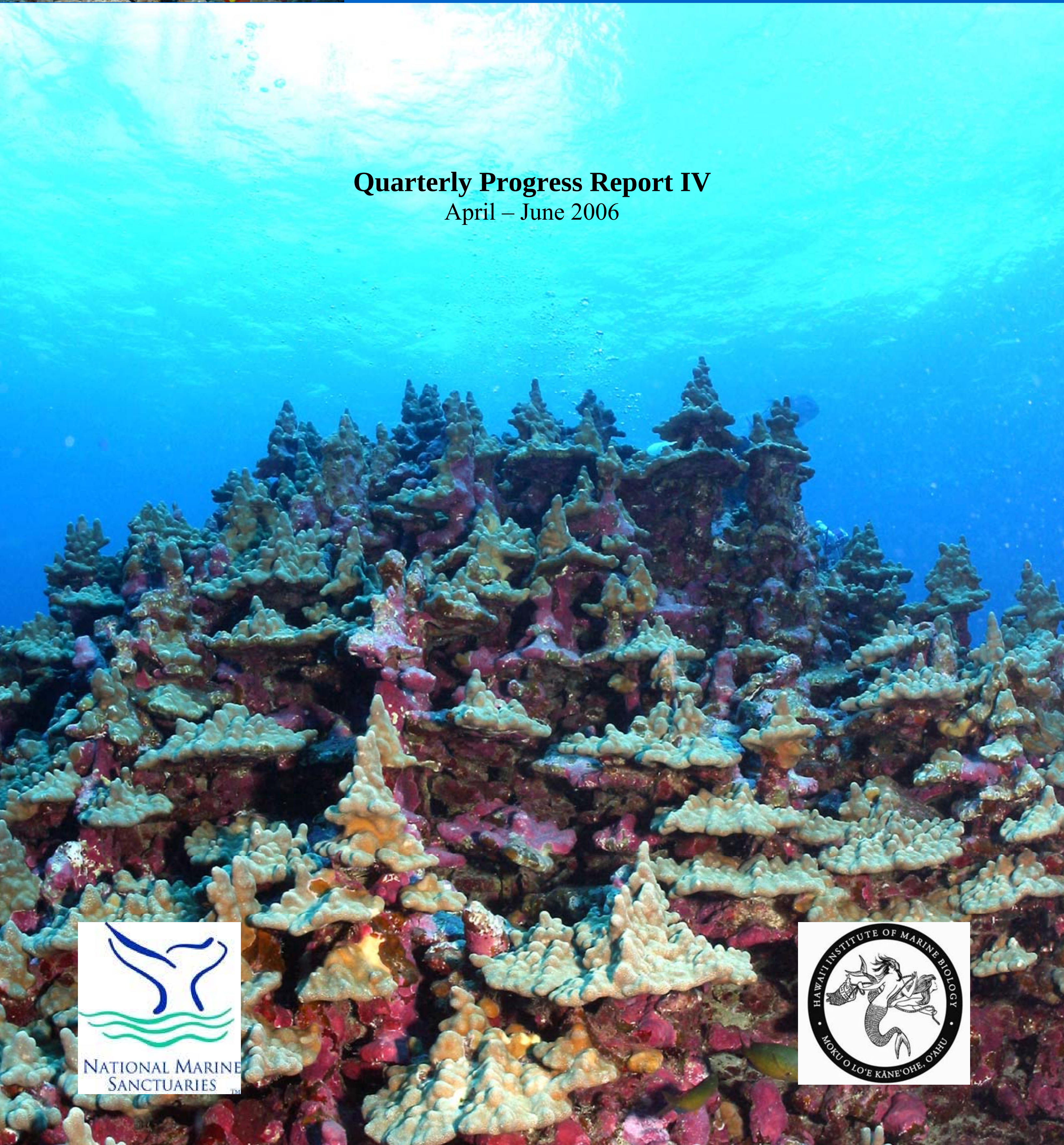
Hawai'i Institute of Marine Biology
Northwestern Hawaiian Islands
Coral Reef Research Partnership



Quarterly Progress Report IV
April – June 2006

Cover photos by James Watt:
Front and back cover – reef at Lisianski
Front cover, upper left – reef at Pearl and Hermes

Hawai‘i Institute of Marine Biology
Northwestern Hawaiian Islands
Coral Reef Research Partnership

Quarterly Progress Report IV
April – June 2006

Report submitted by Jo-Ann Leong, Malia Rivera and Jennifer Barrett

August 21, 2006

Acknowledgments: Hawai‘i Institute of Marine Biology (HIMB) acknowledges the support of Senator Daniel K. Inouye's Office, the National Marine Sanctuary Program (NMSP), the Northwestern Hawaiian Islands Marine National Monument (NWHI MNM), State of Hawai‘i Department of Land and Natural Resources (DLNR) Division of Aquatic Resources, U.S. Fish and Wildlife Service, NOAA Fisheries, and the numerous University of Hawai‘i partners involved in this project. Funding provided by NMSP MOA 2005-008/66832.

Photos provided by NOAA NWHI MNM and HIMB.
This page: Aerial photo of Moku o Lo‘e (Coconut Island) by Brent Daniel.

CONTENTS

Background

The Hawai‘i Institute of Marine Biology signed a memorandum of agreement with the National Marine Sanctuary Program (NOS, NOAA) on March 28, 2005, to assist the Northwestern Hawaiian Islands Coral Reef Ecosystem Reserve (NWHICRER) with scientific research required for the development of a science-based ecosystem management plan. With this overriding objective, a scope of work was developed to:

1. Understand the population structures of bottomfish, lobsters, reef fish, endemic coral species, and adult predator species in the NWHI.
2. Characterize the genetic diversity of corals in the NWHI and determine the background levels of coral health in the NWHI.
3. Support mapping activities that will be used in the Sanctuary designation and management zones.
4. Identify the pool of invasive species in the Main Hawaiian Islands (MHI) and develop measures to prevent the spread of these species to the NWHI.
5. Support sound ecosystem management.

The Hawaiian Archipelago is located at 19°-28° N to 155°-178° E and spans approximately 1,200 miles of the Pacific Ocean. The lower eight Main Hawaiian Islands (MHI) are home to a growing population of more than a million people. In the northern reaches of the Hawaiian Archipelago are a series of tiny islands, atolls, and shoals that make up the Northwestern Hawaiian Islands (NWHI) which are largely uninhabited. The pristine beauty and unique ecosystem of the NWHI was recognized in 2001 when Executive Order 13178 created the Northwestern Hawaiian Islands Coral Reef Ecosystem Reserve, the largest marine protected area in the United States.

On June 15, 2006, President George W. Bush signed a proclamation creating the Northwestern Hawaiian Islands Marine National Monument (NWHI MNM), the world’s largest marine protected area. This proclamation designation overlays the NWHI CRER and affords the NWHI the greatest possible marine environmental protections. It also increases immediacy of the need for an ecosystem management plan. The monument spans nearly 140,000 square miles and is home to an estimated 7,000 species including fishes, invertebrates, marine mammals, and birds. However, many biologists believe that this is a huge underestimate of the actual number of species in the region. While the full extent of its biodiversity is largely unknown, approximately one quarter of these species are likely found nowhere else on Earth.

Hawai‘i is one of the most isolated yet populated areas on Earth and this site provides a unique opportunity to compare relatively pristine coral reefs in the northern end of the Hawaiian Archipelago with those that are influenced by human activities in the southern end. This "gradient" of human disturbance on coral reefs offer scientists an opportunity to examine species diversity and function under different environmental conditions, with the aim of developing best practices for the maintenance of a healthy coral reef ecosystem in the NWHI. The vision of the proposed sanctuary: "*That the vast coral reefs, ecosystems, and resources of the Northwestern Hawaiian islands (NWHI) - unique in the world - be healthy and diverse forever*" is the guiding principle in the design of the research objectives proposed for the NWHI MNM-HIMB partnership.

Summary of Activities

The Hawai‘i Institute of Marine Biology has completed three sampling cruises to the Northwestern Hawaiian Islands since the research partnership was initiated in April of 2005. The most recent cruise aboard NOAA Ship Hi‘ialakai occurred during May–June 2006. Preliminary results of the fourth quarter of research are presented here.

- Population structure across the Hawaiian Archipelago does not fit a simple isolation by distance model, and broad-scale generalizations based on oceanographic currents are poorly supported.
- Closely-related species with similar ecology and reproductive biology, such as opihi and hermit crabs appear to have significantly different patterns of gene flow.
- At French Frigate Shoals, tagging the large predatory fish ulua and monitoring their movements has revealed that these animals generally remain within a small daytime home range. Yet every full moon during the summer months, ulua at FFS travel up to 30 kilometers to congregate at Rapture Reef, a preferred spawning site on the south side of the atoll.
- The use of satellite transmitters has provided new information on the movements of tiger and galapagos sharks. Both species were found to range from the ocean’s surface to depths of 680 meters; that’s over 2,200 feet below the surface, the length of about seven football fields.
- The squirrelfish *Myripristis berndti* shows high genetic connectivity across the Hawaiian archipelago, indication that this species forms a single widespread population.
- *Acropora* white syndrome, a coral disease afflicting giant table coral, was found to be highly virulent. Researchers marked 41 infected coral colonies in 2005 and returned a year later to find 97.6% of the infected colonies suffering partial to complete mortality from the disease.
- Johnston Atoll exhibited a higher occurrence of *Acropora* white syndrome than French Frigate Shoals and may prove to be the pathway from which the disease arrived at FFS. One reef (Donovan’s Reef) at Johnston Atoll showed a dramatic decline in live coral cover in 2006 (~5%) as compared to visual surveys in 2001 and 2003 (~80%).
- Healthy *Porites compressa* (finger coral) and *Porites lobata* (lobe coral) harbor specific bacterial communities with little overlap between the two coral species.
- A particular genetic type of coral-associated microbe is absent from all Northwestern Hawaiian Island samples of finger coral and lobe coral studied to date, but is present in all Kāne‘ohe Bay finger coral and lobe coral samples examined thus far. Continuing research seeks to reveal this microbe’s possible ecological function.
- There appears to be a correlation between disease susceptibility and colonies of giant table coral that harbor a specific type of dinoflagellate symbiont. Preliminary findings reveal that colonies harboring clade A *Symbiodinium* are more susceptible to disease. Continuing

research will confirm whether this association may be relied upon as an indicator for disease susceptibility.

- There appear to be multiple species of the invasive coral *Carijoa* in Hawai‘i, contradicting previous reports that this invasive species was introduced from one source.
- The few alien species known to be present in the NWHI are restricted to the anthropogenic habitats at Midway Atoll and French Frigate Shoals. Only one, the hydroid *Pennaria disticha,* has spread extensively in the NWHI but neither this or other species have exhibited invasive characteristics at this time. Populations of alien marine species that have already colonized areas of the MHI represent the most likely source of invasive species in the NWHI based on the proximity and pattern of ship movements associated with the MHI.
- Mapping data from various sources was integrated into a single framework which can be utilized to produce meaningful ecological insights for the assessment and monitoring of the Monument.
- An Index of Biological Integrity (IBI) for reefs throughout the Hawaiian Archipelago was developed using a relatively small number of biological indicators. Subsequent evaluation of the IBI supported its viability as a management tool.
- A study assessing potential threats to the NWHI revealed that the greatest threats are global climate change effects and marine debris, while fishing and other locally-based activities result in comparatively less impact.
- NOAA IDEA Center awarded $75,000 to HIMB and NWHI MNM through the Pacific Region Integrated Data Enterprise (PRIDE) program for an integrated data visualization website for the HIMB-NWHI Coral Reef Research Partnership.
- HIMB performed a preliminary assessment of vessel traffic patterns in the Northwestern Hawaiian Islands and identified a potential hot spot of activity between Pearl and Hermes Atoll and Lisianski Island.
- An overview of the HIMB-NWHI Coral Reef Research Partnership has been incorporated into the HIMB Continuing Education Program which hosts tours for 6,000-plus community members annually.
- The NWHI Outreach and Education Program successfully competed for a NOAA Office of Education MiniGrant totaling $100,000.
- For the first time, mini-documentaries were created onboard the NOAA Ship Hi‘ialakai during the May-June 2006 research cruise as an outreach tool using “podcast” technology.

The following pages provide detailed descriptions of the research progress.

Northwestern Hawaiian Islands Connectivity Studies

Project:

- ✓ **Connectivity among coral reef invertebrates across the Hawaiian Archipelago (R. Toonen, B. Bowen et al.)**

We are continuing our survey of fish and invertebrate species across the NWHI to assess the level of connectivity between the isolated reef habitats of the NWHI and the Main Hawaiian Islands (MHI). Our research seeks to answer the questions: How connected are populations of invertebrates 1) within the NWHI?; and 2) between the MHI and NWHI? An ongoing debate exists as to whether the NWHI is a series of relatively fragile (isolated) ecosystems, or whether it is a single large and robust ecosystem that can withstand anthropogenic influences such as debris accumulation, pollution and global climate change (Selkoe et al. in prep). Whether the NWHI serves as a source or a sink for the Main Hawaiian Islands has direct management implications. By using assays of population connectivity, we hope to inform this issue in a format that has statistical power and scientific credibility.

We have assembled an outstanding team of scientists, including: Iliana Baums (Ph.D., coral genetics); Kim Selkoe (Ph.D., ecosystem management & threat analyses); Kim Weersing, Chris Bird, Greg Concepcion, Joe O'Malley, Matt Iacchei (graduate students); Van Nicholas Velasco and Carly Allen (undergraduate student assistants). These researchers have participated in three research cruises to the NWHI using the newly-outfitted Hiʻialakai to visit eight locations throughout the NWHI. On these cruises we have collected non-lethal tissue specimens (coral fragments, and small tissue clips) from over 2,000 invertebrates of 30 different species from throughout the Hawaiian archipelago. The multi-species approach to assess population connectivity in the Hawaiian archipelago will be incorporated into a broader framework (funded by the National Science Foundation) to put population structure within the Hawaiian archipelago into context using samples from elsewhere in the Indo-Pacific.

To date, our preliminary results suggest that there are large differences among taxa in their degree of connectivity throughout the archipelago. Some species appear to move between the NWHI and MHI with relative ease (e.g., the opihi species *Cellana exarata* and *C. sandwicensis*), while others (e.g., *C. talcosa*) do not (Bird et al. in prep; see Figure 1). Among both opihi (Hawaiian endemic limpets; Bird et al., in prep) and hermit crabs (Baums et al., in prep), we find that some closely related species show significant population structure across the archipelago while other species show no significant population structure within Hawaiʻi. Our results thus far suggest that population structure across the Hawaiian archipelago does not fit a simple isolation by distance model, and broad-scale generalizations based on oceanographic currents are

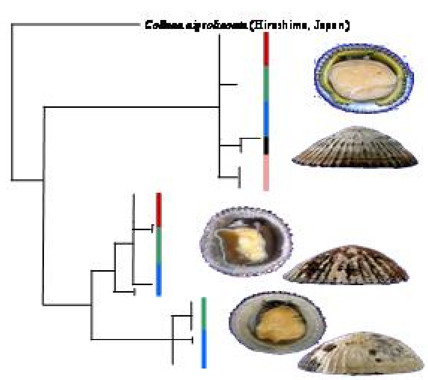


Figure 1: Opihi population structure across Hawaiʻi. Two species show little population structure across the Archipelago, whereas the third shows a distinct genetic break at Kauai, which suggests that there is little or no larval dispersal across that channel for *C. talcosa*.

poorly supported. Also, closely-related species with similar ecology and reproductive biology (such as opihi and hermit crabs as outlined above) appear to have significantly different patterns of gene flow. Together, these results suggest that species-specific differences in reproductive biology or larval behavior may dominate patterns of connectivity in Hawaiʻi.

Figure 2: *Calcinus haigae* commonly found in colonies of *Porites meandrina*. *Photo: Iliana Baums*.

Project:

- ✓ **Movements of Top Predators along the Hawaiian Archipelago (C. Meyer & K. Holland)**

Top predators play a keystone role in many ecosystems. In the Northwestern Hawaiian Islands Marine National Monument (NWHI MNM), this role is filled by sharks (primarily *Galeocerdo cuvier*, *Carcharhinus galapagensis*, *Carcharhinus amblyrhynchos* and *Triaenodon obsesus*) and large teleost fishes (primarily *Caranx ignobilis*). Science-based management of the fish resources of the Hawaiian Archipelago requires that we know whether key species are site-attached to specific areas and, if not, how frequent and extensive are their movements. If the populations of key species are "tied" to individual atolls or islands, different management options may be available than if significant numbers of individuals move between atolls or, indeed, move throughout the archipelago (and between the Main Hawaiian Islands and the Northwest Hawaiian Islands). We are using acoustic and satellite telemetry to quantify the movements of top predators in the NWHIMNM to address three questions relevant to management zoning:

(1) Do top predators move across open ocean between atolls?
(2) How extensive are their intra-atoll movements?
(3) Do top predators exhibit predictable patterns of movement and habitat use?

<u>Transmitter and receiver deployments</u>: To date we have equipped 122 top predators with surgically implanted acoustic transmitters (Table 1) and stationed 19 underwater receivers among eight NWHIMNM atolls, reefs and pinnacles to detect movements of these animals (Table 2). We have also deployed a receiver at Johnston Atoll to detect predator movements between the NWHIMNM and this location.

Table 1. Acoustic transmitter deployments in the NWHI MNM by species.

Species Name	Hawaiian Name	Common Name	Number Equipped with Transmitters
Aprion virescens	Uku	Green Jobfish	28
Caranx ignobilis	Ulua aukea	Giant Trevally	32
Caranx melampygus	Ōmilu	Bluefin Trevally	2
Carcharhinus amblyrhyncos	Manō	Grey Reef Shark	6
Carcharhinus galapagensis	Manō	Galapagos Shark	38
Galeocerdo cuvier	Manō	Tiger Shark	15
Triaenodon obesus	Manō lālākea	Whitetip Reef Shark	1
TOTAL			**122**

Table 2. Acoustic receiver deployments in the NWHI MNM & Johnston Atoll.

Location	Number of Acoustic Receivers Deployed
Kure Atoll	3
Midway Atoll	3
Pearl and Hermes Reef	4
Maro Reef	2
French Frigate Shoals Atoll	5
Mokumanamana	1
Nihoa	1
Johnston Atoll*	1
TOTAL	20

In addition to our acoustic transmitter deployments, we have also equipped nine sharks (five tiger sharks and four galapagos sharks) with satellite transmitters to provide information on shark movements in areas not equipped with acoustic receivers. We are using two types of satellite transmitters: (1) Fin mounted SPOT tags (Figure 1); and (2) Pop-up archiving tags (PAT tags – Figure 2). SPOT tags transmit the shark's location to the Argos satellite array whenever the dorsal fin breaks the surface of the water. PAT tags collect and store temperature, depth and light intensity data as the shark swims, and then detach from the animal on a preprogrammed date and time. The released PAT tags float to the surface where they transmit archived data to the Argos satellite array.

Figure 1. SPOT tag attached to dorsal fin of tiger shark. *Photo: Jill Zamzow*.

Figure 2. Tiger shark with PAT tag. *Photo: Jill Zamzow*.

Acoustic monitoring results: In May 2006, we downloaded data from our receivers stationed at FFS, and now have a 12 month time-series of acoustic monitoring data for this location. Between May 2005 and May 2006, our receivers recorded 192,070 detections of 32 top predators that were equipped with acoustic transmitters at FFS during 2005. Our data suggest that most of these animals are site-attached to FFS, but wide ranging within this location. Several of these species exhibit well-defined patterns of movement and habitat use. For example, ulua were site-attached to small (<4 km^2) daytime home ranges within FFS atoll from which they made monthly excursions of up to 29 kilometers during summer, to reach a spawning site (Rapture Reef) on the south side of the atoll. These summer spawning migrations have a lunar rhythm, with ulua aggregating at Rapture Reef during the full moon and last quarter of the lunar cycle.

On May 23, 2006 (nine days after the full moon), we sighted a large school of ulua containing individuals with characteristic dark spawning coloration while SCUBA diving at Rapture Reef (Figures 3 and 4).

Figure 3. Spawning color dimorphism in ulua from school at Rapture Reef (FFS Atoll). *Photo: Jill Zamzow*.

Figure 4. Ulua school at Rapture Reef (FFS Atoll), May 23, 2006. *Photo: Jill Zamzow*.

Satellite telemetry results:
Since May 2006, we have received 1,526 transmissions from 8 SPOT-equipped sharks, yielding 229 estimates of geographic position. The SPOT data show that while most sharks have remained at FFS, 2 tiger sharks have moved north to submerged banks located approximately 100 kilometers north of FFS atoll (e.g., Figure 5). Preliminary data from released PAT tags have provided unprecedented insight into the depth profiles of tiger and galapagos sharks, revealing that these animals range from the surface to depths of 680 meters in the waters around FFS.

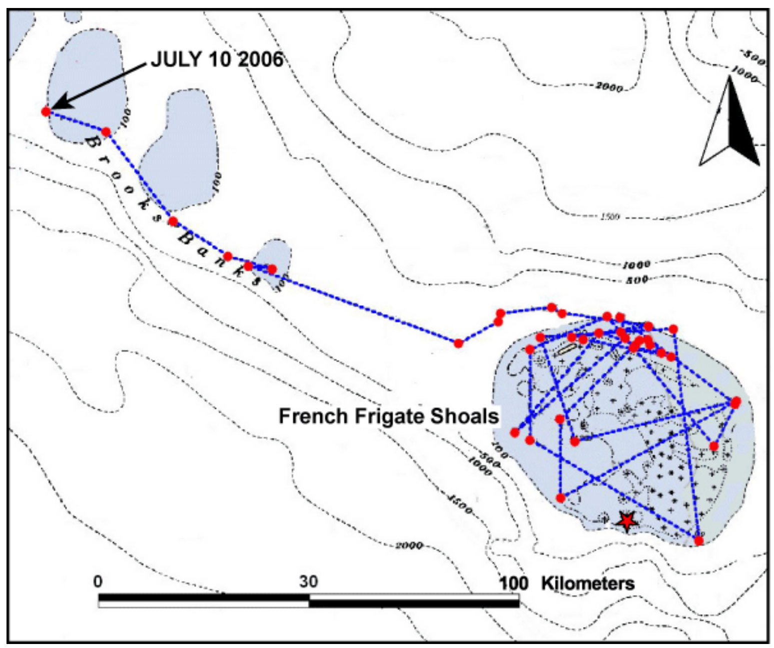


Figure 5. Geographic track of tiger shark #67152 equipped with SPOT tag at FFS atoll on May 26, 2006. Red points = geographic fixes from SPOT tag, dashed blue line = nominal path between fixes, red star = capture location.

Invited Presentations: Sharks of the NWHI at Hanauma Bay. On April 27, 2006 Carl Meyer gave a formal presentation on the sharks of the NWHI to a full house of 60 at the Hanauma Bay Education Center. This presentation concluded a series of public lectures on the NHWI held at Hanauma Bay Nature Preserve and co-sponsored by the NWHI Reserve.

Project:

- ✓ **Connectivity among coral reef fishes and bottomfishes across the Hawaiian Archipelago**
  (B. Bowen, R. Toonen, et al.)

This research component seeks to assess the level of connectivity among isolated reef habitats through a survey of fish species across the NWHI. The survey includes samples of 20+ fish species collected at the major islands and atolls of the NWHI. This research is conducted with advanced molecular genetic methodologies, using the genetics core facility at HIMB.

Ultimately, our work will determine whether reef ecosystems of the NWHI are isolated management units or components of a large interactive ecosystem. In the former case, each reef ecosystem will have to recover from environmental insults (whether human or natural) without significant input from other reef ecosystems. A corresponding conservation mandate would be that each ecosystem is an independent management unit.

Our multi-species approach to assessing population connectivity in the Hawaiian Archipelago will be incorporated into a broader framework (funded by the National Science Foundation) to examine the phylogeography of many of these same species throughout the Indo-Pacific. This will provide a comparative framework to bolster the scientific foundations for implementing the NWHI MNM. The information provided by our connectivity studies is critical to assessing the patterns and magnitude of connection between the NWHI and MHI; knowledge required by decision makers formulating management plans for both the NWHI and MHI.

Progress in the field: Two previous Hawaiian research cruises using the Hiʻialakai (May-June 2005 and September-October 2005) allowed our personnel to visit eight locations in the NWHI. In May-June 2006, we participated in an additional cruise with HIMB personnel collecting over 1,000 fish specimens at Nihoa, French Frigate Shoals, Gardner Pinnacles, and the isolated Johnston Atoll. The collections at Johnston Atoll, approximately 1,000 kilometers south of FFS, will allow us to test for a possible connection to the middle region of the NWHI.

In addition to reef fish collections in the NWHI and at Johnston Atoll, NSF funding was used to make parallel collections at island groups to the west and south of Hawaiʻi. These include: the Marshall Islands (March 2006); Fiji (April 2006); and Christmas Island (June 2006).

The snappers that inhabit the deep reefs are of special concern due to the ongoing bottomfish fishery. In collaboration with Chris Kelley, we have contracted fishermen in the MHI as well as the NWHI to collect specimens in support of microsatellite DNA and mtDNA surveys of ehu (*Etelis carbunculus*), onaga (*Etelis coruscans*), and opakapaka (*Pristipomoides filamentosus*). We have procured independent funding from the NMFS and Western Pacific Fisheries Management Council to advance these projects.

Progress in the lab: As noted in previous reporting periods, we have sequenced mitochondrial DNA from three fish species: the flame angelfish (*Centropyge loriculus*); large-scale squirrelfish (*Myripristis berndti*); and yellow tang (*Zebrasoma flavescens*). The first two are known to disperse on ocean currents as larvae and appear to have widespread Central Pacific populations

(Schultz et al. 2006; Craig et al. 2006), while the yellow tang shows statistically significant genetic structure, indicating that the Hawaiian Archipelago is not a single highly-connected ecosystem.

Hawaiian endemic species require special attention in terms of wildlife management due to the greater risk that accompanies a restricted distribution. In the current research period, we began to examine population structure in three species of endemic Hawaiian butterflyfishes (*Chaetodon miliaris*, *C. fremblii*, and *C. multicinctus*). Thus far, we have collected and sequenced 170 *C. fremblii* and 229 *C. miliaris* (Figure 1), and have made significant progress in collections of *C. multicinctus* from throughout the Hawaiian Islands. Each of these species plays a distinct role in the coral reef ecosystem, based on feeding habits and social behavior. Hence, these three species can provide a unique example of how these factors may alter the degree to which populations are connected over meso-scale distances.

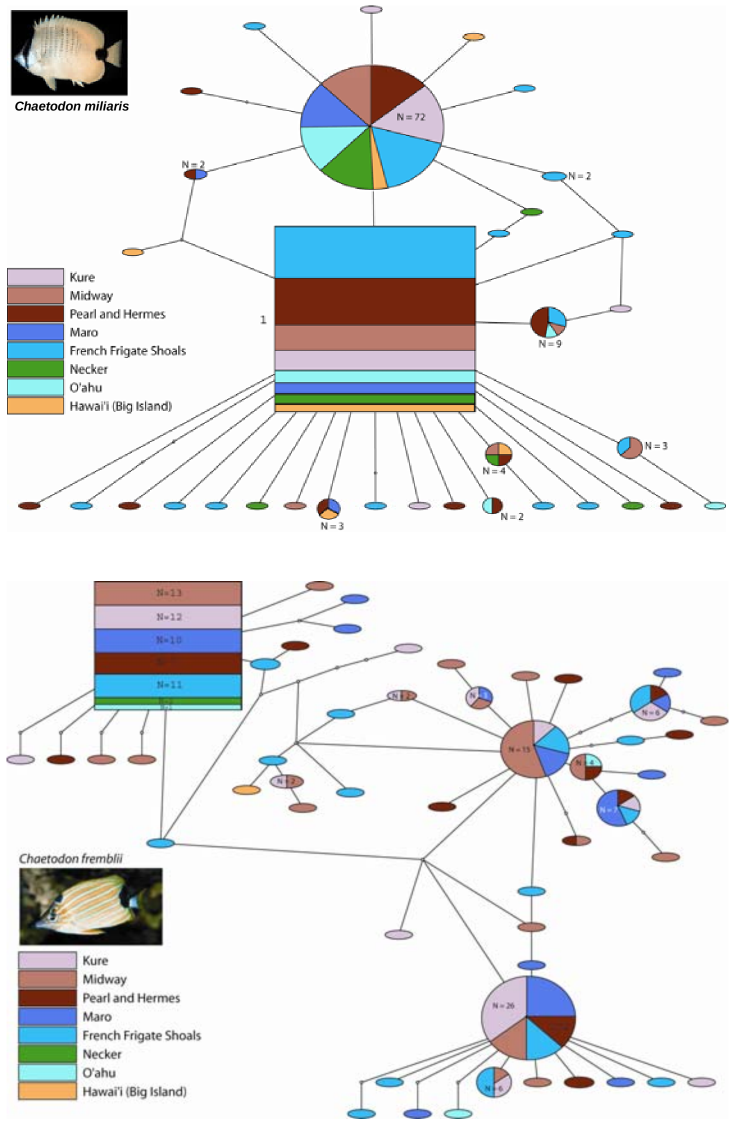


Figure 1. These parsimony networks show the relationship among mtDNA haplotypes for the endemic butterfly fishes *Chaetodon miliaris* and *Chaetodon fremblii*, as well as the geographic distribution of haplotypes. Note that the common haplotypes are distributed across the entire Hawaiian archipelago.

Bottomfish surveys are still in the development stage. Currently, Ph.D. students Tonatiuh Trejo and Michelle Gaither are screening microsatellite loci to determine the most robust and dependable loci.

<u>Introduced Species</u>: The blueline snapper (ta'ape; *Lutjanus kasimira*) was introduced to O'ahu from two locations: French Polynesia and the Marquesas. Previous work by Sergio Planes has demonstrated diagnostic mtDNA markers for these two locations. Using these markers, Ph.D. student Michelle Gaither is surveying across the Hawaiian archipelago, to resolve the pattern of colonization for this introduced species. Her initial results demonstrate that both Polynesian and Marquesan lineages survived the colonization, and occur at proportions close to the size of the initial introductions.

Additional specimens from the NWHI are needed to characterize the invasion of this isolated habitat.

<u>Summary</u>: Questions persist as to whether the NWHI is comprised of a series of relatively fragile (isolated) ecosystems or functions as a single robust ecosystem. There are management implications as to whether the NWHI serves as a source or a sink for the Main Hawaiian Islands. Our assays of population connectivity will inform this issue in a format that has statistical power and scientific credibility. To accomplish this, our greatest bottleneck lies in sample acquisition, and it is here that we will focus our efforts in the coming year.

Coral Health Assessment Program Studies

Project:

- ✓ **Investigation of diseases of corals and fish within the Northwestern Hawaiian Islands and Johnston Atoll (G. Aeby, T. Work & D. Albert)**

Disease in coral reef ecosystems has received great attention, particularly in the western Atlantic where coral disease has been incriminated in the marked degradation of reef habitats (Santavy & Peters 1997, Green & Bruckner 2000). The reefs of the Northwestern Hawaiian Islands (NWHI) are considered to be relatively healthy but are not immune to the conditions that have led to the decline of other reef systems. An outbreak of white syndrome on *Acropora cytherea* was found at French Frigate Shoals (FFS) in 2003 (Aeby 2006) and an unusual disease presenting as skin de-pigmentation was discovered in reef fish (*Ctenochaetus strigosus*) at FFS in May 2005. It is important for management agencies to have an understanding of the vulnerability of these reefs to disease. The first step in managing disease is to develop an understanding of the causes of disease, followed by an assessment of its geographic extent. The objective of our study is to understand the epizootiology, or disease dynamics, of coral and fish disease within the NWHI.

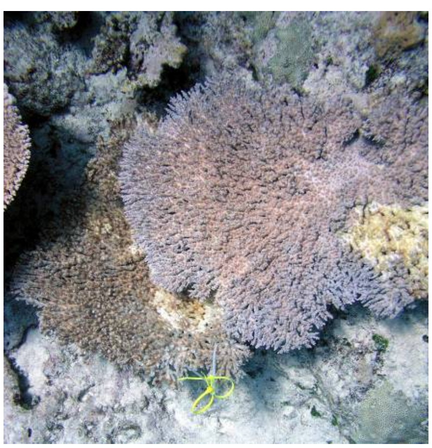


Figure 1. Marked colony with *Acropora* white syndrome at FFS in May 2005.

French Frigate Shoals (FFS) has the highest diversity and abundance of acroporid corals within the NWHI (Maragos et al. 2004). *Acropora* is affected by two diseases of concern (*Acropora* white syndrome and *Acropora* growth anomalies) at FFS (Aeby *in press*). Five permanent sites were set up in May 2005 to follow the temporal and spatial changes in diseases through time. All five permanent sites at FFS were re-surveyed in May 2006 and four other *Acropora*-rich sites were surveyed for disease either quantitatively (Rapture Reef, CRED site# 8) or qualitatively (La Perouse, CRED site# 28). Seven of the nine sites were found to have colonies infected with *Acropora* white syndrome (frequency of occurrence = 66.7%). Prevalence of the disease ranged from zero to 4.98%.

Fifty out of 54 marked colonies of *Acropora cytherea* were re-located and photographed. Analysis of the 41 marked colonies that had *Acropora* white syndrome (AWS) in 2005, revealed partial to total mortality in 97.6% of the colonies in 2006. Forty-six percent of the colonies suffered complete mortality with the remaining colonies exhibiting partial colony mortality that ranged from 10–90%. Twenty-seven new cases of AWS were found and tagged for follow-up surveys. Eight out of nine marked colonies with growth anomalies were also found and re-photographed. Five of the eight colonies (62.5%) had an increased number of growth anomalies that ranged from 2 to 19 new tumors per colony. 57.1% of the colonies had growth anomalies with signs of necrosis. Within the transects, seven new colonies with growth anomalies were found and tagged.

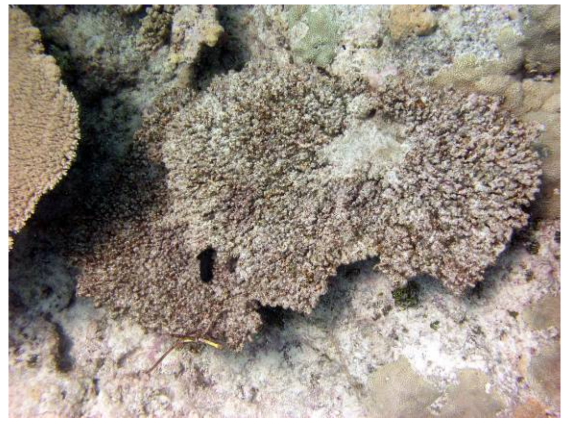


Figure 2. Same colony (Fig. 1) one year later (May 2006) showing complete mortality from disease.

Disease can affect coral communities directly through mortality of colonies (such as we observed with *Acropora* white syndrome) or indirectly through sub-lethal events such as reduced growth, resilience or reproduction. *Acropora* growth anomalies probably result in an energy drain on the colony that could affect reproductive output. *A. cytherea* is known to reproduce during May/June in the NWHI (Kenyon 1992), so we sampled eight tumored and non-tumored *A. cytherea* colonies. These samples will be examined histologically (studying cells and tissues at the microscopic level) and for reproductive status in the lab. Once a disease of concern has been identified, the next step is to understand the disease processes so that management or treatment of the disease can begin. Despite the successful treatment of black-band disease accomplished in the Florida Keys, few other studies have explored the possibility of treating infected corals. The first step in developing a treatment plan would be to determine the etiology, or the cause or origin, of the disease.

Recently, the etiology of *Acropora* white syndrome in the Marshall Islands was determined and found to be a pathogenic bacterial sp. (Sussman et al. *personal communication*). As a first step in elucidating the etiology of *Acropora* white syndrome at FFS we conducted basic studies on the ecology of the bacterial communities on infected versus uninfected corals. Bacteria from colonies with signs of *Acropora* white syndrome and uninfected controls were cultured and colony-forming units per cm^3 coral tissue were found to be much higher in the infected tissue (avg. >15,000) as compared to control regions of the colony (avg. 34.8) or healthy colonies (avg. 25.9). This suggests that the disease front of the infected region has an overgrowth of bacteria that could be due to either pathogenic and/or opportunistic bacteria. The low bacterial numbers on the control regions of the infected colony suggests that the increased bacterial response may be limited to the site of infection with no whole colony response.

<u>Johnston Atoll</u>**:** Johnston Atoll may serve as a stepping-stone for recruitment of organisms into the NWHI including potential pathogens. *Acropora* white syndrome (AWS) has been documented at Johnston Atoll (Aeby *unpublished data*) opening up the possibility that Johnston was the pathway from which the disease entered FFS. While we were unable to test this hypothesis through identification and comparison of the pathogens causing AWS at Johnston

versus FFS during the May-June 2006 cruise due to logistical issues, we were able to examine the condition of the reefs of Johnston that could reflect the potential for future damage from AWS at FFS. Quantitative and qualitative disease surveys were conducted at several sites at Johnston Atoll. *Acropora* white syndrome was found at 8 of 12 sites (frequency of occurrence = 66.7%). Prevalence of the disease ranged from zero to 19.2%. One site (JM 1ap: Donovan's Reef) was found to have high numbers of completely dead table corals with coral cover less than 5%. Coral cover at Donovan's Reef in 2001 and 2003 was visually estimated from photographs to be greater than 80%. This suggests that this area of the reef has experienced a dramatic decline in coral within the past three years.

Fish disease**:** Diseases in marine ecosystems are not limited to corals. A number of diseases of reef fish have now been found in the Main Hawaiian Islands (Work et al 2003; Work & Aeby *unpublished data*) and our objective was to determine whether those diseases also occur in fish in the NWHI. We have examined 336 butterflyfish for cutaneous tumors in the NWHI (May 2005: n = 155; May 2006: n = 181) and found no signs of disease. Butterflyfish with cutaneous tumors have been found in the MHI (Work & Aeby *unpublished. data*) but apparently this disease is not found or is extremely rare on the pristine reefs of the NWHI.

In May 2005, a particular reef fish, kole, was observed to have an obvious skin discoloration. External examination of some of these fish revealed poor body condition and fins with ragged edges. The most significant histological finding in fish with pigment anomalies was excessive growth of skin cells suggestive of pre-cancerous lesions. Other incidental lesions such as muscle parasites or necrosis of the kidney were occasionally found. An additional twenty-three kole were necropsied in May 2006 and will be examined histologically for signs of disease.

Ta'ape were introduced into Hawai'i in the 1950s (Randall 1987) and have spread all the way to Midway Atoll but have not yet reached Johnston Atoll. Ta'ape are closely associated with certain native goatfish (*Mulloidichthys* sp.) (Friedlander et al. 2002) and goatfish from the MHI have been found to be infected with some of the same diseases as ta'ape (Work et al. *unpublished data*). Given that ta'ape were introduced into Hawai'i, there is the concern that the recently documented diseases may also have been introduced and are spreading to native fish species. To address this question we sampled goatfish from the NWHI (n = 11) where ta'ape are present and Johnston Atoll (n = 26) where no ta'ape occur. Tissue samples are being processed and will be examined histologically for signs of disease.

Project:

- ✓ **Assessing the diversity of microbes associated with healthy and health-compromised corals in the Hawaiian Archipelago and Greater Pacific**
  (J. Salerno, E. Hambleton, A. Apprill, & M Rappé)

May 2006 Sample Collection: The main objectives on the May 2006 cruise were to: complete collections from islands that were not visited on previous cruises (Nihoa and Gardner Pinnacles); revisit sites in French Frigate Shoals that were sampled in September 2005 for seasonal sampling of coral-associated microbial communities; and to include Johnston Atoll reefs in our ongoing phylogeographic survey of coral-associated microbial communities in the Greater Pacific. Our sampling strategy focused on collecting from healthy *Porites lobata*, *Porites compressa*, *Pocillopora meandrina*, *Montipora capitata* and *Acropora cytherea* coral colonies, with diseased and/or bleached colonies being sampled when encountered at each atoll/island.

Table 1. Summary of coral and seawater samples collected from the Northwestern Hawaiian Islands in May of 2006.

DATE	ISLAND / ATOLL	SITE	Coral species / SW	TOTALS	UNITS
5/20/2006	Nihoa	North side	*Montipora capitata*	1	colonies
			Pocillopora meandrina	5	colonies
			Porites lobata	10	colonies
			seawater (DNA)	1	L
			seawater (chl a)	125	mL
			seawater (nutrients)	125	mL
5/22/2006	French Frigate Shoals	R16	*Acropora cytherea*	10	colonies
			Porites lobata	10	colonies
			seawater (DNA)	1	L
			seawater (chl a)	125	mL
			seawater (nutrients)	125	mL
5/22/2006	French Frigate Shoals	H6	*Porites lobata*	8	colonies
			Montipora capitata	12	colonies
			seawater (DNA)	1	L
			seawater (chl a)	125	mL
			seawater (nutrients)	125	mL
5/22/2006	French Frigate Shoals	30	*Pocillopora meandrina*	16	colonies
			Acropora cytherea	6	colonies
5/23/2006	French Frigate Shoals	R34	*Acropora cytherea*	2	colonies
			Montipora capitata	1	colonies
			Porites lobata	5	colonies
5/23/2006	French Frigate Shoals	R29	*Pocillopora meandrina*	5	colonies
			Porites lobata	3	colonies
			seawater (DNA)	1	L
			seawater (chl a)	125	mL
			seawater (nutrients)	125	mL
5/23/2006	French Frigate Shoals	37	*Pocillopora meandrina*	9	colonies
			seawater (DNA)	1	L
			seawater (chl a)	125	mL
			seawater (nutrients)	125	mL
5/24/2006	Gardner Pinnacles	7	*Porites lobata*	5	colonies
			Pocillopora meandrina	9	colonies
5/25/2006	French Frigate Shoals	30	*Porites lobata*	3	colonies
			Acropora cytherea	3	colonies
5/25/2006	French Frigate Shoals	48	*Pocillopora meandrina*	5	colonies
			seawater (DNA)	1	L
			seawater (chl a)	125	mL
			seawater (nutrients)	125	mL
5/25/2006	French Frigate Shoals	33	*Porites lobata*	5	colonies
5/27/2006	French Frigate Shoals	R29	*Montipora capitata*	6	colonies
			Porites compressa	5	colonies
5/28/2006	French Frigate Shoals	R11	*Acropora cytherea*	8	colonies
5/28/2006	French Frigate Shoals	48	*Porites compressa*	7	colonies
			seawater (DNA)	1	L
			seawater (chl a)	125	mL
			seawater (nutrients)	125	mL
5/29/2006	French Frigate Shoals	23	*Porites compressa*	7	colonies
			Porites lobata	4	colonies
			seawater (DNA)	1	L
			seawater (chl a)	125	mL
			seawater (nutrients)	125	mL
			total # of colonies sampled	**170**	

Seawater samples were taken adjacent to sampled coral heads for comparison of microbial community composition between corals and the surrounding seawater environment, and to assess other environmental variables including temperature, nutrients, and cholorphyll *a* (see Table 1 for a summary of samples collected). Additionally, three *Porites lobata* and three *Acropora cytherea* colonies at French Frigate Shoals site 30 were tagged, sampled, and photographed to allow for repeat seasonal sampling and to monitor recovery from genetic tissue sampling. Colonies will be resurveyed and tags will be removed in October of 2006. All seawater and coral-associated microbial samples were killed by freezing and stored in the -37°C freezer aboard the research vessel. Samples were then transported to the Hawai‘i Institute of Marine Biology in Kāne‘ohe Bay, O‘ahu for further processing and molecular analysis in the laboratory of Dr. Michael Rappé.

Small fragments of healthy *Acropora cytherea* are placed into a sterile plastic bag for later microbial analysis. All samples collected are photodocumented.

A healthy colony of *Porties compressa* at French Frigate Shoals.

<u>Method Development</u>: A variety of methods have been employed to investigate the diversity of bacterial communities associated with corals (Rohwer et al. 2001, 2002, Knowlton and Rohwer 2003, Kellogg 2004, Lesser et al. 2004, Klaus et al. 2005, Penn et al. 2006). These methods range in form from the initial steps of sample collection and processing, to the molecular techniques that are used to identify bacteria.

We conducted a methods comparison pilot study to determine the best method for extracting bacterial DNA from corals. Frozen coral samples from *Montipora capitata*, *Porites compressa*, and *Porites lobata* colonies (n = 3 per species) were thawed on ice and subjected to either a crushing or airbrushing treatment prior to DNA extraction. Five extraction methods were used to test for the highest yield of PCR-amplifiable bacterial DNA. The five extraction methods included: the DNeasy Tissue Kit (Qiagen Inc., Valencia, CA); the DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA); the UltraClean Soil DNA Kit (MoBio Laboratories Inc,. Carlsbad, CA); the PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA); and a CTAB chemical extraction.

Final DNA yield was assessed through gel electrophoresis. Bacterial DNA from each combination of coral host species, airbrushed/crushed treatment, and DNA extraction method was amplified via Polymerase Chain Reaction (PCR) using fluorescently-labeled universal and bacterial-specific primers. PCR products were cleaned using the QIAquick PCR Purification Kit (Qiagen) and restricted with *HAEIII* enzyme (Promega, Madison, WI). Restriction digests were cleaned using the QIAquick Nucleotide Removal Kit (Qiagen) and subjected to terminal restriction fragment length polymorphism (TRFLP) analysis. TRFLP is a community profiling technique used to assess the diversity and relative abundance of microbial species associated with each individual coral sample. The different sized fragments generated from the restriction digest correspond to natural species variations in base pair lengths of a portion of the 16s rDNA gene. Data are expressed in an electropheregram format where relative fluorescent units are a proxy for species abundance and base pair lengths are a proxy for genetically distinct bacterial ribotypes (see Figure 1).

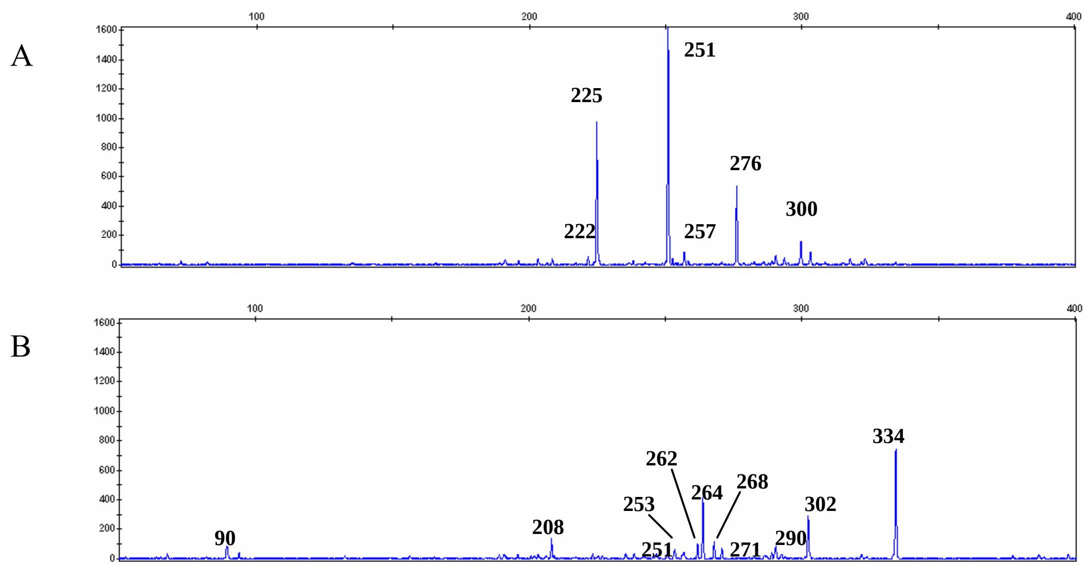


Figure 1. Terminal Restriction Fragment Length Polymorphism electropherograms. X-axis = base pair length (proxy for bacterial ribotype); y-axis = relative fluorescent units (proxy for relative abundance of a particular ribotype). A: Bacterial community profile from airbrushed, PowerSoil Kit-extracted *Porites compressa* collected from Kāne‘ohe Bay, O‘ahu. B: Bacterial community profile from airbrushed, PowerSoil Kit-extracted *Porites lobata* collected from Kāne‘ohe Bay, O‘ahu.

Preliminary results indicate that the combination of airbrushing and extracting with the PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA) result in the highest yield of PCR-amplifiable bacterial DNA and the highest quality TRFLP bacterial community profiles for both *Porites compressa* and *Porites lobata*. We were unable to obtain high quality TRFLP bacterial community profiles for *Montipora capitata* using the methods employed in this study. Further analyses are being carried out to determine the best method for extracting bacterial DNA from this coral species.

Initial TRFLP profile analyses suggest that healthy *Porites compressa* and *Porites lobata* harbor species-specific bacterial communities with little overlap between the two species (see Figure 1). TRFLP analysis of multiple samples taken from individual colonies of *Porites lobata* revealed that although bacterial species diversity is relatively conserved across a colony, species abundance may vary. Preliminary analyses of TRFLP profiles from *Porites compressa* and *Porites lobata* collected in Kāne‘ohe Bay and at French Frigate Shoals (NWHI) revealed the presence of a ribotype (251) that was only found in Kāne'ohe Bay samples. This bacterial ribotype will be identified via cloning and sequencing in order to gain an understanding of its possible ecological function. Overall, results from this study revealed that terminal restriction fragment length polymorphism (TRFLP) analyses can be replicated easily and are useful for comparing bacterial diversity between coral species.

Project:

- ✓ **The diversity of the coral endosymbiont *Symbiodinium* and their role in disease susceptibility (M. Stat & R. Gates)**

Dinoflagellates from the genus *Symbiodinium* form mutualistic associations with coral. This symbiosis allows for the growth and development of coral reefs, which provide ecologically important habitat for marine invertebrates and fish. The genus *Symbiodinium* contains a diverse number of genetic varieties which have been shown to affect the biology of the coral host, including growth rate and tolerance to elevated sea surface temperatures. The genus has been grouped into eight clades (A-H), which in turn contain multiple sub-clade types based on rDNA. The diversity of *Symbiodinium* harbored by corals in the Northwestern Hawaiian Islands (NWHI) and Johnston Atoll (JA) remains unexplored. This research aims to: 1) investigate whether the genotype of *Symbiodinium* affects the health status of coral; and 2) describe the diversity of *Symbiodinium* in corals in the NWHI and JA and in the surrounding reef waters.

<u>1: Investigating whether the genotype of *Symbiodinium* affects the health status of coral</u>.

We have chosen *Acropora cytherea* to investigate whether the *Symbiodinium* genotype affects the health status of the coral host. *A. cytherea* is common at French Frigate Shoals in the NWHI and at JA. We collected samples from colonies of *A. cytherea* that appeared healthy, bleached, and diseased (see Figure 1). Restriction fragment length polymorphism (RFLP) of small subunit

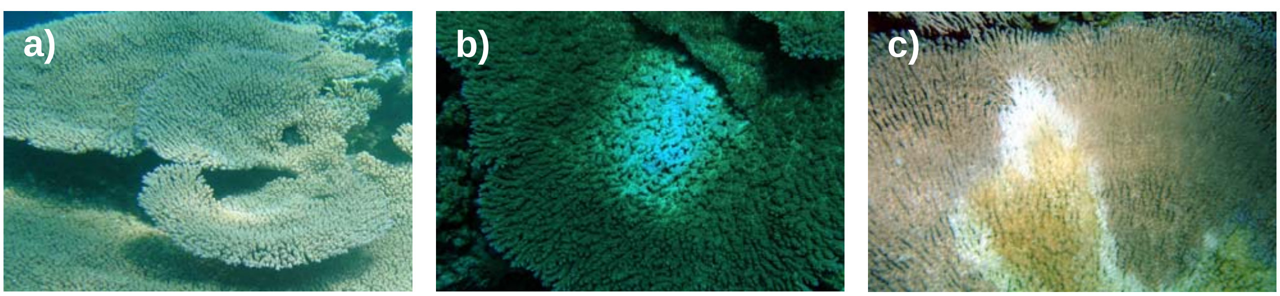


Figure 1. The genotype of *Symbiodinium* will be identified from colonies of *Acropora cytherea* that appear: a) healthy; b) bleached; and c) diseased (white syndrome).

(ssu) rDNA will be used to determine the clade of *Symbiodinium* present in coral hosts. To date, our results indicate that colonies of *A. cytherea* harboring clade A *Symbiodinium* are more susceptible to disease, mainly white syndrome (see Table 1). We are currently processing additional samples that will confirm whether the association between *A. cytherea* and clade A *Symbiodinium* renders a coral more susceptible to disease.

Table 1. *A. cytherea* colonies, health state, and the clade of *Symbiodinium* harbored.

Reef	**Location**	**Date collected**	**No. of colonies**	***Symbiodinium* clade**	**Host Health Status**
FFS	FFS R46	09/05	4	C	Healthy
	FFS R46	09/05	1	A	Healthy
	FFS 12	09/05	5	C	Healthy
	FFS 12	09/05	4	A	Diseased
	FFS R16	05/06	1	A	healthy
	FFS 30	05/06	1	A	healthy
JA	JOH 20	05/06	1	C	healthy

2: Exploring the diversity of *Symbiodinium* from corals in the NWHI and JA and the surrounding reef waters.

We will investigate the diversity of *Symbiodinium* in coral hosts from the NWHI and JA and the surrounding reef waters to determine the biogeographic distribution of symbiont types and potential genetic varieties that render a coral susceptible to disease. RFLP of *Symbiodinium* ssu rDNA from coral hosts show that corals predominantly harbor clade C *Symbiodinium* (Table 2). The *Symbiodinium* sub-clade will be identified by sequence analysis of more variable gene markers. We have sequenced the chloroplast 23S rDNA from *Symbiodinium* in *Pocillopora meandrina* collected at Necker and Kure. The results indicate that a single sub-clade chl23S rDNA genotype is dominant in these coral hosts. We will compare these results to sub-clade genotypes characterized by the Internal Transcribed Spacer region (ITS2) to determine whether: 1) the *Symbiodinium* population in *Pocillopora meandrina* is very low; or 2) the chl23S rDNA gene marker does not resolve *Symbiodinium* into distinct genotypes and has lower resolving power compared to the ITS2 gene marker. The results from this analysis will determine which gene marker we will use to investigate the diversity of *Symbiodinium* in all coral hosts and the water environment.

Table 2: Coral hosts and the *Symbiodinium* clade harbored

Species	**Location, number of host colonies sampled, and clade of *Symbiodinium* harbored**						
	Necker	FFS	Maro	P&H	Mid	Kure	JA
Pocillopora meandrina	4: C					7: C	
Porites compressa				5: C		4: C	
Porites lobata	4: C	9: C	6: C	24: C	12: C	6: C	
Acropora cytherea		10: C 7: A					1: C
Montipora capitata			1: C		3: C		

FFS: French Frigate Shoals, P&H: Pearl & Hermes, Mid: Midway, JA: Johnston Atoll.

Invasive Marine Species Studies

Project:

- ✓ **Molecular tools for invasive species in Hawai‘i**
  (R. Toonen, G. Concepcion, S. Kahng, & M. Crepeau)

Together with funding from Hawai‘i Sea Grant, Hawai‘i Coral Reef Initiative and the Hawai‘i Invasive Species Council, we have recently developed novel molecular tools that provide the greatest phylogenetic resolution of any genetic markers developed to date. We hope that these markers will be able to identify the source of invasive species introductions into the Hawaiian Archipelago. Evaluating the relative risk of introductions requires detailed surveys of the presence and abundance of invasive species on ships hulls, ballast water, and derelict fishing gear (see report by Jokiel et al. below). Although the presence or absence of fouling on any individual ship hull or competent larvae in ballast water tells us that such transport is possible, those data do not provide us with the actual source of any introduction, and in some cases the species identity of such propagules is controversial. Molecular data can provide us with a tool by which to examine the history of species movement, and we are currently developing the tools to go beyond what **might** have happened to what **most likely** happened.

We focused on the invasive octocoral *Carijoa riseii* in Hawai‘i, and used this funding to leverage additional support to develop the necessary tools to examine other species throughout the Hawaiian Archipelago. As the appropriate tools become available, future efforts will expand to include other invasive species. To date, our accomplishments include:

- Developing and testing two new mitochondrial DNA (mtDNA) markers which can provide reliable sequencing results to support our phylogeographic analysis (Concepcion et al. 2006). This is a significant achievement because mtDNA markers commonly used in other organisms have repeatedly proven useless in understanding patterns of colonization by corals (reviewed by Shearer et al. 2002).

- Securing *Carijoa* samples from over 20 locations in the Pacific and Atlantic (see Figure 1) in collaboration with the Bishop Museum; Museum and Art Gallery of the Northern Territory, Australia; Florida Museum of Natural History; Coral Reef Research Foundation, Palau; University of the Virgin Islands; US Geological Survey; University of Puerto Rico; Universidade de Madeira; and Tel Aviv University. We have also sampled *Carijoa* from each of the Main Hawaiian Islands where it is known to occur. We have sequenced over 250 individuals for our mtDNA markers and are currently working to complete sequencing of the nDNA markers as well. Our data thus far clearly indicates a need to revisit the taxonomy of *Carijoa* world-wide, because some samples sent to us and identified by taxonomic experts as *Carijoa riseii* are clearly not the same species. They are more closely related to corals in a completely different **family** of octocorals than they are to other species of *Carijoa* (see Figure 2).

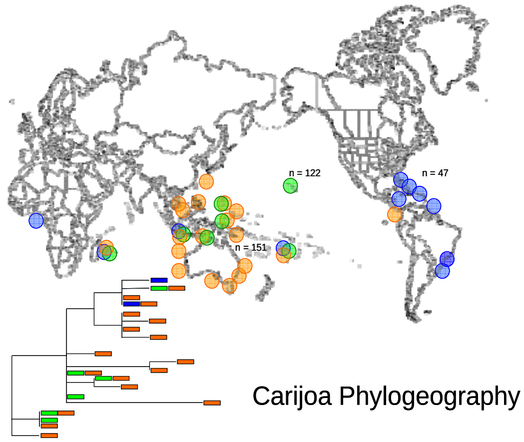


Figure 1. Global sampling of *Carijoa*. To date, 320 samples are included from throughout the Atlantic, Caribbean, Indo-Pacific, and Western Pacific. Samples from throughout the Atlantic and Caribbean show little genetic variation, and none of those genetic variants are found among samples from throughout Hawai'i. In contrast to previously published reports, *Carijoa* in Hawai'i are not from the Caribbean, and instead appear to be derived from multiple Pacific sources.

• Developing a novel nuclear DNA (nDNA) marker: Signal Recognition Protein 54 intron (Concepcion et al. in prep). Despite considerable effort, no lab in the world has successfully isolated nuclear intron markers for phylogenetic studies in corals, and this is the first such marker to be used in a coral study. Among *Carijoa* samples sequenced to date, these genetic markers are roughly ten times more variable than previously available markers, have the potential to discriminate species identity, and will hopefully serve to identify the source from which *Carijoa*

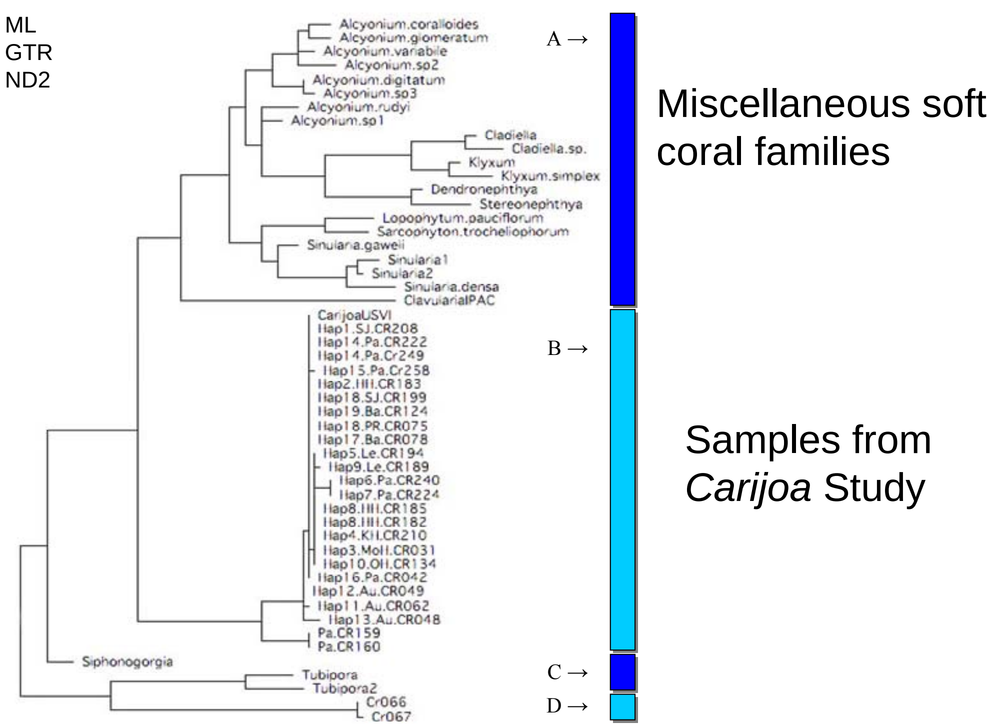


Figure 2. Phylogenetic tree of *Carijoa* samples. Some individuals (D) identified by taxonomic experts as *Carijoa* are more closely-related to our outgroup, *Tubipora musica* (C), than they are to the other samples of *Carijoa* (B) or to various representative species from other octocoral families (A).

first colonized Hawai'i. Our results thus far have led to interest in collaboration from the Cnidarian Tree of Life consortium. Offers of collaboration are coming in from researchers around the world based on our recent successes. One such example is a collaborative proposal currently in development with Yehuda Benayahu (Tel Aviv University) & Cathy McFadden (Harvey Mudd) for submission to the U.S.-Israeli Binational Science Foundation.

Our study has already uncovered some very surprising results with regard to the dispersal history and biogeography of *Carijoa* in Hawai'i:

A. *Carijoa* appears to be native to both the Atlantic and the Western Pacific, each with high and unique genetic diversity (i.e., the Indo-Pacific population of *Carijoa* is not a modern introduction from the Caribbean or Atlantic, and vice-versa).
B. The coral identified as *Carijoa riisei* in Hawai'i does not appear to be all the same species. Preliminary data suggests that there are multiple species of *Carijoa* in Hawai'i which are currently unidentified because of poor taxonomic resolution in the group worldwide. We are beginning to sequence our novel nDNA markers from all samples to resolve the taxonomic status of the group.
C. Several specimens identified as *Carijoa riisei* by taxonomic experts are not closely related to any other *Carijoa* samples in our database. The taxonomy of *Carijoa* clearly requires a major revision based on our research findings.
D. In contrast to published reports, *Carijoa* in Hawai'i almost certainly originated from a Pacific source, not the Caribbean or Atlantic as previously reported.
E. There appear to be multiple introductions of *Carijoa* into Hawai'i; sources of these introductions were likely locations throughout the Indo-Pacific, although based on the current molecular data we cannot exclude the possibility that *Carijoa* invaded the Hawaiian Archipelago prior to human colonization.

Project:

- ✓ **Reducing potential impact of invasive marine species in the NWHI MNM**
  (P. Jokiel, R. Toonen, & S. Godwin)

This project was completed within the planned time frame of Year 1. The fourth quarter involved completion of field effort, processing of samples and archiving of material in the Bishop Museum, and the completion of the final document covering what is known about introduced marine species in the NWHI.

The final document provides recommendations on future courses of action to prevent introduction of other species:

> Scott Godwin, Ku'ulei S. Rodgers and Paul L. Jokiel. 2005. Reducing Potential Impact of Invasive Marine Species in the NWHI Marine National Monument. 66 pp.

Examples of the information summarized in the final document:

Marine introduced species in the NWHI.
NIH = Nihoa, NEC = Necker Island, FFS = French Frigate Shoals, MAR = Maro Reef, PHR = Pearl and Hermes Reef, LAY = Laysan Island, LIS = Lisianski Island, MID = Midway Atoll, KUR = Kure Atoll

Species	Native Range	Present Status in NWHI	Mechanism of Introduction
Hypnea musciformes (algae)	Unknown; Cosmopolitan	Unknown; in drift and on lobster traps (MAR and NEC)	Intentional introduction to MHI (documented)
Diadumene lineata (anemone)	Asia	Unknown; on derelict net only (PHR)	Derelict fishing net debris (documented)
Pennaria disticha (hydroid)	Unknown; Cosmopolitan	Established (FFS, PHR, LAY, LIS, KUR and MID)	Fouling on ship hulls (hypothesized)
Amathia distans (bryozoan)	Unknown; Cosmopolitan	Established (MID)	Fouling on ship hulls (hypothesized)
Schizoporella errata (bryozoan)	Unknown; Cosmopolitan	Established (MID)	Fouling on ship hulls (hypothesized)
Balanus reticulates (barnacle)	Atlantic	Established (FFS)	Fouling on ship hulls (hypothesized)
Balanus venustus (barnacle)	Atlantic and Caribbean	Not Established; on vessel hull only (MID)	Fouling on ship hulls (documented)
Chthamalus proteus (barnacle)	Caribbean	Established (MID)	Fouling on ship hulls (hypothesized)
Lutjanus fulvus (fish)	Indo-Pacific	Established (NIH, FFS)	Intentional introduction to MHI (documented)
Lutjanus kasmira (fish)	Indo-Pacific	Established (NIH, NEC, FFS, MAR, LAY, and MID)	Intentional introduction to MHI (documented)
Cephalopholis argus (fish)	Indo-Pacific	Established (NIH, NEC and FFS)	Intentional introduction to MHI (documented)

Marine introduced species in the NWHI.
NIH = Nihoa, NEC = Necker Island, FFS = French Frigate Shoals, MAR = Maro Reef, PHR = Pearl and Hermes Reef, LAY = Laysan Island, LIS = Lisianski Island, MID = Midway Atoll, KUR = Kure Atoll

Species	Native Range	Present Status in NWHI	Mechanism of Introduction
Hypnea musciformes (algae)	Unknown; Cosmopolitan	Unknown; in drift and on lobster traps (MAR and NEC)	Intentional introduction to MHI (documented)
Diadumene lineata (anemone)	Asia	Unknown; on derelict net only (PHR)	Derelict fishing net debris (documented)
Pennaria disticha (hydroid)	Unknown; Cosmopolitan	Established (FFS, PHR, LAY, LIS, KUR and MID)	Fouling on ship hulls (hypothesized)
Amathia distans (bryozoan)	Unknown; Cosmopolitan	Established (MID)	Fouling on ship hulls (hypothesized)
Schizoporella errata (bryozoan)	Unknown; Cosmopolitan	Established (MID)	Fouling on ship hulls (hypothesized)
Balanus reticulates (barnacle)	Atlantic	Established (FFS)	Fouling on ship hulls (hypothesized)
Balanus venustus (barnacle)	Atlantic and Caribbean	Not Established; on vessel hull only (MID)	Fouling on ship hulls (documented)
Chthamalus proteus (barnacle)	Caribbean	Established (MID)	Fouling on ship hulls (hypothesized)
Lutjanus fulvus (fish)	Indo-Pacific	Established (NIH, FFS)	Intentional introduction to MHI (documented)
Lutjanus kasmira (fish)	Indo-Pacific	Established (NIH, NEC, FFS, MAR, LAY, and MID)	Intentional introduction to MHI (documented)
Cephalopholis argus (fish)	Indo-Pacific	Established (NIH, NEC and FFS)	Intentional introduction to MHI (documented)

Major points covered in the final document are:

1. Populations of alien marine species that have already colonized areas of the MHI represent the most likely source of invasive species in the NWHI based on the proximity and pattern of ship movements associated with the MHI.
2. The majority of alien marine species that are currently known from the Hawaiian Archipelago are found in harbor and bay habitats and have not colonized high energy fore reef habitats.
3. The few alien species known from the NWHI are restricted to the anthropogenic habitats at Midway Atoll and French Frigate Shoals. Only one, the hydroid *Pennaria disticha* has spread extensively in the NWHI but neither this or other species has exhibited invasive characteristics at this time.
4. Marine debris has been shown to have the ability to transport nonindigenous species to the NWHI. Modes of transport such as derelict fish nets are a challenge to manage but the impact of other anthropogenic debris, such as Fish Attraction Devices (FAD) deployed by the State of Hawai'i, can be minimized.

Figure 1. The barnacle *Chthamalus proteus*. is an example of an introduced invertebrate. It was well established on O'ahu, Maui, and Kaua'i by the time it was noticed in 1995 and was recorded in the NWHI in the harbor at Midway Atoll in 1998. This barnacle is native to the Caribbean, Gulf of Mexico, and the Western Atlantic, and is a common fouling organism on ship hulls. A potential threat of this species is alteration of natural substrates through dense colonization. This would alter settlement patterns of native species and exclude algal grazers such as opihi. *Photo: C. Zabin.*

Figure 2. Blue-Lined Snapper (also known as ta'ape or *Lutjanus kasmira*) was introduced in the Main Hawaiian Islands during the 1960s but has spread to all islands in the Hawaiian Archipelago including the NWHI. *Photo: K. Stender*

Recommendations covered in the final document:

1. It will be important to establish formal administrative rules and codes of conduct to minimize exposure from the variety of potential transport mechanisms for nonindigenous transport to the NWHI.
2. Continue activities pertaining to species richness and diversity as part of establishing baseline information, and pursue research pertaining to biogeography focused on connectivity and larval transport.
3. Include the issue of marine nonindigenous species in education and outreach activities
4. Integrate the concepts of marine nonindigenous species and invasive behavior into the mindset of monitoring and assessment activities occurring in the NWHI.

Northwestern Hawaiian Islands Mapping

Project:

- ✓ **Mapping Support (P. Jokiel)**

This quarter was devoted to integrating data sets from many sources onto our primary platform which is a GIS Work Station (64 bit, Xenon Dual Processor with 8 Gig RAM, 1.2 Terabytes of storage) suited to handle the demands of the mapping work. Available data are being integrated into GIS layers needed for any future ecosystem work. A major portion of the work this quarter was directed at pulling together all available benthic data from various sources so that we can provide synthesized summary data relevant to the management of the NWHI Marine National Monument. For example, Figure 1 presents a chart of area at each 1 meter (m) depth increment at depths shallower than 30 meters. This type of chart reveals that the total shallow area is dominated by habitats at 2m–5m in depth. Such data will be used to define many ecological characteristics of the NWHI marine ecosystem.

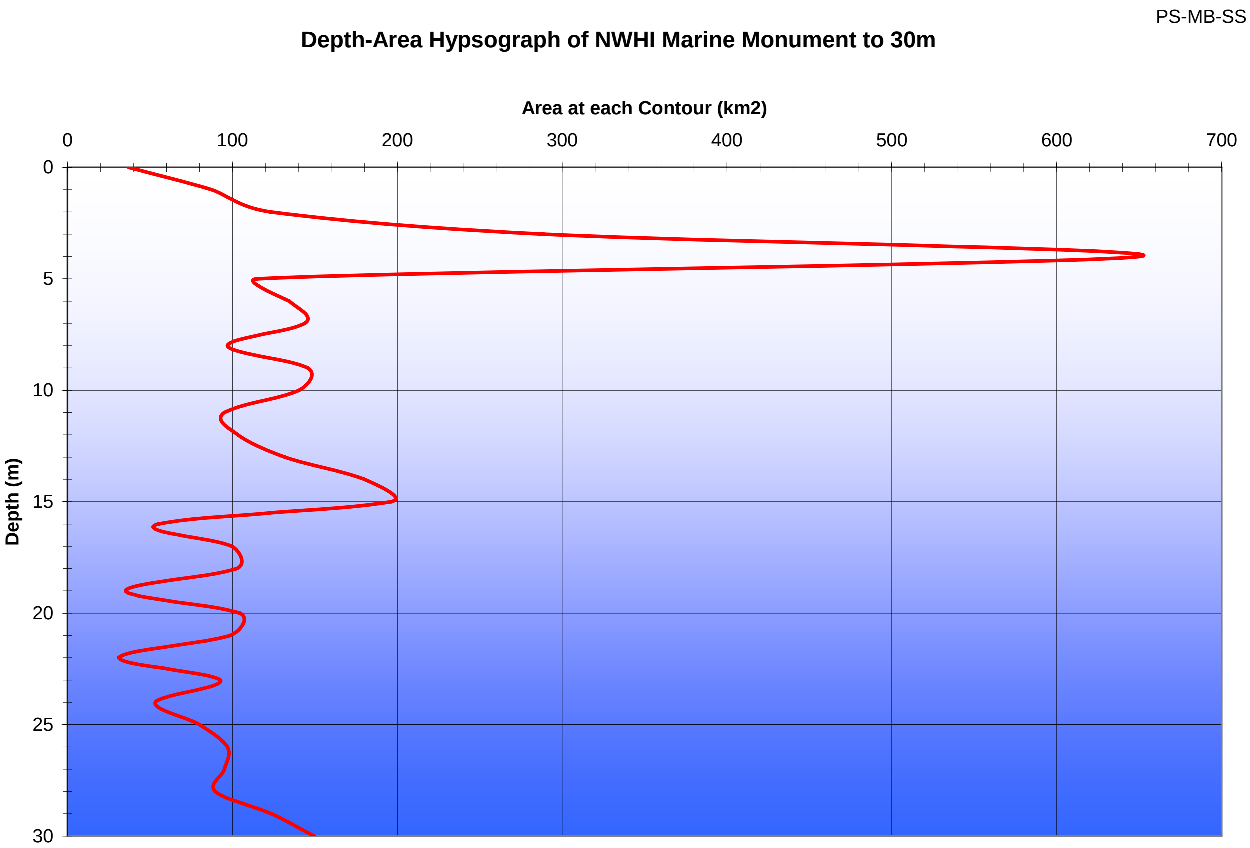


Figure 1. Hypsometric curve showing amount of real estate at each 1 m increment of depth from surface to 30 m.

Other activities initiated during the fourth quarter include further compilation of all GIS watershed, boundary, population, bathymetric and other data into the system. An analysis of NMFS tow-board data is being undertaken by Ben Richards in collaboration with the CRED and Dr. Jerry Ault. A major socioeconomic investigation of the “existence” value of the NWHI Marine National Monument is being initiated in collaboration with Pieter van Beukering

(Institute for Environmental Studies, Vrije University, Amsterdam) who is a leading expert on coral reef valuation, and Dr. Wolfgang Haider (Simon Frazer University, Canada) who is a leader in choice analysis for establishing coral reef value. We will also combine information in the habitat maps with information based on climate change scenarios to identify possible changes in ecosystem health and value.

Ecosystems Management Studies

Project:

- ✓ **Evaluating ecosystem 'health and value' (P. Jokiel & K. Rodgers)**

During the fourth quarter we continued to gather and compile existing data, develop methods comparisons, and synthesize available information. Our ranking scheme comparing the health and value of islands in the Northwestern Hawaiian Islands (NWHI) with islands in the Main Hawaiian Islands (MHI) was accepted for publication in the journal Pacific Conservation Biology with very favorable reviews:

> Jokiel, P. L. and K. S. Rodgers. (in press). Ranking coral ecosystem "health" and "value" for the islands of the Hawaiian Archipelago. Pacific Conservation Biology.

This paper forms an important basis for an expanded future ecosystem evaluation in the NWHI which will be expanded as we increase the data base. This report describes the development and evaluation of what is essentially an index of biological integrity (IBI). Sufficient data on five vitally important biological indicators were developed for both the NWHI and the MHI. These include: reef fish biomass; reef fish endemicity; total living coral cover; population of the endangered Hawaiian monk seal (*Monachus schauinslandi*); and number of female green sea turtles (*Chelonia mydas*) nesting annually on each island. The index of relative biological "health" and "value" of the coral reefs of the NWHI was developed in the context of the entire Hawaiian Archipelago. The resulting index showed that the "worst" of the NWHI is better than the "best" of the MHI. Only five metrics were used, but a sensitivity analysis revealed that all five metrics are equally significant in defining relative health and value. The same pattern emerges when we use any four of the five metrics (see Figure 1). Thus all five biological metrics show essentially the same pattern, suggesting that numerous biological characteristics of ecosystems are impacted in a similar manner under anthropogenic stress.

The composite scoring developed using these data shows that the ecological status of the MHI is poor compared to the NWHI. A growing body of information demonstrates that the reefs of the NWHI are an integral component of the Hawaiian Archipelago ecosystem and are an extremely valuable ecological resource. Thus, the proper management of the NWHI is important to the ecological vitality of the Hawaiian Archipelago as a whole. The NWHI should not be viewed as a separate entity from the MHI because the two areas are clearly interdependent. The migration of turtles from feeding grounds in the MHI to nesting grounds in the NWHI provides an excellent example of the interdependence of the two areas. Movement of large fish and endangered Hawaiian monk seals provide other examples. The fact that the same species of fish, corals and other marine organisms occur along the entire Archipelago with high rates of endemism provides evidence that the NWHI and the MHI represent a single ecosystem with a long evolutionary history.

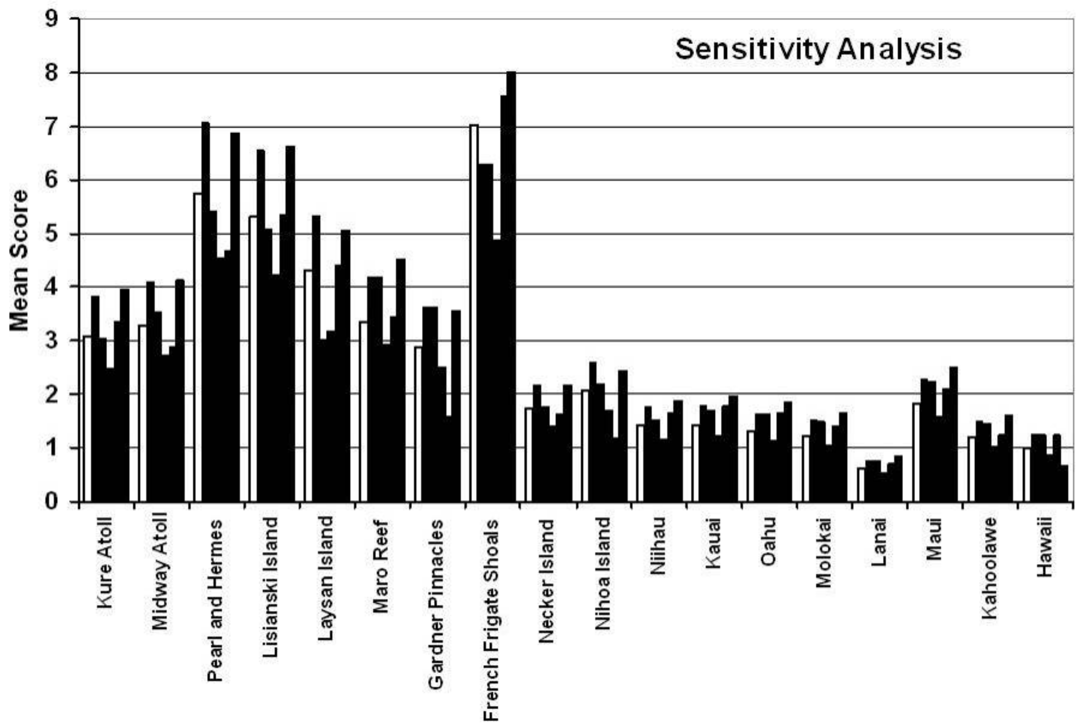


Figure 1. Results of sensitivity analysis with index of biological integrity (i.e. "health" or "value") ranking recalculated using only 4 of the 5 metrics. From left to right in the figure: the first bar (open) is the index calculated with all 5 metrics for use as a reference; the second bar was calculated with no turtle data; the third bar with no seal data; the fourth bar with no fish biomass data; the fifth bar with no fish endemism data. The same pattern emerges even if we eliminate one of the metrics.

Project:

- ✓ **Ecosystem management of the NWHI Marine National Monument (R. Toonen, K. Selkoe & B. Halpern)**

During the last quarter, we have focused primarily on our threat analysis project. As with any natural resource management situation, the degree and nature of human impacts on the NWHI ecosystem must be determined so that the worst of these can be identified and, if possible, regulated or mitigated. The need to understand the effects of human activities on the NWHI ecosystem is particularly critical at this time as management plans are currently being drafted for the NWHI MNM. While exhaustive lists of anthropogenic threats to the NWHI have been generated in the past (e.g., Maragos and Gulko 2002, Friedlander et al. 2005), understanding how and why those anthropogenic activities threaten the ecosystem has not been systematically addressed. The goals of this project are: (1) to understand which anthropogenic threats have had the greatest and least impact on the NWHI ecosystem; (2) to systematically compare the way in which each threat impacts the NWHI with five metrics; (3) to understand the different vulnerabilities of select habitats in the NWHI (e.g., lagoon, intertidal or deep banks) to each threat; and (4) identify which threats and habitats are poorly understood and require further study.

Our approach is based on surveying expert opinion and was developed in a previous study (Halpern et al. 2006) that sought to assess all anthropogenic threats to marine ecosystems on a global scale. Twenty-five academic, state, federal and NGO scientists with a mean of 391 days spent working in the NWHI completed the survey between February and May 2006. We have completed multiple analyses of the data and have written up two manuscripts for publication: a shorter one focused on the management and policy implications of our results; and a longer one focused on presenting the details of our methodology and analyses which is geared toward local natural resource managers and those in other areas of the world that may want to adopt our approach for their region. We will submit both manuscripts within the next few weeks.

Our initial findings on the vulnerability of the NWHI to different anthropogenic threats are presented in Figure 1. Effects of global climate change, such as sea level rise and sea temperature rise, were ranked to have the most profound and widespread impacts on the ecosystems of the NWHI. Marine debris and associated ghost fishing – threats caused by a handful of Pacific-wide human activities – rank a close second.

The greatest locally-generated threats were identified to be alien species invasions and ship groundings, both of which result from ship-based human visitation to the NWHI. Coastal engineering and runoff of pollutants, land-based threats generated by past military use of certain areas of the NWHI, were also identified as significant threats. In contrast, fishing, diving, and research scored relatively low presumably because they occur on much smaller scales and impact only few members of the marine communities with fast recovery times. These results have important implications for the management and funding priorities of the new Marine National Monument.

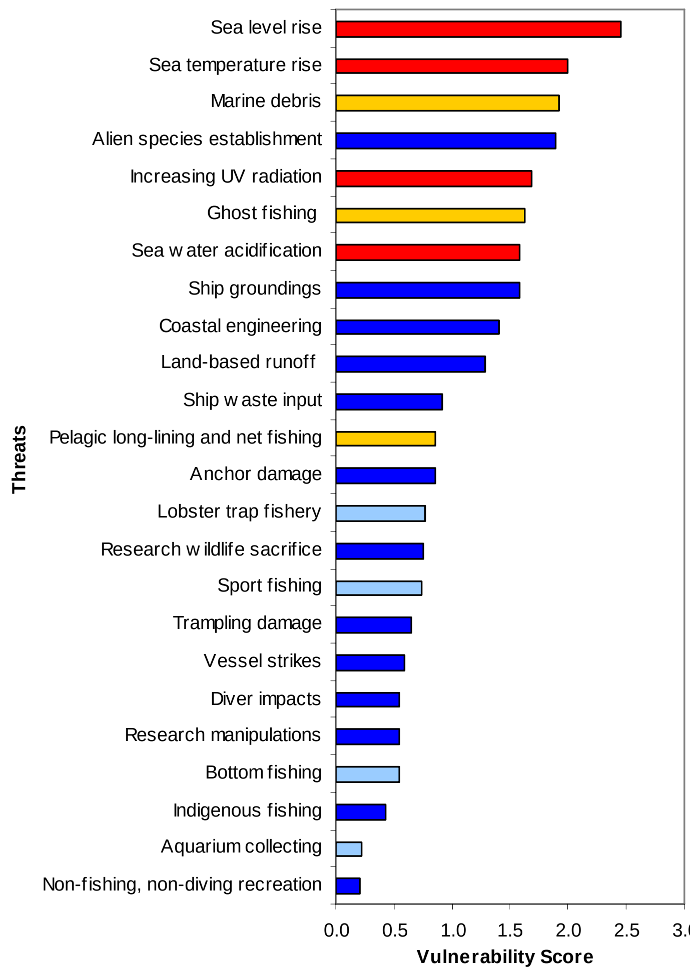


Figure 1. Relative ranking of threats by the 5-factor vulnerability score (possible values range 0-4), based on pre-Monument conditions in the NWHI. Threats generated on a local scale (within the NWHI) are coded blue; light blue identifies threats that have been eliminated by the new Monument status; red = threats with a global scale source; gold = Pacific-wide.

The lagoon/back/patch reef ecozone was identified as the most vulnerable environment, followed by the reef crest and slope, while the pelagic zone appears to be the least vulnerable to the present suite of threats. By asking respondents to indicate the certainty, or depth of knowledge, with which they estimated the vulnerability of ecozones to threats, we identified where knowledge gaps exist. Scientists had highest uncertainty in the way that climate change factors (increasing ultraviolet radiation, seawater acidification, rising sea level and sea temperature) affect the ecosystem. The effects of ship-based waste and land-based runoff are also poorly understood, and algal beds are the most poorly studied of the ecozones.

Our next goal is to build a map of all anthropogenic impacts to the NWHI. This map will provide a strong visual impact of how threats are distributed across the area, and where the most and least impacted areas are. It will also be a useful tool for managers in setting use and management priorities for different areas, and provide a data repository that allows these datasets to be easily accessed and utilized. We have begun to identify and track down appropriate datasets for 18 principle threats from researchers around the world. We will work closely with Erik Franklin, HIMB GIS Specialist, to create an interactive, web-based map using Arc GIS software tools.

Figure 2. Bleaching will increase as sea temperature increases due to global climate change in the coming years. Photo shows *Pocillopora meandrina* (bleached), with initial overgrowth by turf algae in the central patch reef of Kure Atoll, Sept. 2002. *Photo: J. Kenyon, reproduced from Friedlander et al. 2005*.

In terms of our other ecosystem management project, modeling patterns of connectivity within and among the main islands and NWHI, we have primarily focused on gathering parameter values for species, some of which have only recently become available with recent publications. We have focused on lobster and coral life history, reproduction and demographic information. We have also continued refining our design of the population array based on updated habitat maps. Now that the threat analysis is nearly complete, we will spend more time doing preliminary model runs in the next few months.

Geospatial Technologies and Data Analysis (E. Franklin)

Erik Franklin was hired as the GIS/Data Analyst in November 2005, near the end of the second quarter of the HIMB-NWHICRER Research Partnership. During the second and third reporting periods, a number of accomplishments regarding geospatial technologies and data analysis have been achieved.

<u>NOAA IDEA Center Award of a Pacific Region Integrated Data Enterprise Grant</u>

In April 2006, the HIMB-NWHI Coral Reef Research Partnership was awarded a $75,000 grant for the "Integration of Biological Data and Visualization Tools for the Northwestern Hawaiian Islands" through the Pacific Region Integrated Data Enterprise (PRIDE) program sponsored by NOAA's Integrated Data and Environmental Applications (IDEA) Center. The award will facilitate the management and dissemination of data from the HIMB-NWHI Coral Reef Research

Partnership through the development and maintenance of an online searchable website and geospatial database, and will serve as a prototype for integrating biological data into an information management system for the NWHI MNM.

HIMB Research in the Northwestern Hawaiian Islands ArcIMS Website

To communicate and share information about the scientific activities of the Coral Reef Research Partnership, the development of an online, interactive mapping ArcIMS website (see Figure 1) and associated spatial database has been initiated. HIMB has partnered with the Hawai‘i Biodiversity Mapping Program (HBMP) who will host the database and web services on an NSF Experimental Program to Stimulate Competitive Research (EPsCOR) server with available digital storage. The database will also be searchable through the Census of Marine Life’s (CoML) Ocean Biogeographic Information System (OBIS) web portal.

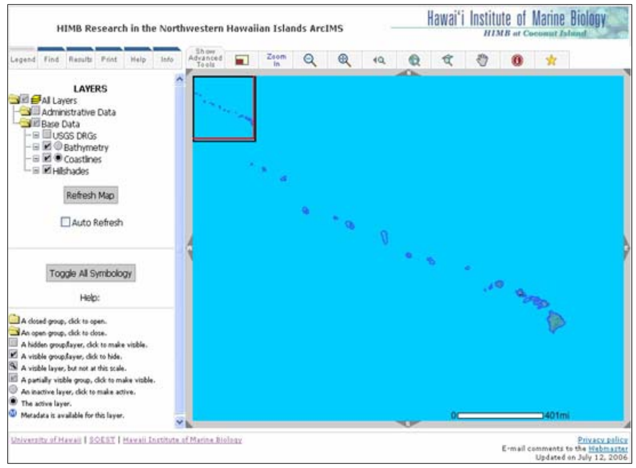


Figure 1. Screen shot of the interactive mapping ArcIMS website currently under development.

Northwestern Hawaiian Islands Coral Reef Ecosystem Reserve Planning Support

HIMB provided analytical and GIS support to the preparation of the Northwestern Hawaiian Islands Coral Reef Ecosystem Reserve (NWHI CRER) Draft Environmental Impact Statement and NWHI CRER Draft Management Plan. At the time, the NWHI CRER lacked the staff expertise to create, manage, and analyze GIS layers relevant to the EIS and Plan development and requested that HIMB supply those services.

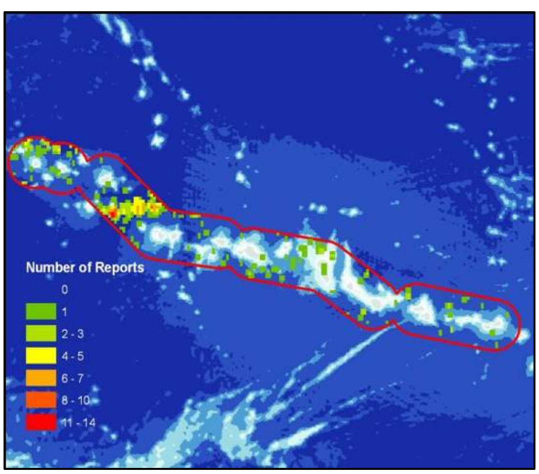
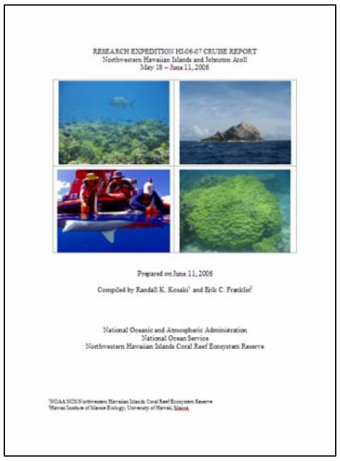


Figure 2. The cluster of red and orange areas denote a potential hot spot of vessel activity between Pearl and Hermes Atoll and Lisianski

NWHI Vessel Threat Assessment

A preliminary assessment of vessel traffic patterns was performed to determine baseline ship activity in the Northwestern Hawaiian Islands. Vessel location data was provided by the NOAA ICOADS program and the U.S. Coast Guard provided descriptive information about the size, tonnage, and country of the vessels. From data collected between March 1, 2004 and November 22, 2005, a potential hot spot of vessel activity was identified between Pearl and Hermes Atoll and Lisianski Island (see Figure 2). Analysis of vessel traffic patterns will continue in the next reporting period to meet the information needs of the new NWHI Marine National Monument. The results of this study will also be incorporated into the regional threat assessment in the "Ecosystem management of the NWHI reserve project" (R. Toonen, K. Selkoe & B. Halpern).

Northwestern Hawaiian Islands and Johnston Atoll Cruise Report


RESEARCH EXPEDITION HI-06-07 CRUISE REPORT
Northwestern Hawaiian Islands and Johnston Atoll
May 18 – June 11, 2006

Prepared on June 11, 2006

Compiled by Randall K. Kosaki[1] and Erik C. Franklin[2]

National Oceanic and Atmospheric Administration
National Ocean Service
Northwestern Hawaiian Islands Coral Reef Ecosystem Reserve

[1]NOAA NOS Northwestern Hawaiian Islands Coral Reef Ecosystem Reserve
[2]Hawaii Institute of Marine Biology, University of Hawaii, Manoa

A 35-page cruise report that included a cruise summary, daily activity log, and research project descriptions was prepared by Randy Kosaki (NWHI MNM Chief Scientist) and Erik Franklin (HIMB) during the cruise and made available upon return to Honolulu on June 11, 2006. This rapid preparation of operational and research activities is essential to communicate preliminary scientific accomplishments to marine resource managers, permitting agencies, collaborators, and the general public. The document provides a singular reference for anyone interested in reviewing events from the cruise. For example, the 20 scientists onboard performed over 500 SCUBA dives which added up to over 18 days underwater!

Outreach and Education (M. Rivera)

The Outreach and Education component of the HIMB-NWHI Coral Reef Research Partnership continues to develop and expand the already existing outreach and education programs at HIMB, and successfully initiated a number of new projects during the fourth quarter:

HIMB Community Education Program (CEP):
Information about the HIMB-NWHI research program is disseminated through the ongoing HIMB CEP. A cadre of volunteer docents host up to 6,000 visitors annually, introducing students, community groups, families and others to the wide array of marine research programs conducted at HIMB. The Outreach Coordinator for the NWHI program sponsors a large proportion of these visitors, each of which receives a brief oral introduction to NWHI research either through a presentation or walking tour, the tri-fold informational brochure (see quarterly report II-III), and views the NWHI video *Islands in the Sea—the Northwestern Hawaiian Islands* (available at www.hawaiireef.noaa.gov). The following groups, totaling over 600 individuals, have been hosted directly by the Outreach Coordinator during this reporting period:

- Kaimuki High School
- Waipahu High School
- Kahuku High School (Career Day)
- University of Hawai‘i Lab School
- Myron B. Thompson Academy
- West Hawai‘i Explorations Academy
- UH Scientific Divers Class
- QuickScience High School Students (California)
- Sea Perch Teachers Workshop (UH and MIT Sea Grant)
- The Nature Conservancy Legacy Club
- Undergraduate Mentoring in Evolutionary Biology Pacific Instructors
- UH Windward Community College Seniors Continuing Education Program
- Hawai‘i Aquaculture Association

- Conservation International Seascape Program
- UH Water Resources Research Center
- Honolulu Board of Water Supply Water Resources

Agency and government visitors:
Briefing tours and events for agency and government at the state, federal, and international levels is a regular part of the NWHI outreach efforts at HIMB. In the last six months, HIMB has hosted visitors and held briefings and/or tours to disseminate information on the NWHI research project to the following:

Figure 1. Visit from the U.S. House of Representatives Science Committee to HIMB in January 2006.

- NOAA Leadership
- NOAA Coastal Services Center
- NOAA Pacific Services Center
- NOS Leadership
- NMSP Leadership
- NOAA Maritime Archeology
- Senator Daniel K. Inouye's Office
- U.S. House Science Committee
- Office of Senator Clayton Hee
- Hawai'i State Department of Land and Natural Resources
- Hawai'i State Department of Health, Water Quality Division
- NOAA International Program Office – U.S.-China Science & Technology Agreement

Invited presentations and events:
The Outreach Coordinator continues to provide seminars that detail HIMB's research in the NWHI to various Rotary Club divisions in Honolulu. The Outreach Program also participated with numerous partners, led by the Division of Aquatic Resources, to develop an informational exhibit at the first annual Hawai'i Ocean Expo in February of 2006 at the Hawai'i Convention Center. Hundreds of Hawai'i residents and visitors to the State came to the event.

May 18 – June 11, 2006 NOAA Ship Hi'ialakai-HIMB Research Cruise:
Building on existing outreach techniques utilized by the NWHI MNM Education Coordinator, HIMB successfully relayed "podcasts" of the research activities onboard Hi'ialakai this field season. Eight 1-2 minute mini-documentaries based on interviews with HIMB scientists were created and posted to both a newly created HIMB website for the NWHI (http://www.hawaii.edu/himb/HiialakaiNWHI2006/hiialakai_may-jun06.html) and the already existing education website for the NWHI MNM (www.hawaiianatolls.org). In addition, nine in-depth articles were posted with accompanying images on both web pages. The final component of onboard outreach involved interacting with high school biology students from Mililani High School. As part of their coursework, students were given an introduction to the NWHI from their teacher, Mrs. Sandy

Figure 2. Example of the web-based delivery of "podcasts" from NOAA Ship Hi'ialakai.

Webb, a member of the August 2005 NWHI CRER Teachers at Sea Voyage. The students then followed the daily stories and podcasts posted on the aforementioned websites, as well as the daily coverage of the research expedition in the local newspaper *The Honolulu Advertiser*. The students were then required to develop a series of questions which were posed to, and later answered by, the researchers onboard. This exchange between students and researchers was facilitated by the Outreach Coordinator. We also attempted a live question and answer session via iridium phone connection, but technical difficulties prevented us from accomplishing this goal on the May-June 2006 voyage.

<u>NMSP "Telepresence" demonstration</u>: In partnership with the NMSP, NWHI CRER (now MNM) and HIHWNMS, we successfully demonstrated a proof of concept project using telepresence technology. HIMB was supported by UH's ITS department and LOEA Corporation to utilize HIMB's new Internet2 capacity to bring real time video and two-way audio capability between an interactive underwater diver and a biology class at Moanalua High School. The students were prepared with materials provided by Mrs. Patty Miller of HIHWNMS, with technical support provided from Mystic Aquarium's Institute of Exploration.

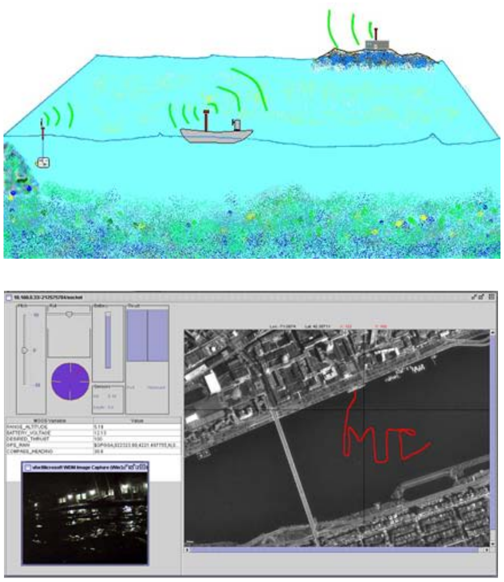


Figure 3. Schematic of the wireless AUV concept and interactive web-based interface (by Victor Polidoro, MIT AUV Lab).

<u>Extramural grants</u>: One of the goals of the NWHI Outreach and Education Program is to bolster formal education curriculum for Hawai'i schools that focus on marine science by using locally based examples as case studies. To this aim, the Outreach Coordinator submitted and received a competitive grant from NOAA's Office of Education to develop four marine science curriculum units that focus on ecological and water quality subjects in Kāne'ohe Bay, while making conceptual connections to the NWHI ecosystems. *Reef Missions: Exploring Remote Underwater Habitats with Autonomous Vehicles* will bring real-time video, still images, and water quality data via a wireless underwater vehicle (AUV designed by MIT AUV Lab) that will be controlled by students through and web-based interface. This will allow students to develop hypotheses-based research questions and collect associated data. The units will be incorporated into a larger effort to develop a formal State of Hawai'i approved marine education curriculum being developed by the HIHWNMS and HIMB. The HIHWNMS marine education project is also being coordinated by the NWHI Outreach Coordinator at HIMB. HIMB has hired three graduate students who will provide science-based content to curriculum writers for educational unit development. The Outreach Coordinator has also partnered on numerous pending research proposals to NSF that will incorporate informal education components to HIMB funded research.

Literature Cited

Aeby, G. S. 2006. Outbreak of coral disease in the Northwestern Hawaiian Islands. Coral Reefs 24(3):481.

Aeby, G.S. in press. Baseline levels of coral disease in the Northwestern Hawaiian Islands. Atoll Research Bulletin.

Craig, M.T., J.A. Eble, D.R. Robertson, B.W. Bowen. High genetic connectivity across the Indian and Pacific Oceans in the reef fish *Myripristis berndti* (Holocentridae). Marine Ecology Progress Series *Accepted pending revision*

Friedlander, A., G. Aeby, R. Brainard, A. Clark, E. DeMartini, S. Godwin, J. Kenyon, R. Kosaki, J. Maragos, and P. Vroom. 2005. The State of Coral Reef Ecosystems of the Northwestern Hawaiian Islands. In: Waddell J. (Ed). The State of Coral Reef Ecosystems of the United States and Pacific Freely Associated States: 2005. NOAA/NCCOS Center for Coastal Monitoring and Assessment's Biogeography Team, Silver Spring.

Friedlander, A.M., J.D. Parrish, and R.C. DeFelice. 2002. Ecology of the introduced snapper *Lutjanus kasmira* (Forsskal) in the reef fish assemblage of a Hawaiian Bay. J. Fish Biol 60:28-48.

Green, E. and A. Bruckner. 2000. The significance of coral disease epizootiology for coral reef conservation. Biological Conservation. 96: 347-361.

Halpern, B. S., K. A. Selkoe, F. Micheli, and C. V. Kappel. 2006. Evaluating and ranking the vulnerability of marine ecosystems to anthropogenic threats. Ecological Applications in press.

Kellogg, C. 2004. Tropical Archaea: diversity associated with the surface microlayer of corals. Marine Ecology Progress Series 273:81-88.

Kenyon, J. 1992. Sexual reproduction in Hawaiian *Acropora*. Coral Reefs 11:37-43.

Klaus, J.S., J. Frias-Lopez, G.T. Bonheyo, J.M. Heikoop, and B.W. Fouke. 2005. Bacterial communities inhabiting the healthy tissues of two Caribbean reef corals: interspecific and spatial variation. Coral Reefs 24:129-137.

Lesser, M.P., C.H. Mazel, M.Y. Gorbunov, and P.G. Falkowski. 2004. Discovery of symbiotic nitrogen-fixing cyanobacteria in corals. Science 305: 997-1000.

Maragos, J., and D. Gulko, editors. 2002. Coral Reef Ecosystems of the Northwestern Hawaiian Islands: Interim Results Emphasizing the 2000 Surveys. U.S. Fish and Wildlife Service and the Hawai'i Department of Land and Natural Resources, Honolulu, Hawai'i. 46 pp.

Maragos, J., G. Aeby, D. Gulko, J. Kenyon, D. Potts, D. Siciliano, and D. VanRavensway. 2004. The 2000-2002 Rapid Ecological Assessment of Corals in the Northwestern Hawaiian Islands, Part I: Species and Distribution. Pacific Science 58(2):211-230 .

Penn, K., D. Wu, J.A. Eisen, and N. Ward. 2006. Characterization of bacterial communities associated with deep-sea corals on gulf of Alaska seamounts. Applied and Environmental Microbiology 72(2):1680-1683.

Randall J.E. 1987. Introduction of marine fishes to the Hawaiian Islands. Bull Mar Sci 41:490-502.

Rohwer, F., M. Breitbart, J. Jara, F. Azam, and N. Knowlton. 2001. Diversity of bacteria associated with the Caribbean coral Montastraea franksi. Coral Reefs 20: 85-91.

Rohwer, F., V. Seguritan, F. Azam, and N. Knowlton. 2002. Diversity and distribution of coral-associated bacteria. Marine Ecology Progress Series 243:1-10.

Santavy, D. and E. Peters. 1997. Microbial pests: Coral disease in the Western Atlantic. Proc. 8th Int. Coral Reef Symp. 1:607-612.

Schultz, J.K., R.L. Pyle, E. DeMartini, and B.W. Bowen. 2006. Genetic homogeneity among color morphs of the flame angelfish, *Centropyge loriculus*. Marine Biology *In press*

Wafar, M., S. Wafar, and J.J. David. 1990. Nitrification in reef corals. Limnology and Oceanography 35(3):725-730.

Work, T., R.A. Rameyer, G. Takata, and M. Kent. 2003. Protozoal and epitheliocystis-like infections in the introduced blueline snapper *Lutjanus kasmira* in Hawaii. Diseases of Aquatic Organisms 37:59-66.