<0.001	nit = -0.6344(sal) + 20.617
ortho-phosphate & salinity (6)	-0.977	<0.001	o-pho = 0.0123(sal) + 0.4624
temperature & salinity (4)	0.004	0.986	tem = -0.0039(sal) + 25.25
total-nitrogen & salinity (6)	-0.978	<0.001	t-nit = 0.017(sal) + 0.7079
total-phosphorus & salinity (6)	-0.642	0.002	t-pho = 0.0008(sal) + 0.0507
silica & salinity (6)	-0.984	<0.001	sil = 21.578(sal) + 805.92
sulfate & salinity (5)	1.000	<0.001	sul = 1337.8(sal) + 1598

Table 4. Algal Observations
This table summarizes observations of algal species within the lagoon being investigated at Waiopae.

Algae	
species	**patterns**
Amansia glomerata	relative densities are greater toward the back of the lagoon; inhabits crevices, escarpments, and open flats
Dichotomaria marginata	relative densities are greater toward the back of the lagoon; inhabits crevices
Galaxaura rugosa	relative densities are greater toward the back of the lagoon; inhabits crevices
Gelidiella acerosa	relative densities are greater toward the back of the lagoon; inhabits open flats

Table 5. Coral Observations
This table summarizes observations of coral species within the lagoon being investigated at Waiopae.

Coral	
species	**patterns**
Montipora capitata	once could be found throughout the lagoon in comparable densities, but since a coral die back in 2008 relative densities are greater toward the front of the lagoon
Porities lobata	relative densities are greater toward the front of the lagoon

Table 6. Fish Observations

This table summarizes observations of fish species within the lagoon being investigated at Waiopae.

Fish		
species	**size**	**patterns**
Abudefduf abdominalis	adult	found throughout the lagoon
Abudefduf sordidus	adult	found throughout the lagoon
Acanthurus nigrofuscus	adult	no pattern observed
Acanthurus triostegus	juvenile, adult	found throughout the lagoon
Calotomus carolinus	adult	no pattern observed
Cephalopholis argus	juvenile	relative densities are greater toward the front of the lagoon
Chaetodon lunula	adult	relative densities are greater toward the front of the lagoon
Ctenochaetus strigosus	adult	no pattern observed
Fistularia commersonii	adult	relative densities are greater toward the front of the lagoon
Gomphosus varius	adult	no pattern observed
Hemitaurichthys polylepis	adult	no pattern observed
Lutjanus fulvus	adult	no pattern observed
Mulloidichthys flavolineatus	juvenile	no pattern observed
Parupeneus multifasciatus	juvenile	no pattern observed
Scarus rubroviolaceus	adult	relative densities are greater toward the front of the lagoon
Scaridae species	juvenile	found throughout the lagoon
Stegastes fasciolatus	adult	no pattern observed
Stethojulis balterata	adults	no pattern observed
Thalassoma duperrey	juvenile, adult	found throughout the lagoon

Table 7. Mega-Fauna Observations
This table summarizes observations of mega-fauna species within the lagoon being investigated at Waiopae.

Mega-Fauna		
species	**size**	**patterns**
Chelonia mydas	adult	only observed twice toward the front of the lagoon

Table 8. Between Site Comparisons of Treatments, Biomass
This table displays algal biomass comparisons of the same treatment, during the same time period, between Kaawaloa and Kahauloa (n = 5). The first column shows the treatment being compared between sites. The remaining column shows the *p*-values of algal biomass comparisons between sites for each treatment after three months (second column), five months (third column), and seven months (fourth column). If a significant difference was determined between sites the site with the greater biomass of a given treatment during a time period is signified within prentices. Within prentices "awa" means Kaawaloa and "hau" means Kahauloa.

Between Site Comparisons of Treatments During Each Time Period, Biomass			
treatment	**after three months**	**after five months**	**after seven months**
control	$p < 0.01$, (awa < hau)	$p < 0.01$, (awa < hau)	$p = 0.011$, (awa < hau)
nutrients	$p < 0.01$, (awa < hau)	$p = 0.028$, (awa < hau)	$p = 0.050$, (awa < hau)
cage	$p = 0.338$	$p = 0.389$	$p = 0.704$
nutrient-cage	$p < 0.01$, (awa < hau)	$p = 0.186$	$p = 0.448$

Table 9. Comparison of Treatments at Kaawaloa After Three Months, Cover
This table displays the results of an ANOSIM within site pair-wise analysis of algal species percent cover on tiles from Kaawaloa (n = 5). This table represents tiles exposed to treatments for three months. Within the table the sample statistic (r-value) and the significance level of sample statistic (p-value) are shown.

Within Site Comparison of Treatments at Kaawaloa After Three Months, Cover					
	control	**cage-control**	**nutrients**	**cage**	**nutrient-cage**
control		$p = 0.764$	$p < 0.010$	$p < 0.010$	$p < 0.010$
cage-control	$r = -0.092$		$p < 0.010$	$p < 0.010$	$p < 0.010$
nutrients	$r = 0.612$	$r = 0.436$		$p < 0.010$	$p < 0.010$
cage	$r = 0.992$	$r = 1.000$	$r = 1.000$		$p < 0.010$
nutrient-cage	$r = 1.000$	$r = 1.000$	$r = 1.000$	$r = 0.764$	

Table 10. Comparison of Treatments at Kaawaloa After Five Months, Cover
This table displays the results of an ANOSIM within site pair-wise analysis of algal species percent cover on tiles from Kaawaloa (n = 5). This table represents tiles exposed to treatments for five months. Within the table the sample statistic (r-value) and the significance level of sample statistic (p-value) are shown.

Within Site Comparison of Treatments at Kaawaloa After Five Months, Cover					
	control	**cage-control**	**nutrients**	**cage**	**nutrient-cage**
control		$p = 0.103$	$p = 0.222$	$p < 0.010$	$p < 0.010$
cage-control	$r = 0.292$		$p = 0.127$	$p < 0.010$	$p < 0.010$
nutrients	$r = 0.116$	$r = 0.148$		$p < 0.010$	$p < 0.010$
cage	$r = 0.956$	$r = 0.840$	$r = 0.996$		$p = 0.024$
nutrient-cage	$r = 0.960$	$r = 0.888$	$r = 0.968$	$r = 0.436$	

Table 11. Comparison of Treatments at Kaawaloa After Seven Months, Cover
This table displays the results of an ANOSIM within site pair-wise analysis of algal species percent cover on tiles from Kaawaloa (n = 5). This table represents tiles exposed to treatments for seven months. Within the table the sample statistic (*r*-value) and the significance level of sample statistic (*p*-value) are shown.

Within Site Comparison of Treatments at Kaawaloa After Seven Months, Cover					
	control	**cage-control**	**nutrients**	**cage**	**nutrient-cage**
control		$p = 0.119$	$p < 0.010$	$p < 0.010$	$p < 0.010$
cage-control	$r = 0.140$		$p = 0.040$	$p < 0.010$	$p < 0.010$
nutrients	$r = 0.492$	$r = 0.308$		$p < 0.010$	$p < 0.010$
cage	$r = 1.000$	$r = 0.996$	$r = 1.000$		$p = 0.032$
nutrient-cage	$r = 1.000$	$r = 1.000$	$r = 1.000$	$r = 0.352$	

Table 12. Comparison of Treatments at Kahauloa After Three Months, Cover
This table displays the results of an ANOSIM within site pair-wise analysis of algal species percent cover on tiles from Kahauloa (n = 5). This table represents tiles exposed to treatments for three months. Within the table the sample statistic (*r*-value) and the significance level of sample statistic (*p*-value) are shown.

Within Site Comparison of Treatments at Kahauloa After Three Months, Cover					
	control	**cage-control**	**nutrients**	**cage**	**nutrient-cage**
control		$p = 0.516$	$p < 0.010$	$p < 0.010$	$p < 0.010$
cage-control	$r = -0.016$		$p < 0.010$	$p < 0.010$	$p < 0.010$
nutrients	$r = 0.764$	$r = 0.660$		$p < 0.010$	$p < 0.010$
cage	$r = 0.980$	$r = 0.936$	$r = 0.948$		$p < 0.010$
nutrient-cage	$r = 1.000$	$r = 0.992$	$r = 1.000$	$r = 0.964$	

Table 13. Comparison of Treatments at Kahauloa After Five Months, Cover
This table displays the results of an ANOSIM within site pair-wise analysis of algal species percent cover on tiles from Kahauloa (n = 5). This table represents tiles exposed to treatments for five months. Within the table the sample statistic (*r*-value) and the significance level of sample statistic (*p*-value) are shown.

Within Site Comparison of Treatments at Kahauloa After Five Months, Cover					
	control	**cage-control**	**nutrients**	**cage**	**nutrient-cage**
control		$p = 0.016$	$p = 0.087$	$p = 0.016$	$p < 0.010$
cage-control	$r = 0.440$		$p = 0.016$	$p < 0.010$	$p < 0.010$
nutrients	$r = 0.140$	$r = 0.488$		$p = 0.032$	$p < 0.010$
cage	$r = 0.568$	$r = 0.720$	$r = 0.716$		$p < 0.010$
nutrient-cage	$r = 0.984$	$r = 1.000$	$r = 0.948$	$r = 0.700$	

Table 14. Comparison of Treatments at Kahauloa After Seven Months, Cover
This table displays the results of an ANOSIM within site pair-wise analysis of algal species percent cover on tiles from Kahauloa (n = 5). This table represents tiles exposed to treatments for seven months. Within the table the sample statistic (*r*-value) and the significance level of sample statistic (*p*-value) are shown.

Within Site Comparison of Treatments at Kahauloa After Seven Months, Cover					
	control	**cage-control**	**nutrients**	**cage**	**nutrient-cage**
control		$p < 0.010$	$p < 0.010$	$p < 0.010$	$p < 0.010$
cage-control	$r = 0.368$		$p < 0.010$	$p < 0.010$	$p < 0.010$
nutrients	$r = 0.944$	$r = 0.872$		$p < 0.010$	$p < 0.010$
cage	$r = 1.000$	$r = 1.000$	$r = 0.676$		$p = 0.024$
nutrient-cage	$r = 1.000$	$r = 1.000$	$r = 0.996$	$r = 0.336$	

Table 15. Contributing Species to Similarity Within Treatments at Kaawaloa, Cover

This table displays the contributing species to similarity within each treatment during each time period at Kaawaloa as determined from algal species percent cover data by the SIMPER analysis (n = 5). The first column lists the treatments. The second column shows the number of months tiles were exposed to treatments. The third column lists the major contributing species or functional group to similarity within each treatment during each time period. Often the top three contributing species or functional groups are listed, but on a few occasions, only two species or functional groups are listed because the SIMPER analysis produced only two contributing species. Within the third column the average percent cover of each major contributing species are shown in prentices. The abbreviation "CCA" in the third column represents coralline crustose algae.

Major Contributing Species to Similarity Within Each Treatment During Each Time Period at Kaawaloa, Cover		
treatment	**mo**	**major contributing species (average percent cover)**
control	3	CCA (47%), open space (27%), *Ulvella* sp. (8%)
control	5	CCA (49%), *Calothrix* sp. (15%), *Ulvella* sp. (7%)
control	7	CCA (49%), open space (23%), *Ulvella* sp. (14%)
cage-control	3	CCA (40%), open space (23%)
cage-control	5	CCA (40%), *Ulvella* sp. (14%), open space (9%)
cage-control	7	CCA (51%), *Ulvella* sp. (23%), open space (13%)
nutrients	3	CCA (27%), *Ulvella* sp. (21%), open space (21%)
nutrients	5	CCA (43%), *Calothrix* sp. (14%), *Ulvella* sp. (12%)
nutrients	7	CCA (64%), *Ulvella* sp. (19%)
cage	3	*Jania pumila* (16%), *Centroceras* sp. (12%), Vaughaniella stage (10%)
cage	5	*Jania pumila* (32%), CCA (7%), *Cladophora* sp. (6%)
cage	7	*Jania pumila* (24%), *Centroceras* sp. (12%), *Chondria* sp. (11%)
nutrient-cage	3	*Centroceras* sp. (20%), *Jania pumila* (13%), CCA (9%)
nutrient-cage	5	*Jania pumila* (25%), *Centroceras* sp. (33%), *Sphacelaria* sp. (7%)
nutrient-cage	7	*Jania pumila* (24%), *Centroceras* sp. (20%), *Amphiroa* sp. (14%)

Table 16. Contributing Species to Similarity Within Treatments at Kahauloa, Cover

This table displays the contributing species to similarity within each treatment during each time period at Kahauloa as determined from algal species percent cover data by the SIMPER analysis (n = 5). The first column lists the treatments. The second column shows the number of months tiles were exposed to treatments. The third column lists the major contributing species or functional group to similarity within each treatment during each time period. Often the top three contributing species or functional groups are listed, but on a few occasions, only two species or functional groups are listed because the SIMPER analysis produced only two contributing species. Within the third column the average percent cover of each major contributing species are shown in prentices. The abbreviation "CCA" in the third column represents coralline crustose algae.

Major Contributing Species To Similarity Within Each Treatment During Each Time Period At Kahauloa, Cover		
treatment	**mo**	**major contributing species (average percent cover)**
control	3	CCA (27%), open space (27%), *Ulvella* sp. (19%)
control	5	CCA (37%), *Calothrix* sp. (14%), *Sphacelaria* sp. (11%)
control	7	CCA (42%), *Calothrix* sp. (20%), *Ulvella* sp. (11%)
cage-control	3	CCA (33%), open space (25%), *Sphacelaria* sp. (10%)
cage-control	5	CCA (35%), *Ralfsia expansa* (19%), *Calothrix* sp. (12%)
cage-control	7	CCA (36%), *Ralfsia expansa* (14%), *Ulvella* sp. (13%)
nutrients	3	CCA (23%), open space (18%), *Ralfsia expansa* (9%)
nutrients	5	CCA (35%), *Ralfsia expansa* (11%), *Sphacelaria* sp. (8%)
nutrients	7	*Sphacelaria* sp. (24%), CCA (18%), *Ralfsia expansa* (16%)
cage	3	CCA (26%), *Sphacelaria* sp. (19%), *Distromium flabellatum* (11%)
cage	5	CCA (32%), *Sphacelaria* sp. (23%), *Distromium flabellatum* (16%)
cage	7	*Jania pumila* (31%), Vaughaniella stage (12%), *Ceramium* sp. (13%)
nutrient-cage	3	*Jania pumila* (12%), open space (15%), *Sphacelaria* sp. (12%)
nutrient-cage	5	*Jania pumila* (31%), Vaughaniella stage (12%), *Sphacelaria* sp. (13%)
nutrient-cage	7	*Jania pumila* (33%), *Ceramium* sp. (13%), *Dictyota friabilis* (13%)

APPENDIX 2. FIGURES

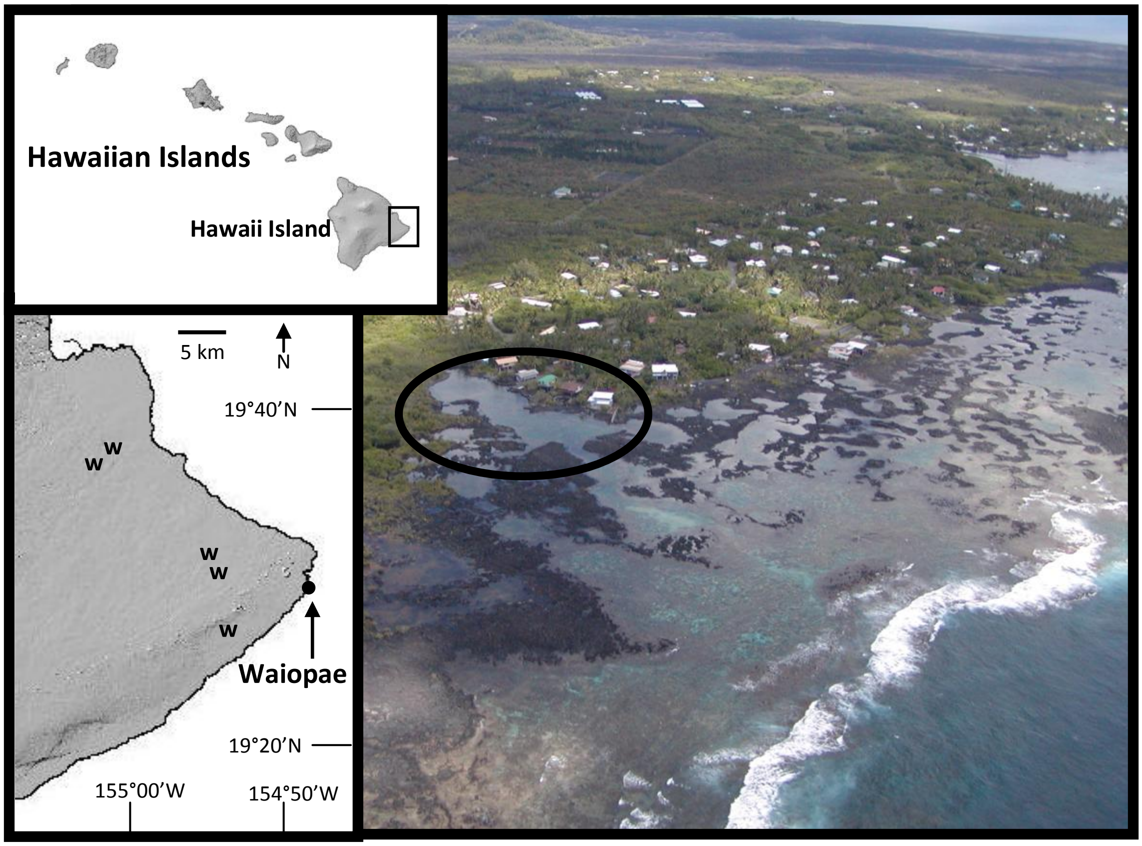


Figure 1. Site Map of Waiopae, Puna, Hawaii
The map to the top left displays the Hawaiian Islands. The largest island on this map is Hawaii Island. The rectangle section on Hawaii Island is represented in more detail by the map to the bottom left. The map to the bottom left displays the eastern section of Hawaii Island. Waiopae is pointed out as a dot. The "w"s on this map identifies the locations of the Department of Water Supply, County of Hawaii wells in the district of Puna. The photo to the right exhibits the tide-pools and lagoons of Waiopae. The body of water identified with the black oval is the lagoon being investigated in this study.

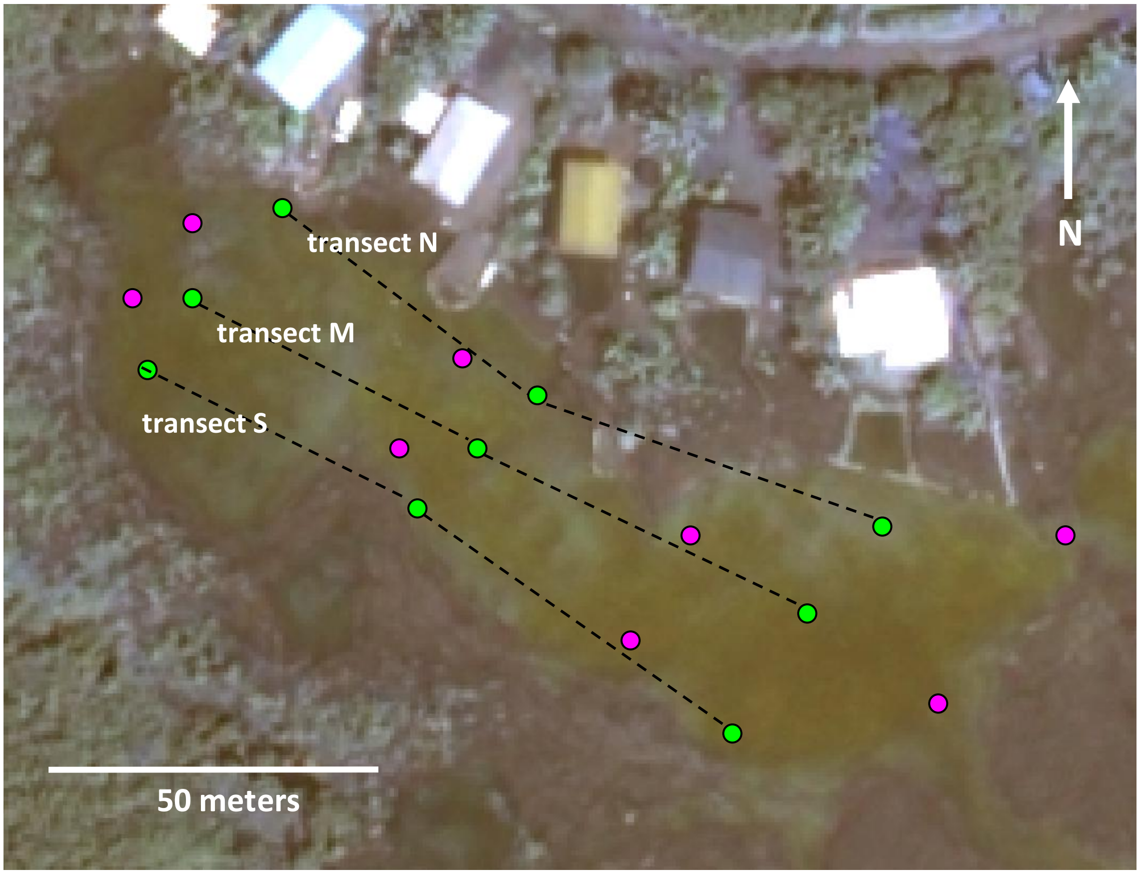


Figure 2. Waiopae Sample Points
copyright DigitalGlobe Inc. 2005. This orthorectified satellite image displays the lagoon being investigated. Green dots identify where salinity profiles were taken during sample events 1, 2, and 3. The dashed lines represent transects connecting salinity profile points (green dots) that will be referenced by salinity contour graphs in the results section. Pink dots identify where water quality samples were taken during sample events 4, 5, and 6.

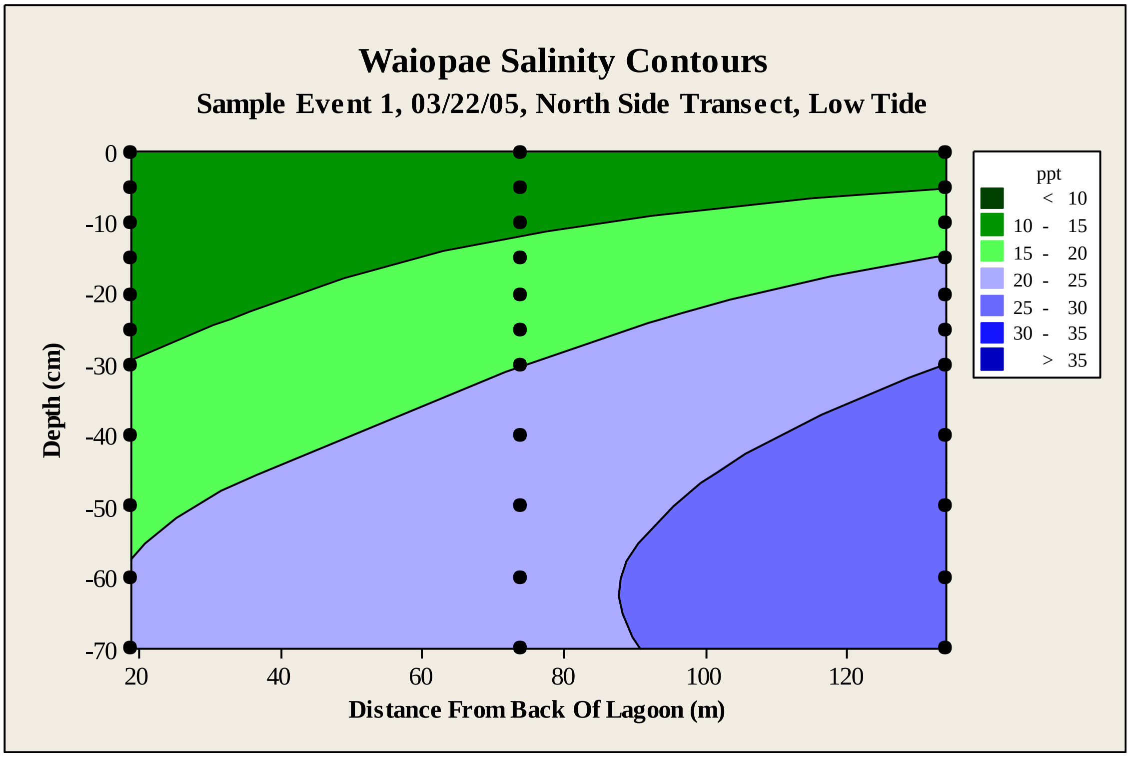


Figure 3. Salinity Contours, Event 1, North Side, Low Tide
This salinity contour plot presents the salinity profile data from sample events 1 during low tide. This plot represents the north side transect as shown in figure 2. Black dots on the contour plots show the position where salinity values were documented. The legend to the right of the plot displays the range of salinity values that are represented by the various colors within these plots.

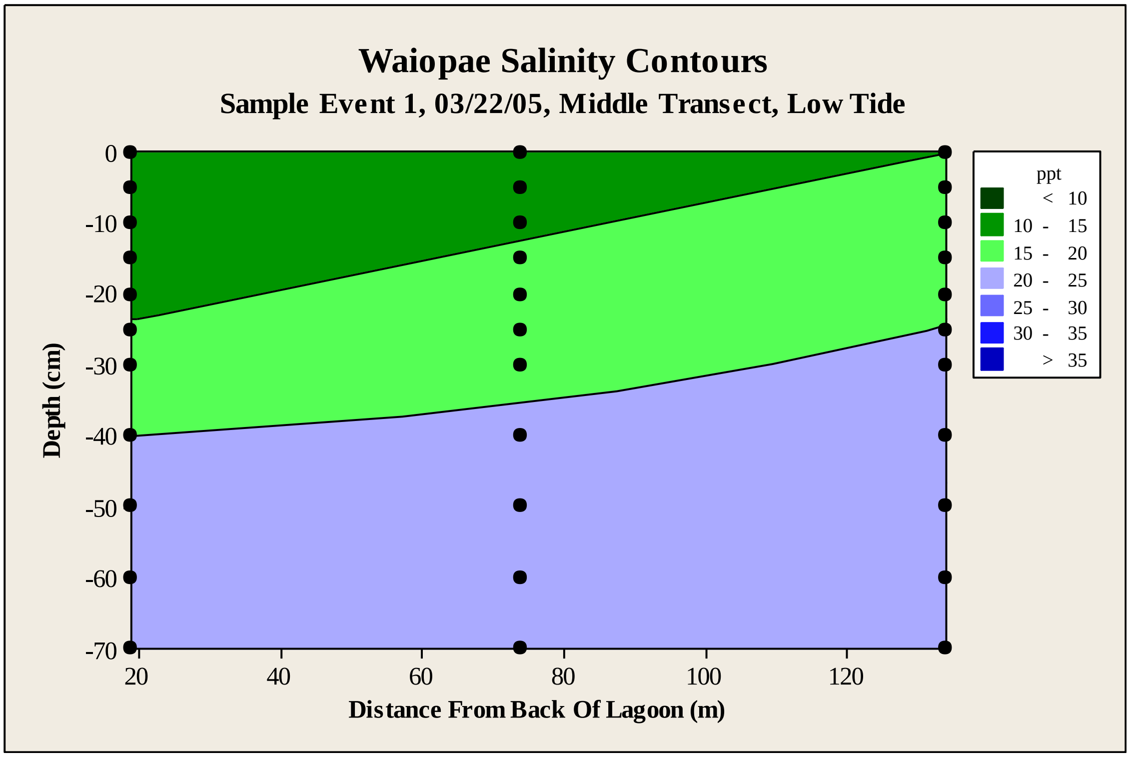


Figure 4. Salinity Contours, Event 1, Middle, Low Tide
This salinity contour plot presents the salinity profile data from sample events 1 during low tide. This plot represents the middle transect as shown in figure 2. Black dots on the contour plots show the position where salinity values were documented. The legend to the right of the plot displays the range of salinity values that are represented by the various colors within these plots.

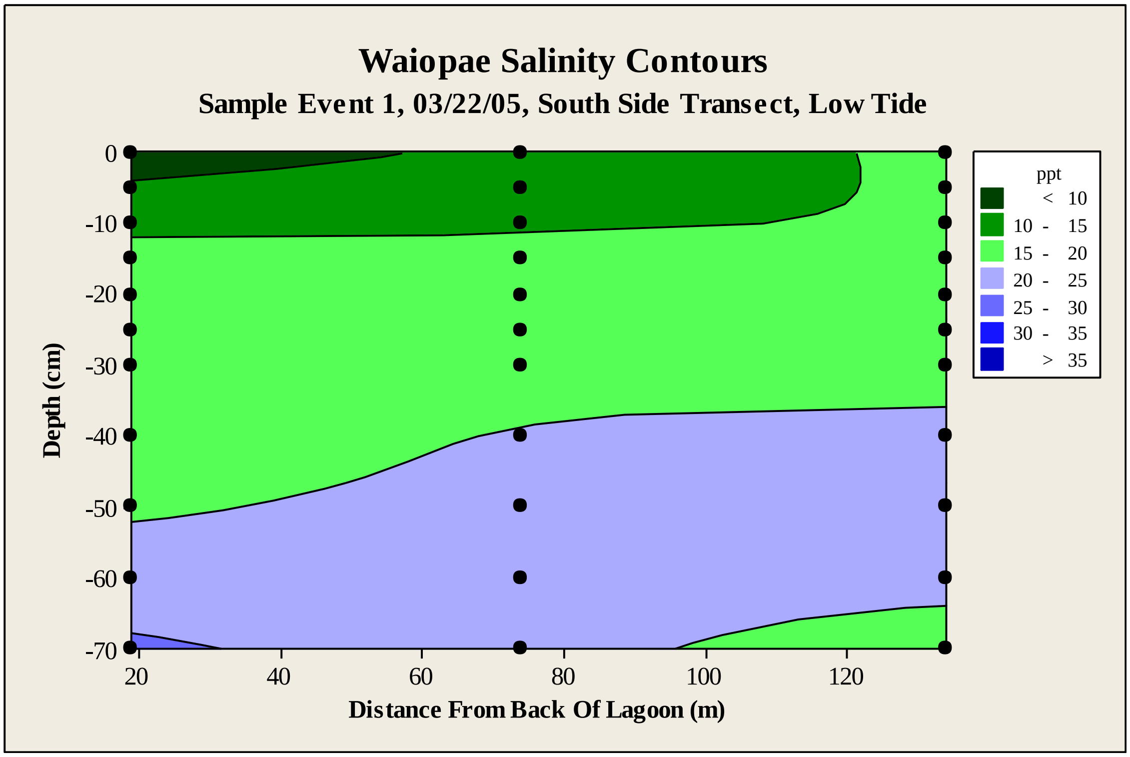


Figure 5. Salinity Contours, Event 1, South Side, Low Tide
This salinity contour plot presents the salinity profile data from sample events 1 during low tide. This plot represents the south side transect as shown in figure 2. Black dots on the contour plots show the position where salinity values were documented. The legend to the right of the plot displays the range of salinity values that are represented by the various colors within these plots.

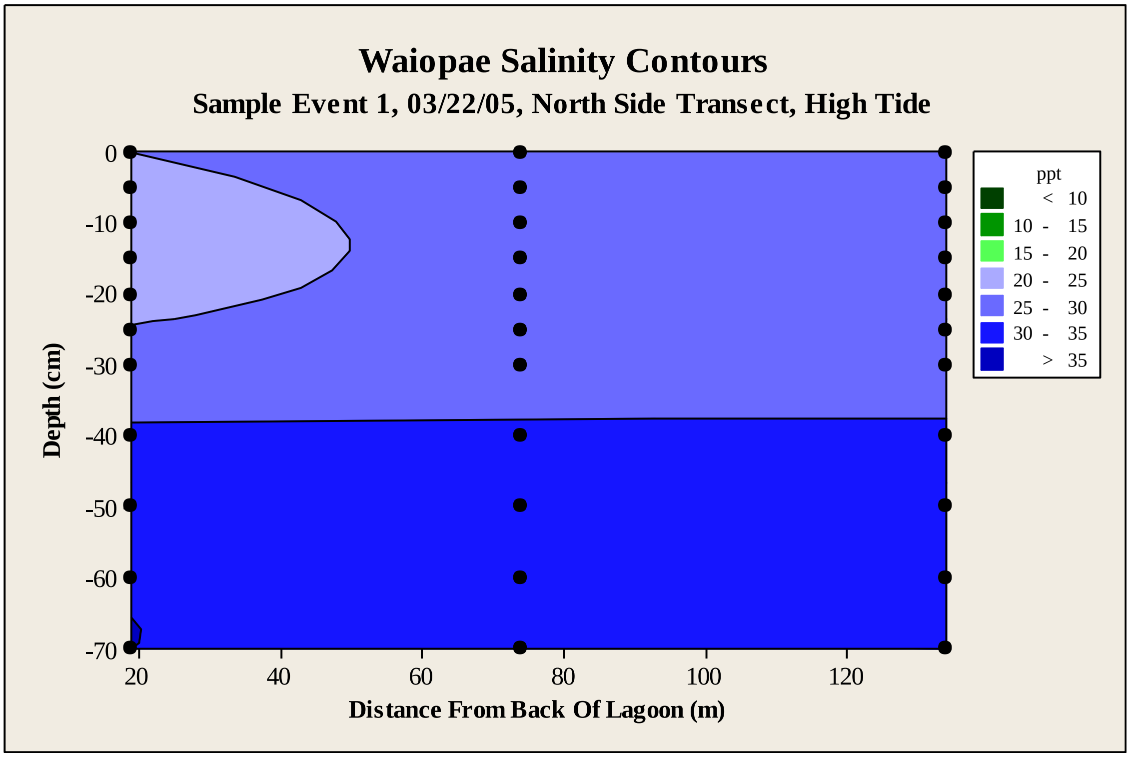
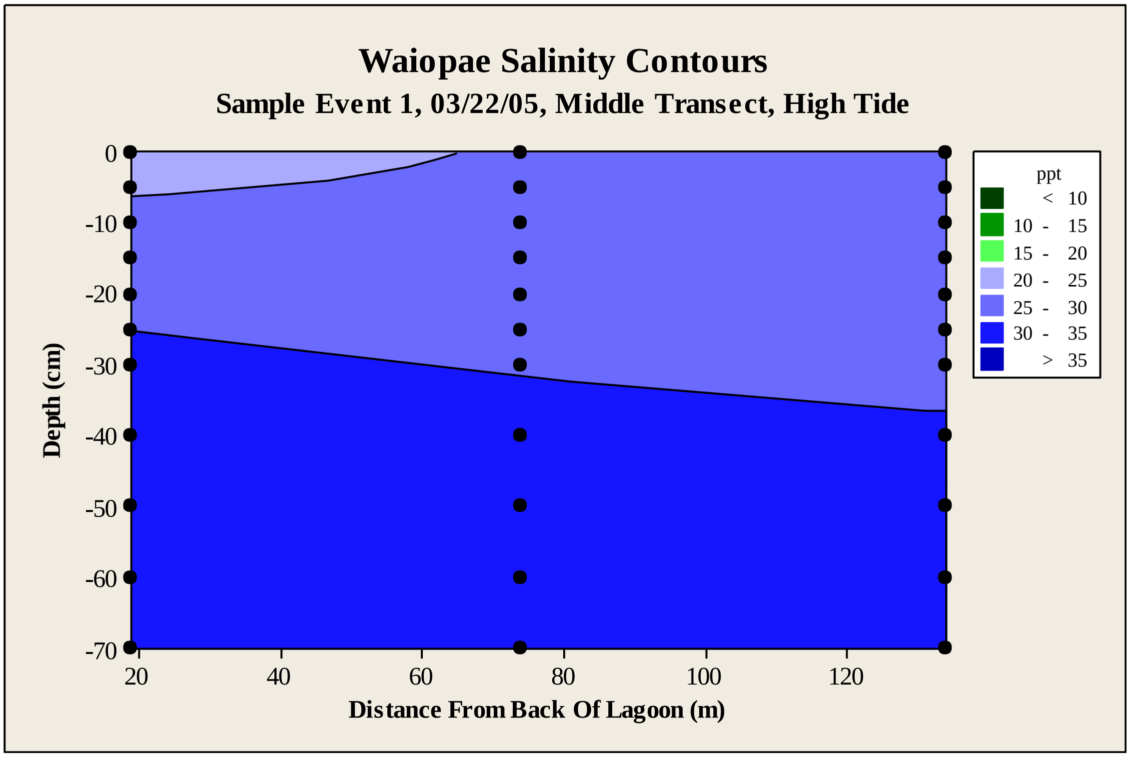


Figure 6. Salinity Contours, Event 1, North Side, High Tide
This salinity contour plot presents the salinity profile data from sample events 1 during High tide. This plot represents the north side transect as shown in figure 2. Black dots on the contour plots show the position where salinity values were documented. The legend to the right of the plot displays the range of salinity values that are represented by the various colors within these plots.



<u>Figure 7. Salinity Contours, Event 1, Middle, High Tide</u>
This salinity contour plot presents the salinity profile data from sample events 1 during High tide. This plot represents the middle transect as shown in figure 2. Black dots on the contour plots show the position where salinity values were documented. The legend to the right of the plot displays the range of salinity values that are represented by the various colors within these plots.

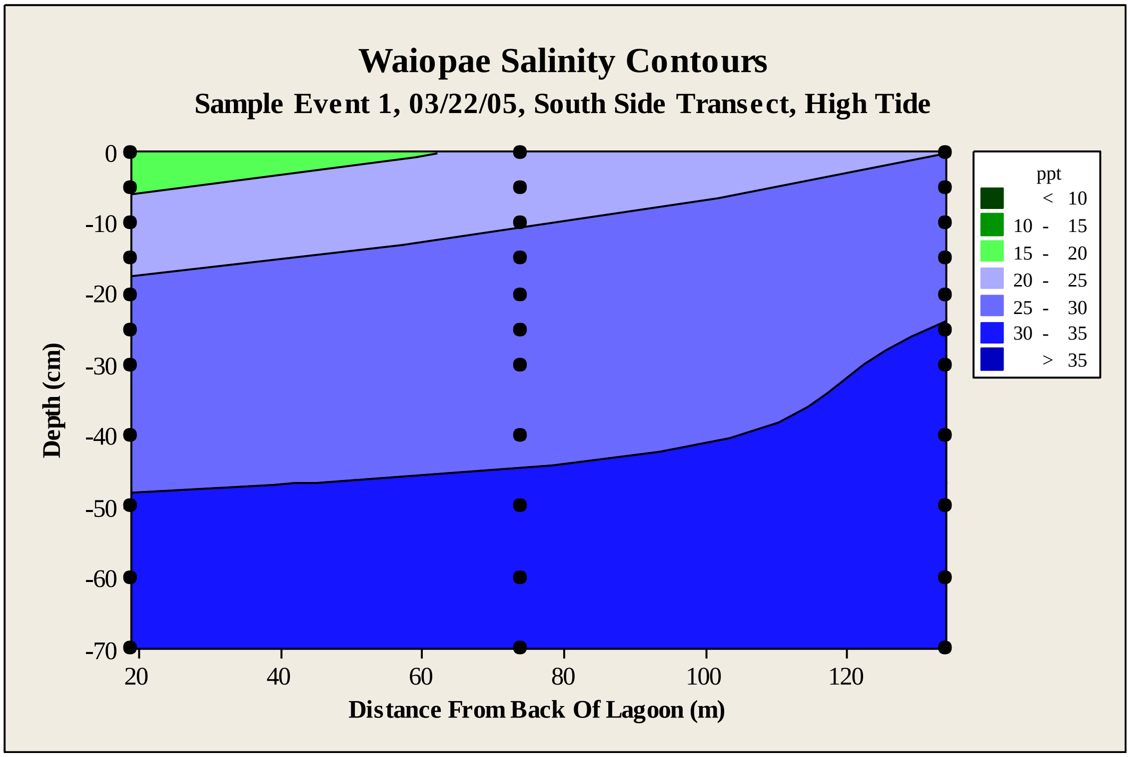


Figure 8. Salinity Contours, Event 1, South Side, High Tide
This salinity contour plot presents the salinity profile data from sample events 1 during High tide. This plot represents the south side transect as shown in figure 2. Black dots on the contour plots show the position where salinity values were documented. The legend to the right of the plot displays the range of salinity values that are represented by the various colors within these plots

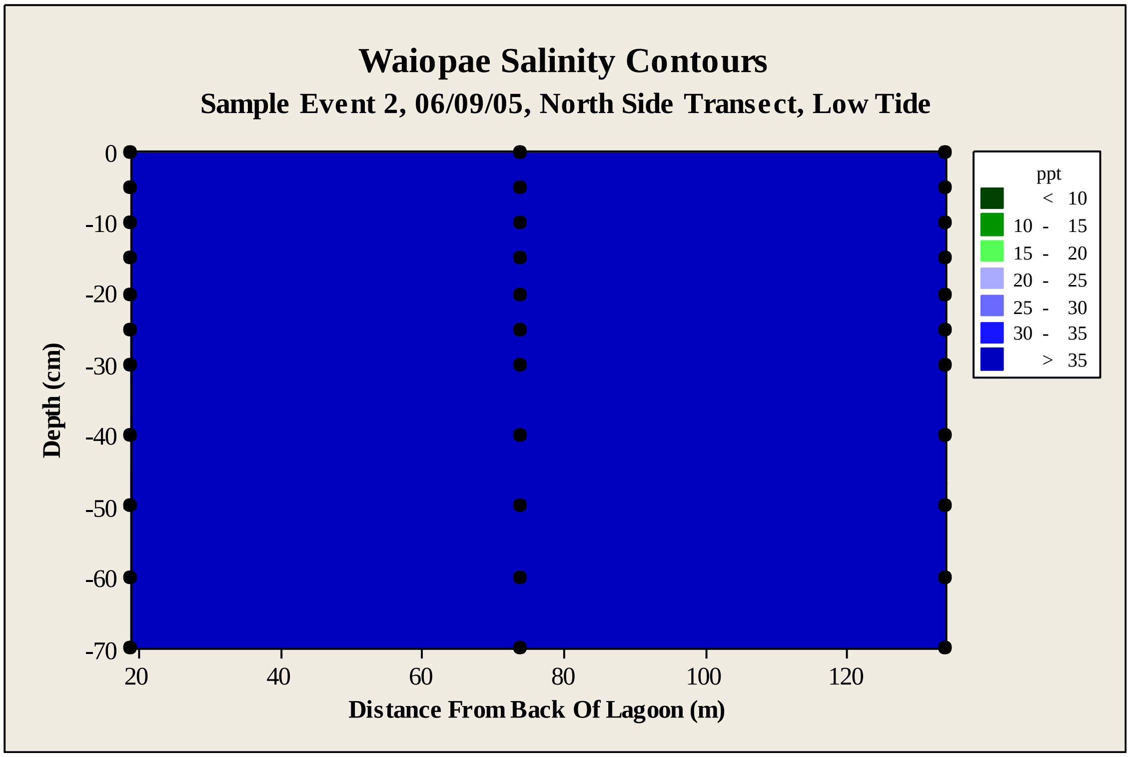


Figure 9. Salinity Contours, Event 2, North Side, Low Tide
This salinity contour plot presents the salinity profile data from sample events 2 during low tide. This plot represents the north side transect as shown in figure 2. Black dots on the contour plots show the position where salinity values were documented. The legend to the right of the plot displays the range of salinity values that are represented by the various colors within these plots.

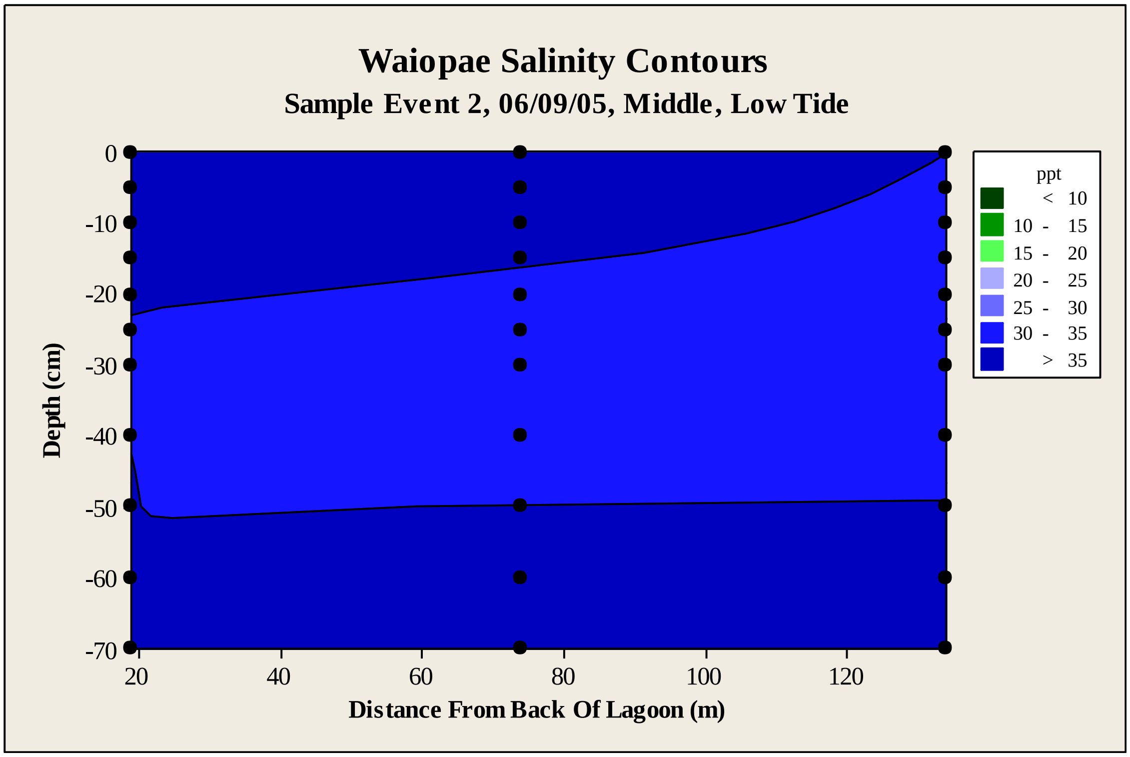


Figure 10. Salinity Contours, Event 2, Middle, Low Tide
This salinity contour plot presents the salinity profile data from sample events 2 during low tide. This plot represents the middle transect as shown in figure 2. Black dots on the contour plots show the position where salinity values were documented. The legend to the right of the plot displays the range of salinity values that are represented by the various colors within these plots.

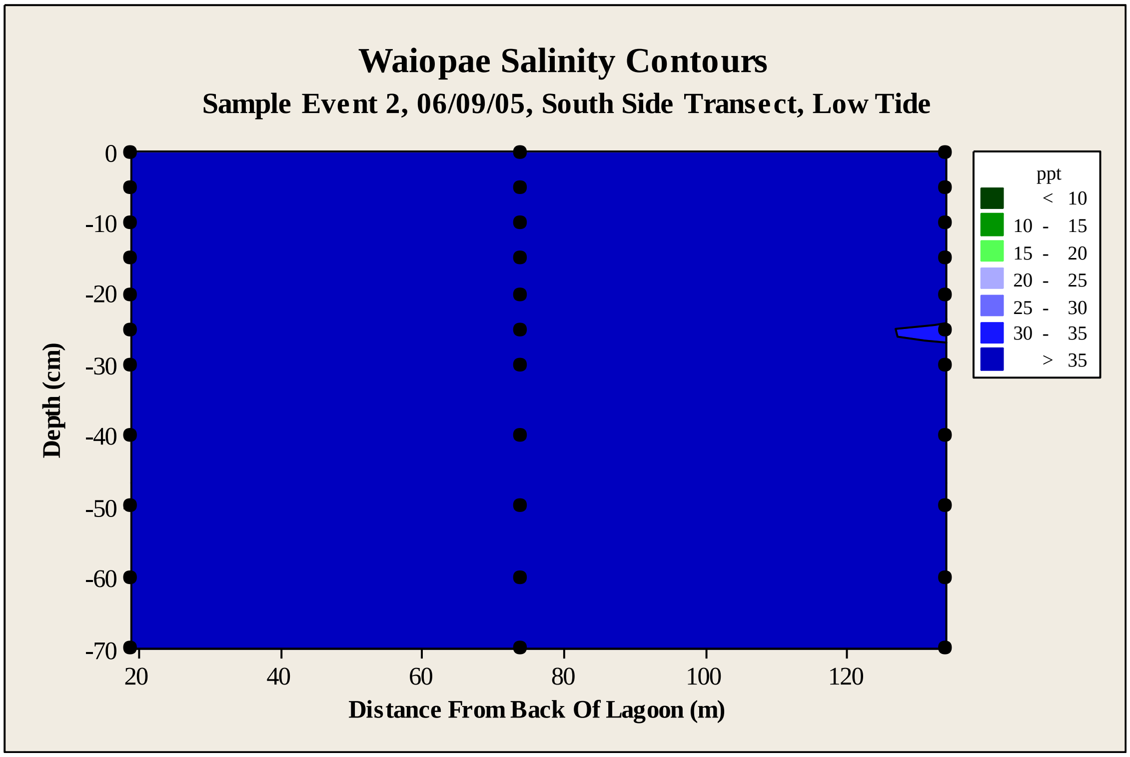


Figure 11. Salinity Contours, Event 2, North Side, Low Tide
This salinity contour plot presents the salinity profile data from sample events 2 during low tide. This plot represents the south side transect as shown in figure 2. Black dots on the contour plots show the position where salinity values were documented. The legend to the right of the plot displays the range of salinity values that are represented by the various colors within these plots.

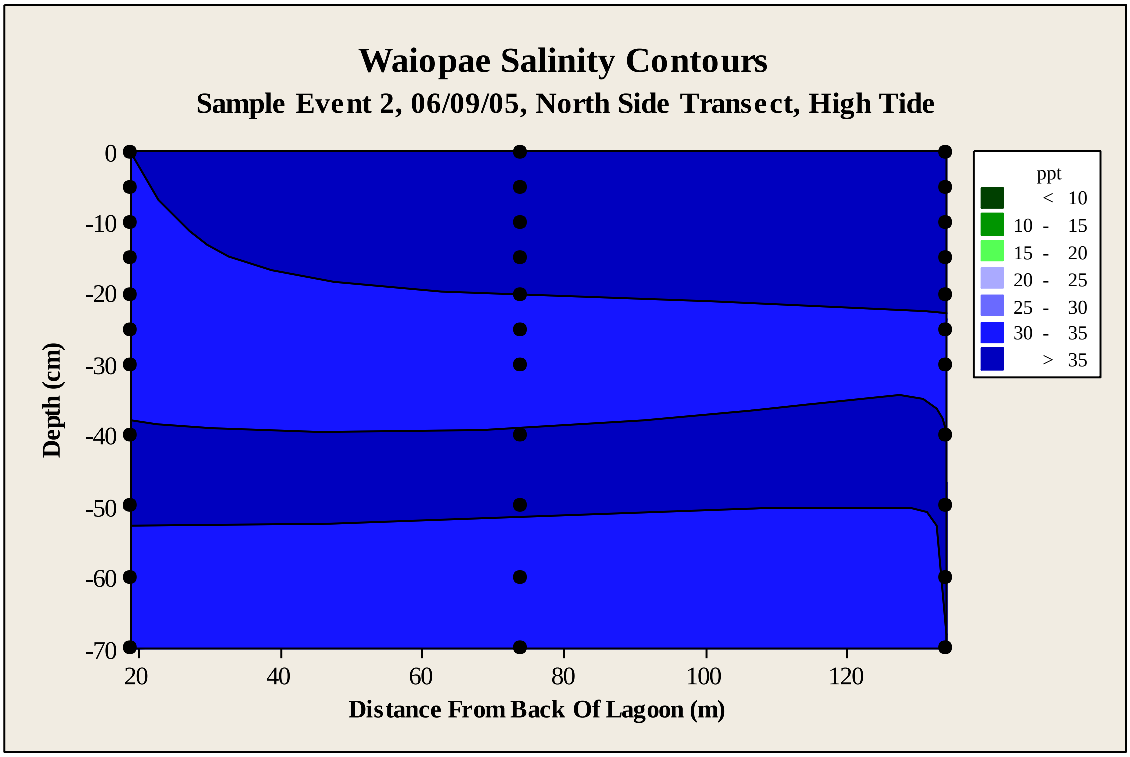


Figure 12. Salinity Contours, Event 2, North Side, High Tide
This salinity contour plot presents the salinity profile data from sample events 2 during high tide. This plot represents the north side transect as shown in figure 2. Black dots on the contour plots show the position where salinity values were documented. The legend to the right of the plot displays the range of salinity values that are represented by the various colors within these plots.

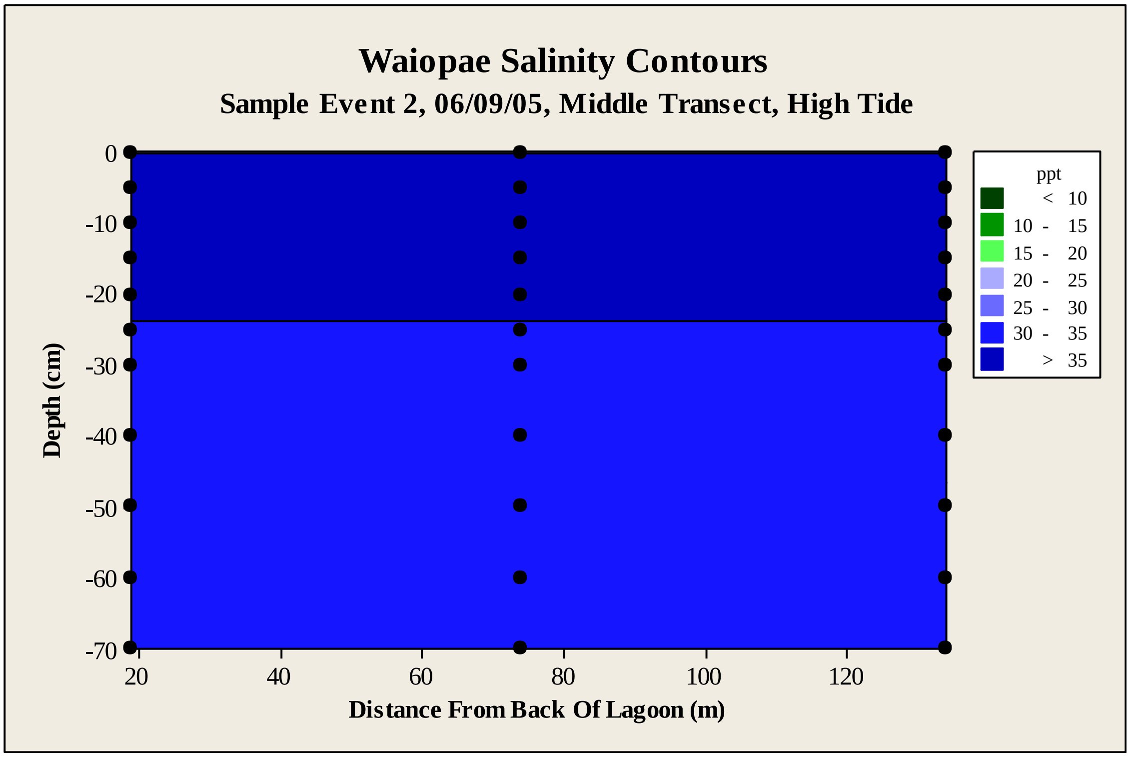


Figure 13. Salinity Contours, Event 2, Middle, High Tide
This salinity contour plot presents the salinity profile data from sample events 2 during high tide. This plot represents the middle transect as shown in figure 2. Black dots on the contour plots show the position where salinity values were documented. The legend to the right of the plot displays the range of salinity values that are represented by the various colors within these plots.

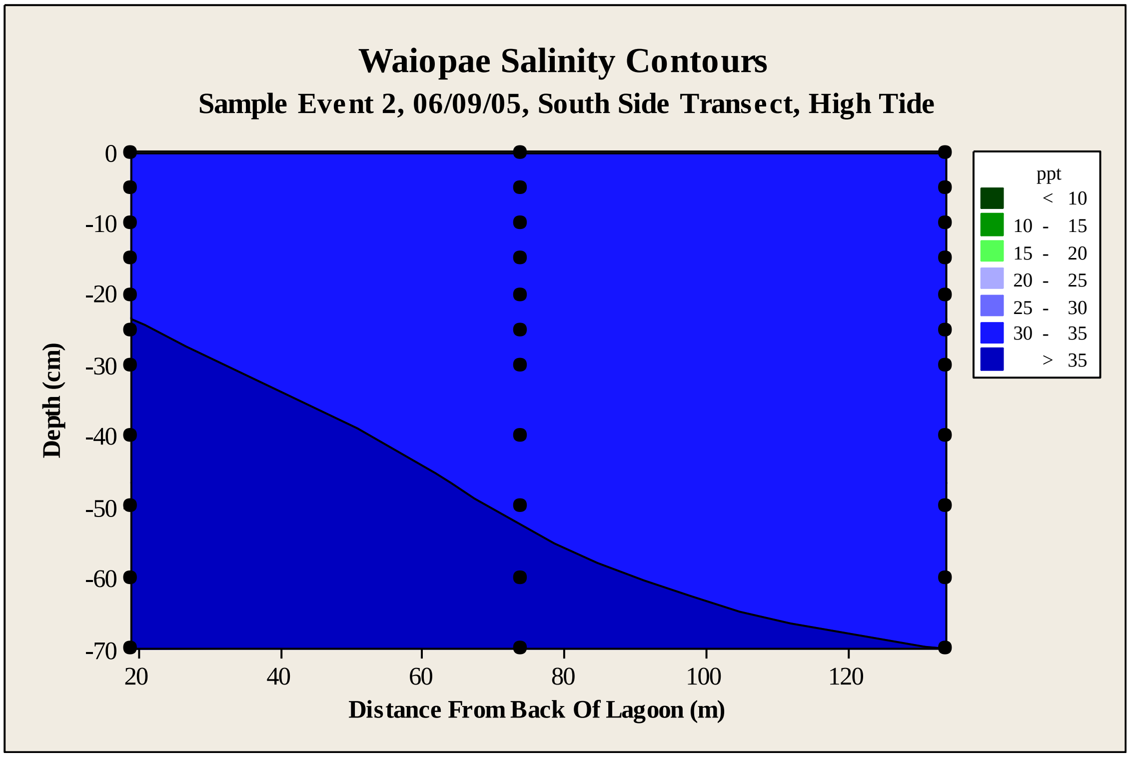


Figure 14. Salinity Contours, Event 2, South Side, High Tide
This salinity contour plot presents the salinity profile data from sample events 2 during high tide. This plot represents the south side transect as shown in figure 2. Black dots on the contour plots show the position where salinity values were documented. The legend to the right of the plot displays the range of salinity values that are represented by the various colors within these plots.

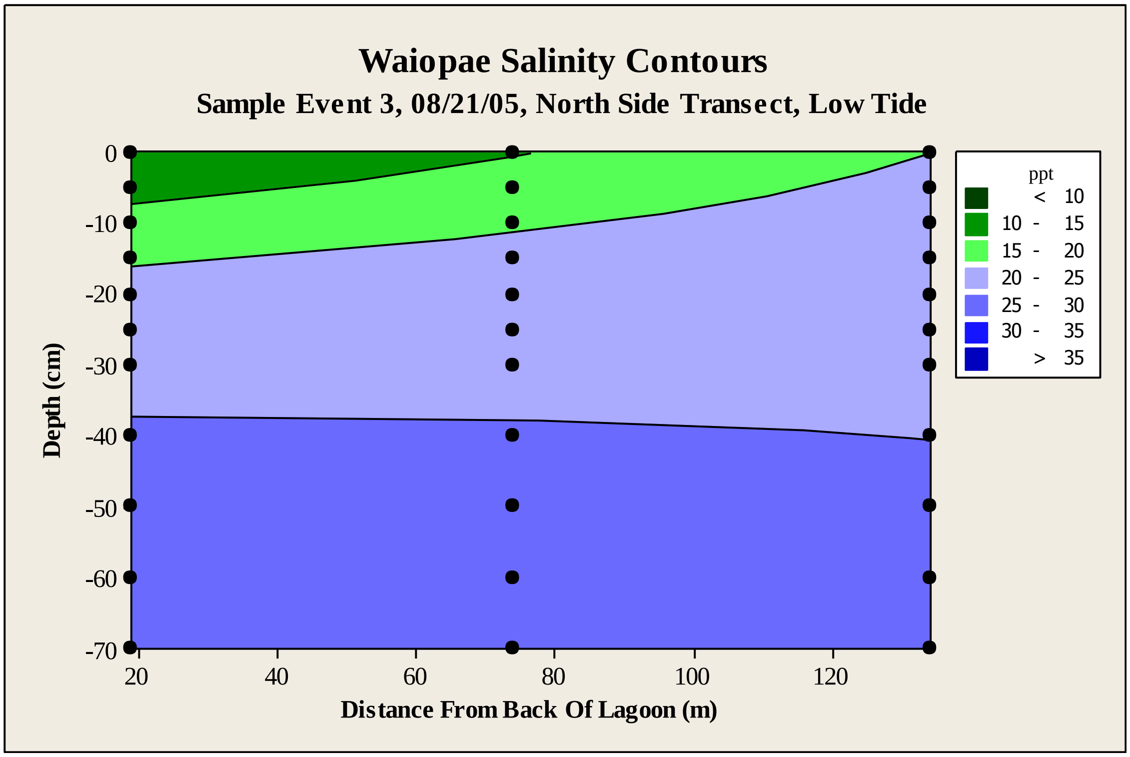


Figure 15. Salinity Contours, Event 3, North Side, Low Tide
This salinity contour plot presents the salinity profile data from sample events 3 during low tide. This plot represents the north side transect as shown in figure 2. Black dots on the contour plots show the position where salinity values were documented. The legend to the right of the plot displays the range of salinity values that are represented by the various colors within these plots.

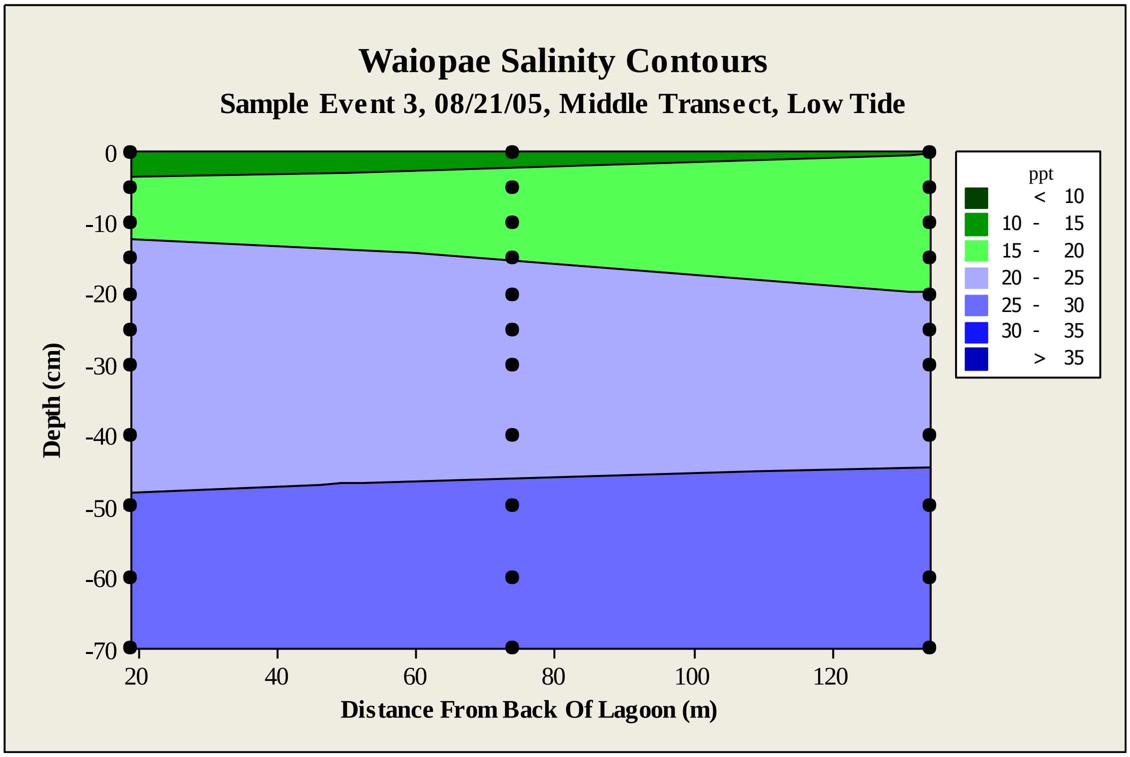


Figure 16. Salinity Contours, Event 3, Middle, Low Tide
This salinity contour plot presents the salinity profile data from sample events 3 during low tide. This plot represents the middle transect as shown in figure 2. Black dots on the contour plots show the position where salinity values were documented. The legend to the right of the plot displays the range of salinity values that are represented by the various colors within these plots.

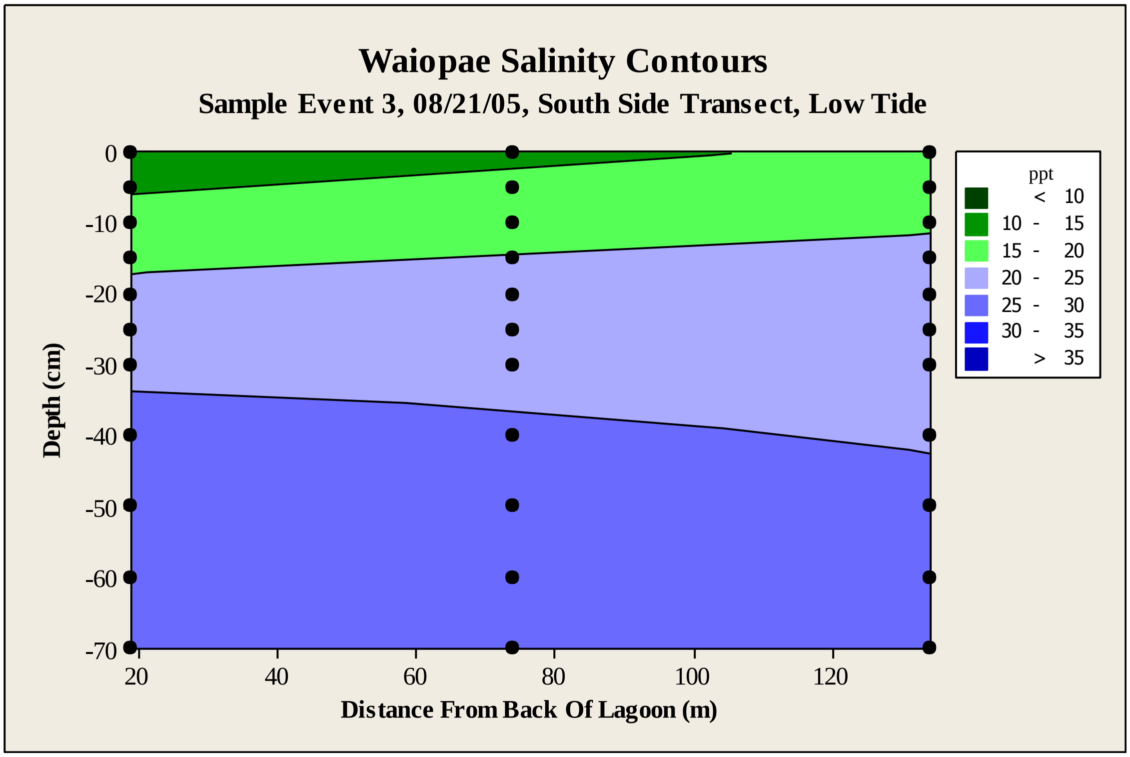


Figure 17. Salinity Contours, Event 3, South Side, Low Tide

This salinity contour plot presents the salinity profile data from sample events 3 during low tide. This plot represents the south side transect as shown in figure 2. Black dots on the contour plots show the position where salinity values were documented. The legend to the right of the plot displays the range of salinity values that are represented by the various colors within these plots.

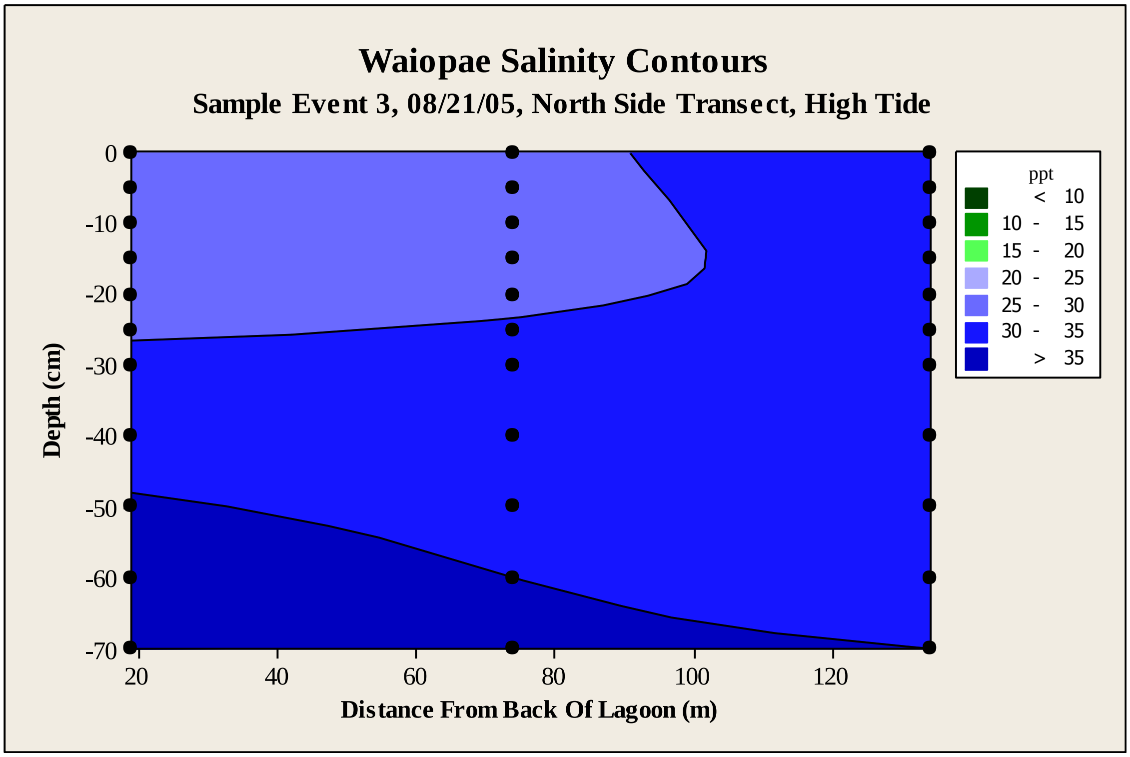


Figure 18. Salinity Contours, Event 3, North Side, High Tide
This salinity contour plot presents the salinity profile data from sample events 3 during how tide. This plot represents the north side transect as shown in figure 2. Black dots on the contour plots show the position where salinity values were documented. The legend to the right of the plot displays the range of salinity values that are represented by the various colors within these plots.

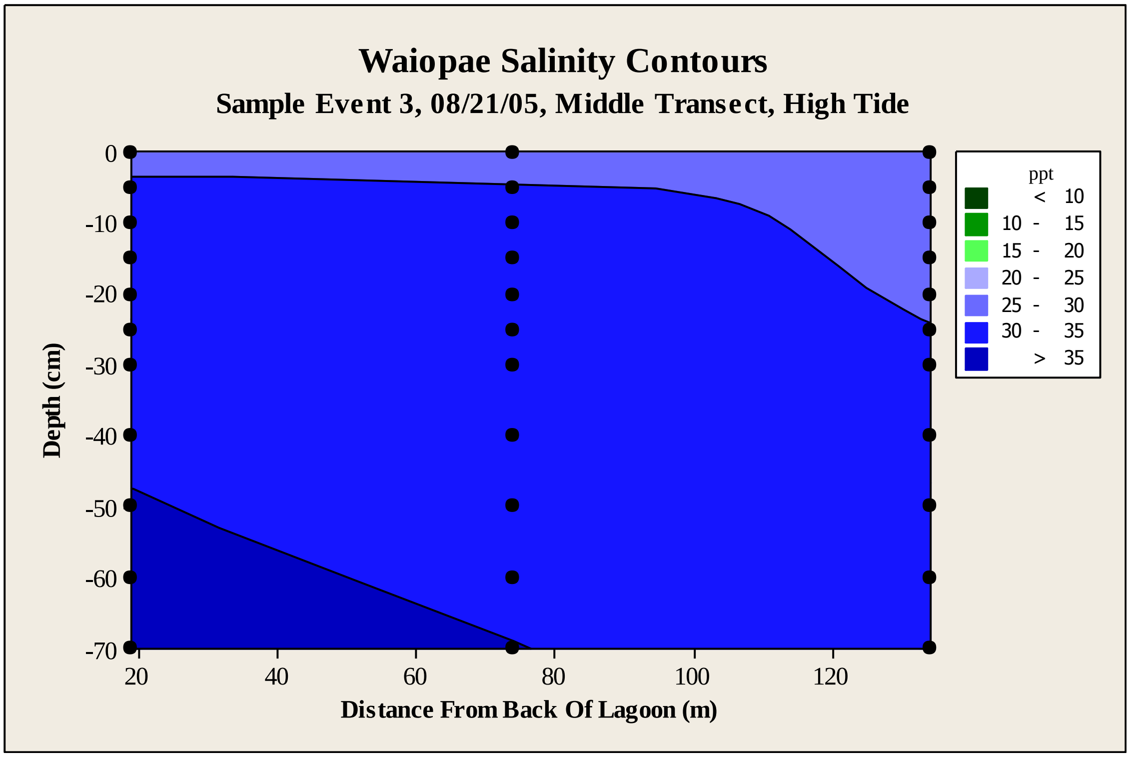


Figure 19. Salinity Contours, Event 3, Middle, High Tide
This salinity contour plot presents the salinity profile data from sample events 3 during how tide. This plot represents the middle transect as shown in figure 2. Black dots on the contour plots show the position where salinity values were documented. The legend to the right of the plot displays the range of salinity values that are represented by the various colors within these plots.

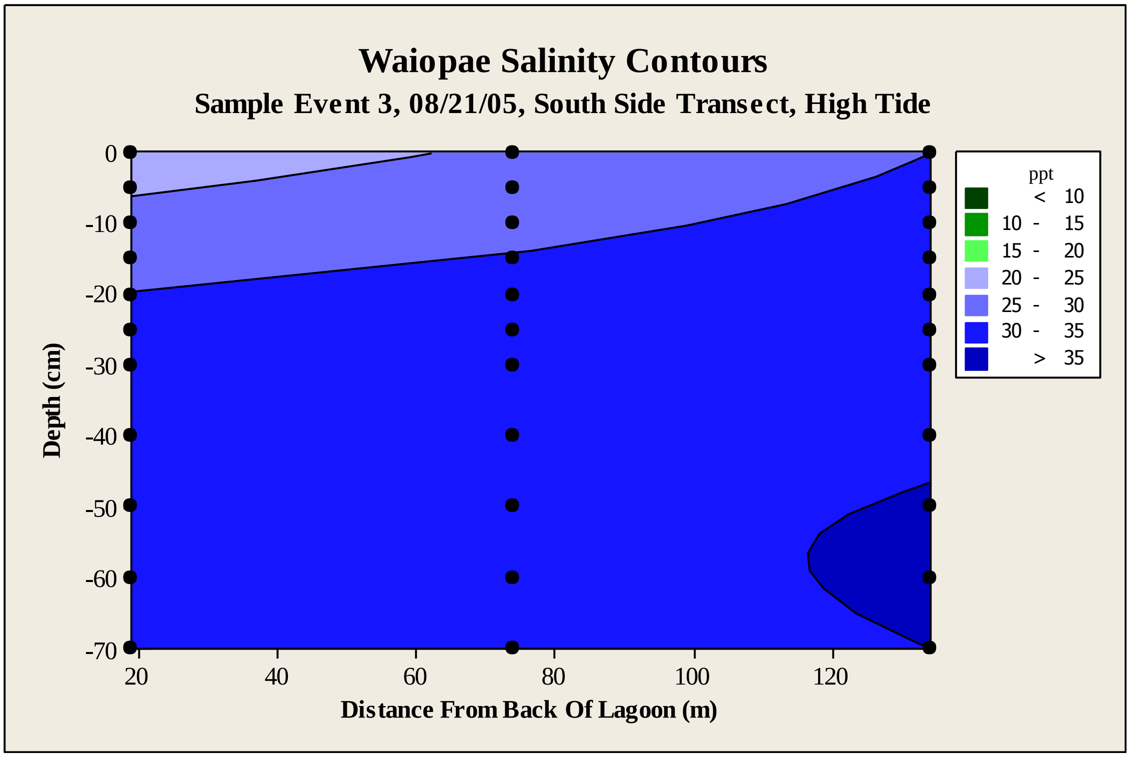


Figure 20. Salinity Contours, Event 3, South Side, High Tide
This salinity contour plot presents the salinity profile data from sample events 3 during how tide. This plot represents the south side transect as shown in figure 2. Black dots on the contour plots show the position where salinity values were documented. The legend to the right of the plot displays the range of salinity values that are represented by the various colors within these plots.

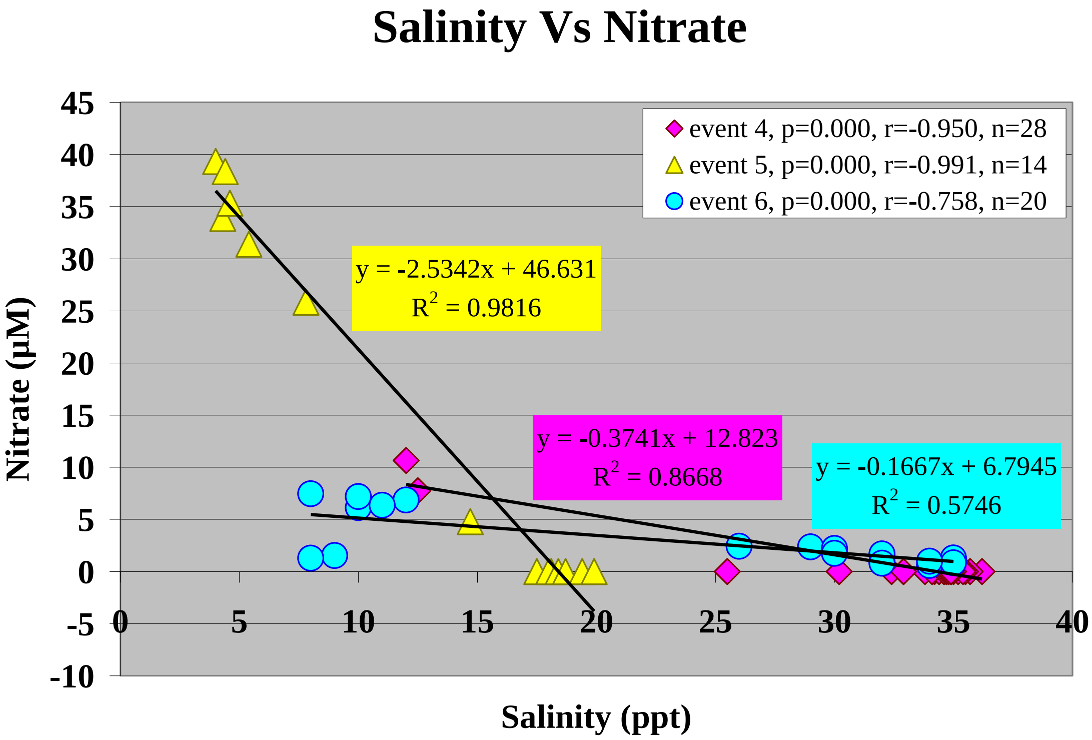


Figure 21. Salinity Vs Nitrate at Waiopae

This scatter plot represents correlations between salinity and nitrate. The legend found at the top right of the plot displays which symbol represents what sample event. The legend also presents the *p*, *r*, and n value of each sample event. The three colored data labels match the color of the appropriate sample event. These labels display the equation of the appropriate trend line and the R^2 value of that particular correlation.

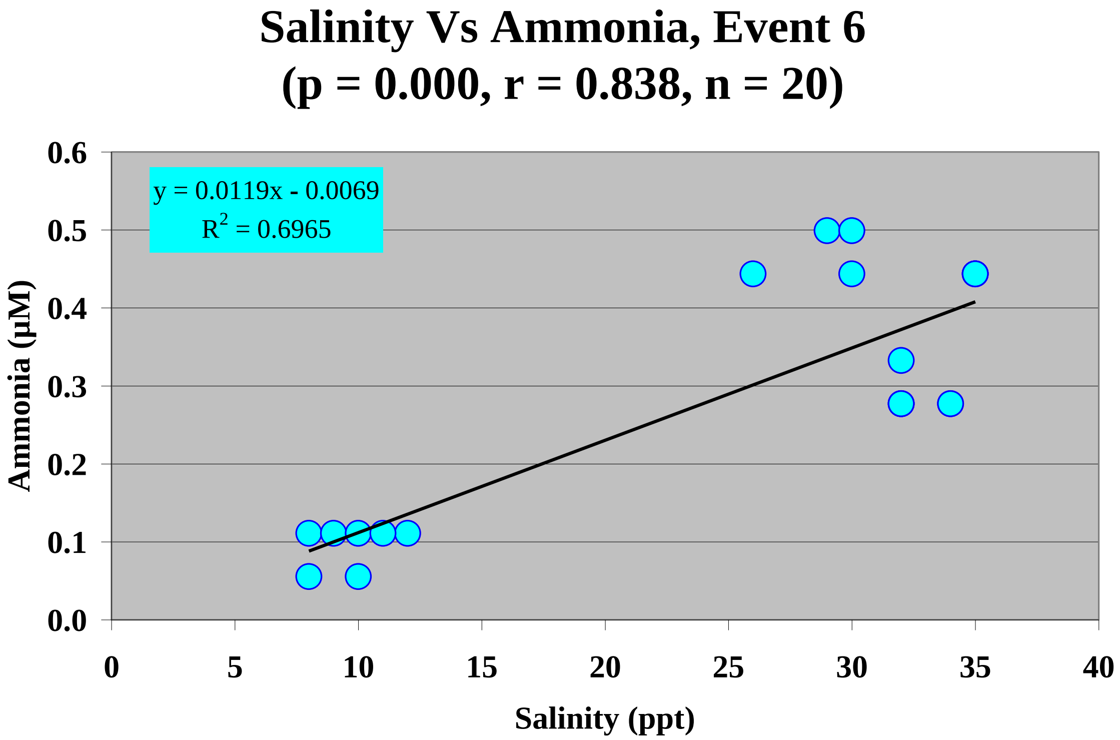


Figure 22. Salinity Vs Ammonia at Waiopae
This scatter plot represents a correlation between salinity and ammonia from water quality samples. The data label found within this plot displays the trend line equation and the R^2 value of this correlation.

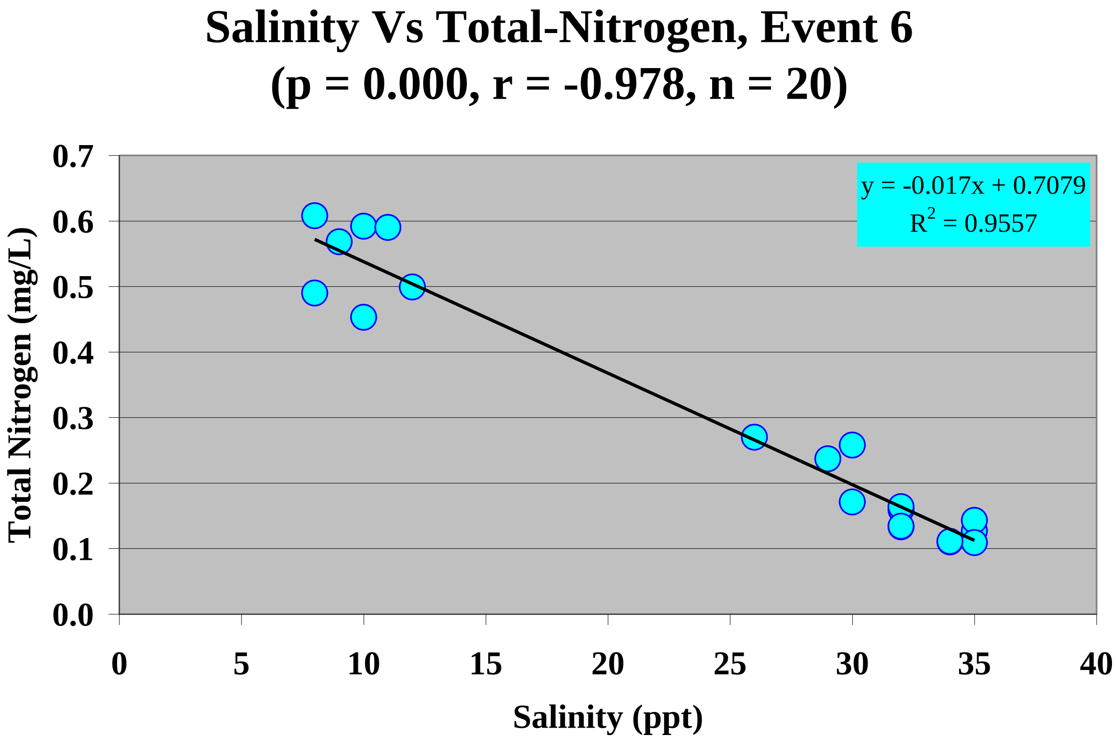


Figure 23. Salinity Vs Total-Nitrogen at Waiopae
This scatter plot represents a correlation between salinity and total nitrogen from water quality samples. The data label found within this plot displays the trend line equation and the R^2 value of this correlation.

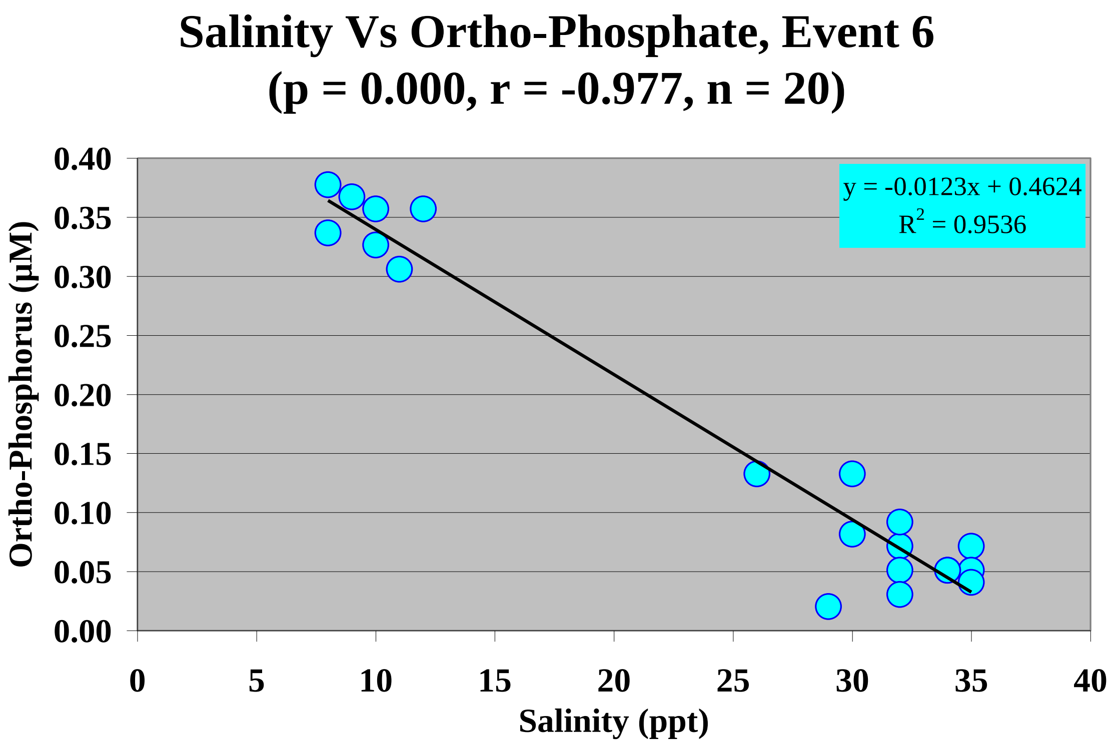


Figure 24. Salinity Vs Ortho-Phosphate at Waiopae
This scatter plot represents a correlation between salinity and ortho-phosphate from water quality samples. The data label found within this plot displays the trend line equation and the R^2 value of this correlation.

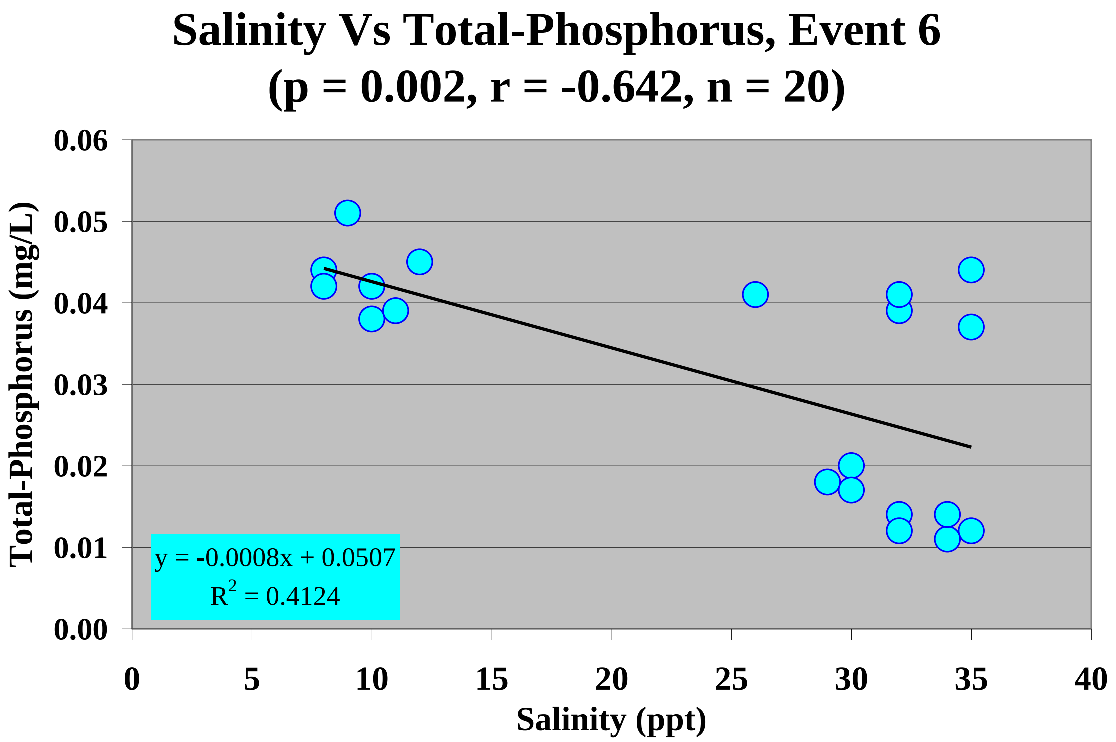


Figure 25. Salinity Vs Total-Phosphorus at Waiopae
This scatter plot represents a correlation between salinity and total-phosphorus from water quality samples. The data label found within this plot displays the trend line equation and the R^2 value of this correlation.

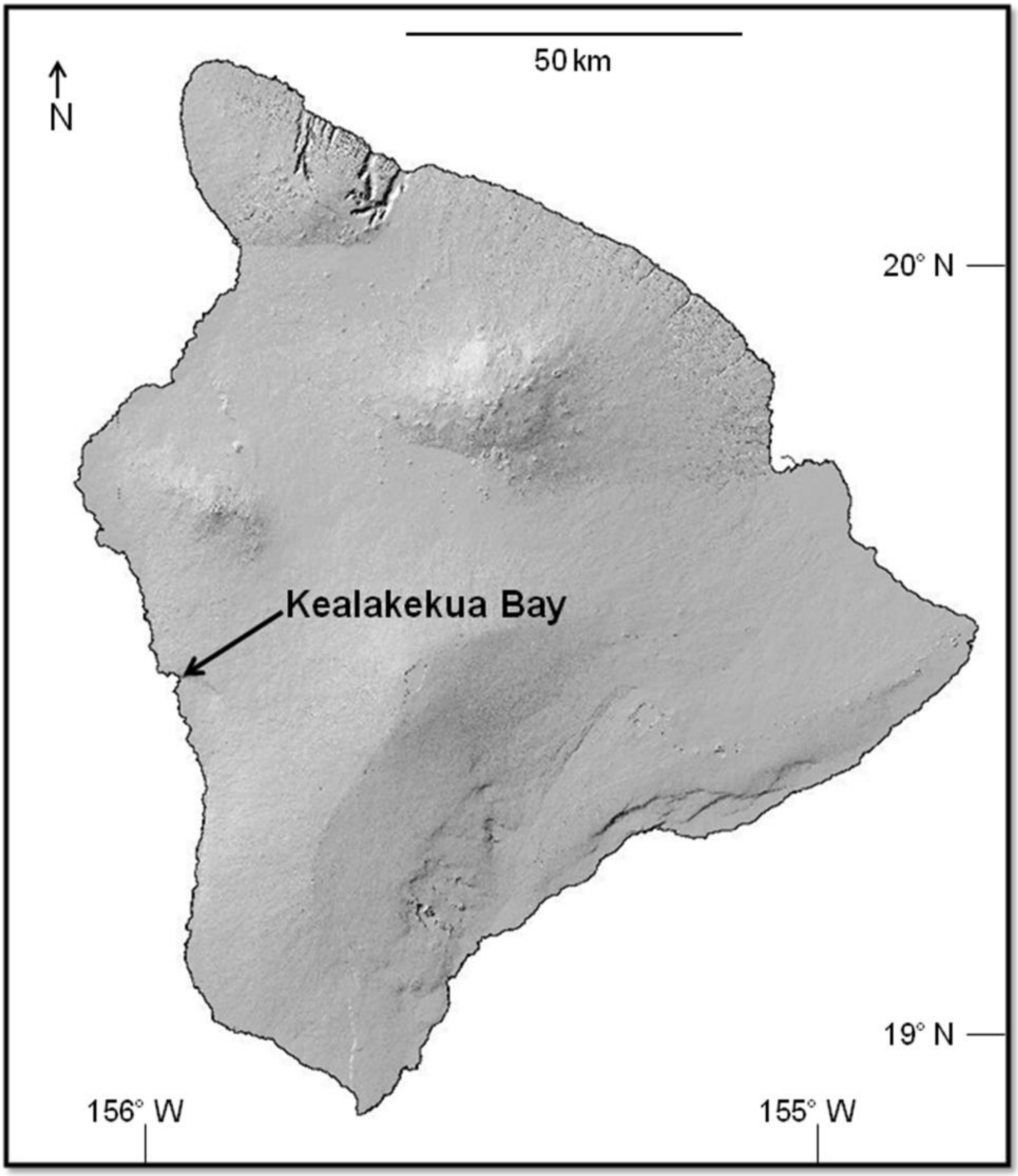


Figure 26. Map of Hawaii Island
This figure is a map of Hawaii Island, pointing out the location of Kealakekua Bay.

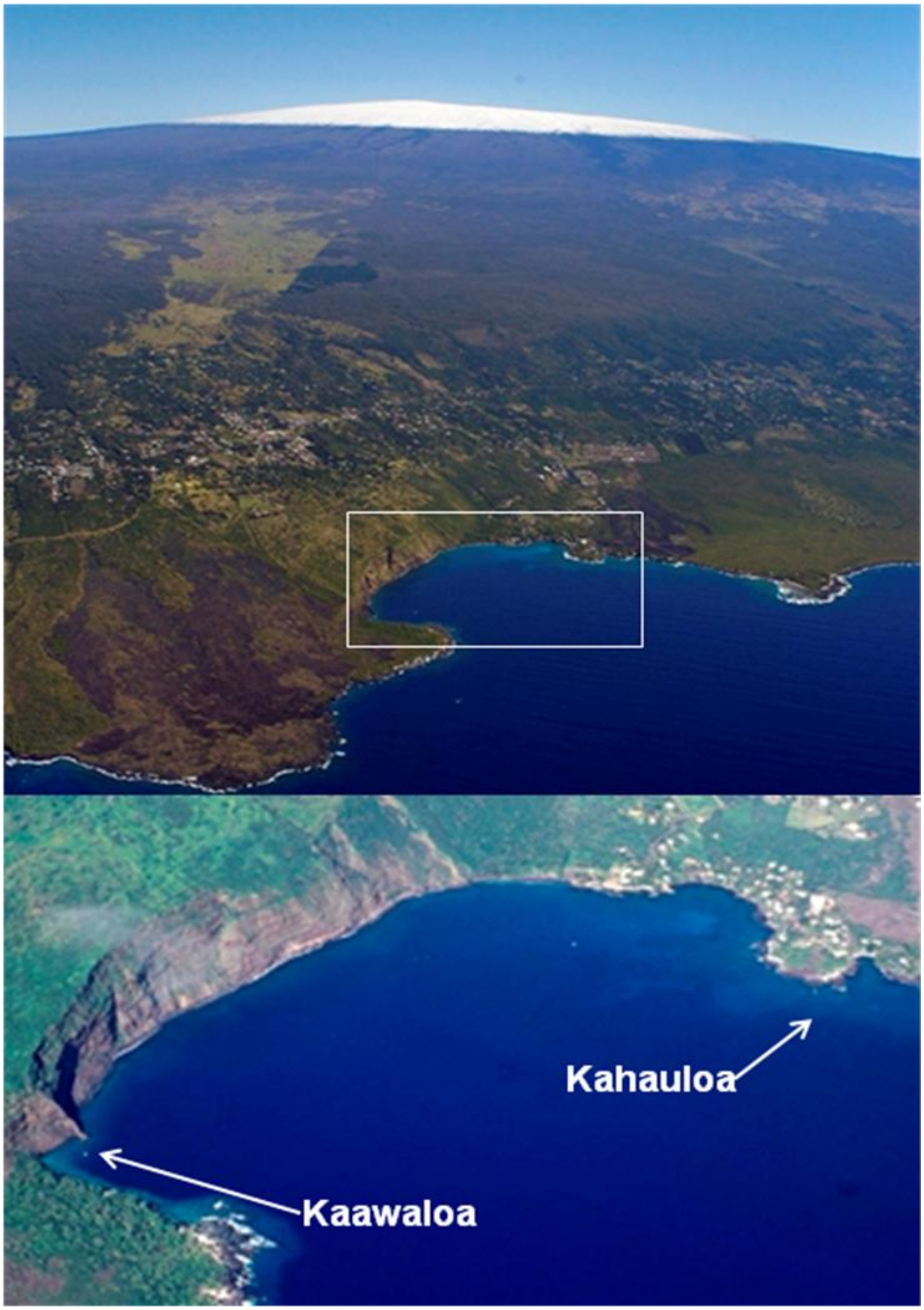


Figure 27. Location of Sites
The top image displays Kealakekua Bay, the rectangled are in this image is shown in greater detail by the bottom image. The bottom image shows the location of Kaawaloa and Kahauloa. These images are credited to Hawaiian Images Photography & Video.

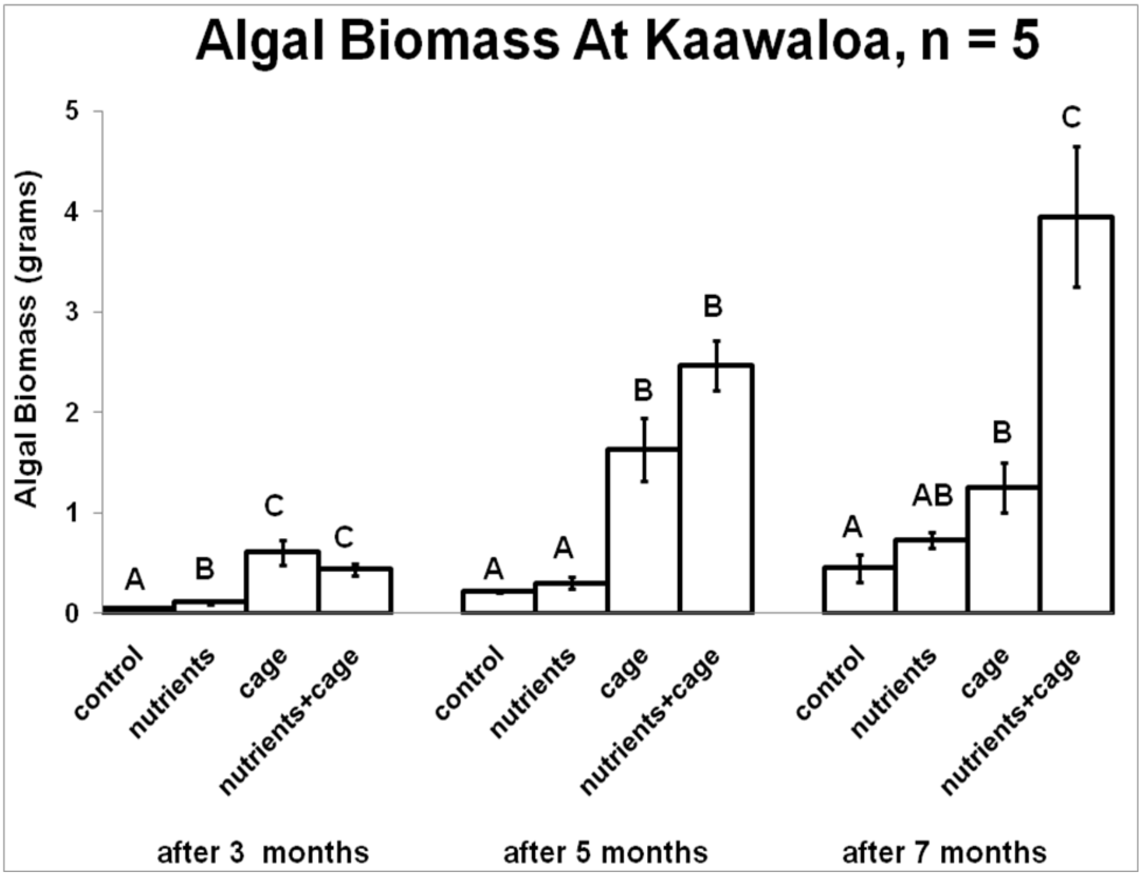
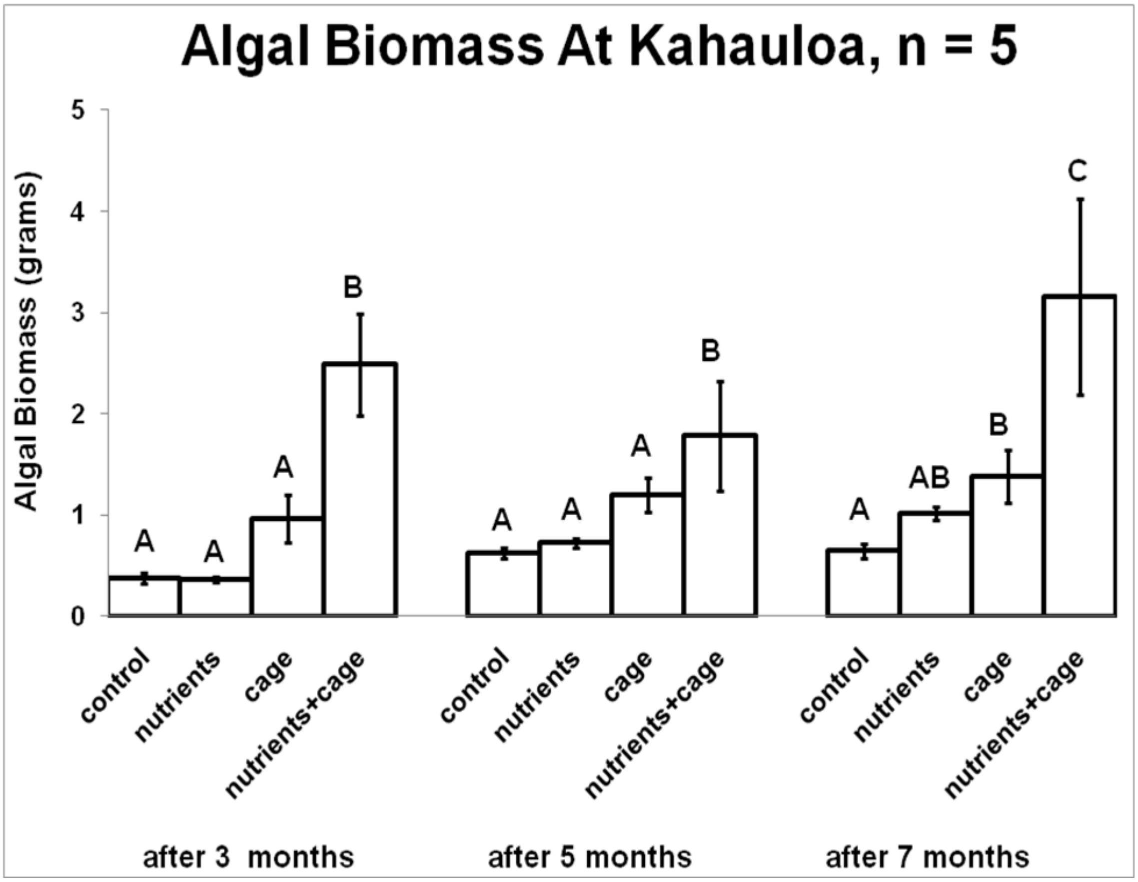


Figure 28. Algal Biomass on Tiles at Kaawaloa
This graph displays the algal biomass on tiles of different treatments during different time periods at Kaawaloa (n = 5). The x-axis represents treatments and time in months. The y-axis represents grams of dry algal biomass on tiles. Error bars represent standard error. Letters above bars display significance between treatments within time periods.



<u>Figure 29. Algal Biomass on Tiles at Kahauloa</u>
This graph displays the algal biomass on tiles of different treatments during different time periods at Kahauloa (n = 5). The x-axis represents treatments and time in months. The y-axis represents grams of dry algal biomass on tiles. Error bars represent standard error. Letters above bars display significance between treatments within time periods.

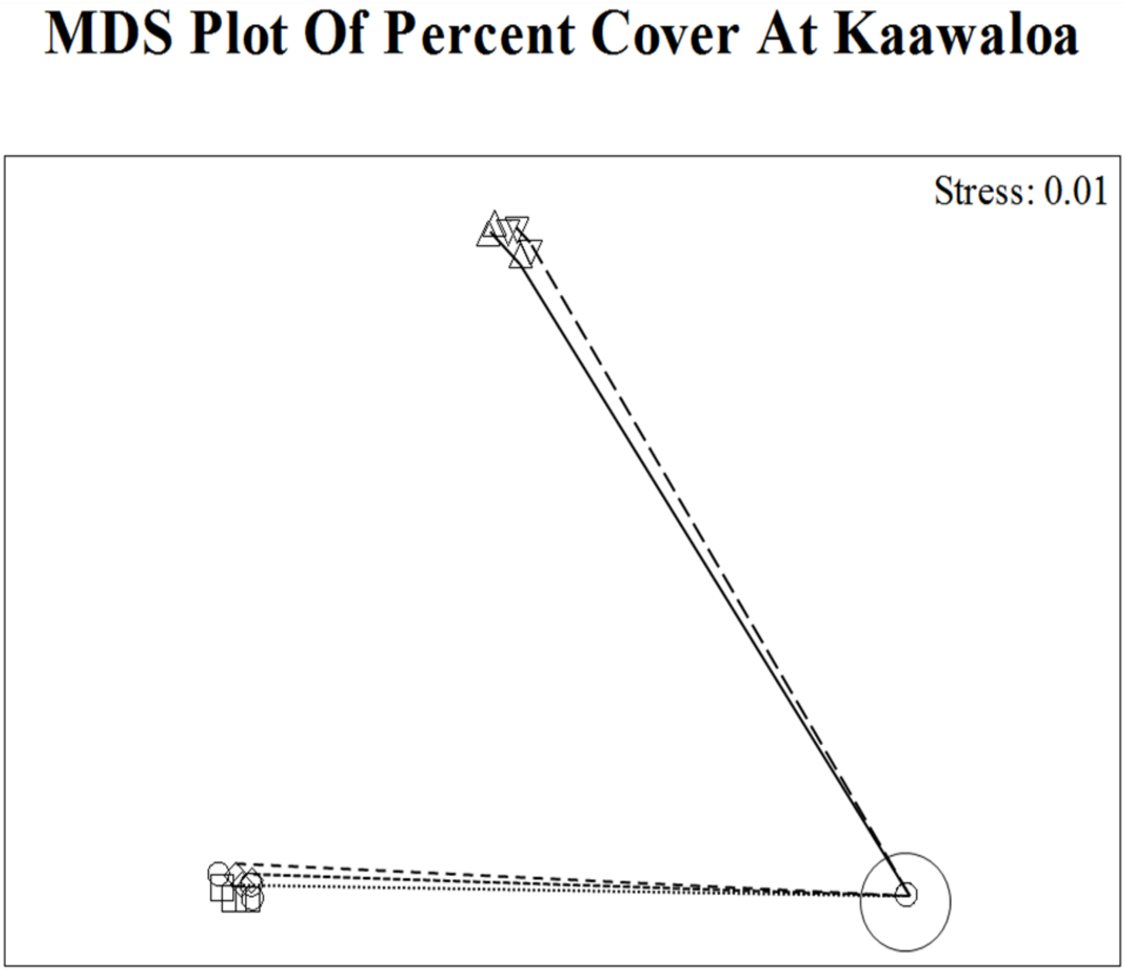


Figure 30. MDS Plot of Percent Cover at Kaawaloa

This MDS plot displays the average algal percent cover of tiles at Kaawaloa. Each point represents the average percent cover of five tiles for each time period during each time period. The closer points are together the more similar the algal communities. The further points are from each other the more dissimilar the algal communities. Lines display the successional trajectory of each treatment. Each trajectory begins at the double circle. As the line moves from the double circle, the first point it intersects is the average percent cover of a treatment after three months, the second point after five months, and the third point after seven months. Due to the closeness of point on this plot the trajectory line are difficult to visualize after the first or second point. Key for points: double circle = tiles prior to treatment, single circles = control, diamonds = cage control, squares = nutrient treatment, downward pointing triangles = cage treatment, upward pointing triangles = nutrient-cage treatment.

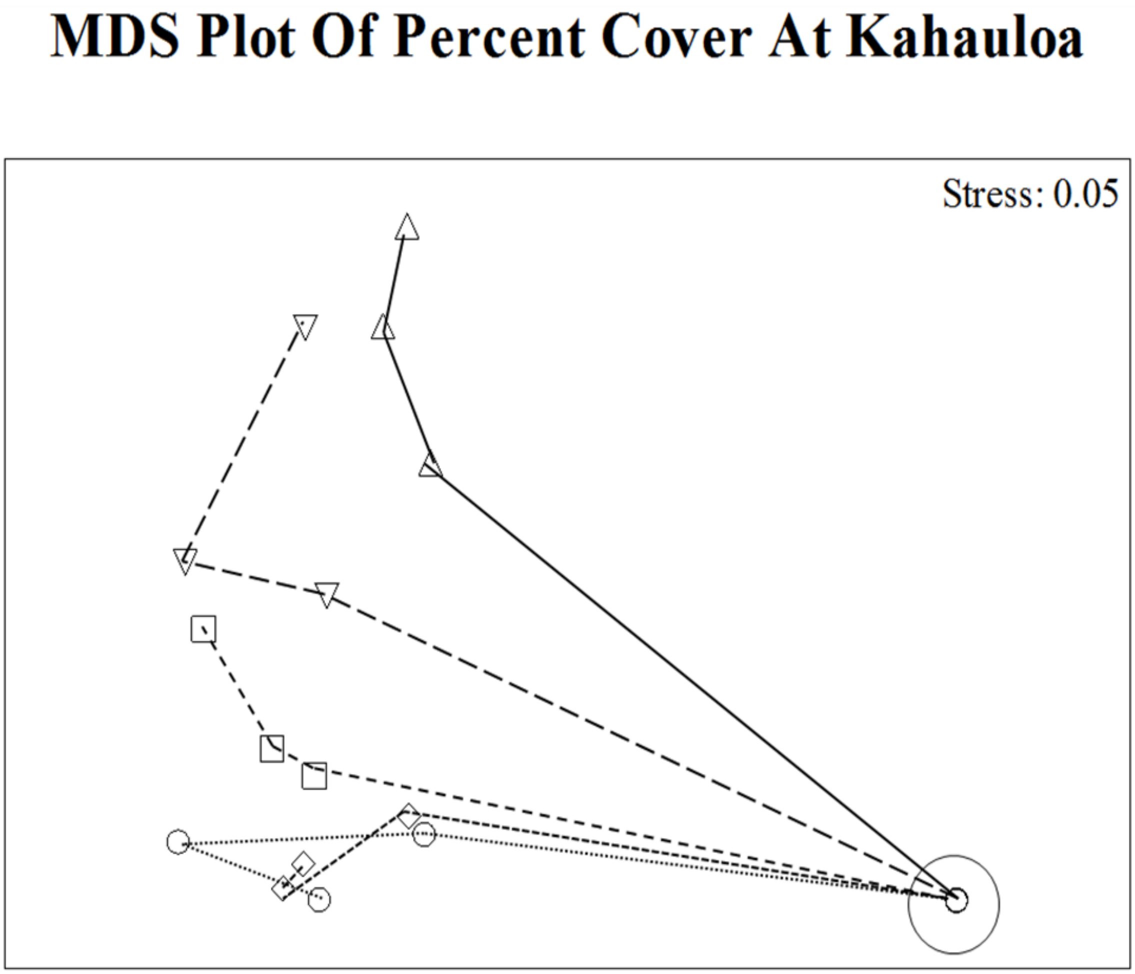

MDS Plot Of Percent Cover At Kahauloa

Figure 31. MDS Plot of Percent Cover at Kahauloa
This MDS plot displays the average algal percent cover of tiles at Kahauloa. Each point represents the average percent cover of five tiles for each time period during each time period. The closer points are together the more similar the algal communities. The further points are from each other the more dissimilar the algal communities. Lines display the successional trajectory of each treatment. Each trajectory begins at the double circle. As the line moves from the double circle, the first point it intersects is the average percent cover of a treatment after three months, the second point after five months, and the third after seven months. Key for points: double circle = tiles prior to treatment, single circles = control, diamonds = cage control, squares = nutrient treatment, downward pointing triangles = cage treatment, upward pointing triangles = nutrient-cage treatment.

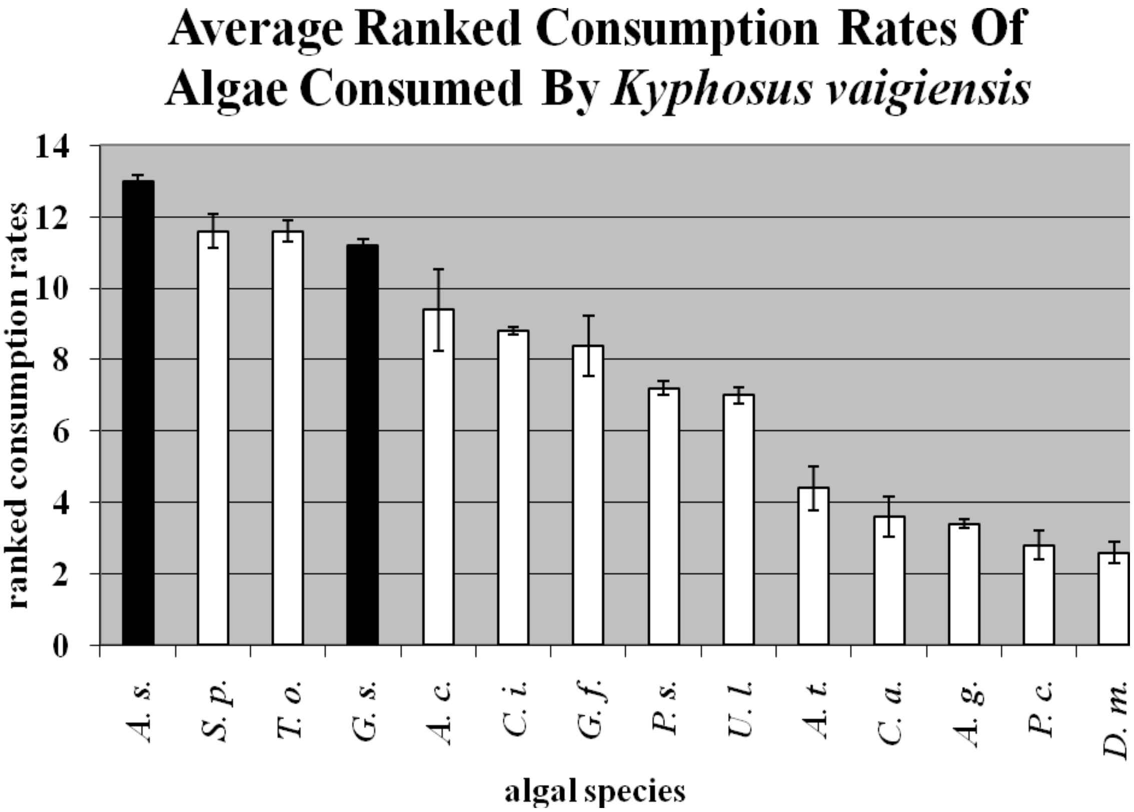


Figure 32. Consumption Rates of Algae Consumed by *Kyphosus vaigiensis*
Average ranked consumption rates of algae consumed by *K. vaigiensis* (n = 5). Algal species are represented by their initials. Black bars represent alien algal species, while white bars represent indigenous algal species. Error bars represent +/- 1 SE.

Kyphosus vaigiensis

Differences In Algal Consumption Rates

black = no significant difference

the initials of the algae with the greater consumption rate is

displayed in the matrix when a significant difference ($p < 0.05$) does exist

	A.s.	**S.p.**	**T.o.**	**G.s.**	**A.c.**	**C.i.**	**G.f.**	**P.s.**	**U.l.**	**A.t.**	**C.a.**	**A.g.**	**P.c.**	**D.m.**
A.s.		■	■	■	■	■	■	*A.s.*	*A.s.*	*A.s.*	*A.s.*	*A.s.*	*A.s.*	*A.s.*
S.p.	■		■	■	■	■	■	■	■	*S.p.*	*S.p.*	*S.p.*	*S.p.*	*S.p.*
T.o.	■	■		■	■	■	■	■	■	*T.o.*	*T.o.*	*T.o.*	*T.o.*	*T.o.*
G.s.	■	■	■		■	■	■	■	■	*G.s.*	*G.s.*	*G.s.*	*G.s.*	*G.s.*
A.c.	■	■	■	■		■	■	■	■	*A.c.*	*A.c.*	*A.c.*	*A.c.*	*A.c.*
C.i.	■	■	■	■	■		■	■	■	■	*C.i.*	*C.i.*	*C.i.*	*C.i.*
G.f.	■	■	■	■	■	■		■	■	■	■	*G.f.*	*G.f.*	*G.f.*
P.s.	*A.s.*	■	■	■	■	■	■		■	■	■	■	■	■
U.l.	*A.s.*	■	■	■	■	■	■	■		■	■	■	■	■
A.t.	*A.s.*	*S.p.*	*T.o.*	*G.s.*	*A.c.*	■	■	■	■		■	■	■	■
C.a.	*A.s.*	*S.p.*	*T.o.*	*G.s.*	*A.c.*	*C.i.*	■	■	■	■		■	■	■
A.g.	*A.s.*	*S.p.*	*T.o.*	*G.s.*	*A.c.*	*C.i.*	*G.f.*	■	■	■	■		■	■
P.c.	*A.s.*	*S.p.*	*T.o.*	*G.s.*	*A.c.*	*C.i.*	*G.f.*	■	■	■	■	■		■
D.m.	*A.s.*	*S.p.*	*T.o.*	*G.s.*	*A.c.*	*C.i.*	*G.f.*	■	■	■	■	■	■	

Figure 33. *Kyphosus vaigiensis* Differences in Algal Consumption Rates
Results of Tukey's test of differences in consumption rates of algae by *K. vaigiensis* (n = 5). Algal species are represented by their initials. Black signifies no significant difference between the consumption rates of the two species being compared. Where differences are significant ($p < 0.05$), the initials of the preferred species are shown within the matrix.

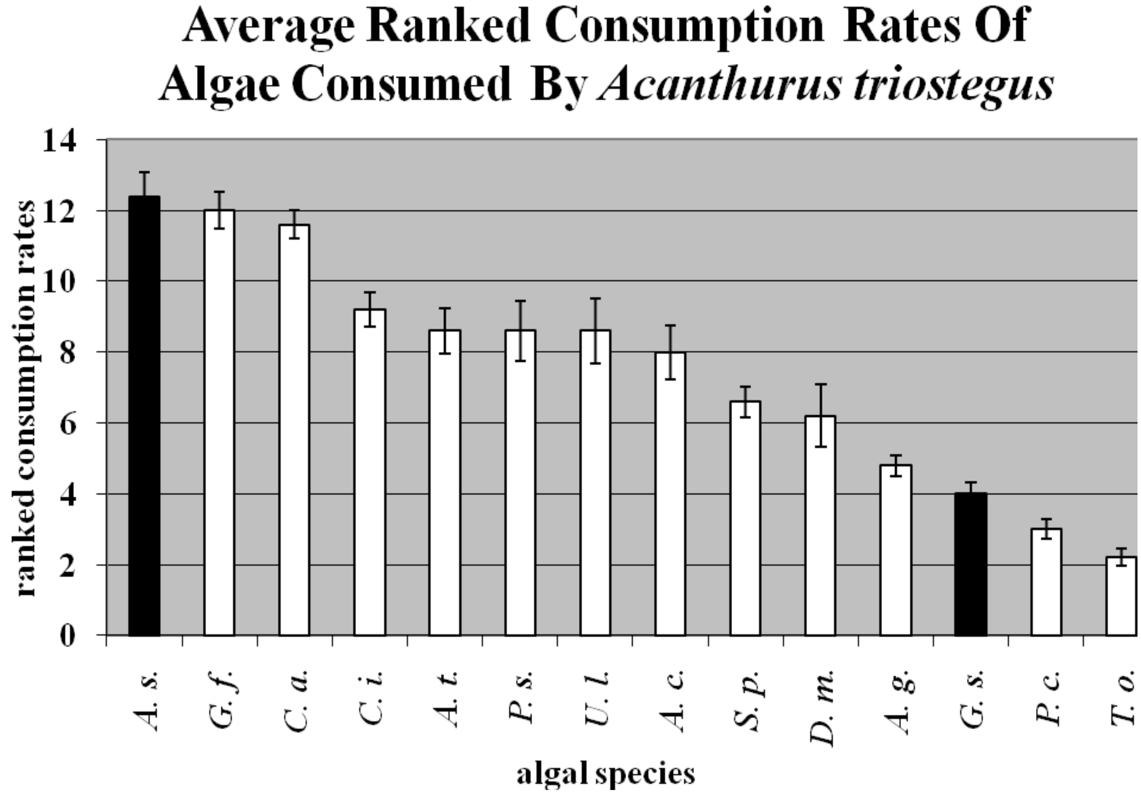


Figure 34. Consumption Rates of Algae Consumed by *Acanthurus triostegus*
Average ranked consumption rates of algae consumed by *A. triostegus* (n = 5). Algal species are represented by their initials. Black bars represent alien algal species, while white bars represent indigenous algal species. Error bars represent +/- 1 SE.

Acanthurus triostegus

Differences In Algal Consumption Rates

black = no significant difference

the initials of the algae with the greater consumption rate is

displayed in the matrix when a significant difference (*p* < 0,.05) does exist

	A.s.	**G.f.**	**C.a.**	**C.i.**	**A.t.**	**P.s.**	**U.l.**	**A.c.**	**S.p.**	**D.m.**	**A.g.**	**G.s.**	**P.c.**	**T.o.**
A.s.		■	■	■	■	■	■	■	■	*A.s.*	*A.s.*	*A.s.*	*A.s.*	*A.s.*
G f.	■		■	■	■	■	■	■	■	■	*G.f.*	*G.f.*	*G.f.*	*G.f.*
C.a.	■	■		■	■	■	■	■	■	■	*C.a.*	*C.a.*	*C.a.*	*C.a.*
C.i.	■	■	■		■	■	■	■	■	■	■	■	*C.i.*	*C.i.*
A.t.	■	■	■	■		■	■	■	■	■	■	■	■	*A.t.*
P.s.	■	■	■	■	■		■	■	■	■	■	■	■	*P.s.*
U.l.	■	■	■	■	■	■		■	■	■	■	■	■	*U.l.*
A.c.	■	■	■	■	■	■	■		■	■	■	■	■	■
S.p.	■	■	■	■	■	■	■	■		■	■	■	■	■
D.m.	*A.s.*	■	■	■	■	■	■	■	■		■	■	■	■
A.g.	*A.s.*	*G.f.*	*C.a.*	■	■	■	■	■	■	■		■	■	■
G.s.	*A.s.*	*G.f.*	*C.a.*	■	■	■	■	■	■	■	■		■	■
P.c.	*A.s.*	*G.f.*	*C.a.*	*C.i.*	■	■	■	■	■	■	■	■		■
T.o.	*A.s.*	*G.f.*	*C.a.*	*C.i.*	*A.t.*	*P.s.*	*U.l.*	■	■	■	■	■	■	

Figure 35. *Acanthurus triostegus*'s Differences in Algal Consumption Rates
Results of Tukey's test of differences in consumption rates of algae by *A. triostegus* (n = 5). Algal species are represented by their initials. Black signifies no significant difference between the consumption rates of the two species being compared. Where differences are significant (*p* < 0.05), the initials of the preferred species are shown within the matrix

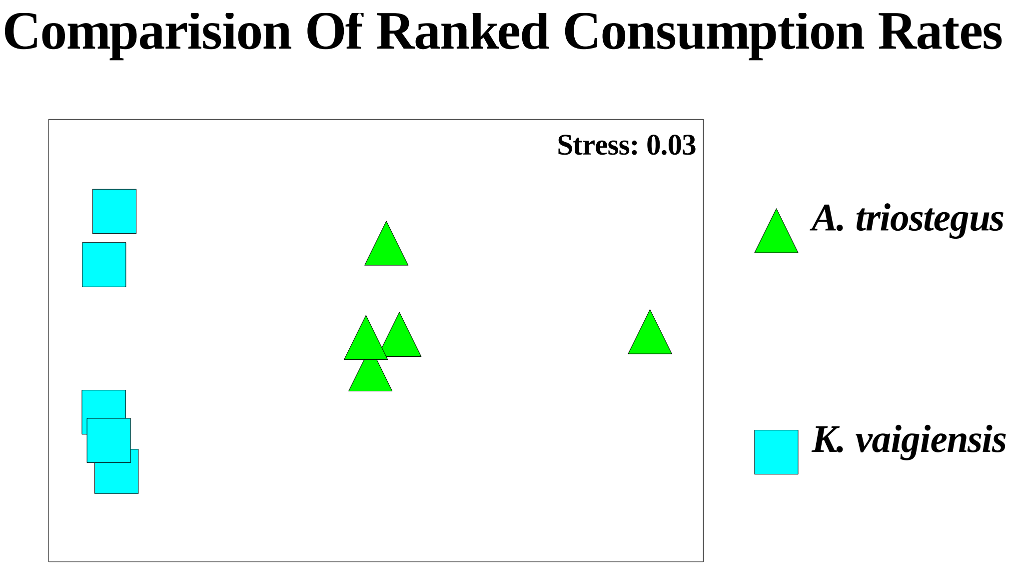


Figure 36. Comparison of Consumption Rates
MDS plot of ranked consumption rates of *A. triostegus* and *K. vaigiensis* from multiple-choice feeding assays. The closer points are the more similar consumption rates. The farther points are the more dissimilar consumption rates are.

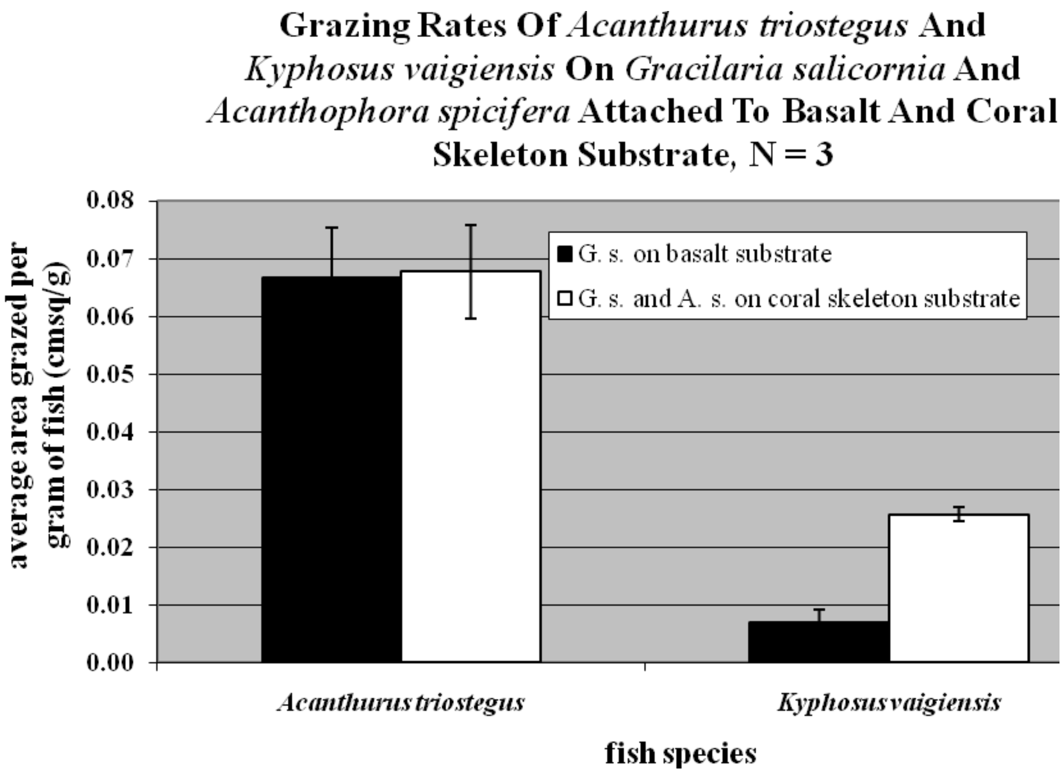


Figure 37. Grazing Rates of Fish on Alien Algae Attached to Rocks
Average cm^2 of alien algae attached to basalt and coral substrate grazed per gram of *A. triostegus* and *K. vaigiensis* (n = 3). Black bars show the area of *G. salicornia* grazed off basalt substrate. White bars show the area of *G. salicornia* and *A. spicifera* grazed off coral skeleton substrate. Error bars represent +/- SE.

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