

Papahānaumokuākea Marine National Monument
RESEARCH Permit Application

NOTE: *This Permit Application (and associated Instructions) are to propose activities to be conducted in the Papahānaumokuākea Marine National Monument. The Co-Trustees are required to determine that issuing the requested permit is compatible with the findings of Presidential Proclamation 8031. Within this Application, provide all information that you believe will assist the Co-Trustees in determining how your proposed activities are compatible with the conservation and management of the natural, historic, and cultural resources of the Papahānaumokuākea Marine National Monument (Monument).*

ADDITIONAL IMPORTANT INFORMATION:

- Any or all of the information within this application may be posted to the Monument website informing the public on projects proposed to occur in the Monument.
- In addition to the permit application, the Applicant must either download the Monument Compliance Information Sheet from the Monument website OR request a hard copy from the Monument Permit Coordinator (contact information below). The Monument Compliance Information Sheet must be submitted to the Monument Permit Coordinator after initial application consultation.
- Issuance of a Monument permit is dependent upon the completion and review of the application and Compliance Information Sheet.

INCOMPLETE APPLICATIONS WILL NOT BE CONSIDERED

Send Permit Applications to:

Papahānaumokuākea Marine National Monument Permit Coordinator
6600 Kalaniana'ole Hwy. # 300
Honolulu, HI 96825
nwhipermit@noaa.gov
PHONE: (808) 397-2660 FAX: (808) 397-2662

SUBMITTAL VIA ELECTRONIC MAIL IS PREFERRED BUT NOT REQUIRED. FOR ADDITIONAL SUBMITTAL INSTRUCTIONS, SEE THE LAST PAGE.

Papahānaumokuākea Marine National Monument Permit Application Cover Sheet

This Permit Application Cover Sheet is intended to provide summary information and status to the public on permit applications for activities proposed to be conducted in the Papahānaumokuākea Marine National Monument. While a permit application has been received, it has not been fully reviewed nor approved by the Monument Management Board to date. The Monument permit process also ensures that all environmental reviews are conducted prior to the issuance of a Monument permit.

Summary Information

Applicant Name: Michael S. Rappe

Affiliation: SOEST, Hawaii Institute of Marine Biology, University of Hawaii at Manoa

Permit Category: Research

Proposed Activity Dates: May 15, 2008 - September 15, 2008

Proposed Method of Entry (Vessel/Plane): Vessel, NOAA Ship HIALAKAI

Proposed Locations: Shallow water habitat around Kure Atoll, Midway Atoll, Pearl and Hermes Atoll, and Nihoa Island.

Estimated number of individuals (including Applicant) to be covered under this permit:

7

Estimated number of days in the Monument: 30

Description of proposed activities: (complete these sentences):

a.) The proposed activity would...
enable researchers to 1) assess the community structure (diversity and relative abundance) of microbes, particularly Bacteria, associated with different species of healthy and health-compromised (e.g. diseased or bleached) corals and reef water across the Papahānaumokuākea Marine National Monument, 2) determine if coral-associated microbial communities vary seasonally and/or geographically, 3) determine if coral-associated bacterial community assessments can be used to indicate a coral's health state and to predict its susceptibility to bleaching and/or disease, and 4) determine the extent to which microbial pathogens are present on Monument reefs.

b.) To accomplish this activity we would
continue our ongoing effort to thoroughly sample and characterize microbial communities from diverse coral species encountered in various health states throughout the Papahānaumokuākea Marine National Monument. Additionally, we will collect surface seawater samples within the Monument to determine the spatial and geographic variability of these microbial communities compared to those associated with corals. All sampling will be conducted using minimally-invasive, non-lethal techniques and sample sizes are limited to the amount needed to perform meaningful statistical analyses.

c.) This activity would help the Monument by ...

Gaining a solid understanding of the possible roles that Bacteria play in maintaining and/or destabilizing coral health is essential if we are to effectively prevent disease from causing widespread harm to Hawaii's coral reef ecosystems. The methods employed in this study may enable us to determine a coral's health state at a given point in time and to predict a coral's susceptibility to disease under different environmental variables. If we are able to determine which coral species are the most susceptible to disease, and how this susceptibility changes under varying environmental conditions, we can take special steps to enhance the survival or recovery of this species. Disease prevention management may include establishing protected areas for more sensitive, disease prone coral species, as well as for coral species that are more robust and resilient to disease, reducing human impacts that may increase a coral's susceptibility to disease, prohibiting the introduction of any foreign materials or pollutants that may alter coral-associated microbial communities, and other measures that will help to prevent the spread of disease. Most importantly, we believe that our study methods will enable us to detect physiological stress in a coral before the onset of disease occurs. By identifying the early warning signs of disease, we can potentially prevent disease from spreading to epizootic proportions by developing methods to effectively treat or quarantine diseased corals. Finally, this study should enable us to identify invasive microbial pathogens, determine their source, and begin to develop methods that will prevent them from entering the pristine coral reef ecosystems of the Papahānaumokuākea Marine National Monument.

Other information or background:

Samples for this study have been collected on four previous cruises to the Papahānaumokuākea Marine National Monument in May 2005, September 2005, May 2006, and September 2007. The goals and objectives for the 2008 cruise period are to continue our assessment of microbial community structure and invasive microbial pathogens in corals over spatial and temporal scales within the Monument. Specifically, we would like to return to sites at Kure Atoll, Midway Atoll, and Nihoa island where we have previously sampled coral-associated microbes to assess how these communities vary seasonally and also to increase our spatial coverage of these reefs. These atolls were previously visited during the September 2005 cruise. Most importantly, we want to focus on collecting samples from health compromised (e.g. bleached and diseased) coral colonies to determine whether or not microbial communities differ between healthy and health-compromised corals of the same species and to identify the microorganisms that may be associated with coral disease in the monument.

Section A - Applicant Information

1. Applicant

Name (last, first, middle initial): Rappe, Michael S.

Title: Assistant Researcher/Professor, SOEST, Hawaii Institute of Marine Biology, University of Hawaii at Manoa

1a. Intended field Principal Investigator (See instructions for more information):

Jennifer Salerno, Ph.D. student/graduate research assistant, Zoology Department/Hawaii Institute of Marine Biology, University of Hawaii at Manoa

2. Mailing address (street/P.O. box, city, state, country, zip):

Hawaii Institute of Marine Biology

Phone:

Fax:

Email:

For students, major professor's name, telephone and email address:

Michael S. Rappe

3. Affiliation (institution/agency/organization directly related to the proposed project):

Hawaii Institute of Marine Biology, University of Hawaii at Manoa

4. Additional persons to be covered by permit. List all personnel roles and names (if known at time of application) here (e.g. John Doe, Research Diver; Jane Doe, Field Technician):

Jennifer Salerno, Ph.D. student (diver/technician), Hawaii Institute of Marine Biology, University of Hawaii at Manoa; Megan Huggett, post-doctoral researcher (diver/technician), Hawaii Institute of Marine Biology, University of Hawaii at Manoa; Michael Stat, post-doctoral researcher (diver), Hawaii Institute of Marine Biology, University of Hawaii at Manoa; Xavier Pochon, Hawaii Institute of Marine Biology, University of Hawaii at Manoa; Nadiera Sukhraj, Zoology Ph.D. student (diver), University of Hawaii at Manoa; Anderson Mayfield, Ph.D. student (diver/technician), Hawaii Institute of Marine Biology, University of Hawaii at Manoa; Ashley Smith, Masters Student

Section B: Project Information

5a. Project location(s):

<input checked="" type="checkbox"/> Nihoa Island	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> Necker Island (Mokumanamana)	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> French Frigate Shoals	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> Gardner Pinnacles	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> Maro Reef			
<input checked="" type="checkbox"/> Laysan Island	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> Lisianski Island, Neva Shoal	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> Pearl and Hermes Atoll	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> Midway Atoll	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> Kure Atoll	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input type="checkbox"/> Other			

Ocean Based

NOTE: There is a fee schedule for people visiting Midway Atoll National Wildlife Refuge via vessel and aircraft.

Location Description:

We plan to focus our research efforts at Kure Atoll, Midway Atoll, Pearl and Hermes Atoll, and Nihoa Island. However, at this time, we also request permission to sample at additional locations listed below in order to retain flexibility in our sampling strategy (e.g. due to unforeseen opportunistic events and/or changes in schedule or accessibility due to inclement weather).

Id	Location	Longitude	Latitude
1	Kure Atoll	-178.19706492000	28.55825235580
1	Kure Atoll	-178.19623585400	28.29958375730
1	Kure Atoll	-178.45987884800	28.29958375730
1	Kure Atoll	-178.46070791400	28.55742328970
2	Midway Atoll	-177.19638223300	28.37419969920
2	Midway Atoll	-177.19721129900	28.13377055310
2	Midway Atoll	-177.52800864100	28.13459961920
2	Midway Atoll	-177.52800864100	28.37419969920
3	Pearl and Hermes Atoll	-176.08850981800	28.04643025580
3	Pearl and Hermes Atoll	-175.63289162600	28.04539944540
3	Pearl and Hermes Atoll	-175.63289162600	27.70729363750
3	Pearl and Hermes Atoll	-176.08954062900	27.70626282710
4	Lisianski Island	-173.67292570900	26.25150771120
4	Lisianski Island	-173.67292570900	25.83942708400
4	Lisianski Island	-174.23095155800	25.83942708400
4	Lisianski Island	-174.23095155800	26.25150771120
5	Laysan Island	-171.47900122300	25.96027179830
5	Laysan Island	-171.47725234300	25.65596666490

5	Laysan Island	-171.97918092500	25.65771554490
5	Laysan Island	-171.97918092500	25.96202067840
6	Maro Reef	-170.18133220600	25.69968866680
6	Maro Reef	-170.17958332600	25.21524888540
6	Maro Reef	-171.00505472200	25.21524888540
6	Maro Reef	-171.00505472200	25.69968866680
7	Gardner Pinnacles	-167.74832319300	25.26070709440
7	Gardner Pinnacles	-167.75087047400	24.34878019150
7	Gardner Pinnacles	-168.36221811900	24.35132747340
7	Gardner Pinnacles	-168.36476540100	25.26070709440
8	French Frigate Shoals	-165.93465851400	23.94630965900
8	French Frigate Shoals	-165.93465851400	23.56421738120
8	French Frigate Shoals	-166.45685129400	23.56421738120
8	French Frigate Shoals	-166.45685129400	23.94630965900
9	Necker Island	-164.13627752700	23.71705429230
9	Necker Island	-164.13373024500	23.20505064020
9	Necker Island	-164.92084033700	23.20505064020
9	Necker Island	-164.92338761900	23.71960157420
10	Nihoa Island	-161.66031956700	23.23816530420
10	Nihoa Island	-161.66286684900	22.94013332760
10	Nihoa Island	-162.05005369100	22.94268060940
10	Nihoa Island	-162.05260097200	23.23561802240

5b. Check all applicable regulated activities proposed to be conducted in the Monument:

- Removing, moving, taking, harvesting, possessing, injuring, disturbing, or damaging any living or nonliving Monument resource
- Drilling into, dredging, or otherwise altering the submerged lands other than by anchoring a vessel; or constructing, placing, or abandoning any structure, material, or other matter on the submerged lands
- Anchoring a vessel
- Deserting a vessel aground, at anchor, or adrift
- Discharging or depositing any material or matter into the Monument
- Touching coral, living or dead
- Possessing fishing gear except when stowed and not available for immediate use during passage without interruption through the Monument
- Attracting any living Monument resource
- Sustenance fishing (Federal waters only, outside of Special Preservation Areas, Ecological Reserves and Special Management Areas)
- Subsistence fishing (State waters only)
- Swimming, snorkeling, or closed or open circuit SCUBA diving within any Special Preservation Area or Midway Atoll Special Management Area

6 Purpose/Need/Scope *State purpose of proposed activities:*

Microbial pathogens have been identified as the causative agents for a variety of diseases that have devastated coral reefs world wide; however, healthy corals are also known to host diverse and unique communities of microorganisms, including both Bacteria and Archaea. Determining coral disease etiologies is problematic because little is known about the types of microbes found on healthy corals, the functional role that these microbes may play in maintaining host health during disease-free periods, and how these communities may respond to environmental changes. The purpose of this study is to assess the community structure (diversity and relative abundance) of microbes associated with different species of healthy and health-compromised corals in the Papahānaumokuākea Hawaiian Island Marine National Monument and to determine the extent to which invasive microbial pathogens are present on Monument reefs. We will investigate if certain microbes and corals form species-specific associations and whether or not these associations vary on spatial and/or temporal scales. We will also examine if healthy and health-compromised corals of the same species harbor similar or different microbial communities. The ultimate goal of this study is to determine if the assessment of microbial community structure can be used as a biological indicator of coral health and a predictor of disease and/or bleaching susceptibility. This is the first large scale study of coral associated microbial communities in the monument and is part of a biogeographical survey that includes the Main Hawaiian Islands and other locations in the greater Pacific. Microbial communities from these other locales will be compared with those within the monument to examine if community structure varies with geographic location and/or anthropogenic input.

Throughout this investigation, the following hypotheses are being tested:

H01: Coral associated microbial (CAM) community structure (species diversity and relative abundance) does not differ between different coral species.

H02: CAM community structure does not vary between different reefs and/or between different islands/atolls.

H03: CAM community structure does not vary on a seasonal scale.

H04: Health-compromised (e.g. bleached or diseased) corals do not have different CAM communities than healthy conspecifics.

H05: Invasive microbial pathogens are not present in the Papahānaumokuākea Marine National Monument.

Thus far, our research indicates that bacterial community structure differs between coral species and appears to be species-specific. Additionally, preliminary results suggest that bacterial communities associated with *Porites lobata* from French Frigate Shoals are more similar to one another than to other atolls, but with some overlap with Kaneohe Bay, Oahu samples. We observed significant overlap amongst samples taken from the remaining islands/atolls. Corals with different assemblages of resident bacteria may respond differently to environmental changes. Therefore, it is important to identify the particular groups of bacteria that may be contributing to the differences we have observed between the atolls. We will continue to sample coral colonies from the Papahānaumokuākea Marine National Monument and the main eight Hawaiian Islands in order to increase our spatial coverage of these areas, establish a baseline of coral-associated bacterial communities, determine the geographic scale on which coral-

associated bacterial communities differ across the Hawaiian archipelago, and identify the environmental factors that may be causing these differences.

7. Answer the Findings below by providing information that you believe will assist the Co-Trustees in determining how your proposed activities are compatible with the conservation and management of the natural, historic, and cultural resources of the Monument:

The Findings are as follows:

a. How can the activity be conducted with adequate safeguards for the cultural, natural and historic resources and ecological integrity of the Monument?

All researchers will participate in a cultural briefing before entering the Papahānaumokuākea Marine National Monument so that they may gain a greater understanding of the cultural significance of the region, its importance to the Hawaiian people, and pledge to respect its sacred waters. As a scientist studying corals, I like to keep the Kumulipo Hawaiian creation myth in mind. Before the advent of "modern" technology ancient Hawaiians had an understanding of simple organisms giving rise to more complex organisms and, in particular, the importance of the coral polyp and how other organisms depended upon it for life. We too depend on the reef and must respect it. We must recognize that every action that we take has a consequence. As scientists, we must ensure that our actions are well thought out and are of minimal impact to the surrounding environment. I personally choose to conduct research in these waters not only because of their scientific importance, but because of an intrinsic value that cannot be enumerated or clearly defined, because of something that transcends people of all cultures - a desire to learn about and protect something that is simply a natural wonder to us all. I hope that we can evolve as a group through open discussion and additional cultural training.

To safeguard our research practices within the Monument, all equipment involved in sampling corals, including diving gear (e.g. wet suits, BCDs, masks, fins, snorkels, regulator, dive computers, etc.) and sampling gear (e.g. mesh bags, plastic clip boards, underwater cameras, metal chisels, forceps) are soaked in an antimicrobial disinfectant solution on a daily basis. Furthermore, separate equipment is used to sample healthy versus health-compromised (e.g. bleached and diseased) corals and these tools are soaked in a freshwater bleach solution between dives. The personnel listed on this permit are trained to sample coral colonies with minimal damage and are familiar with the procedures used to ensure that all biological specimens (corals and microbes) are killed upon collection. Our sampling protocol involves removing 3 small fragments of coral, totaling 6 grams in weight, from each colony. The cumulative size of these chunks are no larger than a parrot fish bite.

b. How will the activity be conducted in a manner compatible with the management direction of this proclamation, considering the extent to which the conduct of the activity may diminish or enhance Monument cultural, natural and historic resources, qualities, and ecological integrity, any indirect, secondary, or cumulative effects of the activity, and the duration of such effects?

Our proposed research activities parallel the research priorities drafted in the current NOAA research plan for the Monument. Additionally, all of our sampling is conducted using minimally-invasive, non-lethal techniques and sample sizes are limited to the amount needed to

perform meaningful statistical analyses. To determine the longer-term effects of our sampling method, we tagged, sampled, and photographed three *Porites lobata* and three *Acropora cytherea* colonies at French Frigate Shoals site 30 during the May 2006 cruise. During the September 2007 cruise, the colonies were re-located, photographed, and tags were removed. The coral colonies did not show any recognizable evidence of previous sampling and appeared to be fully recovered. We will continue to minimize our footprint in the monument by practicing responsible sampling techniques.

c. Is there a practicable alternative to conducting the activity within the Monument? If not, explain why your activities must be conducted in the Monument.

There is not a practical alternative to conducting the proposed research activity within the Monument due to its unique ecosystem and location along an extensive gradient of anthropogenic disturbance. This area is truly a natural laboratory. Sampling within the monument is of paramount importance in obtaining a baseline assessment of bacterial communities associated with Hawaiian corals that are relatively removed from human disturbance. By obtaining these data over the wide geographical expanse of the Monument, we can begin to understand the natural variability of these associations, how they respond to seasonal and environmental changes, and the role that they play in maintaining and/or destabilizing coral health.

d. How does the end value of the activity outweigh its adverse impacts on Monument cultural, natural and historic resources, qualities, and ecological integrity?

Any potential adverse impacts that our presence may have on the Monument would be minimal compared to the anthropogenic disturbances that will effect it whether we are present or not. In other words, global-scale disturbances such as climate change, pollution, and disease will continue to negatively affect the Monument in our absence. Given the opportunity, we can begin to understand the underlying processes that shape coral-bacterial associations and recommend the best management practices to protect and conserve corals in the Monument and Hawaiian archipelago. The cost of removing the approximate weight of one adult (*Pocillopora meandrina*) coral colony per year to obtain new and valuable scientific data that has implications for the entire archipelago is undoubtedly beneficial for this resource.

e. Explain how the duration of the activity is no longer than necessary to achieve its stated purpose.

Unfortunately, we do not have the opportunity to sample at regular intervals on a seasonal basis due to weather conditions and other issues regarding accessibility; however, we optimize the time that we are granted to conduct research within the Monument by focusing on our specific research goals and priorities for that particular cruise. During the 2008 cruise period, we will focus on collecting at Kure Atoll, Pearl and Hermes Atoll, Midway Atoll, and Nihoa Island; spending just a few days at each of these chosen locations.

f. Provide information demonstrating that you are qualified to conduct and complete the activity and mitigate any potential impacts resulting from its conduct.

The personnel listed under this permit are knowledgeable of the sampling techniques involved with this research and have been trained by those who have expertise in scientific diving and

microbial laboratory practices. All personnel listed here have previous experience conducting this type of research in the Papahānaumokuākea Marine National Monument or in the main Hawaiian Islands.

g. Provide information demonstrating that you have adequate financial resources available to conduct and complete the activity and mitigate any potential impacts resulting from its conduct. Ship time aboard the Hialakai has been reserved for Hawaii Institute of Marine Biology researchers and funding for this project is supported by a partnership between the National Marine Sanctuary program and the Hawaii Institute of Marine Biology (NMSP MOA 2005-008/66882).

h. Explain how your methods and procedures are appropriate to achieve the proposed activity's goals in relation to their impacts to Monument cultural, natural and historic resources, qualities, and ecological integrity.

Our methods and procedures have been designed to impart minimal impact on the coral organisms that we must interact with in order to obtain scientific samples. We collect the minimum number of non-lethal samples needed to obtain data that can statistically validated.

i. Has your vessel has been outfitted with a mobile transceiver unit approved by OLE and complies with the requirements of Presidential Proclamation 8031?

The NOAA ship HIALAKAI is equipped with a NOAA OLE Vessel Monitoring System.

j. Demonstrate that there are no other factors that would make the issuance of a permit for the activity inappropriate.

I certify that there are no other factors that would make the issuance of a permit for the activity inappropriate.

8. Procedures/Methods:

Coral species that are common and ecologically important in the Papahānaumokuākea Marine National Monument, and throughout the Pacific, have been selected for microbial community analysis. These include: *Porites lobata*, *Porites compressa*, *Pocillopora meandrina*, *Montipora capitata*, and *Acropora cytherea*. Using SCUBA and underwater tools, three non-lethal sub-samples are taken from each coral colony, with 10 colonies sampled per species per site (10 healthy colonies or 5 healthy plus 5 bleached/diseased colonies, if present). A sub-sample consists of a 6-mm diameter, 6-mm deep core or fragment (approximately 2 grams in weight) that includes the coral tissue, overlying mucus layer, and underlying skeleton (these cores are fragments are no larger than a parrot fish bite). The minimum size needed for analysis is taken when possible and any extra tissue is frozen and archived for future analysis. All sampled colonies are photodocumented. During the 2008 cruise period, our sampling strategy will focus on collecting from health-compromised coral colonies. Health-compromised colonies include colonies with discoloration or signs of bleaching and colonies that are positively identified as having symptoms indicative of one of the previously documented diseases (e.g. *Montipora* white spot, *Acropora* white syndrome, *Porites* brown necrotizing disease, tumors, etc.). These colonies

will be sampled opportunistically when they are encountered and surrounding healthy colonies of the same species will be sampled for comparison.

All field work will take place during daylight hours. Our specific site locations will be reached using a 17.5 foot Zodiac inflatable boat with a 50-hp, 4-stroke, outboard gasoline engine. The boat and coxswain will be provided by the NOAA R/V Hi'ialakai. Specimens will be collected on SCUBA. Samples will be collected from various habitats (e.g. fore reef, back reef, lagoon) at each atoll/island. Seawater samples will be taken adjacent to sampled coral heads for comparison of coral-associated microbial (CAM) community composition between corals and the surrounding seawater environment. Other environmental variables including temperature, salinity, nutrients, and chlorophyll a will be measured in the water column. CAM samples are processed and killed by freezing in the -37°C freezer aboard the research vessel. Seawater samples are filtered and filters are then frozen. Samples will be stored in the freezer on board the ship and then transported to the Hawaii Institute of Marine Biology (HIMB) on Coconut Island for further processing and molecular analysis in the laboratory of Dr. Michael Rappé.

In the laboratory, CAM communities associated with each coral species and seawater are assessed through molecular analyses of microbial 16S rRNA genes. Molecular tools enable us to identify microorganisms that are not detected using traditional culturing methods. Techniques for CAM DNA extraction and amplification have been optimized for each target coral species. Polymerase chain reaction-amplified CAM DNA is used in a community fingerprinting technique known as terminal restriction fragment length polymorphism (TRFLP) to assess the diversity and relative abundance of microbial species associated with each individual coral sample. CAM PCR products will also be used in cloning and sequencing to identify microbes down to species or phylotype. In addition, currently available nucleic acid sequence and community fingerprinting data is being compiled into a database for the rapid identification of microbial taxa based on phylogenetically-informative and discriminative signatures.

NOTE: If land or marine archeological activities are involved, contact the Monument Permit Coordinator at the address on the general application form before proceeding, as a customized application will be needed. For more information, contact the Monument office on the first page of this application.

9a. Collection of specimens - collecting activities (would apply to any activity): organisms or objects (List of species, if applicable, attach additional sheets if necessary):

Common name:

1. table coral
2. rice coral
3. cauliflower coral
4. finger coral
5. lobe coral
6. seawater

Scientific name:

1. Acropora cytherea
2. Montipora capitata
3. Pocillopora meandrina
4. Porites compressa
5. Porites lobata
6. seawater

& size of specimens:

For coral samples:

= up to 10 colonies per site (10 healthy colonies or 5 healthy colonies + 5 diseased/bleached colonies)

size = 6 grams per colony (approximate combined wet weight of mucus, tissue, and skeleton)

* NOTE: not all 5 species will be collected at each site. We typically sample 1 to 3 species per site.

For seawater samples:

= 1 sample per site

size = 1.185 L of seawater

Collection location:

Coral and seawater samples will be collected from various lagoon, back reef, patch reef, and fore reef habitats within Kure Atoll, Pearl and Hermes Atoll, Midway Atoll, Nihoa Island, and possibly other island/atolls. Specific sites will be determined daily amongst the participating scientists and will be based on the research priorities and needs of each project and the weather conditions. We estimate that we will visit 60 sites during the cruise.

Whole Organism Partial Organism

9b. What will be done with the specimens after the project has ended?

The majority of the coral sample will be completely consumed during processing. Any left over tissue and skeleton will be kept frozen at -80°C and archived for future analysis in the laboratory of Dr. Michael Rappe at the Hawaii Institute of Marine Biology.

9c. Will the organisms be kept alive after collection? Yes No

• General site/location for collections:

• Is it an open or closed system? Open Closed

• Is there an outfall? Yes No

• Will these organisms be housed with other organisms? If so, what are the other organisms?

- Will organisms be released?

10. If applicable, how will the collected samples or specimens be transported out of the Monument?

Samples will be killed and stored frozen in the -37 C freezer on board the NOAA R/V Hiāialakai. Upon reaching the port of Honolulu, samples will be packaged in sealed coolers and transported to the Hawaii Institute of Marine Biology in Kaneohe.

11. Describe collaborative activities to share samples, reduce duplicative sampling, or duplicative research:

The Rappé and Gates laboratory personnel are interested in collecting samples from health-compromised coral colonies and will collaborate on collecting said samples through a combined sampling effort. Samples collected for the species *Acropora cytherea*, *Porites lobata*, *Pocillopora meandrina* and *Montipora capitata* will be shared with the Gates lab. In these instances, a single 1cm² piece will be taken and split for downstream experiments, thus reducing the number of samples by half for those that overlap between these research teams.

12a. List all specialized gear and materials to be used in this activity:

SCUBA equipment:

Buoyancy control device

2 regulators (extra for back up)

dive computer

dive watch

dive weights

dive bag

mask, fins, and snorkel

whistle

safety sausage

wet suit

dive boots (2 pairs)

hood

dive gloves (3 pairs)

extra fin and mask straps

tools for equipment repair

Field equipment:

GPS

brass clips

fishing weights

plastic zip ties (black and colored)

stainless steel hammers (2)

stainless steel chisels (2 plus extras)

core borers

refractometer
flagging tape
1-liter nalgene sample bottles
250 mL amber bottles
digital camera with underwater housing
medium-sized action packer
medium-sized cooler
plastic clipboards (2)
underwater paper
pencils
whirl-paks
small and large zip-loc bags
Laboratory equipment:
DNA lysis buffer
peristaltic pump motor and parts
peristaltic pump cartridges
sterile surgical tubing
plastic tri pour beakers (5)
metal filter adapters
13 mm 0.2 micron Supor filters
13 mm filter cartridges
small and large tupperware containers
stainless steel tweezers (2)
2.5 mL cryovials
no skid rubber mats
paper towels
blue ice packs
Dry laboratory equipment:
pencils
sharpies (fine point and larger)
3-ring binder with maps
AA batteries
blank rewritable CDs
flash drive

12b. List all Hazardous Materials you propose to take to and use within the Monument:

A 4% paraformaldehyde solution will be stored in microfuge tubes for sample preservation.

13. Describe any fixed installations and instrumentation proposed to be set in the Monument:

n/a

14. Provide a time line for sample analysis, data analysis, write-up and publication of information:

Ongoing: Processing and molecular analyses of coral associated microbial communities in the laboratory

April 2008 – June 2008: Data analysis and write-up of results for publication in a peer-reviewed scientific journal

15. List all Applicants' publications directly related to the proposed project:

Salerno, J., E. Hambleton, and M. S. Rappé. In prep. Optimization of nucleic acid extraction and PCR methods for coral-associated microorganisms. In preparation for submission to Applied and Environmental Microbiology.

With knowledge of the penalties for false or incomplete statements, as provided by 18 U.S.C. 1001, and for perjury, as provided by 18 U.S.C. 1621, I hereby certify to the best of my abilities under penalty of perjury that the information I have provided on this application form is true and correct. I agree that the Co-Trustees may post this application in its entirety on the Internet. I understand that the Co-Trustees will consider deleting all information that I have identified as “confidential” prior to posting the application.

Signature

Date

SEND ONE SIGNED APPLICATION VIA MAIL TO THE MONUMENT OFFICE BELOW:

Papahānaumokuākea Marine National Monument Permit Coordinator
6600 Kalaniana'ole Hwy. # 300
Honolulu, HI 96825
FAX: (808) 397-2662

DID YOU INCLUDE THESE?

- Applicant CV/Resume/Biography
- Intended field Principal Investigator CV/Resume/Biography
- Electronic and Hard Copy of Application with Signature
- Statement of information you wish to be kept confidential
- Material Safety Data Sheets for Hazardous Materials