Final Report to BRD, USGS, on the Global Climate Change Project –

Developing a coral conservation strategy for the global warming era in the National Park of American Samoa

Principal Investigator: Charles Birkeland
Project Duration: 1 July 2006 to 31 August 2009
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EXECUTIVE SUMMARY

Anthropogenic increases in atmospheric concentrations of CO_2 and other greenhouse gases are contributing to a decrease in seawater pH and an increase in seawater temperatures, and secondarily through increased seawater temperatures, to a rise in sealevel, an increased intensity of storms, and increased sedimentation. Although corals survived more extreme levels of these factors in the late Cretaceous, the present rates of global changes of these factors are unprecedented, i.e., it is the rate of change in stress rather than the ultimate level of stress that is the unprecedented aspect of anthropogenic greenhouse gas increase. About 80 species of corals on the reef flat coral communities on Ofu Island near American Samoa tolerate extreme daily fluctuations in water temperature, seawater pH, and levels of dissolved oxygen. In view of the global climate changes coming on to us, it is important for managers of coral-reef resources to know the mechanisms by which these corals on the reef flat at Ofu gain the capability of tolerating these extreme fluctuations in the physical environment, while those a few tens of meters away on the forereef slope are vulnerable.

Findings

We determined that corals can resist stress by **acclimatization** (behavioral, physiological, and biochemical mechanisms in the individual colony) and by **adaptation** (genetic changes in the population of colonies), but some **local aspects of the physical environment** such as water motion can locally ameliorate stresses from the global changes. An example of a **behavioral** mechanism of acclimatization by corals to temperature change is to expel symbiotic algae that perform best at moderate temperatures and take up symbiotic dinoflagellates that perform better at slightly higher temperatures (Smith et al. 2008 attachment A; Smith 2008 attachment B; Baker et al. in prep attachment C). **Physiological** conditioning or morphological adjustments to changing environmental conditions enables coral colonies to sustain themselves through some environmental changes (Smith et al. 2007 attachment D). **Biochemical** mechanisms of corals to adjust to abnormal temperature changes include heat-shock proteins that brace or support the structure of enzymatic or structural proteins when they would otherwise become denatured and lose their three-dimensional shape; ubiquitin that labels proteins that are wearing out for proteasomal degradation or for stabilization; and antioxidants that absorb toxic oxygen radicals

that build up when metabolism is too intense in response to stress from the physical environment (Barshis et al. in review attachment E). Whether changes in morphology, symbiotic relationships, physiological conditioning, or production of biochemicals are the mechanisms to shift the threshold for survival from climate change, acclimatization costs the coral in terms of energy and materials that would otherwise be available for growth and successful competition.

Acclimatization can be approached by **robustness or plasticity**. The mound-shaped species such as *Porites lobata* are robust and live in a wide range of habitats. They are the last to drop out of the coral community near a river mouth or in bays with increasing turbidity. The relatively rapidly growing brancing species such as *Pocillopora eydouxi* display plasticity and can differ substantially among habitats in rates of growth, colony morphology, and types of zooxanthellae hosted (Smith et al. 2008 attachment A) . *Pocillopora* are generally more vulnerable to the physical environment and so their growth rates vary among habitats and they are more likely to bleach [expel zooxanthellae and/or photosynthetic pigments] with higher than usual water temperatures and with more intense UV radiation.

Studies of **adaptation** of the coral-dinoflagellate-bacteria-cyanobacteria holobiont to varying environmental conditions have previously been focused on the dinoflagellate (zooxanthellar) clades. Our studies indicate that local adaptation (genetic selection) of the coral hosts play a significant role in the success of corals in the environmentally extreme back reef pools of Ofu (Barshis et al. in review attachment E).

A number of previous studies have determined that **water motion** ameliorates physical environmental stress by mechanisms of mass transfer, advection, and breaking boundary layers, but this appeared to be contradicted by our findings that back reef pools survived harsh physical environmental factors such as intense UV, high seawater temperatures, low levels of dissolved oxygen, low salinity, nutrient pulses, and low seawater pH, and yet 80 species of corals did well. With controlled experiments at Ofu, we determined that even intermittent water flow can effectively reduce photoinhibition and bleaching in corals (Smith and Birkeland 2007 attachment F).

Relevance

The objective of this project was to provide guidance to managers for maintaining coral-reef ecosystems and resources during global environmental changes. Our findings suggest that managers should provide protection to coral populations in areas under environmental stress for reproductive stock for corals that have the greater potential for survival under conditions of rapid climate change. “What doesn’t kill you makes you stronger” (Friedrich Nietzsche) changes. In the past, we have tended to favor protection of “pristine” and attractive areas of reef from destructive human activities. In contrast, surveys in Palau and elsewhere during the 1997/1998 El Niño indicated that healthy, unstressed corals are more vulnerable to unprecedented

environmental changes than are some of those in marginal habitats. The reason for protecting coral populations from destructive activities of humans is that these corals with potential for survival of unprecedented rates of change in the physical environment may provide the reproductive stock for future generations of corals.*

Accomplishments

This project provided major support for two PhD dissertations. Lance Smith graduated in May 2008 (Smith 2008 attachment B) and Dan Barshis is expected to graduate about May 2009. Lance Smith's dissertation is:

Smith, L.W. 2008. Environmental and biological characteristics of shallow back reef pools: implications for coral resilience. A dissertation submitted to the Graduate Division of the University of Hawaii in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Zoology. University of Hawaii at Manoa. 143 p.

This project at Ofu compelled the organizers of the 11th International Coral Reef Symposium to request that I and Barbara Brown, the preeminent expert on mechanisms of coral resilience, organize a mini-symposium on Reef Resilience. Our session on Reef Resilience hosted 28 oral presentations and six posters.

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* John C. Briggs (2008. Atlantic coral reefs: the transplantation alternative. Biological Invasions in press) referred to (USGS. 2007. Strategic science for coral ecosystems 2007 – 2011. U.S. Geological Survey, Reston, Virginia) "…corals on Ofu Island, American Samoa, have become adapted to temperature extremes. Such corals would also appear to be promising" to introduce new species into the western Atlantic reefs to provide more resilient corals to maintain the coral-reef community structure in the face of global environmental change. We do not endorse this approach of transplanting species among geographic provinces. Our study recommends to management to protect from unnecessary disturbances from human activities those populations of corals that are likely to be most resilient to climate change so as to allow those species to provide the reproductive stock for future corals as the climate change occurs. We do not suggest transplanting corals to other locations. We recommend allowing the resilient corals to reproduce and provide larvae for transport by natural water currents to damaged areas.

Peer-reviewed publications from this project to date are:

Garrison, V., K. Kroeger, D.Fenner, P. Craig. 2008. Identifying nutrient sources to three lagoons at Ofu and Olosega, American Samoa using $\delta^{15}N$ of benthic macroalgae. Marine Pollution Bulletin 54: 1813 - 1838

Smith, L.W., D. Barshis, and C. Birkeland. 2007. Phenotypic plasticity for skeletal growth, density and calcification of *Porites lobata* in response to habitat type. Coral Reefs 26: 559-667

Smith, L.W., and C. Birkeland. 2007. Effects of intermittent flow and irradiance level in back reef *Porites* corals at elevated seawater temperatures. Journal of Experimental Marine Biology and Ecology 341: 282-294

Smith, L.W., H. Wirshing, A.C. Baker, and C. Birkeland. 2008. Environmental versus genetic influences on growth rates of the corals *Pocillopora eydouxi* and *Porites lobata*. Pacific Science 62: 57-69

Accepted and in press in peer-reviewed journals:

Galkiewicz, J.P., and C.A. Kellogg. 2008 (published online ahead of print on 17 October 2008). Cross-kingdomamplification using bacterial-specific primers: complications for coral microbial ecology. Applied Environmental Microbiology

Piniak, G.A., and E.K. Brown. 2009. Seasonal variability of in situ chlorophyll fluorescence of back reef corals in Ofu, American Samoa. Biological Bulletin

Submitted and in review:

Barshis, D.J., J.H. Stillman, R.D. Gates, R.J. Toonen, L.W. Smith, and C. Birkeland. Protein expression and genetic structure of the coral *Porites lobata* in an environmentally extreme Samoan back reef: evidence for host local adaptation. Molecular Ecology

Websites:

Piniak G.A., C. Birkeland, and G. Garrison (2004). Persistence of coral reefs under extreme environmental stress in American Samoa. USGS SoundWaves web article. http://soundwaves.usgs.gov/2004/10/fieldwork3.html

Kellogg, C.A. (2005). Coral microbial ecology. USGS Fact Sheet 2005-3039 http://pubs.usgs.gov/fs/2005/3039/

Kellogg, C., V. Garrison, and J. Lyle Global Climate Change-Microbial Communities as a Diagnostic Tool?- Coral Microbial Ecology, a USGS website presentation that explains their participation and progress in this project at http://coastal.er.usgs.gov/coral-microbes/climate-change.html

C. Kellogg and V. Garrison just agreed to set up a database of all the coral photos from our sampling expeditions with links to the metadata of each sample. When complete, a link to this database will be put on the web page listed below for the project.

Oral presentations:

Barshis, D., J. Stillman, R. Gates, R. Toonen, L. Smith, and C. Birkeland. Corals in hot water: physiological responses of *Porites lobata* in a diurnally fluctuating environment. 9 July 2008

Boeing, B. "A reanalysis of the oxidative stress paradigm of coral bleaching: the effects of nitric oxide" 2 February 2007 (successfully defended his master of Science in Oceanography thesis, partially supported by this project)

Piniak G.A. and E.K. Brown. "Seasonal patterns in chlorophyll fluorescence of back reef corals in Ofu, American Samoa". 15 Feb 2007. NOAA Center for Coastal Fisheries and Habitat Research, Beaufort, NC (invited)

Piniak G.A. and E.K. Brown. "Seasonal variability in back reef coral chlorophyll fluorescence". 21-25 March 2007. 2007 Benthic Ecology Meetings, Atlanta, GA

Stillman, J. "Coral reefs: How they survive – Will they survive?" April 2007 Steinhart Aquarium BioForum on Corals (sponsored by the California of Sciences) (invited)

Smith, L.W. 2007. "Developing a coral conservation strategy for the global warming era in The National Park of American Samoa" 26 March 2007 Tester Symposium, University of Hawai'i at Manoa, Honolulu

Poster presentations:

Lance Smith "Effects of Intermittent Flow and Irradiance Level on Porites Corals at High Temperatures" 26 February 2006 American Society of Limnology and Oceanography symposium in Honolulu, Hawaii

Daniel Barshis "Physiological conditioning by a short term stress regime: stress biomarker levels in the reef-building coral *Porites lobata*" 26 February 2006 American Society of Limnology and Oceanography symposium, Honolulu, Hawaii

Daniel Barshis "Acclimation of thermal and oxidative stress responses to environmental fluctuation in the reef building coral *Porites lobata*" 10 January 2007 International Conference on Ecophysiology of Marine Organisms (Coping with change: physiological rssponses of marine organisms) Swire Institute of Marine Science, Hong Kong

In preparation:

Baker AC, Smith LW, and Wirshing HH (in prep for Coral Reefs) Coral symbiont diversity along a temperature gradient. Hoped to be submitted next month.

Baker AC (invited article in special issue) Thermally-tolerant coral-algal symbioses as bioindicators of climate change on coral reefs. Journal of Environmental Bioindicators (the idea of a temperature threshold for "D" has been substantially informed from work at Ofu). This should be out in the first half of 2008.

Baker AC, Kenyon J, Jones P (in prep, likely target will be Coral Reefs or MEPS) *Symbiodinium* diversity in reef corals of the central Pacific (this includes Ofu, Tutuila, Jarvis, Kingman etc)

A paper addressing seasonal shifts in the coral-associated bacterial communities of four Pacific stony coral species in the Ofu lagoon; comparing the communities in the mucus to those in the tissue, comparing between pools 300 and 400, and comparing within and between species. [Authors: CA Kellogg, JT Lisle, VH Garrison]

Creation of an active cooperative marine laboratory:

There was no marine laboratory on Ofu Island and so the graduate students constructed a portable flow-through seawater system in which replicate experiments with controlled environmental conditions could be accomplished. The system is portable and can be broken down and stored in the National Park building on Ofu. One of the graduate students composed a detailed 9-page instruction manual for getting the flow-though seawater system set up (Smith 2006 attachment G). The American Samoa National Park has provided storage for the flow-through seawater system, the generator, scuba tanks and compressor, the -80° C freezer for protein studies, and other marine laboratory equipment. This has allowed the American Samoa National Park to host not only our 4-year project, but also hosts our cooperators from Stanford, Rosenstiel School of Marine and Atmospheric Science (University of Miami), Romberg Tiburon Marine Laboratory (San Francisco State University), St. Petersburg USGS Science Center, and the Hawaii Institute of Marine biology (University of Hawaii). We expect to be able to work together with the American Samoa National Park and other agencies and institutions into the future with the portable marine laboratory this project initiated on Ofu.

FINAL REPORT

Background

Coral reefs are not just exotic places for tourists. Although coral reefs occupy less than one thousandth of the ocean surface area, they have an especially important role in the shape of the surface of Earth, the chemistry of the ocean, and marine fisheries yield. Coral reefs host the greatest concentrations of diversity on Earth. Coral reefs host 31 animal phyla, while all the land and freshwater habitats on all continents and islands together have 17 phyla. The only phylum found on the land on Earth that is not found on coral reefs is the obscure little velvet worm Phylum Onychophora. Coral reefs are also among the most biologically productive ecosystems

on Earth in terms of gross productivity, with the global potential for coral-reef fisheries estimated to be 9 million metric tons per year, or nearly 10 % of the global marine fisheries from about 0.089 % of the ocean surface. However, the net productivity of coral reefs is relatively low among ecosystems and so the fisheries are especially vulnerable to overharvest and must be managed with an understanding of the life-histories of the exploited species and the biology of the corals that create their ecosystem. Corals have also shaped the surface of the planet more than any other organism, including humans. If not for the limestone deposited by coral reefs, entire nations such as the Marshalls, the Maldives, the Tuamotus and most of the Carolines would not exist. With each atom of calcium deposited, a molecule of CO_2 is also deposited, with gross CO_2 fixation estimated on the order of 700 billion kg of carbon per year. Over millions of years, corals have deposited limestone structures sometimes over 1,300 m thick (atolls in the Marshall islands) or over 2,000 km long (Great Barrier Reef). Roughly half the calcium that enters the sea each year around the world, from the north to south poles, is taken up and temporarily bound into coral reefs. Yet despite creating the most massive biogenic structures on Earth and despite promoting such gross productivity and providing habitat for the greatest diversity of phyla, the living coral is especially delicate, being a film only millimeters thick, like peanut butter on bread.

Coral reefs are economically and ecologically very important, but they are now on a severe decline. On a global scale, coral reefs are currently affected by an interrelated suite of anthropogenically-generated environmental changes, including increasing temperature related to increasing greenhouse gas emissions, ocean acidification due to increasing atmospheric CO_2 concentration, eutrophication resulting from increasing supply of nutrients, and increasing incidence of disease related at least in part to increasing nutrient inputs and seawater temperatures. The severity and geographical distributions of all of those impacts are expected to increase in the future.

Global processes that are affecting coral reefs, which are related to the increased concentration of atmospheric CO_2, are sea level rise, the decline in pH of seawater, and the increase in seawater temperature. The concentration of CO_2 in the atmosphere is generally expected to reach two times the preindustrial (late 18th century) levels by 2065. As CO_2 concentration increases in the atmosphere, the surface seawaters take up more CO_2. The increased uptake of atmospheric CO_2 by the surface waters of the ocean leads to a decrease in pH of surface waters, an increase in the proportion of bicarbonate ions ($3HCO-$), and a decrease in the proportion of carbonate ions. The overall effect is on the rate of precipitation of coral skeleton,

$$CO_2 + H_2O \quad \Leftrightarrow \quad HCO_3^- + H^+ \quad \Leftrightarrow \quad CO_3^{2-} + 2\,H^+$$

The oceans have already taken up an additional one-third to one-half of industrial-age emissions of CO_2, and the concentrations of carbonate ions in the oceans have decreased from 11% (preindustrial), to 9% (now) and are projected to decrease to 7% when carbonate concentrations are double the preindustrial concentrations, perhaps in 3 to 5 decades. Doubled atmospheric CO_2 will lead to a 14% to 30% decrease in reef calcification rates. This has been estimated to be a

general tipping point from net carbonate accretion to net carbonate. Net reef accretion is potentially reduced to zero when increased CO_2 in the atmosphere reaches about 500 to 600 ppm. On the other hand, CO_2 is less soluble in seawater at higher temperatures. While increased concentrations of atmospheric CO_2 may be accelerating the uptake of CO_2 by surface seawater, global warming may be slightly damping the uptake. But of more substantial influence in accelerating the tipping point of net reef accretion are the synergistic biological effects on corals of reduced growth in the face of natural and anthropogenic stressors.

Uptake of anthropogenic CO_2 by subtropical Atlantic waters has been greater than by Pacific waters. The north Atlantic stores 23% of the total anthropogenic (fossil-fuel and cement-manufacturing emissions) CO_2 taken up by the world oceans, even though the north Atlantic occupies only 15% of the world's total ocean area. Pacific waters are less receptive to the uptake of CO_2 and therefore are buffered from a decrease in pH because of higher concentrations of dissolved inorganic carbon. As seawater becomes warmer coral reef net accretion will probably become slightly more restricted in latitude because of the changes in chemistry from CO_2 uptake in the world's oceans.

The thresholds in tolerance of corals to an increase in water temperature and its duration before "bleaching" (expelling the symbiotic zooxanthellae) is predicted by the degree heating week (DHW) record, 12-week accumulations measured as °C weeks. The DHW product is an accumulation of hotspot values over the bleaching threshold [1°C over the maximum monthly mean (MMM)]. The threshold values of DHW vary from site to site because the MMM varies from site to site; thus, corals are likely adapted to their own threshold temperatures at each site. Furthermore, the past history of events in the physical environment and local characteristics of the physical environment can modify the actual location of the threshold or tipping point. Based on our knowledge of tolerances and the gaps in the literature on thresholds, corals are *likely* to reach a threshold with an increase in sea water temperatures.

In contrast to the increase in atmospheric CO_2, the doubling of fixed nitrogen on Earth in the past few decades by use of fossil fuels is unprecedented. Industrial agriculture has increased the production of fixed nitrogen from pre-industrial levels of approximately 1 – 3 kg ha^{-1} yr^{-1} to 7 kg ha^{-1} yr^{-1} over central and eastern USA, 17 kg ha^{-1} yr^{-1} over central Europe and to as much as 100 kg ha^{-1} yr^{-1} over parts of the Netherlands. The production of CO_2 is generally an easy exergonic (downhill) byproduct of the burning of materials created by photosynthesis, whether by metabolism of biota or through the use of fossil fuels. The ups and downs of global atmospheric CO_2 because of volcanism and use of photosynthetic products are natural. But nitrogen fixation by combination of nitrogen with hydrogen, oxygen, or carbon is an expensive energy-consuming endergonic (uphill) process. Our industrial production of food is replacing agricultural production by subsidizing traditional solar energy with the use of fossil fuels to produce fertilizers by fixing nitrogen. By using fossil fuels, we are compounding the climate-change problem by adding more CO_2 as well as N_2O to provide the greenhouse effect as we produce fertilizer. Nitrous oxide has 296 times more influence on global warming than the same per mass unit of carbon dioxide.

Fixed nitrogen has been shown to be the nutrient most influential in structuring most temperate terrestrial, freshwater, and marine ecosystems, affecting diversity, species composition and

functioning. In the 1990s, the Mississippi carried 10 times the nitrates and phosphates that it did in the 1960s. Each year the Mississippi transports 1.6 million tons of nitrogen from fertilizer runoff in the Midwest USA into the Gulf of Mexico. By fertilizing algae (phytoplankton), the excess fertilizer has created at least 146 large-scale hypoxic zones in coastal oceans of the world in which fishes and crustaceans and other animals suffocate from oxygen depletion. The hypoxic zone south of Louisiana, fertilized by the Mississippi, occasionally gets to be as large as the area of Massachusetts. In the Baltic Sea, industrial agriculture has produced a hypoxic area of 70,000 km^2.

With surplus nutrients, time and yield are everything, and survival in competition for space by efficiency with recycling loses in a major way. This is especially true for coral reefs. Coral holobionts are excellent at living in oligotrophic situations because of efficiency and recycling, but surplus nutrients from upwelling and runoff favor profligate growth and biomass accumulation, both to the detriment of the individual coral recruit and at the level of geographic patterns of community structure and ecosystem processes of coral reefs. Surplus nutrients are considered by some to favor major evolutionary innovations. Under conditions of surplus nutrients, profligate yield would favor rapid growth of "nuisance algae" and heterotrophic benthic animals over efficient recycling of nutrients by autotrophic holobionts. The terrestrial habitats are similar, in that fixed nitrogen produces accelerated losses of biological diversity, especially losses of plants adapted to efficient use of nitrogen, and losses of the animals and microorganisms that depend on them.

The resilience of corals to environmental changes is largely determined by their capacity to acclimatize (adjust physiologically and behaviorally). The thresholds of resilience of corals to environmental factors, such as water temperature and ultraviolet (UV) radiation, are altered by changes in symbiotic interactions. Reef-building corals are dependent on symbiotic dinoflagellate algae (zooxanthellae) in their endodermal cells for their nutrition and proficiency in deposition of skeleton. There are a number of clades or types of zooxanthellae, and the physiological and ecological attributes of zooxanthellae vary among clades. The symbiotic relationship breaks down under stressful conditions of extra warm seawater or strong UV radiation. Under these conditions, corals sometimes expel much of the zooxanthellae of clade C and allow the buildup of clade D, with which the coral growth rate is slower but survival under stressful conditions may be greater. As with morphological adjustments, the symbiotic adjustments of corals may be determined by a balance between the stresses imposed by the physical environment and by ecological interactions with other species. In addition to adjustments in morphology and symbiotic relationships, acclimatization can occur through biochemical conditioning. Increased water temperature triggers a substantial increase in biochemical activity in corals. Intense biochemical activities resulting from changes in water temperature may indicate a processes of acclimatization that might increase the distance to the threshold for mortality of the coral from seawater temperature.

Whether changes in morphology, symbiotic relationships, physiological conditioning, or production of biochemicals are the mechanisms to shift the threshold for survival from climate change, acclimatization costs the coral in terms of energy and materials that would otherwise be available for growth and successful competition. Acclimatization can be approached by robustness or plasticity. The mound-shaped species of *Porites* (such as *P. lobata*) are robust and

live in a wide range of habitats. They are the last to drop out of the coral community near a river mouth or in bays with increasing turbidity. Species of *Acropora* dominated the reef front at the municipal sewer outfall for Koror, Palau, until predation on corals by the crown-of-thorns starfish and bleaching by the large-scale seawater warming of 1997–98 killed the *Acropora* spp. but not the *Porites* spp. *Porites* can maintain itself rather constantly despite fluctuations in the external physical environment, but at a metabolic cost.

Our study site at Ofu is where corals are subjected to extreme daily fluctuations in seawater temperature, pH, and level of dissolved oxygen (Figs. 1, 2, and 3).

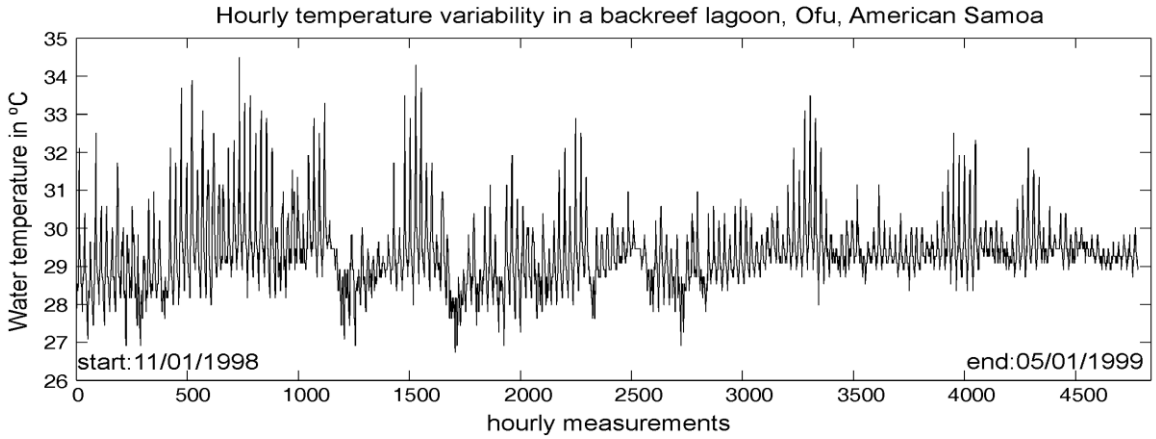

Fig. 1 Seawater temperature in the study site in a backreef lagoon on Ofu.

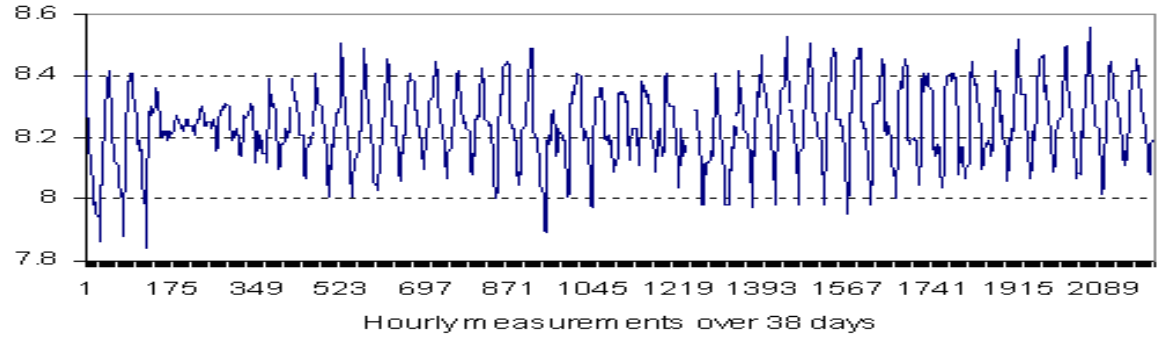

Fig. 2 Seawater pH in the study site in a backreef lagoon on Ofu.

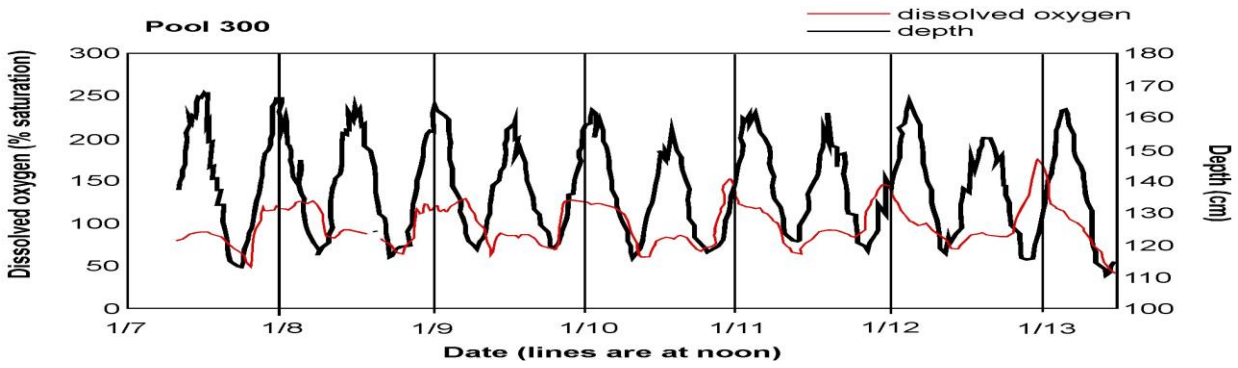

Fig. 3 percent saturation of dissolved oxygen in a backreef lagoon in Ofu.

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The relatively rapidly growing *Pocillopora eydouxi* display plasticity and can differ substantially among habitats in rates of growth, colony morphology, and types of zooxanthellae hosted. *Pocillopora* are generally more vulnerable to the physical environment and so their growth rates vary among habitats and they are more likely to bleach [expel zooxanthellae and/or photosynthetic pigments] with higher than usual water temperatures and with more intense UV radiation.

Corals are most vulnerable to infrequent or very frequent environmental changes. As explained in the previous section, corals can acclimatize (physiological or behavioral response) or adapt (genetic response) to environmental changes of intermediate frequency. If the phenomena, such as extraordinarily warm seawater, are infrequent enough to be unpredictable, then the corals will not be able to acclimatize or adapt. However, if the events are too frequent, the corals will not have time to recover between events.

Corals are a “fundamental species” because they provide the structural framework that defines their ecosystem. In addition to the complexity of the diverse and productive ecosystem, corals themselves are a holobiont—a composite organism that includes symbioses between closely associated but evolutionarily distant organisms from different kingdoms, including bacteria and cyanobacteria, fungi, algae and the animal. Each component organism of the holobiont

contributes to the maintenance and function of the holobiont in taxon-specific ways, and interactions among components may determine the holobiont's resilience from (or vulnerability to) environmental perturbation from the influence of changing conditions, both global and local. To understand how coral reefs will persist under future climate conditions, we must understand how those conditions impact each component of the coral holobiont, and we also need to understand how the changing environment, whether natural or anthropogenic, affects the interactions among these components.

A further complication arises because each of the symbiotic components of an individual coral has a variety of genotypes within the taxon. For example, the zooxanthellae found within coral are the organisms that provide most of the caloric food input by photosynthesis to the individual coral. These zooxanthellae are dinoflagellate algae in the species *Symbiodinium microadriaticum*, but they come in a variety of genetically distinguishable clades and each clade fares better under different environmental conditions. Clade C of *Symbiodiinum microadriaticum* provides more photosynthetic input to the coral's metabolism for less net cost and favors growth of the host coral, possibly enhancing its fitness through more energy to enhance fecundity and better ability to compete for space. On the other hand, clade D of *Symbiodinium microadriaticum* is more of a metabolic cost to the coral host, but is more tolerant of the stressful conditions of exceptionally warm seawater and thereby allows the host coral to survive the stressful conditions by maintaining the symbiotic relationship.

A new challenge to the study of the coral holobiont is the complexity of the system being investigated in combination with new constraints. Each site in which coral are found can be considered unique in the combination of environmental factors affecting the survival and success of the coral, and therefore the only way to control for the variation the environmental factors are reciprocal transplant experiments. Yet this approach is frowned upon by many scientists because introducing new genotypes into new environments might mix genes and/or introduced alien microbes or other species. Whereas reciprocal transplants and other controlled manipulative experiments in the field have been very productive in the past, we must now design studies to address these types of questions in new ways.

In addition, we must examine the resilience of less physiologically robust, younger coral lineages that have arisen since the Cretaceous/Cenozoic mass extinction and how they must acclimatize to changing environmental conditions though the uptake of novel symbiont lineages from the environment or sequestered in alternative host organisms. This is to be compared to the mechanisms for resilience of robust lineages that had survived the Cretaceous / Cenozoic mass extinction. Recognition of these lineages is an original way of approaching the question of how the corals and coral reefs will survive global changes in climate and human activities.

Marine reserves have been documented in over 80 studies to have enhanced the abundance, size distribution and, in some cases, diversity of the fish associations. However, there have been essentially no studies that have determined whether or not marine reserves have enhanced ecosystem processes such as reef framework accretion, bioerosion, and predation on corals, and very few that have experimentally determined the effects of marine reserves on living coral cover and nature of herbivory. We have examined these effects with original controlled manipulative field experiments.

The objective of this project is to accomplish Goal 4 of the U.S. Climate Change Science Program Strategic Plan, i.e., to "Understand the sensitivity and adaptability of different natural and managed ecosystems and human systems to climate and related global changes" (Abraham et al. 2003). This goal is particularly pertinent to coral reefs because they are especially vulnerable to the effects of global warming. Coral reefs are important economically, providing approximately $ 375 billion annually to the world economy) and $805 million annually to Hawaii. In these times of more frequent periods of extra warm seawater, mass coral bleachings and mortality have also become more frequent. NOAA, AIMS and other agencies and institutions are attempting to compile and produce a guide to managing reefs in the face of global warming, including procedures to prevent corals from bleaching, promoting survivorship of corals during bleaching, recovery after bleaching, and most importantly, implementing measures to promote long-term reef resilience. But there is not sufficient knowledge at this time. We found a two-mile stretch of reef flat in the National Park of American Samoa where about 80 species of reef-building scleractinian corals appear to live well although the water temperatures frequently reach 35.5° C and the temperatures often fluctuate by 6.5° C daily. This site on American Samoa is providing an exceptional opportunity to examine coral adaptation, the mechanisms for acclimatization by coral, and the possible factors in the physical environment that can ameliorate the stress to coral of especially warm seawater.

ONGOING PROJECTS

1) Coral transplant experiments

Dan Barshis and Lance Smith have, altogether, set up 754 transplanted corals as part of two main experiments described below. One experiment is to determine the biochemical aspects of acclimatization of corals to more stressful conditions (e.g., higher water temperatures) and the second is to test the hypothesis that water motion enhances the resistance of corals to the stress of higher water temperatures. We also transplanted twelve *Pocillopora damicornis* colonies to Pool 300 from the forereef (Sili) and six from the lagoon (Pool 300, partially to control for the stress of transplantation). These colonies were for brood stock for an experiment concerning genetic adaptation to warmer seawater temperatures.

In order to obtain standard cores of massive corals such as *Porites lobata*, it was necessary to invent and construct an underwater coring devise, powered by air pressure from a scuba tank. Dan was assigned this task and he accomplished it in a timely manner so the equipment was used this year.

Dan (with field assistance of colleague Lance Smith) was able to perform 418 coral transplants to establish a set of corals from which the biochemical changes could be monitored hourly at first and then over longer periods of time as they were transferred from a benign to stressful environment (i.e., from the forereef with daily temperature maxima of about 31° C and daily fluctuations of < 1° C, to a reef flat pool with daily maxima of about 33° C and daily fluctuations of 2 - 4° C) or, conversely, from stressful to benign (from the forereef to the reef flat pools). The transplanted cores or nubbins were from 4 source colonies per site.

Dan constructed a grid of transplanted corals for the time series sampling (Fig. 4). Sampling was done every 4 hrs for a 72-hour period. Dan also had a stress recovery experiment set up whereby two plugs from three source colonies were sampled every four to five days to examine at post-coring recovery

levels. Pulse Amplitude Modulation (PAM) measurements were taken on both the transplants and the source colonies at approximately 6 and 30 hours after coring and a significant decrease in fluorescent yield was found at 6 hours ($p=0.02$), but there was no decrease after 30 hours. It will be important to determine how the PAM recovery correlates with the change in proteins. Dan expects the damage to the algal photosystem may be minimal, but the damage to the coral tissue may be longer lasting.

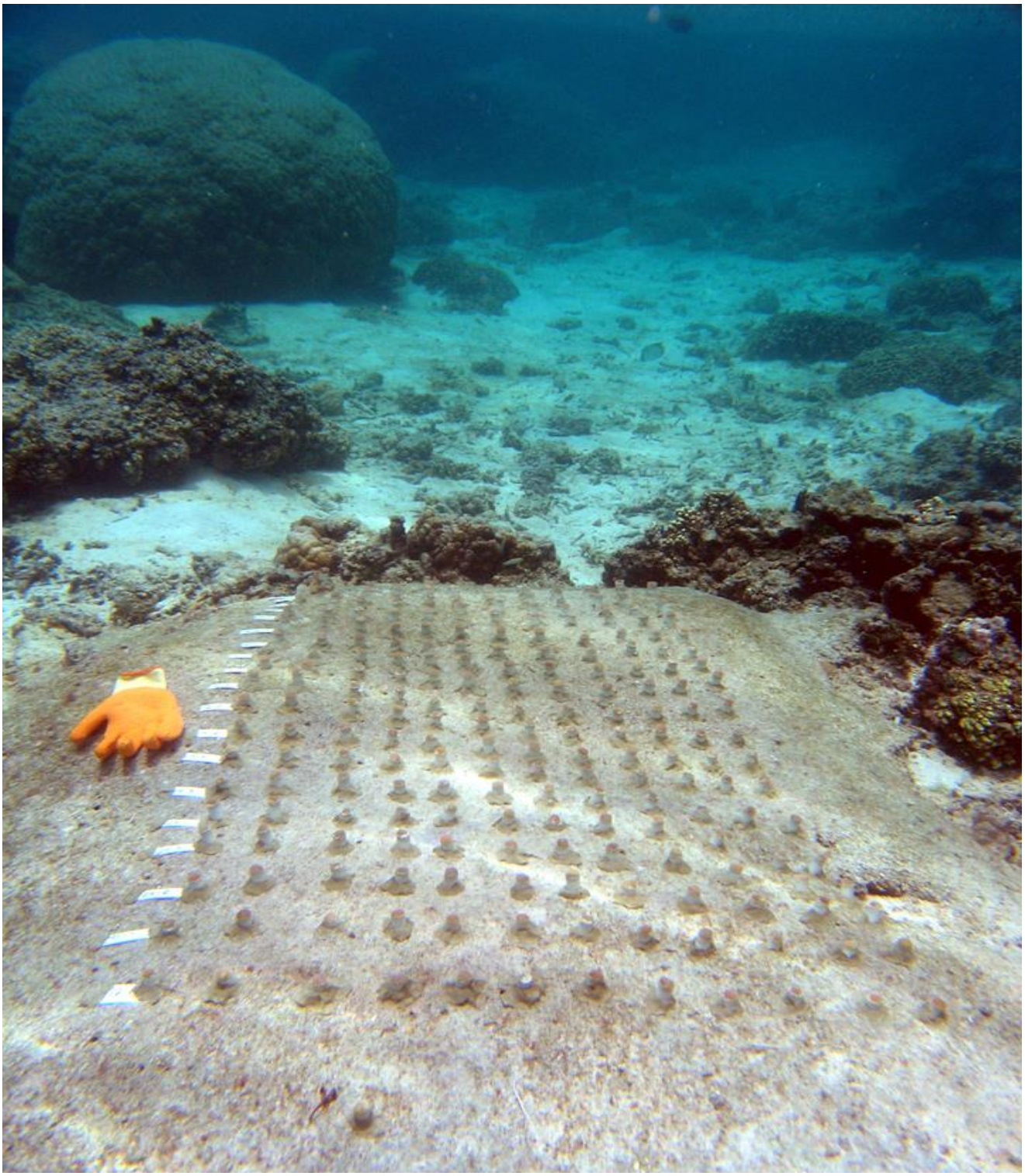


Fig. 4. One set of Dan's transplanted corals for monitoring biochemical changes during the process of acclimatization.

Fig. 5. A comparison of growth of transplanted corals from the forereef (left) with those from the lagoon (right).

With assistance from Dan, Lance set up a transplant experiment in which a total of 336 transplants (56 each for six replicate groups of corals) were obtained from 84 source colonies using underwater pneumatic drilling tools operated with a spare scuba tank. *Porites lobata, Porites cylindrical, Pocillopora eydouxi,* and *Acropora gemmifera* were the species used. All transplants were weighed and stained so that growth was able to be determined at the end of the 6-month transplant period. A first sets of reciprocal transplants were between the forereef (exposed to the open ocean) and the pools of the lagoon (exposed to a wide range of physical environmental factors). All replicate groups of corals had four sets of corals transplanted: from Sili forereef to Pool 400, from Sili forereef to Sili forereef (for control of effects of transplanting and handling), from Pool 400 to Sili forereef, and from Pool 400 to Pool 400. In February 2005, the surviving transplants were collected. The data show that those originating from the lagoon had superior growth compared with those from the forereef, regardless of where they were transplanted (Fig. 5). The second set was between the large Pool 400 (relatively moderate water movement and temperature fluctuations) and the smaller Pool 300 (exposed to more water motion and more extreme fluctuations of water temperature). The pattern of replication from Pool 300 to Pool 400, Pool 300 to Pool 300, Pool 400 to Pool 300, and Pool 400 to Pool 400 was similar in design to the first set concluded in February. But this ongoing experiment involves 224 transplants, 56 each for four species: *Porites lobata, Porites cylindrical, Pocillopora eydouxi,* and *Acropora gemmifera.*

At the same time as the transplant experiments were running, variables of the physical environment were recorded with Hobotemps and remote data loggers. Water temperatures were recorded every 30 minutes at three of the study sites over a 1-year period. Light intensity, turbidity, dissolved oxygen, and pH were all recorded for a 1-month period at three study sites in August – September 2004 and February – March 2005.

2) Flow studies of transplant sites

Lance has determined the comparative magnitude of water currents among the pools on the Ofu reef flat by use of clod cards that were composed of plaster of Paris (gypsum, calcium sulfate). Rate of dissolution of plaster of Paris is a measure of water motion. He also studied the flow patterns with drogues (Smith 2004 attachment attachment H).

In order to be able to further control and test separately the several factors in the physical environment, Lance designed and constructed a running seawater system on Ofu (Fig. 6) for water table experiments. The system on Ofu required a deep well pump to lift water from the lagoon approximately 25 ft to the headtank that supplies the water to the water tables. This high suction lift and the requirement that the system be constructed so that it can be easily disassembled for storage made the design difficult. Lance spent most of this past field season conducting experiments in these water tables to test the effect of intermittent water motion on the tolerance of corals to high seawater temperatures and strong irradiance. Branches of *Porites cylindrica* and plugs of *Porites lobata* were used for these experiments. Effects were evaluated with PAM (using a fluorometer borrowed from USGS Pacific Science Center, Santa Cruz, California), and effects of bleaching are being evaluated by measuring zooxanthella densities from frozen samples brought back to Hawaii.

The open seawater system devised by Lance has added an effective marine laboratory to the facilities of the National Park at American Samoa on Ofu. It should be mentioned that as Hurricane Olaf was approaching Ofu, Lance and Dan dismantled the seawater system. Category 5 (sustained winds of over 150 mph) Hurricane Olaf made a direct hit on Ofu, but lance and Dan had the seawater system up and running about 12 hours after Olaf passed.

Fig. 6. The portable seawater system with a pump created by Lance Smith. A holding tank (left), shade or reducing intense sunlight, and experimental water tables (right).

3) Development of methods to analyze antioxidant enzymes and heat shock proteins

Jonathan Stillman, Ruth Gates, and Rob Toonen worked with Dan Barshis to develop coral protein extraction procedures, protein quantification methods, techniques of gel electrophoresis, and Western blotting to determine binding specificity of antibodies. Dan prepared a Coral Protein Extraction Protocol and Molecular Biomarker Coral Transplant Protocol. Heat-shock proteins help other proteins retain their three-dimensional structure and thereby prevent the denaturing of the other proteins and maintain the integrity of the system during stress. Antioxidants deactivate the toxic O^{++} radicals that accumulate during times of activity in reaction to stress. Malondialdehyde (MDA) and 4-Hydroxynonenal (HNE) are end products of oxidative damage to lipids and so their presence is indicative of defensive activities of the coral to environmental stresses.

Dan has completed four separate experiments using *Porites lobata*, investigating host tissue response to environmental variability. From each of six host colonies on the forereef, standardized coral cores were transplanted to Pool 400 and from six host colonies in Pool 400, standardized coral cores were transplanted to the forereef. A total of 120 individual cores were transplanted in August, the cooler season in American Samoa, south of the equator. The whole procedure was repeated six months later in the warmer season, this time using eight source colonies and 160 individual cores. In addition, a 72-hour time-series profile of the daily natural variation in protein concentrations in the lagoon was taken for *Porites lobata* using 228 cores from six different coral colonies. Finally, a core recovery project was undertaken by examining protein recovery from coring stress using three source colonies and a total of 30 cores.

Pulse-Amplitude-Modulation (PAM) readings were taken on the recovery project. The pre-coring and post-coring PAM readings indicated that the photosystem was stressed 8 hours after the coring compared with the source colonies, but the photosystem recovered in as little as 30 hours. The host coral tissue may take longer to recover and the biochemical samples are being analyzed to determine the answer to this question.

It was necessary to keep the tissue samples at -50° C in order to fix the proteins so for field work on the tiny isolated island of Ofu we had to acquire a -80° C freezer, as well as superinsulated boxes and a bicycle for rapid transportation of the samples from the coral transplants to the freezer. Although the freezer was delicate and vulnerable to any shocks or bumps, and to being turned on its side, Lance and Dan were successful in getting the freezer to Ofu. Dan and lance were also successful in the challenging adventure of transporting the coral tissue samples from the small island of Ofu, from which transportation is by small propeller aircraft that are very unpredictable in schedule, via Tutuila which also has an erratic flight schedule, across 35° of latitude while somehow keeping the samples ultracold (-80° C, not allowing to raise to warmer than -50° C). It is remarkable that they were both successful. Dan was stuck on Tutuila for several days because of unexpected circumstances and he was able to find an entomologist with an ultracold freezer to hold his samples while stuck on Tutuila.

Greg Piniak, formerly of the USGS Pacific Science Center, Santa Cruz, and now with the Center for Coastal Fisheries and Habitat Research, NOAA, 101 Pivers Island Road, Beaufort, NC has been assessing the stress on zooxanthellae and corals by using a submersible pulse-amplitude-modulated (PAM) fluorometer to study the fluorescence yield of the zooxanthellae. The fluorescence yield is a measure of

the relative portion of light energy that does not go into photosynthesis or heat. This measurement can be used to estimate how well the photosynthetic system is working in the symbiotic relation with the coral colony. He has collected over 600 PAM readings on numerous species in Ofu lagoon. At least 15 yield measurements were collected for at least 5 colonies of 10 coral species, in each of two pools (300 and 400). In addition, rapid light curves were measured for 5 colonies of *Goniastrea retiformis* in both of the pools. Measurements were made at night and during mid-day (10 am to 2 pm) to compare diel differences in photosynthetic state. A seasonal comparison was also performed—winter measurements were made in August 2004 with Eric Brown, and summer measurements in February 2005 with Lance Smith.

4) Sampling of microbial symbionts

Coral colonies are made up of three realms of genetic programs: the scleractinian coral (host and structural framework), the symbiotic dinoflagellate (*Symbiodinium microadriaticum* complex, i.e., zooxanthellae), and the associated microbial community (nonphotosynthetic bacteria, Cyanobacteria, Rhodophyta, Fungi and other biota) on the surface tissue and mucus of the coral. These three genetic realms interact in different ways as the environment changes.

Virginia Garrison and Christina Kellogg (USGS at St. Petersburg) sampled the microbial biota on corals in Ofu lagoon Pools 300 and 400 to assess the changes in microbial community structure. Sampling times were selected to correspond to the periods of coolest water temperatures (August 2004), warmest water temperatures (January 2005), and at the end of the warm water period (April 2005) when the most bleaching would be likely to occur. The final field sampling is planned for August/September 2005. The selection of the coral species sampled was based on species other researchers were using in related projects, The importance of the species in the community (abundance, ecological importance), and availability of previously unsampled / undamaged colonies. Six colonies of *Pocillopora eydouxi*, *Pocillopora damicornis*, *Porites* sp. (massive, probably *P. lobata*), and *Acropora gemmifera* were selected from both Pools 300 and 400. Each colony was photographed, mucus and a tissue sample were collected for microbial community analysis, and at the time of the first sampling, a second tissue sample was collected for DNA. Samples of tissue and mucus were preserved in the field in a DMSO/saturated EDTA/salt solution and in ethanol for DNA investigation. Samples of massive *Porites* were collected, bleached and SEM (scanning electron microscope) images were taken in order to help identify the species.

5) Sampling of algal symbiont communities

Eric Brown, Greg Piniak, and Lance Smith collected 220 samples of zooxanthellae in August and September 2004, which included samples from about a dozen representative species, as well as from the source colonies Lance used for his forereef-lagoon transplant experiment. In February and March 2005, Lance once again collected samples from the source colonies, as well as from the *Porites lobata* transplants. These samples will be analyzed in Andrew Baker's laboratory to determine differences in phylotypes or clades of zooxanthellae among different species under different environmental conditions.

6) **Water table experiments on effects of intermittent flow and irradiance level on *Porites* corals at elevated seawater temperatures**

The mandate to the projects funded by the USGS BRD Climate Change Science Program is to provide understanding and necessary data to facilitate actions by resource managers to ameliorate the effects of unnaturally rapid climate change. The management of marine biological resources differs from that of terrestrial biological resources in that the adults of coral-reef communities, including fishes, are relatively stationary, and the placement of populations is determined by the movement and selection of the larval stages in the life histories. In terrestrial systems, the adults usually have the capability of relocating and choosing the spot to deposit eggs or raise offspring. In the marine environment, the critical decision is to determine where to focus the effort to save the source populations. To do this, the managers need to know what local aspects of the physical environment ameliorate the effects of global climate change. To put realistic limits on the time frame and on the thresholds of abilities of the key organisms, it is necessary to determine the mechanisms of acclimatization and the capacity for adaptation of the organisms. We have made substantial progress towards these objectives.

The focus and the overall goals of this project were developed in conjunction with NPSA staff to address the National Park Service's management needs in the face of sustained elevated seawater temperatures and high irradiance levels that are likely to occur due to global warming. The first two long-term goals of the USGS BRD Climate Change Science Program is to "determine the sensitivity and response of ecosystems and ecological processes to environmental factors, including existing impacts at the local, landscape and continental level" and "to assess and predict how future environmental conditions may affect structure. function and long-term viability of natural and human impacted ecosystems".

- What are the pathways by which previous experience with sublethal stress allows corals to acclimatize and become better prepared physiologically for additional stress?

- Is selection strong enough that adaptation to local environmental stresses can occur under usual levels of connectivity?

- How does the physical environment (e.g., climate/ocean interactions, local water motion, shade) enhance the ability of corals to endure thermal stress in some localities more than others?

- How is the capacity of coral communities to recover affected by initial community composition (e.g., branching, massive or mixed species) and by morphological characteristics of the reef (e.g., topographic complexity and solidified substrata)?

Corals inhabiting the Ofu back reef pools are often simultaneously exposed to elevated seawater temperatures and high irradiance levels, conditions known to cause coral bleaching. Water flow in the pools and other back reef coral reef ecosystems is tidally influenced, resulting in semi-diurnal flow patterns. Controlled experiments were conducted to test effects of semi-diurnally intermittent water flow on photoinhibition and bleaching of the corals *Porites lobata* and *P. cylindrica* kept at elevated seawater temperatures and different irradiance levels. In the high irradiances caused by turbidity or shading, may reduce photoinhibition and bleaching of back reef corals during warming events (Smith and Birkeland 2007 attachment F).

7) Contrasting environmental variance in skeletal growth of two reciprocally transplanted

That the skeletal growth rates of *Pocillopora damicornis* and *Porites cylindrica* were greater in the warmest Ofu back reef pool (Pool A) than in other pools (Pool B and others), suggesting that something about the environment in Pool A enhanced skeletal growth even though it has (presumably more stressful) higher maximum seawater temperatures than the other pools. To investigate environmental vs. non-environmental variance in coral skeletal growth in these two pools, an 18-month reciprocal transplant experiment (RTE) was carried out using 5 species: *Pocillopora damicornis, Pocillopora eydouxi, Porites cylindrica, Porites lobata,* and *Acropora gemmifera.* Since 5 species were used, and each species in a RTE consists of four groups, about 200 transplants were completed for this experiment. However, there was inadequate survival of *Pocillopora damicornis, Porites cylindrica,* and *Acropora gemmifera*, thus only the results from *Pocillopora eydouxi* and *Porites lobata* are presented below.

The results of skeletal growth for both surviving species indicate environmental variance in skeletal growth between the two pools for each species. There was no genetic or other non-environmental variance between the transplant groups from the different pools that were transplanted side-by-side. That is, intraspecific variation in skeletal growth was due to differences in the environments of the two pools, not to intrinsic differences between the coral populations of the two pools, an example of phenotypic plasticity. Thus each species' reaction norm arrows are sloped and nearly overlapping, but oppositely for the two species: While both grew about the same in Pool B, *P. eydouxi* grew more in Pool A than in Pool B, and *P. lobata* grew less in Pool A than in Pool B.

To determine which environmental factor may have resulted in opposite growth results for the two species in Pool A, data were collected from the two pools on temperature, light, salinity, dissolved oxygen, turbidity, nutrients, and water velocity during a variety of weather conditions. The only factor besides temperature that differs substantially between the two pools is water velocity during rough conditions: During calm conditions, velocity is slightly higher in Pool A than Pool B, but in rough conditions, Pool A velocity is often double that in Pool B, and can exceed 1 m. *Pocillopora eydouxi* is most commonly found in high velocity habitats, such as shallow forereef areas, whereas *P. lobata* is most commonly found in low velocity habitats such as back reef pools. Thus, the consistently slightly higher velocities, and occasionally much higher velocities, in Pool A may enhance *P. eydouxi* growth and suppress *P. lobata* growth.

8) Acclimatization of reef-building corals by shifting populations of their symbiotic zooxanthellae

Goniastrea retiformis was used for a double reciprocal transplant experiment between a shallow, relatively warm, fluctuating site (Pool 300) and a deep, relatively cool, stable site (Sili 17 m depth). The first part of the RTE was standard, in that 4 groups (5 individuals apiece) were used: 1) from Site A to Site A; 2) from Site A to Site B; 3) from Site B to Site B; and 4) from Site B to Site A. The second part of the RTE used an additional 20 colonies (10 from each site) and was the same as the first, except that the colonies were first bleached in the water tables, then transplanted. So a total of 40 colonies were used for

the double RTE. The colonies will be retrieved approximately one year after transplanting. The experiment is designed to test for zooxanthellae switching by habitat. The artificial bleaching component was added to increase the likelihood of detecting zooxanthellae switching, since this phenomenon may not occur in the absence of a natural bleaching event.

9) Physiological responses to a short term stress regime: stress biomarker level in the reef-building coral *Porites lobata*

The Ofu lagoon in the National Park of American Samoa (NPSA) hosts a wide variety of reef-building corals that undergo large daily fluctuations in temperature and dissolved oxygen. Despite these known stressors, these corals appear healthy, exhibiting limited bleaching in the face of high levels of environmental disturbance. This part of the study aims to answer whether the habitual exposure to the short-term stress regimes of the Ofu lagoon enhances the corals abilities to cope with environmental stress. To address this question, a comprehensive analysis of heat and oxidative stress protein biomarkers is currently being completed for corals that were reciprocally transplanted between the Ofu lagoon and the neighboring fore-reef. Transplants were conducted in Jan 2005 and sampled every 24 hrs over a 4 day period. After an initial 24hr acclimation period, samples were collected at mid-day low tide to coincide with the hottest part of the temperature cycle. On collection day 1 relative levels of Heat Shock Protein 70 (Hsp70) had significantly increased from pre-transplant baseline levels in the lagoon transplants but not in the fore-reef transplants ($p < 0.01$). Fore-reef transplants both to the lagoon and back to the fore-reef had significantly lower levels of expression than lagoon transplants in each location ($p < 0.01$). This trend was consistent with relative levels of ubiquitin expression. However, on sampling day 2 fore-reef transplants to the lagoon showed increased levels of Hsp70 expression compared to day 1 and baseline fore-reef samples ($p < 0.01$), while ubiquitin expression remained the same. Heat Shock Protein 60, Super Oxide Dismutase, Rubisco, and 4-Hydroxynonenal levels are currently being analyzed along with days 3 and 4. It is unclear at this point in time whether the reduced ubiquitin expression is evidence of a higher stress tolerance of the fore-reef corals or representative of a lower limit of stress tolerance when compared to the lagoon corals. Controlled laboratory experiments investigating the exact stress thresholds of each group of corals as well as their acclimation potential are planned in the near future. By examining the level and temporal expression of protein biomarkers in transplanted and non transplanted corals we hope to gain a more comprehensive understanding of the potential advantages in terms of physiological range attained by corals that are routinely challenged by abrupt changes in their environment.

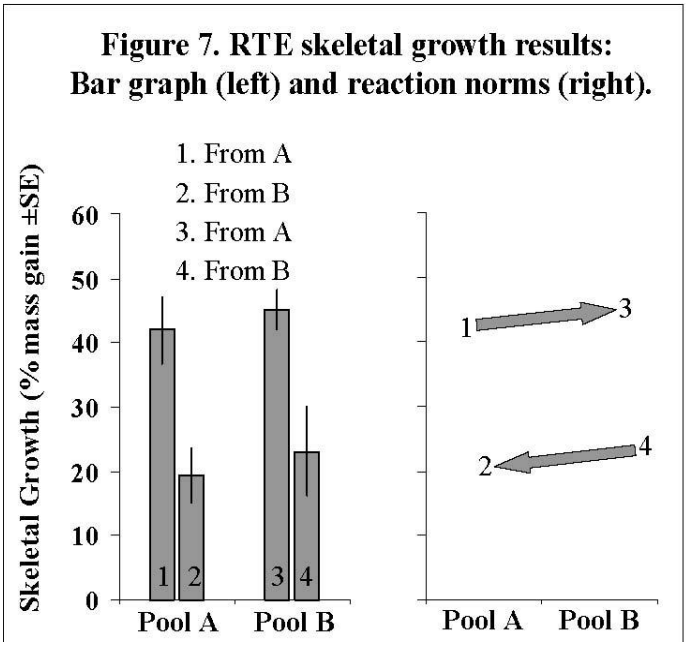
As for sample numbers, we have currently processed an entire transplant experiment, performed in January 2005 which totals ~80 transplants from 4 source colonies by 2 locations for 5 days plus baseline. For these samples I have analyzed Hsp70, Ubiquitin (heat shock and protein degradation), MnSOD and 4-HNE (superoxide defense and lipid peroxide damage) protein expression levels which consistantly show different levels of protein expression between lagoon and forereef transplant sites. This past field season I also collected 228 samples ~25/site of P. lobata for genetic analysis of lagoon/forereef relationships across the entire territory, 6 Tutuila sites, 6 Ofu/Olosega sites, 2 Ta'u sites. The genetic analysis will be completed in the next 6 months of laboratory work. In total we've collected over 750 protein samples to date including 2 reciprocal transplant experiments (1 southern winter, 1 southern summer), 1 background level time series experiment during the southern summer, and a broad baseline survey collection of

background lagoon/forereef protein expression levels during this last southern summer (january-march 2006).

Significant progress has been made in developing a good method for analysis of many coral samples (ePage gels and Western Blot) and we have a number of antibodies that we know give specific cross reactivity to corals for the following proteins: heat shock proteins, ubiquitin, superoxide dismutase, oxidatively damaged proteins (4HNE).

10) Reciprocal transplants show small-scale population structure of the spawning coral *Pocillopora eydouxi*

To investigate whether corals in the back reef pools are genetically distinct from their conspecifics on the nearby forereef, we did a 6-month RTE using *Pocillopora eydouxi* between a forereef and a back reef pool site in 2004 – 2005. A successful RTE can determine whether the phenotypic response is attributable to environmental or non-environmental variance. However, non-environmental variance may be due to unequal effects of colony size, colony shape, competition, predation, disease, zooxanthellae type, or host genetics on the transplants. Colony size, colony shape, and competition effects were eliminated by using the same size and shape of transplants, and adequately spacing the transplants. Surveys during the first and last months of the experiment did not reveal any predation or disease effects. Zooxanthellae samples were taken at the beginning and end of the experiment, and showed that zooxanthellae type was the same between sites and seasons (Type C2). Thus, the flat, non-overlapping skeletal growth reaction norms are most likely due to host genetic differences between these sites, despite their proximity (5 km) and the fact that *P. eydouxi* is a spawning species (Fig. 7).

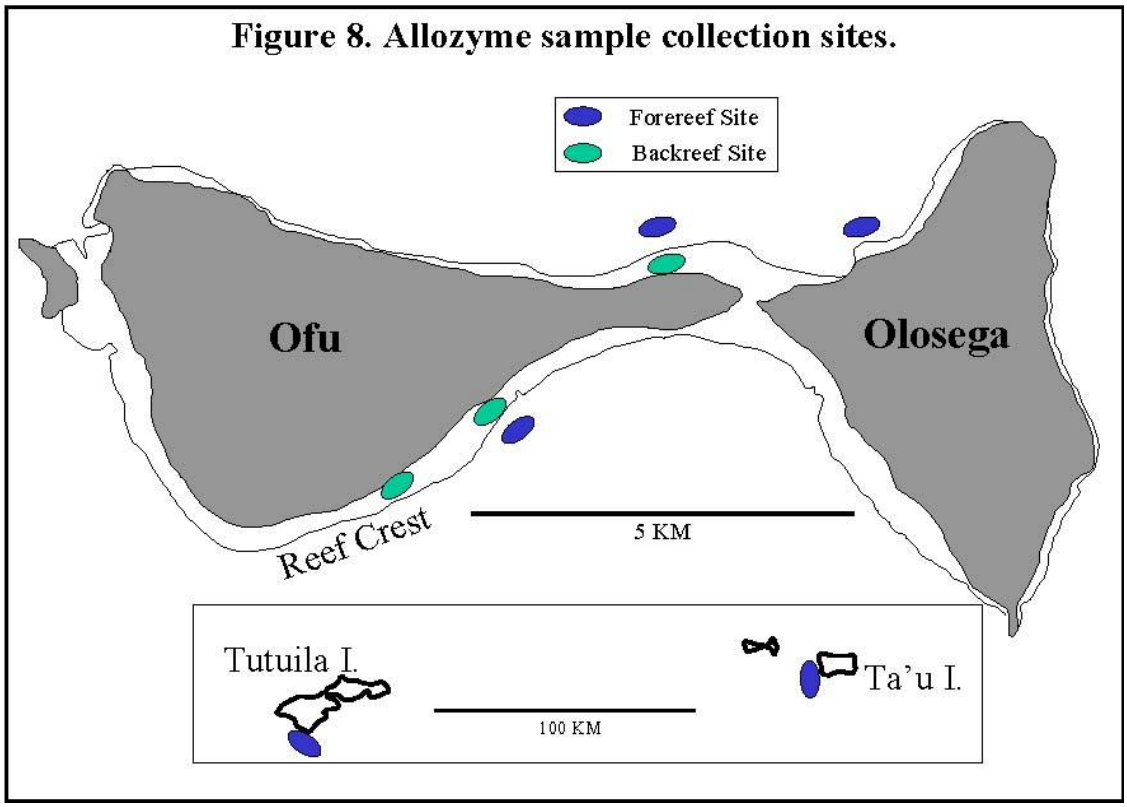


The hypothesis that the forereef and back reef *P. eydouxi* groups used for the RTE are genetically distinct is being tested with an allozyme survey. We intend to use hierarchical surveys of variation at polymorphic allozyme loci to quantify genetic variation and infer gene flow between the two groups. Samples were collected from the two sites in April – May 2006, frozen in the ultracold at our Ofu lab, and transported to the Carlon lab, where the allozyme work was done in Fall 2006.

11) Population structure of *P. eydouxi* in American Samoa

In conjunction with the RTE, allozymes collected from 8 sites in April – May 2006 will be used to study the population structure of *P. eydouxi* in American Samoa at four spatial scales: (1) different habitats on the same reef (< 1 km); (2) adjacent reefs and islands (5 km); (3) within the Manu'a Archipelago (15 km); and (4) within the Territory (100 km; Fig. 8).

Figure 8. Allozyme sample collection sites.



12) Population structure of *Porites lobata* on the forereef and lagoon in Ofu, American Samoa

Host sequences of Porites lobata on the forereef and lagoon on Ofu, American Samoa, were aligned and five (5) sequence groups were resolved. The results were that:

1. Each coral contains more than one sequence type

2. Each core contains more than one sequence type and there is overlap in the sequences found in cores from the same coral colony

3. The ratio of sequence types AY320327.1 (= L28, L31, L34, L36, L39) and AY320311.1 (L35, L40, L42, L43, L45) found in cores A and B of lagoon colony 4 is 4:1 and 1:4, respectively. L44 (similar to AY320318.1) is only found once, in core B.

4. All sequences obtained for the forereef colony are most closely related to *P. lobata* sampled in Easter Island, Chile (E. Pacific) and all the sequence types found in the lagoon coral are most closely related to *P. lobata* sampled in Australia/New Zealand/Tahiti (W. Pacific)

BLAST Comparison with sequences published in GenBank

Sequence Type 1

F8 MATCH 326/326 (100%) **F16** Identities = 321/326 (98%), Gaps = 3/326 (0%)

gi|34979732|gb|AY320300.1| Porites lobata isolate E47-3 18S r... 646 0.0 E = Chile: Easter Island

Sequence Type 2

F4 and F2 320/326 (98%), Gaps = 3/326

gi|34979737|gb|AY320305.1| Porites lobata isolate E121-25 18S... 595 4e-167 E = Chile: Easter Island

Sequence Type 3

L36 MATCH 309/309; **L34 (308/309); L28 short** (288/288)**; L31** (308/309)**; L39** (308/309)

gi|34979759|gb|AY320327.1| Porites lobata isolate T3-4 18S ri... 613 1e-172 T = French Polynesia: Tahiti

Sequence Type 4

L35, L40, L42, L43, L45 – MATCH 315/315; L26 (314/315 (99%), Gaps = 0/315**,**

gi|34979743|gb|AY320311.1| Porites lobata isolate A2-9 18S ri... 624 4e-176 A = Australia

Sequence Type 5

L44 (315/319 (98%), Gaps = 0/319 (0%)

gi|34979750|gb|AY320318.1| Porites lobata isolate R4-9 18S ri... 601 6e-169 R = New Zealand: Cook Islands, Rarotonga

Sample Identifiers – The sequences are for two corals one from the fore reef and one from the lagoon – two cores of each coral were processed.

F01 D1A Forereef colony 1 core A

F01 D1B Forereef colony 1 core B

L04 D1A Lagoon colony 4 core A

L04 D1A Lagoon colony 4 core B

		Sequence Type
F2	F01 D1A	2
F4	F01 D1A	2
F8	F01 D1A	1
F16	F01 D1B	1
L28	L04 D1A	3
L31	L04 D1A	3
L34	L04 D1A	3
L36	L04 D1A	3
L39	L04 D1B	3
L35	L04 D1A	4
L40	L04 D1B	4
L42	L04 D1B	4

L43	L04 D1B	4
L45	L04 D1B	4
L44	L04 D1B	5

13) Global climate change microbial communities as a diagnostic tool?

Kellogg, Garrison and Lisle put together a USGS website presentation that explains their participation and progress in this project at http://coastal.er.usgs.gov/coral-microbes/climate-change.html

Samples collected so far include

Sept 04: Sampled 16 *Pocillopora eydouxi*, 12 *Acropora gemmifera*, 12 *Pocillopora damicornis* (half in Pool 300, half in Pool 400). Samples = 40 mucus, 40 tissue, 40 photos

Jan 05: 14 *P. eydouxi*, 12 *A. gemmifera*, 12 *P. damicornis*, 12 massive *Porites* (aka *Porites lobata*). Samples = 50 mucus, 50 tissue, 50 photos

April 05: 13 *P. eydouxi*, 12 *A. gemmifera*, 12 *P. damicornis*, 12 massive *Porites* (aka *Porites lobata*). Samples = 49 mucus, 49 tissue, 49 photos

Sept 05: 13 *P. eydouxi*, 12 *A. gemmifera*, 12 *P. damicornis*, 12 massive *Porites* (aka *Porites lobata*). Samples = 49 mucus, 49 tissue, 49 photos

May/June 06: 13 *P. eydouxi*, 12 *A. gemmifera*, 12 *P. damicornis*, 12 massive *Porites* (aka *Porites lobata*). Samples = 49 mucus, 49 tissue, 49 photos

That makes for a total of 376 DNA extractions (mucus + tissue samples), plus 4 water controls (Sept 04 sample set to confirm mucus signal was not background water) = 380 DNA extractions.

Of those 380 extractions, 155 mucus samples and 129 tissue samples (284 combined) were positive by PCR amplification for bacterial community DNA (16S rDNA genes). Those 284 samples are being analyzed by DGGE (denaturing gel gradient electrophoresis) right now, so that we can compare bacterial community 'fingerprints' between (1) Individuals of the same coral species, (2) Different coral genera and species, (3) Pool 300 and Pool 400, and (4) mucus and tissue. This will take a couple of months to run all the gels and use the software to interpret the patterns.

14) Structure of microbial communities on corals, coral mucus, and seawater on islands densely and sparsely inhabited by humans in the American Samoan

Hypotheses:

1) H_0: Diverse, healthy coral species inhabiting Ofu lagoon pools do not harbor unique microbial communities (i.e., different coral species do not harbor different microbial communities).

   Microbial community diversity will be assessed for a variety of different coral species to determine if certain corals form species-specific associations with microbes. Microbes will be identified using molecular techniques.

2) H_0: Microbial community diversity in Samoan corals and seawater does not differ from that of geographically separated conspecifics or waters.

   Microbial community diversity of seawater and corals (*Porites lobata, Porites lutea, Pocillopora damicornis, Pocillopora meandrina*) collected from sites around American Samoa will be compared to samples collected from sites around the main and Northwestern Hawaiian Islands and Johnston Atoll.

3) H_0: Seawater and coral-associated microbial communities do not fluctuate over a diel cycle in Ofu lagoon pools.

   Three colonies of *Porites cylindrica* and *Pocillopora damico*rnis were tagged in Ofu lagoon Pool 300. Surrounding seawater and non-lethal biopsies were collected from each colony to assess changes in microbial community structure and function over a diel cycle. Samples were collected over a 24-hour period, during high and low tides and during periods of light and dark.

New findings:

- Preliminary results from flow cytometry cell count data revealed that the waters surrounding American Samoa contain fewer pigmented eukaryotes compared to Kaneohe Bay, Oahu. These data are indicative of the oligotrophic nature of the waters surrounding American Samoa.

- Preliminary analyses of TRFLP profiles from *Porites compressa* and *Porites lobata* collected in Kaneohe Bay and at French Frigate Shoals (Northwestern Hawaiian Islands) revealed the presence of a single ribotype (251) that was only found in Kaneohe Bay samples. This bacterial ribotype will be identified via cloning and sequencing in order to gain an understanding of its possible ecological function. TRFLP profiles from American Samoa corals (*Porites lobata, Porites lutea, Pocillopora damicornis, Pocillopora meandrina*) will be compared with those from the Hawaiian Islands and Johnston Atoll to determine if the corals harbor bacteria that are unique to Samoan reefs.

Sample Collection Sites			
Ofu		**Tutuila**	
Pool 400 Pool 300 Pool 200 Sili fore reef		Vatia Bay Onesosopo Utulei Maloata Fagaitua Bay	
Coral Samples			
species	**# of colonies sampled**	**species**	**# of colonies sampled**
Acropora austera	12	*Acropora abrotanoides*	3
Acropora gemmifera	3	*Acropora humilis*	3
Favia matthias	2	massive *Porites* sp.	9
Favia stelligera	2	*Pavona divaricata*	2
Goniastrea retiformis	3	*Pocillopora damicornis*	10
Heliopora coerulea	1	*Pocillopora eydouxi*	1
Leptastrea purpurea	3	*Pocillopora meandrina*	5
Lobophyllia hemprichii	1	*Pocillopora verrucosa*	3
massive *Porites* sp.	7	*Porites cylindrica*	18
Pocillopora damicornis	9	*Porites lobata*	1
Pocillopora meandrina	8	*Porites lutea*	8
Pocillopora verrucosa	2	*Porites rus*	8
Porites cylindrica	9	*Sarcophyton* sp.	1
Porites lobata	11		
Porites lutea	3		
Porites rus	10		
Seawater Samples			
sample type	**# samples**	**sample type**	**# samples**
chl *a* (125 mL/sample)	7	chl *a* (125 mL/sample)	5
microbial DNA (1 L/sample)	7	microbial DNA (1 L/sample)	5
cell counts (1 mL/sample)	7	cell counts (1 mL/sample)	5
nutrients (125 mL/sample)	7	nutrients (125 mL/sample)	5

Table 1. Summary of sample collections made during the Rappé Laboratory April 2006 field season. A total of 4 sites were sampled around Ofu and a total of 5 sites were sampled around Tutuila, with 86 and 72 coral colonies sampled from each island, respectively. Seawater samples were collected for chlorophyll *a* measurements, microbial DNA analysis, microbial and eukaryotic cell counts, and nutrient concentration measurements.

PERSONNEL and COLLABORATORS:

BRD Principal Contact: Dr. Charles Birkeland, Assistant Leader - Hawai'i Cooperative Fishery Research Unit (BRD), University of Hawai`i at Manoa, Honolulu 96822 telephone 808 956-8350; fax 808 956-4238; e-mail charlesb@hawaii.edu

Reponsibilities included design and supervision of the growth and survival experiments (staining with Alizarin red S and transplantation with Sea Goin' Poxy Putty) on corals and coordinating the interactions among the projects of the cooperators/partners and seeing that everything keeps on schedule.

Facilitator: Dr. Peter Craig - National Park of American Samoa Pago Plaza, Suite #114 Pago Pago, AS 96799 telephone 011 (684) 633-7082 fax 011 (684) 633-7085 e-mail

Responsibilities included overseeing the propriety of experimental procedures, as well as logistics and permits, for working in the National Park of American Samoa. NPSA offered considerable "cost sharing" (estimated at $66 K) through personnel time, equipment provided, office space on Ofu and staging and logistics from the office at Tutuila.

Co-PI: Andrew C. Baker – Marine Biologist, Wildlife Conservation Society, and adjunct Assistant Professor, Center for Environmental Research and Conservation MC5557, Columbia University, 1200 Amsterdam Avenue, New York, NY 10027 telephone (212) 854-8184 fax (212) 854-8188 e-mail abaker@wcs.org

Responsibilities included molecular identification of algal symbiont communities in the moat and on the forereef. Also was responsible for training the resident Research assistant in the techniques of collecting, preserving and shipping coral tissue specimens.

Co-PI: Virginia (Ginger) H. Garrison - USGS Survey Center for Coastal and Watershed Studies, 600 Fourth Street South, St. Petersburg, Florida 33701 telephone 727 803-8747 ext. 3061; fax 727 803-2032; e-mail ginger_garrison@usgs.gov

Responsibilities included design of the experiment to elucidate the bacterial community associated with corals in different areas of the reef and under different environmental conditions and integrating the bacterial community findings with coral

resilience and ecology. Her office contributed $30 K in matching funds over the three years as cost-sharing.

Co-PI: Ruth Gates – Hawaii Institute of Marine Biology, SOEST, University of Hawaii PO Box 1346, Kaneohe, HI 96744 telephone 808 236-7420, fax 808 236-7493 e-mail rgates@hawaii.edu

Responsibilities included determining the levels of heat-shock proteins and antioxidants in the corals in the moat and offshore, as well as the genotype of the corals and their algal symbionts.

Co-PI: Christina Kellogg – USGS Microbiologist/Mendenhall Fellow, USGS Center for Coastal and Watershed Studies, 600 Fourth Street South, St. Petersburg, FL 33701 telephone 727 803-8747 x3128; fax 727 803-2031; e-mail ckellogg@usgs.gov.

Responsibilities included determining the variation in bacterial communities associated with corals in different areas of the reef and under different environmental conditions. She was also responsible for training the resident Research Assistant in the techniques of collecting coral mucus, inducing samples, and stabilizing samples for shipment.

Co-PI: Gregory Piniak - Research Geologist/Mendenhall Fellow, USGS Pacific Science Center (CMG), 1156 High Street, Santa Cruz, CA 95064 telephone 831 427-4729 fax 831 427-4748 e-mail gpiniak@usgs.gov

Responsibilities included PAM fluorometry experiments.

Co-PI: Michael Rappé – Hawaii Institute of Marine Biology, PO Box 1346, Kaneohe, HI 96744 telephone 808 236-7464; e-mail: rappe@hawaii.edu

Responsibilities included the identification of photoprotective pigments and quantification of thermal tolerance within marine microorganisms present in natural and transplanted coral-associated microbial communities.

Co-PI: Robert J. Toonen – Hawaii Institute of Marine Biology, PO Box 1346, Kaneohe, HI 96744 telephone 808 236-7420; e-mail: toonen@hawaii.edu

Responsibilities included determining the levels of heat-shock proteins and antioxidants in the corals in the moat and offshore, as well as genotyping the corals and their algal symbionts.

Co-PI: Jonathan Stillman –Assistant Professor, Department of Zoology, University of Hawaii, Honolulu, Hawaii 96822 telephone 808 956-8350 e-mail stillman@hawaii.edu

Responsibilities included development of methods for analysis of heat-shock protein and antioxidant levels in coral samples collected in this study and contribution of experience involving thermal stress physiology.

Research Assistant: Lance Smith – Department of Zoology, University of Hawaii, Honolulu, Hawaii 96822 telephone 808 956-8350 e-mail lancesmi@hawaii.edu

Responsibilities included setting up the coral growth and survival transplant experiments, maintaining the monitoring equipment for measuring environmental factors, and collecting, preserving and shipping tissue specimens as needed.

FACILITIES/EQUIPMENT/STUDY AREA: Field work was carried out on Ofu Island in the Ofu Unit of NPSA. While in the field, researchers were based at Vaoto Lodge on Ofu Island, where NPSA has a small office with phone and email connections. A laboratory was built on Ofu to conduct some laboratory experiments, and lab work wasl also done at the University of Hawai'i's Hawai'i Institute of Marine Biology on Oahu, Hawai'i.

COORDINATES:

S 14° 10.830′ W 169° 39.111′

S 14° 09.838′ W 169° 37.496′