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: Megan Donahue
Affiliation: Hawaii Institute of Marine Biology

Permit Category: Research
Proposed Activity Dates: 06/01/12-11/15/12
Proposed Method of Entry (Vessel/Plane): R/V Hi'ialakai
Proposed Locations: Shallow water reef (<100 ft depth) focused on bioeroder communities in forereef and lagoon habitats. Specific locations for the study will depend on cruise logistics but will include forereef sites at FFS, LIS, PHR, and KUR and lagoon sites at MID.

Estimated number of individuals (including Applicant) to be covered under this permit: 4
Estimated number of days in the Monument: 50

Description of proposed activities: (complete these sentences):

a.) The proposed activity would…

measure bioerosion rates and bioeroder community composition on reefs in the NWHI to evaluate whether internal bioeroders can serve as indicators of community response to ocean acidification on coral reefs. Taking advantage of variation in pH at large and small spatial scales, we will test whether the total bioerosion rate and/or the community composition of internal bioeroders responds to natural spatial variation in pH or other environmental drivers along the Archipelago. Bioerosion rates will be measured using microCT scans of coral blocks to get a 3D image of the eroded material; this method gives a better estimate of bioerosion rate than the traditional buoyant weight technique and allows characterization of distinct bioeroder groups. Community composition will be measured using a ReefChip, a molecular microarray that will be customized to detect and quantify the bioeroder community. If effective, this method would be an efficient and inexpensive way to detect community level effects of ocean acidification in remote areas.

b.) To accomplish this activity we would ….

(i) measure bioerosion rates by installing small calcium carbonate blocks (5x5x2cm) on reef substrate at each site. Five calcium carbonate blocks were deployed at 15 forereef sites (5 sites each at FFS, LIS, PHR) and 20 blocks were deployed at one lagoon site (MID) during the July-August 2011 cruise to the PMNM (a site is a 20m x 20m area of reef). If cruise logistics allow, we will deploy additional blocks this year at 5 forereef sites at Kure. These blocks act as a settling substrate for bioeroding organisms. Prior to deployment, each block is scanned by microCT (to create a 3D image of the block) and autoclaved. On the upcoming cruise, we will retrieve these calcium carbonate blocks, rescan with microCT, calculate bioerosion rates, and assess the bioeroder community composition at each site. At each forereef site, blocks were attached to calcification acidification units (CAUs) previously deployed by NOAA's Coral Reef Ecosystem Division (CRED). At the lagoon site, blocks were affixed to dead reef substrate.

(ii) measure variation in the natural occurring bioeroder community. On the 2011 cruise to the PMNM, we collected 10 small pieces (5x5x5cm) of dead coral skeleton at each of 16 sites (5 sites on FFS, 5 sites on LIS, 5 sites on PHR, and 1 site on MID). These pieces of reef substrate have been reserved for identification using molecular approaches (the ReefChip microarray). This year, we are requesting permission to take additional samples for traditional taxonomic identification; these additional samples are critical for providing vouchered taxonomic specimens to associate with the molecular sequences we find in our samples from 2011 and put on the ReefChip microarray. To get adequate taxonomic coverage across the Archipelago, we request permission to take up to 10 additional samples (5x5x5cm) at each of the 16 sites from the 2011 cruise; in addition, we request permission to take up to 20 samples from each of five new sites on Kure.

(iii) relate bioerosion rates to environmental data collected by NOAA CRED (including, pH, nitrate, dissolved inorganic carbon, temperature, salinity, and chlorophyll)

c.) This activity would help the Monument by …
evaluating whether internal bioeroders can serve as indicators of community response to ocean acidification on coral reefs. The community structure and function of bioeroding organisms may have a major effect on coral reef resilience: the sponges, polychaete worms, and tiny mollusks that comprise bioeroder communities control the strength and complexity of the coral reef framework, which is the habitat for more charismatic coral reef organisms. Shifts in the composition and functioning of these out-of-sight, but fundamental members of coral reef ecosystems may change the accretion-erosion balance of coral reefs. The methods developed here will help managers anticipate the likely effects of ocean acidification on bioeroder communities and bioerosion rates. If effective, this method would be an inexpensive way to detect community level effects of ocean acidification in remote areas.

Other information or background: All forereef sites are co-located with NOAA-CRED permanent sites. This minimizes the impact to the reefs and faciliates sharing of information.

Section A - Applicant Information

1. Applicant

Name (last, first, middle initial): Donahue, Megan J.

Title: Assistant Researcher, Hawaii Institute of Marine Biology

1a. Intended field Principal Investigator (See instructions for more information):
Nyssa Silbiger, graduate student

2. Mailing address (street/P.O. box, city, state, country, zip): Hawaii Institute of Marine Biology, [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 (HIMB), 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):
Megan Donahue, PI, research diver
Nyssa Silbiger, Field PI, research diver
Holly Bolick, research diver
Scott Godwin, research diver
Un-named Individual, research diver

Section B: Project Information

5a. Project location(s):

		Ocean Based	
☒ Nihoa Island	☐ Land-based	☒ Shallow water	☐ Deep water
☒ Necker Island (Mokumanamana)	☐ Land-based	☒ Shallow water	☐ Deep water
☒ French Frigate Shoals	☐ Land-based	☒ Shallow water	☐ Deep water
☒ Gardner Pinnacles	☐ Land-based	☒ Shallow water	☐ Deep water
☒ Maro Reef			
☒ Laysan Island	☐ Land-based	☒ Shallow water	☐ Deep water
☒ Lisianski Island, Neva Shoal	☐ Land-based	☒ Shallow water	☐ Deep water
☒ Pearl and Hermes Atoll	☐ Land-based	☒ Shallow water	☐ Deep water
☒ Midway Atoll	☐ Land-based	☒ Shallow water	☐ Deep water
☒ Kure Atoll	☐ Land-based	☒ Shallow water	☐ Deep water
☐ Other			

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

Location Description:
Specific locations for the study will depend on cruise logistics, but out target sites are:

Island/Atoll	Site Name	Latitude	Longitude
French Frigate Shoals	FFS-34	23.62792	-166.13538
French Frigate Shoals	FFS-12	23.63835	-166.18005
French Frigate Shoals	FFS-H6	23.88046	-166.27306
French Frigate Shoals	FFS-21	23.84695	-166.32695
French Frigate Shoals	FFS-33	23.83651	-166.26669
Midway Atoll	MID-H11A	28.217667	-177.403217
Midway Atoll	MID-H11B	28.2175	-177.40305
Pearl and Hermes Atoll	PHR-39	27.94045941	-175.8613056
Pearl and Hermes Atoll	PHR-44	27.91026	-175.90483
Pearl and Hermes Atoll	PHR-42	27.75312882	-175.9489414
Pearl and Hermes Atoll	PHR-R26	27.78583	-175.78028
Pearl and Hermes Atoll	PHR-R33	27.78546679	-175.82355
Lisianski Island Marine Area	LIS-09	25.9580487	-173.8823619
Lisianski Island Marine Area	LIS-R10	25.94461746	-173.9536197
Lisianski Island Marine Area	LIS-18	26.00425931	-173.99409
Lisianski Island Marine Area	LIS-R14	26.07838458	-173.9970317
Lisianski Island Marine Area	LIS-R9	26.03954921	-174.0124643
Kure Atoll	KUR-12	28.382308	–178.324479
Kure Atoll	KUR-33	28.416767	–178.378433
Kure Atoll	KUR-02	28.453633	–178.344017
Kure Atoll	KUR-04	28.426650	–178.285870
Kure Atoll	KUR-06	28.386780	–178.347920

However, cruise logistics will influence the specific locations for our study, so I have listed all possible sites below. This ensures maximum flexibility due to weather or unforeseen changes to our cruise schedule. All activities will occur within the area outlined by the following coordinates.

Location:	Longitude	Latitude
Kure Atoll	-178.19706492000	28.55825235580
Kure Atoll	-178.19623585400	28.29958375730
Kure Atoll	-178.45987884800	28.29958375730
Kure Atoll	-178.46070791400	28.55742328970
Midway Atoll	-177.19638223300	28.37419969920
Midway Atoll	-177.19721129900	28.13377055310
Midway Atoll	-177.52800864100	28.13459961920
Midway Atoll	-177.52800864100	28.37419969920
Pearl and Hermes Atoll	-176.08850981800	28.04643025580
Pearl and Hermes Atoll	-175.63289162600	28.04539944540
Pearl and Hermes Atoll	-175.63289162600	27.70729363750
Pearl and Hermes Atoll	-176.08954062900	27.70626282710
Lisianski Island	-173.67292570900	26.25150771120
Lisianski Island	-173.67292570900	25.83942708400
Lisianski Island	-174.23095155800	25.83942708400
Lisianski Island	-174.23095155800	26.25150771120
Laysan Island	-171.47900122300	25.96027179830
Laysan Island	-171.47725234300	25.65596666490
Laysan Island	-171.97918092500	25.65771554490
Laysan Island	-171.97918092500	25.96202067840
Maro Reef	-170.18133220600	25.69968866680
Maro Reef	-170.17958332600	25.21524888540
Maro Reef	-171.00505472200	25.21524888540
Maro Reef	-171.00505472200	25.69968866680
Gardner Pinnacles	-167.74832319300	25.26070709440
Gardner Pinnacles	-167.75087047400	24.34878019150
Gardner Pinnacles	-168.36221811900	24.35132747340
Gardner Pinnacles	-168.36476540100	25.26070709440
French Frigate Shoals	-165.93465851400	23.94630965900
French Frigate Shoals	-165.93465851400	23.56421738120
French Frigate Shoals	-166.45685129400	23.56421738120
French Frigate Shoals	-166.45685129400	23.94630965900

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:***
Bioerosion, the removal of CaCO3 reef structure by biological agents (Neumann 1966), is a natural process that influences the mechanical stability, structural complexity, and net accretion rate of coral reefs. Extensive bioerosion can compromise the mechanical stability and structural complexity of reefs, thereby increasing susceptibility to storm damage (Hutchings 1986) and decreasing habitat availability for other reef organisms (Hoegh-Guldberg et al. 2007), and organisms that rely on emergent land, including Hawaiian monk seals, sea turtles, and sea birds. Bioeroders may be classified into three functional groups: microborers (e.g., euendoliths), macroborers (e.g., sponges, polychaetes, and bivalves), and grazers (e.g., urchins and fish). Micro- and macroborers erode the interior of reef substrate and are typically more abundant in dead coral substrate than live coral (Highsmith 1981). In the PMNM, micro- and macro-borers communities have remained largely unstudied and, although grazer density has been estimated on a few reefs, erosion rates due to bioeroders of any group have never been measured directly.

The community of bioeroders are a good target for detecting community changes in response to ocean acidification: (i) bioerosion is integral to long-term reef sustainability (Grigg 1982), (ii) bioerosion rates are sensitive to pH (Tribollet et al 2009), (iii) bioeroder community composition may shift in response to changes in pH, and (iv) applying new technologies will allow the efficient measurement of bioerosion rates and community composition that is critical for managers. The effective use of bioerosion rates as a monitoring and management tool requires distinguishing the effects of ocean acidification from other environmental parameters; this is the challenge that motivates this project.

Anthropogenic climate change is an environmental threat that challenges conventional management solutions. The 38 Gt of anthropogenic carbon dioxide (CO2) emitted each year has resulted in the highest concentrations of atmospheric CO2 in the last 740,000 years (Petit et al 1999), resulting in increased sea-surface temperature, sea-level rise, and alteration of the carbon cycle in our oceans (IPCC 2007). In Hawai'i, the Hawai'i Ocean Timeseries has detected a 0.075 decrease in mean annual pH at Station Aloha over the past 20 years (Doney et al 2009); globally, a further decrease of 0.14-0.35 pH units is predicted for the 21st century (IPCC 2007). Despite these predictions, the effects of ocean acidification on coral reef communities are unknown and unregistered because we lack effective monitoring tools.

Available predictions of pH in the coastal zones (Orr et al 2005, IPCC 2007) are based on models of open ocean values. Applying these predictions to coral reef ecosystems is complicated by new data highlighting the temporal and spatial variability of pH in coastal waters (Gagliano et al 2010). These new studies show substantial small scale variation in pH within and between reef habitats, including a range of natural variation that can be as large as predicted changes in ocean acidification at the global scale (Gagliano et al 2010, K. Anthony, pers comm). For instance, recent work shows pH variation from 7.82 to 8.12 in the shallow waters of Kane'ohe Bay (Miles 2010). This is not unexpected: studies of reef metabolism

indicate that these differences in pH may be influenced by relative abundance of respiring and photosynthesizing organisms, flushing rate of the overlying water mass (and, therefore, the presence and thickness of boundary layers), and the history of the water mass. While this variation in pH complicates our predictions of coral reef response to ocean acidification, it also provides an opportunity to examine community-level responses to pH variation and, further, how communities may respond to future change.

In the proposed project, we take advantage of natural variation in the pH over small spatial scales in lagoonal reefs and at large scales over the Hawaiian Archipelago to examine how bioeroding communities may respond to ocean acidification and to test the effectiveness of using bioerosion rates and bioeroder communities as indicators of climate change in remote coral reef systems. We include forereef sites to decrease the within-site variation and examine Archipelago-wide patterns. Bioeroder community composition will be assessed using a combination of taxonomic and molecular techniques, including the ReefChip microarray, which is under development in Dr. Rob Toonen's laboratory. The ReefChip allows species-specific identification of organisms in a mixed environmental sample. Concomitant benefits of the project include accurate measurement of crustose coralline algal growth using microCT and tests for the presence of undetected alien species in the NWHI using the ReefChip technology.

The specific objectives identified for this project are:
1) Characterize variation in bioeroder community composition within reefs and across the Archipelago using ReefChip
 a)Contribute sequences to ReefChip to customize it to the bioeroding community of the Hawaiian Archipelago
 b)Using ReefChip, test for undetected alien species among bioeroders of the NWHI
2) Measure bioerosion rates using microCT technology
 a) Compare $CaCO_3$ loss within and between reefs across the Archipelago
 b) Measure $CaCO_3$ accretion by CCA
 c) Use 3-dimensional reconstructions to associate specific patterns of erosion with specific taxa
3) Evaluate the relationship between pH, bioerosion rate, and bioeroder community composition

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?

We are a team of conservation biologists, teaching and studying the science of how best to manage and conserve the ecological integrity of marine ecosystems. Therefore, minimizing our impact to the ecosystem we are trying to conserve is a natural and inherent part of any research we conduct within the Monument. It is my goal to inculcate in students and trainees that work with me a respect for the resources that we study. This respect requires that we carefully consider the impact of our study design, that our study design is robust and will produce useful results, and that our work is disseminated to scientists and managers to improve the conservation efforts in these systems. In developing our research methods, we have taken care to minimize any potential negative impacts to the system as outlined in the methods section below. We believe that we have implemented every reasonable safeguard for the natural resources and ecological integrity of the Monument in our research, and we do not expect any detectable impact from our research sampling. As outlined in detail below, our sample size and methodologies have all been selected to provide robust and scientifically rigorous information to managers with the least possible impact to the natural resources of the Monument.

Our work will not impact historic resources: we do not set foot on land within the Monument, and we report but do not touch any submerged artifacts discovered during our diving activities.

As in previous years, each participant is required to participate in a Cultural Briefing prior to departure on the Hi'ialakai. Each member of my team is aware of the unique ecological status of the Monument, and this briefing reminds all team members of the cultural significance of the place. However, this separation of natural, cultural, and historic resources is itself a western construct. Stewardship of natural resources is a central theme in the relationship that Hawaiians have with the natural world and, thus, there is no difference between a natural and cultural resource.

Papahanaumokuakea is a sacred place to native Hawaiians; a place that is included in the oral history of chants and meles; a place where native Hawaiians have travelled for hundreds of years. We strive to approach our work in the Monument with the same humility, wonder, and regard for the natural world as these travelers. We intend that our research in the Monument will give a strong foundation to stewardship practices that best manage and protect the coral reefs ecosystems of Papahanaumokuakea. Native Hawaiians learned when and where important food fish were spawning and, understanding their potential impact on fish populations, protected these times and areas. In a similar way, we will be learning about the bioeroding communities of the Monument and trying to understand and mitigate the impacts of anthropogenic climate change

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 research we propose here is the type of research directly mandated by the Proclamation: it is "research designed to further understanding of monument resources and qualities… [and] will assist in the conservation and management of the monument". The research we propose is necessary to both maintain ecosystem integrity and provide for adaptive ecosystem management

in the face global climate change. As outlined above and below, our activities have no detectable effect to diminish Monument resources, nor have any known indirect, secondary or cumulative effects on the ecosystem or resources therein. Because of concerns about cumulative impacts, a threat assessment of the activities in the Monument have been conducted (Selkoe et al. 2008), and a compiled cumulative impact threat map of the Monument (Selkoe et al. 2009) has been provided to the co-trustees for use in future management decisions.

Our proposed activities are minimally invasive. On forereefs, coral blocks were attached to permanent transect stakes and CAUs (artificial units that measure accretion rates) with cable ties that were previously installed by NOAA's Coral Reef Ecosystem Division (CRED). On lagoon reefs, coral blocks were attached to dead substrate with marine epoxy, carefully avoiding live coral. These blocks were deployed on the July-August, 2011 NOAA cruise to the PMNM. This year, we will retrieve the blocks deployed last year and, if cruise logistics permit, deploy blocks at five forereef sites at Kure, where we were unable to deploy last year. The small samples of dead coral skeleton (5x5x5cm) that we plan to collect from reefs are a tiny fraction of the reef substrate removed naturally by external bioeroders (e.g., urchins, parrotfish). Negative impacts on the reefs, atoll, and Monument are exceedingly small, while the positive impacts of the results of our research are Monument-wide.

Our overriding goal is to provide scientific information to managers so that the Papahanaumokuakea Marine National Monument can be managed and protected based on policy grounded in sound science. Our divers are experienced in moving in and around coral and coral reefs so as to not cause damage. Each diver has been through intensive dive training and is a certified scientific diver with the American Association of Underwater Scientists. We are conducting these activities already in Kane'ohe Bay, allowing us to hone our methods to minimize impacts on the Monument.

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 are no alternatives to conducting this activity within the monument. Our research is aimed at understanding how bioerosion processes shift along the Hawaiian Archipelago. There is no practicable alternative to doing this in the Monument because it is the reefs in the Monument that will need to be managed. For example, the same information from reefs in the main Hawaiian Islands is interesting – indeed, we are pursuing a similar study in Kaneohe Bay-- but there is no basis upon which to say that the reefs in the Monument are like the Main Hawaiian Island reefs. In fact, we know they are not the same -- Kaneohe Bay has many introduced species that are not present in the Monument, and a concomitant benefit of our study is understanding the potential impacts of these introduced species.

d. How does the end value of the activity outweigh its adverse impacts on Monument cultural, natural and historic resources, qualities, and ecological integrity?
We anticipate truly negligible impact of our study on the resources of the Monument and, therefore, believe that the end value of this research clearly outweighs that imperceptible impact. Further, an understanding of bioerosion rates across this region will greatly increase the decision

making capacity of the co-trustees in dealing with the potential impacts of global climate change within the Monument

e. Explain how the duration of the activity is no longer than necessary to achieve its stated purpose.
It is anticipated that retrieving the coral blocks, collecting pieces of dead coral skeleton, and collecting associated data will take 2-3 days per atoll with 2-4 divers. We are proposing to study 5 forereef sites at each of 3-4 atolls (retrieval at FFS, LIS, PHR; deployment at KUR) and one lagoon site at Midway Atoll. Given preliminary cruise itineraries, we will need to participate on two cruises to retrieve currently deployed blocks. As such, the estimated number of days in the monument (50 days) is necessary to accomplish the research goals outlined in this permit application.

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 an AAUS certified scuba diver and NAUI instructor for 18 years. I have used diving for research and trained others to dive on projects in the Gulf of Maine, California, and Hawaii, including research in other protected areas like the Channel Islands National Park. I have a PhD in Ecology from the University of California, Davis and have publications on marine ecology and spatial population dynamics relevant to this study. This is my second permit applicatio for work in the Monument. I was privileged to enter the Monument on the July-August, 2011 cruise to deploy calcium carbonate blocks for the project outlined in this application and on the May 2010 cruise to support other projects, including Scott Godwin's (PMNM) surveys of invasive species and Rob Toonen's connectivity sampling. My experience on previous cruises has been excellent preparation for the study proposed here.

The field PI for the July cruise (anticipating collection at PHR, LIS, FFS, and deployment at KUR) is Nyssa Silbiger. She was a field PI on the July-August 2011 cruise and also assisted Derek Smith on the May 2010 cruise. Nyssa is a graduate student in my laboratory and an experienced coral reef diver; her masters research was performed at Aquarius, an underwater ocean laboratory located in the Florida Keys National Marine Sanctuary.

The field PI for the June cruise (anticipating collection at MID) is Scott Godwin. Scott serves as Resource Protection Specialist for PMNM and has been chief scientist on multiple cruises to PMNM.

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 project proposed here is a collaboration between the Donahue (sampling of bioeroders) and Toonen (ReefChip) laboratories at the Hawaii Institute of Marine Biology, and NOAA CRED (site coordination and environmental data). We anticipate funding through Hawaii SeaGrant for this project: we have been notified of our inclusion in the 2012-2014 Hawaii SeaGrant omnibus to support this work. In addition, there are adequate finances in the Donahue lab and through the PMNM-HIMB partnership to conduct and complete all the research outlined

herein. This research is currently or has been previously funded by a combination of the NWHI PMNM-HIMB partnership, the University of Hawaii, and NSF.

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 choice of sites will be guided by the vessel and Monument staff while aboard the NOAA vessel Hi'ialakai. We generally avoid any sites that are identified as culturally significant, and focus our activities in regions that maximize the safety of the crew while ensuring that the proposed work will be completed. The questions we are addressing are central to understanding reef erosion processes and the Monument's response to global climate change. Any negative impacts of our study are minimal and temporary and should not alter the Monument's cultural, natural and historic resources, qualities or ecological integrity. The positive impacts of our study will help guide appropriate stewardship practices to preserve and manage the qualities and integrity of the Monument's cultural and natural and historic resources. Our data is necessary to provide a strong scientific understanding of coral reef ecosystem processes by which proper management protocols can be designed. These data also are invaluable in providing a baseline with which to monitor the success of management efforts.

i. Has your vessel has been outfitted with a mobile transceiver unit approved by OLE and complies with the requirements of Presidential Proclamation 8031?
We will be on board NOAA vessel Hi'ialakai

j. Demonstrate that there are no other factors that would make the issuance of a permit for the activity inappropriate.
In 2011, Donahue held a permit for similar research activities in the Monument and demonstrated compliance with all permit and reporting requirements.

8. Procedures/Methods:
There are two aspects to the study(i) characterizing the bioeroding community in dead coral substrate (ReefChip) and (ii) measuring bioerosion rates using experimental coral blocks (microCT). Each aspect of the study leverages cutting edge technology to rapidly advance our understanding of bioerosion on reefs and accelerate the development of effective tools for managers.

For the overall study, we expect to spend 1 day the Midway lagoon site and a half-day at each of 20 forereef sites (FFS, LIS, PHR, KUR), depending on cruise logistics. Below we describe the sampling methodology for each of these sites.

SAMPLING
On the 2011 cruise to the PMNM, we collected 10 small pieces (5x5x5cm) of dead coral skeleton at each of 15 sites (5 sites on FFS, 5 sites on LIS, 5 sites on PHR), and 20 small pieces at 1 site on MID. These pieces of reef substrate have been preserved and will be used for

identification using molecular approaches (the ReefChip microarray). This year, we are requesting permission to take additional samples for traditional taxonomic identification; these additional samples are critical for providing vouchered taxonomic specimens to associate with the molecular sequences we find in the 2011 samples and put on the ReefChip microarray. To get adequate taxonomic coverage across the Archipelago, we request permission to take up to 10 additional samples (5x5x5cm) at each of the 16 sites from the 2011 cruise; in addition, we request permission to take up to 20 samples from each of five new sites on Kure. For each Kure site, 10 of these samples will be preserved for identification using molecular approaches and up to 10 separate samples will be collected specifically for traditional taxonomic identification and vouchering. The maximum number of dead coral skeleton samples (5x5x5cm) proposed for the Monument in 2012: 260 = 10 x 16 sites (5 sites FFS, 5 sites PHR, 5 sites LIS, 1 site MID) + 20 x 5 sites (KUR) = 0.0325 cubic meters.

For the taxonomic collections taken at all sites, these samples will be taken in seawater back to the ship. These samples of dead coral skeleton contain numerous epibiotic and bioeroding organisms. Shipboard, each sample will be placed in approximately 250 mL of seawater; a drop of clove oil (5% clove oil solution suspended in 95%EtOH, MSDS attached) will be added to the sample to evacuate the organisms from the coral skeleton. The sample will then be dyed with rose bengal (MSDS attached) and preserved. This process facilitates taxonomic preservation and identification. The samples will be will be stored in vials of >70% ethanol or saturated salt buffer at room temperature, given a unique sample number, and archived in a database. Upon return to HIMB, organisms will be carefully extracted from the coral skeleton. These organisms will be identified, vouchered, and sequenced in cooperation with Holly Bolick of the Bishop Museum, Scott Godwin of PMNM, and the laboratory of Rob Toonen at HIMB. For a small number of organisms, particularly sponges, we may need to send samples to other taxonomic experts for identification. As this need arises, we will contact the Monument to facilitate permissions for appropriate transfer of Monument material.

For the collections to be used for molecular sequencing (only at Kure in 2012), samples will be brought back to the ship and preserved in >70% ethanol. Upon return to HIMB, these samples will be crushed or dissolved in an acid solution to extract the bioeroding organisms from the coral skeleton and homogenized for analysis on the ReefChip (see below). All samples will be maintained in perpetuity and future permit requests for DNA sampling of species in the NWHI can be redirected to the existing tissue sample "museum" that will result from our collections.

COMMUNITY COMPOSITION USING REEF CHIP

The ReefChip is a specialized microarray composed of an array of short DNA fragments (25-35 nucleotides), each of which differentially binds to the DNA of a specific species, that are attached to a specially polished and chemically coated glass microscope slide in a process called "printing". Each unique DNA fragment, or probe, is placed in a specific position on the slide so that we can keep track of identity of the probes. Next, an environmental sample is collected (here, a piece of coral skeleton with its associated bioeroding community that has

been collected and homogenized) and the DNA of all species present is isolated and extracted from the sample. The extracted environmental DNA sample is broken into smaller fragments and fluorescent molecules are attached, thereby labeling the environmental DNA. The labeled, double-stranded environmental DNA is separated into single strands and hybridized to the DNA "capture" probes on the ReefChip, forming double stranded DNA where one strand is a capture probe and the other strand is the labeled environmental DNA. The excess labeled environmental DNA that did not match and, therefore, did not bind to the capture probes is washed away, leaving only the targeted environmental DNA that has been captured by the ReefChip. Finally, the ReefChip is dried and scanned at the wavelengths emitted by the fluorescent labels attached to the environmental DNA, producing an image of fluorescing (positive identification of a targeted species) and non-fluorescing spots (absence of a targeted species). Further, the intensity of the fluorescence of each spot can be used to estimate the quantity of the targeted species in the environmental sample.

ReefChip technology will allow us to detect, quantify, and compare species assemblages throughout the Hawaiian Archipelago at a scale beyond what would be possible using traditional taxonomic methods. This technology does not obviate the need for taxonomic expertise; like all barcoding approaches, individual specimens must be vouchered and identified to species so that a DNA sequence can be used to generate a species-specific probe. An important limitation of this technology is that it will not detect a species for which there is no species-specific probe. To manage this limitation, ReefChip also includes probes for higher taxonomic categories; e.g, a species-specific probe will detect only Mycale armata but the Demospongiae probe will detect any sponge in Class Demospongiae. This makes it possible to know when a taxonomic group is present that does not have a species-specific probe.

BIOEROSION RATES

At each site16 sites (5 at FFS, 5 at PHR, 5 at LIS, 1 at MID), we will retrieve previously installed calcium carbonate blocks (5x5x2cm). In addition, we will deploy 5 blocks at each of 5 sites at KUR. These blocks are attached to Calcification Acidification Units (CAUs), previously installed by NOAA Coral Reef Ecosystem Division (CRED), with cable-ties. CAUs are used by CRED to measure coral accretion rates and are composed of small pieces of rebar and a 5cm x 5cm piece of plastic. In 2011, a single block was attached to each CAU (there were 5 CAUs per site) at each of 15 forereef sites. At Midway, our lagoon site, we attached 20 blocks to non-living parts of the reef. These blocks were cut from dead P. lobata heads that wash up on exposed shores of the main Hawaiian islands. These blocks will act as settling substrate for bioeroders Prior to deployment, calcium carbonate blocks were scanned using an eXplore CT120 µCT scanner at Cornell University. Micro computer-aided tomography is a powerful technology for visualizing the internal structure of solid objects. The exceptional resolution of this technology allows for precise examination of coral skeletal density and the size, shape, and location of each bore hole in a given coral block. By performing pre- and post-deployment scans of the coral blocks, we can accurately measure of the amount of CaCO3 removed to

calculate bioerosion rate, as well as any accretion of CaCO3 by crustose coralline algae. Pre- and post-deployment scans will be aligned and subtracted to show the total volume of lost substrate and the size, shape, and location of excavation sites. The size, shape, and location of the excavation sites will be used to associate particular taxa with particular excavation types. Depending on the organism and the quality of preservation, organisms associated with particular excavation sites will be identified by morphology and/or DNA sequence. We will develop a model based on excavation characteristics that will predict the taxon associated with a particular excavation based on its location and dimensions. Finally, the entire block and all associated tissue will be homogenized and run on ReefChip to describe bioeroder community composition.

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:
We will be collecting pieces of dead Porites spp. skeleton. Dead coral skeleton harbors a diverse community of bioeroding organisms that has not been systematically targeted for study previously in the PMNM. One of the goals of this project is to more thoroughly document the composition of the bioeroding community in the PMNM. Based on studies in the MHI and previous work of the Census of Marine Life, we anticipate a wide variety of sponges and marine worms, as well as hydrozoans, bryozoans, barnacles, tiny mollusks, and turf algae. We expect a subset of these organisms will settle on our deployed blocks of calcium carbonate. Although we cannot give a specific list of the numbers of individual species we will find in samples, we have attached an excel sheet with a list of bioeroders and and other organisms that commonly settle on coral skeleton in Kaneohe Bay, Oahu (based on White 1980 and our own observations).

Scientific name:
dead Porites spp skeleton

& size of specimens:
up to 260 pieces, 5x5x5 cm each (total: 0.0325 cubic meters)

Collection location:
up to 10 pieces per site at 16 sites (5 sites on FFS, 5 sites on PHR, 5 sites on LIS, 1 site at MID); up to 20 pieces per site at 5 sites on KUR

☒ Whole Organism ☒ Partial Organism

9b. What will be done with the specimens after the project has ended?
Preserved samples remain the property of the Monument and will be made available to others requesting access to these materials through the appropriate permit process. PI Donahue will maintain a database of samples and provide for the storage of all samples collected at HIMB until they are consumed by the study or such time as the Monument co-trustees request that they be returned to them. Taxonomic voucher specimens will be submitted for permanent inclusion in the Bishop or Smithsonian museum collections as per the terms of material transfer agreement.

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?
Calcium carbonate blocks and samples of dead coral tissue will be preserved for taxonomic and genetic analyses (in ethanol or saturated salt buffer) and transported back to HIMB aboard the R/V Hi'ialakai. See attached MSDS sheets.

11. Describe collaborative activities to share samples, reduce duplicative sampling, or duplicative research:
All HIMB researchers working on similar species have coordinated to share samples and avoid duplicate sampling. This project reflects this coordination, as a joint effort between the Donahue and Toonen laboratories at HIMB, and NOAA CRED. HIMB and NOAA monument staff hold semiannual meeting and annual meetings with other agencies working in the monument so that research projects and resources available are widely known. To my knowledge, no other

systematic collections of internal bioeroders and measures of bioerosion rates have been made in the Monument.

Anticipated sharing of collections:
Samples of bioeroders in dead Porites spp. skeleton: We anticipate doing most of the sample processing at HIMB, including extracting bioeroding organisms from the samples, most morphological inspection, DNA extraction, and running on the ReefChip microarray. DNA extracted from some samples may be sent out for sequencing to facilities on UH Manoa main campus or off campus. We request permission to share samples with our collaborator, Holly Bolick, at the Bishop Museum, who will be working with us on taxonomic identification (contact information below). For some number of organisms, we may need to send samples to taxonomic experts outside of Hawaii for identification. Taxonomic specialists are few and far between; therefore, we request the flexibility to share specimens with the appropriate specialists to help identify difficult taxa that we cannot identify on our own. These taxonomic specialists are listed below.

Holly Bolick
Collections Manager- IZ
Bishop Museum
[redacted]

Barbara Calcinai
Dipartimento di Scienze del Mare
Universita Politecnica delle Marche
[redacted]

Dale R. Calder
Department of Natural History
Royal Ontario Museum
[redacted]

Rolando Bastida Zavala
Universidad del Mar LABSIM
[redacted]

Leslie Harris, Collections Manager
Research & Collections

Natural History Museum of Los Angeles County
[redacted]

Daphne Fautin
Haworth Hall
[redacted]

Kevin J. Tilbrook
Museum of Tropical Queensland
Queensland Museum
[redacted]

Roger J. Cuffey
Dept. of Paleontology
Pennsylvania State University
[redacted]

12a. List all specialized gear and materials to be used in this activity:
• Divers will use standard open-circuit SCUBA and snorkling equipment.
• We will retrieve calcium carbonate blocks by cutting cable-ties with clippers or a dive knife
• On the ship, samples of dead coral skeleton and calcium carbonate blocks will be placed in plastic containers filled with ethyl alcohol or salt-saturated dimethyl sulfate and dyed with rose bengal.

12b. List all Hazardous Materials you propose to take to and use within the Monument:
Tissue preservative solutions for DNA analyses include: 95% ethanol (EtOH) and saturated salt buffer with dimethylsulfoxide (DMSO). Shipboard, clove oil (5% solution suspended in 95% ethanol) is added to taxonomic samples before preservation. Rose bengal is also added to taxonomic samples shipboard at the time of preservation. MSDS sheets are attached.

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

In 2011, a total of 95 calcium carbonate blocks were attached to either CAUs or bare rock with cable-ties and marine epoxy, respectively. In 2012, we will remove every block deployed in 2011. If the cruise schedule permits, we will also deploy 25 blocks at Kure Atoll to existing NOAA-CRED CAU sites identical to the 2011 deployment.

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

Analysis of bioeroders in the pieces of dead coral skeleton will take up to a year, as it requires dissolution of the calcium carbonate, vouchering of specimens, DNA extraction and sequencing, and running the entire sample on the ReefChip. We anticipate that extraction of organisms, vouchering of specimens, and DNA extraction and sequencing will take place within one year of returning from the cruise, followed by ReefChip microarray analysis of dead coral skeleton samples. Once the calcium carbonate blocks are retrieved in August, 2012, we will immediately send them to the microCT laboratory at Cornell University to be scanned. Upon return, these coral blocks will be homogenized and run on the ReefChip microarray to identify organisms. The analysis of bioeroder communities in environmental samples of dead coral skeleton will be completed and submitted for publication within two years of the cruise. Analysis of bioerosion rates along the Archipelago and analysis of bioeroder community composition will be completed within 1-2 years of this cruise. Regardless of the time to publication, the results from these studies are made available to Monument managers as quickly as possible through the brown-bag luncheons, semi-annual reports, and semi-annual mini symposium during which all researchers involved in this project present the most current findings from their ongoing research to the broader management community. We also reach the NGO community and general public each year with presentations at the Hawaii Conservation Conference, Hanauma Bay seminar series, and other education and outreach venues. In sum, these efforts ensure that research results are provided to the Monument co-trustees almost as quickly as they become available, and made available to the greater management community within no more than 6 months of the data being collected.

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

This is a new project, and we do not yet have published results. Please see the attached CVs for other publications that are not directly related to the project.

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