

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: Ruth D. Gates

Affiliation: Hawaii Institute of Marine Biology

Permit Category: Research

Proposed Activity Dates: 06/01/08-08/31/08

Proposed Method of Entry (Vessel/Plane): Hi'ilakai NOAA research vessel

Proposed Locations: Cruise Itinerary is unknown at this time and therefore this permit will cover activity for all atolls in the Monument to provide flexibility.

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

4

Estimated number of days in the Monument: 45

Description of proposed activities: (complete these sentences):

a.) The proposed activity would...
identify and monitor biological indicators of coral disease and/or bleaching and assess the diversity of coral-endosymbionts in Papahānaumokuākea.

b.) To accomplish this activity we would
sample coral colonies and other marine invertebrates and single-celled protists that harbor the same dinoflagellate symbionts as well as the surrounding reef waters and sediment to determine the molecular diversity of coral endosymbionts. These activities will be performed using SCUBA from small boat operations launched from the Hi'ilakai NOAA vessel.

c.) This activity would help the Monument by ...
identifying biological indicators of disease, revealing the diversity of coral-endosymbionts in Papahānaumokuākea, and developing capacity to predict the effects of global climate change on coral reef ecosystems in the monument.

Other information or background:

The scleractinian corals provide the structural and biological framework that supports the high diversity of marine organisms that inhabit coral reef ecosystems. As such, the health status and functional integrity of coral has profound ramifications for other members of these environments (Hoegh-Guldberg 1999). Corals are susceptible to a variety of environmental disturbances that include changes in seawater temperature, pH, salinity, UV light, pollution, and increased sedimentation (Brown 1997, Williams & Bunkley-Williams 1990, Barber et al 2001, Hoegh-Guldberg 1999). Many of these disturbances are predicted to increase in frequency and magnitude due to changes in the global climate, and these coupled with direct anthropogenic pressure has implications for even the most remote coral reef ecosystems (Hoegh-Guldberg 1999, Bellwood et al 2004). Corals respond to these environmental insults by losing their symbionts (bleaching) and/ or by exhibiting an increased susceptibility to coral diseases. These compromised biological states culminate in reduced growth and reproduction and ultimately, the death of the coral and the degradation of the habitat (Hoegh-Guldberg & Smith 1989, Jokiel & Coles 1977, Gleason & Wellington 1993, Goreau TF 1964, Kushmaro et al 1996, Glynn 1993). One of the most striking facets of corals that are exposed to deleterious conditions or exposed to disease agents is that they do not respond uniformly, that is, different species are differentially sensitive and members of the same species are not equally impacted or susceptible. At this point, our understanding of the biological factors that drive this variation in response is not well developed although we know that corals form intimate intracellular relationships with a variety of dinoflagellate symbionts, and the taxonomic specifics of these unions potentially influence the vulnerability of corals to environmental disturbance and disease causing agents. These symbionts belong to the genus Symbiodinium, a highly diverse group that show geographic, depth, and host specificity (LaJeunesse 2005, Stat et al 2006). Given the fundamentally important role that these symbionts play in coral biology, it is not surprising that the type of Symbiodinium that a coral hosts affects the growth rate and thermal tolerance of the colony (Little et al. 2004, Rowan 2004). Other biological traits that have been discussed as contributing to the vulnerability of specific corals to environmental disturbance and or disease agents include colony morphology and coral size (Loya et al 2001). Thus, a detailed understanding of morphological characteristics of the corals combined with a thorough characterization of the types of symbionts they harbor has the potential to be extremely informative about the sensitivity of the specific corals and reef assemblages to environmental shifts and disease agents.

Our previous work conducted on samples collected during September 2005 and May 2006 show that diseased *Acropora cytherea* contain a specific type of symbiotic dinoflagellate that is quite different from the type found in their healthy counterparts (Stat et al, submitted). The type of symbiont found in the diseased corals is extremely rare in the Pacific and has most likely been introduced from the Caribbean (Stat & Gates 2007). Given the implications for coral health, it is critical that we obtain an understanding of the prevalence and geographic spread of this rare symbiont in Papahānaumokuākea. The work covered by this permit request specifically addresses this need by examining the symbiont types harbored by a range of healthy and diseased corals across the Monument. In addition, we will test the hypothesis that morphological traits that can be non-destructively evaluated are informative with regard to the disease and/or bleaching susceptibility of a coral with a view to using these traits for monitoring the health of

corals on the monument and elsewhere in the future. We have collected samples from a variety of coral species within the Monument to determine if the symbiont type harbored in diseased *A. cytherea* is present in other coral species, and to understand the overall diversity of coral endosymbionts harbored by corals within the monument. This permit requests the continuation of this diversity study by sampling coral, marine invertebrates, protists, sediment, and water from atolls within Papahānaumokuākea.

REFERENCES:

- Barber R, Hilting A & Hayes M. 2001. The changing health of coral reefs. *Human and Ecol. Risk Assessment* 7(5):1255-1270.
- Bellwood DR, Hughes TP, Folke C, Nystrom M. 2004. Confronting the coral reef crisis. *Nature* 429 (6994): 827-833.
- Brown B. 1997. Coral bleaching: causes and consequences. *Coral Reefs* 16: S129-138.
- Gleason DF & Wellington GM. 1993. Ultraviolet radiation and coral bleaching. *Nature*. 365: 836-838.
- Goreau TF. 1964. Mass expulsion of zooxanthellae from Jamaican reef communities after hurricane Flora. *Science*. 145: 383-386.
- Glynn PW. 1993. Coral reef bleaching: ecological perspectives. *Coral reefs*. 12: 1-17.
- Goreau TF. 1964. Mass expulsion of zooxanthellae from Jamaican reef communities after hurricane Flora. *Science*. 145: 383-386.
- Hoegh-Guldberg O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. *Mar. Freshwater Res.* 50:839-866.
- Hoegh-Guldberg O & GJ. 1989. The effect of sudden changes in temperature, light and salinity on the population density and export of zooxanthellae from the reef corals *Stylophora pistillata* Esper and *Seriatopora hystrix* Dana. *J. Exp. Mar. Bio. Eco.* 129: 279-303.
- Hoegh-Guldberg O, Smith GJ (1989) The effect of sudden changes in temperature light and salinity on the population density and export of zooxanthellae from the reef corals *Stylophora pistillata* espera and *Seriatopora hystrix* Dana. *Journal of Experimental Marine Biology and Ecology* 129: 279-304.
- Jokiel PL & Coles SL. 1977. Effects of temperature on the mortality and growth of Hawaiian reef corals. *Mar. Bio.* 43: 201-208.
- Kushmaro A, Loya Y, Fine M, Rosenberg E. 1996. Bacterial infection and coral bleaching. *Nature*. 380: 396.
- LaJeunesse TC. 2005. "Species" radiations of symbiotic dinoflagellates in the Atlantic and Indo-Pacific since the Miocene-Pliocene transition. *Mol. Biol. Evo.* 22: 570-581.
- Little AF, van Oppen MJH, Willis BL. 2004. Flexibility in algal endosymbiosis shapes growth in reef corals. *Science*. 304: 1492-1494.
- Loya Y, Sakai K, Yamazato K, Nakano Y, Sambali H, van Woesik R. 2001. Coral bleaching: the winners and the losers. *Ecology Letters* 4 (2): 122-131.
- Rowan R. 2004. Thermal adaptation in reef coral symbionts. *Nature*. 430: 742.
- Stat M, Carter D, Hoegh-Guldberg O (2006) The evolutionary history of Symbiodinium and scleractinian host – Symbiosis, diversity and the effect of climate change. *Perspectives in Plant Ecology, Evolution and Systematics* 8: 23-43.
- Stat M & Gates R (2007) Vectored introductions of marine endosymbionts into Hawaii. *Biological Invasions*, online first.

Williams E & Bunkley-Williams. 1990. The worldwide coral reef bleaching cycle and related sources of coral mortality. *Atoll Res. Bull.* 335: 1-63.

Section A - Applicant Information

1. Applicant

Name (last, first, middle initial): Gates, Ruth, D.

Title: Associate Research Professor

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

Michael Stat

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

Phone:

Fax:

Email:

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/University of Hawaii

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):

Michael Stat (Field PI)

Xavier Pochon (Research Diver)

Anderson Mayfield (Field Technician)

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			

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

Location Description:

The following GPS coordinates bound the region surrounding each atoll where sampling will occur. The activity will be ocean based in shallow (5 ft) to deep (80 ft) coral reef waters. The exact locations are unknown, which is dependent on coral location, presence of disease/bleaching, and the final NOAA cruise itinerary. Exact coordinates will be provided in the post cruise summary.

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:*

The purpose of this research is to identify robust biological indicators of coral disease and bleaching susceptibility. Specifically, we will characterize the diversity of symbiotic dinoflagellates (Symbiodinium) harbored by corals and examine morphological traits in healthy, diseased and bleached corals in order to identify biological traits that correlate with health state. In addition, we will evaluate the diversity of Symbiodinium in corals, other marine invertebrates and protists (Foraminifera) from Papahānaumokuākea, and free-living in the surrounding waters and sediment to examine the prevalence and geographic spread of Symbiodinium that render corals disease and/or bleach susceptible and to understand the diversity present in the Monument.

We hypothesize that:

- 1) Specific coral-Symbiodinium assemblages render corals susceptible to disease and bleaching in Papahānaumokuākea
- 2) Morphological traits in corals correlate with disease and bleaching in Papahānaumokuākea
- 3) A high diversity of Symbiodinium exist in corals, Foraminiferan, seawater and sediment surrounding the reef in Papahānaumokuākea

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 personnel listed on this permit have attended cultural briefings on the significance of Papahānaumokuākea. Our research aims to learn more about this ecosystem and provide the science to help conserve and protect it. Our impact on the environment is minimal, and does not involve the removal of whole marine invertebrate organisms. We will only take a small biopsy of each animal sampled and therefore preserve the integrity of life and the ecosystem in the monument.

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 sampling technique will not impact the ecological integrity of the coral reef ecosystem. The small biopsy that we remove from each colony will heal in a short period of time (weeks) and is significantly less impact than what is caused by natural predators of these organisms. We have already shown the wealth of information that can be returned from this sampling through peer reviewed publications (Stat & Gates 2007, Stat et al, in review). Our research has identified a type of endosymbiont that has been introduced into Hawaii that is associated with diseased

compromised coral. We are identifying indicators of coral disease that can be used to monitor the health state of the ecosystem over time.

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.

Our research aims at identifying the causes of coral reef degradation and its distribution in Papahānaumokuākea. These causes and their effects on ecosystem health can only be conducted and monitored in the location in which you want to manage. Therefore our research can only be performed 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?

The end value of our activity is to contribute to the knowledge base necessary to effectively manage the Monument. The benefits of attaining this knowledge far outweigh the minimal costs of taking small non-lethal biopsies from the marine invertebrate, and the collections of protists, water and sediment collected. Our sampling has a very minor effect on the reef invertebrates who recover in a short period of time (weeks).

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

Our diversity study will map the patterns of coral endosymbionts in the Monument and provide a baseline for future comparisons to understand community shifts. To achieve this we need to sample at all the atolls. Monitoring of the coral endosymbiont community can then be achieved by sampling the water column and colonies showing disease/bleaching. This is an ongoing monitoring project, where the sampling is non-lethal, and will provide data to management on changes in the community structure of the ecosystem. Once the baseline diversity is determined, monitoring the environment can be achieved on a single cruise on an annual basis.

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

The PI and other scientists on the application are qualified coral reef biologists, recognised on an international level. They have the background and resources to conduct and complete the stated activity. There are no potential impacts from the outlined activities that we foresee.

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.

The science conducted in this project is performed at the Hawaii Institute of Marine Biology which houses all the facilities and infrastructure needed to perform the data analysis which is supported by funding from a partnership with Papahānaumokuākea Monument Office.

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 research focuses on using Molecular biology and DNA analysis. To achieve this we only need small biopsies in order to extract DNA. We understand that all life is sacred in the

Monument, and that coral represents the first life in Hawaiian culture. Our sampling is non-lethal and will not have an ongoing or long term impact on the environment.

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 vessel is a federal owned NOAA ship, Hi'ialakai.

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

The applicant can not foresee any other factors that find issuance of the permit inappropriate.

8. Procedures/Methods:

A detailed sample list and the collection sites are provided in Appendix 1.

To satisfy hypothesis 1: A maximum of 30 coral colonies (5 species; 10 healthy, 20 unhealthy) will be sampled from reefs surrounding each atoll to determine if there is a correlation between disease/bleaching susceptibility and the type of Symbiodinium harbored. This number is much greater than what the actual "take" number will be, as the occurrence of bleaching and disease in all the listed species at all atolls is extremely rare. In fact, the previous 2 cruises on which we had requested this sample size, resulted in a "take" of approximately 20% of the requested total. This sample list requested thus provides flexibility and represents a perfect world scenario that will most likely never be encountered in nature.

To satisfy hypothesis 2: Coral colonies sampled that are unhealthy vs healthy will be photographed for further morphological analysis.

To satisfy hypothesis 3: A total of 5 colonies per marine invertebrate species, 20 Foraminifera, 20 5L water samples, and 20 1ml sediment samples from reefs surrounding each atoll will be sampled to determine the diversity of Symbiodinium inhabiting Papahānaumokuākea.

The samples taken from corals is a very small biopsy (<1cm). This sampling strategy is extremely minimal and poses no threat to the health and survival of the sampled coral. Infact the removal of each biopsy is significantly less damaging than the tissue damage inflicted by marine organisms that feed on coral tissue, such as in the area parrot fish.

Marine invertebrates and Foraminifera will be collected using SCUBA with a chisel, pliers or scissors and placed into plastic collection bags. The samples will be frozen and transported to the Hi'ialakai. Foraminiferan, which are single-celled calcifying protists that resemble a grain of sand, will be identified using microscopy. The tissue from all samples will be placed into individual 1.5 ml microcentrifuge tubes containing DNA preservation buffer which destroys the integrity of the living material and poses no threat for the spread of living organisms. The remaining calcium carbonate skeleton and tissue will be bleached to kill all living material. The samples and skeletons will be stored at the Hawaiian Institute of Marine Biology for further downstream analyses upon return to Honolulu.

For the detection of free-living Symbiodinium, water samples will be collected using a plastic container and sediment samples using a syringe. The samples will be filtered on board the Hi'ilakai and the filter paper containing the organic material will be stored in individual 1.5 ml microcentrifuge tubes in DNA preservation buffer.

All SCUBA operations will be conducted off small vessels launched from the Hi'ilakai NOAA research vessel.

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:
see Appendix 1

Scientific name:
see Appendix 1

& size of specimens:
see Appendix 1

Collection location:
see Appendix 1

Whole Organism Partial Organism

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

DNA and the remaining material will be archived at the Hawai 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 stored in 1.5 ml microcentrifuge tubes containing DNA preservation buffer that destroys living material. Remaining skeletons will be bleached to destroy left-over living material and stored in sealed bags. These samples will remain on board the Hi'iialakai and then transported to the Hawaii Institute of Marine Biology upon return to Honolulu.

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

Samples that overlap with the Rappe, Toonen, and Karl lab will be shared.

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

SCUBA equipment
pliers
hammer
chisel
fishing collection bag to hold samples
plastic collection bags
DNA preservation buffer
ethanol
bleach
MQ water
filter unit and vacuum pump
plastic containers for water
syringes
razor blades
filter paper
camera and underwater housing
laptop computer

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

bleach
ethanol
DNA preservation buffer (contains Guanadinium isothiocyanate, 2-mercaptoethanol)

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:

Sample, data analysis, and manuscript submission will be completed by the end of 2009.

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

Stat M and Gates RD. (2007) Vectored introductions of endosymbiotic dinoflagellates into Hawaii. Biological Invasions, DOI:10.1007/s10530-007-9167-0.

Stat M, Morris E, Gates RD. Functional diversity in coral-dinoflagellate symbiosis. Submitted to PNAS.

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