

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: Greta Smith Aeby

Affiliation: HIMB

Permit Category: Research

Proposed Activity Dates: May 1 - Sept 30, 2012

Proposed Method of Entry (Vessel/Plane): NOAA research vessel Hiialakai

Proposed Locations: shallow water reefs throughout the Monument (Nihoa, Necker, FFS, Gardner, Pearl and Hermes, Maro, Laysan, Lisianski, Midway, Kure,)

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

8

Estimated number of days in the Monument: 21-28 days

Description of proposed activities: (complete these sentences):

a.) The proposed activity would...

Determine the prevalence and incidence (change in levels through time) of coral disease within the Monument. Examine degree of recovery of colonies or reefs affected by disease. Compare the microbial communities between healthy and diseased coral to investigate disease pathogenesis and etiology. If Montipora white syndrome is found, microbial studies will tell us whether the pathogen is the same or different as what is found in the MHI. Determine whether intrinsic factors such as genetic relatedness or zooxanthellae clade may help explain the high prevalence of Acropora growth anomalies (~40% of the colonies) within "tumor city" at FFS as compared to disease levels elsewhere within the Monument and the Indo-Pacific (<1% of the coral colonies).

b.) To accomplish this activity we would

Survey reefs for coral disease, mark and photograph individual colonies exhibiting signs of disease, and repair permanent sites. Coral colonies are tagged by placing a cable tie through a natural hole in the colony thereby producing no harm to the coral. GA-affected and healthy table corals would be sampled for follow-up molecular studies for relatedness and zooxanthellae clade by the Toonen lab. Genetic analysis requires a minimum of 50 small samples from individuals

within a population. White syndrome and healthy table and rice corals will be sampled for follow-up microbial studies by the Callahan lab. If we encounter any new coral diseases then we need to sample these for histological analyses to determine what the disease is doing to the coral at the cellular level. We will collect one healthy and one disease sample per colony. I am asking to be able to sample 20 diseased colonies (1 healthy and 1 diseased sample/colony) per island, which I estimate, based on disease prevalence from past surveys, as being sufficient to cover all the surveys at each of the islands. This request should cover our ability to adequately investigate any disease outbreaks we might encounter.

c.) This activity would help the Monument by ... giving them information as to the health status of their reefs, ability to predict amount of damage to reefs from coral disease through time, and a measure of the degree of resilience (ability to recover) of their reefs. Tissue loss diseases on corals throughout the Indo-Pacific have been found to be caused by pathogenic bacteria with *Vibrio corallyticus* identified in 3 different regions. In the main Hawaiian Islands we have identified three different bacterial pathogens causing tissue loss disease in *Montipora*. Identifying bacteria in healthy and disease corals will tell us whether the same pathogens are killing coral within the PMNM or if they are novel. A comparison of microbial communities is also the first step in identifying the pathogen. Pathogen identification allows us to start to understand where the diseases may be coming from and allows for the development of disease treatment to help contain the diseases. *Montipora* White Syndrome (MWS) has emerged as a serious problem on reefs within the MHI. We will also screen the reefs of PMNM for this disease and if found, the microbial community will be examined to determine if the disease is caused by the same pathogens as in the MHI. Is MWS spreading up into PMNM? The prevalence of disease at "tumor city" is unusually high and could be due to intrinsic factors such as the genetic relatedness or zooxanthellae clade of the table corals at that site and/or extrinsic factors such as contaminants. Molecular studies on coral colonies with this disease will help answer this question.

Other information or background: Coral reef ecosystems are at risk locally and globally due to global climate change and human activities. Mass bleaching events have increased dramatically since the 1980's and have usually been linked to El Nino or global warming-related increases in annual sea surface temperature (Brown 1997, Barber et al. 2001). The El Nino Southern Oscillation (ENSO) conditions during 1997 to 1998 resulted in worldwide bleaching from the Western Atlantic to the Great Barrier Reef. ENSO events have increased in frequency and duration in the past two decades (Barber et al. 2001, Walker 2001) and it has been predicted that the frequency and severity of coral bleaching will also continue to rise (Hoegh-Guldberg 1999).

In the western Atlantic coral disease has been incriminated in the marked degradation of reef habitats (Santavy and Peters 1997, Green and Bruckner 2000). Coral disease is reported to be responsible for the dramatic decline of *Acroporids*, one of the major frame-building corals in the Florida Keys, changing the structure and function of the coral reef ecosystem (Aronson & Precht 2001). Despite the major impact disease can have on reef systems, the etiology of most coral diseases remains unclear (Santavy and Peters 1997, Richardson 1998). The causative agents,

mechanism of pathogenesis and link to environmental or anthropogenic stress are still largely unknown (Richardson 1998, Green & Bruckner 2000).

The reefs of the Northwestern Hawaiian Islands (NWHI) are considered to be relatively healthy but they are not immune to the conditions that have led to the decline of other reef systems. In September 2002, the first mass-bleaching event was recorded on the reefs of the NWHI with a second bleaching event occurring in 2004. In the three northwestern-most atolls of the Archipelago (Pearl & Hermes, Midway and Kure) over half of all sites had significant bleaching (Aeby et al. 2003, Kenyon et al., 2005). Ten coral disease states have now been described from the NWHI (Aeby 2006) and we have established permanent sites which allow us to determine both temporal and spatial changes in diseases through time and the ultimate affect of disease on the health of the ecosystem. We will measure changes in disease levels through time, rates of tissue loss from different diseases, patterns of disease transmission among colonies, rate of spread of disease and evaluate changes in coral cover and coral species composition. In addition, two diseases of concern have been identified, *Acropora* white syndrome and *Acropora* growth anomalies which we are targeting for focused studies.

Acropora white syndrome (AWS) is a disease which causes acute tissue loss in acroporids and has been reported from across the Indo-Pacific. *Acropora* white syndrome appeared on one reef in the northwestern Hawaiian Islands (NWHI) in 2003 (Aeby 2006) and has since spread. Our prior studies in 2005 and 2006 found this disease to be highly virulent having killed over 19 large table acroporids with numerous other colonies suffering massive tissue loss from the disease. The disease occurs predominantly at French Frigate Shoals (FFS) within the NWHI, which is the center of abundance and diversity of acroporids in Hawaii. We plan to continue to follow the dynamics of this disease by re-surveying permanent sites to measure coral mortality and disease spread. We also need to start understanding the underlying etiology of disease and we will be comparing the microbial community of AWS and healthy coral as a first step. Within the MHI, Montipora white syndrome is becoming a problem with two separate disease outbreaks reported from Kaneohe Bay. Our disease surveys will document whether MWS is occurring in the PMNM and if so, microbial comparisons will inform us whether they are caused by the same or different pathogens as found in the MHI. This work will be conducted in collaboration with the Callahan lab.

"Tumor city" at FFS has an unusually high prevalence of *Acropora* growth anomalies (40%) as compared to other areas (<1%). We hypothesize this could be due to intrinsic factors (genetic susceptibility, zooxanthellae clade, etc) or extrinsic factors such as contaminants in the environments. As a first cut in understanding why disease levels are so high on that reef, we will examine the genetic relatedness and zooxanthellae clades of affected vs. unaffected colonies found on that reef. This work will be conducted in collaboration with the Toonen lab and will complement the work proposed by the Karl lab.

It is important for management agencies to have a through understanding of the vulnerability of these reefs to disease and the first steps in managing disease are developing an understanding of the causes of disease, modes of transmission and assessing its geographic extent. Management of disease in wildlife populations usually involves either reducing or removing the source of infection or reducing the spread of the disease. However, before appropriate management plans can be made the epizootiology of diseases must be understood. Corals are the very foundation of the entire coral reef ecosystem and as such threats to their survival must be managed using the best available science. Our studies, past, present and

proposed, are supplying critical information about coral disease and disease dynamics, which are a serious threat, within the NWHI.

Section A - Applicant Information

1. Applicant

Name (last, first, middle initial): Aeby, Greta S.

Title: assistant researcher

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

Dr. Steve Karl

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

[REDACTED]

Phone: [REDACTED]

Fax: [REDACTED]

Email: [REDACTED]

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

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

Hawaii Institute of Marine Biology

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):

Dr. Sean Callahan: co-investigator

Dr. Fenny Cox: co-investigator

Dr. Frank Stanton: co-investigator

Amanda Shore: graduate student

Maya Walton: graduate student

Jonothon Whitney: graduate 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:

shallow reefs throughout the Monument

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:*

1. To re-survey permanent sites for assessment of disease dynamics
2. To conduct new disease surveys at any sites of interest to management
3. To compare microbial communities between healthy and white syndrome corals
4. To determine the zooxanthellae clades of GA-affected vs. healthy coral colonies at FFS with samples received in collaboration with the Karl lab.

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?

PMNM is now considered a World Heritage Site which emphasises the cultural and historic importance of the area as well as the ecological importance. As such, members of this team will be informed of the uniqueness of PMNM and will be trained to conduct all activities in a manner reflecting this importance. All personnel will also attend established cultural training classes to better understand and respect the cultural and spiritual importance of PMNM. From a biological point of view, activities will be conducted in a manner to minimally impact coral reef resources and standard protocols for disease studies developed for the Monument will be used. All gear will be sterilized each day and any collected organisms will be placed in plastic bags at depth before transfer to the small boat. All laboratory work will be conducted using established biosecure protocols including sterilizing all tools and work surfaces. All biological samples will be fixed in solution for transport to our laboratories in Honolulu.

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?

The collapse of coral reefs from disease in other regions points to the critical need to understand disease processes. Our research program is dedicated to studying disease in the Monument so that managers have the information they need to protect these vulnerable resources. All research proposed in this permit application is directly applicable to the management of diseases of coral within the region. All surveys are conducted in a manner causing little to no impact on the environment as they use visual and photographic techniques. We will be collecting the minimal number of coral samples required to complete our laboratory analyses.

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 no alternative to conducting the activity in the Monument. Although, comparative studies of disease in other regions are useful, they cannot replace understanding damage from the specific diseases affecting coral populations in the Monument.

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

If diseases are not managed in the Monument, the coral reefs will suffer the same fate as coral reefs in the Florida Keys and other regions of the Caribbean. In the Keys, their acroporids, which used to be their numerically dominant coral, have been reduced by 90% and are now on the endangered species list (Patterson et al. 2002). Acroporids in the Monument are already in decline due to two different diseases, Acropora white syndrome and Acropora growth anomalies. Current models of global climate change predict a significant increase in sea surface temperature (Kleypas et al. 1999). Elevated temperatures have been shown to accelerate the growth rate and pathogenicity of pathogens and so it is predicted coral disease will become more common and widespread (Porter et al. 2001). On the GBR, increases in White Syndrome are associated with temperature anomalies. Acropora white syndrome is also currently killing corals in the Monument so information of the epizootiology of this disease is critically important for the development of both immediate and long-term management strategies. Montipora white syndrome outbreaks are now occurring in the MHI. We bring a pro-active approach to PMNM in screening reefs for this new potential threat to the corals.

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

We are requesting the absolute minimum amount of time require to condut our studies. We anticipate staying a maximun of 5 days at any one island within the Monument.

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

I have been conducting coral disease surveys and studies in the Monument since 2002. I am familiar with the reefs and methodology required to safely conduct all proposed studies. I was involved in the development of protocols for investigations of coral disease developed for the Monument. I am also a co-author on the book "A coral disease handbook: guidelines for assessment, monitoring and management." and was the lead in developing Hawaii Division of Aquatic Resources "Rapid Response Contingency Plan for unusual events of coral bleaching, disease and COTS outbreaks".

Both of these publications make recommendations for proper procedures involving investigating marine diseases including field techniques, the need for follow-up laboratory investigations and safe handling of samples.

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. I am employed by the University of Hawaii and thus would be covered under University policies.

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.

The Monument's goal is to preserve the integrity of the resources for cultural, historical and ecological reasons. Disease is already established in the Monument and is starting to degrade the coral populations. Corals are the very foundation of the coral reef ecosystem so if the Monument is to prevent irreparable damage from disease it must first have information on the extent and harm from diseases. Our research addresses these needs for the Monument and does so in the most minimally invasive manner as possible. Our methods are predominately visual surveys which do no harm. Marking of individual colonies is also non-invasive. Small samples will be taken for molecular and microbial analysis for the Callahan lab, and Toonen lab and of any new diseases encountered not yet characterized by histology. The acropora GA and GA control samples will be obtained from the Karl lab to minimize overall collections from the reefs.

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

yes

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

I am an established disease scientist who has conducted similar research throughout the Indo-Pacific and in the Florida Keys with no problems. I am familiar with all required protocols in disease assessment and have helped author numerous publications outlining proper response and procedure for investigating disease outbreaks.

8. Procedures/Methods:

Disease surveys: As possible, re-survey of established sites or new sites of interest (up to 6) throughout the Monument will be done following established protocols. Two 25 m lines will be laid out along the permanent pins. A diver will then swim over the lines during which all corals within one half meter of either side of the transect lines will be identified to species, counted, and assigned to a size class (0-5cm; 6-10cm; 11-20cm;

21-40cm; 41-80cm; 81-150cm; >150cm.). In the same manner, a second diver will swim over the lines and examine all corals for signs of bleaching or disease. Bleached colonies will be assigned a bleaching category: 0-no bleaching; 1- 10-30%; 2-30-50%; 3-50-100%; 4- 100%; 5-mortality. For corals exhibiting disease, a general description of the condition will be recorded, the coral will be photographed and a specimen will be collected for histopathological examination. 20 samples/island for other coral diseases are requested and are to be used for histology to examine disease processes at the cellular level. These samples will only be required if we come across undocumented diseased colonies or a disease outbreak within our transects. If we do not encounter a disease outbreak or undocumented diseases then no sampling will occur. I am asking to be able to sample 20 diseased colonies (1 healthy and 1 diseased sample/colony) per island, which I estimate, based on disease prevalence from past surveys, as being sufficient to cover all the surveys at each of the islands. Individual colonies tagged in 2005 or 2006 will be relocated, remarked and photographed. At permanent sites, any new infected colonies along the transect will be photographed and tagged. Coral colonies are tagged by placing a cable tie through a natural hole in the colony thereby producing no harm to the coral. Any lost pins will be replaced and loose pins re-glued.

Zooxanthellae clade of affected and unaffected Acropora

50 samples (1.0 cm) each of tumors and healthy tissue from different colonies will be collected for molecular analysis for the Toonen lab. 50 is the minimum number needed to do genetic analysis. All samples will be fixed in salt (DMSO) buffer for transfer to our labs at HIMB. The requested samples for Acropora cytherea with and without tumors will be used for both genetic and zooxanthellae clade analyses and will be obtained from collections covered under the permit by the Karl lab.

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:
table coral

Scientific name:

Acropora cytherea

& size of specimens:

30 samples @ 3-5 cm². total=30 samples. see attached table

Collection location:

FFS

Whole Organism Partial Organism

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

Samples will be destroyed in the various analyses. Any samples that are not, will be maintained preserved at HIMB.

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

Samples will be kept alive until they can be frozen on the ship which kills microbes preventing disease spread. We will be processing the coral samples upon our return from the small boats so we should be done before leaving a location but that depends on how many samples we have and sometimes the ship leaves early, etc. so wanted to make sure we had some flexibility in processing time. will be frozen or placed in Z-fix in the lab both of which kills microbes preventing any disease spread.

• General site/location for collections:

FFS

• 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?
no

• Will organisms be released?

no

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

Transport of preserved samples (dead samples) out of the Monument would occur during transit between islands and back to the MHI

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

Acroporid samples will be shared with the Callahan (Dr. Sean Callahan, Microbiology Dept, UH Manoa) lab for microbial analyses. We are not requesting samples for the zooxanthellae clad analyses as these will be obtained from sharing samples with the Karl lab. Dr. Karl will serve as my field PI and our teams will be working together at FFS. We will not be doubly collecting *Acropora cytherea* but will be sharing samples when appropriate. Samples from colonies with growth anomalies will be shared between the two labs.

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

See list at end up 11.

Common name: reef coral

Scientific name: *Porites* sp., *Pocillopora* sp., *Pavona* sp., Species will vary depending upon disease occurrence.

& size of specimens: up to 200 samples per coral genera @ 3-5cm². see attached table

Collection location: shallow water reefs throughout the Monument (Nihoa, Necker, FFS, Gardner, Pearl and Hermes, Maro, Laysan, Lisianski, Midway, Kure,)

Whole Organism Partial Organism

1b. What will be done with the specimens after the project has ended?

Samples will be destroyed in the various analyses. Any samples that are not, will be maintained preserved at HIMB.

1c. Will the organisms be kept alive after collection? Yes No

Corals will be transported live in buckets of sea water to the Hi'ialakai where they will be frozen or placed in Z-fix in the lab which kills microbes preventing disease spread.

- Specific site/location:
- 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? No

• Will organisms be released? No

2. If applicable, how will the collected samples or specimens be transported out of the Monument? Samples will be transported in buckets on small boats to the Hiʻialakai which may or may not be within Monument waters. Fixed or frozen samples (dead samples) will be transported back to Honolulu via the Hiʻialakai.

3. Describe collaborative activities to share samples, reduce duplicative sampling, or duplicative research: Samples may be shared with Callahan lab (UH Microbiology) if they need microbial analysis (MWS) or if histology is needed they will be shared with Dr. Thierry Work (USGS).

Common name: Rice coral

Scientific name: Montipora sp., Species will vary depending upon disease occurrence.

& size of specimens: up to 150 samples @ 3-5 cm². see attached table

Collection location: shallow water reefs throughout the Monument (Nihoa, Necker, FFS, Gardner, Pearl and Hermes, Maro, Laysan, Lisianski, Midway, Kure.)

Whole Organism Partial Organism

1b. What will be done with the specimens after the project has ended?

Samples will be destroyed in the various analyses. Any samples that are not, will be maintained frozen at HIMB.

1c. Will the organisms be kept alive after collection? Yes No

Corals will be transported live in buckets of sea water to the Hiʻialakai where they will be frozen in the lab which kills microbes preventing any disease spread.

• Specific site/location:

- 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? No
- Will organisms be released? No

2. If applicable, how will the collected samples or specimens be transported out of the Monument? Samples will be transported in buckets on small boats to the Hiʻialakai which may or may not be within Monument waters. Fixed or frozen samples (dead samples) will be transported back to Honolulu via the Hiʻialakai.

3. Describe collaborative activities to share samples, reduce duplicative sampling, or duplicative research: Samples may be shared with Callahan lab (UH Microbiology) if they need microbial analysis (MWS) or if histology is needed they will be shared with Dr. Thierry Work (USGS).

List all specialized gear to be used in this activity

dive gear

coral collection gear (bone cutters, hammer, chisel, ziplock and whirlpak bags, bag to carry gear)

coral processing gear (plastic jars, z-fix, clorox)

stereo microscope

cameras and underwater housing

sludge hammer, steel pins and underwater glue

field equipment (tape measures, floats, clipboards, underwater paper, cow ear tags, cable ties)

hand held GPS

computer

5 gal buckets with lids

Miscellaneous office supplies (books, tablets, pencils, pens, markers, scissors, stapler, 3-hole punch, etc.)

Personal gear (clothing, personal hygiene items, diet coke, snacks, sunglasses, etc)

Aeby NWHI permit sample collection table 2012

Coral specie	common name	island	#	size	tot #
Acropora cytherea	table coral	FFS	up to 30	3-5cm	30
Montipora sp.	rice coral	all islands(up to 5)	up to 30/is	3-5cm	150
Porites	reef coral	all islands(up to 10)	up to 20/is	3-5cm	200
Pocillopora	reef coral	all islands(up to 10)	up to 20/is	3-5cm	200
Pavona	reef coral	all islands(up to 10)	up to 20/is	3-5cm	200
total					780

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

Clorox- 5 gallons-used for sterilization of equipment and growth anomaly processing for skeletal analyses.

Z-fix-1gallon-used for preserving coral samples for histology

Ethanol-1 gallon-used for preserving samples for molecular analyses

DMSO-1 gallon used for preserving samples for molecular analyses

All chemicals will be contained in bottles within secondary containment and will be transported out of the Monument and sent back to our lab at HIMB

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

Repair or replacement of steel pins at permanent monitoring sites.

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

Fall 2012-Spring 2013: histology, molecular and microbial analyses. Summer - Fall 2012: data analysis and report writing

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

Wilson, B, Aeby, GS, Work, TM and DG Bourne. In press. Bacterial communities associated with healthy and Acropora White Syndrome-affected corals from American Samoa. Environ Micro.

Sudek, M, Aeby, GS, and SK Davy. In press. Localized bleaching in Hawaii causes tissue loss and a reduction of the number of gametes in Porites compressa. Coral Reefs.

Work, TM, Forsman, Z, Szabo, Z, Lewis, T, Aeby, G and R Toonen. 2011. Inter-

specific coral chimerism: genetically distinct metazoa associated with tissue loss in *Montipora capitata*. PLoS ONE 6(7):e22869.

Aeby, GS, Williams, GJ, Franklin, EC, Kenyon, J, Cox, EF, Coles, S and TM Work. 2011. Patterns of coral disease across the Hawaiian archipelago: relating disease to environment. PLoS ONE 6(5):e20370.

Williams, G, Knapp, I, Aeby, G and S. Davy. 2011. Spatial and temporal patterns of scleractinian coral, soft coral, and zoathid disease on a remote, near-pristine coral reef (Palmyra Atoll, central Pacific). Dis Aquat Org 94:89-100.

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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 of 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